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From Molecules To Ecosystems: A Review On Environmental DNA And Metabarcoding In Biodiversity Science

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Abstract

Traditional biodiversity monitoring methods often struggle to detect elusive, rare, or cryptic species due to their reliance on direct observation and specimen collection. In an era of accelerating habitat loss and climate change, there is an urgent need for rapid, reliable, and non-invasive tools to assess biodiversity across ecosystems.This review aims to synthesize the emerging field of environmental DNA (eDNA) and metabarcoding, focusing on their principles, methodologies, and transformative applications in ecological monitoring, species detection, and conservation management.We summarize global research developments on eDNA collection from environmental matrices such as soil, water, and air, explain the integration of PCR-based metabarcoding with high-throughput sequencing, and analyze bioinformatics pipelines used for taxonomic identification and community composition assessment. Additionally, we highlight advances in combining eDNA with remote sensing, machine learning, and conservation genomics.The synthesis demonstrates that eDNA metabarcoding provides a cost-effective, scalable, and highly sensitive framework for biodiversity assessment, enabling detection of rare, invasive, and cryptic species. Its integration with ecological and bioinformatics tools bridges molecular data with environmental management, offering a transformative frontier for global biodiversity monitoring and conservation planning under changing climatic and anthropogenic pressures.

Key Words: e-DNA, Metabarcoding, Remote sensing, Environment,Community, Species

Introduction

Biodiversity underpins the stability and functionality of ecosystems, yet accelerating species loss and habitat fragmentation challenge our capacity to document and conserve life on Earth. Traditional biodiversity assessments, often reliant on direct observation, trapping, or morphological identification, are labor-intensive, invasive, and limited by species detectability, especially for cryptic, rare, or microscopic organisms.In this context, environmental DNA (eDNA)the genetic material released by organisms into their environment through feces, mucous, gametes, sloughed cells, or decomposition has revolutionized ecological monitoring. The concurrent advancement of metabarcoding, which couples eDNA with high-throughput sequencing (HTS), allows simultaneous identification of multiple taxa from complex environmental samples.Together, eDNA and metabarcoding have become central to molecular ecology, bridging genomics and field-based ecology for a new era of rapid, accurate, and scalable biodiversity assessment.

Concept and Principle of Environmental DNA

Environmental DNA (eDNA) refers to the extracellular or intracellular genetic material that organisms release into their surrounding environment through processes such as excretion, secretion, reproduction, and decomposition. These DNA fragments are found in various environmental matrices, including water, soil, sediment, snow, and even air. Once released, eDNA can persist in the environment for varying durations from a few minutes to several weeks depending on physicochemical and biological factors such as pH, temperature, ultraviolet (UV) radiation, microbial activity, and oxygen availability. The persistence and detectability of eDNA are therefore influenced by the interaction of these factors, which determine degradation rates and the potential for recovery in different ecosystems.The process of eDNA analysis follows a well-defined workflow that begins with the careful collection of environmental samples such as water, sediment, soil, or air. Following collection, the total DNA present in the sample comprising both target and background genetic material is extracted using standardized molecular protocols optimized for low-concentration DNA. The extracted DNA is then subjected to polymerase chain reaction (PCR) amplification using either universal primers that target broad taxonomic groups or specific primers designed for a particular species or lineage. Commonly used genetic markers include mitochondrial cytochrome oxidase I (COI) for animals, ribulose-bisphosphate carboxylase (rbcL) and maturase K (matK) for plants, and the internal transcribed spacer (ITS) region for fungi.

After amplification, the PCR products are sequenced using high-throughput sequencing (HTS) platforms such as Illumina, Oxford Nanopore, or PacBio. These next-generation sequencing technologies enable the generation of millions of sequence reads, allowing for the simultaneous detection of multiple species within a single environmental sample. The raw sequence data are then processed through bioinformatics pipelines that include quality filtering, sequence clustering into Operational Taxonomic Units (OTUs) or Amplicon Sequence Variants (ASVs), and taxonomic assignment by comparison with curated reference databases such as GenBank, Barcode of Life Data Systems (BOLD), SILVA, or UNITE.

Through this integrated molecular approach, eDNA analysis facilitates the identification of species even from minute and degraded genetic traces providing a comprehensive and non-invasive genetic fingerprint of the local biota. This method overcomes many limitations of traditional field surveys, offering an efficient, scalable, and highly sensitive tool for detecting biodiversity in both aquatic and terrestrial ecosystems. Ultimately, eDNA metabarcoding serves as a bridge between molecular biology and ecology, enabling researchers to reconstruct community composition and monitor ecosystem changes with unprecedented accuracy and resolution.

Table 1. eDNA vs. Traditional Biodiversity Monitoring.

Feature	Traditional Methods	eDNA-Based Methods
Detection approach	Visual/physical capture	DNA from environment
Invasiveness	Often invasive	Non-invasive
Taxonomic bias	Skilled taxa-specific identification needed	Broad, multi-taxon
Cryptic/rare taxa detection	Often missed	Detectable
Cost/time	High & time-intensive	Rapid and scalable
Environmental sensitivity	Limited detection in complex habitats	Sensitive to fine-scale changes
Quantitative accuracy	Abundance data limited	Relative abundance (semi-quantitative)

Thus, eDNA enhances monitoring resolution, detects elusive or early-invasive species, and enables near-real-time biodiversity assessment.

Metabarcoding: The Molecular Backbone of eDNA

Metabarcoding represents the fusion of environmental DNA (eDNA) analysis with next-generation sequencing (NGS) technologies, forming a powerful molecular framework for biodiversity assessment. This method relies on the amplification of specific, conserved DNA regions across taxa using universal primers such as mitochondrial COI, 12S, and 16S for animals; rbcL and matK for plants and ITS for fungi. High-throughput sequencing technologies then generate millions of sequence reads, enabling the simultaneous detection of hundreds of species from a single environmental sample. The resulting data are processed through bioinformatics pipelines such as QIIME2, OBITools, Mothur, or USEARCH, which classify reads into operational taxonomic units (OTUs) or amplicon sequence variants (ASVs). These are then matched against curated reference databases to achieve accurate species identification. Through this process, metabarcoding offers detailed community-level biodiversity snapshots, capturing taxonomic richness, composition, and relative abundance across spatial and temporal gradients.

Applications of eDNA and Metabarcoding

The applications of eDNA and metabarcoding are diverse, spanning species detection, ecosystem monitoring, and conservation planning. In species detection and biomonitoring, eDNA has proven particularly effective in identifying endangered, cryptic, or invasive species that may otherwise go unnoticed through traditional survey methods. It enables early detection of invasive alien species, providing critical time for management interventions before their establishment. Furthermore, eDNA offers molecular presence absence data that are independent of seasonal constraints or observer bias, greatly improving the accuracy of biodiversity assessments.

In community and ecosystem assessments, metabarcoding reveals patterns of species composition, turnover, and functional diversity across various ecosystems whether aquatic, terrestrial, or marine. This makes it a valuable tool in environmental impact assessments (EIA), where it can be used to compare biodiversity before and after disturbances. Beyond species-level identification, eDNA data help elucidate ecological processes and interactions within communities, contributing to a deeper understanding of ecosystem structure and resilience.Conservation genomics has also benefited immensely from eDNA-based approaches. By tracking genetic diversity and population connectivity in fragmented landscapes, researchers can monitor restoration success and recolonization dynamics in degraded habitats. These insights are vital for formulating evidence-based conservation strategies and ensuring the long-term persistence of vulnerable species. Similarly, in paleoecology and historical reconstruction, ancient DNA preserved in sediments and ice cores provides a window into past ecosystems, revealing ecological baselines prior to anthropogenic influences and helping to contextualize current biodiversity patterns.

A particularly novel application lies in airborne eDNA (aero-genomics), which captures plant and animal genetic material from air particles. This emerging technique has the potential to revolutionize terrestrial biodiversity monitoring by enabling the detection of diverse organisms from pollen to insects and vertebrateswithout direct physical sampling.

Advances and Integrations

Recent advances in eDNA research are expanding its capabilities through interdisciplinary integration. The combination of remote sensing with eDNA enables spatially explicit biodiversity mapping by correlating spectral data with molecular signals. Similarly, the use of artificial intelligence (AI) and machine learning has enhanced species prediction and automated data interpretation. Citizen science initiatives are also gaining traction, as eDNA sampling kits empower non-specialists to contribute to biodiversity monitoring, fostering public participation in conservation science. Moreover, the emerging field of functional metagenomics is shifting focus from merely identifying who is there to exploring what they do, linking gene functions with ecological roles and ecosystem processes. The use of environmental RNA (eRNA) is another promising frontier, offering the ability to detect metabolically active species and distinguish living organisms from relic DNA, thereby improving the accuracy of biodiversity assessments.

Challenges and Limitations

Despite its transformative potential, eDNA metabarcoding faces several challenges that must be addressed to ensure reliable and reproducible results. DNA degradation, influenced by factors such as ultraviolet radiation, pH, temperature, and microbial activity, can limit detection accuracy. Contamination remains a persistent concern, necessitating rigorous field and laboratory protocols to avoid false positives. Additionally, the quantitative interpretation of eDNA data remains imperfect, as the relationship between DNA concentration and organism abundance is not always linear. The incompleteness of reference databases, particularly for tropical and understudied taxa, further constrains species identification. Moreover, the absence of standardized bioinformatics pipelines and thresholds complicates data comparison across studies. Ecological interpretation also poses difficulties, as the presence of eDNA does not necessarily imply an organism's ecological relevance or viability. Ongoing research efforts aim to refine sampling methodologies, develop universal primers, and expand comprehensive genetic reference libraries, such as the Barcode of Life Data System (BOLD), to overcome these obstacles.

Future Prospects and Global Trends

The future of eDNA and metabarcoding is increasingly global and interdisciplinary. Large-scale initiatives such as the Earth BioGenome Project, BIOSCAN, and OceanDNA are working toward integrating eDNA data into global biodiversity databases, fostering a unified framework for monitoring life on Earth. The incorporation of environmental genomics into international and national policy frameworks such as the EU Biodiversity Strategy, the Convention on Biological Diversity (CBD), and other conservation programs demonstrates its growing acceptance as a mainstream monitoring tool. In the Indian context, as one of the world’s megadiverse nations, eDNA-based approaches hold exceptional promise for biodiversity mapping in regions like the Western and Eastern Ghats and the Himalayan wetlands, where traditional surveys face logistical challenges. Looking forward, coupling eDNA with artificial intelligence models can enhance ecosystem forecasting, predicting biodiversity responses to climate change and land-use alterations. Furthermore, the integration of eDNA in one health frameworks highlights its potential for pathogen surveillance, enabling the detection of zoonotic agents and environmental microbiomes linked to human health.

Overall, eDNA metabarcoding stands at the forefront of a new era in biodiversity science, offering an unparalleled molecular lens to observe, monitor, and protect the living world.

Conclusion

Environmental DNA (eDNA) and metabarcoding have transformed biodiversity research by providing rapid, non-invasive, and highly sensitive methods for detecting and monitoring species across diverse ecosystems. By combining universal genetic markers with high-throughput sequencing, metabarcoding allows researchers to capture comprehensive snapshots of community composition and ecosystem dynamics, often revealing species that remain undetected through traditional methods. Integrations with remote sensing, artificial intelligence, and citizen science are expanding the scope and accessibility of eDNA research, while advances such as environmental RNA and functional metagenomics promise deeper insights into ecosystem function and organismal activity.Despite challenges such as DNA degradation, contamination risks, incomplete reference databases, and limited quantification accuracy, ongoing methodological refinements and the growth of global genetic databases continue to strengthen the reliability of eDNA-based approaches. As international initiatives like the Earth Bio Genome Project and OceanDNA align molecular tools with conservation policy, eDNA metabarcoding is emerging as a central pillar of twenty-first-century biodiversity science. For a megadiverse country like India, adopting eDNA technologies can revolutionize species discovery, conservation monitoring, and ecosystem management, bridging data gaps and supporting global sustainability goals.In essence, eDNA metabarcoding represents the molecular future of biodiversity monitoring uniting ecology, genomics, and technology to safeguard the planet’s biological heritage.

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