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Pesticides override other stressors to drive stream macroinvertebrate diversity loss: unraveling interactive effects and ecological thresholds

Jing Fang [a,b], Lin Hou [c,d], Shiqi Jia [a], Jian Xu [b], Andrew C. Johnson [e], Xiaowei Jin [a,*]

[a] China National Environmental Monitoring Centre, Beijing 100012, China
[b] State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China
[c] Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[d] College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100049, China
[e] Centre for Ecology and Hydrology, Wallingford, Oxfordshire OX10 8BB, UK

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ABSTRACT

Freshwater ecosystems worldwide face increasing pressures from chemical pollutants, particularly pesticides, across both agricultural and urban settings. The individual and interactive impacts of pesticide contamination and other environmental stressors on freshwater macroinvertebrates remain insufficiently quantified under real-world, multi-stressor conditions. Here, we conducted seasonal monitoring at 39 sites in the Wei River Basin—one of China's most intensively managed agricultural and urban regions. A multiple-stressor analytical framework was then constructed to examine how pesticide contamination interacts with other environmental stressors to shape macroinvertebrate diversity. Results revealed that pesticides contributed more than 40% to both α-diversity loss (species richness) and β-diversity nestedness, exceeding the combined effect of the other factors that were considered. Interactive effects between pesticides and land use were stronger in the dry season, evidenced by a 2.1-fold increase in functional sensitivity and a 56% rise in interaction contributions. Structural equation modeling revealed that pesticides and land use differentially regulated community structure and function via direct and indirect pathways, respectively, with pronounced seasonal variation and coupled interactive effects. Community-level Threshold Indicator Taxa Analysis (TITAN) was conducted for the four high-priority pesticides. The ecological harm thresholds were estimated at 13.81 ng/L for Imidacloprid (5-95% quantile range: 4.02-104.47 ng/L), 1.78 ng/L for Hexazinone (0.65-4.20 ng/L), 10.70 ng/L for Atrazine (2.87-31.33 ng/L), and 0.34 ng/L for Simazine (0.01-2.29 ng/L). These compound specific thresholds, derived under real-world multiple stressor conditions, provide ecologically grounded benchmarks for regional watershed management and highlight the importance of compound level threshold derivation for pesticide risk assessment and the protection of macroinvertebrate communities. Building on established multiple-stressor frameworks, this study provides quantitative evidence that pesticide contamination is primarily associated with macroinvertebrate diversity loss in a major agricultural watershed, and demonstrates how hydrological seasonality modulates pesticide effects on taxonomic and functional diversity, offering evidence-based support for watershed pesticide regulation and biodiversity conservation.

1. Introduction

Global freshwater ecosystems are subject to multiple, often synergistic, pressures from anthropogenic activities and climate change, potentially causing biodiversity losses and impaired ecological functioning. Among these pressures, chemical pollution—particularly pesticide contamination—in certain landscapes is likely to be one of the more important stressors. Nonetheless, distinguishing the relative contribution and interactive mechanisms of pesticide inputs under multiple-stressor conditions remains a major challenge (Young et al., 2020, Groh et al., 2022, Sigmund et al., 2023). In agriculture-dominated landscapes, pesticide usage is widely recognized as an anthropogenic stressor that disrupts aquatic ecosystem structure and function (Navarro et al., 2024). Cumulative evidence highlights the detrimental influence of organic pollutants and insecticides on freshwater integrity, with adverse effects observed at regional scales, especially for

* Corresponding author.
E-mail address: jinxw@cnemc.cn (X. Jin).

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macroinvertebrate assemblages (Liess et al., 2021, Beketov et al., 2013, Malaj et al., 2014). Such insecticides have been identified as major drivers of community degradation to macroinvertebrate community in rivers (Nowell et al., 2024). Meanwhile, natural ecosystems are commonly exposed to multiple environmental drivers (e.g., climate change, human land use intensity, hydrology alterations, and chemical pollution), whose combined effects often differ from those of single stressors; thus, incorporating multiple stressors is essential for realistic ecological risk assessment (Simmons et al., 2021, Allan et al., 2012, Yang et al., 2023).

Traditional ecological risk assessments typically rely upon single-pollutant, single-species laboratory tests, which may underrepresent the ecological consequences of stressor interactions under field conditions (Jin et al., 2024). Macroinvertebrates, which participate in pivotal ecosystem processes (e.g., nutrient cycling, food-web support), serve as valuable bioindicators sensitive to habitat and water quality changes (Pinna et al., 2024, Melcher et al., 2018). Functional traits and functional diversity, strongly governed by environmental gradients, provide robust, reliable endpoints for detecting stressor impacts (Ding et al., 2017, Hou et al., 2025). Integrating ecological trait-based and environmental data can help disentangle trait-environment linkages and spatiotemporal variability from local to regional scales. Detailed investigations of how macroinvertebrate communities respond to chemical and non-chemical factors could be very helpful to riverine ecological management (Wilkes et al., 2020, Butkas et al., 2010). Functional trait analyses, in particular, have previously been shown to capture distinct community reorganization patterns in response to climate variability (Troia et al., 2019), chemical pollution (Van den Berg et al., 2019, Liess et al., 2021), land use alterations (Scotti et al., 2019) and compound stressor conditions (Lange et al., 2014). Yet, a notable research gap persists: few studies have systematically merged functional trait approaches with multiple-stressor frameworks to quantify the individual and interaction effects of pesticides and other environmental drivers in intensively human-impacted landscapes (de Bello et al., 2025).

The Wei River Basin, characterized by intensive agricultural activities and strong anthropogenic pressures, provides an ideal model system for examining the interactive effects of pesticide pollution and land use on stream macroinvertebrate communities across contrasting hydrological seasons. Compared to agricultural watersheds in Europe and North America, pesticide application in China is generally more intensive and concentrated, driven by land fragmentation and small-scale farming. The predominance of manual and broadcast spraying methods results in relatively low pesticide use efficiency, with a large proportion of applied compounds lost to the surrounding environment rather than reaching target organisms, generating disproportionately high environmental exposure (Pei and Chen, 2024). Limited adoption of ecological buffer measures further underscores the unique challenges and research significance of Chinese agricultural watersheds in pollution management and ecological response mechanisms (Wu et al., 2018, Postigo et al., 2021). Here we hypothesized that pesticide contamination alters macroinvertebrate community structure, leading to detectable changes in both α- and β-diversity across environmental gradients. Meanwhile, pesticide effects are jointly modulated by other environmental factors, and hydrological seasonality is used as a natural framework to examine how these interactions vary under contrasting environmental conditions. By examining the interactive relationships among these environmental factors, we aimed to: (1) quantitatively assess the relative contributions of pesticide contamination and co-occurring environmental stressors (including water quality, hydrology, and land use) to patterns of macroinvertebrate taxonomic and functional diversity; (2) examine whether interactive or modulatory relationships exist between pesticide stress and other environmental stressors, with particular attention to how hydrological seasonality influences the strength and pathways of these effects; (3) identify community-level ecological thresholds along pesticide toxicity gradients, in order to improve the ecological relevance of pesticide risk assessment under realistic, multi-stressor conditions. This study aimed to assess what the ecological repercussions of pesticide pollution are within complex environmental scenarios and to propose frameworks for improving basin-scale ecological conservation strategies.

2. Materials and methods

2.1. Study area and sample collection

The Wei River is the largest tributary of the Yellow River, located in the Yellow River hinterland (103°57′-110°17′E, 33°42′-37°24′N), with a main stem length of 818 km and a watershed area of 134,800 km2. The basin supports a permanent population of approximately 35 million people, with over 4,000 years of agricultural history (Luo et al., 2024). As an important grain producing region, high-intensity agricultural production and rapid urbanization have promoted extensive pesticide application and led to water-quality degradation, imposing compound stressors on local macroinvertebrate communities, including habitat deterioration (Zhang et al., 2024b, Nie et al., 2024, Zhang et al., 2021). Overall, the Wei River Basin is characterized by transitional climatic conditions, pronounced hydrological variability, and strong human disturbance, making it a representative system for investigating pesticide-related ecological effects under multiple stressors. In this study, 39 sites (W1-W39) were established across the Wei River Basin and sampled in September 2023 (normal season) and April 2024 (dry season). The sites were distributed along the main stem and major tributaries, including 25 riverine sites and 14 tributary confluence sites (Fig. 1a). The normal season is represented by September, which falls within the region's main rainfall period (June-September), during which 50-72% of the annual precipitation occurs. The dry season is represented by April, which belongs to the basin's low-precipitation and low-flow period (February-April) (Li et al., 2019). Surface water was collected 20-30 cm below the surface and 3-5 m from the riverbank. A total of 2 L was taken using a water sampler, stored in amber glass bottles, transported at 4℃, and pretreated within 24 h for analysis.

2.2. Chemical Analysis and Quality Control

Ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS, Waters) was employed for quantitative analysis of 49 commonly used and representative pesticides in water bodies, including carbamates (CAs), neonicotinoids (NEOs), triazines (TRIs), and organophosphates (OPPs) (Table S2). These pesticides were selected based on national regulatory priorities, reported contamination patterns in China, and the main pesticide use practices in the Wei River Basin (CCPIA, 2024, Guo et al., 2025, Li et al., 2025, MEP, 2002). Samples were spiked with deuterated surrogate standards prior to SPE to correct for matrix effects and procedural losses during sample pretreatment, separated using a C18 column, and quantitatively detected using MRM mode (Table S1 and Table S2). Standard curves for all pesticide classes showed good linearity ($R^2 > 0.99$) with stable recovery rates. Detailed information on sample processing, instrumental parameters, and quality control is provided in Text S1. Simultaneously, in accordance with the Environmental Quality Standards for Surface Water (GB 3838-2002) and the Technical Specifications for Surface Water Environmental Quality Monitoring of China (HJ 91.2-2022), we measured the key water quality physicochemical parameters (Table S3, Table S7), including ammonia-nitrogen (NH_3-N), total phosphorus (TP), dissolved oxygen (DO), permanganate index (COD_{Mn}), biochemical oxygen demand (BOD_5), and chemical oxygen demand (COD), etc.

2.3. Macroinvertebrates Collection and Identification

Within a 100 m range at each sampling site, three representative macroinvertebrate fauna samples were collected using a Surber net. Samples were screened, sorted, and preserved in 75% ethanol.

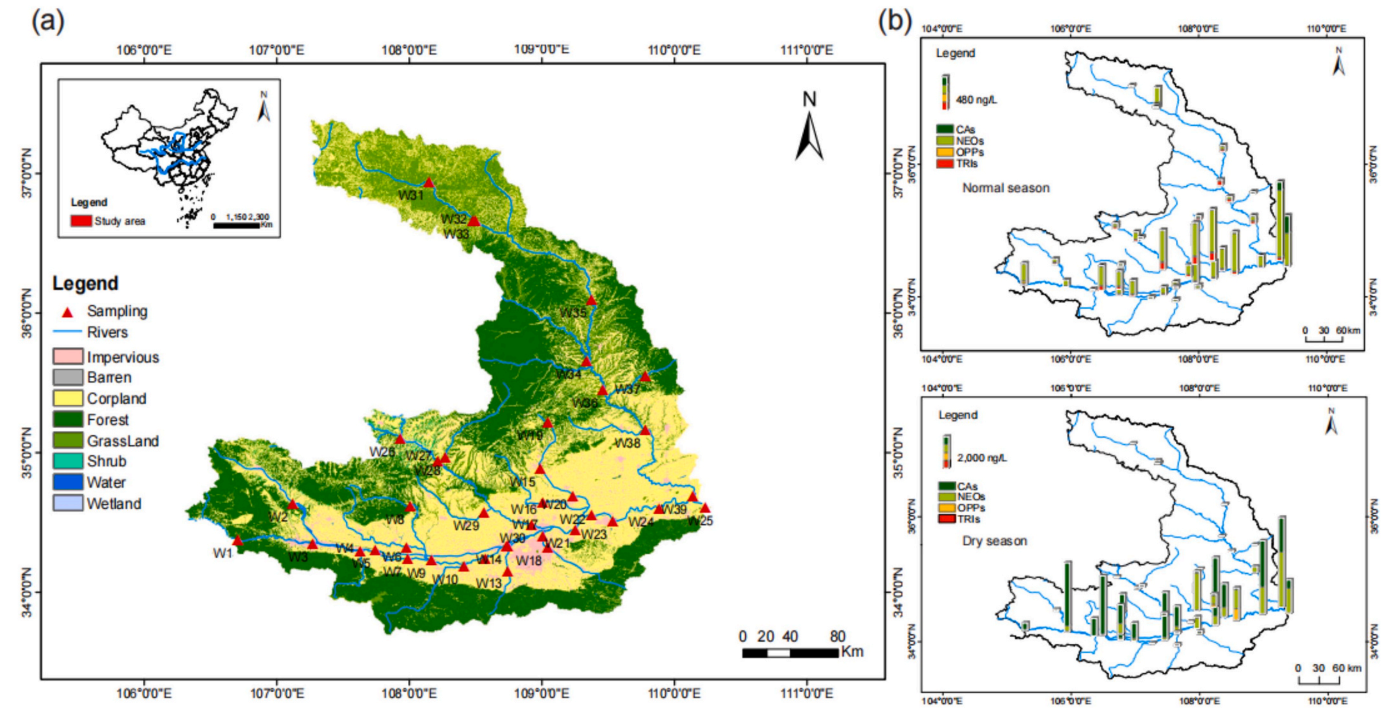

Fig. 1. (a) Locations of 39 sampling sites within various land use patterns. (b) Concentrations of different pesticide categories, including carbamates (CAs), neonicotinoids (NEOs), organophosphates (OPPs), and triazines (TRIs).

Subsequently, specimens were identified, classified, counted, and weighed under a microscope, and density per unit area (ind./m^2) was calculated. Detailed sampling and identification methods are provided in Text S2.

2.4. Environmental Stressors Acquisition

In addition to pesticides, a total of 21 environmental variables across four major categories were collected (Table S3 and Table S7). All candidate variables were initially evaluated in the statistical analyses. After considering collinearity, statistical relevance to biodiversity responses, and variable redundancy, 9 representative environmental variables (flow, cropland, impervious, precipitation, temperature, NH_3-N, TP, COD, DO) were retained together with pesticide indicators for subsequent analyses, including variation partitioning, interaction analysis, and structural equation modeling.

(1) Geographic, climatic, and hydrological variables

Geographic, climatic, hydrological, and land-use variables were incorporated as environmental descriptors representing prevailing conditions during each sampling period and were included as environmental predictors in subsequent statistical analyses. Geographic locations of sampling sites were obtained using a Global Positioning System (GPS, Garmin Legend, Garmin USA). Monthly precipitation (PPT) and temperature (T) data for the study area were acquired through Google Earth Engine (GEE) (https://earthengine.google.com/), while flow (Q) data were sourced from the ERA5 reanalysis dataset of the European Centre for Medium-Range Weather Forecasts (ECMWF) (https://www.ecmwf.int/).

(2) Land use variables

Land use data were derived from the 30 m resolution China Land Cover Dataset (CLCD) based on Landsat remote sensing observations by Wuhan University (Yang and Huang, 2021). Using ArcGIS (Ver 10.7) with the WGS-84 coordinate system, land use conditions within a 2 km radius around each sampling point were extracted, and the percentage coverage of land use types within each buffer zone was calculated. The final land use types were integrated into two categories: human land use (including cropland, impervious surface, and barren land) and natural land use (including forest, grassland, water bodies, shrub, and wetland), with their respective total proportions within buffer zones calculated.

(3) Pesticide toxicity metric

Toxic units (TUs) were calculated following Beketov et al. (Beketov et al., 2013) in which the toxic pressure of each pesticide class is represented by the maximum TU value among all detected compounds within that class:

$TUmax = max(Ci/EC_{50}i)$. Where Ci is the measured concentration of compound i and $EC_{50}i$ is the chronic toxicity value for freshwater macroinvertebrates obtained from the U.S. Environmental Protection Agency (U.S. EPA) Aquatic Life Benchmarks and Ecological Risk Assessments database (https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk), following the principles of reliability, accuracy, and relevance (Liu et al., 2020). This approach captures the dominant toxic pressure within each pesticide class and is consistent with established practices in large-scale field assessments of pesticide effects on stream macroinvertebrate communities (Weisner et al., 2021) (Table S2).

2.5. Biodiversity Index Calculation

To assess macroinvertebrate fauna responses to multiple environmental drivers, 13 biodiversity indices were selected (Table S3). α-taxonomic diversity included Richness, Shannon, Simpson, and evenness (Pielou); functional richness (FRic), functional evenness (FEve), functional divergence (FDiv), functional dispersion (FDis), and RaoQ index. β-taxonomic diversity was calculated using the Sørensen dissimilarity index and further decomposed into species turnover (beta-taxo-sim) and nestedness (beta-taxo-sne); β-functional diversity was divided into functional turnover (beta-func-sim) and functional nestedness (beta-func-sne). Relevant functional trait data were sourced from Freshwaterecology.info (https://www.freshwaterecology.info/) and the North American lotic invertebrate trait database (http://pubs.water.usgs.gov/ds187), encompassing 9 categories and 32 traits (Table S5). Diversity index calculations were performed in R, with the "vegan" package used for α taxonomic diversity analysis, the "FD" package for α functional diversity, and β-diversity analysis implemented through the "betapart" package.

2.6. Statistical Analysis

All statistical analyses were performed in R 4.3.2. Non-metric multidimensional scaling (NMDS) analysis and principal component analysis (PCA) were employed to assess differences in community species composition and environmental factors under different hydrological periods. Multiple linear regression models were constructed with 13 macroinvertebrate diversity indices as dependent variables, and environmental variables as independent variables. Stepwise regression using bidirectional selection was performed separately for each dependent variable to identify the optimal set of explanatory factors for each diversity index individually. To quantify the relative explanatory contributions of individual variables, standardized relative importance values (% R^2) were calculated using the lmg method implemented in the relaimpo package (Groemping, 2006). Diagnostic tests were conducted for each regression model, including normality tests of residuals (Shapiro-Wilk test), homoscedasticity assessment (non-constant variance test), and multicollinearity evaluation (through variance inflation factors, VIF). Model performance was comprehensively evaluated using the coefficient of determination (R^2) and Akaike Information Criterion (AIC). Redundancy analysis (RDA) was first conducted to examine relationships between biodiversity indices and environmental variables, and the rdacca.hp package was employed to decompose the explanatory effects of different environmental factor groups (including water physicochemical parameters, climatic factors, pesticides, land use, and hydrological factors) on biodiversity indices (Lai et al., 2022). Structural equation models (SEM) were constructed using the "plspm" package to investigate direct and indirect pathways through which environmental factors influence diversity indices, incorporating five latent variable groups: pesticides (TUs) (NEOs, CAs, TRIs, OPPs), water quality (NH_3-N, TP, COD, DO, WT), hydrology (Q), climate (PPT, T), and land use (cropland, impervious). Based on principal component analysis (PCA) and generalized linear modeling (GLM), two-factor interaction models were constructed among five environmental factor categories to systematically evaluate their main and interactive effects on multidimensional biodiversity indices (Birk et al., 2020). The interaction terms were classified into seven categories based on the direction and statistical significance of main effects: synergistic, antagonistic, reversal, opposing, additive, dominant, and none (Text S5). Pesticide ecological thresholds were calculated using the TITAN2 package to obtain threshold distributions for positive (z^+) and negative (z^-) response taxa, community-level change points (nCPA), and metrics including observed critical values and confidence intervals (5%, 50%, 95% quantiles) for each response group (Baker and King, 2010, Neale et al., 2024). All figures and tables presented in this study were generated using Origin 2025 and R 4.3.2.

3. Results

3.1. Distribution Characteristics of Pesticides and Macroinvertebrate Communities

Among the 39 sampling sites in the Wei River Basin, a total of 49 pesticides were detected, including 11 CAs, 9 NEOs, 14 OPPs, and 15 TRIs (Fig. 1b, Table S4), At least one pesticide was detected at each sampling site, while 10 pesticides showed detection frequencies exceeding 90% (92.5-100.0%) on average across both sampling periods, comprising 5 NEOs, 4 TRIs, and 1 CAs. Imidacloprid, thiamethoxam, and atrazine achieved 100% detection rates at all sampling sites during both sampling periods, while dichlorvos (OPPs) was not detected in either sampling campaign. Pesticide detection frequencies and concentrations exhibited distinct spatiotemporal distribution patterns. During the normal season, average concentrations of different pesticide categories were ranked as follows: NEOs (18.9 ng/L) > TRIs (2.46 ng/L) > CAs (1.27 ng/L) > OPPs (0.370 ng/L). During the dry season, the ranking shifted to: NEOs (46.0 ng/L) > CAs (34.5 ng/L) > OPPs (1.80 ng/L) > TRIs (1.69 ng/L). NEOs maintained consistently high concentration levels in both normal and dry seasons, while OPPs and CAs exhibited relatively lower concentrations, particularly during the dry season when OPPs showed low detection concentrations (0-17.2 ng/L) and detection rates (2.50-12.50%). Overall pesticide contamination levels were higher during the dry season than the normal season, likely attributed to seasonal pesticide application patterns and season. High-concentration pesticide sites (W5, W11, W27, W28, W35) were primarily distributed along the mainstream Wei River and in the Guanzhong Plain in the southeastern basin.

A total of 257 macroinvertebrate species were identified across the two sampling, belonging to 7 classes, 20 orders, 65 families, and 150 genera, with 127 species recorded during the normal season and 182 species during the dry season (Fig. S2). Taxonomically, macroinvertebrates in the Wei River Basin were predominantly composed of Diptera, Ephemeroptera, and Trichoptera, with their relative abundances accounting for 24.6%, 21.0%, and 39% during the normal season, and 36.9%, 19.9%, and 13.8% during the dry season, respectively. Notably, Tubificida exhibited increased abundance during the dry season, comprising 24.2% of total density. However, Insecta maintained absolute dominance during both hydrological periods, primarily represented by Diptera, Ephemeroptera, and Trichoptera. Due to elevated abundances of Tubificida (mainly Limnodrilus hoffmeisteri) at certain sites, this order emerged as one of the dominant groups during the dry season. NMDS analysis, PCA analysis, and box plots of various factors further demonstrated differences in species composition and environmental conditions between different hydrological periods (Figs. S2-S4). These results indicate that both environmental stressors and macroinvertebrate community structure exhibit seasonal differences.

3.2. Primary Environmental Drivers and Contributions to Community Variation

Multiple linear regression analyses revealed that macroinvertebrate diversity indices responded differently to environmental factors (Fig. 2 and Table S6). Among pesticide classes, CAs showed broader and stronger associations with multiple diversity indices in both seasons, while OPPs demonstrated consistently high explanatory power for functional diversity metrics. NEOs showed significant associations with RaoQ during the dry season. From the perspective of explanatory contribution, during both hydrological seasons, CAs showed stronger associations with β-diversity metrics, whereas OPPs showed consistently higher associations with α-diversity metrics. These results indicate that different pesticide types may influence macroinvertebrate communities through distinct ecological pathways, with CAs mainly affecting species turnover and functional redundancy, while OPPs tend to alter community structure by modifying intra-community species composition. During the normal season, in addition to pesticides, COD also exhibited moderate explanatory power for functional indicators such as Pielou and Simpson, suggesting potential synergistic effects between water quality nutrients and pesticides. During the dry season, pesticide factors also displayed noticeable influences, with CAs significantly affecting β-functional nestedness (beta-func-sne, $p < 0.001$, $R^2 = 0.458$, $\beta > 0$) and β-taxonomic nestedness (beta-taxo-sne, $p < 0.001$, $R^2 = 0.725$, $\beta > 0$), and associating with Richness ($p = 0.003$, $R^2 = 0.365$, $\beta > 0$). OPPs significantly influenced functional diversity indices, including FDiv ($p <$

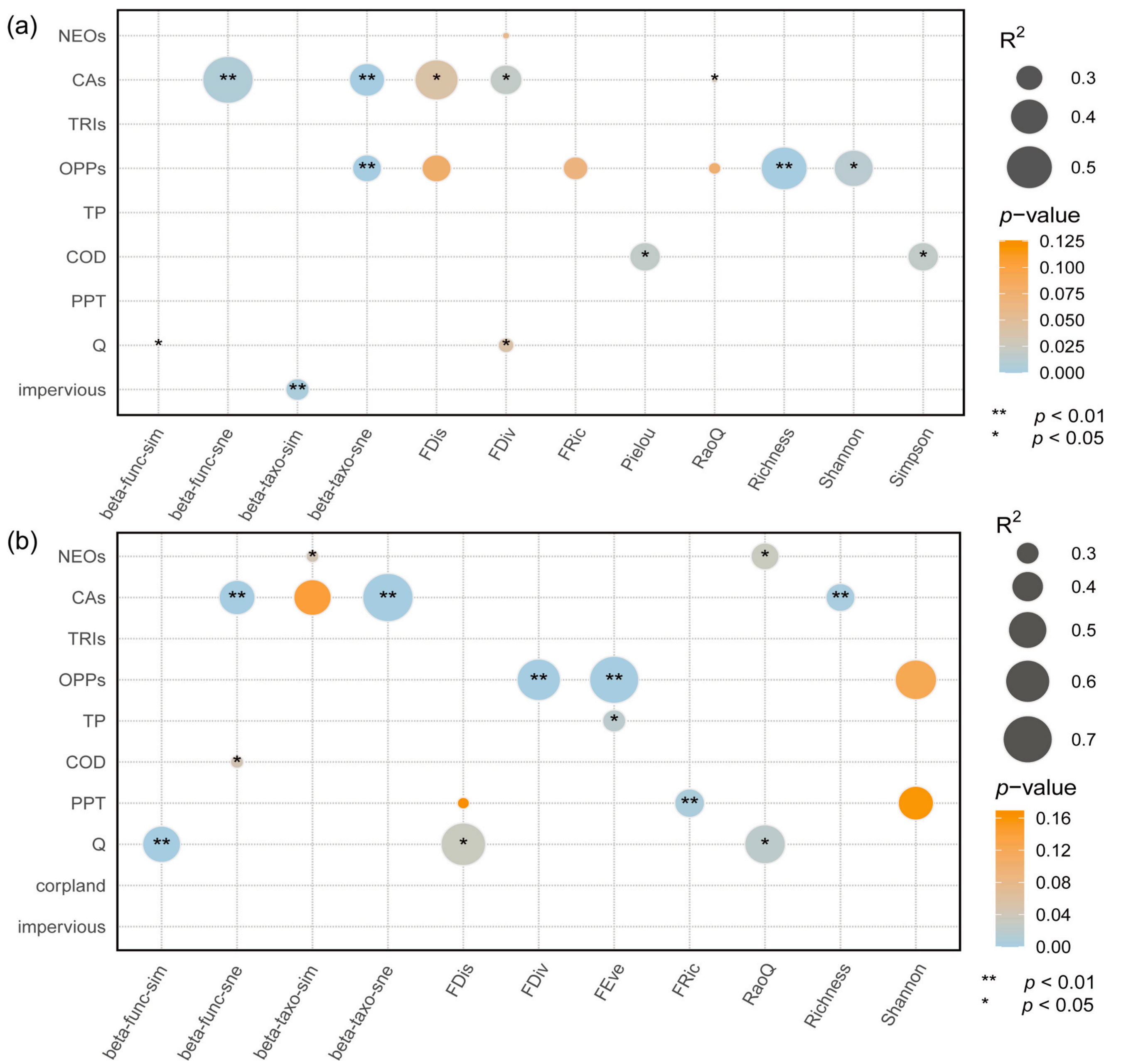

Fig. 2. Relative importance of environmental stressors on biological diversity endpoints during the (a) normal season and (b) dry season. Multiple linear regression models were used to determine the variance explained (R^2), represented by the size of each circle. The significance level (*p*-value) is indicated by color. For visual clarity, only relationships with $R^2 > 0.2$ are displayed to emphasize comparatively stronger associations; complete results including all R^2 values, coefficient estimates, and their signs for all predictor-response combinations across both hydrological seasons are provided in Table S6.

0.001, $R^2 = 0.584$, $\beta < 0$) and FEve ($p < 0.001$, $R^2 = 0.699$, $\beta < 0$). These patterns indicate that pesticide gradients are associated with progressive functional homogenization of macroinvertebrate communities and suggest that functional diversity metrics may serve as more sensitive indicators of pesticide stress than taxonomic indices, with potential implications for ecosystem processes that depend on functional complementarity. Additionally, flow (Q) also exhibited high explanatory power for functional diversity (bate-func-sim, $p < 0.001$, $R^2 = 0.490$, $\beta > 0$; FDis, $p = 0.033$, $R^2 = 0.606$, $\beta < 0$; RaoQ, $p = 0.022$, $R^2 = 0.535$, $\beta < 0$) during the dry season, indicating enhanced regulatory effects of hydrological processes. Overall, the consistently significant relationships between pesticide contamination and both α- and β-diversity metrics indicate that pesticides remain a key driver of macroinvertebrate community structural changes and should receive priority consideration in watershed ecological management. At the same time, functional diversity metrics, particularly β-functional diversity, showed consistently stronger statistical responses to multiple environmental drivers across hydrological seasons compared to taxonomic indices. Therefore, β-functional diversity may provide a more integrative indicator for detecting compound stressor effects under varying hydrological conditions.

The hierarchical partitioning in redundancy analysis (RDA) was applied to quantify the relative importance of different environmental factors on biodiversity indices. Results revealed that during the normal season, pesticides explained the highest proportion of the constrained variance in α-taxonomic diversity (Richness, 42.38%) and in the nestedness component of β-taxonomic diversity (beta-taxo-sne, 41.60%) (Fig. 3, Table S8). These contributions exceeded those of other

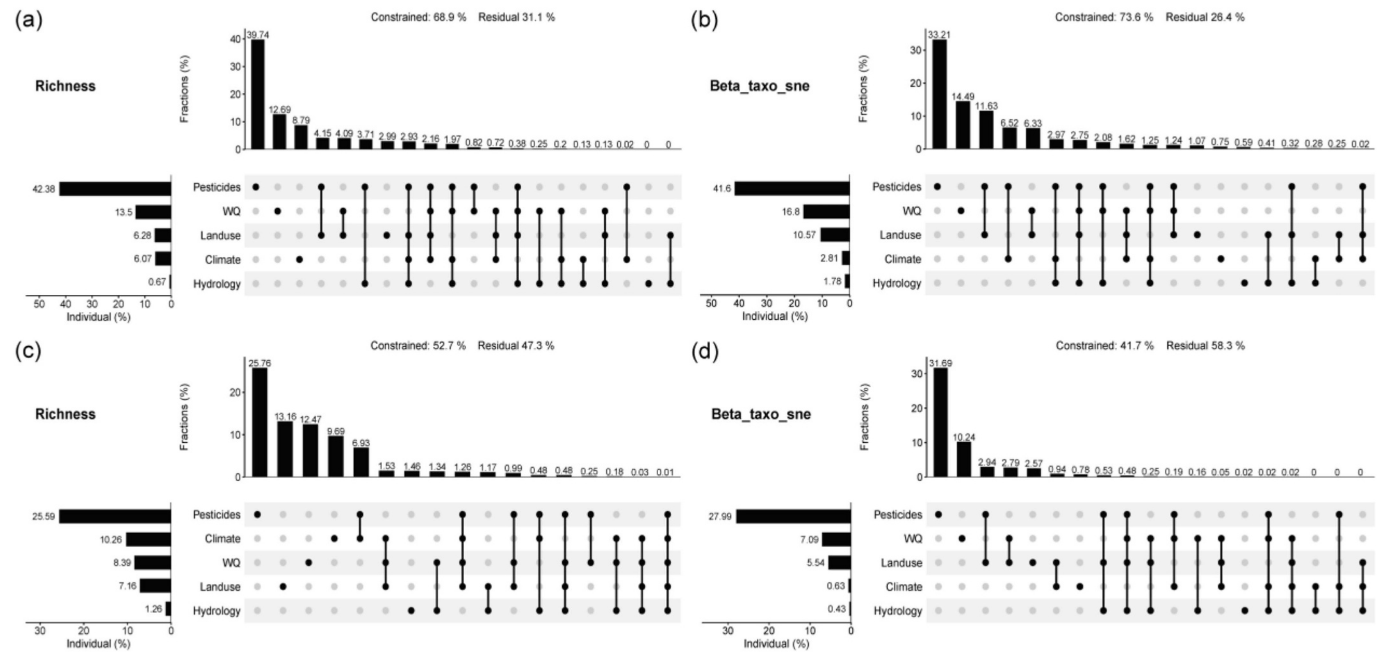

Fig. 3. The relative importance of environmental factors to biodiversity: Richness and beta-taxo-sne (a, b) during the normal season, and Richness and beta-taxo-sne (c, d) during the dry season. The percentage of variation explained by each component, derived from variation partitioning, is displayed in the top column diagram. For each column, the isolated black dot represents the marginal effect of each environmental factor, while the lines connecting multiple dots indicate the shared effect among these corresponding environmental factors. The column diagram on the left illustrates the individual effect of each environmental factor, as determined through hierarchical partitioning. Its value is the sum of its marginal effect and the average shared effect with other environmental factors.

environmental factor groups, including water quality, land use, climate, and hydrology, highlighting pesticide contamination as the dominant driver of community structural variation during this period. Particularly for beta-taxo-sne, the high explanatory power of pesticides highlighted their critical role in community species loss and replacement processes. In contrast, during the dry season, although pesticides remained the primary contributing factor, their individual explanatory rates declined to 25.59% (Richness) and 27.99% (beta-taxo-sne).

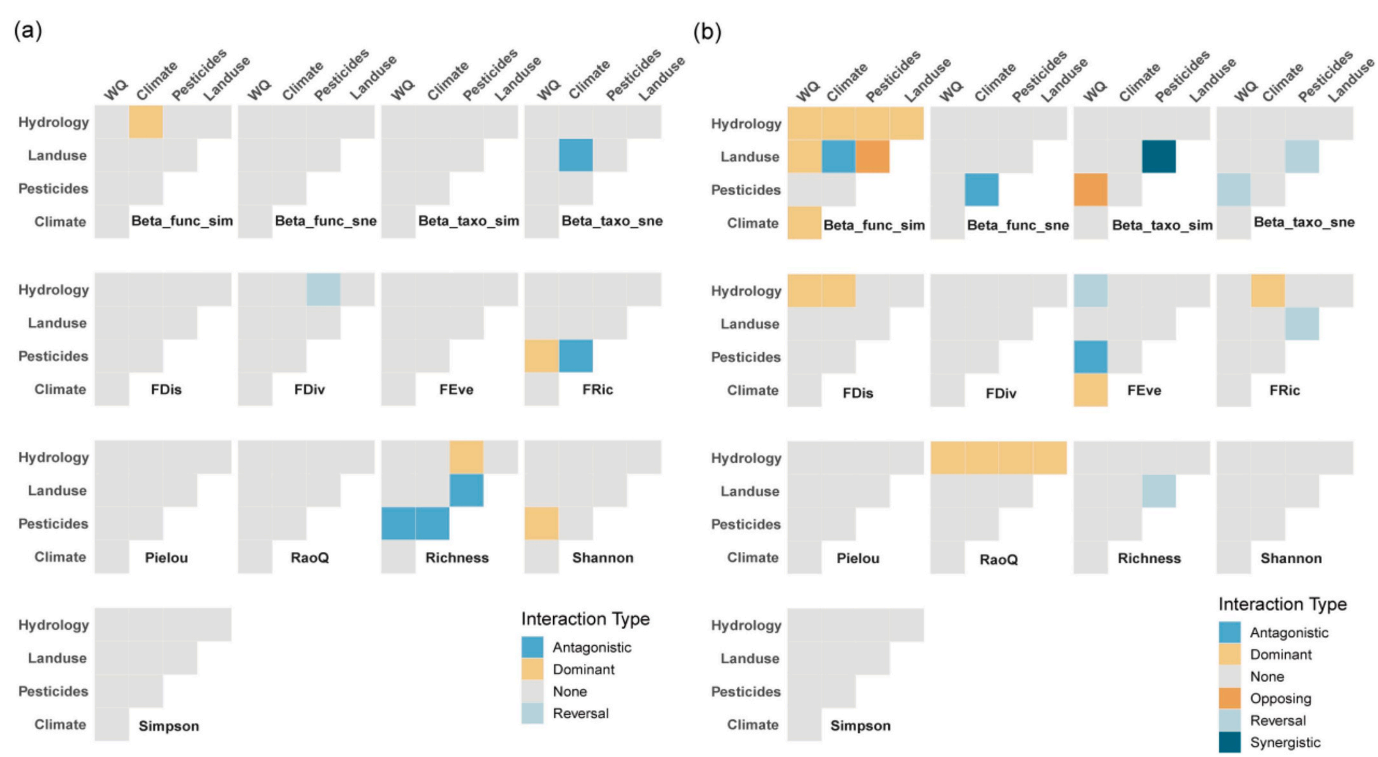

Fig. 4. Interaction types among environmental stressors affecting macroinvertebrate diversity in (a) normal and (b) dry seasons. The x-axis and y-axis represent two interacting stressors (stressor1 and stressor2, respectively). Interaction effects between each stressor pair are visualized at the intersection of the corresponding axes using a color-coded scheme.

3.3. Interactive Effects Analysis and Impact Pathway Identification

The interaction analysis results (Fig. 4) showed that during the normal season, interactions among environmental factors were generally weak, with most indicators not exhibiting significant strong interactions. In contrast, the dry season displayed relatively more complex and extensive interactions, particularly the combinations of “Pesticides × Landuse” and “Pesticides × WQ (water quality),” which demonstrated reversal or dominant effects on multiple diversity indices. These findings suggest that the ecological impacts of pesticide pollution on community structure may be amplified or altered, with the direction of effects depending on other environmental factors. Meanwhile, functional diversity indices (such as RaoQ, FEve, FDis) exhibited more interactive responses during the dry season, indicating that functional diversity is more sensitive to compound environmental stressors. Further plotting of the “Pesticides × Landuse” interaction response revealed the regulatory effects of land use intensity on various diversity indices under pesticide influence (Fig. S8). Overall, diversity indices showed weaker responses to pesticide-land use interactions during the normal season. In the dry season, however, the modulatory role of land use became more evident. These response patterns provide preliminary insights into the potential joint effects of pesticides and land use, suggesting that composite stressors may exhibit certain ecological linkages.

The analysis of interactive effects identified the relationships among key environmental stressors influencing macroinvertebrate community structure, but did not elucidate the directionality of these impacts. To address this issue, partial least squares path modeling (PLS-PM) was employed to construct structural equation models, illustrating the strength and direction of direct and indirect pathways affecting macroinvertebrate diversity. Results revealed (Fig. 5, Table S11) that the action pathways of environmental factors remained generally consistent across different hydrological periods, but their impact magnitudes on community diversity composition and structure differed. The normal season was characterized by direct effects of pesticides and land use, while during the dry season, the direct regulatory role of land use was reduced, and forming coupled pathways with pesticides that jointly dominated changes in diversity patterns. Specifically, during the normal season, macroinvertebrate communities were primarily shaped by the combined effects of land use and pesticides, with pesticides exhibiting negative associations with both α-diversity (path coefficient = -0.702) and β-diversity (-0.539), and land use showing negative associations with α-diversity (-0.610) and β-diversity (-0.419). Moreover, land use showed a strong association with the pesticide pathway (-0.934, $p < 0.001$), indicating its role as a catchment-scale contextual factor shaping anthropogenic disturbance transmission rather than a direct in-stream stressor. During the dry season, land use continued to influence

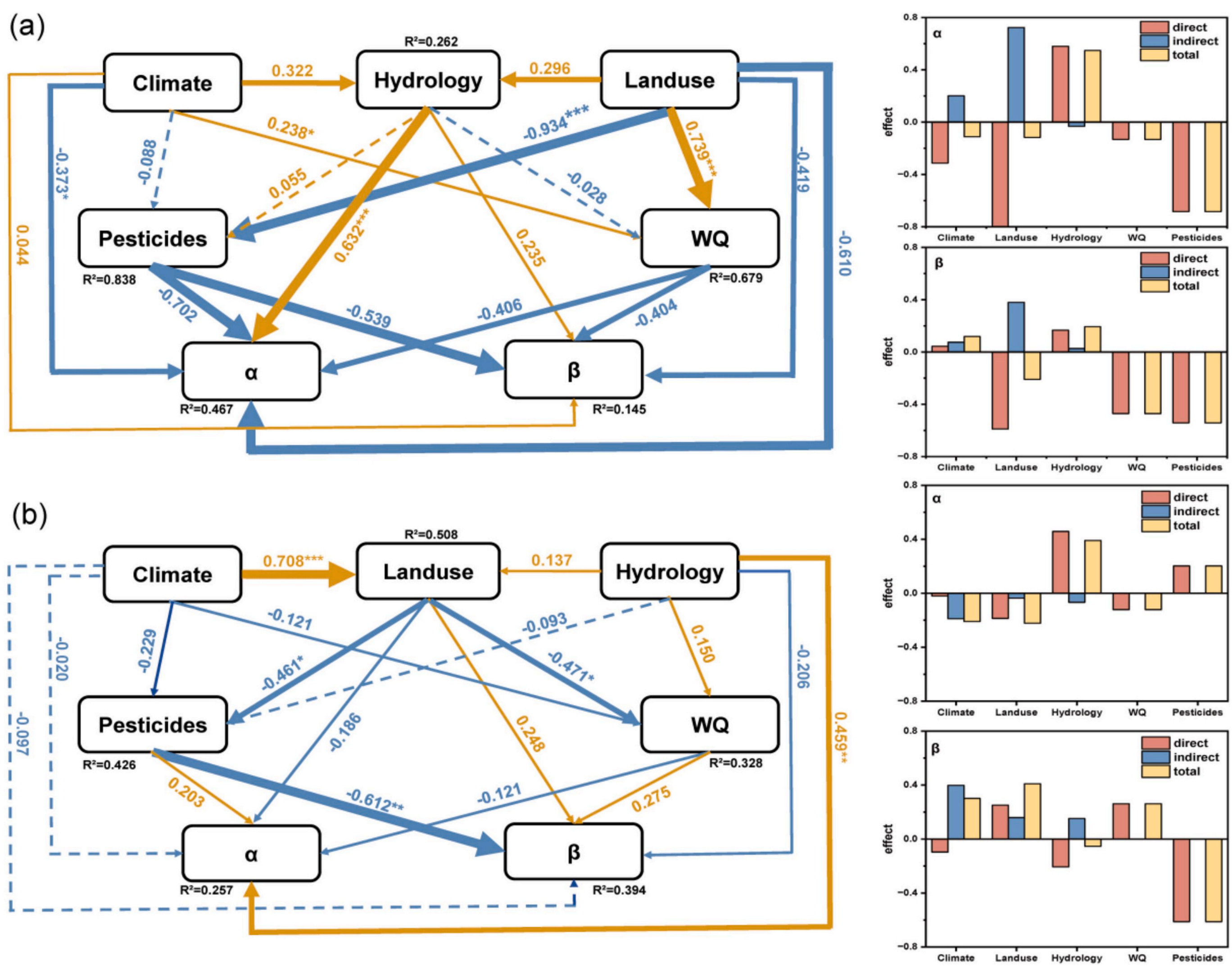

Fig. 5. Structural equation path diagram of the impact of environmental stress factors on the diversity of macroinvertebrates: (a) the normal season (GOF = 0.593); (b) the dry season (GOF = 0.511). The blue and yellow lines represent negative and positive influence, respectively. R^2 represents the proportion of variance explained for every dependent variable. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

pesticide levels and water quality, but the magnitude of these effects decreased under low-flow conditions. Specifically, land use exerted effects on pesticides (-0.461) and water quality (-0.471), which in turn produced pronounced negative effects on β-diversity through the pesticide pathway (-0.612). These linkages reflect tighter coupling between land use, pollutant transport, and community responses in low flow season, the pathways through which land use affected community structure became more mediated by pesticides, hydrology, and water quality, rather than through direct effects. Flow (Q) consistently showed positive effects in both seasons and acted as an important buffering factor during the normal season, likely through increased dilution capacity and enhanced downstream transport of chemical stressors such as pesticides. Overall, macroinvertebrate diversity responded through a dual mechanism of "direct pressures and indirect regulation," with seasonal differences reflected mainly in the intensity rather than the direction of effects. This result reveals the dynamic interactions between pesticides and human land use across different hydrological periods, suggesting the stage-specific dominance of abiotic stressors in shaping benthic community responses and underscoring the complex interactions and feedback mechanisms among multiple environmental factors.

3.4. Derivation of Pesticide Ecological Thresholds Based on Community Effects

Risk screening and prioritization of the 49 contaminants were conducted based on the risk quotient (RQ) and priority ranking index (PI). The RQ was defined as the ratio of the measured environmental concentration to the predicted no-effect concentration (PNEC). The PI integrates the maximum detected concentration, detection frequency, and corresponding toxicity threshold across the basin, characterizing long-term exposure levels and potential ecological risks (Kim et al., 2023, Zhou et al., 2019). RQ and PI screening identified Imidacloprid , Hexazinone, Atrazine, and Simazine as the highest-priority pollutants among the 49 detected pesticides for TITAN threshold analysis (Fig. S11, Table S2). Among these, Imidacloprid showed the highest RQ values in both seasons (normal season: 111.25; dry season: highest among all compounds), and all four compounds exhibited PI values exceeding 1 in both hydrological seasons, indicating consistently high ecological risk across the study period.

TITAN analysis quantified community-level change points for macroinvertebrates along the concentration gradients of the four priority pesticides (Fig. 6, Table 1). Among the four compounds, Imidacloprid exhibited the highest ecological risk, with a negative response threshold of 13.81 ng/L (5-95% quantile range: 4.02-104.47 ng/L) and basin-wide concentrations ranging from 0.50-1991.70 ng/L (mean: 73.70 ng/L), approximately 5.3 times the identified threshold, indicating widespread exceedance of ecologically safe levels across all 39 sampling sites. Atrazine and Hexazinone also showed mean concentrations exceeding their respective thresholds of 10.70 ng/L (mean: 19.83 ng/L, range: 0.60-124.80 ng/L) and 1.78 ng/L (mean: 2.95 ng/L, range: 0-21.00 ng/L), with 100% detection rates at all sites reflecting persistent and widespread exposure throughout the basin. Notably, Simazine exhibited the lowest threshold among the four compounds (0.34 ng/L, 5-95% quantile range: 0.01-2.29 ng/L), yet its basin-wide mean concentration (1.38 ng/L) still exceeded this threshold by approximately 4-fold, suggesting disproportionately strong ecological pressure relative to its absolute concentration levels and warranting particular attention in future pesticide monitoring and regulation. The pattern of threshold exceedance across sites was broadly consistent with the RQ and PI rankings derived from the multi-level risk assessment, with Imidacloprid showing the highest PI values in both seasons (normal season: 17.50; dry season: 108.47), further corroborating its dominant ecological risk status in the Wei River Basin. Compared with traditional risk quotient-based

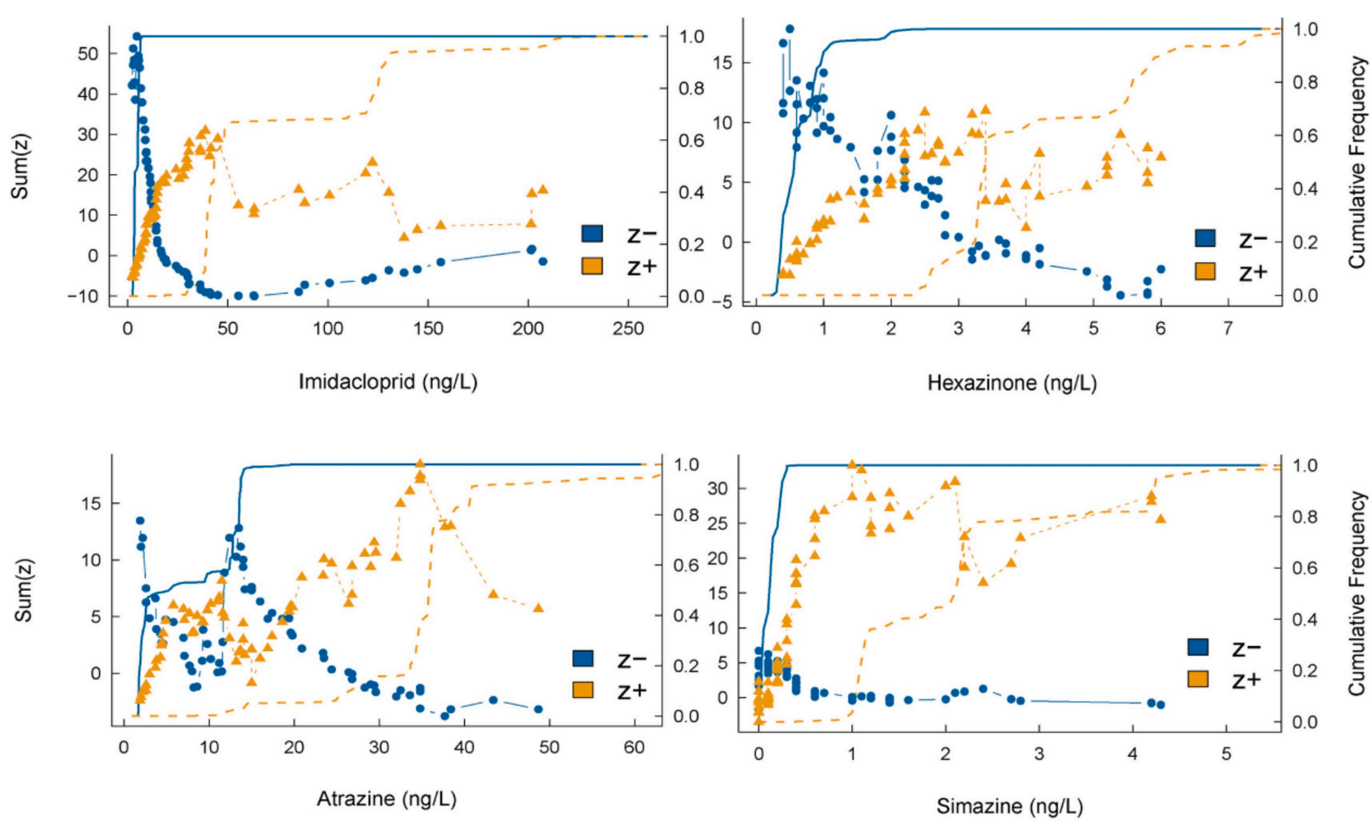

Fig. 6. TITAN-derived thresholds (nCPA, Bray-Curtis distance) to four priority pesticide classes affecting macroinvertebrate communities. Solid lines (z^-) and dashed lines (z^+) represent the cumulative distribution of z^- value peaks obtained from 500 bootstrap iterations for negative and positive indicator groups, respectively (right Y-axis).

Table 1
Thresholds of positive (z^+) and negative (z^-) responding species for four priority pesticides: observed critical values, confidence intervals (5%, 50%, 95% quantiles) for each response group.

	Imidacloprid (ng/L)		Hexazinone (ng/L)		Atrazine (ng/L)		Simazine (ng/L)	
	sum(z^-)	sum(z^+)	sum(z^-)	sum(z^+)	sum(z^-)	sum(z^+)	sum(z^-)	sum(z^+)
Observed	13.81	9.00	1.78	3.72	10.70	25.41	0.34	2.01
5%	4.02	54.20	0.65	1.10	2.87	6.75	0.01	0.10
50%	10.42	209.25	1.65	3.22	9.18	21.76	0.26	1.37
95%	104.47	146.56	4.20	5.99	31.33	44.18	2.29	4.47

approaches, the community-level thresholds derived from TITAN analysis incorporate biological response data directly into threshold derivation, providing ecologically grounded change points that more accurately reflect the concentration ranges at which macroinvertebrate communities undergo measurable reorganization in the field.

4. Discussion

4.1. Environmental factors and macroinvertebrate community heterogeneity

Our research found that the composition and structure of macroinvertebrate communities in the Wei River Basin exhibited significant differences across different hydrological periods, indicating certain sensitivity to seasonal hydrological fluctuations. Pesticide pollution is influenced not only by application patterns but also closely related to hydrological processes (Mitra et al., 2024). The higher concentrations of TRIs and NEOs observed during the normal season are consistent with crop-specific application patterns in the Wei River Basin, where corn and horticultural crops dominate during this period (Wang et al., 2019, Wang et al., 2021b). Meanwhile, due to decreased temperature and reduced flow, pesticide degradation rates slow down, potentially leading to residue accumulation and further elevating pesticide pollution levels (Ouyang et al., 2017). Previous studies have demonstrated that hydrological seasonal changes significantly influence the habitat and resource availability for macroinvertebrates in river ecosystems by altering key environmental factors such as water velocity, depth, DO, and substrate characteristics (Hernández-Carrasco et al., 2025). For example, during the dry season, reduced water flow and exposed substrates may lead to local habitat degradation, subsequently affecting the survival and distribution of sensitive taxonomic groups (Linares et al., 2025, Birk et al., 2020). In addition to natural hydrological processes, anthropogenic disturbances are also important factors driving changes in macroinvertebrate communities. Pollution loads from urbanization and agricultural activities have been confirmed to be closely related to community degradation (de Vries et al., 2021, Qi et al., 2025). Particularly in agriculture-dominated regions, when riparian crop coverage exceeds certain thresholds (such as 8-10%), the diversity and functional diversity of invertebrate communities decline significantly (Paukert et al., 2020). Furthermore, anthropogenic factors and hydrological processes may interact, exacerbating ecosystem responses to disturbances. For example, both pollutant inputs from runoff during flood periods and pollutant concentration during drought periods can affect community structure (van Vliet et al., 2023). Therefore, in watershed-scale ecological assessments, greater attention should be paid to the relationships between aquatic community characteristics and environmental factors, particularly the interactive effects of diversity indices with pollution loads and land use, to enhance the scientific rigor and sensitivity of ecological monitoring and management.

4.2. Community regulation mechanisms under multiple stressor interactions

Pesticide-driven reductions in functional diversity indices suggest not only species loss but also functional homogenization of macroinvertebrate communities, whereby sensitive, functionally distinct taxa are replaced by tolerant generalists. This compression of functional space has potential implications for ecosystem processes such as organic matter decomposition, nutrient cycling, and secondary production, which depend on functional complementarity rather than species richness alone (Heino and Tolonen, 2017, Wang and Han, 2023). However, translating observed changes in functional diversity metrics into quantitative predictions of ecosystem service loss remains challenging. While theoretical frameworks consistently link biodiversity to ecosystem functioning through complementarity and selection effects, functional redundancy, nonlinear diversity-function relationships, and context-dependent environmental conditions all mediate this linkage (Wagg et al., 2022, Wang et al., 2021a). The observed functional diversity losses should therefore be interpreted as early warning signals of potential ecosystem functional impairment, and the direct linkage between metric-level changes and specific ecosystem service outcomes warrants further experimental validation.

The GLM interaction analysis and PLS modeling revealed that pesticide contamination played a dominant role in regulating macroinvertebrate community diversity, while additional environmental factors modulated the strength and expression of these effects across seasons. These results suggest that traditional single-factor analyses may underestimate ecological risks under compound stressor scenarios (Jin et al., 2024). Globally, multiple pressures from natural and anthropogenic disturbances on aquatic ecosystems have become the norm, with aquatic environments universally experiencing interactive effects from multiple stressor sources. Notably, compared to the normal season, the interactive effects between pesticide pollution and land use changes were more significant during the dry season. This confirms that in natural ecosystems, the interactions among stressor factors may exhibit certain variability, with the types and intensities of interactions among stressor factors potentially changing over time (Turschwell et al., 2022). During drought or low flow periods, ecosystem responses to multiple stressors are more intense, and interactive effects are more readily apparent (Piggott et al., 2015). Meanwhile other environmental variables also demonstrated certain predictive capabilities for macroinvertebrate community structure, particularly flow and COD, which exhibited high explanatory power under different hydrological conditions. This is consistent with studies showing that hydrodynamics and oxygen content dominate community distribution patterns at regional scales (Su et al., 2019). Meanwhile, it also indicates that single environmental factors may not accurately explain the response mechanisms of macroinvertebrate communities, emphasizing the need to consider the combined effects of multiple stressor sources in ecological assessments (Simmons et al., 2021).

Urban river ecosystems face long-term disturbances from multiple stressor sources (Chen et al., 2023, Jackson et al., 2015). We found that multi-factor drivers centered on pesticide pollution profoundly influenced macroinvertebrate communities. During different hydrological periods, pesticides and land use dominated community structural and functional responses through direct and indirect pathways, respectively, exhibiting significant seasonal differences and coupled interactive effects. Previous studies have shown that watershed land-use composition influences pesticide inputs and exposure pathways (Zhang et al., 2024a, Schmidt et al., 2019), particularly under the coexistence of drought and anthropogenic disturbances, where the compound action mechanisms become even more complex (Schürings et al., 2024, Tang et al., 2021, von Gönner et al., 2023). By altering hydrological processes and water-quality conditions, land use indirectly shapes macroinvertebrate community patterns (Ma et al., 2023, Newbold et al., 2015). Urban expansion and agricultural intensification can increase non-point source pollution and disturb habitat structure via enhanced runoff and reduced dissolved oxygen, thereby strengthening environmental filtering under seasonal drought (Shi et al., 2024, Stegen et al., 2013, Urban et al., 2024). Consequently, macroinvertebrate diversity patterns in urban rivers are primarily associated with pesticide contamination, with their spatial and seasonal expression further modulated by hydrological conditions and land-use-mediated exposure contexts. Functional diversity, particularly β-functional diversity, serves as a sensitive response indicator that not only reveals changes in community composition but also reflects the disruption of ecological functional structure and decline in system integrity. This highlights the limitations of traditional single-factor analyses, which may underestimate ecological risks under compound stressor scenarios. Future urban river management and ecological restoration efforts should fully consider the interactive mechanisms of multiple stressor sources and incorporate functional indicators into monitoring and assessment frameworks to achieve precise diagnosis and effective intervention of ecosystem health status.

4.3. Threshold identification and pesticide risk management based on community-level effects

TITAN analysis effectively identified response thresholds of macroinvertebrate communities along concentration gradients of the four priority pesticides, revealing community-level change points associated with different pesticide exposure gradients. Among the priority pesticides identified, Imidacloprid showed the highest ecological risk levels across both hydrological seasons, consistent with its well-documented neurotoxic mechanism. As a NEO insecticide, Imidacloprid primarily targets nicotinic acetylcholine receptors in insects, exhibiting high specificity toward aquatic insects such as Diptera, and laboratory studies have demonstrated that exposure at moderate to low concentrations is associated with sharp reductions or complete disappearance of sensitive taxonomic groups (Wang et al., 2022, Hladik et al., 2018). The three TRI herbicides (Hexazinone, Atrazine, and Simazine) all ranked among the highest-priority pesticides, yet their identified community thresholds differed substantially: 1.78 ng/L for Hexazinone, 10.70 ng/L for Atrazine, and 0.34 ng/L for Simazine. The substantial differences in thresholds among compounds within the same pesticide class further highlight that ignoring inter compound variability in ecological risk assessment or regulatory frameworks may result in overly generalized ecological thresholds, thereby reducing their ecological relevance and indicative value. Although TRIs share a common mode of action by inhibiting photosystem II in primary producers and thereby indirectly affecting food resources and oxygen supply for higher trophic levels, including macroinvertebrates (Beasley, 2020, Yang et al., 2019, Zhang et al., 2024c), differences in molecular structure, environmental persistence, and bioavailability among individual compounds can result in markedly different community-level response thresholds. The extremely low threshold identified for Simazine (0.34 ng/L) in particular suggests that this compound may exert disproportionately strong ecological pressure relative to its measured concentrations, and warrants closer attention in pesticide monitoring and regulation. These findings collectively emphasize that compound-level threshold derivation is essential for accurately characterizing pesticide-associated ecological risks, while acknowledging that establishing direct causal linkages between pesticide exposure and community responses requires further experimental validation under controlled conditions.

It should be noted that these field-derived thresholds represent apparent change points identified under real-world multiple-stressor conditions, and are therefore likely lower than those obtained from single-compound laboratory exposures, reflecting the combined effects of mixture toxicity and background environmental stress. In recent years, the widespread use of pesticides in agricultural production and urban landscaping has led to common pesticide pollution risks in many major rivers in China. Pesticide residue problems are particularly prominent in estuarine regions of the Yellow River, Yangtze River, Huai River, and Hai River basins, especially with herbicides represented by TRIs showing high detection frequencies and concentrations in water bodies. Furthermore, with accelerating urbanization, the detection frequency of NEOs such as Imidacloprid has significantly increased in southeastern coastal areas and around major cities, suggesting that urban areas have also become potential pollution input sources. These pesticides may pose continuous and widespread ecological stresses to aquatic organisms, particularly under long-term low-dose exposure conditions, where their potential ecological effects are often underestimated (Li et al., 2023, Miller et al., 2020, Xie et al., 2025). In multi-stressor systems, biological communities often undergo abrupt transitions at specific pollution thresholds rather than linear gradual changes. These abrupt transitions may be collectively driven by factors such as niche filtering, food chain disruption, or habitat quality deterioration (Hillebrand et al., 2020). Meanwhile, changes in species composition may lead to functional homogenization of biological communities, affecting ecosystem functions and services. Species richness exhibits threshold responses to anthropogenic pressures, with sharp declines occurring beyond threshold levels. The existence of such thresholds suggests the need to focus on nonlinear and non-monotonic response patterns (Cazalis, 2022, Liu et al., 2025). Additionally, Rasmussen (Rasmussen et al., 2015) also proposed the viewpoint that “low-dose pesticides may trigger ecosystem-level responses.” Therefore, threshold identification not only provides reference value for risk assessment but also offers theoretical foundation for establishing “ecological safety limits” for pollutants. Current risk assessment frameworks remain primarily focused on toxicological endpoints and have not adequately reflected the compound impacts of pesticides on biological community structure and function, a limitation that becomes even more prominent under rapid urbanization. Future pesticide ecological risk management should be based on scientifically sound monitoring strategies and multi-factor synergistic regulation mechanisms, strengthening the characterization of real exposure scenarios and biodiversity response processes in ecosystems. First, in pesticide ecological risk management, focus on peak pesticide application periods (such as spring planting and summer management phases) and high-risk areas (such as intensive agricultural zones and urban-rural fringes) by implementing high-frequency, multi-site monitoring strategies to enhance the sensitivity and timeliness of pesticide pollution identification. Second, existing research (Guo et al., 2025) has indicated that cropland and urban construction land are the primary land types driving increased pesticide ecological risk, while forests and grasslands possess significant dilution and buffering functions. The influence of land use types on ecological risk exists at specific spatial scales; therefore, land use patterns can be incorporated into ecological buffering mechanism assessments. Finally, models incorporating multi-factor interactions should be integrated into ecological risk assessment frameworks, comprehensively considering the mechanisms of action among different stressor sources under multiple stressor contexts (Alric et al., 2021). Combined with the identification of biological community response thresholds, this helps precisely define pesticide ecological risk critical points, providing support for developing more scientifically sound and targeted management strategies.

5. Conclusion

Based on multi-site monitoring and multi-factor analytical frameworks, this study quantitatively revealed the effects of pesticide and other environmental stressors interactions on macroinvertebrate community structure and function, providing new perspectives for understanding watershed ecosystem response mechanisms under urbanization and agricultural intensification. The research found that pesticide pollution plays a dominant role among the multiple stressors reviewed in the study for this water basin in 2023-2024, with ecological effects exhibiting significant seasonal dependency. This suggests that beyond traditional risk assessments, ecological functional indicators and sensitive response critical points should be further incorporated to enhance the precision of risk identification and the ecological relevance of management. This has been recommended by others as the "bottom up" approach (Johnson et al., 2025). Watershed ecological protection may be more effective and precise if we move from "concentration-oriented" to "mechanism-oriented" and "functional restoration" approaches. This would include constructing comprehensive assessment systems that encompass high spatiotemporal resolution monitoring, remote sensing, and modeling techniques. Meanwhile, risk early warning methods based on functional traits and threshold identification could provide scientific support for pollutant control and ecosystem resilience enhancement at the watershed scale.

Currently, the cumulative impacts of pesticides widely used in agriculture and urban areas on non-target aquatic biological communities remain underestimated. Although our results revealed the central role of coupled effects between pesticides and land use in shaping spatial patterns of river macroinvertebrate diversity, this conclusion still requires further validation with broader regional, multi-seasonal, and multi-annual data, direct validation under region-specific environmental conditions remains necessary. In particular, single-visit sampling at each site inherently limits the ability to capture the full temporal dynamics of pesticide exposure, as surface water concentrations can fluctuate substantially in response to agricultural application schedules, rainfall-driven runoff events, temperature-dependent degradation rates, and seasonal hydrological dynamics. Moreover, potential spatial autocorrelation arising from watershed structure and environmental gradients may influence the observed community-environment relationships, suggesting that future studies should incorporate spatially explicit analytical frameworks to improve inference. The relatively small sample size relative to the number of explanatory variables also limits the statistical power of interaction analyses, and future studies should employ larger datasets to validate the observed patterns. As Daphnia magna is generally less sensitive to insecticides than aquatic insects such as EPT taxa, TU values calculated using Daphnia magna-based EC_{50} data likely represent conservative lower-bound estimates of toxic pressure, and ecological risks associated with insecticide exposure may be underestimated. As ecosystem management under global change increasingly emphasizes "mechanism-based restoration strategies" and the development of "monitorable and early-warning ecological indicators," understanding the coupled relationships between pesticide pollution and land use driving mechanisms is not only significant for predicting biodiversity vulnerability but also provides theoretical support for achieving more precise, ecologically-oriented watershed protection and policy formulation. Future studies should integrate remote sensing, high-resolution ecological monitoring, ecological modeling, and long-term biological response data to further explore restoration windows, threshold mechanisms, and ecological compensation pathways. This includes strengthening the identification and regulation of critical pollutant concentration thresholds and promoting more ecologically-oriented environmental governance practices.

CRediT authorship contribution statement

Jing Fang: Writing – original draft, Software, Methodology, Investigation, Data curation. **Lin Hou:** Writing – review & editing, Software, Methodology. **Shiqi Jia:** Writing – review & editing, Investigation. **Jian Xu:** Writing – review & editing. **Andrew C. Johnson:** Writing – review & editing. **Xiaowei Jin:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Data availability

Data will be made available on request.

References

Allan, J.D., McIntyre, P.B., Smith, S.D.P., Halpern, B.S., Boyer, G.L., Buchsbaum, A., Burton, G.A., Campbell, L.M., Chadderton, W.L., Ciborowski, J.J.H., Doran, P.J., Eder, T., Infante, D.M., Johnson, L.B., Joseph, C.A., Marino, A.L., Prusevich, A., Read, J.G., Rose, J.B., Rutherford, E.S., Sowa, S.P., Steinman, A.D., 2012. Joint analysis of stressors and ecosystem services to enhance restoration effectiveness. Proc. Natl. Acad. Sci. 110, 372–377.

Alric, B., Dézerald, O., Meyer, A., Billoir, E., Coulaud, R., Larras, F., Mondy, C.P., Usseglio-Polatera, P., 2021. How diatom-, invertebrate- and fish-based diagnostic tools can support the ecological assessment of rivers in a multi-pressure context: Temporal trends over the past two decades in France. Sci. Total Environ. 762, 143915.

Baker, M.E., King, R.S., 2010. A new method for detecting and interpreting biodiversity and ecological community thresholds. Methods Ecol. Evol. 1, 25–37.

Beasley, V.R., 2020. Direct and Indirect Effects of Environmental Contaminants on Amphibians. Reference Module in Earth Systems and Environmental Sciences. Elsevier.

Beketov, M.A., Kefford, B.J., Schäfer, B., Liess, M., 2013. Pesticides reduce regional biodiversity of stream invertebrates. Proc. Natl. Acad. Sci. 110, 11039–11043.

Birk, S., Chapman, D., Carvalho, L., Spears, B.M., Andersen, H.E., Argillier, C., Auer, S., Baattrup-Pedersen, A., Banin, L., Beklioğlu, M., Bondar-Kunze, E., Borja, A., Branco, P., Bucak, T., Buijse, A.D., Cardoso, A.C., Couture, R.-M., Cremona, F., de Zwart, D., Feld, C.K., Ferreira, M.T., Feuchtmayr, H., Gessner, M.O., Gieswein, A., Globevnik, L., Graeber, D., Graf, W., et al., 2020. Impacts of multiple stressors on freshwater biota across spatial scales and ecosystems. Nat. Ecol. Evol. 4, 1060–1068.

Butkas, K.J., Vadeboncoeur, Y., Vander zanden, M.J., 2010. Estimating benthic invertebrate production in lakes: a comparison of methods and scaling from individual taxa to the whole-lake level. Aquat. Sci. 73, 153–169.

Cazalis, V., 2022. Species richness response to human pressure hides important assemblage transformations. Proc. Natl. Acad. Sci. U.S.A. 119, e2107361119.

CCPIA, China Crop Protection Industry Association, 2024. Analysis of China's pesticide registration status and trends in 2024 (in Chinese). China Crop Protection Industry Association (accessed 15 May 2025).

Chen, M., Jin, X., Guo, C., Liu, Y., Zhang, H., Wang, J., Dong, G., Liu, N., Guo, W., Giesy, J.P., Wu, F., Xu, J., 2023. Micropollutants but high risks: human multiple stressors increase risks of freshwater ecosystems at the megacity-scale. J. Hazard. Mater. 460, 132497.

de Bello, F., Fischer, F.M., Puy, J., Shipley, B., Verdú, M., Götzenberger, L., Lavorel, S., Moretti, M., Wright, I.J., Berg, M.P., Carmona, C.P., Cornelissen, J.H.C., Dias, A.T.C., Gibb, H., Lepš, J., Madin, J.S., Majeková, M., Pausas, J.G., Segrestin, J., Sobral, M., Zanne, A.E., Garnier, E., 2025. Raunkiæran shortfalls: Challenges and perspectives in trait-based ecology. Ecol. Monogr. 95.

de Vries, J., Kraak, M.H.S., Skeffington, R.A., Wade, A.J., Verdonschot, P.F.M., 2021. A Bayesian network to simulate macroinvertebrate responses to multiple stressors in lowland streams. Water Res. 194.

Ding, N., Yang, W., Zhou, Y., González-Bergonzoni, I., Zhang, J., Chen, K., Vidal, N., Jeppesen, E., Liu, Z., Wang, B., 2017. Different responses of functional traits and diversity of stream macroinvertebrates to environmental and spatial factors in the Xishuangbanna watershed of the upper Mekong River Basin, China. Sci. Total Environ. 574, 288–299.

Groemping, U., 2006. Relative importance for linear regression in r: the package relaimpo. J. Stat. Softw. 17, 1–27.

Groh, K., vom Berg, C., Schirmer, K., Tlili, A., 2022. Anthropogenic chemicals as underestimated drivers of biodiversity loss: scientific and societal implications. Environ.. Sci. Technol. 56, 707–710.

Guo, X., Luo, Y., Xie, H., Chen, M., Xu, J., Wang, Y., Johnson, A.C., Jin, X., 2025. Beyond agriculture: Land use thresholds governing pesticide mixture risks in megacity surface waters. J. Hazard. Mater. 494, 138657.

Guo, X.Y., Luo, Y., Du, C., Li, J.Y., Jia, S.Q., Xu, J., Wang, Y.Y., Jin, X.W., 2025. Characteristics, sources, and ecological risks of typical pesticide contamination in the Dongting Lake Basin. Sci. Bull. 70, 2104–2116.

Heino, J., Tolonen, K.T., 2017. Ecological drivers of multiple facets of beta diversity in a lentic macroinvertebrate metacommunity. Limnol. Oceanogr. 62 (6), 2431–2444.

Hernández-Carrasco, D., Tylianakis, J.M., Lytle, D.A., Tonkin, J.D., 2025. Ecological and evolutionary consequences of changing seasonality. Science 388, eads4880.

Hillebrand, H., Donohue, I., Harpole, W.S., Hodapp, D., Kucera, M., Lewandowska, A.M., Merder, J., Montoya, J.M., Freund, J.A., 2020. Thresholds for ecological responses to global change do not emerge from empirical data. Nat. Ecol. Evol. 4, 1502–1509.

Hladik, M.L., Main, A.R., Goulson, D., 2018. Environmental Risks and challenges associated with neonicotinoid insecticides. Environ.. Sci. Technol. 52, 3329–3335.

Hou, L., Xiong, W., Chen, M., Xu, J., Johnson, A.C., Zhan, A., Jin, X., 2025. Pesticide pollution reduces the functional diversity of macroinvertebrates in urban aquatic ecosystems. Environ.. Scie. Technol. 59, 8568–8577.

Jackson, M.C., Loewen, C.J.G., Vinebrooke, R.D., Chimimba, C.T., 2015. Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. Glob. Chang. Biol. 22, 180–189.

Jin, X., Hou, L., Liu, N., Xu, J., Zhang, X., Hollert, H., Johnson, A.C., Giesy, J.P., Wu, F., 2024. Bridging the gap: advancing ecological risk assessment from laboratory predictions to ecosystem reality. Environ.. Sci. Technol. 58, 18447–18449.

Johnson, A.C., Sadykova, D., Qu, Y., Keller, V.D.J., Bachiller-Jareno, N., Jürgens, M.D., Eastman, M., Edwards, F., Rizzo, C., Scarlett, P.M., Sumpter, J.P., 2025. Zinc and copper have the greatest relative importance for river macroinvertebrate richness at a national scale. Environ.. Sci. Technol. 59, 4068–4079.

Kim, J.Y., Jeon, J., Kim, S.D., 2023. Prioritization of pharmaceuticals and personal care products in the surface waters of Korea: Application of an optimized risk-based methods. Ecotoxicol. Environ. Saf. 259.

Lai, J., Zou, Y., Zhang, J., Peres-Neto, P.R., 2022. Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rdacca.hp R package. Methods Ecol. Evol. 13, 782–788.

Lange, K., Townsend, C.R., Matthaei, C.D., 2014. Can biological traits of stream invertebrates help disentangle the effects of multiple stressors in an agricultural catchment? Freshw. Biol. 59, 2431–2446.

Li, C., Zhang, H., Gong, X., Wei, X., Yang, J., 2019. Precipitation Trends and Alteration in Wei River Basin: Implication for Water Resources Management in the Transitional Zone between Plain and Loess Plateau, China. Water 11.

Li, J., Hou, L., Liu, N., Rao, K., Zheng, J., Xu, J., Giesy, J.P., Jin, X., 2025. A decade-long meta-analysis of risks posed by pesticides in Chinese surface waters. J. Environ. Manage. 387, 125898.

Li, W., Xin, S., Deng, W., Wang, B., Liu, X., Yuan, Y., Wang, S., 2023. Occurrence, spatiotemporal distribution patterns,partitioning and risk assessments of multiple pesticide residues in typical estuarine water environments in eastern China. Water Res. 245, 120570.

Liess, M., Liebmann, L., Vormeier, P., Weisner, O., Altenburger, R., Borchardt, D., Brack, W., Chatzinotas, A., Escher, B., Foit, K., Gunold, R., Henz, S., Hitzfeld, K.L., Schmitt-Jansen, M., Kamjunke, N., Kaske, O., Knillmann, S., Krauss, M., KüSTER, E., et al., 2021. Pesticides are the dominant stressors for vulnerable insects in lowland streams. Water Res. 201.

Linares, M.S., Macedo, D.R., Marques, J.C., Hughes, R.M., Callisto, M., 2025. Biodiversity spatial distribution of benthic macroinvertebrate assemblages is influenced by anthropogenic disturbances at multiple spatial extents. Sci. Total Environ. 960.

Liu, Y., Jin, X., Zhan, A., Liao, J., Johnson, A.C., Xu, J., 2025. Beyond linear Thinking: Redefining chemical pollution impacts on biodiversity. Environ. Sci. Ecotechnol. 26, 100589.

Liu, N., Jin, X., Feng, C., Wang, Z., Wu, F., Johnson, A.C., Xiao, H., Hollert, H., Giesy, J. P., 2020. Ecological risk assessment of fifty pharmaceuticals and personal care products (PPCPs) in Chinese surface waters: A proposed multiple-level system. Environ. Int. 136, 105454.

Luo, X., Le, F., Zhang, Y., Zhang, H., Zhai, J., Luo, Y., 2024. Multi-scenario analysis and optimization strategy of ecological security pattern in the Weihe river basin. J. Environ. Manage. 366.

Ma, Y., Yu, Z., Jia, S., Wu, N., Yin, K., Wang, Y., Giesy, J.P., Jin, X., 2023. Multiple anthropogenic stressors influence the taxonomic and functional homogenization of macroinvertebrate communities on the mainstream of an urban-agricultural river in China. J. Environ. Manage. 341, 118017.

Malaj, E., Von der ohe, Grote, M., Kühne, R., Mondy, C.P., Usseglio-polatera, P., Brack, W., Schäfer, R.B., 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. Proc. Natl. Acad. Sci. 111, 9549–9554.

Melcher, A., Hauer, C., Zeiringer, B., 2018. Aquatic Habitat Modeling in Running Waters. In: Schmutz, S., Sendzimir, J. (Eds.), Riverine Ecosystem Management: Science for Governing Towards a Sustainable Future. Springer International Publishing, Cham.

MEP, Ministry of Ecology and Environment of the People's Republic of China, 2002. Environmental Quality Standards for Surface Water (GB 3838-2002) [in Chinese]. Beijing, China.

Miller, J.L., Schmidt, T.S., van Metre, P.C., Mahler, B.J., Sandstrom, M.W., Nowell, L.H., Carlisle, D.M., Moran, P.W., 2020. Common insecticide disrupts aquatic communities: a mesocosm-to-field ecological risk assessment of fipronil and its degradates in U.S. streams. Sci. Adv. 6, eabc1299.

Mitra, S., Saran, R.K., Srivastava, S., Rensing, C., 2024. Pesticides in the environment: Degradation routes, pesticide transformation products and ecotoxicological considerations. Sci. Total Environ. 935.

Navarro, I., de la Torre, A., Sanz, P., Abrantes, N., Campos, I., Alaoui, A., Christ, F., Alcon, F., Contreras, J., Glavan, M., Pasković, I., Pasković, M.P., NøRGAARD, T., et al., 2024. Assessing pesticide residues occurrence and risks in water systems: a Pan-European and Argentina perspective. Water Res. 254, 121419.

Neale, P.A., Neelamraju, C., Warne, M.S.J., 2024. Derivation of species sensitivity distributions and ecotoxicity threshold values for 66 pesticide active ingredients and the hazard and risk they pose to freshwater waterways that discharge to the Great Barrier Reef, Australia. Sci. Total Environ. 920.

Newbold, T., Hudson, L.N., Hill, S.L.L., Contu, S., Lysenko, I., Senior, R.A., BöRGER, L., et al., 2015. Global effects of land use on local terrestrial biodiversity. Nature 520, 45–50.

Nie, T., Jiang, X., Deng, C., Cai, W., Lei, Y., Gao, S., 2024. Analysis of the evolution of water culture and water security in the Weihe River Basin over a 100 year-period. Sci. Total Environ. 920.

Nowell, L.H., Moran, P.W., Waite, I.R., Schmidt, T.S., Bradley, P.M., Mahler, B.J., van Metre, P.C., 2024. Multiple lines of evidence point to pesticides as stressors affecting invertebrate communities in small streams in five United States regions. Sci. Total Environ. 915.

Ouyang, W., Cai, G., Tysklind, M., Yang, W., Hao, F., Liu, H., 2017. Temporal-spatial patterns of three types of pesticide loadings in a middle-high latitude agricultural watershed. Water Res. 122, 377–386.

Paukert, C.P., Kleekamp, E.R., Tingley, R.W., 2020. Identifying candidate reference reaches to assess the physical and biological integrity of wadeable streams in different ecoregions and among stream sizes. Ecol. Ind. 111.

Pei, B., Chen, S., 2024. The spatio-temporal variations of cultivated land compensation efficiency and its influencing factors in mainland China. Ecol. Ind. 166, 112596.

Piggott, J.J., Salis, R.K., Lear, G., Townsend, C.R., Matthaei, C.D., 2015. Climate warming and agricultural stressors interact to determine stream periphyton community composition. Glob. Chang. Biol. 21, 206–222.

Pinna, B., Laini, A., Negro, G., Burgazzi, G., Viaroli, P., Vezza, P., 2024. Physical habitat modeling for river macroinvertebrate communities. J. Environ. Manage. 358.

Postigo, C., Ginebreda, A., Barbieri, M.V., et al., 2021. Investigative monitoring of pesticide and nitrogen pollution sources in a complex multi-stressed catchment: The lower Llobregat River basin case study (Barcelona, Spain). Sci. Total Environ. 755, 142377.

Qi, Q., He, C., Liu, X., Wang, F., Zhang, Z., Feng, K., Lai, H., 2025. Spatial niche differentiation and key driving factors of dominant benthic macroinvertebrates in Qinhuangdao City. China. Scientific Reports 15, 24072.

Rasmussen, J.J., Wiberg-Larsen, P., Baattrup-Pedersen, A., Cedergreen, N., McKnight, U. S., Kreuger, J., Jacobsen, D., Kristensen, E.A., Friberg, N., 2015. The legacy of pesticide pollution: An overlooked factor in current risk assessments of freshwater systems. Water Res. 84, 25–32.

Schmidt, T.S., van Metre, P.C., Carlisle, D.M., 2019. Linking the Agricultural Landscape of the Midwest to Stream Health with Structural Equation Modeling. Environ. Sci. Technol. 53, 452–462.

Schürings, C., Globevnik, L., Lemm, J.U., Psomas, A., Snoj, L., Hering, D., Birk, S., 2024. River ecological status is shaped by agricultural land use intensity across Europe. Water Res. 251.

Scotti, A., Füreder, L., Marsoner, T., Tappeiner, U., Stawinoga, A.E., Bottarin, R., 2019. Effects of land cover type on community structure and functional traits of alpine stream benthic macroinvertebrates. Freshw. Biol. 65, 524–539.

Shi, X., Mao, D., Song, K., Xiang, H., Li, S., Wang, Z., 2024. Effects of landscape changes on water quality: a global meta-analysis. Water Res. 260.

Sigmund, G., Ågerstrand, M., Antonelli, A., Backhaus, T., Brodin, T., Diamond, M.L., Erdelen, W.R., Evers, D.C., Hofmann, T., Hueffer, T., Lai, A., Torres, J.P.M., Mueller, L., Perrigo, A.L., Rillig, M.C., Schaeffer, A., Scheringer, M., Schirmer, K., Tlili, A., Soehl, A., Triebskorn, R., Vlahos, P., vom Berg, C., Wang, Z., Groh, K.J., 2023. Addressing chemical pollution in biodiversity research. Glob. Chang. Biol. 29, 3240–3255.

Simmons, B.I., Blyth, P.S.A., Blanchard, J.L., Clegg, T., Delmas, E., Garnier, A., Griffiths, C.A., Jacob, U., Pennekamp, F., Petchey, O.L., Poisot, T., Webb, T.J., Beckerman, A.P., 2021. Refocusing multiple stressor research around the targets and scales of ecological impacts. Nat. Ecol. Evol. 5, 1478–1489.

Stegen, J.C., Lin, X., Fredrickson, J.K., Chen, X., Kennedy, D.W., Murray, C.J., Rockhold, M.L., Konopka, A., 2013. Quantifying community assembly processes and identifying features that impose them. ISME J. 7, 2069–2079.

Su, P., Wang, X., Lin, Q., Peng, J., Song, J., Fu, J., Wang, S., Cheng, D., Bai, H., Li, Q., 2019. Variability in macroinvertebrate community structure and its response to ecological factors of the Weihe River Basin, China. Ecol. Eng. 140.

Tang, F.H.M., Lenzen, M., McBratney, A., Maggi, F., 2021. Risk of pesticide pollution at the global scale. Nat. Geosci. 14, 206–210.

Troia, M.J., Kaz, A.L., Niemeyer, J.C., Giam, X., 2019. Species traits and reduced habitat suitability limit efficacy of climate change refugia in streams. Nat. Ecol. Evol. 3, 1321–1330.

Turschwell, M.P., Connolly, S.R., Schäfer, R.B., Laender, D.E.F., Campbell, M.D., Mantyka-Pringle, C., Jackson, Michelle, C., Kattwinkel, M., Sievers, M., Ashauer, R., CôTé, I.M., Connolly, R.M., Van Den Brink, P.J., Brown, C.J., Byers, J., 2022. Interactive effects of multiple stressors vary with consumer interactions, stressor dynamics and magnitude. Ecol. Lett. 25, 1483–1496.

Urban, M.C., Alberti, M., de Meester, L., Zhou, Y., Verrelli, B.C., Szulkin, M., Schmidt, C., Savage, A.M., Roberts, P., Rivkin, L.R., Palkovacs, E.P., Munshi-South, J., Malesis, A. N., Harris, N.C., Gotanda, K.M., Garroway, C.J., Diamond, S.E., Roches, S.D., Charmantier, A., Brans, K.I., 2024. Interactions between climate change and urbanization will shape the future of biodiversity. Nat. Clim. Chang. 14, 436–447.

van den Berg, S.J.P., Baveco, H., Butler, E., de Laender, F., Focks, A., Franco, A., Rendal, C., van den Brink, P.J., 2019. Modeling the sensitivity of aquatic macroinvertebrates to chemicals using traits. Environ. Sci. Technol. 53, 6025–6034.

van Vliet, M.T.H., Thorslund, J., Strokal, M., Hofstra, N., Flörke, M., Ehalt Macedo, H., Nkwasa, A., Tang, T., Kaushal, S.S., Kumar, R., Van Griensven, A., Bouwman, L., Mosley, L.M., 2023. Global river water quality under climate change and hydroclimatic extremes. Nat. Rev. Earth Environ. 4, 687–702.
von Gönner, J., Gröning, J., Grescho, V., Neuer, L., Hänsch, V.G., Laue, B., Molsberger-Lange, E., Wilharm, E., Liess, M., Bonn, A., 2023. The majority of Germany's small agricultural streams are in poor ecological status. bioRxiv 2023, 10.27.564349.
Wagg, C., Roscher, C., Weigelt, A., Vogel, A., Ebeling, A., de Luca, E., Roeder, A., Kleirspehn, C., Temperton, V.M., Meyer, S.T., Scherer-Lorenzen, M., Buchmann, N., Fischer, M., Weisser, W.W., Eisenhauer, N., Schmid, B., 2022. Biodiversity-stability relationships strengthen over time in a long-term grassland experiment. Nat. Commun. 13 (1), 7752.
Wang, L., Han, B.-P., 2023. Non-random species loss under human disturbance increases the nestedness component of macroinvertebrate beta diversity in tropical streams. Ecol. Ind. 154.
Wang, J., Wei, H., Zhou, X., Li, K., Wu, W., Guo, M., 2019. Occurrence and risk assessment of antibiotics in the Xi'an section of the Weihe River, northwestern China. Mar. Pollut. Bull. 146, 794–800.
Wang, S., Isbell, F., Deng, W., Hong, P., Dee, L.E., Thompson, P., Loreau, M., 2021a. How complementarity and selection affect the relationship between ecosystem functioning and stability. Ecology 102 (6), e03347.
Wang, Y., Song, J., Zhang, X., Sun, H., 2021b. Sustainable development evaluation and its obstacle factors of the weihe river basin in Shaanxi Province, China. Front. Earth Sci. 9.
Wang, Y.Y.L., Xiong, J., Ohore, O.E., Cai, Y.-E., Fan, H., Sanganyado, E., Li, P., You, J., Liu, W., Wang, Z., 2022. Deriving freshwater guideline values for neonicotinoid insecticides: Implications for water quality guidelines and ecological risk assessment. Sci. Total Environ. 828.
Weisner, O., Frische, T., Liebmann, L., Reemtsma, T., Roß-Nickoll, M., Schäfer, R.B., Schäffer, A., Scholz-Starke, B., Vormeier, P., Knillmann, S., Liess, M., 2021. Risk from pesticide mixtures – The gap between risk assessment and reality. Sci. Total Environ. 796.
Wilkes, M.A., Edwards, F., Jones, J.I., Murphy, J.F., England, J., Friberg, N., Hering, D., Poff, N.L., Usseglio-Polatera, P., Verberk, W.C.E.P., Webb, J., Brown, L.E., 2020. Trait-based ecology at large scales: Assessing functional trait correlations, phylogenetic constraints and spatial variability using open data. Glob. Chang. Biol. 26, 7255–7267.
Wu, Y., Xi, X., Tang, X., Luo, D., Gu, B., Lam, S.K., Vitousek, P.M., Chen, D., 2018. Policy distortions, farm size, and the overuse of agricultural chemicals in China. Proc. Natl. Acad. Sci. 115, 7010–7015.
Xie, Y., Liu, Q., Xu, X., Lin, L., Wang, W., Wang, D., 2025. Comprehensive Analysis of Pesticide Pollution across the Yangtze River Basin: Spatiotemporal Distribution, Socio-Economic Correlations, and Ecological Risk Assessment. ACS ES&T Water 5, 1971–1981.
Yang, J., Huang, X., 2021. The 30 m annual land cover dataset and its dynamics in China from 1990 to 2019. Earth Syst. Sci. Data 13, 3907–3925.
Yang, L., Li, H., Zhang, Y., Jiao, N., 2019. Environmental risk assessment of triazine herbicides in the Bohai Sea and the Yellow Sea and their toxicity to phytoplankton at environmental concentrations. Environ. Int. 133.
Yang, Z., He, S., Feng, T., Lin, Y., Chen, M., Li, Q., Chen, Q., 2023. Spatial variation in the community structure and response of benthic macroinvertebrates to multiple environmental factors in mountain rivers. J. Environ. Manage. 341.
Young, L., Wright, M., Tockner, K., Thieme, M., Tharme, R.E., Robinson, J., Ormerod, S. J., Olden, J.D., Muruven, D., McClain, M.E., Leonard, P., Lynch, A.J., Leclère, D., Jones, T., Hughes, K., Harrison, I., Edwards, G., Darwall, W., Dalton, J., Cooke, S.J., Bunn, S.E., Arthington, A.H., Acreman, M., Abell, R., Opperman, J.J., Tickner, D., 2020. Bending the curve of global freshwater biodiversity loss: an emergency recovery plan. Bioscience 70, 330–342.
Zhang, L., Song, Z., He, L., Zhong, S., Ju, X., Sha, H., Xu, J., Qin, Q., Peng, J., Liang, H., 2024a. Unveiling the toxicological effects and risks of prometryn on red swamp crayfish (Procambarus clarkii): Health assessments, ecological, and molecular insights. Sci. Total Environ. 951.
Zhang, Q., Yang, T., Wan, X., Wang, Y., Wang, W., 2021. Community characteristics of benthic macroinvertebrates and identification of environmental driving factors in rivers in semi-arid areas – A case study of Wei River Basin. China, Ecological Indicators, p. 121.
Zhang, Y., Huang, D., Jin, X., Li, L., Wang, C., Wang, Y., Pellissier, L., Johnson, A.C., Wu, F., Zhang, X., 2024b. Long-term wetland biomonitoring highlights the differential impact of land use on macroinvertebrate diversity in Dongting Lake in China. Commun. Earth Environ. 5.
Zhang, Y., Wang, M., Zhou, X., Cheng, W., Ren, J., Wan, T., Liu, X., 2024c. Transmission mechanism of antibiotic resistance genes and their differences between water and sediment in the Weihe River Basin. Environ. Res. 252.
Zhou, S., di Paolo, C., Wu, X., Shao, Y., Seiler, T.-B., Hollert, H., 2019. Optimization of screening-level risk assessment and priority selection of emerging pollutants – The case of pharmaceuticals in European surface waters. Environ. Int. 128, 1–10.