

Atmospheric Environmental DNA: Integrating Biodiversity Monitoring with Global Ecological Forecasting

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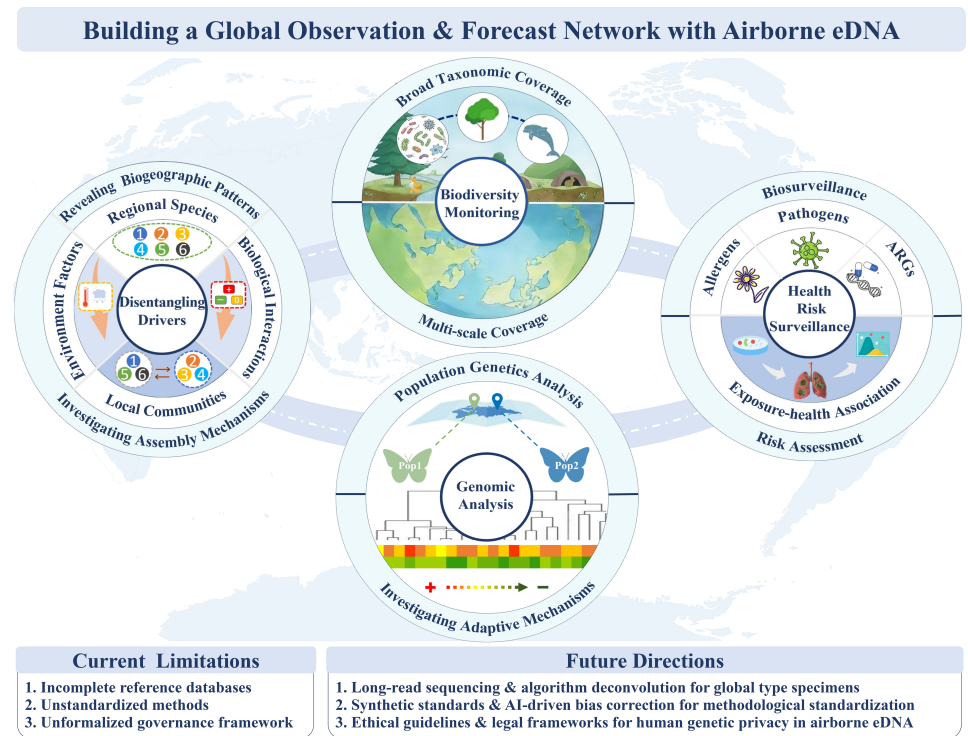
Keywords

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Highlights

- Assessing airborne eDNA capabilities from taxonomy to public health.
- Identifying methodological biases and ecological mechanistic gaps.
- Proposing standardized frameworks for global ecological forecasting.

Abstract: Airborne environmental DNA (eDNA) analysis is emerging as a transformative tool for planetary-scale biodiversity monitoring. This approach enables the capture of genetic material from entire biological communities, spanning viruses to vertebrates within a single air sample. While this technology offers unprecedented potential for conducting non-invasive biodiversity surveys, pathogen surveillance, and population genetic studies, the field remains dominated by descriptive observational studies. Furthermore, it faces significant hurdles, including a lack of mechanistic understanding, methodological biases, and unresolved ethical concerns related to human genetic bycatch. In this perspective, we present the current research landscape of airborne eDNA, highlighting key advancements and persistent limitations. We propose a roadmap for future development aimed at building a predictive framework for global ecological forecasting.



1. Introduction: The Promise of Airborne eDNA

The atmosphere serves as both a medium for microbial dispersal and a dynamic ecosystem in which diverse genetic materials are continuously transported and mixed [1]. This property makes the air a natural reservoir of environmental DNA (eDNA). By capturing suspended genetic signals and retrieving airborne DNA fragments, researchers can conduct comprehensive, non-invasive surveys of entire ecosystems [2–4]. For instance, large-scale aerobiological studies using amplicon sequencing have shown that airborne fungal communities display clear spatial and temporal patterns, varying globally in species richness and composition [5]. However, amplicon-based sequencing focuses on specific taxonomic groups and provides limited coverage of biodiversity. Shotgun metagenomic sequencing of airborne eDNA

overcomes this constraint by recovering genetic information across the tree of life in a single analysis, moving beyond targeted, taxon-specific assays toward an unbiased and “whole-biome” approach [6]. Despite these advances, the field remains largely descriptive [4,7,8], with fragmentary understanding of the ecological processes shaping the observed patterns, including dispersal limitation [9], environmental filtering [3], and species interactions [10]. Most current studies rely on correlative models, with insufficient experimental evidence for causal inference [11]. This limits the development of mechanistic frameworks for predicting biodiversity responses to environmental change using airborne eDNA. To overcome these challenges, we propose a standardized technical framework for airborne eDNA research (Figure 1), aiming to support a transition from descriptive surveys toward mechanistic inference.

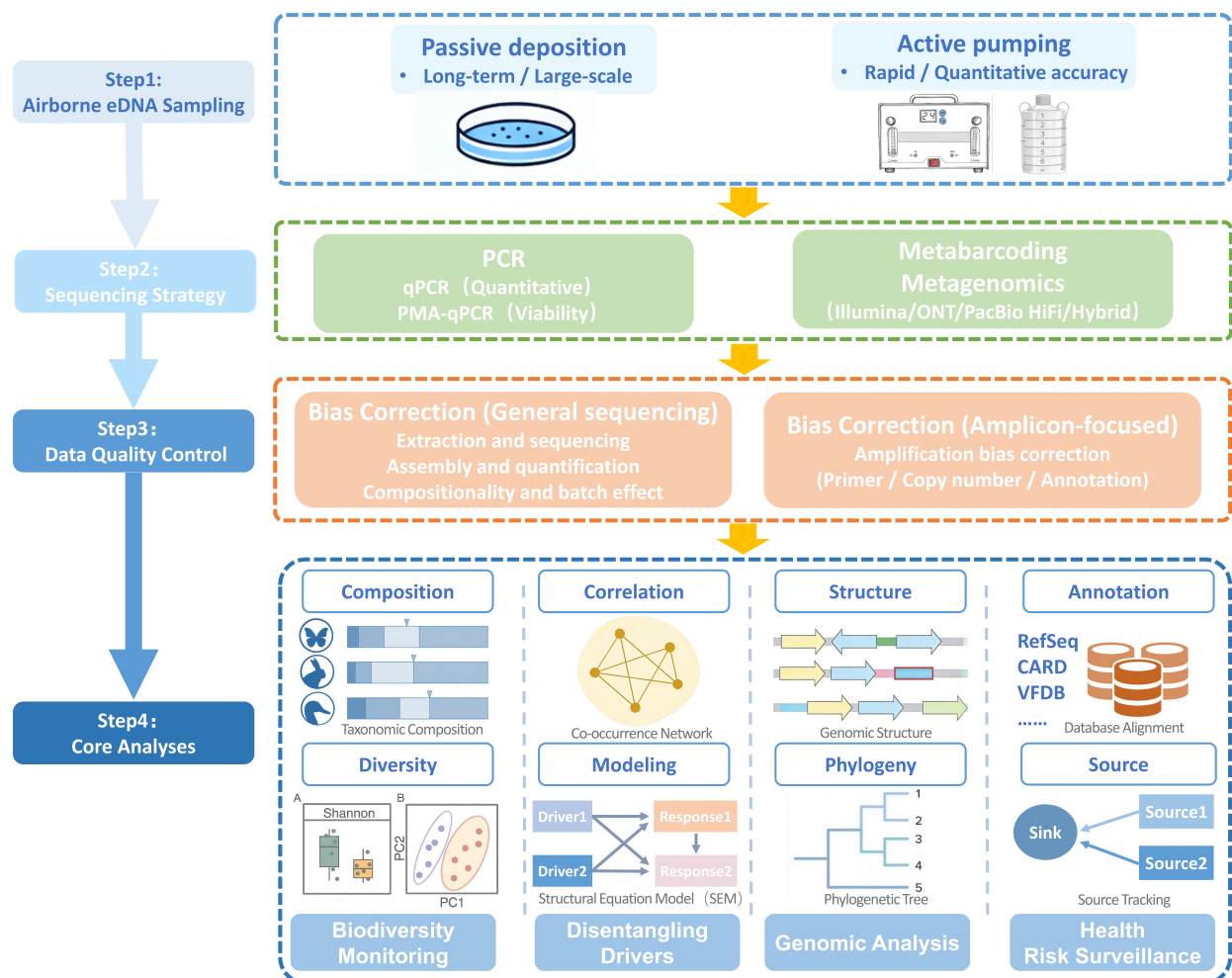


Figure 1. Overview of a standardized technical workflow for airborne eDNA studies. Correlation shows a co-occurrence network (yellow nodes: taxa/variables; edges: correlations). Structure depicts gene clusters (arrows: transcription directions; red frame: SNPs/variants). Annotation profiles potential biological risks by aligning sequences against NCBI Reference Sequence database (RefSeq), the Comprehensive Antibiotic Resistance Database (CARD) for resistome profiling, and the Virulence Factor Database (VFDB).

2. Technical Capabilities: Breadth versus Depth

Airborne eDNA analysis has been broadly used for its extensive taxonomic coverage. DNA of viruses, bacteria,

fungi, plants, invertebrates and vertebrates can be detected from a single air sample using shotgun sequencing which, unlike polymerase chain reaction

(PCR)-based approaches, does not require specific primers and therefore reduces primer bias and enables the detection of unexpected or poorly characterized taxa. It enabled the reconstruction of the 30-year temporal dynamics of biodiversity, spanning microbes to mammals in forest ecosystems [6]. Additionally, shotgun sequencing on 2,700 vertebrate genera recovered abundance indices consistent with those obtained from traditional monitoring methods [12]. The results of Global Spore Sampling Project (GSSP) indicate that airborne eDNA can detect fungal functional groups, including lichenized and ectomycorrhizal fungi, from different climatic zones, indicating its strength in assessing microbial functional profiles across multiple scales [5].

However, the airborne eDNA analysis has limitations in taxonomic and ecological resolution. One of them is the lack of complete reference databases, making it difficult to identify operational taxonomic units (OTUs) beyond the genus level, especially for insects, fungi, and micro-eukaryotes [13,14]. Research conducted in Switzerland revealed that approximately 30% of internal transcribed spacer (ITS) sequences were assigned to incorrect taxon names using Basic Local Alignment Search Tool (BLAST) against the GenBank database [15]. This result confirms that a large proportion of sequences cannot be assignable at the species level. Furthermore, PCR amplification and sequencing often give higher weight to abundant taxa and overlook rare or endangered species, limiting their application in detecting invasive species at the early stages [15,16]. For instance, dominant taxa are usually recovered across PCR replicates, while low-abundance taxa are variable, with 21–29% detected in only one of 24 replicates [17]. Strategies to reduce these biases include application of multiple primer sets, replicated PCRs, and deep learning [18–20].

3. Applications in Health and Disease Surveillance

Airborne eDNA can also be applied in public health. For example, shotgun sequencing has successfully detected DNA from human, animal, and plant pathogens in air samples, including *Alternaria alternata*, vaccinia virus, and antimicrobial resistance (AMR) genes [6]. The airborne fungal communities exhibit allergenic potential. In addition, spores from clinically relevant fungal taxa may increase under certain meteorological conditions [21]. Based on long-term air monitoring across multiple communities, researchers recovered full syndrome coronavirus 2 (SARS-CoV-2) genomes and identified variant lineages [22]. These findings demonstrate that airborne eDNA is an efficient tool for “One Health” surveillance, enabling simultaneous pathogen detection, epidemiological analysis, and viral tracing.

The limitation of airborne eDNA analysis in public health is the difficulty in distinguishing viable, infectious pathogens from non-viable genetic debris. Standard methods such as reverse transcription quantitative PCR

(RT-qPCR) detects genetic material but does not show whether the particles are infectious [23]. Similarly, viral RNA can persist beyond the period of infectivity, emphasizing that nucleic acid detection alone does not indicate viable virus [24]. To translate eDNA signals into meaningful estimates of infection risk, integration with quantitative microbial risk assessment (QMRA) frameworks is essential. Propidium monoazide (PMA)- or propidium monoazide derivative (PMAxx)-based PCR approaches can selectively detect DNA from intact, potentially infectious particles because these dyes penetrate only damaged cells or virions and covalently bind to their DNA, preventing its amplification [25]. By excluding DNA from non-viable particles, these methods provide more accurate inputs for dose-response models that relate airborne eDNA concentrations to exposure and infection risk. Therefore, integrating improved molecular detection with epidemiological data is critical for robust and quantitative assessment of pathogen transmission risk.

4. Advances and Challenges in Population Genomics

Remarkably, airborne eDNA is now sufficiently robust for population-level genetic analysis [26]. Air samples from urban environments showed substantially higher human DNA load, mitochondrial DNA (mtDNA) abundance, and haplotype diversity than rural samples (84–87 vs. 8 haplotypes), demonstrating that airborne eDNA can reflect human population genetic diversity and structure across environments [6]. Similarly, researchers successfully recovered mtDNA from bobcats (*Lynx rufus*) and golden-silk spiders (*Trichonephila clavipes*) through one week of non-targeted short-read sequencing of air samples collected from Florida forest ecosystems. Phylogenetic analyses matched bobcat sequences to regional wild and captive populations, and spider sequences to North American isolates, confirming that airborne eDNA alone can support population-level analyses, including phylogeography and population assignment [6].

However, the resolution of airborne eDNA for population genomics remains limited. Most studies currently rely on mtDNA, which is easily detectable from environmental samples but provides limited genetic information, reflecting only maternal lineages and suitable primarily for coarse-scale population distribution and regional phylogeographic analyses [27–29]. In contrast, nuclear genomes offer more comprehensive genetic data, enabling estimates of effective population size, inbreeding, and fine-scale population structure. Nevertheless, it is hard to reconstruct them reliably from environmental samples [30,31]. In addition, DNA from environmental samples usually comes from many individuals. This creates difficulties in assigning sequences to single organisms and limits kinship or parentage analyses [32]. The advances in target enrichment, long-read sequencing, and bioinformatic

deconvolution methods are required to achieve finer-scale population genomic insights [33–35].

5. Methodological Convergence and Standardization

Results from different sequencing technologies, including long-read and short-read sequencing, show strong agreement in species inventories from the same samples, providing a technical basis for methodological standardization [36]. For example, when the two sequencing methods were compared using six airborne eDNA samples, abundance estimates were strongly correlated for 45 species with direct genome alignments (log-log regression $R^2 = 0.64$) [6]. This agreement is largely due to the consistent detection of dominant taxa with sufficient DNA in the sample. However, the differences among different sequencing platforms mainly stem from variation in read length, error profiles, and assembly efficiency [37]. Shotgun metagenomic data can be used to analyze functional genes, metabolic pathways, and phylogenomic markers. These data help researchers understand ecological functions, evolutionary history, and adaptive traits. For example, phylogenetic metrics derived from whole-genome data revealed that climate responses are often conserved within fungal lineages [6].

A major challenge is the lack of standardization across studies, which severely hinders the integration and cross-validation of airborne eDNA data on a global scale. Unlike soil or host-associated samples, air samples usually contain very little biomass. This means that small

differences in sampling methods, such as active pumping versus passive deposition, as well as pretreatment, sequencing, or bioinformatic analysis, can affect the final results strongly [2,6]. For instance, it is unclear whether differences between fungal diversity from soil and air are real ecological differences or methodological biases [38,39]. Cross-validation studies and bioinformatic tools for correcting methodological biases are essential to integrate eDNA data on a global scale.

6. Disentangling Ecological Drivers

On a broad scale, climatic factors appear to be the primary drivers of airborne eDNA patterns, revealing consistent patterns of variation across different regions (Figure 2) [3]. Mean annual temperature is a particularly powerful predictor, explaining a large proportion of the variance in microbial community composition [5]. Consistent with the effects of climate, both eDNA concentration and species richness decrease from tropical to polar regions across most trophic levels [5]. For example, a two-year airborne monitoring study across a latitudinal gradient (from subarctic to temperate regions) in Sweden revealed significant spatial and seasonal variation in airborne microbial communities. Diversity peaked during warmer periods, with bacterial composition primarily driven by geographic location, while fungal communities were influenced by both location and seasonal climatic changes [3].

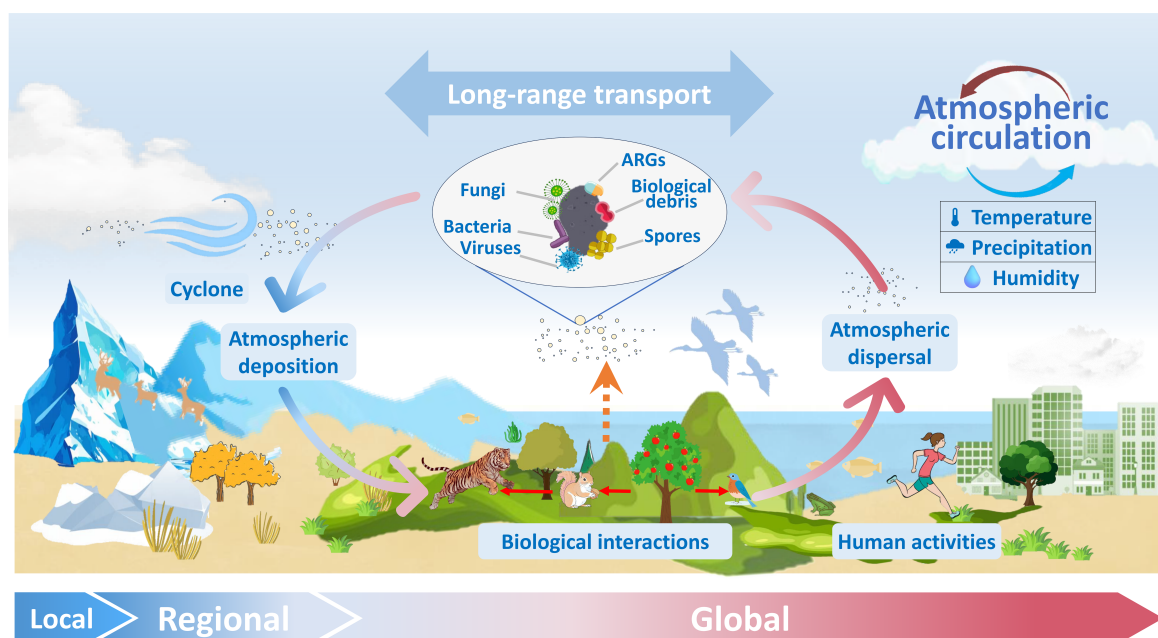


Figure 2. Multi-scale dynamics of airborne eDNA in the atmospheric ecosystem.

Beyond broad-scale climatic effects, airborne eDNA also reflects local ecological processes, but the influence of local-scale factors remains poorly understood [21]. In recent years, local biotic sources have been confirmed as key determinants of airborne eDNA community

composition [38,39]. Emerging studies have begun to explore how resource heterogeneity shapes community structure. For example, in a tropical rainforest ForestGEO plot, airborne eDNA sampling integrated with a comprehensive fruit tree census demonstrated that plant-

vertebrate trophic interactions significantly influence the detection rate and abundance of vertebrate taxa [39]. However, factors such as fine-scale habitat structure, host availability, and interspecific competition are rarely incorporated into the statistical analyses of airborne eDNA community drivers [39]. A more integrated approach, which combines eDNA data with high-resolution remote sensing (e.g., normalized difference vegetation index, land-use layers) and in-situ microclimate measurements, is crucial for separating the effects of climate from those of local ecological drivers [40]. Most current studies rely on correlative models, with limited experimental evidence to support causal interpretation. As a result, it remains difficult to build a mechanistic framework for predicting biodiversity responses to environmental change using airborne eDNA.

7. Future Directions and Ethical Considerations

For the field to mature, several key challenges must be addressed. First, the incompleteness of genomic databases is a fundamental bottleneck that requires global collaboration to sequence type specimens and key taxa. Second, methodological standardization is paramount, including the use of synthetic standards for data normalization and the development of algorithms to correct for technical biases. Third, and perhaps most urgently, scientists, ethicists, and policymakers need to work together to establish ethical guidelines and legal frameworks for handling human genetic information from eDNA samples. The ultimate goal is to create an integrated Earth-observation network that combines airborne eDNA data with climatic models and artificial intelligence (AI)-driven analytics. This system would enable the real-time monitoring and forecasting of planetary life dynamics, providing actionable intelligence for governing the impacts of global change.

Author Contributions

X.W.: conceptualization, writing—original draft & editing; G.W.: writing—review & editing; Z.S.: writing—review & editing. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

Use of AI and AI-assisted Technologies

During the preparation of this work, authors used ChatGPT to correct grammar and optimize language phrasing. After using these tools, authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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