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Research Paper

Relationships between the Seasonal Variations of Macroinvertebrates, and Land Uses for Biomonitoring in the Xitiaoxi River Watershed, China

key words: biomonitoring, macroinvertebrate community, land uses, seasonal variations, Xitiaoxi River watershed

Abstract

The impacts of differences in watershed land uses, and differences in seasonality on benthic macroinvertebrate communities, were evaluated in 12 stream sites within the Xitiaoxi River watershed, China, from April 2009 to January 2010. The composition of macroinvertebrate community differed significantly among three land use types. Forested sites were characterized by high taxa richness, diversity and the benthic-index of biotic integrity (B-IBI), while farmland and urban disturbed stream sites presented contrary patterns. The percentage of urban land use, conductivity, dissolved oxygen, ammonia nitrogen and total phosphorus were the major drivers for the variations. The land use related water quality stress gradients of the four sampling seasons were determined by means of four independent Principal Component Analyses. The responses of macroinvertebrate community metrics, to anthropogenic stressors, were explored using Spearman Rank Correlation analyses. All the selected metrics, including total numbers of taxa, numbers of Ephemeroptera, Plecoptera and Trichoptera taxa, percentage of non-insect abundance, percentage of scrapers abundance, Pielou's evenness index, Simpson diversity index, and the Benthic Index of Biotic Integrity were correlated significantly with environmental gradients (PC1) in autumn. In other seasons such correlations were less pronounced. Our results imply that autumn is the optimal time to sample macroinvertebrate communities, and to conduct water quality biomonitoring in this subtropical watershed.

1. Introduction

The conversion of forest land into agricultural and urban land uses in a watershed was identified as the major stress on stream ecological conditions (WANG and KANEHL, 2003; ALLAN, 2004). For example, agricultural land uses often result in increased inputs of herbicides/pesticides and fine sediments, loss of riparian complexity and instream habitats, and changes in hydrology (HARDING *et al.*, 1998). Some urban land uses may influence stream

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ecosystems by creating impervious areas, modifying channel morphology, and eliminating intermittent and headwater channels. This may result in modified flows and thermal regimes, increased sediment and nutrient loads, and elevated levels of pollutants (LENAT and CRAWFORD, 1994; SNYDER *et al.*, 2003; WALSH *et al.*, 2005). Biological monitoring and assessment of aquatic systems have been widely conducted for evaluating such human impacts, and the benthic macroinvertebrate community is widely used in biomonitoring and bioassessment for a number of reasons (LINKE *et al.*, 1999). Benthic macroinvertebrates are ubiquitous, long-lived, sensitive to disturbance, and cost-effective to sample, making them ideal biological indicators of aquatic system degradation (ROSENBERG and RESH, 1993). They are commonly used in stream biomonitoring and bioassessment worldwide (RESH, 2008). As biological indicators, macroinvertebrates can provide insights into the current and past conditions of waterbodies and integrate the effects of cumulative stressors (BARBOUR *et al.*, 1999; BONADA *et al.*, 2006). The degradation of physical habitats and water quality ultimately result in the reduction of richness or abundance of intolerant taxa of macroinvertebrates (GARIE and MCINTOSH, 1986; KENNEN, 1999). Although much is known of how these anthropogenic activities have affected stream systems in developed countries, such impacts are regionally specific. This is partially due to the strong influence of regional climate and landscape settings on indicator-disturbance relationships (BRENDEN *et al.*, 2008). Additionally, the socio-economic drivers of anthropogenic activities vary from place to place. Therefore, a thorough understanding of these relationships in China is urgently needed.

Seasonal dynamics of macroinvertebrate assemblages are a natural phenomenon. It is well-known that aquatic insects vary in abundance, taxonomic richness, and diversity with seasons of the year (SIMON, 1999; SPORKA *et al.*, 2006). The phenology of benthic macroinvertebrates (LINKE *et al.*, 1999), and seasonal variations in stream thermal and hydrological regimes (BOULTON and LAKE, 1992), were considered the major contributors to such variations. Consequently, the relationships between macroinvertebrate assemblages and measures of human disturbance appear variable among seasons. However, the influence of anthropogenic activities on macroinvertebrate assemblages occurs throughout the year (PAUL and MEYER, 2001; ALLAN, 2004; HELMS *et al.*, 2009). Hence, a thorough evaluation of the temporal variations in relationships between land uses and macroinvertebrate assemblages is critically important for a better understanding of temporal influences and for choosing the "best time" for effective monitoring, assessment, and management of streams that drain degraded watersheds.

The Taihu Lake watershed located in the Yangtze River delta area is characterized by forest, agriculture, and urban land uses. During the past decades, forested land has been changed into cropland and urban land to meet the needs of population growth and industrial development in this rapidly economic-booming area (MA *et al.*, 1997; WU *et al.*, 2008). Consequently, the watershed has been severely impacted by anthropogenic activities since 2000. Nitrogen and phosphorus runoff from croplands, and untreated sewage from cities and towns, are the main sources of eutrophication (LI, 2004). The Xitiaoxi River is one of the major tributaries transporting nutrients to Taihu Lake. Although efforts have been made to assess the impacts of urbanization on surface runoff, such as stage-discharge relationship on lower river reaches of Xitiaoxi River (LIANG *et al.*, 2008). The influence of land-use changes, on storm runoff in the Xitiaoxi River watershed, and the impacts of land-use changes and seasonal variability on the biota of the Xitiaoxi River watershed are still unknown.

The main goal of our study was to evaluate the effects of different land uses and sampling seasons on stream conditions using macroinvertebrate assemblages. Our specific objectives were 1) to examine how macroinvertebrate metrics are related to different land uses, and determine which environmental variables are potential drivers of macroinvertebrate assemblages, 2) to analyze the seasonal variability of benthic habitat, and water physic-chemical stressors associated with land uses, 3) to explore the response of macroinvertebrate community metrics to water quality stress gradient in all seasons, and (4) to identify the best time for detecting anthropogenic impacts in this subtropical region using macroinvertebrates. We hoped that our objectives could provide a tool on ways government agencies could best use relationships concerning land development, water quality, and biological indicators. This approach would in turn help develop adequate policies and effective management actions, and also maintain a healthy environment which could allow sustainable economic development.

2. Methods

2.1. Study Area

The study area spanned 1800 km^2 of the upper Xitiaoxi River watershed (30.39°–30.87° N, 119.23°–119.88° E) in northwestern Zhejiang province, China (Fig. 1). The Xitiaoxi River has a total length of 157 km and an average slope of 0.45‰ (LI *et al.*, 2006). The study area is characterized by a subtropical monsoon climate and the mean annual temperature ranges between 12.2 and 15.6 °C. The annual precipitation of 1485 mm mostly occurs in June and September (Fig. 2). The discharge of the Xitiaoxi River is closely associated with patterns of rainfall and it peaks from June to July and again in September.

The land cover of the Xitiaoxi River watershed was predominantly forest. However, in recent decades, aggregated agricultural practices, urban development, and tourist activities have modified much of the areas (LI *et al.*, 2003). Except for the majority of the south-eastern part of the watershed, where anthropogenic land use is minimal, much of the remaining areas consist of agricultural and urban land uses.

We sampled the physical habitat, water quality, and macroinvertebrates from twelve second to fourth order stream sites in April, August, and October in 2009 and January in 2010. The watershed area of each the 12 sampling sites ranged from 1.5 to 88 km^2 (average of 28 km^2). All of the study sites had a relatively similar geomorphology and climate. However, they differed in watershed size, and land uses, as well as in associated instream physical habitats and water quality.

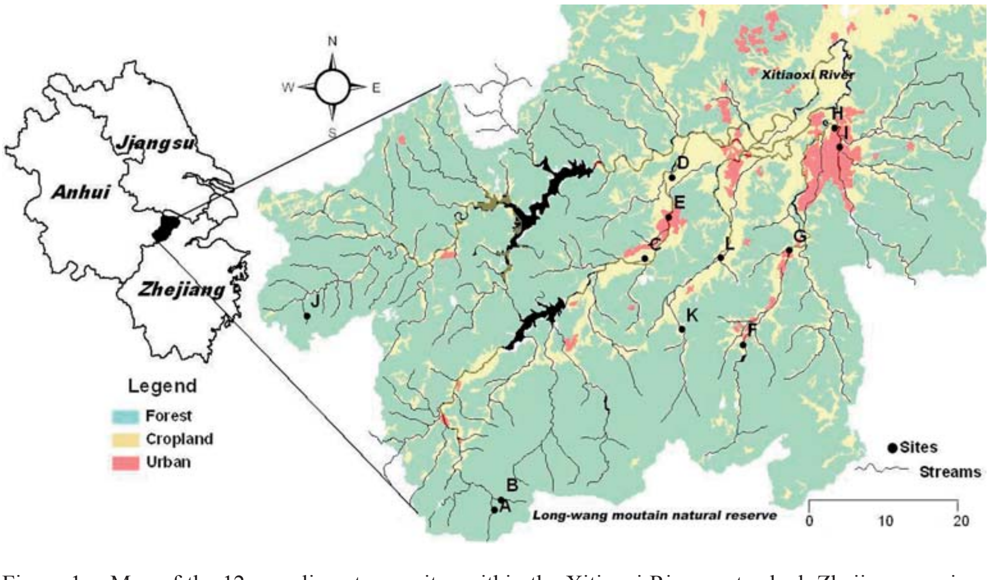

Figure 1. Map of the 12 sampling stream sites within the Xitiaoxi River watershed, Zhejiang province of China.

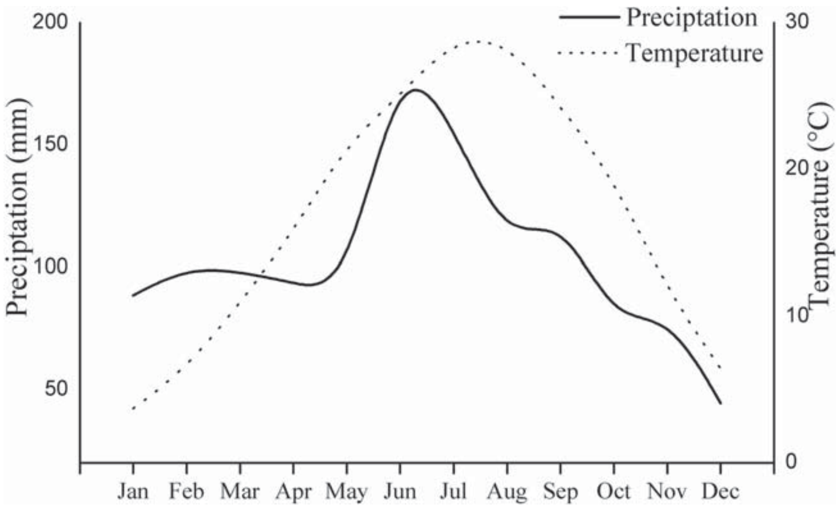

Figure 2. Average monthly precipitation and temperature in the Xitiaoxi River watershed, based on a 5-year average (Jan. 2006–Dec.2010) from Huzhou station.

2.2. Physical Habitat and Water Quality Sampling

At each site, water temperature, pH, and conductivity were measured using a Hanna HI98129 portable meter (HANNA Instrument, Italy). Dissolved oxygen (DO) concentrations were measured using a Hanna HI 9147. Water current velocity, mean depth, and wetted width were quantified on transects with equal distance interval across channel sections (SONG *et al.*, 2009). Current velocity was measured using a flow probe (FP101, Global Water).

Water samples for water chemistry were collected and analyzed for chemical oxygen demand (COD), and for concentrations of total nitrogen (TN), total phosphorus (TP), nitrate nitrogen (NO_3-ubstrate composition was quantified by estimating proportional cover of sand, gravel,cobble,boulder and bedrock.nd HI98130.N), and ammonia nitrogen (NH_4^+). Our analysis followed standard methods (MEPAC 2002). The instream substratum of the study reaches was classified using particle size distributions of surface substrate (WOLMAN, 1954; KONDOLF, 1997). We recorded the estimated percentage of each category: sand (< 2 mm), gravel (2–64 mm), cobble (65–256 mm), and boulder (> 256 mm).

2.3. Macroinvertebrate Sampling

Quantitative macroinvertebrate samples were taken with a modified Surber sampler (0.09 m^2; 250 µm mesh size). Benthic macroinvertebrates were collected from pool and riffle habitats at each sampling site. Riffle samples were collected by placing the sampler on the stream bottom and disturbing the substratum by kicking and rubbing individual rocks in an area (0.09 m^2) immediately upstream. Pool samples were collected by disturbing the substratum and the dislodged material was collected in the Surber net (HILSENHOFF, 1987; WANG and KANEHL, 2003). Three replicate samples were randomly collected from each riffle habitat and two from each pool habitat at each site. All the five samples from riffles and pools were combined in the field.

Samples were rinsed with stream water to remove fine sediments, placed in plastic sampling bags, and then preserved with 4% formaldehyde. In the laboratory, macroinvertebrates were sorted using a dissecting microscope at 10 times magnification. Most organisms were identified to lowest taxonomy possible (usually species or genus). Midges (Diptera: Chironomidae) were mounted on slides in an appropriate medium and identified to subfamily or genus.

2.4. Watershed Land Use Delineation

We delineated watershed boundaries upstream of each sampling site using ARC/INFO 9.3.1 (ESRI, 2008) and automated procedures based on a Digital Elevation Model with a 25-m resolution. These sub-watershed boundaries were then manually verified and corrected by referencing 1:50,000 digital topographic maps. We quantified the land use within each watershed upstream of the sampling site by overlaying watershed boundaries on top of a regional land-use vector layer. This land-use layer is a land cover map that was originated in 2007, for Zhejiang Province, and was collected by the Landsat Thematic Mapper with a 30-m resolution using ERDAS Imagine software, version 9.1 (ESRI, 2006). Additionally, we digitized the land uses of doubtful areas of the watersheds with the aid of Google Maps (2009). The Satellite Maps of the study region were produced in 2007 and 2009, with a ground resolution of 2.5 m or less. By combining ARC/INFO and Google Earth processes, we digitized and refined all the watershed land uses for the study area.

2.5. Statistical Analyses

In order to examine relationships among the twelve sites based on their macroinvertebrate assemblage composition, we first performed a cluster analysis based on the Bray-Curtis similarity calculated from taxon abundance. We then tested the differences in macroinvertebrate metric values among the three groups of sites identified by the cluster analysis using non-parametric ANOVA. To explore the major environmental drivers of macroinvertebrate assemblage composition, the redundancy analysis (RDA) was carried out using the Canoco (version 4.5; Ter Braak and Smilauer, 2002).

Four independent principal component analyses (PCA) were conducted using the physical habitat, water physicochemical features, and land uses to assess environmental stress gradients for the four seasons. The stress gradients were determined by evaluating the principal components that indicated water quality impairment. Variables not highly correlated (Spearman rank correlation coefficient < 0.7) to each other were used in the construction of the stress gradients to reduce redundancy in the PCA axis.

Community responses of macroinvertebrates to water quality stress gradients, were assessed by testing the relationships between the eight selected macroinvertebrate metrics and the PCA score. We used nonparametric correlations (Spearman Rank Correlation Coefficient).

ANOVA and correlation analyses were performed using the SPSS software package (version 16.0; SPSS INC., 2007). The cluster analysis and PCA were carried out using the PRIMER v6 statistical package (Clarke *et al.*, 2006). Data sets were tested for normality, and where necessary, log $(x + 1)$ transformation were performed to minimize the influence of non-normality on statistical results.

3. Results

3.1. Physicochemical Variables and Macroinvertebrate Metrics among Different Groups

Cluster analysis showed three obviously different groups (Fig. 3). Group I was composed of low order sites with their highest proportion of forest land use and narrower channels. Sites in Group II were characterized by wider channel reaches and higher percentages of farmland (average percentage > 20). Group III sites were regarded as those with highest percentages of urban land use (average percentage > 25) (Table 1). Thus, these three groups were referred to as the “reference group”, “agricultural group”, and “urban group”, respectively.

Five sub-watershed variables, seven physical habitat measures, and nine water quality features were statistical summarized (Table 1), and eight macroinvertebrate community metrics were analyzed (Table 2). The study sites had variable levels of anthropogenic land use, physical habitats, and water quality values (Table 1). Non parametric ANOVA analysis showed (Table 2) that all the macroinvertebrate metrics, except for Simpson diversity index (λ’), were significantly different between site groups. Macroinvertebrate numbers of taxa (S),

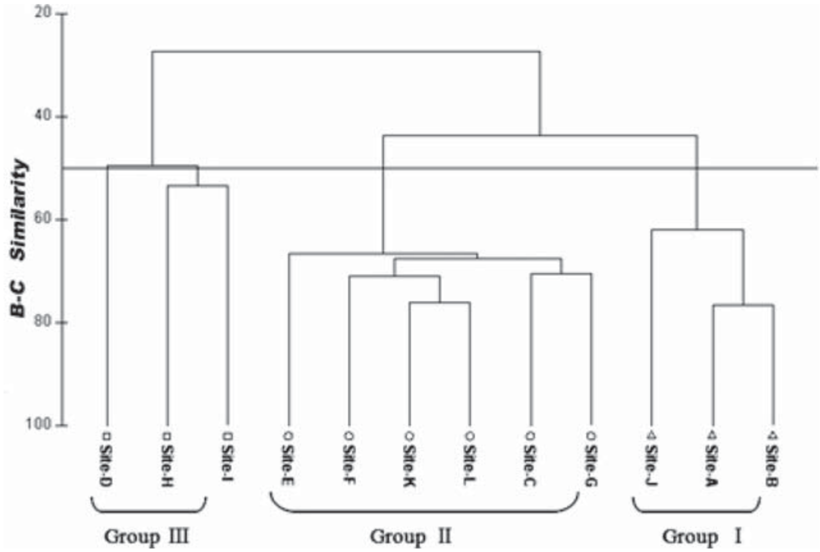

Figure 3. Cluster dendrogram based on Bray-Curtis similarity of the taxon abundance from sites for Xitiaoxi River watershed from April 2009 to January 2010. The line indicates the linkage similarity cut-off level for determining cluster groups (Group I – reference group, Group II – agricultural group and Group III – urban group).

Table 1. Sub-watershed features, physical habitat variables, and water quality characteristics for the three groups of sampling sites identified a gradient analysis for the Xitiaoxi River watershed. Except for the sub-watershed variables, all the other factors were averaged (Mean ± SE).

Name	Abbreviation	Forest	Agriculture	Urban
Sub-watershed variables				
Watershed Area (km^2)	Area	2.3 ± 0.9	24.1 ± 6.3	60.4 ± 25.3
Altitude (m)	Alit	430 ± 116	73 ± 18	21 ± 9
Percentage of Forest Land	% Forest	99 ± 0	67 ± 5	58 ± 0.5
Percentage of Farmland	% Farmland	0.2 ± 0.1	21.4 ± 3.9	16.2 ± 2.1
Percentage of Urban Land	% Urban	0	11.3 ± 3.7	25.8 ± 1.7
Physical Habitat				
Mean Water Depth (m)	MD	0.18 ± 0.05	0.21 ± 0.01	0.23 ± 0.01
Mean Wetted Width (m)	MW	1.7 ± 0.3	10.6 ± 2.0	7.4 ± 2.0
Mean Flow Velocity (m/s)	MV	0.52 ± 0.02	0.73 ± 0.09	0.46 ± 0.30
Percentage of Sand Substrate	% Sand	2.6 ± 2	3.7 ± 1	21.1 ± 15
Percentage of Gravel Substrate	% Gravel	28.5 ± 8	46.2 ± 5	45.4 ± 18
Percentage of Cobble Substrate	% Cobble	32.3 ± 7	39.3 ± 5	26.2 ± 2
Percentage of Boulder Substrate	% Boulder	36.4 ± 18	10.9 ± 4	7.3 ± 4
Water Quality				
Mean Temperature (°C)	T	14.6 ± 0.7	19.1 ± 0.6	19.3 ± 0.2
Mean Dissolved Oxygen (mg/L)	DO	8.6 ± 0.2	6.3 ± 0.3	6.2 ± 1.3
Mean Stream Water pH	pH	7.4 ± 0.2	8.4 ± 0.2	7.7 ± 0.2
Mean Conductivity(μs/cm)	Cond	54.3 ± 29.5	136.5 ± 20.2	205.8 ± 41.7
Mean Total Nitrogen (mg/L)	TN	1.4 ± 0.3	1.5 ± 0.1	4.5 ± 1.3
Mean Total Phosphorus(mg/L)	TP	0.03 ± 0.01	0.06 ± 0.01	0.26 ± 0.16
Mean NH_4^+ Concentration (mg/L)	NH_4^+	0.07 ± 0.01	0.32 ± 0.08	3 ± 0.8
Mean NO_3^- Concentration (mg/L)	NO_3^-	1.4 ± 0.3	1.02 ± 0.1	1.3 ± 0.3
Mean Chemical Oxygen Demand (mg/L)	COD_{Mn}	1.4 ± 0.1	2.1 ± 0.3	4.3 ± 1.3

Table 2. The average value (Mean ± SE) of macroinvertebrate metrics for the three types of streams, and their expected response to water quality deterioration.

Metrics	Acronym	Forest	Agriculture	Urban	Response	reference
Taxonomic Measures						
Total number of macroinvertebrate taxa	S*	54 ± 3	31 ± 1	19 ± 3	Decrease	VINSON and HAWKINS, 1998
Total taxa of Ephemeroptera, Plecoptera and Trichoptera	EPT*	29 ± 2	15 ± 1	4 ± 1	Decrease	SILVEIRA *et al.*, 2005
Percent abundance of Non-insects	% Non-insect*	0.5 ± 0.1	4.4 ± 1.5	44.5 ± 7.2	Increase	MARIO *et al.*, 2010
Community Diversity index						
Simpson diversity index	λ’	0.9 ± 0	0.8 ± 0	0.7 ± 0	Decrease	ROSENBERG and RESH, 1993
Pielou’s evenness index	J’*	0.57 ± 0	0.59 ± 0	0.61 ± 0	Increase	MAGURRAN, 1988
Functional Feeding Group measures						
Percentage of scrapers	% Scrapers*	14.0 ± 1.9	8.8 ± 1.4	2.5 ± 0.9	Decrease	VANNOTE *et al.*, 1980
Percentage of collectors and gatherers	% C-G*	47.0 ± 0.5	58.9 ± 1.8	86.4 ± 2.9	Increase	VANNOTE *et al.*, 1980
Biotic Index						
Benthic-Index of Biotic Integrity	B-IBI*	4.7 ± 0.4	3.1 ± 0.1	2.4 ± 0.1	Decrease	WANG *et al.*, 2005

* indicates significant differences between disturbed (agricultural and urbanized) and reference streams based on nonparametric ANOVA analysis ($p < 0.01$).

numbers of Ephemeroptera, Plecoptera and Trichoptera taxa (EPT), percentage of scrapers (% Scrapers) and benthic-Index of biotic integrity (B-IBI) were higher in the "reference group" than in the "disturbed group" (agricultural and urban groups) ($P < 0.01$). However, the percentage of abundance of non-insect (% Non-insect), Pielou's evenness index (J') and percentage of collectors and gatherers (% C-G) were lower in the "reference group" than in the "disturbed groups" ($P < 0.01$).

3.2. Drivers of Macroinvertebrate Assemblage Composition

The RDA (redundancy analysis) forward selection procedures identified the urban percentage, conductivity, dissolved oxygen, ammonia nitrogen, and total phosphorus (which, of the 19 environmental variables) as significant predictors of macroinvertebrate assemblage measures ($P < 0.05$). These selected environmental variables explained 65% of the variation in macroinvertebrate communities in the consequent constrained ordination analysis of samples. The first RDA axis showed that urban percentage use, ammonia nitrogen and total phosphorus were positively associated with the % Non-insect, J' and % C-G, and were negatively correlated with S, EPT and λ (Fig. 4)'. The second RDA axis indicated that % Scrapers and B-IBI were positively correlated to DO and negatively correlated to conductivity. This analysis implied that these (land use-associated stream water) physicochemical factors are potential drivers of macroinvertebrate assemblage composition.

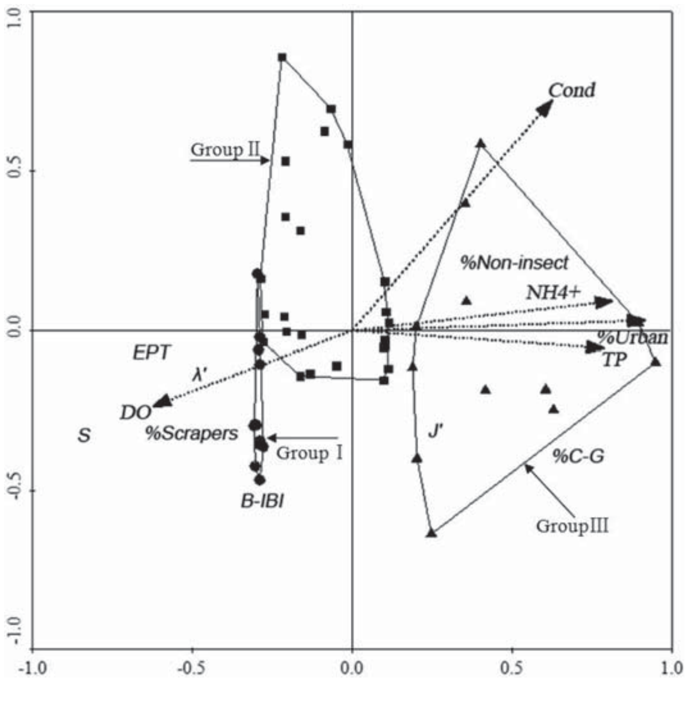

Figure 4. Plots of the first two axes of redundancy analysis between environmental variables and macroinvertebrate metrics of the twelve study sites for the four sampling seasons. Solid circles = sites Group I (reference sites), squares = sites Group II (agricultural sites), and triangles = sites Group III (urban sites). See Tables 1 and 2 for variable explanations.

3.3. Environmental Gradients Among Four Seasons

The first two principal components of the PCA explained 65.1%, 65.1%, 65.6% and 64.5% of the total environmental variance in spring, summer, autumn, and winter, respectively (Fig. 5). For spring, the first principal component (PC1: explained 46.6% variance) showed positive significant correlations with chemical oxygen demand, ammonia nitrogen, and conductivity and negative correlations with the dissolved oxygen concentration. The second principal component (PC2) indicated negative correlations with the concentrations of nitrate nitrogen and total nitrogen, percent urban use, and % Gravel substrate. For summer, PC1 (explained 42.9% variance) showed significant positive correlations with chemical oxygen demand, total nitrogen, and % Sand substrate and negative correlation with dissolved oxygen concentrations. However, PC2 correlated positively with the percentage of forest, and negatively with water temperature and mean flow velocity. For autumn, PC1 (explained 40.2% variance) showed significant positive correlations with temperature, total nitrogen, and % Sand substrate and a negative correlation with the concentration of dissolved oxygen. The PC2 was negatively associated with mean flow velocity and the percentage of farmland. For winter, PC1 (explained 43.9% variance) showed positive significant correlations with total nitrogen, conductivity, mean water depth, % Sand substrate, and percent urban land use

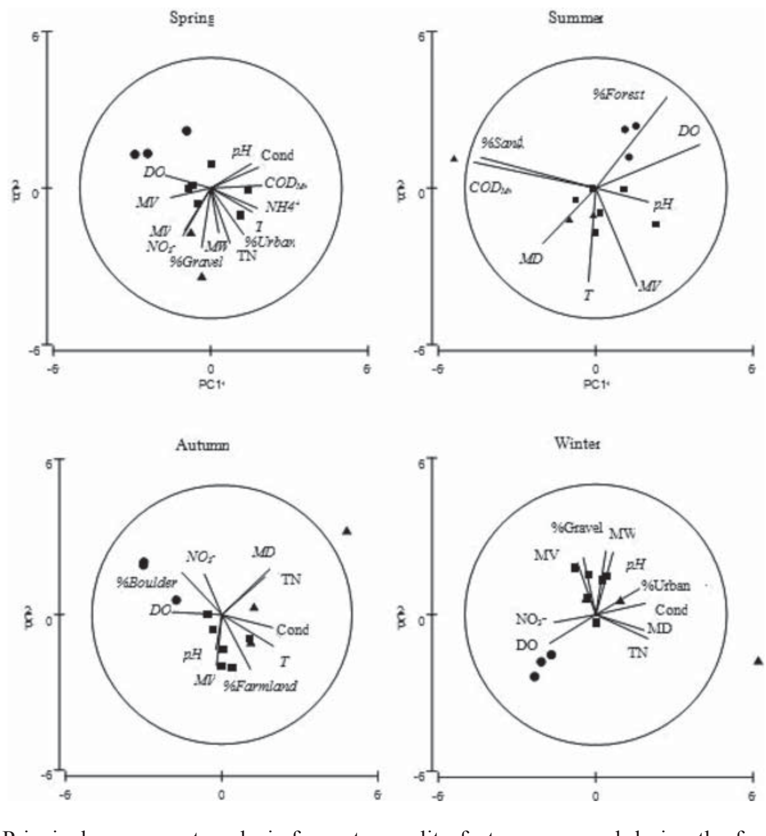

Figure 5. Principal component analysis for water quality factors measured during the four seasons in Xitiaoxi River system. Solid circles = sites Group I (reference sites), squares = sites Group II (agricultural sites), and triangles = sites Group III (urban sites). See Tables 1 and 2 for variable explanations.

Table 3. Coefficients of Spearman's rank correlation between environmental gradient (PC1 score) and macroinvertebrate metrics for the Xitiaoxi River system during the four sampling seasons (*P < 0.05, **P = 0.01).

	Environmental Gradient (PC1 Score)			
	Spring	Summer	Autumn	Winter
S	–0.580*	–0.564	–0.835**	–0.664**
EPT	–0.621*	–0.634*	–0.900**	–0.727**
%Non-insect	0.587*	0.416	0.860**	0.483
%Scrapers	–0.336	–0.832**	–0.727**	–0.517
%C-G	0.469	0.385	0.692*	0.147
λ'	–0.328	–0.331	–0.655*	–0.591*
J'	–0.305	–0.155	0.655*	0.046
B-IBI	–0.762**	–0.678*	–0.790**	–0.636*

and negative correlations with the concentrations dissolved oxygen. The PC2 was negatively correlated with pH, mean wetted channel width, mean flow velocity, and % Gravel substrate. In relation to environmental gradients, sampling sites of different groups were well separated (Fig. 5), indicating that intra-group physical habitat and water quality characteristics were similar.

3.4. Responses of Macroinvertebrate Metrics to Environmental Gradients

The relationships among macroinvertebrate assemblage metrics, and environmental gradients varied seasonally. In autumn, all the eight metrics (S, EPT, % Non-insect, % Scrapers, % C-G, λ', J' and B-IBI) were significantly correlated to the environmental gradient (the first PCA component, Table 3). However, only four metrics (S, EPT, % Non-insect and B-IBI) in spring, four metrics (S, EPT, λ' and B-IBI) in winter, and three metrics (EPT, % Scrapers and B-IBI) in summer were significantly correlated to the environmental gradient.

4. Discussion

Our study showed that urban and agricultural disturbed streams were characterized by lower taxa richness and higher abundance of tolerant macroinvertebrate assemblages compared to forest dominated streams. The percentage of urban land use, conductivity, dissolved oxygen, ammonia nitrogen, and total phosphorus were major drivers in shaping the composition of the macroinvertebrate assemblage. Autumn is the most informative period for macroinvertebrate sampling and bioassessment in Xitiaoxi River watershed. During that period, all the measured macroinvertebrate metrics were significantly related to environmental gradients.

4.1. Land Uses Effects on Stream Physicochemical Characteristics and Macroinvertebrate Assemblages

Agricultural and urban land uses were well documented in the degradation of stream physicochemical conditions and macroinvertebrate assemblages (Meyer *et al.*, 2005). The sites dominated by forest watersheds were characterized by higher dissolved oxygen concentrations. Sites dominated by agricultural or urban watersheds had higher nutrients concentrations, wider stream channels, and finer substrate particles. The predictable physicochemical effects of land uses have been identified as a feature of the "urban stream syndrome" (MEYER *et al.*, 2005). These observations may reflect the removed riparian zone and the altered stream hydrological regime associated with land use. During the field surveys, we observed that sampling sites, with agriculture and urban dominated watersheds, had very little or no vegetated riparian buffer. That presumably reflects the increased sediment load, nutrients, and other contaminants associated with the urban or agricultural lands surface runoff. The positive association of wetted channel width, and the negative association of substrate size with anthropogenic land uses found in this study, were commonly noted within human disturbed landscapes reported in other studies (PAUL and MEYER, 2001; ROY *et al.*, 2003). However, in our study, the nitrate nitrogen concentrations for the sites for forest dominated watersheds were higher (1.4 mg/L) than sites for agricultural (1.02 mg/L) or urban (1.3 mg/L) dominated watersheds. This result differs from the previous findings (*e.g.*, HELMS *et al.*, 2009), implying that our study streams may have higher background concentrations of nitrogen.

From the numerous macroinvertebrate measures that have been used by biomonitoring and assessment worldwide, our study evaluated eight macroinvertebrate assemblage metrics that represent taxonomic measures, diversity indices, functional feeding indices, and indices of biotic integrity. These macroinvertebrate metrics we selected were commonly used in biomonitoring programs in China (WANG *et al.*, 2005; ZHANG *et al.*, 2007; ZHOU *et al.*, 2009), and were selected against the criteria that measure the overall biotic information with minimal redundancy (SURIANO *et al.*, 2011). Although some of the metrics we used appear redundant, they measure different aspects of macroinvertebrate assemblage structure, function, and process. Our results demonstrated that watershed land uses had significant impacts on stream macroinvertebrate communities. These impacts included negative effects of agricultural and urban land uses, and positive effects of forest land use, on the benthic-index of biotic integrity, macroinvertebrate taxa richness, numbers of Ephemeroptera, Plecoptera and Trichoptera taxa, and percentage of scrapers. These findings were consistent with the other studies that assessed impacts of different land uses on macroinvertebrates (*e.g.*, SHAVER *et al.*, 1995; STEPENUCK *et al.*, 2002). However, the major difference between our and previous studies was that we contrasted forest land use with agricultural and urban land use. Other studies only contrasted forest land uses with one type of the anthropogenic land uses, agricultural land uses (BARTON, 1996) or urban land uses (LENAT and CRAWFORD, 1994; HORNER *et al.*, 1996). We think that our study is the first of this kind to assess the impacts of increased anthropogenic land uses (both agricultural and urbanized land uses) of watersheds on the physicochemical conditions of stream and their macroinvertebrate assemblages.

4.2. Potential Drivers of Macroinvertebrate Assemblages

The high taxa richness, and abundance of intolerant taxa of macroinvertebrate assemblages, were found at forest sites dominated by low concentrations of ammonia nitrogen, total nitrogen, and high concentrations of dissolved oxygen (Fig. 4). These results suggest that water physicochemical gradients, associated with land use in the watersheds, directly influence the assemblage of stream macroinvertebrates. This conclusion is supported by numerous studies that suggested that physicochemical conditions are the primary factors influencing the abundance and composition of stream faunal communities (CUFFNEY *et al.*, 2000; ROGERS *et al.*, 2002; FRAPPIER *et al.*, 2007). It has been shown that the impacts of urban land use are mainly from creating impervious areas that increase surface runoff. This, in turn, alters stream thermal and hydrological regimes, and brings in sediment, toxicants, and organic and inorganic pollutants from urban landscapes (Wang *et al.*, 2001; Stepenuck *et al.*, 2002; Wang and Kanehl 2003). The relationships between stream biotic integrity and impervious area have been documented, and the thresholds of percentage of impervious areas, within a watershed, to macroinvertebrate communities, have also been explored in many other studies (Benke *et al.*, 1981; Shaver *et al.*, 1995). In our study, due to the limited sampling sites, we did not quantify the association between percentages of urban land use and macroinvertebrate metrics. This deserves further investigation. Low dissolved oxygen values have been linked with the elimination of sensitive taxa from streams (Closs and Lake, 1994). Connolly *et al.* (2004) found that a reduction in the emergence of aquatic insect taxa at intermediate dissolved oxygen levels (25–35%, and 10–20% saturation). As in other studies, we observed elevated conductivities (Li *et al.*, 2006; Wu *et al.*, 2010), total phosphorus concentrations (Davis *et al.*, 2003; Morgan *et al.*, 2007), ammonia nitrogen (Zhao *et al.*, 2009; Wu *et al.*, 2010), and decreased biotic integrity with increased urban land use. Strong associations between nutrients concentrations and macroinvertebrate measures suggest that these water quality variables are useful proxies for indicating non-point source pollutants and other anthropogenic disturbances. It has been reported that the thresholds of relationships between B-IBI or EPT and total nitrogen are about 1.409 mg/L and between B-IBI or EPT and total phosphorus are about 0.033–0.035 mg/L in the same study area (Wu *et al.* 2010). Beyond these threshold concentrations, stream benthic macroinvertebrate measures degrade dramatically. Therefore, these specific environmental measures could be potentially useful for establishing stream protection and rehabilitation criteria, and for watershed environmental monitoring and assessment.

4.3. Identify the Best Season for Macroinvertebrate Sampling and Biomonitoring

The development of biomonitoring programs of macroinvertebrates requires the understanding of responses of biological communities to anthropogenic stressors (Suriano *et al.*, 2011). However, there is still much debate regarding the predictability of the responses of the macroinvertebrate community to different disturbances (Bonada *et al.*, 2006). The temporal variation of macroinvertebrate metrics might result in different response patterns to a set of existed environmental variables among seasons. Because carrying out biomonitoring in all seasons is resource prohibitive, it is critical to determine the best season, for utilizing the selected maroinvertebrate indicators for the biomonitoring. In our study, all the eight macroinvertebrate assemblage metrics correlated strongly with the measured water quality gradients in the fall. This implied that the fall sampling met our biomonitoring needs. Sampling benthic macroinvertebrate in the fall also improves the identification of macroinvertebrates due to their life stage (Wang *et al.*, 2001). Our study indicated that only three macroinvertebrate assemblage metrics (EPT, % Scrapers, and B-IBI) correlated significantly with water quality gradients for the summer samples. This suggests that summer sampling may be ineffective for biomonitoring using macroinvertebrates. One of the potential reasons, of such week responses of macroinvertebrates to disturbance gradients, is that most precipitation, in the studied subtropical monsoon watersheds, occurs in the summer (Fig. 2). High precipitation could dilute the concentration of pollutants, washed from urban or agricultural watersheds, and could also wash away benthic communities. Similar observations were also reported by others (Morais *et al.*, 2004; Sporka *et al.*, 2006; Mario *et al.*, 2010). Hence, it seems that periods of high precipitation are not reliable for biomonitoring. Our study also showed that four of our evaluated macroinvertebrate metrics were significantly correlated with water quality gradients. Interestingly, none of the functional feeding group measures, nor community diversity indices, correlated with water quality gradients. Although functional feeding metrics have been suggested as useful for assessing stream conditions (Cummins *et al.*, 2005), our results imply that spring sampling alone has some strong limitations for biomonitoring. For winter samples, although four macroinvertebrate metrics (Table 3) were correlated with water quality gradients, we considered winter sampling as not suitable because of the difficulties working in cold weather with snow or ice.

By linking sensitive macroinvertebrate assemblage indicators, with instream physicochemical stressors and watershed land uses for different seasons, our results have some important implications for the biomonitoring and watershed management programs of the subtropical climate region of China in general and for the Xitiaoxi River watershed in particular. First, the eight macroinvertebrate assemblage metrics we evaluated are effective indicators for assessing watershed land uses and their influence on stream physicochemical and biological conditions. Hence, they can be used as criteria for watershed management to protect natural, enhance impacted, and rehabilitate degraded stream systems. Second, the biomonitoring sampling is most effective during the fall because all eight macroinvertebrate metrics we tested are sensitive to watershed land uses and instream condition. When samples are taken from the other seasons, caution is needed when interpreting biomonitoring results, because only some macroinvertebrate metrics are responsive to watershed land uses and instream stressors. Finally, although our study did not evaluate the variation of benthic organisms among years (ROBINSON *et al.*, 2000; MONK *et al.*, 2008), other studies have reported that fluctuations in annual rainfall can be an influential factor on year-to-year variation in macroinvertebrate assemblages (TOWNSEND *et al.*, 1997; MONK *et al.*, 2008). Hence, multiple-year sampling provides more robotic biominitoring results, when resources are available, and can improve our understanding how climate change will influence the design of streams biomonitoring programs and the resultant impact on the interpretation of bioassessment in the subtropical region of China.

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