Decoding Benthic Macroinvertebrate Communities in Freshwater Ecosystems Leveraging Environmental DNA

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Abstract

Benthic macroinvertebrates are key indicator groups within freshwater ecosystems, with their community being closely tied to ecosystem functioning. Environmental DNA (eDNA) technology, with its high sensitivity and non-invasive nature, provides a promising tool for studying the spatiotemporal dynamics of benthic macroinvertebrate communities, their responses to anthropogenic disturbances, and the mechanisms governing community assembly. However, current eDNA-based research on freshwater benthic communities largely concentrates on optimizing sampling and detection methods, along with environmental monitoring applications. Broader ecological investigations using eDNA data remain fragmented and limited. In particular, it remains unresolved whether benthic community ecology theories derived from morphological classification can be reliably reconstructed from eDNA-based molecular signals. We systematically summarize the application of eDNA technology in benthic macroinvertebrate ecology, encompassing diversity assessment, spatiotemporal community dynamics, cascading effects of human-induced disturbances, and multi-trophic interaction networks. Although significant empirical progress has been made, challenges persist, including limited spatiotemporal coverage and methodological constraints. Future developments should prioritize the establishment of long-term monitoring networks, integration of multidimensional data, and deeper engagement with community ecology theories. Such advances will help shift eDNA-based research from descriptive to mechanistic understanding, thereby offering scientific support for the precise management and restoration of river ecosystems.

Keywords: Macroinvertebrates; eDNA; Community ecology; Human disturbance; Multitrophic interactions

1. Introduction

Freshwater ecosystems are fundamental role to maintaining biodiversity and supporting human well-being (Lynch et al., 2023). However, their biological integrity is increasingly threatened by multiple stressors, including climate change, pollution, overexploitation of resources, and the spread of invasive species (Perry et al., 2024; Sayer et al., 2025). Benthic macroinvertebrates are integral components of these ecosystems, performing irreplaceable functions such as detritus decomposition (Tank et al., 2010; Yue et al., 2022). Their diversity is undergoing significant decline under intense anthropogenic disturbances, including urbanization, agricultural expansion, wastewater discharge, aquaculture, and forestry development (Li et al. 2015; Dudgeon and Strayer, 2025; Keck et al., 2025). Owing to their limited mobility and high sensitivity to environmental change, they are widely used as bioindicators across global monitoring programs (Dudgeon et al., 2006; Morse et al., 2007; Thomsen et al., 2011; Jin et al., 2025). Nevertheless, conventional assessments of benthic macroinvertebrate biodiversity which rely on field sampling, sorting, and morphological identification, are time-consuming, labor-intensive, and requires considerable taxonomic expertise (Johnson et al., 1993; Sumudumali and Jayawardana, 2021). This approach often entails extensive specimen sorting and can yield inaccurate results due to difficulties in distinguishing morphologically similar taxa, juvenile stages, and rare species. Ntislidou et al. (2020) highlighted how human error during identification can affect water quality assessments, while Haase et al. (2010) reported that 29% of specimens were overlooked during sorting, identifications varied by more than 30% among analysts, and 16% of samples led to discrepant ecological evaluation. Such methodological limitations may seriously compromise the effectiveness of river management and restoration plans.

In contrast, environmental DNA (eDNA) technology offers considerable technical advantages that are advancing the study of benthic macroinvertebrate biodiversity (Deiner et al., 2017; Múrria et al., 2024). Its non-invasive nature minimizes disturbance to both target species and their habitats, while its cost-effectiveness across temporal and spatial scales facilitates large-scale monitoring of biodiversity dynamics. The high sensitivity of eDNA further allows for effective detection of rare and cryptic species, as well as early-stages biological invasions (Beng and Corlett, 2020; Takahashi et al., 2023; Altermatt et al., 2025; Sander et al., 2025; Yates et al., 2025). Consequently, eDNA is increasingly regarded as the most promising tools for efficient, large-scale species monitoring in aquatic ecosystems.

Environmental DNA (eDNA) studies are conducted more frequently in aquatic ecosystems, such as rivers, lakes, and marine waters, than in terrestrial environments, largely due to the relative ease of sample collection. Among these, freshwater ecosystems dominate the eDNA literature, representing approximately 65% of published studies, whereas marine systems account for about 25% and the remaining 10% pertain to other or multiple habitat types (Takahashi et al., 2023; Altermatt et al., 2025). Notably, more than half of these studies (around 52%) focus on fish species, while benthic macroinvertebrate communities have received comparatively less attention.

In this review, we summarize the application of eDNA technology to key ecological questions regarding benthic macroinvertebrate communities in freshwater ecosystems, including their spatiotemporal dynamics, resilience to anthropogenic disturbance, and multi-trophic interactions. We also synthesize recent technological and methodological advances, outline major international initiatives, and provide a comprehensive overview of published eDNA-based studies in benthic macroinvertebrate community ecology. Current challenges and promising future research directions are discussed. We anticipate that this work will help advance eDNA from a primarily descriptive tool toward a more mechanistic analytical framework, thereby providing actionable insights to support the monitoring and management of freshwater ecosystems under global change.

2. Towards Optimized eDNA-based in Benthic Community Ecology: Sampling Strategies and Methodological Validation

eDNA originates from a variety of biological sources, such as skin, saliva, mucus, secretions, urine, blood, feces, gametes, roots, leaves, pollen, and decomposing tissues (Bohmann et al., 2014). It exists both as extracellular molecules and within cellular debris. The technology enables non-invasive species detection and community assessment by capturing genetic material shed or released by organisms, followed by high-throughput sequencing (HTS) and bioinformatics analysis (Thomsen and Willerslev, 2015; Harrison et al., 2019; Gu et al., 2024). Recently, Sahu et al. (2025) provided an updated overview of the standard eDNA workflow, which outlines the complete pipeline from field sampling and DNA extraction/purification through PCR amplification, HTS, bioinformatic processing, to final taxonomic assignment.

The choice of environmental sample type is crucial in eDNA metabarcoding, as it directly influences taxon detection and the resulting community profile (**Table1**). Water samples are widely used for large-scale biodiversity assessment due to their ease of collection (Vourka et al., 2023); however, local benthic community composition may be underestimated because of signal dilution and hydrological transport (Wang et al., 2021). In contrast, ethanol-preserved samples (e.g., from kick-net sampling) show higher sensitivity for sessile or sedentary taxa and better reflect local biodiversity (Erdozain et al., 2019; Martins et al., 2021), though they can underrepresent rare, small, or heavily sclerotized species (Zizka et al., 2019). Sediment samples accumulate DNA from benthic organisms and are less influenced by transport, making them particularly useful for detecting arthropods, annelids, and mollusks at local scale (Ji et al., 2022). Their high spatial heterogeneity, however, requires careful sampling design and adequate replication to ensure representativeness (Sakata et al., 2021). Biofilm samples also hold promise for monitoring species in complex or structured habitats due to their strong DNA retention capacity (Rivera et al., 2021). Recent evidence suggests that combining water and detritus samples currently represents the most effective strategy for assessing riverine macroinvertebrate diversity. This integrated approach leverages complementary taxonomic coverage and outperforms single-sample methods and other combinations in capturing species richness and community composition (Shi et al., 2025).

Table1. Comparison of Common eDNA Sample Types in Freshwater Ecosystems

Sample Type	Advantages	Limitations	Recommended Applications
Water	Captures broad-scale diversity patterns; easy to collect and process	Prone to dilution; lower resolution for site-specific communities	Regional-scale biodiversity assessment
Ethanol	Accurately reflects local community composition; effective for sessile taxa	May underrepresent rare or small-bodied taxa; potential sampling biases	Local biotic integrity evaluation
Sediment	High DNA concentration; suitable for detecting benthic organisms	High spatial heterogeneity; requires intensive replication for representativeness	Local community structure assessment and taxonomic screening
Biofilm	Long DNA retention time; effective in slow-flow or attached habitats	Methodologically novel; standardization protocols still under development	Monitoring in complex habitats or where conventional methods are limited
Detritus	Superior DNA retention; enhanced detection of key taxa (e.g., Oligochaeta, Diptera)	High operational complexity; limited spatial representativeness	Combined with water samples

Although water sampling remains prevalent in eDNA studies due to its operational simplicity, conducting controlled comparisons with traditional morphological methods are still essential to validate the accuracy and reliability of eDNA-based assessments (**Table 2**).

Table 2. Representative studies comparing benthic macroinvertebrate richness estimates with traditional sampling or historical data for a geographic location to that of eDNA

No.	eDNA Sample Types	Traditional sampling method	Habitat	eDNA efficacy finding	References
1	Water	Dip net	Pond water	◑	(Thomsen et al., 2011)
2	Water	Dip net	Ditches	↑	(Beentjes et al., 2022)
3	Water	Dip net	Ditches	↑	(van der Plas et al.,2025b)
4	Water	Dip net	River	◑	(Marshall and Stepien, 2020)
5	Water	Kick-sweep	Lake and river	◑	(Mächler et al., 2014)
6	Water	Kick-sweep	Hypersaline lake	↑	(Campbell et al., 2023)
7	Water	Kick-sweep	Hypersaline lake	◑	(Saccò et al., 2025)
8	Water	Kick-sweep	River	◑	(Fernández et al., 2019)
9	Water	Kick-sweep	River	↑	(Mächler et al., 2019)
10	Water	Kick-sweep	River	◑	(Brantschen et al., 2021)
11	Water	Kick-sweep	River	↑	(Pereira-da-Conceicoa et al., 2021)

No.	eDNA Sample Types	Traditional sampling method	Habitat	eDNA efficacy finding	References
12	Water	Kick-sweep	River	↑	(Seymour et al., 2021)
13	Water	Kick-sweep	River	◑	(Blackman et al., 2022a)
14	Water	Kick-sweep	River	◑	(Keck et al., 2022b)
15	Water	Kick-sweep	Stream	◑	(Reinholdt Jensen et al., 2021)
16	Water	Kick-sweep	Stream	◑	(Gleason et al., 2021)
17	Water	D-net	River	◑	(Múrria et al., 2024)
18	Water	D-net	River	◑	(Huo et al., 2025)
19	Water	Hand net	Spring	◑	(Blattner et al., 2021)
20	Water	Hand net	River	◑	(Jeunen et al., 2022)
21	Water	Surber net	River	↑	(Uchida et al., 2020)
22	Water	Hess sampling	River	◑	(Penaluna et al., 2026)
23	Water	Visual census, benthic trawling	River	↑	(Hata et al., 2022)
24	Water	Historical data	Lake	◑	(Coghlan et al., 2021)
25	Water	Historical data	Lagoon	◑	(Specchia et al., 2022)
26	Water	Historical data	River	◑	(Wu et al., 2023)
27	Water	Historical data	River	◑	(Qin et al., 2023)
28	Water	Historical data	River and stream	◑	(Aunins et al., 2023)
29	Sediment	Surber net	River	↑	(Ji et al., 2022)
30	Sediment	Surber net	River	↑	(Zhou et al., 2022)
31	Sediment	D-net, Surber net	River	◑	(Li et al., 2024)
32	Detritus	Kick-sweep	River	↑	(Ntislidou et al., 2023)
33	Detritus	D-net	River and stream	↓	(Jijón et al., 2025)
34	Ethanol, Water	D-net	Stream	↑	(Wang et al., 2021)
35	Ethanol, Water	Surber net, kick net	River	↑	(Chen et al.,2025)
36	Biofilm	Historical data	River	↑	(Rivera et al., 2021)

Notes: " ◑ " indicates that the results are complementary; " ↑ " indicates that the eDNA method has obtained higher diversity data compared to the traditional sampling method; " ↓ " indicates that the eDNA method has obtained lower diversity data compared to the traditional sampling method.

In summary, future studies should prioritize multiple sample types, such as water, sediment, and ethanol-preserved materials, to capture complementary ecological niches and improve overall detection efficiency. Even when full integration of all sample types is not feasible, comparative analyses with traditional sampling methods can still offer a robust evaluation of eDNA's strengths and limitations, thereby strengthening the credibility of the findings.

3. Global Insights: eDNA in Setting Research Priorities and Response Indicators

The assessment of ecosystem stress has long relied on diversity metrics, with α diversity (intra-community diversity) and β diversity (inter-community differences) serving as core indicators in ecological research for decades (Daly et al., 2018; van der Plas et al., 2025a). These metrics quantify biodiversity patterns and are essential for detecting shifts in community composition across spatial and temporal scales. Here, we synthesize benthic macroinvertebrate community indices obtained from eDNA data across different countries, illustrating how environmental drivers, such as land use, water quality, and climate factors, influence biodiversity patterns in distinct regions (**Table 3**). This synthesis offers a comprehensive overview of current research trends in this field internationally.

Table 3. Summary of benthic macroinvertebrate community ecological metrics derived from eDNA data, their associated environmental drivers, and representative application studies.

No.	Country	Environmental Variables	Response Variables	References
1	Australia	Salinity	α-diversity, β-diversity	(Campbell et al., 2023)
2	Belarus	Water temperature, Artificial canals	β-diversity, Keystone species screening, Phylogenetic diversity	(Jeunen et al., 2022)
3	Brazil	Land use change	α-diversity, β-diversity	(Faria et al., 2024)
4	Canada	Land use change	α-diversity, β-diversity	(Gleason et al., 2021)
5	Canada	Pollution gradient (herbicide)	α-diversity, β-diversity	(Loria et al., 2025)
6	China	Anthropogenic (pollution gradient)	α-diversity, β-diversity, Functional diversity	(Xu et al., 2023)
7	China	Dams, Nutrient enrichment	α-diversity, β-diversity	(Li et al., 2022)
8	China	Drought	α-diversity, β-diversity	(Feng et al., 2026)
9	China	Elevation	α-diversity, β-diversity	(Shen et al., 2024)
10	China	Flow	α-diversity, β-diversity, Functional diversity	(Li et al., 2024)
11	China	Heavy metal pollution	α-diversity, β-diversity, Phylogenetic diversity	(Zhou et al., 2022)
12	China	Heavy metal pollution (Cu)	α-diversity, β-diversity, Functional diversity, Phylogenetic diversity	(Yang et al., 2018)
13	China	Land use change	α-diversity, β-diversity, Functional diversity	(Li et al., 2020)
14	China	Land use change	α-diversity, β-diversity, Functional diversity, Phylogenetic diversity	(Wu et al., 2023)
15	China	Land use change	α-diversity, β-diversity	(Li et al., 2023a)
16	China	Land use change, Pollutant gradient	α-diversity, β-diversity	(Li et al., 2023b)
17	China	Land use change, Pollution gradient	α-diversity, β-diversity	(Qin et al., 2023)

No.	Country	Environmental Variables	Response Variables	References
18	China	Salinity	α-diversity, Functional diversity, Phylogenetic diversity	(Zhang et al., 2024)
19	China	Seasonal hydrological variation (Water temp., Flow)	α-diversity, β-diversity, Functional diversity	(Liang et al., 2022)
20	China	Trophic status	α-diversity, β-diversity, Functional diversity	(Xiong et al., 2025)
21	China	Urbanization gradient	α-diversity, β-diversity, Functional diversity	(Ji et al., 2022)
22	China	Water temperature, Elevation	α-diversity, β-diversity, Functional diversity, Phylogenetic diversity	(Lu et al., 2023)
23	China	Water temperature, Elevation	α-diversity, β-diversity, Phylogenetic diversity	(Lu et al., 2024)
24	China	Water temperature, Salinity	α-diversity, β-diversity, Functional diversity	(Huo et al., 2025)
25	Denmark	Seasonal hydrological variation (Water temp., Flow)	α-diversity, β-diversity, Functional diversity	(Reinholdt Jensen et al., 2021)
26	Ecuador	Elevation	α-diversity	(Jijón et al., 2025)
27	Germany	Seasonal hydrological variation (Water temp., Flow)	α-diversity, β-diversity	(Hupało et al., 2022)
28	Greece	Hydrological barriers	α-diversity, β-diversity, Functional diversity, Phylogenetic diversity	(Ntislidou et al., 2023)
29	Indonesia	Flow	α-diversity, β-diversity	(Effendi et al., 2023)
30	Italy	Salinity, Anthropogenic pressure	α-diversity, β-diversity, Functional diversity, Keystone species screening	(Specchia et al., 2022)
31	Italy	Salinity, Substrate	α-diversity, β-diversity, Functional diversity	(Pinna et al., 2024)
32	Japan	Water temperature, Flow	α-diversity, β-diversity	(Uchida et al., 2020)
33	Netherlands	Agricultural source (Pesticides)	α-diversity, β-diversity	(Beentjes et al., 2022)
34	Netherlands	Pollution gradient (microplastic)	α-diversity, β-diversity	(van der Plas et al.,2025b)
35	Spain	Anthropogenic (pollution gradient)	α-diversity, β-diversity	(Múrria et al., 2024)
36	Switzerland	Anthropogenic pressure (Non-native species detection)	α-diversity, keystone species screening	(Blackman et al., 2022a)
37	Switzerland	Seasonal hydrological variation (Water temp., Flow)	α-diversity, β-diversity	(Blackman et al., 2022b)
38	Switzerland	Urbanization gradient	α-diversity, β-diversity	(Perrelet et al., 2025)
39	UK	Land use change	α-diversity, β-diversity, Functional diversity	(Seymour et al., 2021)
40	USA	Habitat quality	α-diversity, β-diversity	(Marshall and Stepien, 2020)
41	USA	Land use change	α-diversity, β-diversity, Functional diversity	(Penaluna et al., 2026)
42	USA	Seasonal hydrological variation (Water temp., Flow)	α-diversity, β-diversity	(Aunins et al., 2023)

International research on benthic macroinvertebrates using eDNA technology exhibits distinct regional emphases. For instance, studies in the United States and China frequently examine how environmental variables such as water temperature and nutrient concentrations affect species diversity and community structure, often using classical response indices like α- and β-diversity. In contrast, research in Australia and Brazil tends to focus on functional diversity, species composition, and related ecological processes, commonly applying functional diversity indices to track ecosystem changes. In Germany and Spain, greater attention has been given to pollutant gradients and their interactions with aquatic biota, with species richness and community structure regularly used as response metrics. Notably, as eDNA methods become more accessible and their resolution improves, an increasing number of studies are extending beyond purely taxonomic metrics to quantify ecological function. A significant advance in this direction is the "Fun-eDNA" framework recently proposed by Cantera et al. (2024). This method systematically extracts functional trait information, such as feeding group, body size, and dispersal capacity, from eDNA metabarcoding data, allowing the calculation of functional diversity indices (e.g., functional richness, evenness, and redundancy). By transcending the previous limitation of eDNA to taxonomic identification, this framework supports functional inference at a molecular level, offering considerable potential for both theoretical and applied ecology.

In summary, eDNA technology offers a holistic perspective for assessing aquatic biodiversity. The indices derived from eDNA data provide essential insights into ecosystem health, stability, and resilience, informing targeted conservation and restoration measures (Rowland et al., 2020). Ecosystem health can also be evaluated using biological indices, which are often based on the sensitivity or tolerance of certain taxa to environmental conditions and rely heavily on macroinvertebrate community composition (Sumudumali and Jayawardana, 2021). Moving forward, integrating eDNA with complementary monitoring tools and ecological models will be vital to effectively manage and mitigate the impacts of environmental degradation and climate change.

4. Unraveling Spatiotemporal Dynamics: eDNA Reveals Benthic Community Patterns

The spatiotemporal dynamics of benthic macroinvertebrate communities in freshwater ecosystems are increasingly elucidated through eDNA approaches, capturing their nuanced responses to environmental gradients and seasonal fluctuations.

In the study of temporal dynamics, eDNA technology can elucidate how benthic macroinvertebrate communities respond across multiple timescales. Research on seasonal variation has revealed, for instance, that in temperate rivers, communities undergo functional group restructuring driven by winter low temperatures and summer nutrient inputs (Reinholdt Jensen et al., 2021). Similarly, in Mediterranean regions, high flows during the rainy season alter substrate stability and spatial heterogeneity, promoting a shift toward disturbances-adapted functional groups (Hupało et al., 2022). Over longer timescales, eDNA-based monitoring can track interannual trends in community dynamics. For example, Bista et al. (2017) performed annual eDNA analyses in a lake ecosystem, revealing dynamic biodiversity changes and significant interannual variations in the eDNA abundance of certain benthic macroinvertebrates, which were likely influenced by climate change and anthropogenic activities. Overall, eDNA metabarcoding effectively captures temporal restructuring of benthic communities in response to environmental drivers such as water temperature, nutrient availability, flow regimes, and substrate dynamics. The technique thus provides a sensitive means to trace how these factors shape community composition over time.

Spatial heterogeneity, alongside temporal dynamics, plays a pivotal role in structuring benthic macroinvertebrate communities (**Fig. 1**). Studies employing eDNA have demonstrated that community composition responds strongly to environmental gradients across multiple spatial scales, ranging from broad catchments to microhabitats. At larger (e.g., catchment) scales, complex river networks with high tributary density promote ecological niche diversification and enhance hydrological connectivity, thereby increasing both taxonomic and phylogenetic richness (Chang et al., 2025a). At intermediate (e.g., reach) scales, gradients in temperature and salinity shape distinct distribution patterns, with gradual turnover observed from freshwater inflows to coastal zones (Pinna et al., 2024). In high-altitude systems such as glacier-fed river-lake continua, eDNA has detected continuous transitions in biological assemblages for example from cold-adapted upstream specialists to widely distributed downstream taxa, which are primarily driven by thermal and turbidity gradients (Lu et al., 2023). Notably, such fine-scale community transitions can be detected across distances as short as a few hundred meters (Lu et al., 2024).

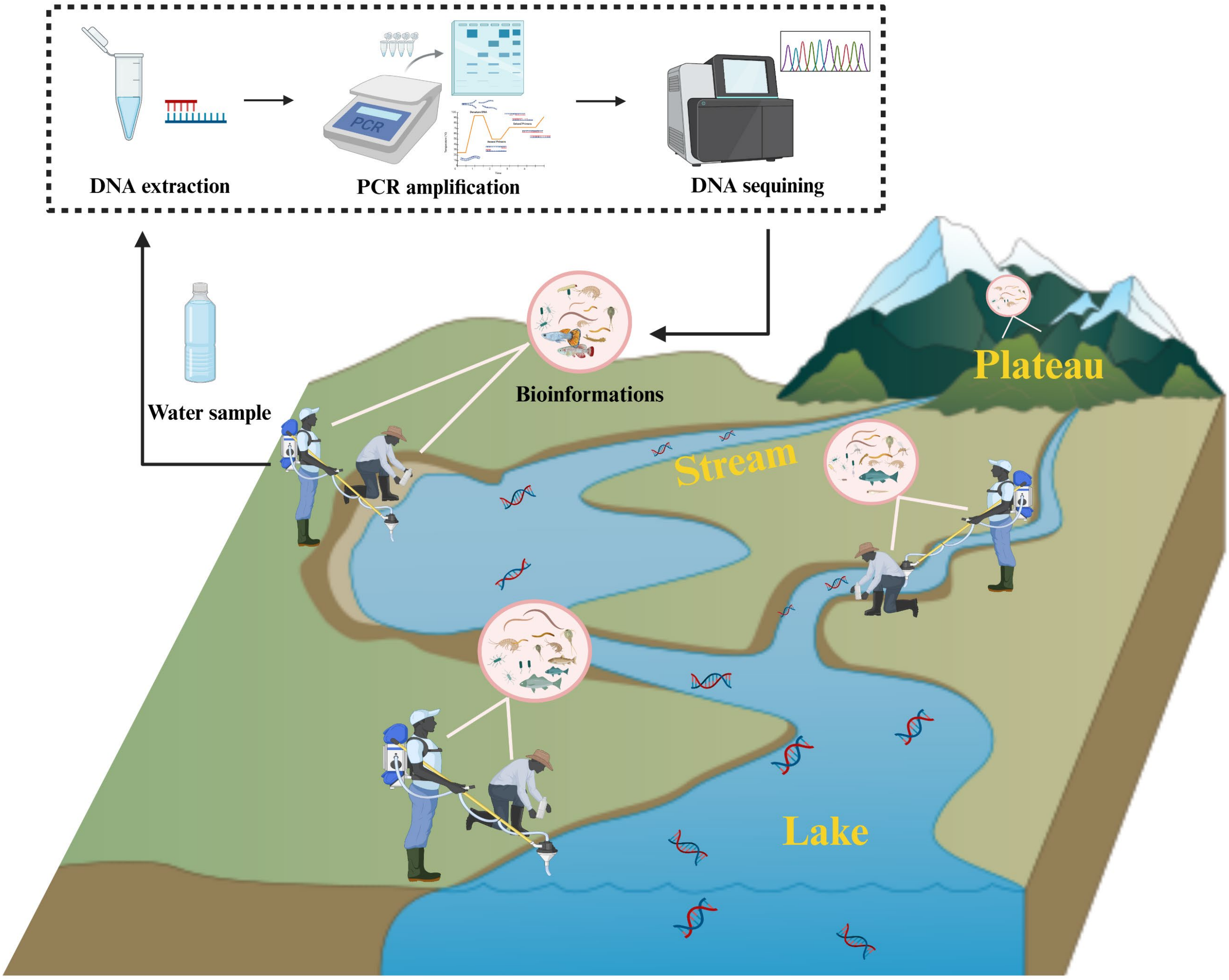

Fig. 1. Representative illustration of spatially explicit eDNA applications for assessing benthic macroinvertebrate communities across freshwater ecosystems.

The observed spatial patterns are driven by a combination of natural environmental gradients and anthropogenic pressures. Land-use changes, for example, indirectly alters community structure through increased nutrient loading and pollutant influx (Li et al., 2020, 2023b; Qin et al., 2023). Agricultural and urbanized areas are frequently dominated by pollution-tolerant taxa, while sensitive groups are diminished, leading to predictable spatial shifts in community composition (Li et al., 2020; Seymour et al., 2021). Overall, eDNA offers a powerful approach for mapping spatial biodiversity patterns and disentangling the complex interplay between natural heterogeneity and human impacts. Its fine spatial resolution also shows strong potential for improving predictive models of benthic community dynamics across multiple scales.

eDNA technology serves as a crucial tool for elucidating spatiotemporal interactions within benthic macroinvertebrate communities. For example, multi-season monitoring in the Weihe River basin demonstrated that hydrological isolation during dry periods and runoff dispersal during wet seasons drive both local adaptation and regional restructuring of these communities (Liang et al., 2022). This indicates that hydrological rhythms regulate community dynamics through coordinated spatiotemporal processes. Collectively, such findings show that eDNA, by integrating data across space and time, provides a reliable foundation for uncovering the assembly mechanisms of benthic macroinvertebrate communities.

Despite its considerable promise for revealing spatiotemporal dynamics, several limitations of eDNA approaches must be addressed in future applications. First, long-term time-series monitoring is often hampered by funding constraints and a lack of methodological standardization, making it difficult to systematically track community changes associated with species phenology. Second, false-negative detections require careful ecological interpretation; a taxon may go undetected due to low abundance (e.g., rare species) or low metabolic activity, resulting in an ecologically meaningful "relative absence". Furthermore, accurately describing spatiotemporal interactions in these communities necessitates the integration of multidimensional datasets (Reinholdt Jensen et al., 2021). Most current studies rely primarily on eDNA data alone and would benefit from deeper integration with hydrological models or machine learning algorithms.

5. Unraveling Cascading Effects: Tracing Anthropogenic Stress in freshwater Ecosystems through eDNA

5.1 Pollution cascades and multi-trophic effects

Freshwater ecosystems face growing pressure from point-source pollutants, including mining runoff, agricultural inputs, and urban drainage. Such stressors can reduce biodiversity through direct toxicity or broader habitat degradation (Reid et al., 2019). eDNA approaches are proving effective in diagnosing the cascading effects of pollutants (such as heavy metals, mining effluents, agrochemicals, and insecticides) on benthic communities across multiple trophic levels.

Pollutants drive systemic reorganization of benthic communities through direct toxicity or indirect alteration habitat, demonstrating characteristic ecological filtering and cascading response patterns. For instance, microcosm experiments and field monitoring show that when specific pollutants (e.g., copper and certain ions) exceed ecological thresholds, they can trigger synchronous collapse across multiple trophic levels, from bacteria and algae to benthic macroinvertebrate communities (Yang et al., 2018; Simonin et al., 2021). In contrast, the effects of agricultural non-point source pollution (e.g., nitrogen, phosphorus nutrients and pesticides) are more complex and cumulative. Evaluations of treated wastewater effluent further reveal that point-source pollution can reorganize multi-trophic communities mainly by altering trophic interactions and control pathways, rather than simply reducing overall diversity (Xiong et al., 2025). eDNA-based analyses indicate that combined stress from agricultural nutrients (e.g., NH_4^+-N and TN) and neonicotinoid insecticides suppresses sensitive groups such as EPT taxa, thereby increasing the dominance of pollution-tolerant groups (e.g., oligochaetes). This shift alters community functional structure and simplifies food-web architecture (Beentjes et al., 2022; Xu et al., 2023). Additionally, pharmaceutical active compounds (PhACs), as emerging contaminants, can significantly disrupt the behavior and reproduction of microbial communities, benthic macroinvertebrates, and fish through chronic low-level exposure, initiating cascading effects across trophic levels (Papaioannou et al., 2023). This process may be accompanied by the spread of resistant bacteria and antibiotic resistance genes, further exacerbating the functional degradation of aquatic communities.

Recent studies show that eDNA metabarcoding effectively captures shifts in community composition and losses of genetic diversity under both acute stressors such as glyphosate exposure (Loria et al., 2025) and diffuse, low-intensity stressors like micro- and nanoplastics, whose impacts are often subtle and transient at the community level (van Der Plas et al., 2025b). Notably, glyphosate-induced effects are strongly amplified under eutrophic conditions, driving rapid decline of sensitive taxa (e.g., crustaceans and rotifers) and resulting in marked structural reorganization of the community.

Together, these findings highlight the need to incorporate both nutrient status and intra-community genetic responses into ecological risk assessments to adequately resolve pollution-driven cascades across trophic levels.

Despite considerable research using eDNA metabarcoding to assess pollutants impacts on benthic macroinvertebrate, several limitations persist. For example, Zhou et al. (2022), combining morphological and eDNA approaches, found that shale-gas wastewater discharge did not significantly alter benthic macroinvertebrate community structure within two years, suggesting that longer-term monitoring may be necessary to detect ecological effects of slow-release pollutants. Furthermore, emerging pollutants such as microplastics and antibiotics remain understudied. More broadly, the current lack of methodological standardization in eDNA research calls for the development of harmonized frameworks to enable large-scale, comparable ecological risk assessments.

5.2 Hydrological alteration and community reorganization

Nearly half of global river volume is affected by flow regulation and/or fragmentation (Grill et al., 2015). Hydraulic infrastructure such as dams alters hydrological regimes and connectivity, driving adaptive restructuring in benthic macroinvertebrate communities (Chan et al., 2025). Reservoirs and cascading dams dampen downstream hydrological pulse; for example, eDNA monitoring in the Wujiang River has shown a shift from fast-flow-adapted taxa (e.g., EPT groups) to standing water-adapted Chironomidae (Shen et al., 2024). In drought-prone regions, prolonged high water levels in reservoirs can exacerbate intermittent streamflow, increasing the risk of habitat desiccation and prompting a transition from seasonal dynamic to stagnant-water, steady-state benthic communities (Li et al., 2020). Furthermore, dams can impede the dispersal of benthic larvae (Ntislidou et al., 2023). Moving beyond single-stressor studies, Li et al. (2022) used eDNA metabarcoding to reveal the synergistic effects of dams and nutrient enrichment on multitrophic community cascades. eDNA-based evidence also indicates that hydrological connectivity buffers multitrophic biodiversity loss during hydrological extremes (e.g., drought), constraining community reorganization beyond the influence of flow regulation alone (Feng et al., 2026).

In summary, future research should integrate hydrological models with eDNA dynamic monitoring and quantify threshold effects of water infrastructure on the resilience of benthic macroinvertebrate communities. Moreover, applying eDNA to decipher complex stressor interactions (such as synergistic, antagonistic, or cumulative effects) will be essential for informing science-based management decisions.

5.3 Effects of land-use change and habitat homogenization

Land-use change is a major driver of biodiversity loss in freshwater ecosystems (Foley et al., 2005). Human activities such as urbanization, agricultural expansion, and deforestation cascade to alter the structure and function of benthic macroinvertebrate communities, largely by increasing nutrient loading (Faria et al., 2024) and reducing habitat heterogeneity (Birk et al., 2020). eDNA offers an effective means of tracking these changes, providing insights into how biodiversity and ecosystem function shift across land-use types changes that ultimately affect human well-being (**Fig. 2**).

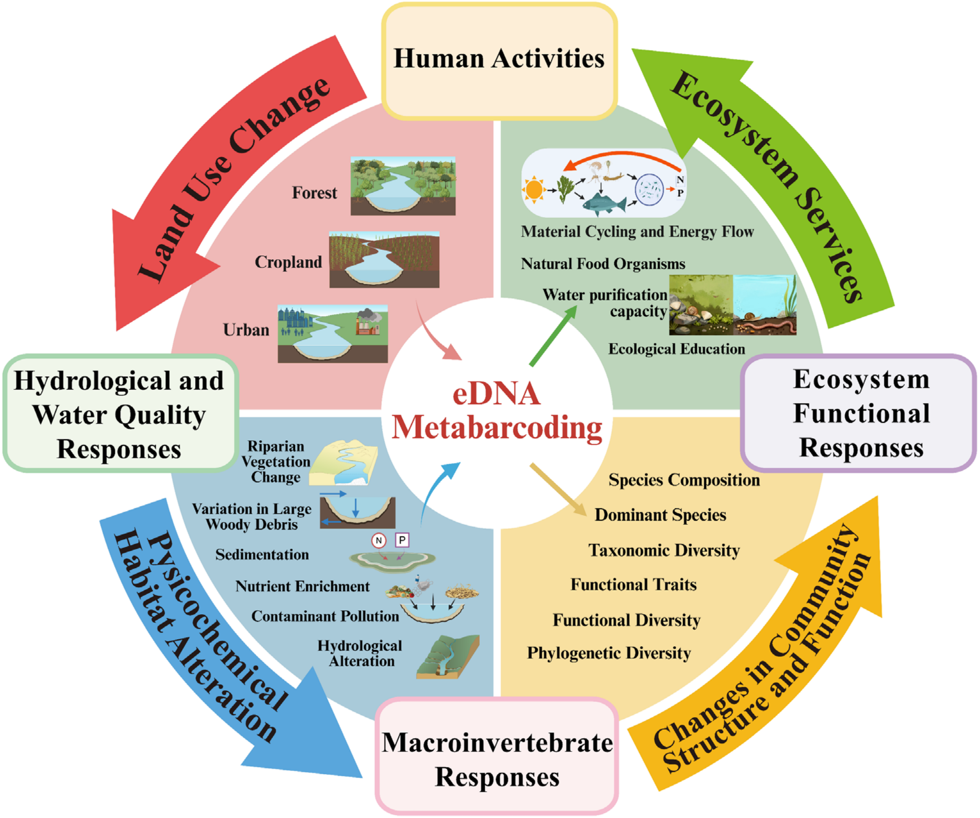

Fig. 2. Responses of benthic macroinvertebrate eDNA to land-use change across river basins.

Accumulating eDNA evidence reveals a consistent pattern: along gradients from forested to agricultural and urban landscapes, benthic macroinvertebrate communities are reshaped by nutrient enrichment, contaminant inputs, and habitat fragmentation, which in turn alter multidimensional biodiversity and interaction network architecture. Forest cover is generally associated with greater community stability, whereas intensive agriculture tends to reduce sensitive EPT taxa and increase the relative dominance of Oligochaeta and Chironomidae (Li et al., 2018; Marshall and Stepien, 2020; Li et al., 2023a; Faria et al., 2024; Zhu et al., 2024). Urbanization and habitat homogenization further reduce β-diversity and functional evenness, leading to compositional convergence and lower functional redundancy (Li et al., 2020; Seymour et al., 2021). These trends are observed across climatic zones and spatial scales—from headwater streams to mainstems and large catchments—and extend to the simplification of multitrophic network, reflected in weakened link density and connectance (Li et al., 2023b; Qin et al., 2023). Beyond broad land-use categories, recent watershed-scale studies also indicate that forest age structure and internal heterogeneity can mediate freshwater community responses independently of overall land-use type (Penaluna et al., 2026).

Importantly, these findings derived from eDNA align with robust morphology-based evidence confirming land use change as a widespread driver of benthic community homogenization and functional erosion (Pratiwi et al., 2024; Vidal-Abarca Gutiérrez, 2024; Xie et al., 2024), while offering greater sensitivity and taxonomic breadth. By resolving finer-scale community across land-use types and detecting cascading effects across trophic levels (Ji et al., 2022), eDNA provides a quantitative means to trace the causal pathway from land-use change, through altered habitat conditions and community structure, to changes in ecosystem functioning and services delivery.

Emerging evidence further suggests that multitrophic network structure exhibits a stronger response to land use than do conventional diversity metrics. Therefore, research on riverine ecosystem responses to anthropogenic pressures should prioritize the analysis of these interaction networks, which can offer a more accurate reflection of a river's ecological condition.

6. Navigating Multitrophic Networks with Environmental DNA

Understanding the structure and dynamics of multitrophic interaction networks, particularly food webs, is essential for assessing ecosystem stability, function, and resilience to disturbances (Delmas et al., 2019; Thompson et al., 2012). Benthic macroinvertebrates occupy pivotal positions within these networks, linking basal resources (e.g., detritus, algae, microbes) to higher trophic levels (e.g., fish).

In the Shaying River and other subtropical river systems, eDNA-based multitrophic monitoring has shown that land-use change reduces the functional evenness of benthic macroinvertebrates and alters their associations with microbial and algal groups (Li et al., 2020, 2023b; Qin et al., 2023). These shifts decrease organic-matter decomposition efficiency and simplify food web architecture. Similarly, Wu et al. (2025) used eDNA in eutrophic lakes to clarify the functional roles of benthic communities and explore regulatory pathways such as bottom-up or top-down control. In extreme habitats like hypersaline lakes, pronounced salinity gradients support unique assemblages of salt-tolerant taxa and markedly reduced network modularity (Zhang et al., 2024). Notably, the spatiotemporal dynamics of food-web structure do not always align with patterns of species diversity (Blackman et al., 2022b), suggesting that understanding ecological networks assembly requires moving beyond taxonomic richness to examine the dynamic configuration of functional groups and their interactions.

By providing high-resolution, multitrophic data, eDNA metabarcoding deepens our understanding of freshwater food webs, revealing changes in connectivity and stability often missed by conventional methods. As a holistic assessment tool, eDNA technology offers the empirical foundation needed to diagnose ecosystem health under global change and to guild effective conservation strategies.

7. Enhanced Detection of Species via eDNA: From Rarity to Invasion

Accurate species detection is fundamental to effective ecological monitoring and biodiversity conservation. In freshwater ecosystems, both rare native species and invasive alien species (IAS) pose critical yet distinct management challenges, with the former often requiring protection and the latter demanding early control (Yates et al., 2025). Conventional survey methods frequently fail to reliably detect these taxa due to their low abundance, cryptic behavior, or early stages of colonization. eDNA technology offers distinct advantages for detecting rare or endangered species (Sigsgaard et al., 2015; Coutts et al., 2022) as well as for tracking biological invasions (Ficetola et al., 2008; Hartle-Mougiou et al., 2023).

7.1 Monitoring Rare and Cryptic Species with eDNA

Monitoring rare and elusive taxa remains a persistent challenge in ecological assessments due to their low abundance, cryptic behaviors, and often benthic or burrowing life histories —traits that frequently lead to under-detection by traditional methods such as visual surveys, benthic trawling, and morphological identification. Owing to its high sensitivity, eDNA has emerged as a transformative tool capable of detecting trace genetic material shed by organisms into the surroundings (Keck et al., 2022; Çevik and Çevik, 2025; Chang et al., 2025b).

For taxa that are difficult to detect, whether due to low abundance, cryptic habits, or morphological ambiguity, eDNA significantly increases detection probability and reduces false-negative rates (Johnsen et al., 2020; Giribet et al., 2023; McCulloch et al., 2025; Zieritz et al., 2025). Its non-invasive nature also supports repeated and ethical sound monitoring of threatened species, aiding in tracking of population declines (Hata et al., 2022) and refining estimates of contemporary distributions (Lor et al., 2020; Preece et al., 2021). Together, these applications deliver timely, actionable data to guide conservation efforts.

However, several limitations affect the detection of rare taxa. Detectability can be influenced by low rates of DNA shedding, constrained environmental dispersion, or PCR primer bias (Coghlan et al., 2021; Schmidt et al., 2021). These issues are particularly evident in complex substrates or lotic (flowing) environments, where DNA undergoes rapid dilution and degradation. Such constraints represent the current sensitivity boundaries of eDNA methods under specific conditions, rather than an inherent flaw of the approach.

7.2 Early Detection and Surveillance of Invasive Species with eDNA

Invasive alien species represent a major threat to freshwater biodiversity, disrupting native communities through competitive exclusion, habitat modification, and resource monopolization (Rahel and Olden, 2008; Pyšek et al., 2020). Early detection and ongoing surveillance are therefore critical for effective management. eDNA has become a widely adopted molecular tool for this purpose, offering particular utility during the initial stages of invasion when traditional surveys often fail.

Often described as a "molecular radar," eDNA can detect target DNA during the earliest stages of invasive, when populations are sparse and not yet visible, or in frontline colonization zones such as ports and water intake points, offering a substantially earlier warning than traditional visual surveys (Thomsen et al., 2011; Goldberg et al., 2013; Dougherty et al., 2016; Jeunen et al., 2022). It can also rapidly characterize macroinvertebrate communities in poorly studied or inaccessible areas, providing initial evidence of invasive presence and securing a critical time window for rapid response (Mauro et al., 2025). Moreover, eDNA enables simultaneous screening of multiple potential invasion sites or extensive water bodies, covering locations that are logistically challenging for conventional methods (Clusa et al., 2021). Notably, quantitative eDNA approaches have been successfully applied to monitor invasive mussels in large-scale water diversion systems (Yang et al., 2025), further demonstrating the practical utility of this technology in invasion surveillance.

Nevertheless, detecting invasive species at their earliest stages remains inherently challenging. There is often a lag between initial colonization and population establishment, during which only a few individuals may be present and shed DNA below current detection thresholds. A further temporal delay can occur between initial DNA deposition and the accumulation of a quantifiable signal, reducing detectability during this critical window. Since eradication efforts are most effective when undertaken early in the invasion process (Fonseca et al., 2023; Harper et al., 2018), enhancing the sensitivity of eDNA detection methods is essential.

A further obstacle to routine implementation is the lack of standardized protocols across jurisdictions for eDNA sampling, laboratory processing, primer selection, and data interpretation. This inconsistency hinders data comparability and limits the regulatory adoption of eDNA evidence in policy-making (Rishan et al., 2023). Addressing these standardization gaps is critical for integrating eDNA into operational invasive species surveillance programs.

In summary, eDNA-based biomonitoring holds transformative potential for detecting both rare native species and invasive taxa, thereby improving biodiversity assessments and enabling proactive ecosystem management. Key challenges include detection limits in low-density populations, primer biases, and the absence of methodological standardization. To fully integrate eDNA into conservation and regulatory practice, future work should prioritize cross-taxa primer optimization, quantitative modeling of detection thresholds, and the international harmonization of sampling and analytical protocols. By bridging the gap between molecular signals and ecological insight, eDNA technology can play a pivotal role in safeguarding freshwater biodiversity amid accelerating global change (**Fig. 3**).

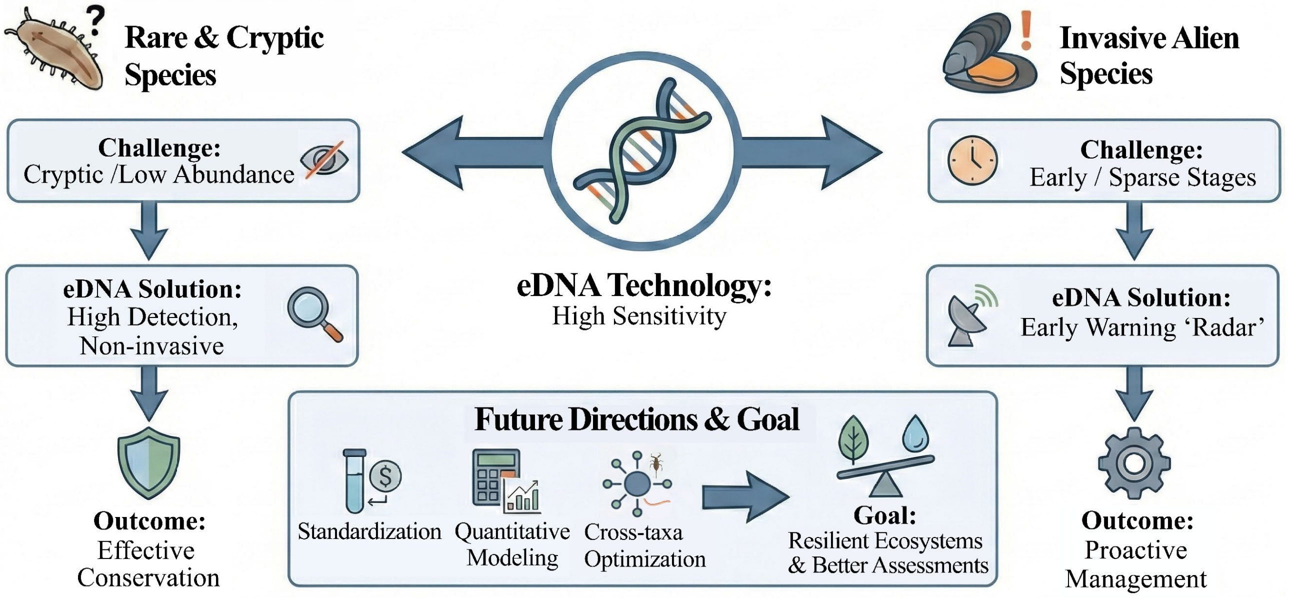

Fig. 3. eDNA as a dual-purpose surveillance tool for benthic macroinvertebrates ecology.

8. From Descriptive to Mechanistic: How eDNA is Unraveling Benthic macroinvertebrate Communities

This review synthesizes current applications of eDNA technology in studying benthic macroinvertebrate communities across global freshwater ecosystems. The continuous and scalable biodiversity monitoring enabled by eDNA metabarcoding in diverse habitats (stream, rivers, lakes, etc.) highlights its transformative role. This approach advances the field by:

(1) Enabling accurate, high-throughput detection of multi-taxa communities, with particularly effective for low-abundance, cryptic, or taxonomically challenging organisms, thus supporting robust assessment of "cryptic biodiversity" often missed by traditional methods;

(2) Capturing spatiotemporal ecosystem dynamics rapidly, including community succession and turnover driven by seasonal/hydrological cycles or environmental gradients;

(3) Detecting early signals of anthropogenic disturbance and evaluating associated ecological impacts, such as biodiversity loss from industrial/agricultural pollution, habitat homogenization due to dams, and functional simplification of communities following land-use change;

(4) Resolving the structure and dynamics of multitrophic interaction networks, revealing how pollutants or habitat alterations affect food-web stability through trophic cascades, or uncovering unique community assembly patterns in extreme environments.

In recent years, eDNA research has progressively shifted from species identification and community description toward a more mechanistic understanding of ecological processes. Breakthroughs have been emerged on several key fronts. For instance, recent studies have parameterized particle-size distributions to simulate the eDNA transport in aquatic systems, thereby improving estimates of its physical detectability (Jo, 2025). Others have shown that data-preprocessing protocols and statistical modeling strategies substantially affect the ecological interpretability of quantitative eDNA results (Bylemans et al., 2025). These advances reflect a broader paradigm shift: eDNA is evolving from a detection tool into a probe for ecological mechanisms, opening new avenues to unravel the assembly rules and functional dynamics of benthic macroinvertebrate communities.

Nevertheless, conceptual validation is required to bridge gaps between eDNA signals (e.g., detection rates, relative read abundance) and the true presence, absence or biomass of source organisms in benthic habitats, especially for rare species, juvenile stages, or metabolically inactive individuals. It is also important to recognize that eDNA constitutes indirect, extracellular genetic material shed by macroinvertebrates. Consequently, any eDNA-based estimates inherently involve uncertainties, including false negatives and false positives. These errors can arise at any stage of the eDNA metabarcoding workflow, from sampling and molecular processing to bioinformatic analysis, and should be systematically calibrated through co-located eDNA and morphological sampling, as well as refined through future technical improvements (Çevik and Çevik, 2025).

Significant technical challenges remain. Integrating eDNA into routine biomonitoring requires further validation of standardized protocols and adaptive modification of existing bioassessment indices (e.g., indices of biological integrity). Detection accuracy can be enhanced through strategies such as seasonal sampling, automated monitoring, and optimized primer design/reference databases design. The resulting large-scale quantitative datasets are essential for capturing fluctuating interspecific interactions and seasonal dynamics in benthic communities. Integrating these data with local hydrological models or machine-learning algorithms offers a promising path to model complex interactions, predict community dynamics from time-series data, and ultimately establish scientific baselines for ecosystem conservation.

Although further technical and methodological refinements are needed, recent national-level initiatives, such as those in China, demonstrate how eDNA monitoring can be institutionalized through standardized protocols and integrated databases (Wang et al., 2022; Chinese Society for Environmental Sciences (CSES), 2023a, 2023b, 2023c; Jiangsu Provincial Administration for Market Regulation (JPAMR), 2023). These efforts highlight the potential for eDNA to become embedded within formal ecological assessment and policy systems.

In conclusion, eDNA is fundamentally reshaping how we understand and conserve freshwater ecosystems. By addressing current methodological limitations and deepening research into ecological mechanisms, eDNA holds strong promise as a core tool for routine monitoring of benthic macroinvertebrate communities. Especially under intensifying global change, its efficiency and sensitivity provide crucial technical support for diagnosing river health and guiding ecological restoration, thereby advancing science-informed decision-making “from DNA to ecosystem management”.

Author Contributions

Yajing Zhang: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review and editing; **Hui Yang:** Investigation, Data curation; **Xiwen Liang:** Investigation, Data curation; **Min Zhang:** Investigation, Data curation; **Wenwen Zhu:** Investigation, Data curation; **Hongxu Yang:** Investigation, Data curation; **Wenze Fan:** Investigation, Data curation; **Kun Li:** Conceptualization, Methodology, Writing – original draft, Writing – review and editing, Supervision, Resources, Funding acquisition.

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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Data Availability

Data will be made available on request.

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