

Reconceptualizing intertidal ecosystem status: integrating eDNA metabarcoding, stable isotopes, and functional indicators within an ecological framework

Ennis Wian Erliani¹, Ali Mashar¹, Majariana Krisanti¹, Irwan Iskandar², and Yusli Wardiatno^{1*}

¹Department of Aquatic Resources Management Study Program, Faculty of Fisheries and Marine Science, 16680 Bogor, IPB University, Indonesia

²Mining Engineering, Faculty of Mining and Petroleum Engineering, 40132 Bandung, ITB University, Indonesia

Abstract. The assessment of intertidal ecosystems presents challenges due to the complex interrelationship between community structure, trophic function, and anthropogenic influences. Current approaches remain methodologically fragmented, with studies typically relying on eDNA-based indicators to characterize biodiversity patterns or stable isotope analysis to infer trophic functioning, while integration between the two remains limited. This study aims to systematically review and synthesize these approaches and evaluate their integration for linking community structure and ecosystem function in disturbed environments. Following PRISMA guidelines, 1,659 articles were screened from ScienceDirect, of which 106 articles fulfilled the inclusion criteria of the PEOS framework. Bibliometric analysis was further performed using VOSviewer software. Of the eligible studies, 88 (83%) employed stable isotope approaches, 17 (16%) applied eDNA metabarcoding, and only one study (1%) explicitly integrated both methods. These findings reveal substantial methodological fragmentation within intertidal ecosystem assessment. This study proposes an integrative ecological framework that links the dynamics of communities, trophic activities, and ecosystem stability in the context of anthropogenic influences. This framework provides a base for a comprehensive ecosystem assessment.

1 Background

Intertidal ecosystems are complex, interconnected systems that are highly influenced by extreme environmental variability, diverse habitats, and terrestrial interactions with marine life. This region represents an ecotone shaped by both oceanographic and terrestrial processes, including tidal movements, salinity fluctuation, and the transfer of nutrients and organic matter across systems [1]. These systems are therefore highly productive but also highly vulnerable to anthropogenic perturbations, including eutrophication, pollution, land use changes, and climate change [2]. Consequently, reliable assessment tools are needed to evaluate ecosystem condition and detect ecological responses to environmental disturbance. However, most current assessment methods rely predominantly on structural indicators, such as species diversity and taxonomic composition. Although these metrics provide valuable information on biodiversity patterns, they do not necessarily reflect ecosystem functioning because changes in community structure may occur independently of changes in trophic interactions, energy flow, and other ecological processes [3]. As a result, assessment based solely on community composition may provide an incomplete picture of ecosystem status, particularly in disturbed intertidal environments.

The link between biodiversity and ecosystem function is complex and is highly influenced by environmental context. Advances in molecular tools, especially environmental DNA (eDNA) metabarcoding, have enabled quick and cost-effective species identification within sensitive communities [4]. eDNA has been widely applied to characterize biodiversity patterns and track community responses to environmental and anthropogenic change. Nevertheless, the information generated by eDNA is primarily structural, providing evidence of species presence and community composition rather than direct information on trophic interactions or ecosystem functioning [5]. Consequently, eDNA alone cannot determine how energy moves through food webs or whether observed changes in biodiversity are accompanied by changes in ecological processes. Addressing these functional dimensions requires complementary approaches capable of tracing resource utilization and trophic relationships across ecological communities.

Stable isotope analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ helps clarify trophic structures and energy flow, revealing ecosystem processes obscured when only community structure is considered, especially under environmental disturbance. Additionally, stable isotopes can be used to monitor pollutants within food webs. Despite their respective strengths, eDNA and stable isotope methods have

* Corresponding author : yusli@apps.ipb.ac.id

largely been developed separately, with minimal integration into ecosystem assessments. This separation reflects the complexity of intertidal ecosystems, which are characterized by multiple interconnected energy pathways and pollutant entry points that may not alter community composition [6]. Furthermore, stable isotope techniques face challenges such as low taxonomic resolution and baseline variability, which hinder detailed species-level associations [7].

Although eDNA metabarcoding and stable isotope analysis have both advanced ecosystem assessment, they have largely been applied independently, resulting in fragmented evaluations of biodiversity and trophic functioning. In addition, disturbance gradients remain inconsistently defined among ecological studies, limiting comparison across ecosystems and reducing the ecological relevance of current assessment frameworks. The bibliometric analysis performed in this review demonstrates that stable isotope studies continue to dominate functional ecosystem assessment, whereas eDNA remains primarily associated with biodiversity characterization. Consequently, an integrated framework that combines structural indicators, trophic processes, and disturbance context is still lacking.

Therefore, this study systematically reviews recent literature and proposes an integrative ecological framework that links community structure, trophic function, and environmental disturbance to support a more comprehensive assessment of intertidal ecosystem status.

2 Methods

2.1 Study design

A systematic literature review methodology based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) was applied for transparency, reproducibility, and traceability during the literature selection [8]. Trustworthy scientific journals in ecology and environmental science were identified or searched using the ScienceDirect database.

2.2 Database search

The search strategies were based on two broad categories: eDNA and stable isotopes. The eDNA method used the keywords “environmental DNA,” “functional indicators,” and “anthropogenic stress,” which produced 429 documents. Limiting the publication years to 2021-2026 produced 363 documents, which were filtered down to 187 research studies and, subsequently, 99 open-access or open-archive articles.

The stable isotope approach employed keywords including “stable isotope,” “trophic level,” and “food web” to produce 16,723 documents. Filtering by publication year 2021-2026 resulted in 5,799 documents, which were ultimately narrowed down to 4,297 research articles. Of these, 1,560 articles were open access or open archives. The total number of

articles retrieved from the two database searches was 1,659.

2.3 Inclusion and exclusion criteria

The study applied the PEOS framework to analyze article inclusion. All articles eligible for inclusion, whether ultimately included or excluded, met the defined criteria, as set out in Table 1.

Table 1. Inclusion and exclusion criteria

Criteria	Category
Inclusion	- Population (P): the study focuses on coastal and intertidal ecosystems, such as rocky shores, sandy beaches, mudflats, and tidal zones within estuarine waters. - Exposure (E): evaluation of systems at the following conditions (physically impacted by anthropogenic pressures such as pollution, dredging, nutrient shifts, or hydrological alteration), (comparatively undisturbed reference/relatively undisturbed). - Outcomes (O): Ecosystem status must be assessed through at least one of the following indicators in studies (structural metrics, such as community composition or biodiversity as determined by eDNA/metabarcoding), (functional indicators, including those derived from stable isotopes that reflect trophic position, carbon pathways, or isotopic niches), (Integrated ecosystem metrics: trophic structure, energy flow, ecosystem function, stability). - Study design (S): Studies are original empirical research such as (Field observations), (Experiments either in situ or ex situ), or (Comparative studies of biodiversity structure and/or trophic function in coastal/intertidal ecosystems).
Exclusion	Studies were excluded if they met one or more of the following conditions: - Not an original research article (reviews, meta-analyses, perspectives, commentaries, or conceptual papers with no empirical data), articles not in English were removed, not consistent with exposure context (Studies which did not assess systems under disturbance or reference conditions (disturbed and relatively undisturbed) were excluded, such as those merely descriptive without the provision of environmental pressures context). - Irrelevant Outcome (studies were excluded if they did not assess ecosystem structure, trophic function, or integrative ecosystem metrics e.g., community composition, biodiversity, trophic structure, energy flow, or ecosystem stability). Studies solely reporting chemical, physical, or contaminant measurements without ecological interpretation were excluded.

2.4 Screening and selection

The screening process was applied systematically and rigorously to 1,659 articles, whereby titles, abstracts,

and full texts were assessed for compliance with the inclusion criteria one at a time. Articles were selected using the PEOS framework (Population, Exposure, Outcomes, and Study design). The assessment results are shown in Figure 1.

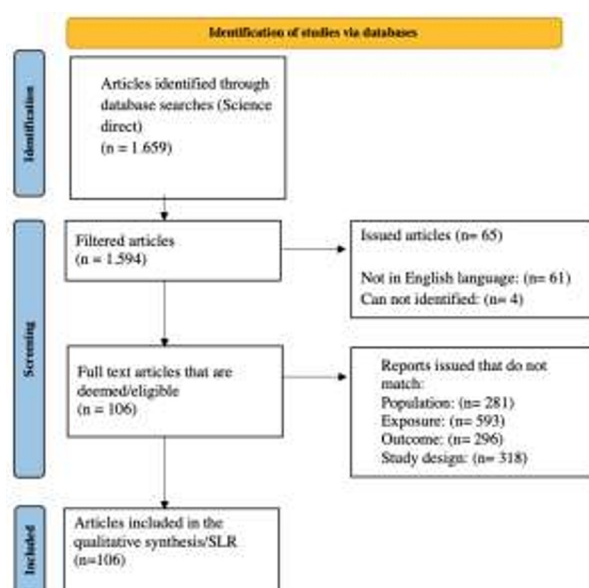


Fig. 1. The process of exclusions with reasons.

This population context includes coastal and intertidal ecosystems such as rocky shores, sandy beaches, mudflats, tidal zones, and estuarine habitats. The exposure consisted of systems that had experienced disturbance (disturbed) and relatively undisturbed (reference) systems. The included outcomes were structural (eDNA-related community composition and biodiversity), functional (e.g., trophic position ($\delta^{15}\text{N}$), carbon pathways ($\delta^{13}\text{C}$), and isotopic niche metrics), and integrative ecosystem metrics (e.g., trophic structure, energy flow, and ecosystem stability).

Field observational studies, experiments, and comparative studies were included in the study design. Articles were excluded if they did not meet the criteria. This method was employed to reduce selection bias and guarantee that the included studies were conceptually and methodologically relevant to this review. After completing all stages of selection, 106 scientific articles met all criteria and were used in the literature synthesis.

3 Results

The synthesis of articles suggests that studies can be divided into three main types based on methodological approaches: eDNA metabarcoding-based, stable isotope-based, and integrated approaches. Studies based on eDNA are the primary focus of the analysis of community structure and biodiversity, specifically identifying species composition in coastal and intertidal ecosystems. In contrast, stable isotope-based studies are predominant in the analysis of trophic structure, organisms' trophic positions, and energy flow across ecosystems. Only one study explicitly integrated both approaches into a single research design, underscoring

the near absence of methodological integration in the literature [9].

Analysis of the research context suggests that most studies integrate disturbance or environmental gradients consistent with exposure criteria. However, the definition of disturbance varies widely, from comparisons between disturbed and reference sites to analyses of pollution or eutrophication gradients. Therefore, while the context of disturbances is gaining attention, there is little consensus on how to use these variables in the design of studies. The distribution of article exclusions at the final stage indicates that the main reason for elimination was exposure factors (38%), followed by outcome criteria (19%), study design (21%), population (18%), and other factors (4%). The details are shown in Figure 2:

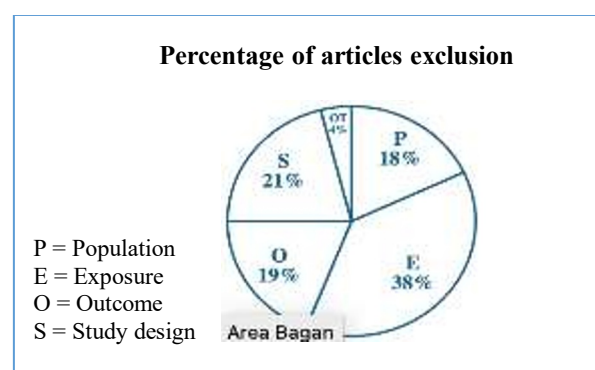


Fig. 2. Percentage of article exclusion by PEOS.

The distribution was highly asymmetric. Stable isotope analysis was the most common method, appearing in 88 studies (~83%). eDNA or metabarcoding approaches were employed in 17 studies (~16%). Only one study (~1%) integrated both methods of assessment. Because the inclusion framework accepted any valid ecological indicator, structural, functional, or integrative, this skewed distribution was not a product of the selection process. This indicates that disturbance-related research in intertidal ecosystems is predominantly conducted using single-method designs that capture either trophic functioning or community structure, but rarely both. Under these conditions, disturbance tends to be treated as a contextual descriptor rather than as a structured analytical variable connecting biodiversity patterns to ecosystem processes.

A bibliometric analysis of the keywords showed similarities with these screening findings. The findings indicate that while advances have been made in methods to understand ecosystems, the existing literature still emphasizes partial methods that separate community structure from trophic function. There is still a lack of integration between the two approaches, and the scientific literature scarcely provides clear references on ecological disturbance.

3.1 Research Trends 2021-2026

The temporal distribution of studies indicates that stable isotope approaches remained the dominant methodology throughout the review period, whereas eDNA applications became increasingly represented in

later years. Despite this gradual diversification of methodological approaches, studies explicitly integrating eDNA and stable isotope analyses remained extremely limited. These patterns suggest that methodological development has progressed unevenly, with advances in biodiversity assessment outpacing efforts to integrate structural and functional ecosystem indicators. In the early part of the study period (2021–2022), research was dominated by stable isotope applications aimed at resolving trophic positions, food web architecture, and energy pathways. The prevailing assumption, often implicit, was that the trophic structure provided a reasonable proxy for the overall ecosystem condition.

A discernible shift emerged around 2023, as environmental DNA (eDNA) metabarcoding studies became more frequent in the field of intertidal research. These studies provided high-resolution community composition data capable of detecting previously overlooked taxa and improving biodiversity characterization in coastal and estuarine ecosystems. The resulting picture of intertidal communities was considerably more complex than trophic models alone suggested, as studies documented instances where high community richness did not correspond to equivalently complex trophic structures.

By 2024, attention had turned to the mechanisms underlying this complexity. Increasing evidence from trophic ecology studies indicates that energy transfer may occur through multiple pathways and resource channels, contributing to spatial and temporal variation in ecosystem functioning. It has been demonstrated that energy in an ecosystem does not move through only one pathway but along multiple, compartmentalized, distinct pathways. The recognition that these pathways can shift independently of species composition introduces a new layer of analytical challenge.

By 2025, studies increasingly emphasized the need to evaluate ecosystem condition using multiple ecological dimensions rather than relying on a single-indicator approach. Studies have increasingly focused on contaminant transfer through food webs and on how environmental stressors influence ecosystem stability and ecological functioning. Food webs must not only transfer energy but also distribute contaminants, such as heavy metals, PFAS, and microplastics. The stage of evidence supports the idea that ecosystem function can undergo radical changes but does not require fundamental transformations in the form of structures, validating the theory of structure-function mismatch. This mismatch arises because eDNA captures compositional diversity at a high taxonomic resolution, whereas stable isotope analysis integrates assimilated energy over time. Therefore, these approaches reflect different ecological dimensions across temporal and functional scales, leading to an apparent decoupling between structure and function.

These patterns suggest an emerging transition toward more integrative ecosystem assessment approaches. Recent studies have emphasized early integration efforts, with several research groups developing approaches that combine eDNA, stable isotopes, and functional indicators within unified study

designs. These initiatives are still in the preliminary stages and lack a standardized theoretical basis; however, they reflect a growing awareness that single-method frameworks cannot fully capture the complexity of disturbed intertidal ecosystems. Published studies are working toward integrated methods for a more comprehensive evaluation of ecosystem health, although this integration remains in its early phases without a solid theoretical foundation. Overall, this phase is part of a broader trend that bridges method- and system-based approaches, aiming to develop a comprehensive scientific framework that connects multiple ecological dimensions for holistic knowledge. The details are shown in Figure 3.

Research trend 2021-2026

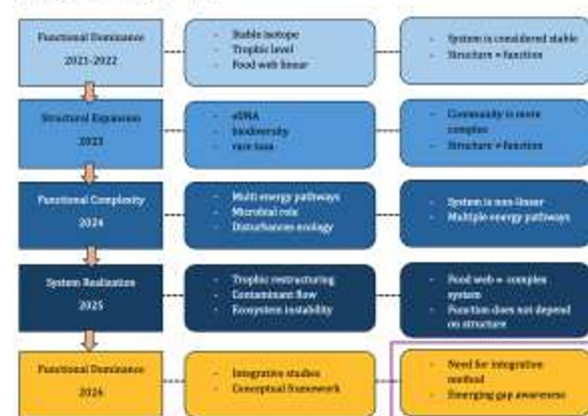


Fig. 3. Research trends 2021-2026.

3.2 Major result

Qualitative synthesis of the reviewed literature revealed three recurring patterns relevant to intertidal ecosystem assessment. These patterns represent interpretive conclusions derived from the collective evidence reported across studies rather than results of a formal meta-analysis. First, the relationship between community structure and ecosystem function is frequently nonlinear and often decoupled under specific environmental conditions. eDNA studies clearly show excellent community complexity, with major contributions of rare taxa and microorganisms. However, these structural complexities do not always result in trophic complexity. Second, stable isotope observations indicate that ecosystem functions, such as energy flow and trophic structure, are stable during severe changes in community composition. This apparent temporal stability, characterized by weak seasonality in trophic structure, reflects a genuine ecological phenomenon rather than a methodological limitation. Third, functional changes occur without detectable structural shifts. These results suggest that ecosystem function is not only a direct function of community structure but also reflects a complex interplay of functional redundancy, organismal plasticity, and environmental dynamics. This is further reinforced by biotic and anthropogenic drivers; invasive species can restructure trophic pathways and benthic-pelagic linkages, altering energy flows independently of

overall community composition. Similarly, legacy contaminants can maintain changing trophic dynamics over time, with biological accumulation reflecting historical inputs rather than the current structure.

In parallel, long-term observations of communities have revealed that species composition can fluctuate over time without necessarily disrupting broader ecosystem organization, highlighting the capacity of ecological systems to maintain functional stability despite compositional turnover. Together, these patterns indicate that ecosystem functioning is governed by multi-layered processes rather than direct structural control [10]. Collectively, these findings challenge the assumption that biodiversity structure can be directly used as a proxy for ecosystem function, highlighting the need for integrative approaches that capture both dimensions simultaneously.

3.3 eDNA-community structure

In eDNA studies, most studies aim to improve the resolving ability of detecting community structures. This facilitates the detection of cryptic taxa, rare taxa, and microbial communities not previously possible by traditional methods. In intertidal habitats, eDNA uncovers community structures that are much more diverse than previously believed, featuring major contributions from microorganisms and meiofauna. eDNA is also used to identify community changes within an ecosystem under anthropogenic stress (i.e., pollution and habitat destruction).

The primary limitation is that it has no direct linear link between structure and function. Because DNA is not indicative of ecological functioning, eDNA-based interpretations are generally descriptive. Uncertainty in data interpretation is associated with DNA transport, environmental degradation, and primer bias. However, it does not include what they do. For example, stable isotope-based studies are the mainstay of ecosystem function analysis in the scientific literature. It provides a good grasp of energy flow, trophic position, and structural information.

3.4 Stable isotopes-pathways

In intertidal systems, stable isotopes demonstrate energy sources from microphytobenthos, phytoplankton, macroalgal detritus, and terrestrial inputs. Microphytobenthos have been identified as the dominant carbon source in the benthic food webs of tropical coastal ecosystems, often surpassing the contribution of pelagic phytoplankton. These energy pathways are not homogeneously linear but are composed of complex and often compartmentalized networks. To perform a comprehensive assessment, it is proposed to combine stable isotope analysis, as the complexity of energy sources within food webs indicates that approaches relying solely on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ risk oversimplifying the relative contributions of various carbon sources, particularly in systems influenced by multiple energy pathways.

Stable isotope records also facilitate the investigation of contaminant biomagnification, showcasing food web-mediated transport of energy and chemical distribution. These limitations are low taxonomic resolution and isotope baseline variability, which make species-based trophic role identification difficult in highly dynamic intertidal ecosystems. Trophic structure and isotope signals are the results of interactions among an organism's trophic flexibility, the complexity of energy sources (benthic versus pelagic), and physical and environmental processes that modulate resource availability.

3.5 Functional indicators

Functional indicator approaches have been developed to connect structure and function. These studies use mechanisms such as functional diversity, trophic redundancy, and isotopic niche to connect community composition with ecosystem function. Overlapping communities do not necessarily imply overlapping functions because partitioning can occur at a finer scale than the resolution of common data. Some scientists suggest that ecosystem function is consistent despite rearrangements of community structures, essentially through functional redundancy. However, this framework is limited by its inferential approach and lack of actual structural and functional data; therefore, it does not achieve a complete bridge between eDNA and stable isotopes.

3.6 Anthropogenic pressure and contaminants

Recent studies have indicated that anthropogenic pressures are significant in the modification of the relation between the structure and function of ecosystems. Pollution, eutrophication, and climate change can alter energy pathways and trophic functions without necessarily affecting community structure directly. However, the presence of contaminants suggests that even food webs act as transporters of chemicals, and agents can interfere with the function of an ecosystem. The results show that ecosystem function is impacted by both biological and complicated environmental processes, which may suggest that structure- and community-level approaches alone are insufficient.

3.7 Multi-layered ecosystem

Energy Literature synthesis suggests that systems of intertidal ecosystems are multi-layered rather than integrated. Sources come from many and are not necessarily directly coupled. In addition, contributions to ecosystem function may not be directly related to species richness, in part because rare taxa and microorganisms play roles as sub-functional layers. Several early attempts have made use of eDNA, stable isotopes, and functional indicators, even though few of these have yet been developed, which shows that this could be a feasible method for an overall understanding of ecosystems. However, such tools present problems in

research design, data integration, and conceptual framework. No standardized approaches show that integration is a project that is still far from being fully established.

3.8 Research-based interpretation

As evident in our survey, the structure-function mismatch is not a contradiction, but rather the mark of an intricate ecological system. eDNA and stable isotopes work on different scales from each other, so they cannot be directly compared. Differences in the scales of the temporal, spatial, and methodological dimensions also complicate this divergence of interpretation. The problem in the literature relevant to this study is not data but conceptual integration.

3.9 Strengths and limitations

A potential limitation of this review concerns whether the low number of integrated studies identified reflects the literature itself or the study selection process. However, because the PEOS framework admitted studies based on ecological outcomes rather than specific methodologies, the inclusion criteria did not inherently favour either eDNA or stable isotope approaches. The observed scarcity of the integrated studies therefore appears to represent a genuine characteristic of the current literature rather than an artifact of the review design. Nevertheless, this interpretation should be considered considering the database, temporal, and eligibility constraints applied during article selection. Additional limitations arise from the inherent characteristics of the assessment approaches reviewed, as each method captures different ecological dimensions and is associated with distinct sources of uncertainty. On a larger scale, eDNA performs well in structural resolution [11], stable isotopes excel in functional insight, functional indicators provide conceptual bridging, and integrative approaches are the direction for future design. This is because no single approach can provide a comprehensive ecosystem picture; thus, the integration of approaches across methods is required.

3.10 Bibliometric analysis

Such a network visualisation analysis indicates the important structure of keywords connected with each other in the literature and directly reflects the conceptual architecture of this research area. Notably, in this network, “stable isotopes” is the biggest node and has the highest connectivity level, suggesting this concept is the primary gravitational centre in the literature. Its extensive links with diet, trophic ecology, nitrogen and biomagnification testify to the fact that stable isotope approaches have historically been a leading tool for understanding ecosystem function, much of it concerned with the functioning of the food webs and associated energy flow. In contrast, clusters related to eDNA metabarcoding, microbiome, and bioindicators show

lower connectivity to the trophic related concepts within the network, indicating that these approaches are developing along partially separate conceptual pathways. This suggests that DNA-based approaches are primarily associated with biodiversity characterization and remain less connected to trophic and functional ecology concepts, which is more concerned with biodiversity characterisation than interfacing with ecosystem functionality. These separate clusters indicate methodological fragmentation in the literature, with structural and functional approaches not evolving within an integrated framework. The data is presented in figure 4:

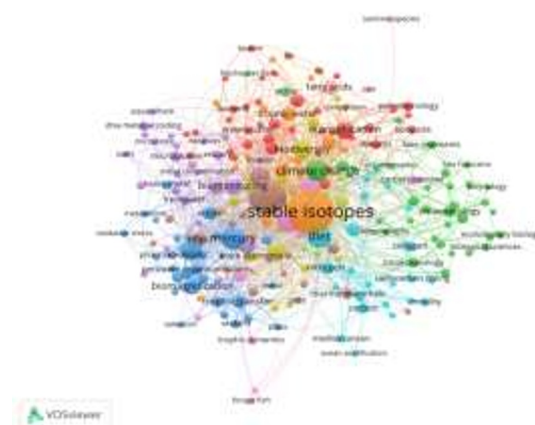


Fig. 4. Network visualization.

That stable isotope cluster correlations correlate with pollution, mercury, and bioaccumulation tells us that this is also a widely employed construct in ecotoxicology work. This explains the importance of stable isotopes in ecosystem function research, but it primarily emphasizes functional aspects of the ecosystem and provides limited direct insight into community structure. The overlay visualisation analysis shows a temporal sequence of the research themes to be presented by colours that are in time with publication. As the visualisation indicates, stable isotopes, trophic ecology, and biomagnification are frequently rendered in an older hue, indicating that these issues have reigned over much of the literature for a longer time. This is consistent with a decades-old trend favouring stable isotope use in trophic ecology. Even new colours for words like 'eDNA', 'metabarcoding', 'microbiome', and 'bioindicators' are emerging.

The increase in these terms marks a paradigm shift towards molecular work within the ecosystem-work context. While eDNA strategies rise in popularity, they show relatively limited association with trophic concepts within the network. The data are presented in Figure 5.

The limited overlap between eDNA and stable isotope clusters suggests that integration between these approaches remains minimal rather than absent within the literature. This overlay visualisation serves not only as an indication of the evolution of research themes but also reveals a temporal lag in the methodological integration between structural and functional approaches. The density visualization shows the distribution of research theme density based on the

frequency and intensity of keyword occurrences. The highest-density areas feature the lines “stable isotopes” and “stable isotope analysis” in the highest colours.

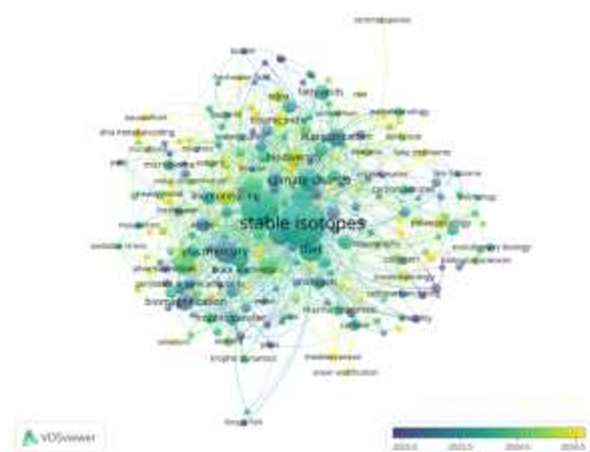


Fig. 5. Overlay visualization.

High density also shows up around trophic function terms like diet, trophic ecology, and nitrogen, suggesting that the dominant study focus is still on ecosystem function analysis. Areas of interest in areas associated with eDNA, metabarcoding, and microbiome have much lower and more dispersed density, which indicates that these methods are still emerging and less widely adopted compared to stable isotope approaches in ecosystem research. There is an intermediate density domain connecting biodiversity, climate change, and eutrophication, which shows that the environmental issues are starting to be embedded in eco-analysis. The data is presented in Figure 6.

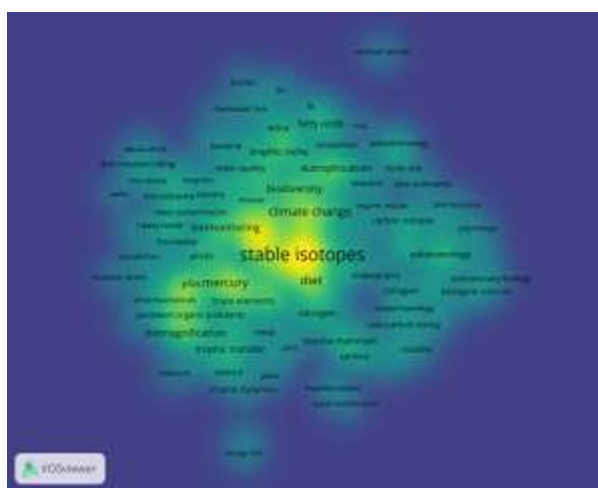


Fig. 6. Density visualization.

Linking this area to eDNA or stable isotope approaches remains weak, though, confirming the existing integration gap in the literature. Taken together, the bibliometric analyses reveal a persistent conceptual separation between biodiversity-oriented and trophic-oriented research themes. While emerging eDNA-related topics are becoming increasingly visible within the literature, their connections with trophic and functional ecology remain comparatively limited. This

pattern supports the need for an integrative framework capable of linking community structure, trophic processes, and ecosystem responses within a unified assessment approach.

4 Discussion

4.1 Revisiting ecological assumptions

The limited integration between structural and functional approaches identified in this review likely reflects fundamental differences in the ecological dimensions measured by each method. eDNA metabarcoding captures patterns of biodiversity and species occurrence, whereas stable isotope analysis integrates assimilated resources and trophic processes over time. Because these approaches operate across different temporal, spatial, and ecological scales, direct correspondence between structural and functional indicators should not necessarily be expected. This perspective suggests that apparent inconsistencies between biodiversity patterns and ecosystem functioning may represent intrinsic properties of complex ecological systems rather than methodological shortcomings.

The consensus results suggest that the link between structure and function is non-linear, context-dependent, and often decoupled. The observed decoupling between community structure and ecosystem function can be explained by several underlying mechanisms. Functional redundancy allows multiple species to perform similar ecological roles, buffering ecosystem processes against compositional changes. Trophic plasticity enables organisms to shift resource use in response to environmental variability, maintaining energy flow stability [12].

Furthermore, external drivers such as disturbance and physical forcing can restructure energy pathways independently of community composition, reinforcing the non-linear nature of ecosystem dynamics. Disturbance factors identified in the literature can be broadly categorized into three groups: (i) chemical stressors, including pollution, heavy metals, and emerging contaminants such as PFAS; (ii) nutrient-driven processes, particularly eutrophication; and (iii) physical alterations, such as hydrological changes and habitat modification. These stressors interact to influence both community structure and trophic function, often producing non-linear and context-dependent ecosystem responses.

In addition, although disturbance is widely represented across studies, it is rarely incorporated as a structured analytical dimension linking community structure and trophic function. Only 1 of 106 studies (~1%) explicitly integrated both eDNA and stable isotope approaches, whereas the majority relied on single-method frameworks. Because the inclusion criteria accepted any valid ecological indicator, not solely eDNA or stable isotopes, this distribution is unlikely to be an artifact of selection bias. Rather, it reveals a structural gap in how disturbance-oriented research conceptualizes ecosystem assessment:

structure and function are investigated in isolation, even when both are acknowledged as relevant.

eDNA-based studies primarily characterize community composition and biodiversity patterns, whereas stable isotope approaches focus on trophic functioning and energy flow. Although both dimensions are widely recognized as important for ecosystem assessment, they are rarely examined within a unified analytical framework. Consequently, disturbance is often treated as contextual information rather than as an explicit factor linking biodiversity patterns with ecosystem processes.

This fragmentation limits the ability of current approaches to evaluate ecosystem responses to anthropogenic stressors and to generate management-relevant indicators. The disconnect between community structure and trophic function identified in this review highlights a broader conceptual gap in ecosystem assessment. Community analyses describe who is present within ecosystems, whereas stable isotope approaches reveal how energy moves through food webs. Consequently, integrating eDNA metabarcoding, stable isotope analysis, and disturbance context provides a more robust basis for ecosystem assessment than reliance on any single category of indicator.

4.2 Integrative ecological framework

The integrative ecological framework developed in this study responds to structural and conceptual fragmentation generated by systematic review and bibliometric analyses. The proposed framework conceptualizes the interactions among community structure, trophic functions, and ecosystem stability in the context of human-induced pressures. It considers eDNA metabarcoding and stable isotope analysis as complementary data sources that collectively offer a comprehensive view of ecosystem dynamics [13]. The data is presented in Figure 7:



Fig. 7. Integrative ecological framework.

By integrating these methods, it becomes possible to discern the relationships between biodiversity and trophic interactions, which facilitates the creation of ecosystem indicators that encompass both structural and functional aspects [14]. Key drivers such as pollution, eutrophication, and changes in land use are recognized as significant influences on these relationships, allowing

for a more nuanced evaluation of how ecosystems respond to environmental stresses.

Operationally, integration is achieved by linking eDNA derived community composition with stable isotope-derived trophic information through functional guild classification. Detected taxa can be assigned to ecological guilds using trait-based informations, while isotope data are used to estimate trophic position, resource utilization, and energy pathways. The combined interpretation of these datasets enables assessment of whether observed changes in biodiversity are accompanied by corresponding changes in ecosystem functioning. Examples of commonly used analytical tools include QIIME2 or DADA2 for eDNA processing and MixSIAR or SIBER for isotope-based trophic analyses. Furthermore, the framework incorporates feedback mechanisms through repeated ecosystem assessments and long-term monitoring programmes [15], which aid in the adaptive refinement and implementation of strategies for dynamic ecosystem management.

Although specific monitoring intervals should be adapted to local ecological conditions and management objectives, the framework is intended to support periodic assessment of both structural and functional ecosystem indicators. Potential proxy metrics include biodiversity and community composition derived from eDNA, trophic position and isotopic niche characteristics derived from stable isotope analysis, and indicators of environmental disturbance such as nutrient enrichment or contaminant exposure. Adaptive management responses may be triggered when persistent divergence is observed between structural and functional indicators or when substantial shifts in trophic organization and biodiversity patterns are detected over time.

By bridging previously isolated approaches, this framework establishes a coherent foundation for comprehending the connections among biodiversity, trophic dynamics, and environmental change.

4.3 Guidance for Future Empirical Application

The proposed framework can be operationalized through several complementary analytical pathways in future empirical studies. The data is presented in Figure 8. First, structural indicators derived from eDNA metabarcoding provide high-resolution information on community composition and biodiversity patterns. Second, functional indicators based on stable isotopes quantify trophic structure, energy pathways, and isotopic niche metrics. Third, environmental context, including disturbance gradients such as pollution and eutrophication, is incorporated as a modulating factor. The integration of these components enables the derivation of composite ecosystem indicators, including trophic stability, energy flow complexity, and ecosystem resilience. This operational structure allows the framework to move beyond conceptual synthesis toward practical application in ecosystem assessment.

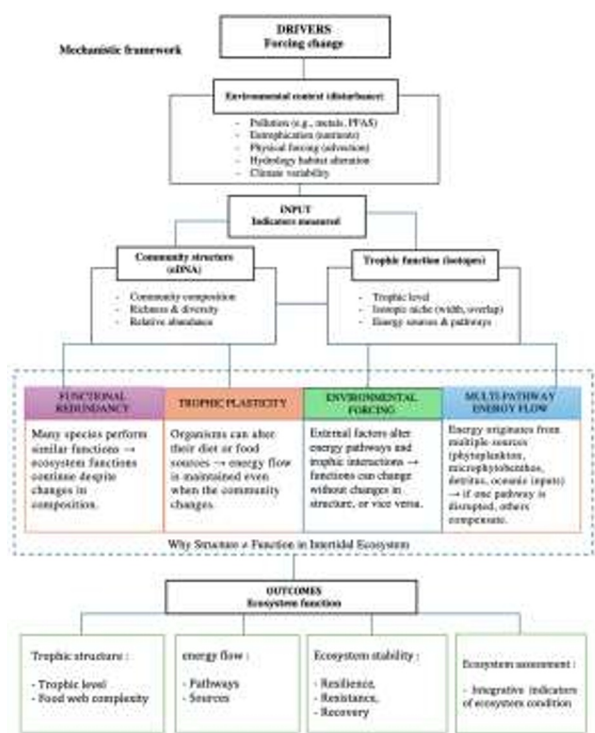


Fig. 8. Mechanistic framework.

The framework is structured as a process-based system, initiating with structural and functional data obtained from eDNA and stable isotope analyses, which are influenced by environmental factors like disturbance gradients, etc. These data undergo processing through essential ecological mechanisms such as functional redundancy, trophic plasticity, and various energy pathways, leading to the development of emergent ecosystem functions including energy flow and stability. Crucially, feedback loops demonstrate that the outcomes within the ecosystem can subsequently alter community structure, emphasizing the system's dynamic characteristics.

4.4 Potential Operationalization Pathways

Species with known ecologies may introduce errors at the foundation of the analytical chain, and these errors can propagate through all subsequent calculations. Stable isotope analysis provides an independent line of evidence. Values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ integrate assimilated diet over periods determined by tissue turnover rates, typically weeks to months, and thus capture ecological processes operating on timescales quite different from the instantaneous or near-instantaneous snapshot provided by eDNA. In intertidal environments, additional complications arise. Isotopic baselines can shift seasonally, with freshwater input, and across small spatial gradients. eDNA is subject to transport by tides and currents, meaning the location of detection may not correspond to the location of ecological activity. These temporal and spatial mismatches between the two data streams are not methodological flaws; they are inherent properties of working in dynamic coastal systems.

Integration is achieved by mapping functional groups identified through eDNA onto trophic positions estimated from isotopic data. Where taxa have well-

characterized trophic ecologies, this mapping is relatively secure. Where ecological knowledge is incomplete, as is often the case for the rare and cryptic taxa that eDNA is particularly effective at detecting, assumptions must be substituted for empirical evidence. A pragmatic response to this uncertainty is to conduct analyses at the resolution of trophic guilds rather than individual taxa. Guild-level resolution sacrifices some taxonomic specificity but gains reliability in functional interpretation. In most intertidal systems, distinguishing among deposit feeders, suspension feeders, herbivores, and predators provides sufficient functional resolution to characterize major energy pathways while minimizing misclassification risk.

Bayesian mixing models (e.g., MixSIAR and related approaches) can then be applied to estimate the proportional contributions of distinct basal resources to each consumer guild. Microphytobenthos frequently dominates benthic energy channels in tropical and subtropical intertidal systems; phytoplankton may support a separate pelagic pathway; macroalgal detritus and terrestrial inputs contribute additional dimensions in specific settings. The critical analytical comparison occurs when these mixing model outputs are examined alongside eDNA-based community composition data. Convergence between structural and functional signals strengthens confidence in the interpretation. Divergence is analytically informative in a different way.

Several mechanisms can produce divergence. Functional redundancy, multiple taxa performing similar ecological roles, can maintain trophic structure despite compositional turnover. Trophic plasticity organisms shifting resource use in response to changing conditions, can stabilize energy flow pathways while community composition fluctuates. Disturbance can also act directly on energy channels: eutrophication may shift basal resource reliance from benthic to pelagic sources without immediately altering species assemblages, or pollution may eliminate sensitive taxa while resistant species expand their trophic niches to compensate. The framework does not automatically distinguish among these mechanisms. What it provides is an empirical basis for detecting when structure and function are coupled or decoupled, and for asking what ecological processes might account for the observed pattern.

Several limitations constrain the framework's application. The accuracy of functional assignments depends on the quality and regional coverage of trait databases, which remain uneven. Isotopic baseline variation introduces uncertainty into trophic position estimates that mixing models address only partially. eDNA detection probability varies with environmental conditions and primer bias, and transport processes can decouple detection from ecological function. Integrating two data streams, each with its own uncertainties, can compound rather than cancel errors. The framework does not resolve these limitations. Its contribution is to make them analytically visible, so that interpretations can be calibrated against what is known and what is assumed at each step.

The broader significance of the framework lies in how it restructures the logic of ecosystem assessment.

Conventional approaches tend to treat agreement between structural and functional indicators as the expected norm, and disagreement as evidence of a problem requiring explanation. The present framework adopts a different stance. It treats the relationship between structure and function as an empirical variable rather than a theoretical constant. When both dimensions align, the case for a particular interpretation is strengthened. When they diverge, the framework directs attention toward the ecological mechanisms of redundancy, plasticity, compartmentalization, and disturbance-driven energy rerouting that may be responsible. In intertidal ecosystems, where multiple stressors interact, and energy pathways are rarely simple, this process-oriented approach may provide a more realistic foundation for assessment and management than reliance on any single category of indicator.

5 Future Research Direction

Future studies should focus on incorporating eDNA metabarcoding and stable isotope-based analyses in a consolidated study design. Integrating disturbance gradients into the basis of research will enhance the ecological validity of ecosystem assessment. There is a need for integrated ecosystem indicators that characteristically reflect biodiversity, trophic structure, and ecosystem stability.

Standardization of methodologies and interdisciplinary modelling across spatial and temporal scales will further advance our understanding of ecosystem responses to environmental change.

6 Conclusion

This study demonstrates that the apparent mismatch between community structure and ecosystem function is not a limitation of current methodologies, but a fundamental property of complex ecological systems. By integrating eDNA metabarcoding, stable isotope analysis, and disturbance context.

From a practical perspective, the proposed framework may support ecosystem monitoring and management by integrating biodiversity assessment with trophic and functional indicators. Future research should prioritize empirical validation of the framework across different intertidal ecosystems and disturbance gradients to evaluate its robustness and broader applicability.

In addition, the development of standardized indicators that combine eDNA metabarcoding and stable isotope analysis may strengthen long-term ecosystem assessment and adaptive management strategies. The proposed framework provides a conceptual pathway from descriptive biodiversity assessment toward a process-based understanding of trophic dynamics, ecosystem functioning, and ecological responses to environmental disturbance.

References

1. D. Siegert, B. Konar, M. R. Lindeberg, S. Saupe, K. Iken, Trophic structure of key taxa in rocky intertidal communities in two contrasting high-latitude environments. *Deep Sea Research Part II. Topical Studies in Oceanography*. **198**, 105050 (2022). <https://doi.org/10.1016/j.dsr2.2022.105050>
2. Z. Ke, R. Li, D. Chen, Y. Tan, Spatial distributions of zooplankton stable isotopes and their trophic positions in a bay under serious anthropogenic influences: Daya Bay, China. *Ecol Indic*. **139**, 108950 (2022). <https://doi.org/10.1016/j.ecolind.2022.108950>
3. I. Perez-Posada, N. Cabanillas-Teran, L. F. Carrera-Parra, D. J. Lizcano, A. Sanchez, Trophic ecology of Caribbean polychaetes: responses to environmental changes driven by massive *Sargassum* arrivals. *Food Webs*. **44**, e00411 (2025). <https://doi.org/10.1016/j.fooweb.2025.e00411>
4. S. Fernandez, S. Aclé, E. Dopico, P. Masia, D. Menendez, J. Rick, A. Ardura, E. Garcia-Vazquez, Anthropogenic stressors that favour nuisance species. A study from environmental DNA in marine plankton samples. *Science of The Total Environment*. **973**, 179194 (2025). <https://doi.org/10.1016/j.scitotenv.2025.179194>
5. Y. Kang, I.S. Kwak, C. K. Kang, Co-occurrence of Bacillariophyceae-based- and Cryptophyceae-based planktonic food webs in a temperate estuarine ecosystem revealed via eDNA. *Estuar Coast Shelf Sci*. **264**, 107661 (2022). <https://doi.org/10.1016/j.ecss.2021.107661>
6. M. E. M. Walton, R. Browne, J. N. Griffiths, D. Cartwright, P. Robins, S. K. Malham, L. Le Vay, Kelp detritus: unutilized productivity or an unacknowledged trophic resource?. *Science of The Total Environment*. **820**, 153191 (2022). <https://doi.org/10.1016/j.scitotenv.2022.153191>
7. S. Xu, Y. Zhou, P. Wang, F. Wang, X. Zhang, S. Yue, Y. Zhang, Y. Qiao, M. Liu, Temporal-spatial variations in the elemental and stable isotope contents of eelgrass (*Zostera marina* L.) in the Bohai Sea and Yellow Sea, northern China: sheath as a novel ecological indicator for geochemical research. *Ecol Indic*. **121**, 107181 (2021). <https://doi.org/10.1016/j.ecolind.2020.107181>
8. N. Keeley, K. Dunlop, O. Laroche, P. K. Hansen, I. L. Angell, K. Rudi, An approach for quantifying the influence of fish farm waste on hard-bottom habitats. *Mar Pollut Bull*. **217**, 118039 (2025). <https://doi.org/10.1016/j.marpolbul.2025.118039>
9. D. Moher, A. Liberati, J. Tetzlaff, D. G. Altman, Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *International Journal of Surgery*. **8(5)**, 336–41 (2010). <https://doi.org/10.1016/j.ijsu.2010.02.007>
10. H. Xie, Y. Liu, Y. Xiao, C. Li, X. Ao, J. Sun, J. Shen, Y. Wang, Y. Liu, T. Wang, Species composition and long-term dynamics of coral reef fishes at Meiji Reef, Nansha Islands. *Reg Stud Mar Sci*. **91**, 104497 (2025). <https://doi.org/10.1016/j.rsma.2025.104497>

11. S. E. Janssen, J. C. Hoffman, R. F. Lepak, D. P. Krabbenhoft, D. Walters, C. A. Eagles-Smith, G. Peterson, J. M. Ogorek, J. F. DeWild, A. Cotter, M. Pearson, M. T. Tate, R. B. Yearley, M. A. Mills, Examining historical mercury sources in the Saint Louis River estuary: how legacy contamination influences biological mercury levels in Great Lakes coastal regions. *Science of The Total Environment*. **779**, 146284 (2021).
<https://doi.org/10.1016/j.scitotenv.2021.146284>
12. C. M Marie-Anne Le Guen, A. Raoux, S. Tecchio, N. Chauvel, T. Merzi, E. Bellemain, A. Bonin, J. P. Pezy, Environmental DNA as a method to reconstruct food webs and assess ecosystem health. *Ecol Indic*. **173**, 113399 (2025).
<https://doi.org/10.1016/j.ecolind.2025.113399>
13. G. L. Lescord, Simard J, Johnston TA, Seguin J, Farrell CE, O'Driscoll NJ, O'Connor CM, The long-term effects of water resource development on blood mercury levels and the trophic ecology of Namew (lake sturgeon, *Acipenser fulvescens*). *FACETS*. **9(1)**, 1–13 (2024).
<https://doi.org/10.1139/facets-2023-0151>
14. V. Marcuk, A. Pina-Ortiz, J. A. Castillo-Guerrero, J. F. Masello, P. Bustamante, S. Griep, P. Quillfeldt, Trophic plasticity of a tropical seabird revealed through DNA metabarcoding and stable isotope analyses. *Mar Environ Res*. **199**, 106627 (2024).
<https://doi.org/10.1016/j.marenvres.2024.106627>
15. D. Jones, M. Ovegard, H. Dahlgren, S. Danielsson, M. Greger, T. Landberg, A. Garbaras, M. L. Karlson, A multi-isotope approach to evaluate the potential of great cormorant eggs for contaminant monitoring. *Ecol Indic*. **136**, 108649 (2022).
<https://doi.org/10.1016/j.ecolind.2022.108649>