Evaluating ecosystem functioning following river restoration: the role of hydromorphology, bacteria, and macroinvertebrates

Running head: Ecosystem function in restored rivers

Qiaoyan Lin[a,b,c], Yixin Zhang[d*], Rob Marrs[b], Raju Sekar[e], Xin Luo[c] and Naicheng Wu[a*]

[a] *Department of Health and Environmental Sciences, Xi'an Jiaotong-Liverpool University, Suzhou, Jiangsu 215123, China*

[b] *School of Environmental Sciences, University of Liverpool, Liverpool L69 3GP, UK*

[c] *Zhejiang Institute of Research and Innovation, The University of Hong Kong, Hangzhou, Zhejiang 311305, China*

[d] *Department of Landscape Architecture, Gold Mantis School of Architecture, Soochow University, Suzhou, Jiangsu 215123, China*

[e] *Department of Biological Sciences, Xi'an Jiaotong-Liverpool University, Suzhou, Jiangsu 215123, China*

*Corresponding authors

Yixin Zhang[d]

Email address: yixin.zhang2019@suda.edu.cn (Y.X. Zhang)

No. 268 Dongping Street, Department of Landscape Architecture, Gold Mantis School of Architecture, Soochow University, Suzhou, Jiangsu, 215123, China

Naicheng Wu[a] Email address: Naicheng.Wu@xjtlu.edu.cn (N.C. Wu)

Department of Health and Environmental Sciences, Xi’an Jiaotong-Liverpool University, Suzhou, Jiangsu, 215123, China

Author Contributions

Qiaoyan Lin: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Project administration. **Naicheng Wu**: Resources, Writing - Review & Editing, Supervision, Funding acquisition. **Yixin Zhang**: Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Funding acquisition. **Rob Marrs**: Writing - Review & Editing, Supervision. **Raju Sekar**: Writing - Review & Editing, Supervision. **Xin Luo**: Writing - Review & Editing, Funding acquisition.

ABSTRACT

Ecological restoration of freshwater ecosystems is now being implemented to mitigate anthropogenic disruption. Most emphasis is placed on assessing physico-chemical and hydro-morphological properties to monitor restoration progress. However, less is known about the structural integrity and ecosystem health of aquatic ecosystems. In particular, little is known about how ecosystem function changes following river habitat restoration, especially in China. Leaf litter decomposition can be used as an indicator of stream ecosystem integrity. Therefore, the leaf breakdown rate was measured to assess the ecosystem function of restored rivers. By comparing leaf breakdown rates in urban rivers undergoing habitat restoration with that in degraded urban rivers and rivers in forested areas (i.e., reference conditions), we aimed to determine: (i) how habitat restoration affected leaf litter decomposition? (ii) the relationship between leaf litter decomposition to both environmental (habitat and physico-chemical variables) and biological factors (benthic communities), and (iii) identify the factors that contribute most to the variance in leaf litter breakdown rates. The results demonstrated a significant increase in leaf breakdown rate (120% in summer and 28% in winter) in the restored rivers compared to the degraded rivers. All environmental and biotic factors evaluated contributed synergistically to the differences in leaf litter decomposition among the three river types. The role of macroinvertebrates, mainly shredders, appeared to be particularly important, contributing 52% (summer) and 33% (winter) to the variance in decomposition, followed by habitat characteristics (e.g. substrate diversity, water velocity; 17% in summer, 29% in winter), physico-chemical variables (e.g. nutrient and organic pollutants; 11% in summer, 1% in winter) and biofilm bacteria (0% in summer, 15% in winter). Habitat restoration positively affected the structure and function of the previously degraded streams. Knowledge on controlling variables and their attribution to changes of ecosystem functioning provides guidance to assist the future planning of ecological restoration strategies.

Keywords: habitat restoration, ecosystem function, leaf litter breakdown, river ecosystems, freshwater management

1. Introduction

With the increasing use of habitat restoration to manage freshwater ecosystems around the world, an abundance of publications emerged about the monitoring and evaluation of restoration projects (Bernhardt et al. 2005; Palmer and Ruhi 2019). Evaluation of restoration not only monitors the progress of the restoration, but the experience gained can be used as a basis to form more systematic and efficient restoration strategies for future endeavors (Knodolf and Micheli 1995; Zan et al. 2017). Within the overall assessment process, it is important to include both structural and functional variables when evaluating the response of ecosystem condition to human activities (Matthews et al. 1982; Gessner and Chauvet 2002; Pascoal et al. 2005). Currently, water quality and hydro-morphological aspects of study receive the greatest attention for monitoring the restoration progress in freshwater systems. A few studies included biological indicators such as measures of microbes, algae, invertebrates, and fish to assess the structural integrity and ecosystem health (Coe et al. 2009; Frainer et al. 2017; Schmutz et al. 2016). However, few studies have been conducted to assess the functional ecosystem response to freshwater management by examining processes such as primary production, ecosystem respiration (Niyogi et al. 2002; Colangelo 2007; Aldridge et al. 2009), or leaf litter decomposition (Dangles et al. 2004; Wenger et al. 2009; Flores et al. 2011).

Anthropogenic disturbances (such as logging and damming) impact freshwaters in many ways, including geomorphology, hydrology, water quality, riparian plant communities, aquatic communities, and many other factors (Little and Altermatt 2018; Hashemi et al. 2019; Zhang et al. 2019). River ecological restoration, in turn, may reverse this damage through restructuring heterogeneous habitat, re-introducing aquatic plants, riparian zone re-forestation, etc., all of which can directly or indirectly affect organic matter breakdown in streams.

Organic matter breakdown is important ecosystem function in aquatic system in terms of nutrient cycling and energy flow (McKie et al. 2006; Tiegs et al. 2019), driving the stream food-web interactions (Zhang et al. 2004). Organic matter breakdown has been proposed as a good indicator of ecosystem integrity (Pascoal et al. 2005; McKie and Malmqvist 2009), and an alternative measure of stream health (Young et al. 2008; Niyogi et al. 2013). Such ecosystem functioning is regulated by both physico-chemical and biological factors (Pascoal and Cassio 2004). Environmental factors such as pH (Dangles et al. 2004), temperature (Ferreira and Chauvet 2011; Martínez et al. 2014), current velocity (Martínez et al. 2015), organic matter input (Graça et al. 2015), and leaf nutrient status (Greenwood et al. 2007; Pérez et al. 2012) can play important roles in influencing leaf litter decomposition. Elevated temperature and dissolved nutrients (N and P) in streams speed up leaf decomposition through stimulating microbial activity (Gulis et al. 2006; Hladyz et al. 2010; Ferreira and Chauvet 2011) and increase the abundance and biomass of shredders (Robinson and Gessner 2000). High inputs of nutrients and organic matter, however, slow down leaf decay rate by reducing the activity of microbial and invertebrate decomposers as a result of a reduction in dissolved oxygen (Medeiros et al. 2008). Faster flow velocity may enhance leaf decomposition through increasing shear force on leaf litters (Paul et al. 2006). In contrast, acidification can slow leaf breakdown by affecting the diversity and activity of aquatic organisms (Dangles and Chauvet 2003) and pollution with heavy metals can harm both microbes and macroinvertebrates (Niyogi et al. 2001).

Leaf litter decomposition may also be influenced by the interactions of those aquatic organisms that convert leaf litter mass to fine particulate organic matter (FPOM), dissolved organic matter, and CO_2 (Gessner et al. 1999; Zhang et al. 2003). Starting from microbial colonization, micro-organisms spread over the leaf surface, then penetrate the leaf interior, reducing leaf toughness through hydrolytic processes, and contribute to leaf litter mineralization (Hieber and Gessner 2002; Gessner et al. 2010). Both softened leaves and colonized microbes enhances the food quality, provides important nutrients for invertebrates (Graça 2001). Macroinvertebrates can be classified into five functional feeding groups (FFGs; Mandaville 2002), collector-gatherers (C-G), collector–filterers (C-F), scrapers (Scr), shredders (Shr), and predators (Prd). Among these FFGs, the feeding activity of leaf-shredding insects were thought to be most important in accounting for differences in leaf breakdown rates between streams (Benfield et al. 1991). Shredding invertebrates speed up leaf decomposition by breaking coarse particulate organic matter into smaller fragments (Suberkropp 1998; Gulis and Suberkropp 2003; Martínez et al. 2015). The enhanced surface area of recalcitrant compounds, in turn, stimulates the colonization of microbial species favoring the metabolism of such compounds (Gessner et al., 2010; Noël et al. 2020), contributing to the subsequent decomposition and overall mineralization (Palmer and Ruhi, 2019).

In summary, environmental and biological factors contribute synergistically to leaf decay in the aquatic ecosystems. However, the relative importance of environmental and aquatic organisms on leaf breakdown has rarely been studied (Encalada et al. 2010), particularly in streams of shifting habitat status. In this study, we compared leaf litter breakdown rates, as a measure of ecosystem function in three contrasting river types: i.e. (1) degraded urban rivers, (2) urban rivers undergoing habitat restoration and (3) rivers in forested areas (i.e., reference conditions) (Fig. 1). In each we assessed the importance of the habitat composition, water chemistry and both benthic bacterial and macroinvertebrate communities in two seasons (winter and summer). We aimed to determine: (i) how habitat restoration affects leaf litter decomposition?

(ii) the relationship between leaf decomposition to both habitat factors (substrate diversity, water velocity) and physico-chemical variables, (iii) the relationship between leaf litter breakdown and benthic organisms, and (iv) which factors contribute to most of the variance in leaf litter breakdown rate within these three river types. Our first hypothesis is that stream habitat restoration would enhance the leaf breakdown rate and be a useful indicator of success. Our second hypothesis is that leaf decomposition would be affected by both abiotic and biological factors. Habitat restoration will lead to faster current velocity that speed the leaf decay through physical process. Improved substrate diversity, dissolved oxygen and living space would shift the community composition and stimulate the microbial and macroinvertebrate activities in decomposing leaf litters. Our third hypothesis is that microbes and macroinvertebrate will contribute more on leaf mass loss than abiotic factors through microbial degradation and feeding activity of shredders.

Practically, knowledge derived from this study will enrich our understanding on the response of ecosystem function to river ecological restoration and linked important controlling variables, which will be useful for policymakers and water managers in future planning of ecological restoration strategies for degraded freshwater streams (Solangi et al. 2019).

2. Methods

2.1. Study sites

This study investigated three stream types (Fig. S1), each with three replicates in both winter (December 2017 to January 2018) and summer (June to August 2018). The stream types were a reference forest stream, a restored urban stream, and a degraded urban stream. The nine streams are located in the same watershed (the Shaoxi River), Zhejiang Province PRC within the Anji City Region.

The degraded rivers possessed similar conditions to those in the pre-restored urban rivers (Lin et al. 2019). The degraded urban rivers were canalized with concrete, had high cover of mud, and high pollutant loads and were classified recently as “rivers to be restored” by the local water conservancy bureau. The three restored urban rivers had been restored for up to seven years using an ecological restoration strategy in an attempt to recover a more natural river form. This involved re-connection and re-meandering the river channels, natural reconstruction of the riverbed using diverse substrates (e.g. boulders, cobbles, and pebbles), construction of floating islands, transplant of submerged macrophytes and emergent plants, and riparian zone re-afforestation. The three undisturbed forest streams were 40-km upstream of these urban rivers within the Tianmu Mountains and were viewed as approximations to reference sites, for the pre-urban landscape form they represented (Violin et al. 2011).

2.2. *Habitat characteristics (denoted Habitat)*

Habitat surveys were performed in both winter and summer. Within each river, we visually estimated the reach canopy cover, counted the river-bed types, measured the water velocity across the channel by Teledyne flow meters (ISCO, Lincoln, NE, USA), and tested the substrate composition by selecting 100 sediment particles on the riverbed randomly and counting the percentage of substrate classes (boulders, cobbles, pebbles, sand grains), according to Kondolf (1997). The substrate diversity was calculated for each site by means of the Shannon-Weiner diversity index H’ (Shannon 1997) for each site.

2.3. *Physico-chemical parameters of stream water (denoted ENV)*

Physico-chemical characteristics of surface water were measured in three sampling spots in each stream in both experimental seasons. pH, dissolved oxygen (DO) and turbidity were *in situ* measured with a HACH pH/temperature meter (LA-pH 10, HACH, Loveland, CO, USA), a YSI (Professional Plus, YSI Incorporated, Yellow Springs, OH, USA), and a turbidity meter (DR2100Q, HACH, Loveland, CO, USA) respectively. One liter of water sample was collected from each location, filtered through 0.45 μm filters, and analysed within 48 hour for a range of chemical measures, these included ammonium nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), total phosphorus (TP) with a Lachat flow injection analyzer (QuickChem 8500, Hach, USA), total organic carbon (TOC), total nitrogen (TN) with a total organic carbon analyzer (Multi N/C3100, Jena, Germany), and chemical oxygen demand (COD) with a COD analyzer (DR1010, HACH, Loveland, CO, USA).

2.4. Spatial factors (denoted Spatial factor)

Geographical position and dispersal across the rivers were assessed using Principal Coordinates of Neighborhood Matrices (PCNM) (Guo et al., 2019). An euclidean distance matrix was calculated using geographic coordinates (latitude and longitude) with the ‘earth.dist’ function in the ‘fossil’ R package. PCNM matrices were then derived using the ‘pcnm’ function in the ‘vegan’ R package (Jyrkänkallio-Mikkola et al., 2017). Seven PCNMs were generated, and those with positive eigenvalues (PCNM2-5) together with latitude and longitude were used as spatial factors (Guo et al., 2019).

2.5. Macroinvertebrates

Macroinvertebrates were sampled in three randomly-distributed sampling quadrats (1m x 1m) close to leaf bags in each river using a kick net (opening: 9.5 cm x 14.5 cm; mesh size: 500 mm) in both winter (January 2018) and summer (August 2018) to coincide with the end of the litter breakdown studies. After disturbing substrates for around ten minutes, macroinvertebrate samples were collected and *in situ* stored in 70% ethanol. Macroinvertebrates were then sorted and identified to family level according to Merritt et al. (2008). Alpha-diversity indices (α-diversity, i.e. total abundance, total richness, Shannon-Wiener diversity) were calculated; all macroinvertebrates except Chironomidae were classified into functional feeding groups (FFGs) at family level according to Mandaville (2002), i.e. shredder, collector-gather, predator, scraper, collector-filterer. The relative abundance of each FFG was calculated and analyzed.

2.6. Biofilm Bacteria

Biofilm colonized on three 10 cm × 10 cm autoclaved unglazed ceramic tiles at 0.3 water depth of rivers were collected from each river after 39 days experiment in both seasons. After scraping and filtering on 0.22 μm pore size polycarbonate membrane filters (Millipore, MA, USA), DNA was extracted (MO BIO PowerBiofilm® DNA Isolation Kit, MO BIO Laboratories, Carlsbad, CA, USA) for each sample based on these filtrates, the V3-V4 region of bacterial 16S rRNA genes were amplified using PCR primer pairs 237F/802R according to protocol described in Lin et al. 2019, purified via MagPure Gel Pure DNA Mini Kit (Magen, Guangzhou, China) and sequenced on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) at Suzhou Genewiz Company.

Sequences were treated and analysed via QIIME 1.8.0. Following removal of the primer, all low-quality reads that containing ambiguous characters, a sequence length less than 200 bp, and having an average quality score < 20 were discarded. After removal of chimeras detected using the UCHIME algorithm (Edgar et al. 2011), the high-quality reads were clustered into OTUs (Operational Taxonomic Units) via USEARCH (1.9.6) with a 97% similarity (Edgar 2010). All OTUs were then assigned to taxonomic category using the Ribosomal Database Project (RDP) classifier at a confidence threshold of 0.8. Bacterial α-diversity indices (i.e. Shannon-Weiner index; Chao1 richness) were calculated based on the results of the operational taxonomic units (OTUs).

2.7. Leaf Litter Decomposition

Leaves of *Cinnamomun camphora* (Camphor), an evergreen and widely distributed tree in Southern China, were collected just after abscission around the Xi'an-Jiaotong Liverpool University campus (31°16′28″ N, 120°44′17″ E) in November 2017 and May 2018 for winter and summer experiment, respectively. After gently removing small, attached particles, intact leaves were oven dried at 60°C for 48 hours, weighed into 5g groups, and placed in coarse-mesh (8-mm mesh) bags (16/20 cm). Six leaf bags were prepared and distributed at the bottom of each river on the first day of the experiment in each season. Four leaf bags were retrieved from each river after 39d of leaf immersion, with the other two bags missing. The collected leaves were gently rinsed with deionized water, dried at 60°C to constant mass (48 h), and weighed to the nearest 0.001 g. The leaf breakdown rate was calculated according to the formula:

$$\ln (W_t/W_0) = - kt + b$$

Where Wt is the leaf weight remaining at time t, W_0 is the initial leaf weight, *t* is the time in d, and *b* is the y-intercept.

2.8. Statistical analysis

All data were analyzed using R (version 3.6.1, R Core Team 2019). Differences in habitat characteristics, water chemistry, α-diversity of bacteria, macroinvertebrate, relative abundance of macroinvertebrate FFGs, and leaf breakdown rate in three stream types as well as the temporal difference of leaf litter decomposition were analyzed using one-way analysis of variance (Torres-Mellado et al. 2012), followed by the Tukey–Kramer post hoc test for comparison of means. To explore relationships between habitat characteristics, physico-chemical features, biofilm bacterial community, macroinvertebrate community, and leaf breakdown rate, Spearman’s correlation coefficients were calculated. Environmental factors and leaf decomposition rate were ln (x + 1) transformed if the residuals deviated from normality, and explanatory factor that reflects notable multi-collinearity (Spearman correlation coefficient ≥ 0.85) was excluded from further analysis (Cai et al. 2017). Stepwise multiple regression analysis was implemented to determine the best model that best explained the difference in leaf breakdown rate. We selected explanatory variables by performing forward selection using the ‘adespatial’ package in R. Monte Carlo permutation tests was then used to test the response significance of litter breakdown rates to abiotic (physico-chemical and habitat variables) and biotic (bacterial and macroinvertebrate taxonomic variables) indices. Finally, variables selected by forward selection in the ‘packfor’ R package were assigned into three factor groups (Habitat, ENV, Spatial), all variables were grouped into four explanatory factor groups: habitat, environmental, bacteria, and macroinvertebrate, variation partitioning was performed to test the contribution of spatial factors to the variance in leaf mass loss, and to explore the contribution of abiotic and biotic factors to the variation of leaf breakdown rate using the ‘varpart’ function in the ‘vegan’ R package (Oksanen et al. 2019).

3. Results

3.1. Abiotic variables

The variations of abiotic variables in winter 2017 and summer 2018 are displayed in Table 1. Briefly, forest and restored rivers exhibited a substantially greater substrate diversity than degraded rivers ($p < 0.05$). In summer, rivers undergoing habitat restoration have a faster current velocity than the other two river types ($p < 0.05$). Degraded rivers had notable greater concentrations of TN, TOC, COD, and turbidity ($p = 0.003$, $p = 0.047$, $p = 0.032$, and $p = 0.014$, respectively) than the forest rivers. Restored rivers had increased turbidity and TN concentrations ($p = 0.013$ and $p = 0.060$, respectively) when compared to forest rivers and a lower concentration of TN than the degraded ones ($p = 0.073$). In winter, forest rivers had greater DO concentrations, lesser TOC, lower turbidity than that in the degraded rivers ($p = 0.029$, $p = 0.002$, $p = 0.018$, respectively), and lower TOC than the restored river ($p = 0.027$); compared with degraded rivers, restored rivers had greater DO and slightly reduced TOC concentration after habitat restoration ($p = 0.049$, $p = 0.122$, respectively).

3.2. Biotic variables

As summarized in Fig. 2a-e and Table 2b, the taxonomic diversity of macroinvertebrate as well as the relative abundance of shredder and collector-gatherer tested in summer 2018 were much smaller in degraded rivers, and greater in forest and restored rivers ($p < 0.05$). No difference of these indices was recorded between restored rivers and reference forest rivers ($p > 0.05$). No differences were detected among three river types with regard to biofilm bacterial taxonomic compositions (Table 2, Chao1 richness ($p > 0.05$). Bacterial Shannon-Wiener diversity was much greater in restored and degraded rivers than forest ones ($p < 0.05$); restored rivers had comparable bacterial diversity with degraded ones ($p > 0.05$) (Fig 2f).

In winter 2018, the taxonomic diversity of macroinvertebrates had a similar trend as that collected in summer investigation (Table 2b). Macroinvertebrate α-diversity presented considerable heterogeneity for total abundance ($F_{2,6}$ = 18.19, $p = 0.0037$), total richness ($F_{2,6}$ = 19.14, $p = 0.0033$), Shannon-Wiener diversity ($F_{2,6}$ = 17.91, $p = 0.0039$), relative abundance of shredder ($F_{2,6}$ = 12.9, $p = 0.0088$) and relative abundance of collector-gatherer ($F_{2,6}$ = 21.07, $p = 0.0025$). Forest and restored rivers have far more macroinvertebrate abundance, richness, and Shannon-Wiener diversity than degraded rivers ($p < 0.05$; Fig. 2a-c), restored rivers have similar taxonomic diversity to forest rivers ($p > 0.05$). The relative abundance of shredder and collector-gatherer species were greater in restored and forest rivers ($p < 0.05$) than in degraded rivers (Fig. 2d-e). In terms of winter bacterial α-diversity, as shown in Table 2a, a greater diversity of bacteria was found in degraded rivers than restored rivers, restored and forest rivers had fewer and comparable bacterial diversity (Fig. 2f).

3.3. Leaf breakdown rate in winter and summer

In both winter and summer, significant differences of leaf breakdown rate were found among river types (Winter: $F_{2,6}$ = 13.58, $p < 0.01$; Summer: $F_{2,6}$ = 20.79, $p < 0.01$). Forest and restored rivers possessed faster leaf decay rates than degraded rivers in either winter or summer ($p < 0.01$; Fig. 3). No difference in leaf decomposition rate was observed when comparing forest with restored rivers during both experiment periods ($p > 0.05$).

Temporally, leaf litter decay faster in summer than winter ($F_{5,12} = 0.001$, $p < 0.01$). In contrast to winter leaf litter decomposition, the leaf breakdown rates were greater in summer in either forest river ($p = 0.005$), or restored rivers ($p = 0.003$). No difference in leaf decomposition, however, was found in degraded rivers between winter and summer ($p > 0.05$).

3.4. Correlation between environmental factors and leaf breakdown rate

The correlation coefficients between abiotic factors (including habitat features and physico-chemical variables) and leaf litter decomposition rate in both summer and winter experiment period are displayed in Table 3. Leaf litter decomposition rate in summer periods had strong, positive correlations with habitat characteristics (substrate diversity) and negative correlations with surface water chemical variables (TOC, TN, and NH_4-N). In winter 2018, leaf litter decompositions were correlated positively with DO, water velocity, substrate diversity, and negatively with water turbidity, TOC, and COD concentrations.

Stepwise regression analysis indicated a greater correlation with substrate diversity ($r^2 = 0.567$, $p < 0.05$) than physico-chemical variable TOC ($r^2 = 0.489$,

$p < 0.05$) in summer (Fig. 4a-b). In winter, leaf decomposition rates were highly correlated with substrate diversity ($r^2 = 0.456$, $p < 0.05$) and COD ($r^2 = 0.711$, $p < 0.01$; Fig. 4c-d).

3.5. *Correlation between benthic organisms and leaf breakdown rate*

Leaf decay rate was positively related to the abundance, richness, Shannon-Wiener diversity index of macroinvertebrate, and relative abundance of functional feeding groups such as shredder in both winter and summer (Table 4). Though the leaf breakdown rate was more related to macroinvertebrate richness in summer and macroinvertebrate abundance in winter, stepwise regression indicated that the summer litter decay rate was multi-linearly linked to total abundance, total richness and relative abundance of collector-gatherer. The predicted values generated based on the model (k = 0.00003*Abundance+0.00046*Richness-0.00004*cg+0.00954) showed a strong fit ($r^2 = 0.925$, $p < 0.01$; Fig. 5a). In terms of winter decomposition, it was strongly related to macroinvertebrate richness ($r^2 = 0.543$, $p < 0.05$; Fig. 5b).

3.6. *Contribution of abiotic and biotic factors in leaf decomposition*

To determine the influence of environmental factors on leaf breakdown rate, spatial factors in particular, abiotic factors were assigned to three factor groups: Habitat, ENV, and Spatial. The results demonstrated that environmental factors explained 68% of variance in summer leaf decomposition and 33% of variance in winter leaf decay, respectively (Fig. 6ab). Most of the variation were explained by habitat variables (44% in summer, 15% in winter), spatial factors explained the lest of variation (6% in summer, 0% in winter).

To explore the driver of leaf decomposition in freshwater ecosystems, abiotic and biotic variables tested were assigned into four sets of explanatory factor groups: habitat characteristics (denoted Habitat), physico-chemical variables (denoted ENV), macroinvertebrate matrix (denoted Macroinvertebrate) and bacterial alpha diversity (denoted Bacteria). Variation partitioning revealed that 99% of the variation of the summer leaf breakdown rate was explained; macroinvertebrate taxonomic matrix accounted for most of the variance of decomposition (52%), followed by habitat factors (17%) and physico-chemical variables (11%) (Fig. 6a). 59% of the total variation was shared by ENV, Habitat, and Macroinvertebrate, additionally, Habitat and Macroinvertebrate accounted for 4% of the decomposition variance. Bacteria explained nothing on its own, however, 11% of the variation was shared by ENV, Macroinvertebrate, and Bacteria, 5% shared by ENV, Habitat, and Bacteria and 5% shared by Habitat, Macroinvertebrate, and Bacteria. No shared effect was found among four sets of factor groups (Fig. 6c).

In terms of winter litter breakdown, 80% of the variation was explained by the four-factor groups. Macroinvertebrates still contributed most to leaf decomposition among river types (33%), Habitat accounted for comparable variance (29%), followed by Bacteria (15%) and ENV (1%). Moreover, 55% of the total variance was shared by all four factors, 34% shared by ENV and Bacteria, 30% shared by Habitat, Macroinvertebrates and Bacteria, 20% by ENV, Habitat, and Macroinvertebrates, 11% by ENV and Habitat, and 1% by ENV and Macroinvertebrates (Fig. 6d).

4. Discussion

4.1. Leaf decomposition in degraded-restored-forest streams

Our overarching result that significant differences in leaf breakdown rate were found among the three river types in both winter and summer support our first hypothesis that stream habitat restoration would enhance the leaf breakdown rate significantly. Indeed, leaf breakdown happened much faster in the restored rivers than the degraded ones, in accordance with previous research that increasing habitat heterogeneity following habitat restoration drove elevated litter decomposition rates (Frainer et al. 2014, 2017). This suggests that habitat restoration can assist in reversing river degradation by enhancing habitat heterogeneity and improving the ecosystem function. Leaf litter decomposed at comparable speeds in the restored and the forest rivers indicated that the ecosystem function has been recovered to natural status under river management. A further important result was that environmental factors, including habitat characteristics, physico-chemical variables in the surface water and spatial factors, contributed to the differences in leaf decomposition rates among the river types. Habitat factors appeared to be more important in controlling leaf decomposition than physico-chemical variables. These results are in line with Frainer et al. (2017) who showed leaf decomposition was positively related to habitat heterogeneity. Spatial factors had the least contribution in both experiment periods, indicating that the spatial variation in sampling sites has little influence on our experiment, rather than spatial factors, local environmental conditions (i.e. longitude or latitude) best determined the variance of leaf mass loss.

In the winter, the restored rivers had a more diverse substrate mix and faster leaf mass loss rate than degraded rivers, results similar to those of Rasmussen et al. (2012), indicating that streams with more heterogeneous physical habitats had faster litter decomposition rates than streams with uniform physical habitats. Riverbed reconstruction and aquatic macrophytes re-introduction implemented in the restored rivers enhanced the habitat heterogeneity (Taniguchi et al. 2003), providing living habitat for periphyton, which in turn increased the activity of microbes and the abundance of shredding invertebrates (Ledger and Hilldrew 2005; Jarno et al. 2018) on leaf decomposition. Moreover, restored rivers possessed higher DO and lower TOC concentration than the degraded urban rivers, which also led to faster decomposition in restored rivers (Medeiros et al.

2008; Graça et al. 2015). With saturated DO induced by hydraulic connection and the re-introduction of aquatic plants in the restored rivers, an reduce of previous concentrated organic matter provides energy and nutrients resources for both microbes and macroinvertebrates, hence stimulating leaf litter decomposition (Graça 2001). However, very high concentrations of organic matter including complex pollutants caused by urbanization depletes DO (Allan 2004), which in turn reduces the activity of microorganisms (fungal, bacteria) and shredder abundance, both of which affect leaf decomposition in degraded rivers (Wantzen and Wagner 2006; Lujan et al. 2013; Graça et al. 2015).

In the summer, the restored rivers had a greater substrate diversity and faster flow velocity than degraded ones. The faster flow was caused by channel reconnection, which increases the shear force on leaf litters (Paul et al. 2006), and along with the enhanced substrates produced during riverbed reconstruction stimulates the growth of abundant microbial and shredding decomposers (Shi et al. 2019), which all combine to produce faster leaf litter decomposition in the restored rivers. Moreover, due to increased flow and developed nutrient cycling, the TN concentration in the restored rivers was lower than that in degraded rivers, but greater than the TN concentration in forest rivers. These moderate dissolved nutrient concentrations in rehabilitated streams provide aquatic biotas with abundant food resources, which in turn promote the metabolism activities (including organic matter breakdown) of biotas in the form of microbial decomposition (Hladyz et al. 2010; Ferreira and Chauvet 2011) and invertebrate decomposition (Gulis et al. 2006). On the contrary, leaf decomposition was reduced in the degraded rivers where habitat diversity was low and eutrophication was present, presumably by the depletion of dissolved oxygen (Allan 2004), and reduced abundance and activity of leaf associated aquatic organisms (Couceiro et al. 2006), here measured as total macroinvertebrate abundance and leaf-shredding species, such as shredders and collector-gatherers, which led to the greatest decomposition in the first phase of leaf litter decay (Gingerich et al. 2015; Tiegs et al. 2013).

Leaf decay much faster in summer in both forest and restored rivers, which is in line with Follstad Shah et al. (2016) who suggested that warming could result in a dramatic increase in leaf breakdown rates. It is possible that this could in part be attributed to the enhanced shear force on leaf litters due to the speed flow velocity in summer in both river types, and increased water temperatures which together stimulates the metabolism of microbial and macroinvertebrate decomposers in streams with heterogenous habitat (Gonçalves et al. 2013; Follstad Shah et al. 2016). It is notable that, no difference in leaf breakdown rate was observed in different seasons in degraded rivers. Relative low abundance, richness and diversity of detritivores presented in the degraded rivers might diminished the litter breakdown increases with temperature (Boyero et al. 2011).

4.2. Bacteria on leaf decomposition

Biofilm bacteria play an important role in the initial decomposition of organic matter such as leaf litters (Bärlocher et al. 2005) as they break down large molecules (cellulose, chitin, and lignin) within leaf litters into smaller compounds through biochemical and physiological processes (Das et al. 2007). Here, bacteria contributed less than macroinvertebrate to the variance of leaf decomposition rates. Bacterial α-diversity accounted for none in the summer and 15% in winter leaf decomposition. This result is in accordance with Baldy et al. (1995) who showed that bacteria contributed little to leaf litter breakdown in a large river and another study which indicated that bacteria contribute less (4.2 to13.9%) to overall leaf carbon loss in a polluted river (Pascoal and Ca´ssio 2004). The aerobic atmosphere in the studied rivers studied here might limit the contribution of bacteria in leaf litter decomposition as bacteria contribute more to leaf decay under anoxic or hypoxic conditions (Pascoal and Ca´ssio 2004). Biofilm samples collected from the ceramic tiles rather than leaf litters might also interpreted the less contribution of bacteria to some extent, for the difference in bacterial community compositions between epilithic biofilm and biofilm associated with plant litter, although the difference is less pronounced (Buesing et al. 2009). However, bacteria account for more variance in winter leaf decomposition than summer ones. Less diverse bacteria in winter may enhance their contribution, as bacterial diversity was linked negatively to the leaf decomposition (*r* = -0.1674 in summer, *r* = -0.3766 in winter, respectively). However, litter decomposition can be controlled by the biodiversity, biomass, and activities of bacteria (Lecerf et al. 2005), evaluating α-diversity alone in this study may obscure the contribution of bacteria in leaf mass loss (Gulis et al. 2006).

Moreover, aquatic fungi, mainly hyphomycetes, have been reported to be more important in the early stages of leaf litter decomposition than bacteria (Rasmussen et al. 2012). Although microbial leaf decomposition results from the combined actions of fungi and bacteria (Das et al. 2007), fungi are more efficient than bacteria in leaf breakdown through invasion and enzymatic hydrolysis of leaf material and lysed hyphae (Chamier 1985; Shearer 1992; Das et al. 2007). Here, unfortunately, fungi were not taken into consideration and this limits the comprehensive interpretation of leaf litter decay.

4.3. Role of Macroinvertebrates on leaf mass loss

Apart from physical abrasion and microbial degradation, invertebrate fragmentation is one of the most important processes in leaf decomposition (Graça 2001; Zhang et al. 2003). Here, the abundance, richness, and diversity of macroinvertebrate in conserved rivers (forest and restored rivers) were greater than those in urban degraded rivers, attributing to the enhanced habitat substrate diversity, faster water current flows, and improved water quality (Iñiguez-Armijos et al. 2016; Turley et al. 2016) in the restored rivers. Among all factors tested, macroinvertebrate indices account for most of the leaf decomposition variance, 52% in summer and 33% in winter, respectively, and are similar to those of Gingerich, Panaccioneb and Andersona (2015). The macroinvertebrate contribution to leaf decay was greater than physico-chemical and microbial factors. Invertebrates play dominant roles in the later stage of breakdown (Webster and Benfield 1986), mainly due to the increased macroinvertebrate abundance, diversity, and subsequent macroinvertebrate associated leaf-shredding activities, as leaf decomposition had significant positive correlations with macroinvertebrate α-diversity indices.

Aquatic decomposition is often driven by invertebrates known as shredders (Encalada et al. 2010; Chara-Serna et al. 2012; Iñiguez-Armijos et al. 2016). Here, leaf breakdown rate in both summer and winter were all associated positively with shredder abundance (*r* = 0.8787 in summer, *r* = 0.7468 in winter, respectively). Consistent with researches which demonstrated a weakened leaf decay due to a decreased shredder abundance (Wallace et al. 1996; Sponseller and Benfield 2001). The relative abundance of shredders was greater in heterogeneous habitat rivers (forest and restored rivers) than degraded rivers (Frainer et al. 2017), demonstrating the reasons for faster leaf decay in the forest and restored rivers compared to degraded rivers. However, elsewhere it has been shown that shredders play a minor role in leaf litter breakdown in neotropical streams (Mathuriau and Chauvet 2002; Goncßalves et al. 2007). Further studies might help to explore the cause of variations.

5. Conclusions

This study indicates that habitat restoration had an important positive effect on leaf breakdown rates in river ecosystems, hence enhancing ecosystem function. Leaf litter decayed faster in rivers under positive management (forest and restored rivers) than degraded urban rivers. Leaf decomposition rate can, therefore, be a good indicator of successful ecological restoration. All factors measured here (i.e., physico-chemical factors, habitat factors, macroinvertebrate, and bacteria) made an appreciable contribution to the leaf litter breakdown process in our study streams. Our results suggest that under habitat restoration, faster water and a more diverse substrate increased the physical abrasion of the leaf litter by stronger shear forces, enhanced the metabolism of leaf litter by active benthic biological decomposers such as macroinvertebrates and bacteria. Accelerated nutrient dilution and cycling declined excessive nutrient and organic pollutants in the surface water of the restored rivers, which in turn promoted the productivity and activity decomposers by providing moderate nutrient and appropriate living habitat. The biofilm bacteria present can break down large molecules of leaf litter into smaller compounds for macroinvertebrates and the greater abundance of shredders can combine to produce a faster leaf decay rate in the forest and restored rivers compared to degraded rivers through feeding activities. To summarize, all factors evaluated in this study played a synergetic contribution to the change in leaf litter decomposition rates among the three river types. The role of macroinvertebrates, mainly shredders appeared to be particularly important, followed by habitat factors, physico-chemical variables, and biofilm bacteria. For the comprehensive evaluation of the stream ecosystem function, leaf-associated fungal community and microbial production should also be tested in future determinations.

Our findings show that the habitat restoration of streams can improve degraded streams by increasing habitat elements, enhancing channel connectivity, changing water chemistry and aquatic communities (e.g., microbe, macroinvertebrate), all of which combine to improve energy and nutrient cycling process, here measured using leaf litter decomposition rates. Habitat restoration positively affected the structure and function of the deteriorate stream ecosystems. The overall findings of this study contribute to our understanding of the responses of ecosystem function to habitat restoration in urban rivers, providing useful evidence that habitat restoration can be used as an effective measure of freshwater management via recovering ecosystem structure and function. For future water conservation and management, we recommend that habitat features, physico-chemical properties and aquatic organisms should be taken into consideration in ecological restoration strategies to restore the ecosystem integrity and related ecosystem process.

Funding

This work was supported by the Natural Science Foundation of Jiangsu Province (BK20171238) and XJTLU Research Development Fund (RDF-15-01-50) to Yixin Zhang; the Foundation of Key Laboratory of Southwest China Wildlife Resources Conservation, China West Normal University (XNYB18-06) to Naicheng Wu; and the seed fund programme in HKU-ZIRI granted to Xin Luo.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Acknowledgments

We thank Eduardo Medina-Roldan, Zheng Chen, and Yi Zou for their comments for this work, thank Hongyong Xiang, Noel Juvigny-Khenafou, Qingsheng Zhu, HuCheng Chang, and LiangPing Long for their assistance with field research, and thank Kun Guo for his assistance in data analysis.

References

Aldridge KT, Brookes JD, Ganf GG (2009) Rehabilitation of stream ecosystem functions through the reintroduction of coarse particulate organic matter. Restoration ecology 17(1):97–106 https://doi.org/10.1111/j.1526-100X.2007.00338.x

Allan DJ (2004) Landscapes and riverscapes: the influence of land use on stream ecosystems. Annual Review of Ecology, Evolution, and Systematics 35:257–284 https://doi.org/10.1146/annurev.ecolsys.35.120202.110122

Baldy V, Gessner M, Chauvet GE (1995) Bacteria, fungi and the breakdown of leaf litter in a large river. Oikos 74(1):93–102 https://doi.org/10.2307/3545678

Bärlocher F (2005) Freshwater fungal communities. In: Deighton J, Oudemans P, White J, editors. The fungal communitiy: its organization and role in the ecosystem. Florida, United States: CRC Press; 39–59

Benfield EF, Webster SW, Golladay GT, Peters GT, Stout BM (1991) Effects of forest disturbance on leaf breakdown in southern Appalachian streams. Ecology 24:1687–1690 https://doi.org/10.1080/03680770.1989.11899049

Bernhardt ES, Palmer MA, Allan JD, Alexander GJ, Barnas KA, Brooks S, et al. (2005) Synthesizing US river restoration efforts. Science 308 (5722):636–637 https://doi.org/10.1126/science.1109769

Boyero L, Pearson RG, Gessner MO, et al. (2011) A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. Ecology Letters 14, 289–294 https://doi.org/10.1111/j.1461-0248.2010.01578.x

Buesing N, Filippini M, Bürgmann H, Gessner MO (2009) Microbial communities in contrasting freshwater marsh microhabitats. FEMS Microbiology Ecology 69(1):84–97 https://doi.org/10.1111/j.1574-6941.2009.00692.x

Colangelo DJ (2007) Response of river metabolism to restoration of flow in the Kissimmee River, Florida, U.S.A. Freshwater Biology 52, 459–470 https://doi.org/10.1111/j.1365-2427.2006.01707.x

Couceiro SRM, Forsberg BR, Hamada N, Ferreira RLM (2006) Effects of an oil spill and discharge of domestic sewage on the insect fauna of Cururu stream, Manaus, AM, Brazil. Brazilian Journal of Biology 66 (1A):35–44 http://doi.org/10.1590/S1519-69842006000100006

Chamier AC (1985) Cell-wall degrading enzymes of aquatic hyphomycetes: a review. Botanical Journal of the Linnean Society 91:67–81 https://doi.org/10.1111/j.1095-8339.1985.tb01136.x

Chara-Serna AM, Chara JD, Zu~niga MDC, Pearson RG, Boyero L (2012) Diets of leaf litter-associated invertebrates in three tropical streams. International Journal of Limnology 48:139–144 https://doi.org/10.1051/limn/2012013

Coe HJ, Kiffney PM, Pess GR, Kloehn KK, Mchenry ML (2009) Periphyton and invertebrate response to wood placement in large pacific coastal rivers. River research and applications 25(8):1025–1035 https://doi.org/10.1002/rra.1201

Gonçalves AL, Graça MAS, Canhoto C (2013) The effect of temperature on leaf decomposition and diversity of associated aquatic hyphomycetes depends on the substrate. Fungal Ecology 6:546-553 https://doi.org/10.1016/j.funeco.2013.07.002

Das M, Todd VR, Laura GL (2007) Diversity of fungi, bacteria, and actinomycetes on leaves decomposing in a stream. Applied and Environmental Microbiology 73(3):756–767 https://doi.org/10.1128/AEM.01170-06

Dangles O, Chauvet E (2003) Effects of stream acidification on fungal biomass in decaying beech leaves and leaf palatability. Water Research 37(3):533–538 https://doi.org/10.1016/S0043-1354(02)00359-7

Dangles O, Gessner MO, Guerold F, Chauvet E (2004) Impacts of stream acidification on litter breakdown: implications for assessing ecosystem functioning. Journal of Applied Ecology 41:365–78 https://doi.org/10.1111/j.0021-8901.2004.00888.x

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461 https://doi.org/10.1093/bioinformatics/btq461

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194–2200 https://doi.org/10.1093/bioinformatics/btr381

Encalada AC, Calles J, Ferreira V, Canhoto CM, Graça MAS (2010) Riparian land use and the relationship between the benthos and litter decomposition in tropical montane streams. Freshwater Biology 55:1719–1733 https://doi.org/10.1111/j.1365-2427.2010.02406.x

Ferreira V, Chauvet E (2011) Synergistic effects of water temperature and dissolved nutrients on litter decomposition and associated fungi. Global Change Biology 17:551–564 https://doi.org/10.1111/j.1365-2486.2010.02185.x

Flores L, Larrañaga A, Díez J, Elosegi A (2011) Experimental wood addition in streams: Effects on organic matter storage and breakdown. Freshwater Biology 56:2156–2167 https://doi.org/10.1111/j.1365-2427.2011.02643.x

Follstad Shah JJ, Kominoski JS, Ardón M, et al. (2017) Global synthesis of the temperature sensitivity of leaf litter breakdown in streams and rivers. Global Chang Biology 23(8):3064-3075 https://doi.org/10.1111/gcb.13609

Frainer A, McKie BG, Malmqvist B (2014) When does diversity matter? Species functional diversity and ecosystem functioning across habitats and seasons in a field experiment. Journal of Animal Ecology 83:460–469 https://doi.org/10.1111/1365-2656.12142

Frainer A, Polvi LE, Jansson R, Mckie BG (2017) Enhanced ecosystem functioning following stream restoration: the roles of habitat heterogeneity and invertebrate species traits. Journal of Applied Ecology 55:377–385 https://doi.org/10.1111/1365-2664.12932

Gessner MO, Chauvet E (2002) A case for using litter breakdown to assess functional stream integrity. Ecological Applications 12:498–510 https://doi.org/10.1890/1051-0761(2002)012[0498:ACFULB]2.0.CO;2

Gessner MO, Chauvet E, Dobson M (1999) A perspective on leaf litter breakdown in streams. Oikos 85:377–384 http://dx.doi.org/10.2307/3546505

Gessner MO, Swan CM, Dang CK, McKie BG, Bardgett RD, Wall DH, et al. (2010) Diversity meets decomposition. Trends in Ecology & Evolution 25:372–380 https://doi.org/10.1016/j.tree.2010.01.010

Gingerich RT, Panaccione DG, Anderson JT (2015) The role of fungi and invertebrates in litter decomposition in mitigated and reference wetlands. Limnologica - Ecology and Management of Inland Waters 54:23–32 https://doi.org/10.1016/j.limno.2015.07.004

Goncßalves JF, Graça MAS, Callisto M (2007) Litter decomposition in a Cerrado savannah stream is retarded by leaf toughness, low dissolved nutrients and a low density of shredders. Freshwater Biology 52:1440–1451 https://doi.org/10.1111/j.1365-2427.2007.01769.x

Graça MAS (2001) The role of invertebrates on leaf litter decomposition in streams – a review. International Review of Hydrobiology 86:383–393 https://doi.org/10.1002/1522-2632(200107)86:4/5<383::AID-IROH383>3.0.CO;2-D

Graça MAS, Ferreira V, Canhoto CM, Encalada AC, Guerrero-Bola~no F, Wantzen KM, et al. (2015) A conceptual model of litter breakdown in low order streams. International Review of Hydrobiology 100:1–12 https://doi.org/10.1002/iroh.201401757

Gulis V, Ferreira V, Graça MAS (2006) Stimulation of leaf litter decomposition and associated fungi and invertebrates by moderate eutrophication: implications for stream assessment. Freshwater Biology 51(9):1655–1669 https://doi.org/10.1111/j.1365-2427.2006.01615.x

Gulis V, Suberkropp K (2003) Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. Freshwater biology 48(1):123–134 https://doi.org/10.1046/j.1365-2427.2003.00985.x

Guo K, Wu NC, Wang C, Yang DG, He YF, Luo JB, et al. (2019) Trait dependent roles of environmental factors, spatial processes and grazing pressure on lake phytoplankton metacommunity. Ecological Indicator 103:312–320 https://doi.org/10.1016/j.ecolind.2019.04.028

Greenwood JL, Rosemond AD, Wallace JB, Cross WF, Weyers HS (2007) Nutrients stimulate leaf breakdown rates and detritivore biomass: bottom-up effects via heterotrophic pathways. Oecologia 151:637–49 https://doi.org/ 10.1007/s00442-006-0609-7

Hashemi M, Zadeh HM, Arasteh PD, Zarghami M (2019) Economic and Environmental Impacts of Cropping Pattern Elements Using Systems Dynamics. Civil Engineering Journal 5(5):1020-1032 https://doi.org/10.28991/cej-2019-03091308

Hieber M, Gessner MO (2002) Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. Ecology 83:1026–1038 https://doi.org/ 10.2307/3071911

Hladyz S, Tiegs SD, Gessner MO, Giller PS, R^ısßnoveanu G, Preda E, et al (2010) Leaf-litter breakdown in pasture and deciduous woodland streams: a comparison among three European regions. Freshwater Biology 55:1916–1929 https://doi.org/10.1111/j.1365-2427.2010.02426.x

Iñiguez-Armijos C, Rausche S, Cueva A, Sánchez-Rodríguez A, Espinosa C, Breuer L (2016) Shifts in leaf litter breakdown along a forest–pasture–urban gradient in Andean streams. Ecology and Evolution 6:4849–4865 https://doi.org/10.1002/ece3.2257

Jarno T, Pauliina L, Heikki M, Jukka A, Emmi P, Ari H, et al (2018) Combined effects of local habitat, anthropogenic stress, and dispersal on stream ecosystems: a mesocosm experiment. Ecological Applications 28:1606–1615 https://doi.org/10.1002/eap.1762

Jyrkänkallio-Mikkola J, Meier S, Heino J, Laamanen T, Pajunen V, Tolonen KT, et al. (2017) Disentangling multi-scale environmental effects on stream microbial communities. Journal of Biogeography 44:1512–1523 https://doi.org/10.1111/jbi.13002.

Lecerf A, Dobson M, Dang CK, et al (2005) Riparian plant species loss alters trophic dynamics in detritus based-stream ecosystems. Oecologia 146:432–442 https://doi.org/10.1007/s00442-005-0212-3

Ledger ME, Hildrew AG (2005) The ecology of acidification and recovery: changes in herbivore-algal food web linkages across a stream ph gradient. Environmental Pollution 137(1):0–118 https://doi.org/10.1016/j.envpol.2004.12.024

Lin QY, Raju S, Marrs R, Zhang YX (2019) Effect of River Ecological Restoration on Biofilm Microbial Community. Water 11, 1244 https://doi.org/10.3390/w11061244

Little CJ, Altermatt F (2018) Species turnover and invasion of dominant freshwater invertebrates alter biodiversity-ecosystem function relationship. Ecological Monographs 88:461–480 https://doi.org/10.1002/ecm.1299

Lujan NK, Roach KA, Jacobsen D, Winemiller KO, Meza Vargas V, Rimarachın Ching V, et al. (2013) Aquatic community structure across an Andes-to-Amazon fluvial gradient. Journal of Biogeography 40:1715–1728 https://doi.org/10.1111/jbi.12131

Kondolf GM (1997) Application of the pebble count: Notes on purpose, method, and variants. Journal of the American Water Resources Association 33:79–87 https://doi.org/10.1111/j.1752-1688.1997.tb04084.x

Mandaville SM (2002) Benthic Macroinvertebrates in Freshwaters – Taxa Tolerance Values, Metrics, and Protocols. Soil & Water Conservation Society of Metro Halifax. Mathuriau C, Chauvet E (2002) Breakdown of leaf litter in a neotropical stream. Journal of The North American Benthological Society 21:384–396 https://doi.org/10.2307/1468477

Matthews RA, Buikema AL, Cairns J, Rodgers JH (1982) Biological monitoring. Part IIA. Receiving system functional methods, relationships and indices. Water Research 16:129–139 https://doi.org/10.1016/0043-1354(82)90102-6

Martínez A, Larrañaga A, Pérez J, Descals E, Pozo J (2014) Temperature affects leaf litter decom- position in low-order forest streams: field and microcosm approaches. FEMS Microbiology Ecology 87:257–67 https://doi.org/10.1111/1574-6941.12221

Martínez A, Pérez J, Molinero J, Sagarduy M, Pozo J (2015) Effects of flow scarcity on leaf-litter processing under oceanic climate conditions in calcareous streams. Science of the Total Environment 503:251–257 https://doi.org/10.1016/j.scitotenv.2014.06.018

McKie BG, Malmqvist B (2009) Assessing ecosystem functioning in streams affected by forest management: increased leaf decomposition occurs without changes to the composition of benthic assemblages. Freshwater Biology 54:2086–2100 https://doi.org/10.1111/j.1365-2427.2008.02150.x

McKie BG, Petrin Z, Malmqvist B (2006) Mitigation or disturbance? Effects of liming on macroinvertebrate assemblage structure and leaf-litter decomposition in the humic streams of northern Sweden. Journal of Applied Ecology 43:780–791 https://doi.org/10.1111/j.1365-2664.2006.01196.x

Medeiros AO, Pascoal C, Graca MAS (2008) Diversity and activity of aquatic fungi under low oxygen conditions. Freshwater Biology 54:142–9 https://doi.org/10.1111/j.1365-2427.2008.02101.x

Merritt RW, Cummins KW, Berg MB (2008) An introduction to the aquatic insects of North America. Kendall Hunt Publishing Company, Dubuque, IA

Niyogi DK, Harding JS, Simon KS (2013) Organic matter breakdown as a measure of stream health in new zealand streams affected by acid mine drainage. Ecological indicators 24:510–517 https://doi.org/10.1016/j.ecolind.2012.08.003

Niyogi DK, Lewis JWM, McKnight DM (2001) Litter breakdown in mountain streams affected by mine drainage: biotic mediation of abiotic controls. Ecological applications 11(2):506–516 https://doi.org/10.1890/1051-0761(2001)011[0506:LBIMSA]2.0.CO;2

Niyogi DK, Lewis WM, McKnight DM (2002) Effects of stress from mine drainage on diversity, biomass, and function of primary producers in mountain streams. Ecosystems 5:554–567 https://doi.org/10.1007/s10021-002-0182-9

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D et al. (2019) Package ‘vegan’ Community Ecology Package Version 2.5-5

Palmer M, Ruhi A (2019) Linkages between flow regime, biota, and ecosystem processes: Implications for river restoration. Science 365 (1264), eaaw2087 https://doi.org/10.1126/science.aaw2087

Pascoal C, Cássio F (2004) Contribution of fungi and bacteria to leaf litter decomposition in a polluted river. Applied and Environmental Microbiology 70(9):5266–5273 https://doi.org/10.1128/AEM.70.9.5266-5273.2004

Pascoal C, Cássio F, Marvanová L (2005) Anthropogenic stress may affect aquatic hyphomycete diversity more than leaf decomposition in a low-order stream. Archiv für Hydrobiologie 162:481–496 https://doi.org/10.1127/0003-9136/2005/0162-0481

Pascoal C, Pinho M, Ca´ssio F, Gomes P (2003) Assessing structural and functional ecosystem condition using leaf breakdown: studies on a polluted river. Freshwater Biology 48:2033–2044 https://doi.org/10.1046/j.1365-2427.2003.01130.x

Paul MJ, Meyer JL, Couch CA (2006) Leaf breakdown in streams differing in catchment land use. Freshwater Biology 51:1684–1695 https://doi.org/10.1111/j.1365-2427.2006.01612.x

Pérez J, Descals E, Pozo J (2012) Aquatic hyphomycete communities associated with decomposing alder leaf litter in reference headwater streams of the Basque Country (northern Spain). Microbial Ecology 64:279–90 https://doi.org/10.1007/s00248-012-0022-1

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org

Rasmussen JJ, Peter WL, Annette BP, Rikke JM, Brian K (2012) Impacts of pesticides and natural stressors on leaf litter decomposition in agricultural streams. Science of the Total Environment 416:148–155 https://doi.org/10.1016/j.scitotenv.2011.11.057

Robinson CT, Gessner MO (2000) Nutrient addition accelerates leaf breakdown in an alpine springbrook. Oecologia 122:258–263 https://doi.org/10.1007/PL00008854

Schmutz S, Jurajda P, Kaufmann S, Lorenz AW, Muhar S, Paillex A, et al. (2016) Response of fish assemblages to hydromorphological restoration in central and northern European rivers. Hydrobiologia 769:67–78 https://doi.org/10.1007/s10750-015-2354-6

Shearer CA (1992) The role of woody debris, p. 77–98. In F. Ba¨rlocher (ed.), The ecology of aquatic hyphomycetes. Springer-Verlag, Berlin, Germany

Shi X, Liu J, You X, Bao K, Meng B (2019) Shared effects of hydromorphological and physico-chemical factors on benthic macroinvertebrate integrity for substrate types. Ecological Indicators 406–414 https://doi.org/10.1016/j.ecolind.2018.02.028

Solangi GS, Siyal AA, Siyal P (2019) Analysis of Indus Delta Groundwater and Surface water Suitability for Domestic and Irrigation Purposes. Civil Engineering Journal 5(7):1599–1608 https://doi.org/10.28991/cej-2019-03091356

Sponseller RA, Benfield EF (2001) Influences of land use on leaf breakdown in Southern Appalachian headwater streams: a multiple-scale analysis. Journal of the North American Benthological Society 20(1):44–59 https://doi.org/10.2307/1468187

Srinivasan MC, Laxman RS, Deshpande MV (1991) Physiology and nutrition aspects of actinomycetes-an overview. World Journal of Microbiology and Biotechnology 7:171–184 https://doi.org/10.1007/BF00328987

Suberkropp K (1998) Effect of dissolved nutrients on two aquatic hyphomycetes growing on leaf litter. Mycological Research 102(8):998–1002 https://doi.org/10.1017/S0953756297005807

Taniguchi H, Nakano S, Tokeshi M (2003) Influences of habitat complexity on the diversity and abundance of epiphytic invertebrates on plants. Freshwater Biology 48:718–728

Tiegs S, Costello DM, Isken MW, et al. (2019) Global patterns and drivers of ecosystem functioning in rivers and riparian zones, Science Advances 5, no1, eaav0486 https://doi.org/ 10.1126/sciadv.aav0486

Tiegs SD, Entrekin SA, Reeves GH, Kuntzsch D, Merritt RW (2013) Litter decomposition, and associated invertebrate communities, in wetland ponds of the Copper River, Delta, Alaska (USA). Wetlands 33 http://dx.doi.org/10.1007/s13157-013-0485-y

Turley MD, Bilotta GS, Chadd RP, Extence CA, Brazier RE, Burnside NG, Pickwell AGG (2016) A sediment-specific family-level biomonitoring tool to identify the impacts of fine sediment in temperate rivers and streams. Ecological Indicators 70:151–165 https://doi.org/10.1016/j.ecolind.2016.05.040

Violin CR, Cada P, Sudduth EB, Hassett BA, Bernhardt PES (2011) Effects of urbanization and urban stream restoration on the physical and biological structure of stream ecosystems. Ecological Applications 21:1932–1949 https://doi.org/10.1890/10-1551.1

Wantzen KM, Wagner R (2006) Detritus processing by invertebrate shredders: a neotropical – temperate comparison. Journal of the North American Benthological Society 25:216–232 https://doi.org/10.1899/0887-3593(2006)25[216:DPBISA]2.0.CO;2

Wallace JB, Webster JR (1996) The role of macroin-vertebrates in stream ecosystem function. Annual Review of Entomology 41:115–139 https://doi.org/10.1146/annurev.en.41.010196.000555

Webster JR, Benfield EF (1986) Vascular plant breakdown in freshwater ecosystems. Annual Review of Ecology, Evolution, and Systematics 17:567–594 https://doi.org/10.1146/annurev.es.17.110186.003031

Wenger SJ, Roy AH, Jackson CR, Bernhardt ES, Carter TL, Filoso S, et al. (2009) Twenty-six key research questions in urban stream ecology: an assessment of the state of the science. Journal of the North American Benthological Society 28(4):1080–1098 https://doi.org/10.1899/08-186.1

Young RG, Matthaei CD, Townsend CR (2008) Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. Journal of The North American Benthological Society 27:605–625 https://doi.org/10.1899/07-121.1

Zan RB, Kondolf GM, Riostouma B (2017) Evaluating stream restoration project: What do we learn from monitoring? Water 9(3) https://doi.org/ 10.3390/w9030174

Zhang YX, Negishi J, Richardson JS, Kolodziejczyk RI (2003) Impacts of marine-derived nutrients on stream ecosystem functioning. Proceedings of the Royal Society B: Biological Sciences 270:2117–2123 https://doi.org/ 10.1098/rspb.2003.2478

Zhang YX, Richardson JS, Negishi JN (2004) Detritus processing, ecosystem engineering and benthic diversity: a test of predator-omnivore interference. Journal of Animal Ecology 73:756–766 https://doi.org/10.1111/j.0021-8790.2004.00849.x

Zhang YX, Juvigny-Khenafou N, Xiang HY, Lin QY, Wu ZJ (2019) Multiple stressors in China’s freshwater ecoregions. In: Sabater S, Elosegi A, Ludwig R (eds) Multiple stress in river ecosystems: status, impacts and prospects for the future, 1st edn. Elsevier, pp 193–204

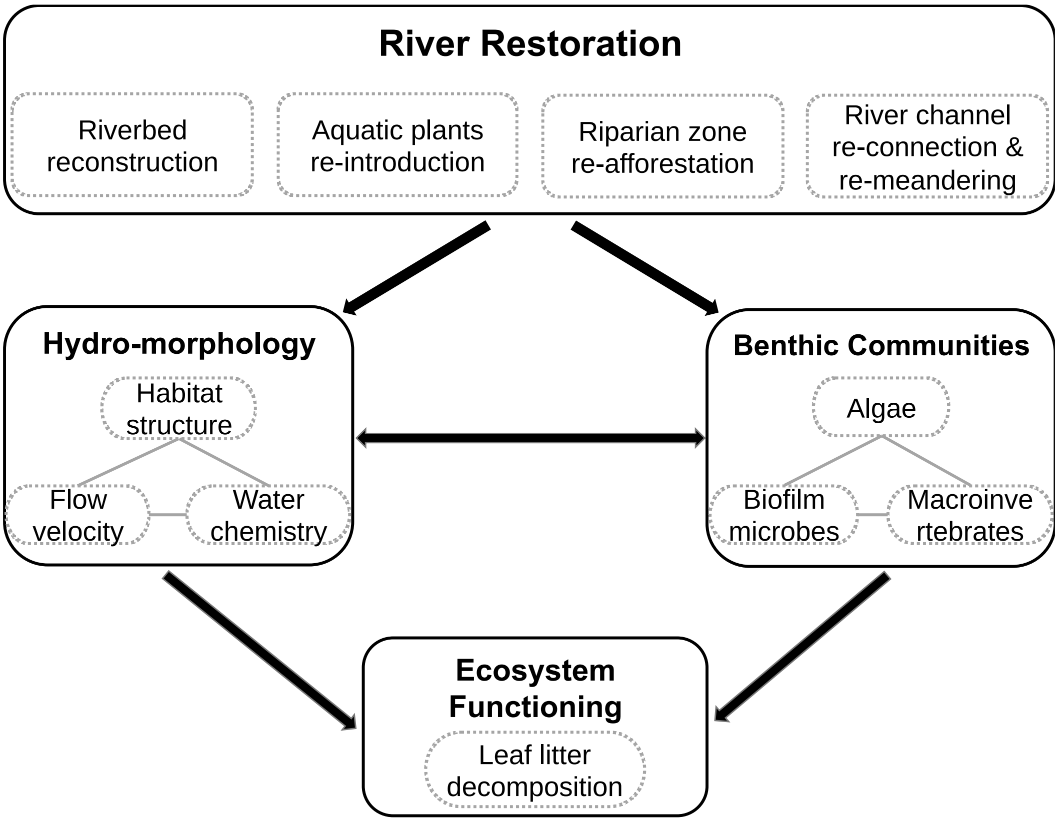

Fig. 1. Conceptual model of the experiment. River ecological restoration induced the variance of a host of environmental conditions (habitat structure, flow velocity and water chemistry), which in turn influence the ecosystem structure (benthic communities) and ecosystem functioning (leaf litter decomposition) of the river ecosystem.

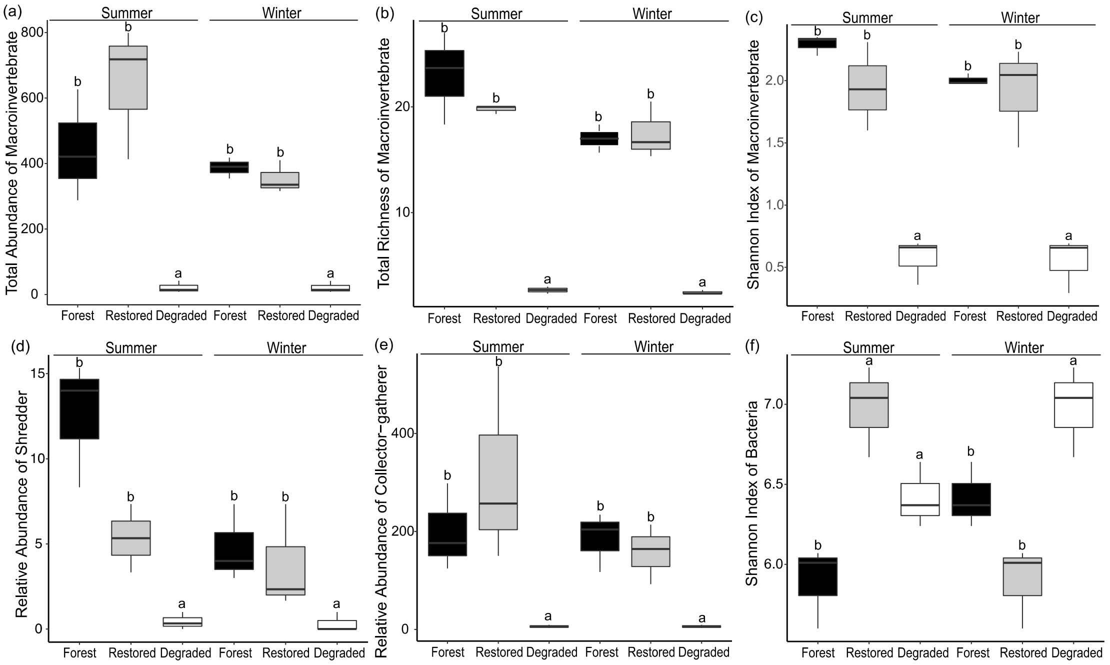

Fig. 2. Comparison of the macroinvertebrate taxonomic diversity (a) total abundance, (b) total richness, (c) Shannon-Wiener diversity, (d) relative abundance of shredder, (e) relative abundance of collector-gatherer and bacterial diversity (f) Shannon-Wiener diversity of bacteria in three river types in summer and winter within Anji City Region, PRC. Mean values (± SE, n = 3) are presented; different lower case letters indicate a significant difference observed at *p* = 0.05 level.

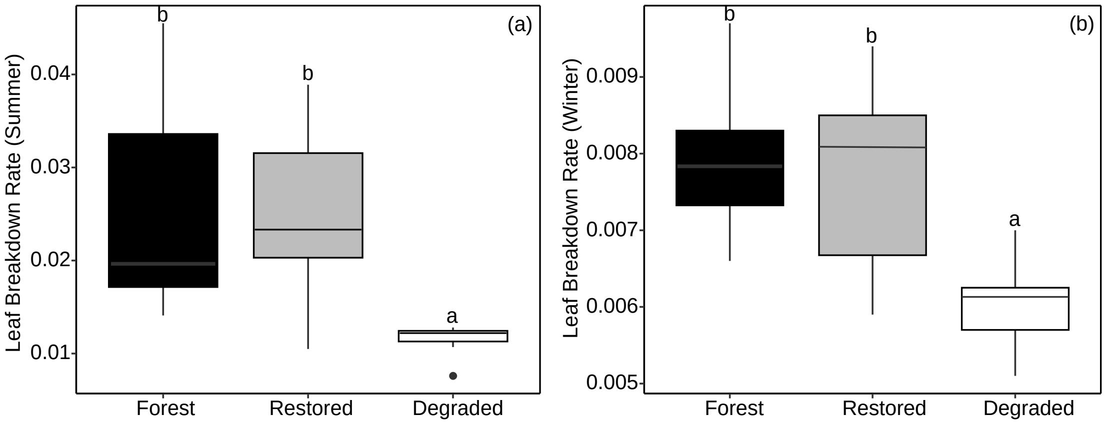

Fig. 3. Boxplots illustrating leaf breakdown rates in summer (a) and winter (b) in forested, restored, and degraded rivers within the Anji City Region, PRC. Blackline: median value; box: quartile interval; whiskers: minimum and maximum value. Different lowercase letters indicate the significant difference observed at the *p* = 0.05 level.

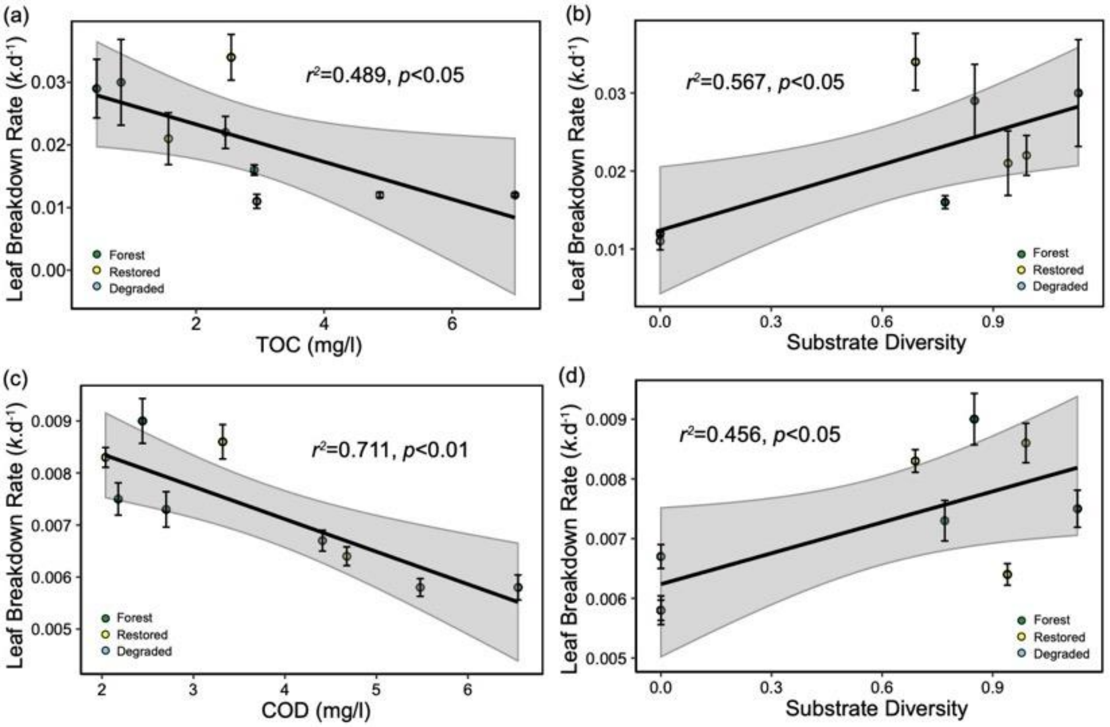

Fig. 4. Stepwise multiple regression analysis to identify the relationship between leaf litter breakdown rates by days (*k* d^{-1}) and physicochemical variable TOC (a), habitat factor Substrate diversity (b) in summer and Physico-chemical variable COD (c), habitat factor Substrate diversity (d) in winter. The coefficients of determination (r^2) and p are shown in each panel. Each data point represents the mean value of each treatment in each stream.

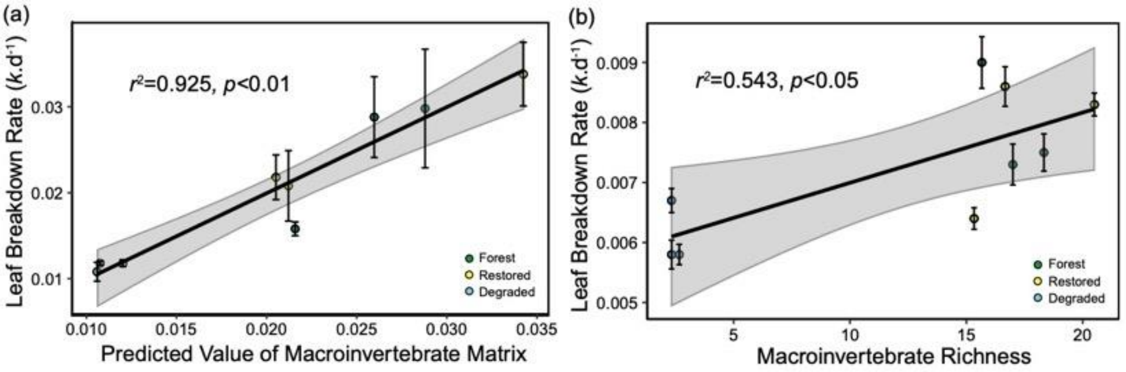

Fig. 5. Stepwise multiple regression analysis to identify the relationship between leaf litter breakdown rates by days (k d^{-1}) and (a) predicted value of macroinvertebrate matrix in summer and (b) macroinvertebrate richness in winter. The coefficients of determination (r^2) and p are shown in each panel. Each data point represents the mean value of each treatment in each stream.

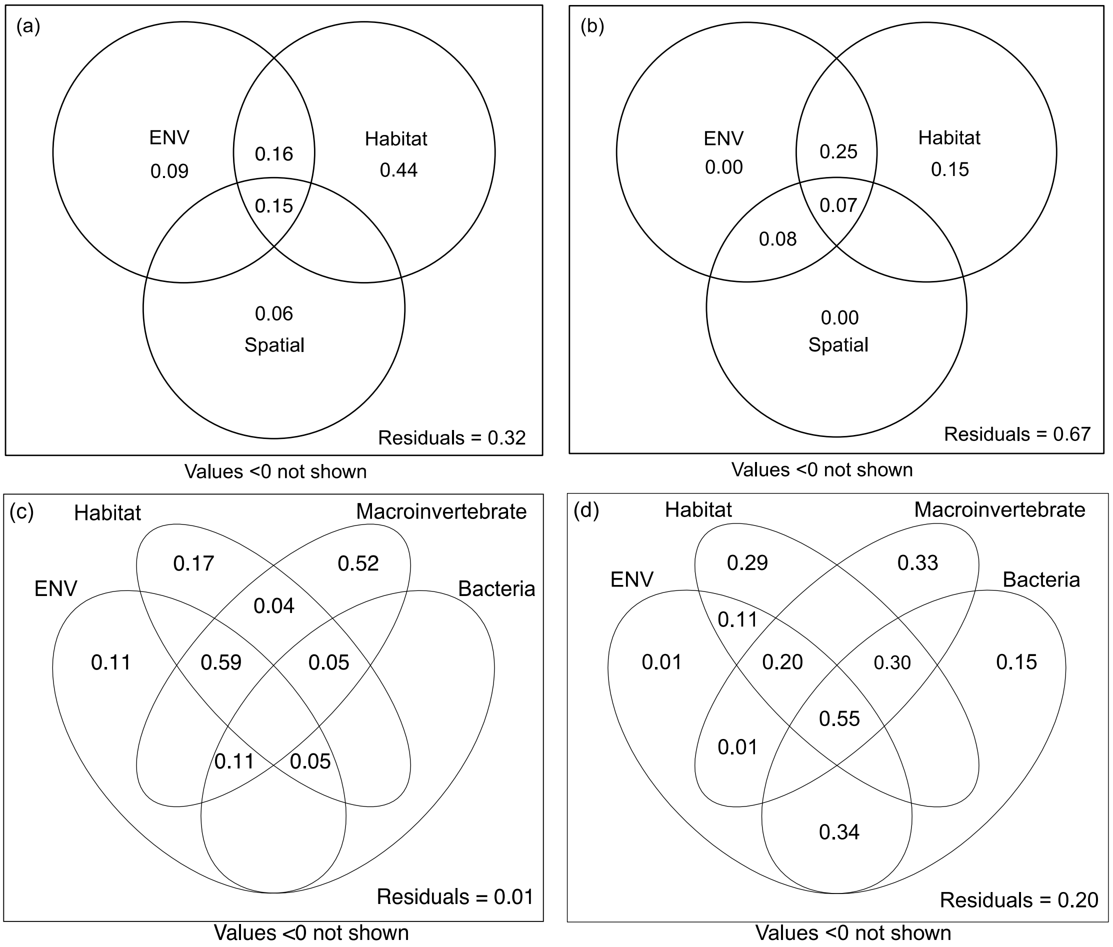

Fig. 6. Venn diagrams illustrating the variation partitioning analysis for leaf litter breakdown rates by days (*k* d^{-1}) in (a,c) summer and (b,d) winter. Habitat, ENV, Spatial, Macroinvertebrate, and Bacteria are sets of explanatory factor groups representing habitat variables, physico-chemical variables, spational factors, taxonomic diversity of macroinvertebrate, and taxonomic diversity of biofilm bacteria, respectively. Residuals are shown in the lower right corner. All fractions based on adjusted R^2 are shown as percentages of the total variation.

Table 1

Mean values of habitat and physico-chemical variables in different types of rivers in winter and summer within the Anji City Region, PRC. The values represent the mean ± standard error of three replicate samples.

Environmental Variables	Winter			Summer		
	Forest	Restored	Degraded	Forest	Restored	Degraded
Width (m)	8.83 ±1.64	13.17 ±3.09	11.57 ±5.72	8.83 ±1.64	13.17 ±3.09	11.57 ±5.72
Mean Depth (cm)	35.87 ±7.97	28.13 ±7.22	22.87 ±3.86	35.87 ±7.97	28.13 ±7.22	22.87 ±3.86
Substrate	0.92 ±0.11	0.87 ±0.09	0.00 ±0.00	0.92 ±0.11	0.87 ±0.09	0.00 ±0.00
Velocity	0.04 ±0.01	0.20 ±0.10	0.00 ±0.00	0.01 ±0.00	0.41 ±0.17	0.00 ±0.00
Canopy	71.67 ±6.01	3.33 ±1.86	21.73 ±14.76	81.67 ±6.01	4.33 ±2.85	27.40 ±17.47
Dissolved Oxygen (mg/L)	7.48 ±0.18	7.23 ±0.31	4.26 ±1.92	7.48 ±0.18	7.23 ±0.31	4.26 ±1.92
pH	7.18 ±0.10	7.12 ±0.13	7.06 ±0.10	7.18 ±0.10	7.12 ±0.13	7.06 ±0.10
Turbidity	1.54 ±0.46	14.13 ±4.74	12.84 ±3.38	1.54 ±0.46	14.13 ±4.74	12.84 ±3.38
NH4-N (mg/L)	0.52 ±0.09	0.61 ±0.13	2.3 ±1.29	0.52 ±0.09	0.61 ±0.13	2.30 ±1.29
NO3-N (mg/L)	0.16 ±0.01	0.69 ±0.22	0.85 ±0.40	0.16 ±0.01	0.69 ±0.22	0.85 ±0.40
TN (mg/L)	1.09 ±0.05	2.07 ±0.47	3.24 ±0.16	1.09 ±0.05	2.07 ±0.47	3.24 ±0.16
TP (mg/L)	0.08 ±0.04	0.07 ±0.01	0.15 ±0.05	0.08 ±0.04	0.07 ±0.01	0.15 ±0.05
Chemical Oxygen Demand (mg/L)	6.29 ±1.96	10.38 ±0.88	16.22 ±2.80	6.29 ±1.96	10.38 ±0.88	16.22 ±2.80
Total Organic Carbon (mg/L)	1.40 ±0.76	2.19 ±0.31	4.93 ±1.16	1.40 ±0.76	2.19 ±0.31	4.93 ±1.16

Table 2

Mean values of (a) bacterial indices and (b) macroinvertebrate taxonomic metrics in different types of rivers in winter and summer within the Anji City Region, PRC. The values represent the mean ± standard error of three replicate samples.

(a)

Bacteria Indices	Winter			Summer		
	Forest	Restored	Degraded	Forest	Restored	Degraded
OTU	615.22 ±41.90	585.00 ±19.86	666.89 ±69.17	338.44 ±60.81	566.00 ±217.7	574.83 ±55.71
Chao1	715.45 ±36.27	708.84 ±21.19	769.73 ±72.81	423.27 ±78.52	661.97 ±236.1	724.64 ±60.45
Shannon	6.42 ±0.12	5.89 ±0.15	6.98 ±0.16	5.89 ±0.15	6.98 ±0.16	6.42 ±0.12

(b)

Macroinvertebrate	Winter			Summer		
	Forest	Restored	Degraded	Forest	Restored	Degraded
Abundance	387.44 ±18.42	353.78 ±28.66	21.11 ±10.26	445.11 ±98.6	643.55 ±117.44	21.33 ±10.48
Richness	17.00 ±0.77	17.50 ±1.55	2.44 ±0.11	23 ±2.53	19.78 ±0.22	2.67 ±0.19
Shannon Diversity	2.00 ±0.03	1.91 ±0.23	0.55 ±0.13	2.29 ±0.05	1.95 ±0.21	0.57 ±0.11
Shredder	4.78 ±1.31	3.78 ±1.79	0.33 ±0.33	12.55 ±2.15	56.22 ±50.9	0.44 ±0.29
Collector-gatherer	185.22 ±35.06	156.89 ±35.32	6.11 ±1.94	199.77 ±51.49	314.67 ±115.19	6.11 ±1.94

Table 3

Correlations between environmental variables (i.e. habitat characteristics, physico-chemical variables) and leaf litter breakdown rates by days (k d^{-1}) for three types of rivers within Anji City Region, PRC. Negative coefficients are specified in capturing parentheses.

ENV Variables	Summer Leaf Breakdown Rate	Winter Leaf Breakdown Rate
pH	0.3933	0.0084
Turbidity	(0.1925)	(0.6946)$^{\cdot}$
DO	0.3933	0.5523$^{\cdot}$
NH_4-N	(0.7113)$^{\cdot}$	(0.5774)
NO_3-N	(0.4435)	0.3347
TN	(0.5439)*	(0.3766)
TP	(0.1681)	(0.2343)
TOC	(0.7448)*	(0.5272)$^{\cdot}$
COD	(0.6092)	(0.8117)*
Velocity	0.7969	0.7010*
Substrate	0.6809*	0.5958*
Canopy	0.3598	0.3766

Note: The one superscript asterisks and dots show the significant level at $p < 0.05$ and 0.1, respectively.

Table 4

Spearman correlation coefficients between biotic factor (i.e. bacterial taxonomic diversity, macroinvertebrate -diversity and the relative abundance of shredders) and leaf litter breakdown rates by days (k d^{-1}) for different types of rivers within Anji City Region, PRC. Negative coefficients are specified in capturing parentheses.

Biotic Indices	Summer Leaf Breakdown Rate	Winter Leaf Breakdown Rate
Bacterial Richness	(0.0167)	(0.1674)
Bacterial Diversity	(0.1674)	(0.3766)
Invertebrate Abundance	0.8285*	0.8619*
Invertebrate Richness	0.8992*	0.6513*
Invertebrate Diversity	0.8536*	0.6778*
Shredder	0.8787*	0.7468$^{\cdot}$
Collector-gatherer	0.6862	0.5774$^{\cdot}$

Note: The one superscript asterisks and dots show the significant level at $p < 0.05$ and 0.1, respectively.

Appendix

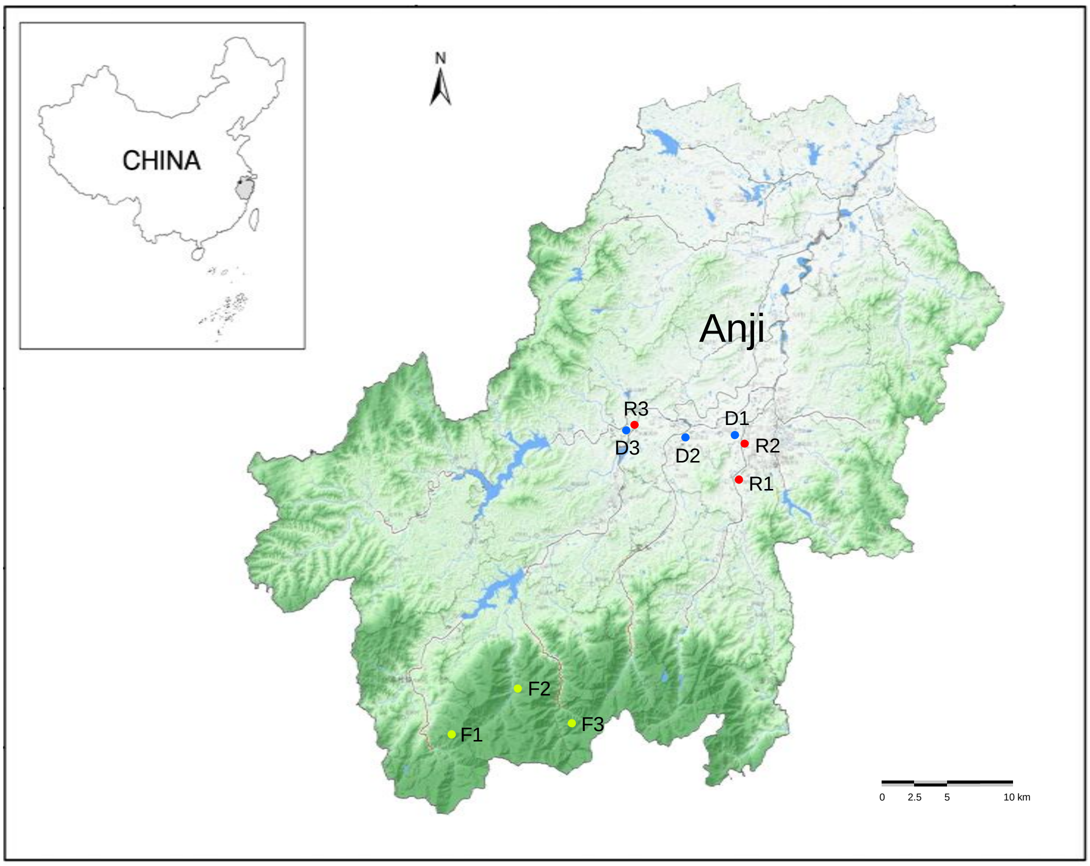

Fig. S1. Study area and locations of sampling sites within the Anji City Region, People's Republic of China (PRC), including three degraded urban rivers (D), three rivers under habitat restoration (R), and three Forested rivers (F).

Table S1 Nomenclature and Abbreviation List

N	Nitrogen
P	Phosphorus
CO2	Carbon dioxide
DO	Dissolved oxygen
NH4-N	Ammonium nitrogen
NO3-N	Nitrate-nitrogen
TN	Total nitrogen
TP	Total phosphorus
TOC	Total organic carbon
COD	Chemical oxygen demand
FPOM	Fine particular organic carbon
FFGs	Functional feeding groups
C-F	Collector-filterer
C-G	Collector-gatherer
Scr	Scraper
Shr	Shredder
Prd	Predator
PRC	People republic of China
PCNM	Principal Coordinates of Neighborhood Matrices
OTUs	Operational Taxonomic Units
RDP	Ribosomal Database Project
Camphor	Cinnamomun camphora
Habitat	Habitat variable
ENV	Physico-chemical variables
Spatial	Spatial factors
Macroinvertebrate	Macroinvertebrate matrics
Bacteria	Bacterial alpha-diversity
Alpha-diversity	α-diversity