

Review

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 based on eDNA data remain fragmented and limited in scope. 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.

1. Introduction

Freshwater ecosystems are fundamental 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., 2012; 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

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plans.

In contrast, environmental DNA (eDNA) technology provides significant advantages in studying benthic macroinvertebrate biodiversity (Deiner et al., 2017; Múrria et al., 2024). eDNA refers to genetic material shed by organisms into the environment, which can be extracted from environmental samples such as water, sediment, or biofilms. It is non-invasive, causing minimal disturbance to species and habitats. Additionally, its cost-effectiveness enables large-scale biodiversity monitoring over time and space. 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 and ecosystem health assessment in aquatic ecosystems (Wu et al., 2026).

One of the key applications of eDNA is metabarcoding, which enables the simultaneous detection of a broad range of species from environmental samples, making it particularly useful for community-level biodiversity assessments (Ruppert et al., 2019). In contrast, single-species detection techniques, such as qPCR, focus on detecting the presence or abundance of a specific species, offering a more targeted approach (McCull-Gausden et al., 2023).

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. This genetic material is shed or released by organisms into their environment, enabling non-invasive species detection and community assessment. eDNA technology, when coupled with high-throughput sequencing (HTS) and bioinformatics analysis, allows for comprehensive profiling of aquatic biodiversity, making it a powerful tool for understanding species distribution, community composition, and ecosystem dynamics (Thomsen and Willerslev, 2015; Harrison et al., 2019; Gu et al., 2024). Recent advancements in this field, as summarized by Sahu et al. (2025), have optimized the standard eDNA workflow, which involves field sampling, DNA extraction, PCR amplification, sequencing, and bioinformatic analysis. These improvements have enhanced the sensitivity and resolution of eDNA, allowing for more accurate detection of both common and rare species, and facilitating large-scale monitoring of biodiversity.

By integrating eDNA with ecological monitoring, researchers can now track community shifts over time, assess ecosystem health, and even identify early signs of anthropogenic disturbances, such as pollution or habitat degradation.

However, the lack of standardized methodologies for sampling, processing, and bioinformatics analysis across studies has significantly hindered the comparability of results. Although both eDNA and bulk DNA, typically referred to as "community DNA" when sampled directly from biomass, are used for biodiversity assessments, they provide distinct ecological insights (Hänfling et al., 2016; Goldberg et al., 2016). Therefore, selecting the appropriate sampling method is critical (Table 1). eDNA, extracted from water or sediment, offers the advantage of providing an integrated signal of biodiversity across larger spatial scales, which is particularly valuable in studies involving large watersheds or ecosystems with broad species distributions (Vourka et al., 2023; Ji et al., 2022). However, water eDNA is susceptible to transport-related issues such as dilution and hydrological movement, and it may yield false positives due to upstream sources, complicating the interpretation, especially in highly connected aquatic systems (Altermatt et al., 2026). The transport of DNA through water can cause significant spatial heterogeneity in the detection of benthic species, as the DNA signal is often diluted or moved away from the original source (Chen et al., 2025). This dynamic is further influenced by drainage area, as larger catchments are more likely to disperse eDNA, thus complicating the detection of benthic organisms that often shed lower quantities of DNA (Carraro et al., 2023).

Moreover, benthic organisms, due to their lower mobility and exoskeletal structures, contribute to this spatial variability by retaining DNA in the sediment layer, which water samples fail to capture accurately (Pawlowski et al., 2022). Consequently, sediment sampling provides a more reliable representation of local species composition compared to water eDNA, which tends to reflect broader, catchment-wide biodiversity patterns. Sediment samples are less influenced by hydrological transport and are particularly useful for capturing benthic invertebrates, annelids, and mollusks (Ji et al., 2022). Biofilm samples, due to their strong DNA retention capability, hold promise for monitoring species in complex or structured habitats (Rivera et al., 2021).

On the other hand, bulk DNA (such as from ethanol-preserved

Table 1
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(eDNA) |
| Sediment | High DNA concentration; suitable for detecting benthic organisms | High spatial heterogeneity; requires intensive replication for representativeness | Local community structure assessment and taxonomic screening(eDNA) |
| 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 (eDNA) |
| Detritus | Superior DNA retention; enhanced detection of key taxa (e.g., Oligochaeta, Diptera) | High operational complexity; limited spatial representativeness | Combined with water samples (eDNA) |
| Ethanol | Accurately reflects local community composition; effective for sessile taxa | May underrepresent rare or small-bodied taxa; potential sampling biases | Local biotic integrity evaluation (Bulk DNA) |

samples) typically provides a more accurate reflection of local species composition, as it is directly sampled from the tissues of benthic invertebrates or other organisms (Erdozain et al., 2019; Martins et al., 2021). While this method has the advantage of reflecting community composition more accurately at a specific location, it lacks the broader catchment-wide integration that eDNA offers (Zizka et al., 2019). Together, these sampling strategies, including both eDNA and bulk DNA demonstrate that each approach has distinct advantages but also limitations. The heterogeneity of sampling methodologies in eDNA and bulk DNA research underlines the importance of harmonizing these methods to enable direct comparisons across studies.

The methodological discrepancies between sampling strategies underscore the necessity of integrating multiple sample types to capture comprehensive biodiversity information. Recent evidence suggests that combining water and detritus samples currently represents the most effective strategy for assessing riverine macroinvertebrate diversity. This integrated approach leverages the complementary taxonomic coverage of water and detritus, as they detect distinct but overlapping sets of taxa. Water samples, while effective at detecting species like bivalves, have limitations due to dilution and DNA degradation in the water column, especially in rivers with fast-moving water. Detritus samples are particularly effective at detecting oligochaetes and other benthic organisms, as detritus accumulates eDNA from species residing in sediment layers, where DNA is more likely to be retained. Combining these two sample types allows for a more complete detection of macroinvertebrate diversity, as they complement each other by covering both benthic and water-column species. This approach outperforms single-sample methods by addressing the limitations of each sampling

type and capturing a broader range of species, especially in complex or heterogeneous river ecosystems (Shi et al., 2025).

In summary, the combined use of eDNA and bulk DNA may provide a more comprehensive understanding of biodiversity, with eDNA offering region-scale diversity information, while bulk DNA provides more localized, higher-resolution data. This integrated approach enhances the accuracy of ecological assessments and offers a more nuanced view of benthic community dynamics, especially in heterogeneous environments where transport dynamics and spatial variability play a significant role.

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).

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

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., 2012) |
| 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-Conceicao et al., 2021) |
| 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 eDNA and traditional sampling method 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.

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.

Literature Search Strategy

To compile the studies summarized in Table 3, we developed and executed a systematic literature search strategy across multiple authoritative scholarly databases, including Web of Science (Core Collection) and Scopus. Search terms were constructed based on key concepts related to environmental DNA (eDNA) assessment of freshwater benthic communities and associated ecological stressors. Specifically, combinations of controlled vocabulary and free-text keywords were used, such as (“environmental DNA” OR “eDNA”) AND (“freshwater” OR “river” OR “stream” OR “lake” OR “reservoir” OR) AND (“benthic macroinvertebrate” OR “macroinvertebrate”) AND (“diversity” OR “community” OR “disturbance”). Boolean operators (AND, OR) were applied to capture variations and synonyms of relevant concepts. Searches were limited to peer-reviewed journal articles published up to the current review period, with no geographic restrictions applied at the search stage. Retrieved records were then screened by title and abstract, followed by full-text review to ensure relevance to our thematic focus. We also performed backward citation tracking (“snowballing”) on key studies to identify additional pertinent publications that met inclusion criteria. We documented all search terms, databases, and screening decisions to ensure transparency and reproducibility of the retrieval process. This approach ensured comprehensive coverage of empirical eDNA studies relevant to freshwater ecosystems, while acknowledging that the regional distribution of published research reflects the current state of the field rather than a restricted retrieval design.

International research on benthic macroinvertebrates using eDNA technology exhibits distinct regional emphases. The body of research in China predominantly focuses on specific environmental stressors such as heavy metal pollution and land-use change, which are particularly relevant to the region's freshwater ecosystems. These stressors are driven by local environmental challenges, including industrial pollution and large-scale infrastructure development. In contrast, studies from other regions, such as Australia and Brazil, focus more on different stressors like salinity gradients and land-use change. This variation in research focus results in an imbalance in the representation of global freshwater ecosystem stressors, potentially leading to a skewed perception of the most pressing environmental issues. This focus on specific environmental challenges reflects the current state of the literature, with no intentional bias in study selection. As eDNA research expands, future studies should aim to include a wider range of environmental variables from underrepresented regions to better capture the global diversity of freshwater stressors.

Another notable observation is the prevalence of α/β diversity indices in Chinese studies. While these indices are effective in capturing species richness and community composition, they do not fully account for the functional roles or evolutionary relationships among species. In comparison, European studies, particularly from countries like Italy and Germany, increasingly incorporate emerging metrics such as functional diversity and phylogenetic diversity. These indices allow for a more comprehensive assessment of biodiversity by evaluating the ecological roles of species and their evolutionary history. To ensure a more holistic approach to biodiversity assessment, future research in China should integrate these emerging metrics, providing a broader and deeper understanding of biodiversity in freshwater ecosystems.

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) |
| 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) |

(continued on next page)

Table 3 (continued)

| No. | Country | Environmental Variables | Response Variables | References |
|-----|-------------|---|---|---------------------------------|
| 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 | (Hupaio 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) |

Tropical ecosystems, including those in Brazil and Indonesia, are underrepresented in the current body of eDNA research, comprising only 10 % of the studies in Table 3. This limited representation may lead to an underestimation of the unique ecological responses of low-latitude ecosystems, which face distinct environmental pressures, including higher temperatures, seasonal hydrological variations, and anthropogenic influences such as deforestation and land-use change. These

factors significantly affect the structure and function of tropical benthic macroinvertebrate communities. The lack of tropical studies reflects logistical and infrastructural challenges in these regions, as well as limited research funding. However, as eDNA technology becomes more accessible and research funding increases, we expect to see more research from tropical regions, providing a more balanced representation of these ecosystems. It is crucial that future research prioritizes tropical ecosystems to expand the global database and gain a more complete understanding of the ecological response mechanisms in these regions.

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 (Hupaio 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

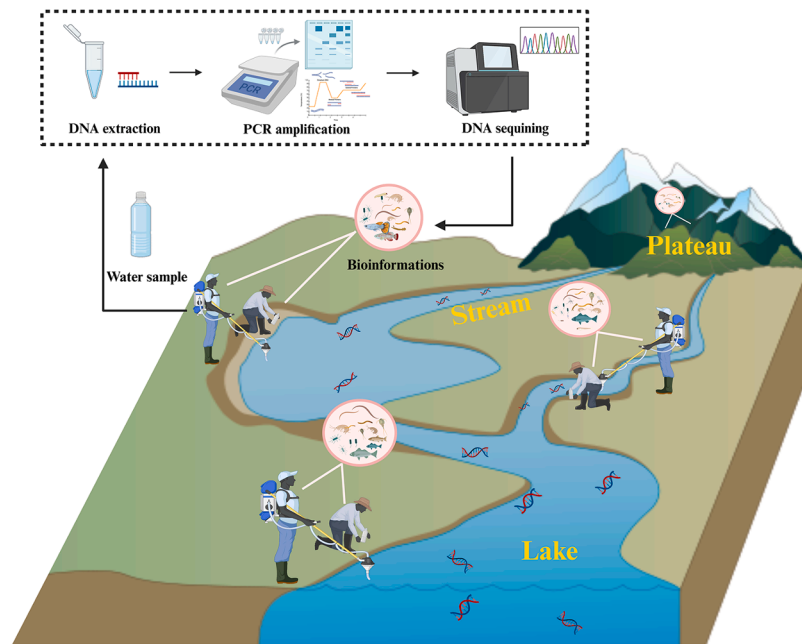


Fig. 1. Representative illustration of spatially explicit eDNA applications for assessing benthic macroinvertebrate communities across freshwater ecosystems. This figure illustrates the use of eDNA technology in studying spatial heterogeneity in benthic macroinvertebrate communities across different freshwater ecosystems, including high-altitude systems (e.g., glacier-fed rivers), stream ecosystems, and lake ecosystems.

short as a few hundred meters (Lu et al., 2024).

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. Long-term time-series monitoring remains challenging due to funding constraints and a lack of methodological standardization, hindering systematic tracking of community changes, especially in response to slow-release pollutants. The integration of long-term, multi-year monitoring plans is essential for capturing chronic ecological effects, including those from pollutants like shale gas wastewater. Additionally, false-negative detections, often caused by low abundance or low metabolic activity, complicate ecological interpretation. Accurately describing spatiotemporal interactions in benthic communities requires the integration of multidimensional datasets, and most current studies would benefit from combining eDNA data with

hydrological models or machine learning algorithms. To address these challenges, future research should prioritize long-term monitoring, especially in data-deficient regions such as high-altitude rivers, to fill existing knowledge gaps and enhance eDNA's utility in ecological assessments.

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., $\text{NH}_4^+\text{-N}$ 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).

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.

In conclusion, while eDNA offers advantages such as high sensitivity and non-invasiveness, its spatiotemporal resolution limitations make it challenging to fully capture the lagged effects of human activities. To address this limitation, we suggest that future studies can improve eDNA monitoring by increasing sampling frequency, integrating multidimensional data such as hydrological models and climate data, and expanding the spatial and temporal coverage of the study area. These measures will help better reveal the long-term impacts of human activities on ecosystems and provide more comprehensive information on long-term ecological changes. With continuous technological advancements, eDNA analysis will become more refined and capable of capturing non-linear shifts in ecosystems more effectively.

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

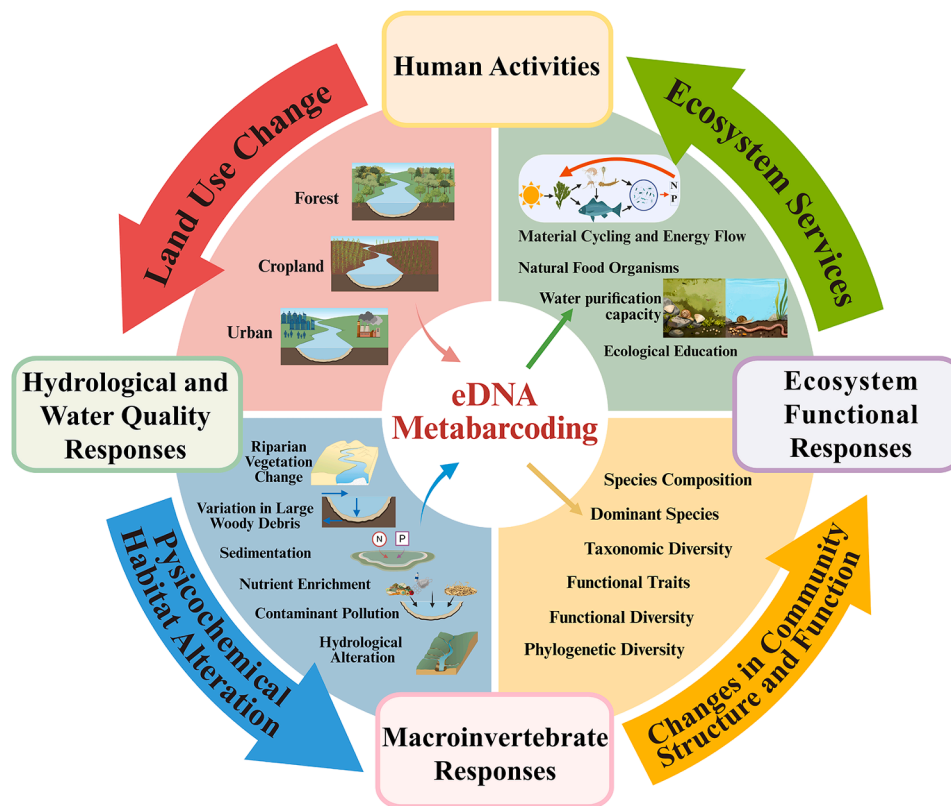


Fig. 2. Responses of benthic macroinvertebrate eDNA to land-use change across river basins. Human activities drive land use change, which alters hydrology and water quality through physicochemical habitat modification. Macroinvertebrate communities respond via shifts in structure and function, which can be effectively detected through eDNA metabarcoding. These biological responses influence ecosystem processes, functional changes then affect ecosystem services that ultimately feedback to human well-being, forming a coupled human–ecosystem feedback loop.

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; Harle-Mougiou et al., 2024).

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., 2022a; 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 (Coughlan 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 invasion, 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., 2012; 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).

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

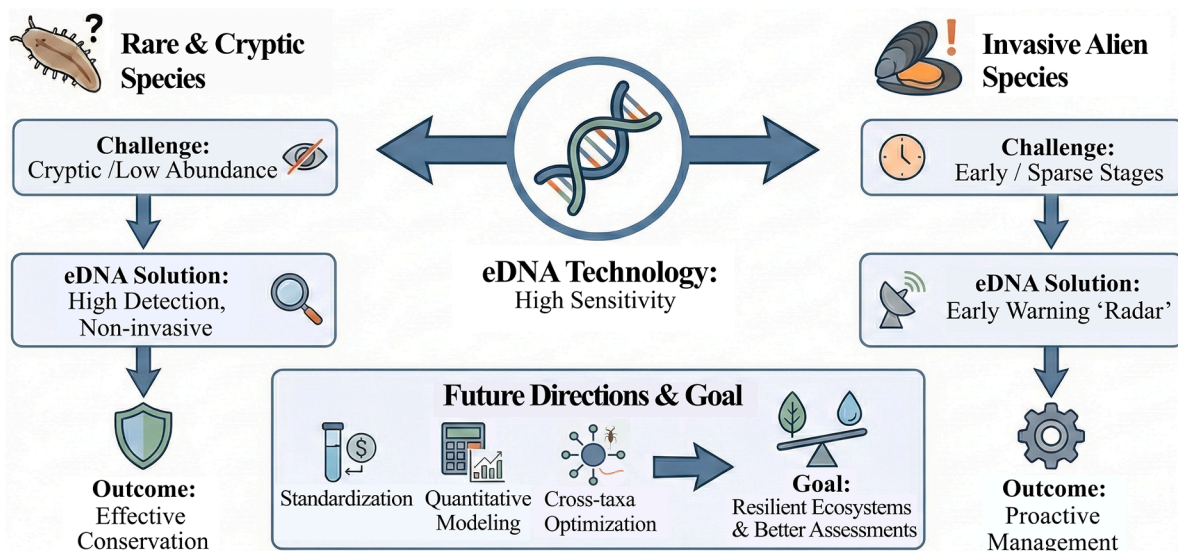


Fig. 3. eDNA as a dual-purpose surveillance tool for benthic macroinvertebrates ecology. This figure illustrates the dual role of eDNA as both a species detection tool and an ecological monitoring tool. eDNA provides early detection of invasive species (e.g., non-native crayfish) at low abundance and enables continuous monitoring of native species, especially rare or cryptic taxa. Additionally, eDNA-based monitoring can assess the ecological health of freshwater ecosystems by tracking community changes across spatial and temporal scales. By offering high sensitivity and broad taxonomic coverage, eDNA is a powerful tool for detecting anthropogenic disturbances, such as pollution or habitat alteration, and guiding timely management actions.

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.

These advances signal a broader paradigm shift where eDNA is evolving from a detection tool into a probe for ecological mechanisms. This evolution not only enhances our ability to track biodiversity but also opens new avenues to unravel the assembly rules and functional dynamics of benthic macroinvertebrate communities. Specifically, eDNA's high-resolution data can now be used to test and refine ecological theories such as Metacommunity Theory. With the capacity to detect hidden or cryptic diversity, eDNA challenges traditional ecological frameworks by providing insights into how species sorting and mass effects influence community assembly, particularly in disturbed or complex environments. The detection of cryptic diversity also offers a novel lens through which we can assess community resilience and ecosystem recovery, revealing species that were previously overlooked and their roles in maintaining ecological stability under stress.

Despite significant advances in the application of eDNA technology for species detection and biodiversity assessment, its use in understanding ecological processes such as community assembly, functional diversity, and interaction networks is still in its early stages. Recent studies have demonstrated that eDNA can effectively capture community composition and diversity across spatial and temporal scales. However, its ability to fully reconstruct functional diversity and ecological interactions remains an active area of research. To apply traditional ecological theories to eDNA data, systematic validation through comparative studies with morphological classification and other ecological data is essential. A promising approach is the integration of functional traits into eDNA-based analyses. The "Fun-eDNA" framework, proposed by Cantera et al. (2025), provides a new method to extract functional trait information from eDNA metabarcoding data, enabling the calculation of functional diversity indices such as functional richness, evenness, and redundancy. This method shows great potential in reconstructing ecological relationships beyond species identification, facilitating the understanding of functional networks and ecosystem processes at a molecular level.

However, several challenges remain in the application of eDNA, particularly regarding the quantification of eDNA in relation to biomass and abundance. For benthic macroinvertebrates, factors such as body size variation, molting, and DNA copy number variation complicate the direct correlation between sequence reads and actual biomass. Furthermore, the differences between qPCR/dPCR and metabarcoding methods, which use distinct approaches to detect species abundance, lead to discrepancies in relative abundance estimates. While qPCR/dPCR provides species-specific measurements, metabarcoding offers broader community profiles but is influenced by amplification biases and sequencing depth (Bylemans et al., 2025). These challenges must be considered when interpreting eDNA data for biomass and abundance in benthic communities.

Another significant limitation of metabarcoding-based eDNA studies is the incompleteness of reference databases, particularly for taxa such as Chironomidae and EPT groups, which are underrepresented in current databases like BOLD and GenBank. These gaps introduce biases in biodiversity estimates and species identification, complicating community composition analysis (Keck et al., 2023). To address this issue, "taxonomy-free" methods, such as Amplicon Sequence Variants (ASVs) and Operational Taxonomic Units (OTUs), offer promising alternatives. These methods classify organisms based on sequence variation, reducing biases caused by incomplete reference libraries. Recent studies have shown that ASVs and OTUs can provide accurate biodiversity estimates even in the absence of comprehensive reference data (Diniz-Filho et al., 2024; Inoue et al., 2026).

Quantifying eDNA in relation to ecological processes requires more than just improving sampling and analytical methods (Çevik and Çevik, 2025). Long-term time-series monitoring remains a significant challenge due to funding constraints and the lack of methodological standardization, limiting the ability to track community changes, especially in response to slow-release pollutants. The integration of multi-year, long-term monitoring plans is essential for detecting chronic ecological effects, such as those from pollutants like shale gas wastewater. Additionally, false-negative results, often caused by low DNA abundance or low metabolic activity, complicate ecological interpretation.

A critical gap in current eDNA studies is the insufficient integration of environmental variables, hydrological models, and machine learning algorithms. These factors, which influence community dynamics, should be incorporated into future eDNA research to provide mechanistic understanding. Recent advancements have shown the value of combining eDNA data with hydrological models and environmental covariates (e.g., temperature, flow velocity) to improve understanding of community assembly and distribution patterns across spatial and temporal scales. Incorporating machine learning algorithms can further enhance these models' predictive power, offering better insights into the interactions between environmental factors and biodiversity.

Despite these challenges, future research should prioritize integrating these approaches into long-term monitoring plans, particularly in data-deficient regions like high-altitude rivers, where eDNA can fill significant knowledge gaps. To improve the detection accuracy, especially for rare and cryptic species, further optimization of primer design is crucial. Optimized primers, tailored to target specific taxa, can reduce amplification biases and improve the reliability of eDNA-based assessments. By incorporating multidimensional datasets, hydrological models, and machine learning techniques, eDNA research can move toward a more mechanistic and reliable understanding of benthic community dynamics, providing more accurate ecological assessments. Furthermore, linking eDNA data with ecological theories such as Metacommunity Theory and community resilience can enhance the predictive power of eDNA-based models and improve our understanding of the ecological processes shaping biodiversity in freshwater ecosystems.

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 (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".

CRediT authorship contribution statement

Yajing Zhang: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **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. **Yuan Ping:** Writing – review & editing. **Kun Li:** Writing – review & editing, Writing – original draft, Supervision, Resources, 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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Data availability

Data will be made available on request

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