

1 **The effect of habitat restoration on macroinvertebrate communities in Shaoxi rivers, China**

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36 **Abstract**

37 In recent decades, the biodiversity of freshwater environments has decreased sharply due to anthropogenic
38 disturbances that damaged ecosystem structures and functions. Habitat restoration has emerged as an important
39 method to mitigate the degradation of river ecosystems. Although in many cases a post-project monitoring has been
40 promoted to access the restoration progress, it is still unclear how aquatic community changes following river habitat
41 restoration in China. Macroinvertebrate communities intermediately positioned within ecosystem food webs play a
42 key role in ecosystem processes within river ecosystem, driving energy flow and nutrient cycling. Here, benthic
43 macroinvertebrates is used as bio-indicators to assess the ecosystem health of degraded urban rivers, restored urban
44 rivers, and undisturbed rivers. This study aims to determine: (i) how habitat restoration influence macroinvertebrates
45 diversity and how this compared to degraded and reference conditions; (ii) how did macroinvertebrate community
46 compositions differ in restored, degraded and reference sites; (iii) the environmental factors shaping macroinvertebrate
47 communities. Habitat restoration significantly increased the diversity and richness of macroinvertebrate community
48 and intolerant species, and shifted the community composition towards reference status. Habitat characteristics and
49 water chemistry, including substrate diversity, water velocity, and both nutrients (TN) and organic pollutants (TOC),
50 appeared to shape the turnover of these communities. Habitat characteristics contributed to most of the variation of
51 the entire macroinvertebrate community. Our research indicates that habitat restoration is an efficient approach to
52 restore the aquatic community and hence improve river ecosystem health for freshwater conservation and sustainable
53 management in Zhejiang province. This study strengthens our understanding of the changes of macroinvertebrate
54 community after habitat restoration and important controlling variables that attribute to these changes, which provides
55 an important guidance for future freshwater management.

56

57 **Keywords:** macroinvertebrate community compositions, bio-indicator, habitat restoration, monitoring, river
58 ecosystem, Zhejiang Province, China

59

60 **Introduction**

61 Anthropogenic disturbances, such as urbanization, damming, water withdrawal and pollution, have sharply increased
62 in the past centuries, which markedly damaged freshwater ecosystem structure and decreased biodiversity (Zhang et
63 al. 2019). To mitigate and prevent the degradation of river ecosystems, habitat restoration has emerged as a key activity
64 around the world (Geist and Hawkins 2016). The aim of habitat restoration is to improve the ecosystem health of
65 freshwater systems through enhancing habitat complexity and heterogeneity, thus sustain the ecosystem from human
66 disturbance. To this end, process-based restoration that focuses on correcting anthropogenic disruptions to driving
67 processes arose as important measure to recover the river habitats in the last 20 years (Beechie et al. 2012). Restoration
68 approach such as river channel re-meandering is applied in some projects to shape the heterogenous river habitat
69 indirectly (Garcia et al. 2012; Lorenze et al. 2016), channel reconfiguration measures including riverbed
70 reconstruction, adding both in-stream islands and aquatic vegetation, and increasing flood plain areas are widely
71 included in restoration strategies of urban rivers to reconstruct the river habitat directly (Bernhardt et al. 2007; Palmer
72 et al. 2014; Martín et al. 2018). In combination, these treatments should enhance substrate and hydraulic heterogeneity,
73 increasing both specific aquatic habitat and food availability (Laasonen et al. 1998; Lepori et al. 2005; Miller et al.
74 2010).

75 Different types of riverine habitats are known to influence the community composition of aquatic organisms such as
76 fish and macroinvertebrates, attributing to the variance of river hydromorphology, substrate composition and
77 environmental condition at the reach scale (Zhang et al. 2009; Kail et al. 2015). Many studies measured benthic
78 biological indicators (i.e. microbes, algae, invertebrates) to assess the structural integrity and ecosystem health
79 following habitat restoration (Frainer et al. 2017; Schmutz et al. 2016; Kail et al. 2016). Evidence accumulated
80 indicated that aquatic rehabilitation would improve habitat condition and water quality for aquatic biota through
81 restructuring heterogeneous habitat, re-introducing aquatic plants, riparian zone re-forestation, etc. (Miller et al. 2010;
82 Kail et al. 2015). However, evidence of ecological improvements associated with habitat restoration have been highly
83 varied due to the natural variability of the system studied (Miller et al. 2010; Louhi et al. 2011; Zan et al. 2017), the
84 response of benthic aquatic communities to habitat restoration remains unclear in China. Therefore, it is imperative to
85 obtain a better understanding of restoration effects and the underlying ecological mechanisms. Some information
86 could be gained to better understand this restoration progress using a before-after (BA), before- after-control-impact
87 (BACI), or control-impact (CI) approach, hence provide sufficient evidence for post river management and
88 improvement of future endeavors.

89 Macroinvertebrate communities are composed of a range of species that tolerate a wide range of environmental
90 conditions (Plafkin et al. 1989). Intermediately positioned within ecosystem food webs in river ecosystems,
91 macroinvertebrate play a key role in ecosystem processes such as nutrient cycling and energy flow (Zhang et al. 2004;
92 Strayer 2006; Duan et al. 2010). Stream macroinvertebrates are generally recognized as good biological indicators of
93 water quality (Hilsenhoff 1988) and ecosystem health (Karr 1999), because of their availability in most freshwater
94 ecosystems, and their sensitivity to environmental changes such as disturbance, deterioration, and improvement
95 (Zhang et al. 2010; Li et al. 2015). They can reflect the relative long-term temporal and spatial changes of river

96 ecosystems and can be early warning indicators of environmental pressures given that they are such a diverse group
97 containing a high number of species with a large variability in ecological requirements (Smith et al. 1999; Shao et al.
98 2006; Dos et al. 2011). Hence, macroinvertebrates are frequently used as indicators of restoration efficiency (Spänhoff
99 and Arle 2007; Besacier- Monbertrand et al. 2014).

100 The use of macroinvertebrates as bio-indicators for restoration have been studied in Europe and North America (Kail
101 et al. 2015; Zan, Kondolf and Rioustouma 2017), but there have been few assessments of restoration in Asia and, in
102 particular China (Li et al. 2015; Lin et al. 2020). Although the restoration-related effect on macroinvertebrate
103 communities should be theoretically positive with the increase of habitat heterogeneity (Miller et al. 2010), as features
104 of river habitat may influence detritus (Douglas and Lake 1994; Taniguchi and Tokeshi 2004), epiphytic algae (Dudley
105 et al. 1986), and form ‘refuges’ from high flow conditions for predators (Lake 2000; Taniguchi and Tokeshi 2004),
106 observed changes have been inconsistent with the scale and specific metrics assessed (Palmer et al. 2010; Ernst et al.
107 2012). The results may also differ when investigating rivers with diverse and complex conditions, especially in China,
108 where land use change posed varying degree of habitat degradation and water pollution in river ecosystems (Zhang et
109 al. 2010; Knouft and Chu 2015).

110 In this study, macroinvertebrate communities of three river groups were compared, (1) degraded urban rivers, (2)
111 urban rivers undergoing habitat restoration and (3) undisturbed rivers (i.e., reference conditions), essentially providing
112 a gradient from severely damaged to near-natural conditions. Within each river, a range of habitat features, physico-
113 chemical factors, spatial factors were measured, and macroinvertebrate communities were sampled. Through
114 comparing the relationship between macroinvertebrate community composition and environmental variables along
115 this simple gradient, this study intends to determine: (i) how habitat restoration impacts on benthic macroinvertebrates
116 diversity and how this compared to degraded and reference conditions; (ii) how did macroinvertebrate community
117 compositions differ in restored relative to degraded and reference sites; (iii) the environmental factors shaping
118 macroinvertebrate communities across the three river groups. We hypothesized habitat restoration would shift the
119 benthic macroinvertebrate community, the macroinvertebrate diversity and richness would increase, and there would
120 be an improvement in both water quality and availability of aquatic habitats following the restructuring of
121 heterogeneous habitat, re-introducing of aquatic macrophytes and riparian zone re-forestation. Moreover, some
122 tolerant species that are dominants in degraded urban rivers will be replaced by Ephemeroptera, Plecoptera, and
123 Trichoptera species (EPT) that are sensitive to external disturbance. Substrate composition, water flow velocity and
124 physico-chemical variables were hypothesized to be the main factors affecting any change in macroinvertebrate
125 community composition.

126

127 **Materials and Methods**

128 **Study sites**

129 Control-impact approach was used for this study. Accordingly, three groups of rivers selected from the same
130 catchment (Shaoxi River) in Anji, Zhejiang Province PRC were investigated from July 8th to August 15th, 2018, each

131 group with three different rivers. Three river groups (Fig. 1, Table S1) include (i) undisturbed rivers (reference sites,
132 denoted F), (ii) urban rivers undergoing habitat restoration in the last seven years (denoted R); and (iii) degraded urban
133 rivers (denoted D). Spatial factors of each river were derived using geographic coordinates (latitude and longitude)
134 measured by a handheld global positioning system (GPS, Trimble Juno SA; Guo et al. 2019; Lin et al. 2020). The
135 investigation was authorized by director Yun Xiang in the general office of Anji County Water Resources Bureau. In
136 summer 2018, the average day/night temperatures of the region were 29°C/ 21°C and the average precipitation was
137 133 mm.

138 Both degraded rivers and pre-restored urban rivers had similar hydromorphological conditions, stream order, slope,
139 temperature regime (Lin et al. 2019), and were located in the same ecoregion. Straitened and hardened with concrete,
140 these three degraded rivers were covered with mud and were listed as rivers to be restored in the future by the local
141 water conservancy bureau. Two of the degraded rivers are surrounded by suburban areas, and another one is located
142 in the city center. The three restored rivers located in urban areas were at the same elevation with those degraded
143 rivers. With reference to the habitat conditions of reference sites, these rivers have been restored using a similar
144 ecological restoration strategy for up to seven years. This involved natural reconstruction of the riverbed using diverse
145 substrates (e.g. cobbles and pebbles), the channel was re-connected and re-meandered, floating islands were
146 constructed, aquatic plants including submerged macrophytes and emergent plants were re-introduced, and the riparian
147 zone was re- afforested in an attempt to recover a more natural river form based on their specific river type. Three
148 undisturbed rivers were 40-km upstream of these urban rivers within the same catchment, and these undisturbed rivers
149 were considered as approximations to reference sites.

150

151 **Habitat characteristics**

152 Habitat surveys were performed in July and August 2018. At each river, habitat characteristics (denoted Habitat) were
153 measured within a 50 m sampling reach as described in Lin et al. (2019). After visually estimating the reach canopy
154 cover, the water velocity across the channel was measured by Teledyne flow meters (ISCO, Lincoln, NE, USA), the
155 river-bed types including riffle, pool, and island were counted, the substrate composition was described by random-
156 selecting 100 sediment particles on the riverbed and counting the ratio of substrate classes (boulders, cobbles, pebbles,
157 sand grains) according to Kondolf (1997). The substrate diversity was then calculated by means of the Shannon-
158 Wiener diversity H' (Shannon 1997) for each site.

159

160 **Physico-chemical variables**

161 A 100 m tape was used to measure the river width. The river depth was measured at five-evenly spaced points across
162 the channel. Three sampling positions were randomly selected within a 50 m sampling reach in each river and physico-
163 chemical variables (denoted ENV) was monitored by standard methods (Lin et al. 2019). Briefly, (1) temperature, pH,
164 dissolved oxygen (DO), and turbidity were measured in situ using handheld water quality analyzers, and (2) a one liter
165 water sample was taken from three sampling points, filtered through a 0.45 μm filter and tested within 48 hours for

166 ammonium nitrogen (NH₄-N), nitrate-nitrogen (NO₃-N), total nitrogen (TN), total phosphorus (TP), total organic
167 carbon (TOC) and chemical oxygen demand (COD).

168

169 **Macroinvertebrates sampling procedure**

170 In each river, three samples of benthic macroinvertebrates in each studied river were sampled according to Chinese
171 Technical Guidelines for Species Monitoring Freshwater - Benthic Macro-invertebrates (HJ 710.8—2014). Samples
172 were collected from 8th July to 15th August 2018 in three sampling sites in each river using a 1 m × 1 m quadrat
173 distributed randomly along a 50 m stretch. Within each quadrat macroinvertebrates were sampled using a D-frame
174 aquatic dip net (opening: 25.4 cm L × 30.5 cm W; mesh size: 500 μm) by disturbing vegetation and substrate; the
175 samples were then preserved in 70% ethanol for storage, sorted and all macroinvertebrates then identified to family
176 level using Merritt et al. (2008), and classified into groups according to their ability to water pollution using the Family
177 Tolerance Value (Mandaville 2002).

178 Differences in the structure of benthic macroinvertebrate communities were then assessed by calculating total
179 abundance, total richness, Shannon-Wiener diversity (H'), Pielou's evenness (Shannon 1997), the abundance and
180 richness of EPT (Ephemeroptera, Plecoptera, and Trichoptera) and richness of intolerant taxa for each river group. To
181 further investigate specific community composition changes, indicator taxa for each group of river was selected using
182 Multilevel pattern analysis at significance level of $p < 0.05$.

183

184 **Statistical analysis**

185 Differences in habitat features, physio-chemical parameters, and macroinvertebrate alpha (α) diversity properties in
186 three river groups were evaluated through analysis of variance with post hoc Tukey–Kramer test (Torres-Mellado et
187 al. 2012). Environmental factors and α -diversity indexes were $\ln(x + 1)$ transformed if the residuals deviated from
188 normality. The similarity in macroinvertebrate community among three river groups was then assessed by analysis of
189 similarities using the 'anosim' function in 'vegan' in R statistical environment (R Core Team 2017). A p -value of 0.05
190 was used as the cutoff for significance.

191 To explore relationships between habitat characteristics, physio-chemical features, spatial factors and α -diversity of
192 macroinvertebrate, respectively, Spearman's correlation coefficients were calculated, explanatory variable that
193 indicates significant multi-collinearity (Spearman correlation coefficient ≥ 0.70) was excluded from further analysis
194 (Cai et al. 2017). The macroinvertebrate abundance matrices were Hellinger-transformed and detrended
195 correspondence analysis (DCA) was then carried out using 'decorana' function in R package vegan to choose response
196 model (linear or unimodal) for the macroinvertebrate community data. The length of the first DCA ordination axis
197 was less than four, which indicated that RDA was suitable for taxonomic composition. Accordingly, RDA was
198 performed, and the significance was tested using the 'anova.cca' function in 'vegan'. Explanatory variables were
199 selected by performing forward selection using function 'forward.sel' in the 'packfor' R package. Monte Carlo
200 permutation tests was then applied to test the contribution significance of each variables. Finally, variation partitioning

201 was performed to explore the pure contribution of each group (i.e. habitat, environmental data, and spatial factors) to
202 the variation of macroinvertebrate community using the ‘varpart’ function in the ‘vegan’ R package (Borcard et al.
203 2018). Multivariate analysis including DCA, RDA, forward selection and variation partitioning were performed
204 according to Borcard, Gillet and Legendre (2018).

205

206 **Results**

207 **Habitat characteristics**

208 Significant differences in water velocity ($F_{2,6} = 6.661, p = 0.030$) and substrate diversity ($F_{2,6} = 71.18, p < 0.001$) were
209 detected between the three river groups; restored rivers had a higher water velocity than both degraded rivers and
210 undisturbed rivers (Fig. 2e); the substrate diversity in the undisturbed and restored rivers was remarkably higher than
211 degraded rivers ($p < 0.001$) (Fig. 2f). Four types of sediment sizes (boulder, cobble, peddle, granule) formed the
212 riverbed of restored and undisturbed rivers, whereas degraded rivers have only one kind of particles (2-4 mm granule).
213 The habitat diversity in undisturbed and restored rivers was also much higher than that in degraded rivers. Riffles,
214 pools, and islands constituted the habitat structure of the undisturbed and restored rivers, whereas degraded rivers
215 were formed by pools and a few islands. No significant difference was observed in canopy cover between the three
216 river groups ($F_{2,6} = 4.198, p = 0.072$).

217

218 **Physico-chemical properties of surface water**

219 Analysis of variance indicated no significant differences among three river groups in river width ($F_{2,6} = 0.336$), and
220 mean river depth ($F_{2,6} = 0.791$), and no difference in water variables such as pH ($F_{2,6} = 0.325$), DO ($F_{2,6} = 1.716$), NH_4 -
221 N ($F_{2,6} = 2.619$), NO_3 -N ($F_{2,6} = 2.498$), and TP ($F_{2,6} = 1.609$). However, variables exhibited significant differences in
222 water turbidity ($F_{2,6} = 11.75, p = 0.008$), TN ($F_{2,6} = 16.17, p = 0.004$), COD ($F_{2,6} = 5.965, p = 0.038$) in different river
223 groups. Undisturbed rivers had significantly lower concentrations of TN, TOC and COD and turbidity than the
224 degraded rivers ($p = 0.003, p = 0.047, p = 0.032$, and $p = 0.014$, respectively; Fig. 2a-d). Restored rivers possessed a
225 higher turbidity ($p = 0.013$) and a slightly increased TN concentration ($p = 0.060$) than undisturbed rivers (Fig. 2a,
226 Fig. 2b), whereas, a weak reduction in TN was found in restored rivers compared to degraded rivers ($p = 0.073$) (Fig.
227 2b).

228

229 **Benthic macroinvertebrate community**

230 In total, 9,990 specimens of macroinvertebrates were identified, 4,006 individuals in undisturbed rivers, 5,792 in
231 restored rivers, and 192 in degraded rivers. Macroinvertebrate α -diversity values (Table 1, Table 2) showed that there
232 were significant differences among river types for total abundance ($F_{2,6} = 37.32, p < 0.001$), total richness ($F_{2,6} =$
233 $222.20, p < 0.001$), EPT abundance ($F_{2,6} = 90.40, p < 0.001$), EPT richness ($F_{2,6} = 67.41, p < 0.001$), intolerant species
234 richness ($F_{2,6} = 122.10, p < 0.001$) and Shannon-Wiener diversity ($F_{2,6} = 49.00, p < 0.001$). Both reference sites and

235 restored sites had significantly higher total abundance, total richness, EPT abundance, EPT richness, Shannon-Wiener
236 diversity and intolerant taxa richness than degraded rivers ($p < 0.001$) (Table 1, Table 2, Fig. 3), whereas no significant
237 difference of taxonomic diversity was detected between undisturbed rivers and restored rivers ($p > 0.05$). No
238 difference was found among three river groups for the evenness of macroinvertebrates ($F_{2,6} = 0.532$).

239 The analysis of similarities (ANOSIM) based on the macroinvertebrate samples showed a significant difference of
240 macroinvertebrate community compositions among the three river groups ($R = 0.845$, $p = 0.001$). Among the 46
241 families of macroinvertebrates identified in this survey, thirteen taxa were selected as indicator taxa (Table 3). Eight
242 species were highly associated with undisturbed rivers, including dominant family Leptophlebiidae (22.35%), Perlidae
243 (7.43%), and some other species like Dytiscidae, Scirtidae, Coenagrionidae, Hydrophilidae, Leptoceridae, Tipulidae.
244 Leptophlebiidae, Perlidae, Leptoceridae, Dytiscidae and Coenagriidae were significantly more distributed in the
245 reference sites than both urban river groups ($p < 0.05$ in all cases), no difference of these taxa was found between
246 restored rivers and urban degraded rivers ($p > 0.05$). Five indicator taxa (Corbiculidae, Glossiphoniidae, Erpobdellidae,
247 Lymnaeidae and Heptageniidae) were found in restored rivers, dominant species were the Caenidae (31.21%),
248 Chironomidae (14.95%) and Baetidae (12.39%). Of the EPT taxa sampled, Caenidae was the most dominant family
249 in the restored sites, and was significantly more abundant than that in degraded urban rivers ($p = 0.05$) and comparable
250 to undisturbed rivers ($p > 0.05$), Baetidae and Heptageniidae were also presented in the restored rivers in greater
251 numbers than in degraded rivers ($p = 0.088$, $p = 0.066$, respectively), although these trends were not significant. Two
252 of the tolerant taxa (Corbiculidae and Glossiphoniidae), however, were significantly greater in restored rivers
253 compared to both degraded and undisturbed rivers ($p < 0.05$). No indicator taxon was allocated to degraded rivers,
254 but degraded rivers had a higher abundance of Tubificidae (46.92%), Chironomidae (32.36%) and Viviparidae
255 (12.26%) (Table 3).

256

257 **Correlation between environmental variables and macroinvertebrate community**

258 The correlation between macroinvertebrate α -diversity and environmental variables (i.e. habitat characteristics, and
259 physico-chemical variables) are listed in Table 4. The relationship among environmental variables, spatial factors and
260 total macroinvertebrate community structure were examined by constrained redundancy analysis (RDA), eigenvalues
261 of 0.500 and 0.249, respectively for axis one and two were generated (Fig. 4). The environmental variables including
262 habitat characteristic, physico-chemical variables and spatial variables, explained 74.9% of the variance in
263 macroinvertebrate community structure. Monte Carlo permutation tests revealed that substrate diversity, water
264 velocity, COD and longitude significantly affected the macroinvertebrate community ($p < 0.05$ in all cases). The
265 macroinvertebrate assemblages of undisturbed rivers were mainly structured by diverse substrates ($F_{2,6} = 3.472$, $p =$
266 0.004) and low COD concentration ($F_{2,6} = 2.285$, $p = 0.022$). COD in the surface water ($F_{2,6} = 25.599$, $p = 0.006$) was
267 also a major factor influencing macroinvertebrate community in degraded rivers. In restored rivers, the
268 macroinvertebrate communities showed a strong correlation with water velocity ($F_{2,6} = 3.801$, $p = 0.014$), substrate
269 diversity ($F_{2,6} = 9.843$, $p = 0.018$) and longitude ($F_{2,6} = 5.687$, $p = 0.026$).

270

271 **Relative importance of environmental, spatial and habitat factors**

272 Variation partitioning showed that 44% of the community taxonomic composition was explained by three sets of
273 environmental variables; habitat factors explained 22%, followed by physico-chemical variables (ENV, 5%) and
274 spatial factors (4%); 12% of the variation was shared by all three sets, 4% between habitat and ENV and 2% between
275 ENV and spatial factors (Fig. 5a). No shared effect was found between habitat and spatial factors (Fig. 5a). In terms
276 of indicator taxa, 36% of the total variation was explained by the three explanatory sets of variables. Habitat features
277 was still the main factor explaining 10%, spatial factors explained 2% and physico-chemical variables explained
278 nothing; 4% of the variation was shared by all three sets, 11% between ENV and spatial factors, 9% between spatial
279 factors and ENV and 5% between habitat and ENV (Fig. 5b).

280

281 **Discussion**

282 **Taxonomic diversity of macroinvertebrate communities**

283 Overall, there were significant differences in macroinvertebrate community composition between the restored and
284 degraded rivers. The taxonomic diversity and composition of macroinvertebrate community in restored rivers were
285 distinct from degraded rivers and strongly associate with habitat characteristic substrate diversity and water velocity,
286 indicating that habitat restoration had impacted the structure of the communities. Compared with degraded rivers,
287 there was a significant increase in macroinvertebrate diversity and total richness in restored rivers, meanwhile, EPT
288 richness and intolerant taxa richness also increased under habitat restoration. These results are in accordance with the
289 stated hypothesis and in line with previous studies in northern Poland and elsewhere (Matthaei and Diehl 2005; Miller
290 et al. 2010; Obolewski et al. 2016), indicating that habitat heterogeneity had significant, positive effects on
291 macroinvertebrate richness and diversity. In-stream habitat restoration enhanced the macroinvertebrate richness and
292 diversity (Flores et al. 2017).

293 The difference in macroinvertebrate diversity reflects the variation of habitat characteristics and physico-chemical
294 variables (Shi et al. 2019). As demonstrated previously, increased depth and frequency of pools should increase species
295 richness through higher habitat heterogeneity (Brasher 2003). Obolewski et al. (2016) also suggested that restoration
296 approach rehabilitation induced hydrological connectivity, improved water quality and increased the diversity and
297 abundance of macrozoobenthos. Here, substrate composition, organic carbon TOC and nutrient TN were important in
298 influencing macroinvertebrate diversity. Riverbed reconstruction and aquatic macrophytes re-introduction applied to
299 the restored rivers enhanced the substrate diversity, diverse substrate and large size particle (e.g., cobbles) can enhance
300 the stability of habitats and form abundant interstitial spaces for macroinvertebrates (Luo et al. 2018). Some
301 macroinvertebrates are very sensitive to organic pollutants and water quality degradation (Kalyoncu and Gülboy 2009;
302 Patang et al. 2018). The decline in organic carbon and nutrient level in restored rivers may improve the water quality
303 and stimulate the development of macroinvertebrates of low tolerance value. This finding differs with many habitat
304 restoration schemes which resulted in modest /unsuccessful ecological responses for the persist of constraints such as
305 degraded hydrological regimes, rare food availability, high pollutant loads (Tullos et al. 2009; Palmer et al. 2010).

306 Jähnig and Lorenz (2008) declared that the diminish of diverse source populations under multiple-factor impairments
307 and cumulative alterations of streams might be another reason for the failed response under ecological restoration.

308 Relative abundance of EPT and intolerant species also increased in restored rivers compared to degraded rivers. Many
309 pollution-intolerant taxa belong to the EPT insect orders Ephemeroptera, Plecoptera and Trichoptera. The observed
310 increase in sensitive EPT taxa agree with earlier observations in field studies and mesocosm experiments, suggesting
311 that EPT taxa are sensitive to environmental degradation and habitat simplification (Cabria et al. 2011; Ilarri et al.
312 2018), EPT taxa often decline where there is a reduction in flow velocity accompanied by clearing of coarse substrates
313 including coarse woody debris (CWD), and excess fine sediment deposition, which reduced food availability (Ryan
314 1991), ruined sheltering areas of specific macroinvertebrate taxa such as stonefly (Kärnä et al. 2018), and physically
315 damages gills and filter-feeding apparatus by abrasion or clogging (Jones et al. 2012; Piggott et al. 2015).

316

317 **Determinants of Macroinvertebrate Community Composition**

318 Distinct macroinvertebrate communities were found among river types. These differences were closely related to the
319 changes in water velocity and substrate diversity, COD, and longitude of the rivers. These results support the
320 hypothesis that macroinvertebrate community composition was driven by habitat characteristics, river discharge,
321 physico-chemical variables and spatial factors, and in line with a summarized concept that benthic macroinvertebrate
322 species are sensitive to both hydromorphology and water quality factors in their environment (Mandaville 2002; Shi
323 et al. 2019). Habitat characteristics contributed to most of the variation of the entire macroinvertebrate community
324 and the structure variation of indicator taxa, followed by ENV and spatial factors (Englund et al. 1997). This supports
325 the view of Jähnig and Lorenz (2008) and Luo et al. (2018), that habitat specific habitat variables explained the major
326 variation in macroinvertebrate community composition. Macroinvertebrate fauna can always be classified into flow
327 exposure groups (obligate, facultative, and avoiders) and habit groups (clinger, burrowers, sprawlers, and swimmers)
328 in accordance with their preference towards hydromorphology conditions that is guided by their flow exposure
329 preferences and behavioral activities (Merritt et al. 2008). Rivers with diverse substrates can provide a high variability
330 of micro-habitats and heterogeneous food resources for macroinvertebrates (Mandaville 2002), especially as water
331 velocity varies at different seasons; hence a diverse species assemblage, adapted to various natural flows can be
332 maintained. In our study, the changes in substrate diversity and flow velocity induced by habitat restoration were
333 important in shaping the macroinvertebrate communities in restored rivers compared to those in degraded rivers. The
334 increase in substrate diversity and flow velocity in the restored rivers induced a more diverse habitat type, which
335 sustain the development of macroinvertebrate taxa with preferences for each particular habitat and hydrology
336 condition (Dewson et al. 2007, Elbrecht et al. 2016).

337 Differences in physico-chemical variables (e.g., TN and TOC) further contributed to the shifts in macroinvertebrate
338 community composition among three river types, though the influence is not as strong as habitat characteristics. Given
339 that water quality conditions are a product of catchment-wide processes which act as large scale filter of the regional
340 species pool (Poff 1997), but habitat-scale variation drives differences in macroinvertebrate communities within the
341 species pool, which yield a greater statistical influence (White et al. 2019). In our study, heavy organic pollutants in

342 the degraded rivers led to higher abundance of tolerant families Tubificidae, Chironomidae and Viviparidae (Al-Shami
343 et al. 2011; Arimoro 2009), whereas, restored rivers improved habitat heterogeneity, declined the nutrient and organic
344 pollutants, provided more favorable conditions for the development of sensitive EPT taxa (including abundant taxa
345 Baetidae and indicator taxon Heptageniidae; Patang et al. 2018; Luo et al. 2018), facilitated the establishment of some
346 low organic pollutant tolerant taxa that live in specific habitats, such as indicator taxa Glossiphoniidae and
347 Corbiculidae (Luo et al. 2018). These results are similar to those reported for the river Danube and illinois streams
348 (Heatherly et al. 2007; Rico et al. 2016) and an indoor experiment (Beermann et al. 2018). Implying that habitat
349 restoration shifted the dominant pollution-tolerant macroinvertebrates to sensitive EPT taxa with the improvement of
350 river habitat and water quality, facilitated the establishment of some low tolerant taxa that live in specific habitat such
351 as sediment, riffle, pool, aquatic plant, and exist under low level of pollution in restored rivers, and this distinguishes
352 the macroinvertebrate community in restored rivers from the community in the other two river types.

353 The shared effects of hydro-morphological and water chemical factors (ENV vs. Habitat vs. Spatial factor), however,
354 had greater influences on macroinvertebrate communities than single effect of physico-chemical or spatial factors.
355 Consistent with Rico et al. (2016), who indicated that chemical pollution had a lower contribution to invertebrate
356 community than shared effect of habitat characteristics and physico-chemical conditions. Spatial factors have a lower
357 contribution on the macroinvertebrate community variance than physico-chemical and habitat variables. The
358 biological communities in rivers may change along the variation of spatial factors (Vannote et al. 1980). However,
359 habitat and water quality conditions, rather than spatial factors, best explained the variance of invertebrate community
360 and diversity (Rico et al. 2016).

361 Overall, the macroinvertebrate community clustered in the restored rivers possessed greater diversity and richness,
362 the community composition was distinct from that in the degraded and undisturbed rivers, and these changes were
363 caused mainly by improved habitat characteristics, followed by physico-chemical variables and lastly spatial factors.
364 Habitat restoration recovered the macroinvertebrate community composition in urban rivers in a positive way, which
365 is in line with a meta-analysis result performed by Miller et al. (2010), whereas, some studies showed small or none
366 ecological effect of improved habitat conditions (Jähnig et al. 2010; Palmer et al. 2010). Restoration response may be
367 varied both spatially and temporally, the restoration approaches applied also influence the variance. Further study and
368 evaluation of the river restoration programs would help to form an integrated view of restoration progress and
369 efficiency of different restoration approaches, which provides water managers and policy makers an integrated
370 guidance for future planning of ecological restoration and management strategies.

371

372 **Conclusions**

373 In this study, we examined the effect of habitat restoration on macroinvertebrate community composition in the urban
374 rivers with and without restoration by comparing them to undisturbed rivers. The results support our hypothesis that
375 habitat restoration positively altered the benthic macroinvertebrate community structure in comparison to that in
376 degraded rivers. Attributing to the increase in substrate diversity, flow velocity, and accompanying decline in total
377 nitrogen, total organic chemical in the surface water, habitat restoration induced higher values in diversity, in richness

378 and abundance of macroinvertebrate, and higher richness and abundance of less tolerant EPT taxa. This study supports
379 the hypothesis that applying habitat restoration in river management enhances habitat heterogeneity and improve the
380 water quality, which can in turn stimulate the shift of macroinvertebrate community composition in urban rivers.
381 Accordingly, habitat restoration is an efficient approach to recover the aquatic biodiversity in degraded urban rivers
382 and to enhance river ecosystem health for freshwater conservation and management.

383

384 **Compliance with ethical standards**

385 **Conflict of interest**

386 The authors declare that they have no conflict of interest.

387

388 **Ethics approval**

389 Not applicable

390

391 **Consent to participate**

392 Not applicable

393

394 **Consent for publication**

395 Not applicable

396

397 **Data availability**

398 The data and materials used to support the findings of this study are shared by the requesting author.

399

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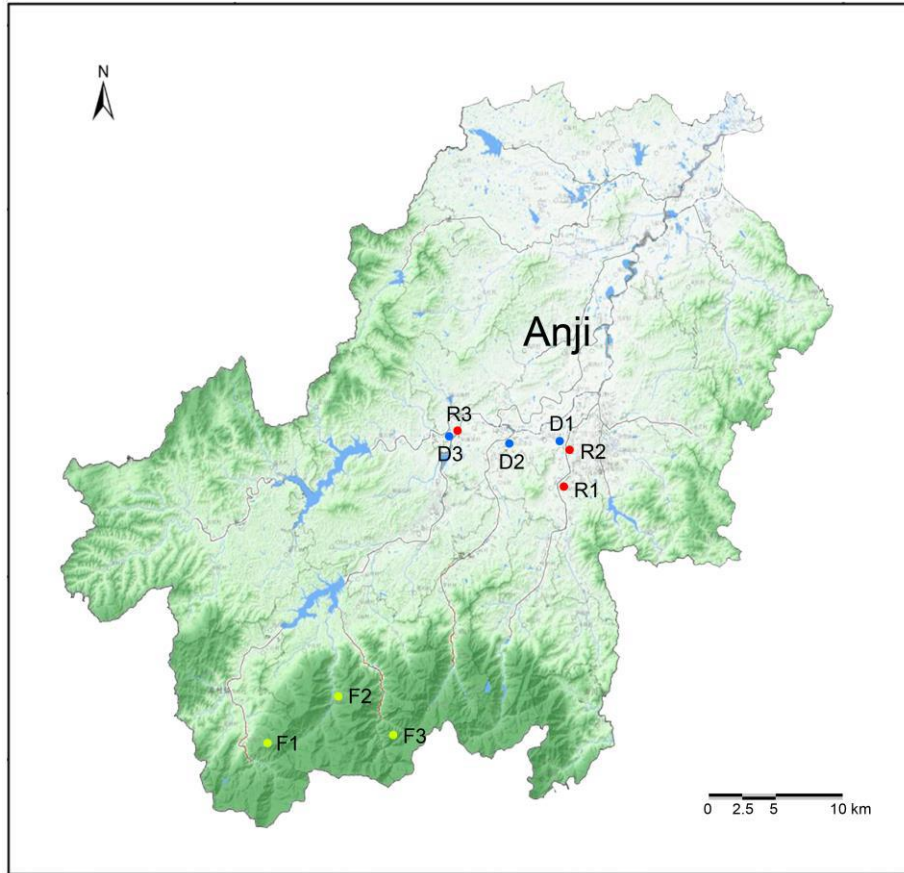
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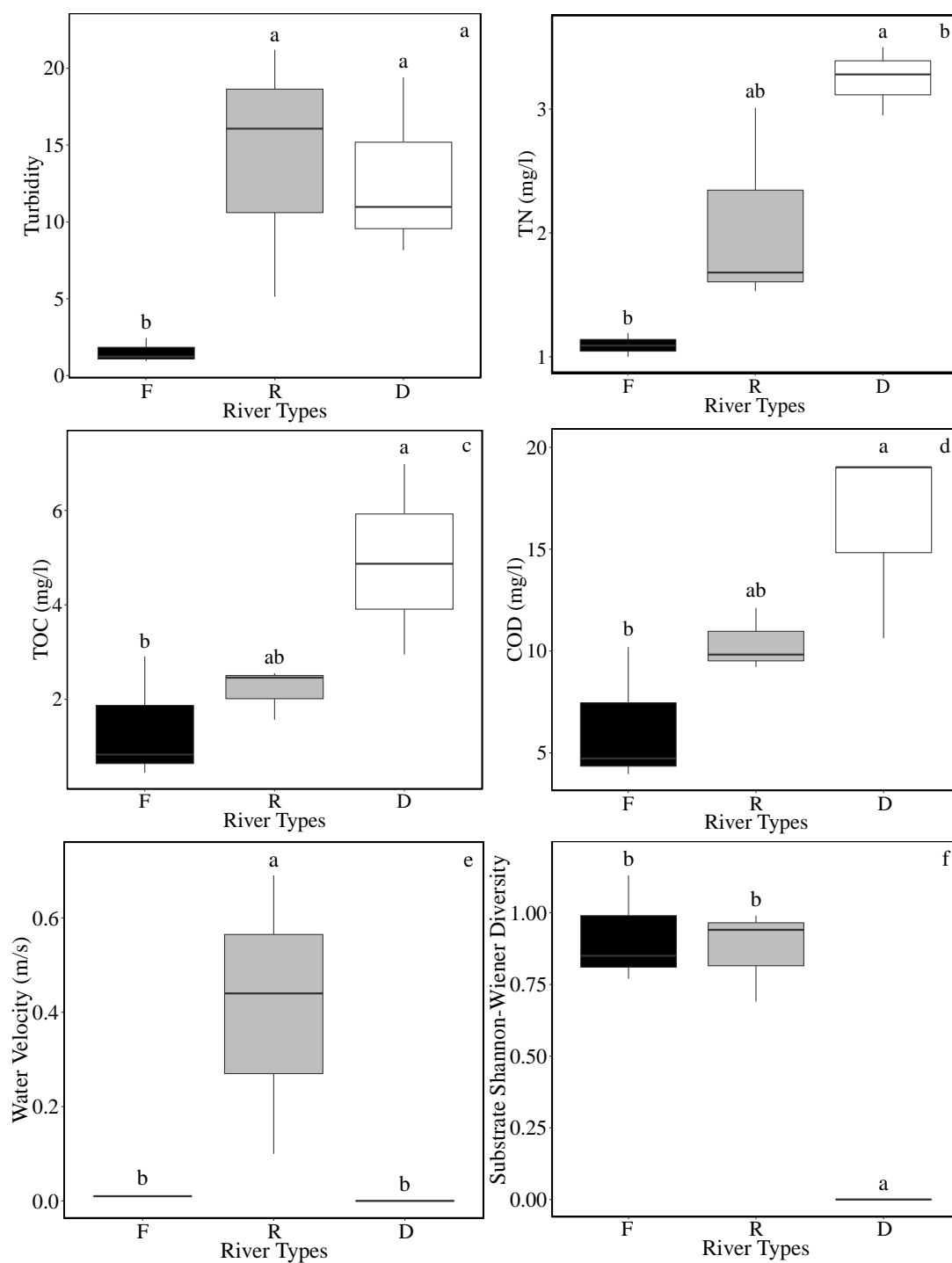
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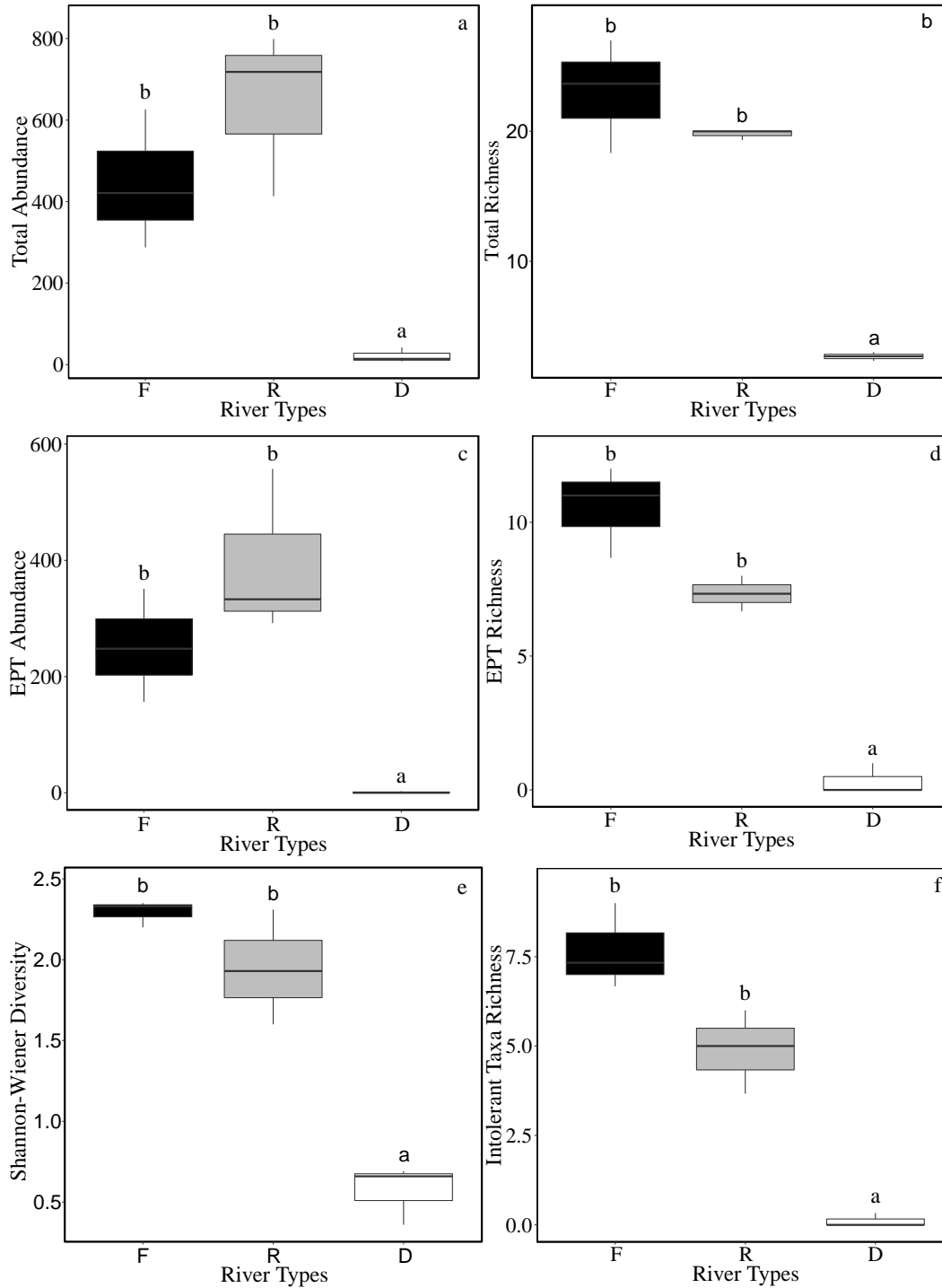


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Fig. 1 Sampling sites within the Anji City Region, PRC; three degraded urban rivers (D), three restored rivers (R) and three undisturbed rivers (F)

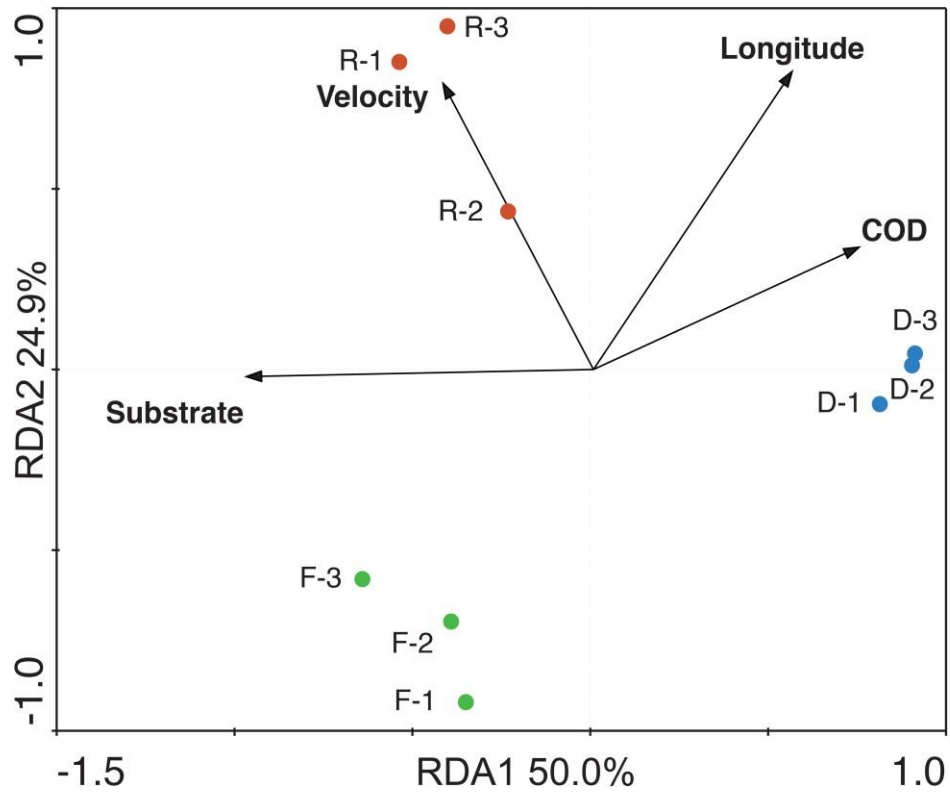


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 620 **Fig. 2** Box-plots of the (a) turbidity, (b) total nitrogen (TN), (c) total organic carbon (TOC), (d) chemical oxygen
 621 demand (COD), (e) water velocity and (f) substrate Shannon-Wiener diversity in three contrasting river types within
 622 Anji City Region, PRC. Mean values (\pm SE, $n = 3$) are presented; different lower-case letters indicate a significant
 623 difference observed at $p = 0.05$ level
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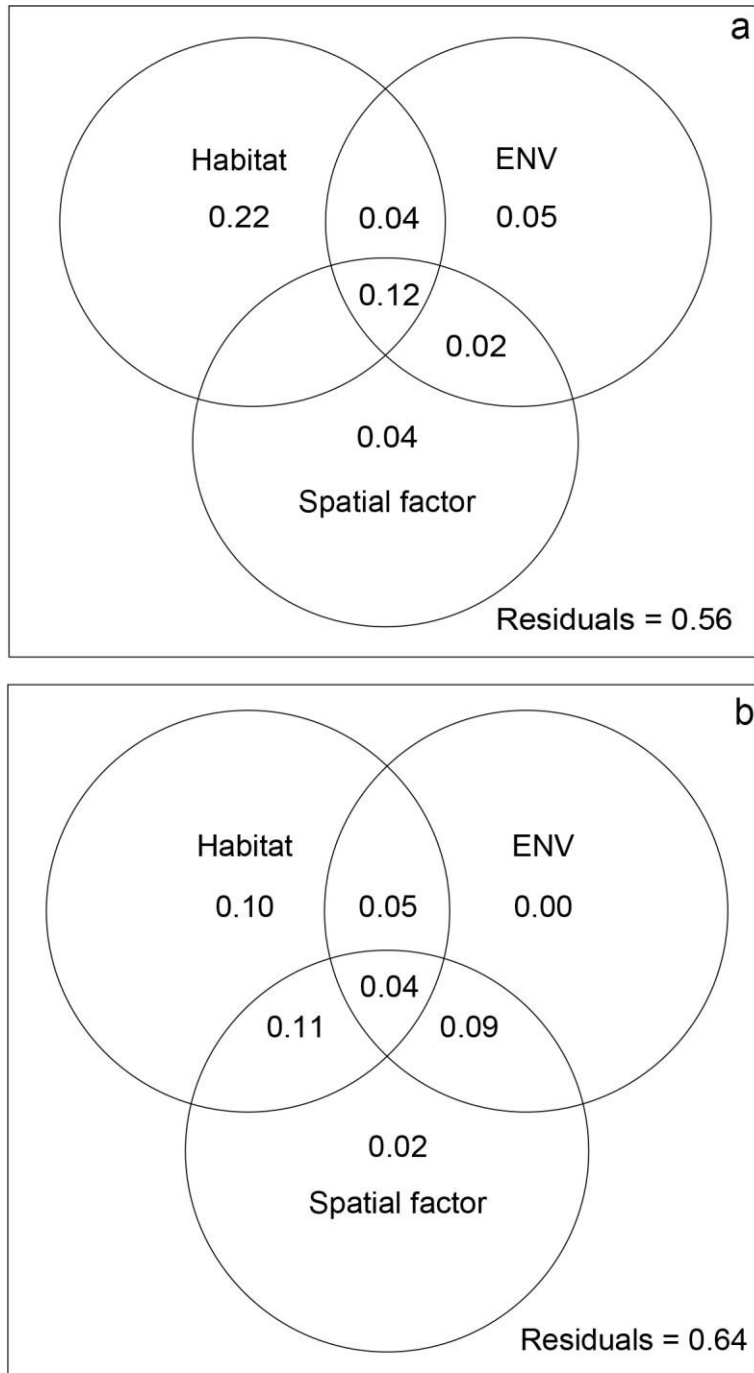
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626 **Fig. 3** Box-plots of macroinvertebrate alpha-diversity (a) total abundance, (b) total richness, (c) EPT tax abundance,
 627 (d) EPT tax richness, (e) macroinvertebrate diversity (Shannon-Wiener diversity) and (f) intolerant taxa richness in
 628 undisturbed, restored and degraded rivers within the Anji City Region, PRC. Mean values (\pm SE, $n = 3$) are presented;
 629 different lower-case letters indicate a significant difference observed at $p = 0.05$ level
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Fig. 4 Redundancy analysis (RDA) of benthic macroinvertebrate community assemblages in undisturbed (F, green circles), restored (R, red circles) and degraded (D, blue circles) rivers with different environmental variables within the Anji City Region, PRC



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637 **Fig. 5** Venn diagrams illustrating the variation partitioning analysis for (a) taxonomic composition and (b) indicator
 638 taxa (taxa at family level). Habitat, ENV, and Spatial factor are sets of variables representing habitat variables,
 639 physico-chemical variables, and spatial factors, respectively. Residuals are shown in the lower right corner. All
 640 fractions based on adjusted R^2 are shown as percentages of total variation

641

642 **Table 1** Mean values of macroinvertebrate taxonomic metrics in different groups of rivers summer within the Anji
 643 City Region, PRC. The values represent the mean \pm standard error of three replicate samples.

River Type	Total abundance	Total richness	EPT abundance	EPT richness	Intolerant taxa richness	Pielou's Evenness	Shannon-Weiner Diversity
Forest	445.11 \pm 98.60	23.00 \pm 2.53	251.89 \pm 56.13	10.56 \pm 0.99	7.67 \pm 0.69	0.74 \pm 0.01	2.29 \pm 0.05
Restored	643.55 \pm 117.44	19.78 \pm 0.22	394.11 \pm 82.46	7.33 \pm 0.38	4.89 \pm 0.67	0.65 \pm 0.07	1.95 \pm 0.21
Degraded	21.33 \pm 10.48	2.67 \pm 0.19	1.0 \pm 1.00	0.33 \pm 0.33	0.11 \pm 0.11	0.61 \pm 0.14	0.57 \pm 0.11

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Table 2 (M)ANOVA results of macroinvertebrate metrics for different rivers types. Significant *p* - values (<0.05) are printed in bold

Macroinvertebrate	F Value	<i>p</i> Value	F vs. D		F vs. R		R vs. D	
			<i>p</i>	difference	<i>p</i>	difference	<i>p</i>	difference
Total abundance	37.32	0.0004	0.0010	3.1620	0.6928	-0.3791	0.0005	3.5410
Total richness	222.20	2.4e-06	3.2e-06	1.8700	0.4259	0.1327	5.0e-06	1.7373
EPT abundance	90.40	3.3e-05	7.8e-05	5.0184	0.5957	-0.4582	4.7e-05	5.4767
EPT richness	67.41	7.7e-05	9.5e-05	2.2085	0.3298	0.3214	0.0002	1.8871
Intolerant richness	122.10	1.4e-05	1.5e-05	2.0574	0.0683	0.3939	5.3e-05	1.6635
Shannon-Wiener Diversity	49.00	0.0002	0.0002	0.7440	0.3868	0.1154	0.0006	0.6286
Pielou's evenness	0.53	0.6130	0.5894	0.0841	0.8193	0.0502	0.9114	0.0339
Dytiscidae	62.87	9.5e-05	0.0002	0.0047	0.0002	0.0047	1.0000	0.0000
Leptophlebiidae	33.32	0.0006	0.0007	0.2008	0.0015	0.1757	0.6390	0.0251
Perlidae	12.59	0.0071	0.0115	0.0713	0.0115	0.0713	1.0000	0.0000
Leptoceridae	10.69	0.0105	0.0151	0.0567	0.0185	0.0542	0.9823	0.0025
Coenagriidae	56.06	0.0001	0.0002	0.0144	0.0002	0.0137	0.8848	0.0007
Caenidae	5.00	0.0528	0.7387	0.0655	0.1357	-0.1960	0.0519	0.2615
Corydalidae	7.89	0.0209	0.0201	0.0071	0.0688	0.0052	0.5898	0.0019
Corbiculidae	13.89	0.0056	1.0000	0.0000	0.0091	0.0249	0.0091	0.0249
Gossiphonidae	6.06	0.0363	1.0000	0.0000	0.0534	0.0084	0.0534	0.0084
Hydrophilidae	5.16	0.0496	0.0633	0.0095	0.0816	0.0088	0.9778	0.0007
Baetidae	3.56	0.0958	0.6854	0.0304	0.2603	-0.0625	0.0882	0.0929
Heptageniidae	4.19	0.0727	0.6355	0.0100	0.2192	0.0201	0.0663	0.0301
Scirtidae	3.37	0.1040	0.1405	0.0340	0.1405	0.0340	1.0000	0.0000
Tipulidae	0.78	0.5000	0.6685	-0.0693	0.9456	0.0251	0.4927	-0.0944
Chironomidae	0.18	0.8370	0.9912	-0.0217	0.8969	0.0768	0.8378	-0.0984
Lymnaeidae	0.86	0.4690	0.4833	-0.0652	0.9785	-0.0106	0.5898	-0.0546
Tubificidae	4.00	0.0787	0.1089	-0.3552	1.0000	0.0000	0.1089	-0.3552
Viviparidae	2.39	0.1720	0.1971	-0.1081	0.9799	-0.0105	0.2506	-0.0977

647

648 **Table 3** Indicator taxa (taxa at family level) of macroinvertebrate communities in three contrasting river types within
 649 the Anji City Region, PRC. IV = Indicator value

River Type	Taxa	IV	<i>p</i> - value
F	Dytiscidae	1.000	0.035*
	Scirtidae	1.000	0.035*
	Perlidae	1.000	0.035*
	Coenagrionidae	0.991	0.035*
	Hydrophilidae	0.982	0.035*
	Leptoceridae	0.974	0.035*
	Tipulidae	0.964	0.035*
	Leptophlebiidae	0.941	0.035*
R	Corbiculidae	1.000	0.039*
	Gossiphonidae	1.000	0.039*
	Erpobdellidae	0.985	0.039*
	Lymnaeidae	0.977	0.039*
	Heptageniidae	0.871	0.039*

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651 **Table 4** Spearman correlation coefficients between environmental variables (i.e. habitat characteristics, physico-
 652 chemical variables) and macroinvertebrate alpha diversity for studied rivers. Asterisks are significant level at $p < 0.05$.

	Total Abundance	Total Richness	EPT abundance	EPT richness	Intolerant richness	Shannon- Wiener Diversity
pH	0.23	0.41	0.08	0.44	0.44	0.50
Turbidity	-0.13	-0.13	-0.05	-0.17	-0.13	-0.10
DO	0.57	0.65	0.55	0.66	0.64	0.62
NH ₄ -N	-0.63	-0.64	-0.59	-0.61	-0.60	-0.59
NO ₃ -N	-0.22	-0.35	-0.12	-0.40	-0.35	-0.35
TN	-0.68	-0.79	-0.62	-0.79	-0.77	-0.80
TP	-0.57	-0.72	-0.62	-0.76	-0.77	-0.65
TOC	-0.73	-0.90	-0.72	-0.90	-0.89	-0.85
COD	-0.44	-0.72	-0.40	-0.79	-0.73	-0.74
Water velocity	0.50	0.30	0.39	0.16	0.08	0.40
Substrate diversity	0.84	0.97*	0.85	0.95*	0.95*	0.90
Canopy cover	-0.04	0.35	-0.09	0.47	0.49	0.39

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654 **Appendix**

655 **Table S1** Location and habitat information for the nine study sites within the Anji City Region, PRC; Habitat
 656 information include canopy cover, habitat types, substrate composition and substrate Shannon-Wiener diversity (H').
 657 F = undisturbed rivers; R = restored rivers; D = degraded rivers.

Site code	River name	Location (Longitude Latitude)	Canopy cover (%)	Habitat types present			Substrate composition (%)				Substrate Shannon-Wiener Diversity (H')
				Island	Pool	Riffle	Boulder	Cobble	Pebble	Granule	
F-1	Longwang Mountain	30°25'3.93"N 119°24'30.52"E	70	✓	✓	✓	20.7	72	7	0.3	0.77
F-2	Yangjiao Mountain	30°26'59.18"N 119°27'55.03"E	90	✓	✓	✓	22.4	68.3	8.1	1.2	0.85
F-3	Zhebei Valley	30°25'24.05"N 119°30'33.60"E	85	✓	✓	✓	13.3	45.3	36.9	4.5	1.13
R-1	Shima Port	30°37'52.98"N 119°41'57.03"E	1	✓	✓	✓	0	13.3	38.7	48	0.99
R-2	Depu Gang	30°36'22.34"N 119°41'39.80"E	2	✓	✓	✓	0	14.9	59.5	25.6	0.94
R-3	Wuxiangba	30°38'43.04"N 119°36'32.29"E	10	✓	✓	✓	0	68.5	29.7	1.8	0.69
D-1	Tongxin	30°38'13.96"N 119°41'28.86"E	20	-	✓	-	0	0	0	100	0
D-2	Wuzhuang	30°38'7.99"N 119°39'2.36"E	0.2	✓	✓	-	0	0	0	100	0
D-3	Chiyi	30°38'28.69"N 119°36'12.85"E	60	-	✓	-	0	0	0	100	0