Environmental DNA

WILEY

SPECIAL ISSUE Environmental DNA Traveling by Air

Environmental DNA
Open Access
Dedicated to the study and use of environmental DNA for basic and applied sciences

ORIGINAL ARTICLE OPEN ACCESS

Winds of Change: Charting a Pathway to Ecosystem Monitoring Using Airborne Environmental DNA

Rachel L. Tulloch[1] | Clare I. M. Adams[2] | Matthew A. Barnes[3] | Elizabeth L. Clare[4] | Henrik C. van de Ven[5] | Andrew Cridge[6,7] | Francisco Encinas-Viso[1] | Kristen Fernandes[8] | Dianne M. Gleeson[9] | Erin Hill[10] | Anna J. M. Hopkins[11] | Anna M. Kearns[1] | Gracie C. Kroos[12] | Anna J. MacDonald[13] | Francesco Martoni[14] | Angela McGaughran[15] | Todd G. B. McLay[1] | Linda E. Neaves[16] | Paul Nevill[17] | Andrew Pugh[6,7] | Kye J. Robinson[18] | Fabian Roger[19,20] | Tracey V. Steinrucken[21] | Mieke van der Heyde[17] | Cecilia Villacorta-Rath[22] | Jenny Vivian[23] | Erin E. Hahn[1]

[1]National Research Collections Australia, Commonwealth Scientific Industrial Research Organisation, Canberra, Australian Capital Territory, Australia | [2]Coastal People, Southern Skies Centre of Research Excellence, Department of Mathematics and Statistics, University of Otago, Dunedin, Otago, New Zealand | [3]Department of Natural Resources Management, Texas Tech University, Lubbock, Texas, USA | [4]Department of Biology, York University, Toronto, Ontario, Canada | [5]Netherlands Organization for Applied Scientific Research (TNO), Utrecht, the Netherlands | [6]Scion (New Zealand Forest Research Institute), Rotorua, New Zealand | [7]Better Border Security (B3, B3nz. Org. Nz), Christchurch, New Zealand | [8]Biodiversity and Conservation Science, Department of Biodiversity, Conservation and Attractions, Kensington, Western Australia, Australia | [9]Faculty of Science and Technology, University of Canberra, Canberra, Australian Capital Territory, Australia | [10]Health & Biosecurity, Commonwealth Scientific Industrial Research Organisation, Canberra, Australian Capital Territory, Australia | [11]Molecular Ecology and Evolution Group (MEEG), School of Science, Edith Cowan University, Joondalup, Australia | [12]Department of Anatomy, University of Otago, Dunedin, New Zealand | [13]Australian Antarctic Division, Department of Climate Change, Energy, The Environment and Water, Kingston, Tasmania, Australia | [14]Agriculture Victoria Research, AgriBio Centre, Bundoora, Victoria, Australia | [15]Te Aka Mātuatua—School of Science, University of Waikato, Hamilton, New Zealand | [16]Fenner School of Environment and Society, The Australian National Univ., Canberra, Australian Capital Territory, Australia | [17]Minesite Biodiversity Monitoring With eDNA (MBioMe) Research Group, Trace and Environmental DNA Laboratory, School of Life and Molecular Sciences, Curtin University, Perth, Western Australia, Australia | [18]Manufacturing, Commonwealth Scientific Industrial Research Organisation, Melbourne, Victoria, Australia | [19]Institute of Biogeochemistry and Pollutant Dynamics (IBP), Zurich, Zurich, Switzerland | [20]DNAir, Winterthur, Switzerland | [21]Health & Biosecurity, Commonwealth Scientific Industrial Research Organisation, Brisbane, Australia | [22]Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER), James Cook University, Townsville, Queensland, Australia | [23]Forest Research Institute, University of Sunshine Coast, Sippy Downs, Queensland, Australia

Correspondence: Erin E. Hahn (erin.hahn@csiro.au)

Received: 5 March 2025 | **Revised:** 11 May 2025 | **Accepted:** 23 May 2025

Funding: This work was supported by Centre for Biodiversity Analysis, Synthesis Group Grant.

Keywords: aerobiology | airborne eDNA | biodiversity | biosecurity | conservation | eolian | implementation | monitoring | southern eDNA society | terrestrial

ABSTRACT

Airborne environmental DNA (airborne eDNA) analysis leverages the globally ubiquitous medium of air to deliver broad species distribution data and support ecosystem monitoring across diverse environments. As this emerging technology matures, addressing critical challenges and seizing key opportunities will be essential to fully realize its potentially transformative impact. In June 2024, the Southern eDNA Society convened over 100 researchers, industry leaders, and biodiversity management stakeholders in a landmark workshop to evaluate the current state of airborne eDNA research and chart a course for future development. Participants explored opportunities for integrating airborne eDNA into existing monitoring systems, but they unanimously agreed that research must first be applied to improving understanding of airborne eDNA ecology. The workshop emphasized the importance of collaborative engagement with stakeholders—including government agencies, Indigenous communities, and

citizen scientists—to ensure practical and ethical implementation. This summary highlights current challenges and actionable recommendations, including improving our understanding of airborne eDNA ecology, harmonizing sampling methodology (e.g., devices, materials, sampling density, duration), identifying and mitigating sources of error, and fostering early, sustained stakeholder collaboration. By addressing these challenges, airborne eDNA analysis can become a transformative tool for biodiversity, biosecurity, and conservation monitoring on a global scale. Its ability to detect diverse taxonomic groups—including fungi, plants, arthropods, microbes, and vertebrates—positions airborne eDNA as a pivotal technology for holistic terrestrial biodiversity assessments that transcend traditional, species-focused monitoring approaches.

1 | Introduction

Amid a growing global biodiversity crisis, decision-makers require accurate and timely species distribution and occurrence data. Over the last decade, environmental DNA (eDNA) analysis has become a widely used surveillance tool, particularly within aquatic ecosystems. Sequencing DNA shed by organisms in the environment has enabled time- and cost-effective, non-invasive biodiversity assessments (Ficetola et al. 2008; Pawlowski et al. 2020; Rodriguez-Ezpeleta et al. 2021). As the field evolves, new eDNA methods continue to emerge, with airborne eDNA analysis being one of the latest additions (Bohmann and Lynggaard 2023; Johnson and Barnes 2024).

Airborne eDNA is derived from bioaerosols, which encompass a diverse array of organic materials. These include (1) microorganisms such as viruses, bacteria, microalgae, and unicellular fungi; (2) propagules like pollen and spores released by plants and fungi; and (3) biological fragments, including excretions, cells, and tissue pieces from plants, animals, and microbes (Després et al. 2012). While the definition of "airborne eDNA" remains an unresolved point in the field, for practical purposes, we define it here as DNA extracted from any biological material captured in air samples. This broad definition acknowledges the methodological consistency of approaches used to collect and analyze airborne biological material, whether targeting pollen, fungal spores, microbes, plant fragments, or vertebrate DNA.

Given its ability to capture DNA from diverse sources, airborne eDNA analysis has been applied across multiple fields, including detection of invasive species (Trujillo-González et al. 2022; Sanders et al. 2023), biodiversity assessments (Clare et al. 2022), detection of rare or elusive species (Garrett, Watkins, Francis, et al. 2023), and tracking of allergenic pollen (Kraaijeveld et al. 2015). Emerging applications in airborne environmental RNA (eRNA) further extend potential use cases, particularly for pathogen surveillance (Chia et al. 2020; Bossers et al. 2024). Together, these advances enable cross-disciplinary ecological and evolutionary research and support comprehensive ecosystem health monitoring.

Airborne eDNA analysis holds immense promise for monitoring applications across a wide range of terrestrial environments, with the ability to capture genetic material from air to complement substrate-restricted eDNA sampling methods. This unique potential could enable broad-scale biodiversity assessments in locations where traditional field monitoring methods are impractical. However, the methodology remains nascent, sharing many challenges with established eDNA sources like water, such as imperfect detection and sensitivity to environmental conditions (Johnson, Cox, et al. 2021; Rowney et al. 2021). Rather than deterring progress, these challenges underscore the need for targeted research and methodological innovation. Variation in sample collection and analysis, although expected in an emerging field, has prompted studies on sampling method effects (Johnson et al. 2019a), detection limits (Foster et al. 2023), and source estimation for airborne eDNA (Lennartz et al. 2021; Gusareva et al. 2022), emphasizing the importance of quantifying methodological impacts on data robustness, repeatability, and reliability. Recognizing this momentum, Johnson and Barnes (2024) recently reviewed the field's growth, challenges, and potential future directions, identifying key hurdles still to be addressed.

In June 2024, over 100 researchers, industry leaders, and management stakeholders convened in Canberra, Australia, both in person and virtually, for a pivotal two-day workshop hosted by the Southern eDNA Society (SeDNAS, https://sednasociety.com/, accessed 13 September 2024). Participants from 30 institutions and eight countries evaluated the current state of airborne eDNA research, identified key challenges, and outlined strategic pathways for future development.

While acknowledging the long-standing use of eDNA metabarcoding and targeted species detection in airborne microbial community and pollen and fungal spore studies, the workshop primarily focused on the use of airborne eDNA for detecting macro-organisms. Discussions revealed many overlapping challenges with other forms of eDNA, such as aquatic or soil-based methods, but workshop participants acknowledged that a subset of challenges including exceptionally low total DNA concentrations and the establishment of appropriate field controls is unique to the medium of air. The workshop centred around four key questions: (1) What might airborne eDNA data be used for? (2) How is airborne eDNA currently collected and processed? (3) What are the key questions about airborne eDNA ecology that need to be answered? (4) How do we as researchers engage effectively with airborne eDNA stakeholders? Here, we summarize the workshop outputs, provide insights into the advances and future directions of airborne eDNA technology, and offer a workshop statement to summarize current community consensus on the emerging field (see Box 1).

2 | Airborne eDNA Applications

Interest in airborne eDNA has grown rapidly following proof-of-concept studies demonstrating its utility in detecting vertebrates (Clare et al. 2021) and plants that rely on insect or animal pollination rather than wind dispersal (Johnson et al. 2019b). These studies paved the way for early applications of airborne eDNA analysis in terrestrial biodiversity assessments (Clare et al. 2022;

BOX 1 | Southern eDNA society airborne eDNA workshop joint statement.

"Airborne eDNA analysis is a potentially powerful biomonitoring tool, however we must improve our understanding of airborne eDNA ecology, sampling strategy impacts, signal variability, and sensitivity. With validation, airborne eDNA tools may become standard in biodiversity, biosecurity, and conservation applications."

Lynggaard et al. 2022, 2024; Bohmann and Lynggaard 2023). The utility of airborne eDNA extends beyond targeted species detection to monitoring across the tree of life. Its ability to simultaneously identify microorganisms, plants, and animals allows for the development of comprehensive biodiversity baselines and offers unparalleled opportunities to detect shifts in community composition and biodiversity health. When paired with traditional survey techniques such as camera traps, manual handling, and visual surveys (Johnson, Fokar, et al. 2021; Roger et al. 2022) and complementary forms of eDNA (Runnel et al. 2024), airborne eDNA may improve the detection of terrestrial and arboreal species that may otherwise be underrepresented or undetected (Banchi et al. 2020).

In the context of a changing climate and an increasingly interconnected world, airborne eDNA analysis enables rapid detection of plant and animal pests and identification of incursion pathways, offering valuable data for biosecurity applications (Kestel et al. 2022; Trujillo-González et al. 2022; Sanders et al. 2023). Its potential spans all phases of the invasion curve—from pre-biosecurity breach and early detection to containment and eradication monitoring—highlighting its future role as a critical tool in biosecurity monitoring (Bell et al. 2024). For example, airborne eDNA has been shown to complement visual monitoring approaches for detecting pest species incursions, such as the successful detection of hemlock woolly adelgid populations in eastern North America (Geller and Partridge 2025), a species native to Japan that has established itself as an invasive pest in affected regions (Havill et al. 2016). Airborne eDNA is also being tested in agricultural settings, such as honeybee colonies, to evaluate colony health and foraging behavior, highlighting its potential for broader applications in agroecological monitoring and biosecurity (Pepinelli et al. 2025).

Airborne eDNA collection offers an opportunity to sample in inaccessible regions and monitor biodiversity at spatial, temporal, and replication scales that were previously unattainable using traditional field-based methods. Like other eDNA approaches, airborne eDNA analysis can facilitate access to remote or challenging locations, including burrows and mountain tops, and enhance monitoring of sensitive or cryptic species (Lynggaard et al. 2024). The possible simplicity of airborne eDNA capture lends kindly to the expansion of sampling density through citizen scientist initiatives (Madden et al. 2016), mirroring those currently in use in aquatic systems (Biggs et al. 2014). To increase sampling scale affordably, an opportunity is emerging in repurposing existing sample collection infrastructure—such as pollen, spore, or pollution monitoring stations (Littlefair et al. 2023), which can generate biodiversity data coupled with environmental and meteorological datasets. Many of these infrastructures archive samples, providing the potential for retrospective analysis of biodiversity trends and historical species presence using airborne eDNA.

3 | Airborne eDNA Collection

Platforms used to collect airborne eDNA vary widely in their design and material composition, generally falling into two categories: passive or active samplers. The choice between these methods depends on the monitoring goal and project resources.

Passive samplers rely on natural air movement to collect eDNA. With simple designs requiring low maintenance, they can be deployed at high density to increase temporal and spatial replication, delivering precise detection probabilities and occupancy estimates while reducing random variation due to fluctuating environmental conditions (Whittington et al. 2015; Burian et al. 2021). Passive sampling is particularly advantageous for cost-effective, mobile deployments, supporting flexible sampling campaigns across many sites. However, passive methods depend on ambient air movement and may require long deployment times to accumulate sufficient DNA, especially in environments with low particulate loads. Examples of passive samplers include Big Spring Number Eight dust traps (Johnson et al. 2023), modified Wilson and Cooke towers, marble-filled pan traps (Johnson et al. 2019a), filter and funnel sedimentation traps (Schlegel et al. 2024), and sticky traps (Runnel et al. 2024). Opportunistic methods, such as collecting spiderwebs to capture airborne eDNA, have also been explored (Xu et al. 2015; Gregoric et al. 2022; Newton et al. 2024).

In contrast, active samplers use powered equipment, such as fans, to intentionally draw air through or onto a particle collection system, like filters, impingers, or cyclonic separators. These systems may increase the volume of air sampled over a given time period, impacting the effective test area and detection probability, though further research is needed to quantify this effect. Although more complex and power-dependent than passive devices, active samplers enable controlled, standardized sampling and can deliver higher temporal resolution over extended periods. Examples of active samplers include cyclonic air-samplers (Brennan et al. 2019; Roger et al. 2022), dry cyclone samplers (Brennan et al. 2019), computer fan-powered 3D-printed filter frames (Lynggaard et al. 2022; Garrett, Watkins, Francis, et al. 2023), and repurposed pollution monitoring stations (Littlefair et al. 2023).

As new collection systems are developed and tested, platform design variation is expected to increase. To guide this innovation, workshop attendees identified key attributes for airborne eDNA samplers (Figure 1). The desired features of a sampling platform directly relate to the monitoring scale, context, and longevity of use.

We define long-term monitoring platforms as those designed for continuous or repeated sampling at fixed sites, typically supported by permanent or semi-permanent infrastructure (e.g., pest monitoring in agricultural systems or biodiversity assessments at long-term research sites). Such platforms should be

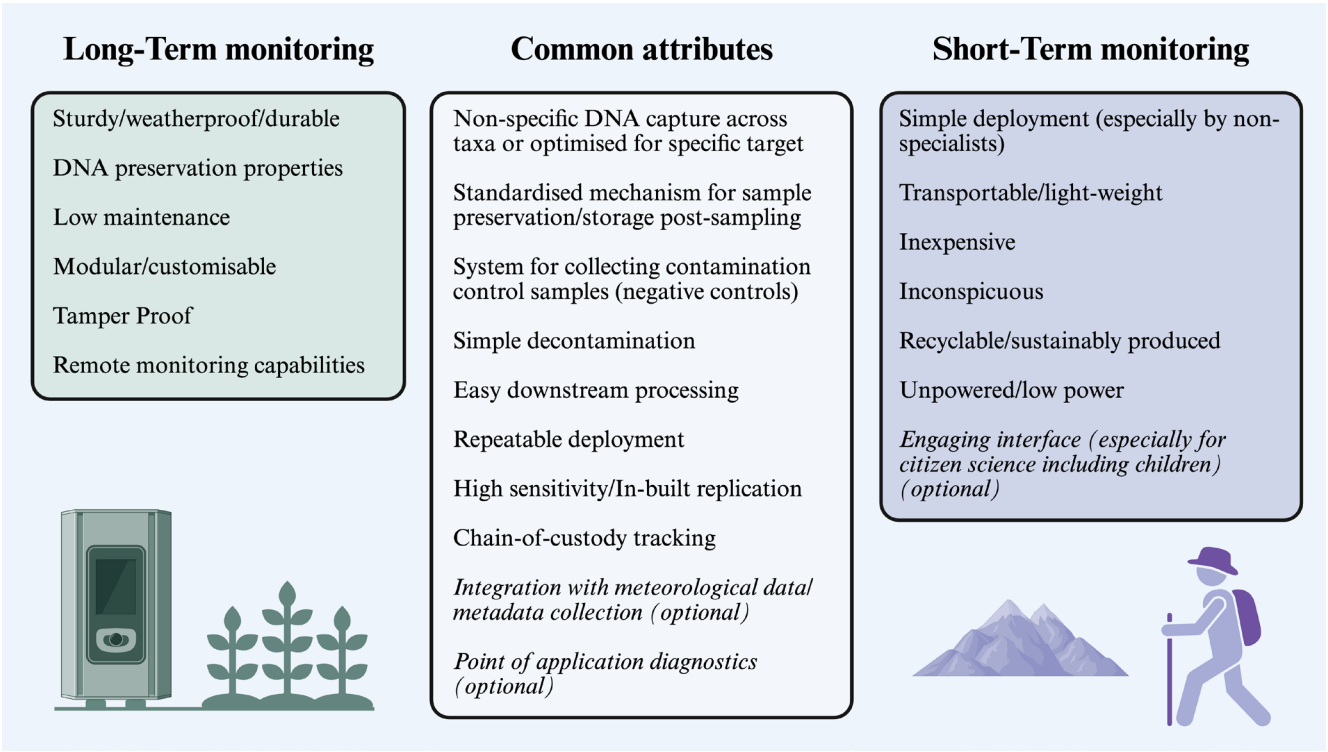

FIGURE 1 | Key attributes of airborne eDNA collection platforms. Ideal airborne eDNA collection devices balance core common attributes with fit-for-purpose design tailored to specific monitoring needs. Long-term monitoring platforms, typically supported by permanent or semi-permanent infrastructure, should prioritize durability, sample integrity, low maintenance, and modular/customizable components that enable evolving monitoring goals. Remote monitoring capabilities can enhance operational efficiency by providing real-time data on environmental conditions or device performance. Short-term monitoring platforms are designed for mobile, temporary use and should emphasize simplicity, portability, and cost-effectiveness, especially when used for rapid-response surveys or citizen science initiatives. In such contexts, engaging packaging and intuitive data interfaces may encourage participation. Across all platforms, standardized mechanisms for sample preparation and preservation, systems for collecting contamination controls, straightforward decontamination, and compatibility with high-throughput downstream processing are critical for ensuring data reliability and usability.

durable, low-maintenance, and tamper resistant, with modular or customizable components that allow different sampling modules, filters, or environmental sensors to be swapped in or upgraded as monitoring objectives evolve. This flexibility can extend the operational lifespan of devices and support multi-purpose sampling, for example, switching between general biodiversity monitoring and targeted surveillance of specific taxa. Features that support DNA preservation, such as in situ drying, chemical stabilization, or refrigeration modules, are critical for maintaining sample integrity during long deployments. Remote monitoring capabilities, such as real-time environmental sensing, airflow or filter performance tracking, and automated alerts for maintenance needs, can further enhance data reliability and operational efficiency.

Short-term monitoring platforms are designed for temporary, mobile deployments—ranging from hours to weeks—for episodic, opportunistic, or event-based monitoring needs like in the case of establishing invasion fronts in biosecurity control efforts or supporting citizen science initiatives. These platforms benefit from simple, low-cost designs that are lightweight, easy to deploy, and ideally inconspicuous in the field. Their portability makes them especially useful for rapid-response surveys or distributed sampling by non-specialists, such as volunteers. Where these devices are used in citizen science or for educational purposes, they may be designed with user-friendly packaging and engaging data exploration interfaces to encourage participation.

Across both long- and short-term applications, all platforms should include core attributes such as standardized sample preparation and storage, systems for collecting contamination controls, and straightforward decontamination processes. Easy downstream sample processing, such as automated filter handling and DNA extraction, helps minimize manual handling and accelerates sample throughput. Additional key features include high sensitivity with in-built replication, repeatable deployment, and chain-of-custody tracking to ensure data integrity. Optional enhancements may include integration with meteorological data or point-of-application diagnostics to improve system utility in specific contexts.

In practice, the distinction between long- and short-term platforms is closely linked to the choice between passive and active samplers, each offering advantages and limitations depending on the deployment context. Passive samplers may be preferable for short-term or opportunistic deployments because of their low cost, ease of transport, and minimal infrastructure needs, especially when broad spatial coverage is required. However, challenges such as maintaining exposure

consistency and ensuring sufficient DNA accumulation limit their suitability for continuous long-term monitoring. Active samplers, though more resource-intensive, provide controlled, standardized sampling and the potential for higher temporal resolution, making them well-suited to long-term monitoring. Active systems may also be preferred for short-term use when rapid DNA collection is essential, such as during time-sensitive biodiversity or biosecurity events. An integrated approach combining both methods, such as deploying passive samplers across broad landscapes while maintaining active systems at key sentinel sites, may optimize monitoring outcomes. Despite their complementary roles, direct performance comparisons between passive and active systems remain limited (but see Jager et al. 2025), highlighting the need for further comparative studies.

Regardless of the sampling approach, attendees underscored that critical sampling parameters must be validated before any method or device can be widely adopted for monitoring purposes to ensure reliable and accurate data generation.

4 | Advancing Understanding of Factors Influencing Airborne eDNA Detection

A comprehensive understanding of environmental, ecological, and technical parameters is critical for optimizing airborne eDNA monitoring. Table 1 summarizes key factors identified by workshop participants that require validation to strengthen confidence in airborne eDNA data.

It is well established that eDNA generation, persistence, and degradation (i.e., eDNA ecology) can be influenced by temperature, humidity, UV exposure, and other environmental factors, which introduce variability in species detection (Barnes et al. 2014, 2021; Shogren et al. 2017; Harrison et al. 2019; Jo and Minamoto 2021). Airborne eDNA studies have begun to explore these influences, demonstrating, for example, the impact of weather and human activity on detection probabilities (Johnson, Cox, et al. 2021; Hanson et al. 2024). Species seasonality (e.g., pollen release, insect emergence, bird migration) and fluctuating air currents have also been identified as variables with potential to skew biodiversity assessments if not properly accounted for (Caliz et al. 2018; Aalismail et al. 2021). These environmental parameters should be routinely recorded alongside sampling to better contextualize results and allow for identification of potential sources of variability.

Beyond environmental factors, technical elements such as sampler type, deployment strategy, and analytical workflows play pivotal roles in shaping data composition. Workshop attendees identified critical technical parameters requiring validation, including sampling methods, sampling density, and replication, sample preservation, bioinformatic parameters, and controls (Table 1). For example, sampling methods encompass device design choices such as filters versus sticky traps, passive versus active systems, or impingement versus filtration, all of which may yield differing efficiencies (Johnson et al. 2019a; Chang et al. 2023). Sampling density refers to the number of independently deployed units across a site, while replication reflects technical and experiment repeats (e.g., number of filters collected per unit or number of qPCR replicates) within each sample. Sample preservation is especially important in airborne contexts, where low biomass and environmental exposure can rapidly degrade or contaminate the DNA sample. Field and laboratory controls are critical for detecting contamination events, while bioinformatic parameters such as filtering thresholds and taxonomic assignment strategies must be appropriately selected and transparently described to ensure data comparability.

Although aquatic and soil eDNA studies provide valuable starting points, their insights do not fully mirror the challenges of airborne eDNA sampling. For example, aquatic-focused studies on DNA particle size, degradation kinetics, and extraction methods (Barnes et al. 2014, 2021; Deiner et al. 2015) offer transferable knowledge but require confirmation under airborne conditions. Airborne eDNA also presents unique challenges, including potentially very low DNA concentrations, rapid particle sedimentation, and the influence of complex air currents, all of which require dedicated investigation. Without insight into these factors, conservation or biosecurity actions informed by airborne eDNA data may risk misinterpretation and inefficiency due to uncharacterized detection error. Thus, investigation and validation of a diverse range of parameters will be essential for progressing the utility of airborne eDNA analysis (Atkinson and Roy 2023; Bohmann and Lynggaard 2023).

The need for parameter validation will depend on study objectives. While the field works toward understanding these factors, it is important that airborne eDNA studies clearly communicate study limitations. Importantly, airborne eDNA studies should clearly articulate their experimental design, use of controls, and data analysis approach to further facilitate identification of potential sources of detection error.

Airborne eDNA, like other eDNA approaches, is prone to error from several major sources, including contamination of DNA in the workflow, inefficient DNA capture, PCR inhibition, misidentification of DNA, and changing taxonomies (Furlan et al. 2020; Burian et al. 2021; Garrett, Watkins, Francis, et al. 2023; Garrett, Watkins, Simmons, et al. 2023). Detection sensitivity and inhibition are particularly critical considerations, as environmental samples often contain low DNA concentrations alongside potential inhibitors such as dust, soot, and pollen (McDevitt et al. 2007). These factors can suppress amplification efficiency, leading to underestimation of biodiversity. To improve confidence in results, validation of detection thresholds, identification of likely inhibitors, and rigorous error estimation, such as using internal controls and mock community trials, are essential steps (Klymus et al. 2020; Burian et al. 2021).

To address these challenges systematically, the workshop developed a four-part framework articulating the main sources of error in eDNA datasets and outlining tailored mitigation strategies (Figure 2). The framework divides the eDNA workflow into two stages: capture (physical collection of environmental DNA) and analysis (identification and interpretation of DNA). Errors arising during capture are classified as detection errors, while those arising during analysis are classified as identification errors. Together, these stages yield four distinct error types: (1) false negative detections, where DNA is present in the environment but is not captured; (2) false negative identifications, where

TABLE 1 | Key parameters requiring validation for reliable airborne environmental DNA (eDNA) monitoring.

Category	Parameters	Validation required	Examples
Technical/ experimental	• Sampling methods • Sampling density • Technical and experimental replication • Sample preservation • Bioinformatics • Field and laboratory controls	Comparisons of sampling methods (e.g., active versus passive). Optimisation of sampling materials. Effects of sampling design (e.g., height of sampler, sampling duration, air volume), DNA preservation solutions and contamination. Selection of bioinformatic parameters. Identification of appropriate controls.	Sampling and processing effects on terrestrial plant detection (Johnson et al. 2019a) Sampling impacts on airborne viral detection (Chang et al. 2023) Aquatic study recommendations (Goldberg et al. 2016)$^{\delta}$ Sampling and extraction effects in freshwater systems (Deiner et al. 2015)$^{\delta}$
Environmental factors	• Weather • UV irradiance • Human activity	Impact of humidity, temperature, wind direction and speed, UV index, precipitation, air pressure, and local human activity on DNA transport and persistence.	Seasonal weather impact on tree species detection (Hanson et al. 2024) Combined influence of seasonality and human activity on plant detection (Johnson, Cox, et al. 2021) Environmental influence over eDNA particle size in freshwater systems (Barnes et al. 2021)$^{\delta}$ eDNA persistence in controlled freshwater system (Barnes et al. 2014)$^{\delta}$
Ecology of target species	• Habitat • Behavior • Life cycle • Species mobility • DNA shedding rates • Shed DNA form	Influence of species biology on DNA shedding, DNA distribution and detection.	Source locations of eukaryotic species detected in atmospheric dust (Aalismail et al. 2021) Influence of tree species biology on detection (Johnson et al. 2019b) Influence of land-use type and seasonality on airborne bacterial and fungal community composition (Bowers et al. 2011; Caliz et al. 2018; Anees-Hill et al. 2022)
Detection limits	• Sensitivity • Inhibition • Error Estimation	Minimum detection thresholds. Identification of likely inhibitors. Estimating and accounting for error using analysis tools.	qPCR inhibition in indoor air samples (McDevitt et al. 2007) Defining detection limits (Klymus et al. 2020) PCR inhibition in freshwater systems (Jane et al. 2015; Buxton et al. 2017)$^{\delta}$ Improving reliability of eDNA data interpretation using statistical models (Burian et al. 2021)

Note: A non-exhaustive list of critical parameters requiring validation to ensure the reliability of airborne eDNA monitoring. Parameters are grouped into four categories: Technical/experimental, Environmental factors, Ecology of target species, and Detection limits. For each category, specific parameters, the validation required, and examples of relevant studies are provided. The δ symbol indicates studies or recommendations made for aquatic eDNA, highlighting transferable knowledge from existing eDNA research.

DNA is captured but cannot be accurately identified; (3) false positive detections, where DNA is correctly identified but originates from outside the target area; and (4) false positive identifications, where DNA is misidentified as the wrong species. Each error type requires tailored mitigation strategies; for example, improving detection methods may address false negatives, while enhanced bioinformatic pipelines and reference databases can reduce the likelihood of false positive identifications.

eDNA datasets are often complicated by false positive detections from laboratory contaminants and ubiquitous signals from humans, agricultural plants and animals, and common fungi. While detection of common contaminants is not unique to airborne eDNA (Sepulveda, Hutchins, et al. 2020), sampling air presents a unique challenge in that every step of the collection and analysis process is unavoidably exposed to ambient air, increasing the risk of contamination at every stage. This underscores the need for robust controls at both field and laboratory stages, as current methodologies may not adequately mitigate contamination risks specific to air sampling.

While most airborne eDNA studies have included standard blank extraction controls, some have instituted negative filter controls (e.g., filters not exposed to air in the field) see Roger et al. (2022), and others also include laboratory air controls (e.g., filters exposed to laboratory air) see Littlefair et al. (2023). In addition to

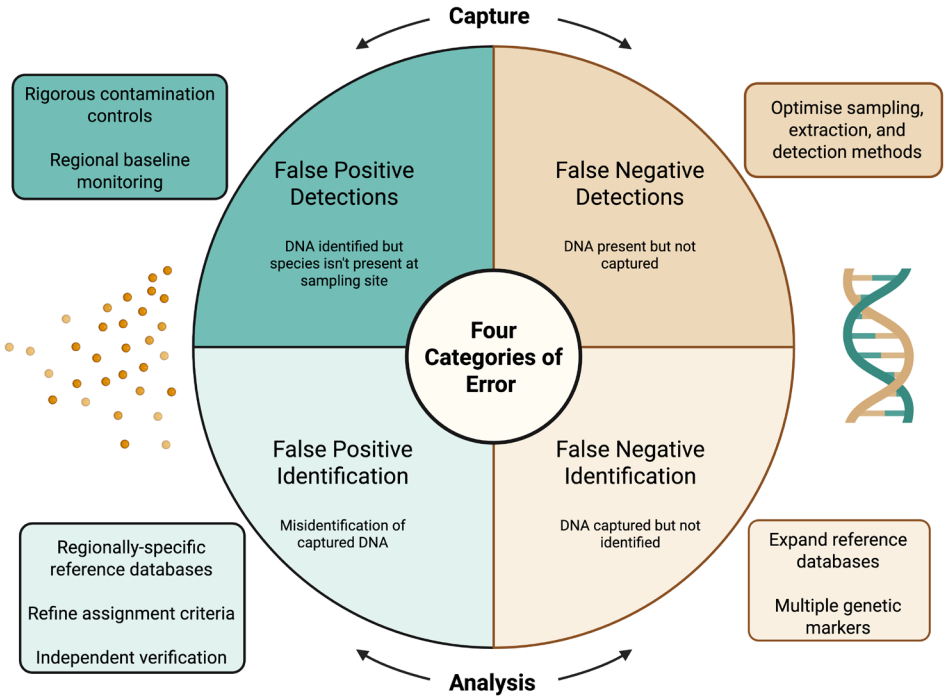

FIGURE 2 | Framework for addressing errors in airborne eDNA analysis. Framework for understanding error in eDNA analysis, distinguishing four categories of error arising from two key stages of the workflow: Detection (during sample collection) and identification (during data analysis). The upper and lower halves of the figure represent detection and identification errors, respectively, while green (left) and tan (right) indicate positive and negative conclusions. Each error type stems from distinct sources and requires tailored mitigation strategies, illustrated around the perimeter.

these controls, regional baseline monitoring, through both targeted eDNA surveys and conventional biodiversity assessments, can help contextualize detections by establishing a reference of species expected to be present in a given area. This approach is particularly valuable for distinguishing between true local detections and potential false positives arising from long-distance DNA transport or unexpected environmental contamination.

A further complication is defining the "target ecosystem" for airborne eDNA. Unlike freshwater aquatic eDNA studies, where the sampling area is often clearly bounded (e.g., a specific pond or stream), airborne eDNA may reflect biological signals from a much broader or ambiguous source area. DNA can accumulate from both local and distant sources, complicating the interpretation of whether a species is truly present at the sampling site. Recent work by Tournayre et al. (2025) has provided tentative estimates of airborne eDNA transport distances, using a network of 15 repurposed air pollution monitors. They reported a median estimated travel distance of approximately 18 km, though these estimates are preliminary and specific to the sampler type used (Digitel 392 DPA-14) and particle size collected (particles $\leq 10\,\mu m$). Smaller particles likely disperse farther, and wind and landscape features may generate complex patterns, underscoring the need for further empirical research to clarify airborne DNA transport dynamics and the spatial resolution of airborne eDNA detections.

To support more reliable interpretation, regional datasets could be developed by leveraging existing environmental monitoring programs, such as national air quality (Littlefair et al. 2023) and large-scale pollen and fungal spore monitoring networks such as the European Aeroallergen Network (ean.polleninfo.eu), the US National Allergy Bureau (pollen.aaaai.org), and the Australian Pollen Allergen Partnership (auspollen.edu.au). Large-scale microbial and dust monitoring initiatives (Barberán et al. 2015; Tignat-Perrier et al. 2019) also present opportunities to cross-reference airborne eDNA detections with broader atmospheric biodiversity trends.

Errors related to DNA identification can also have broad-reaching impacts on biodiversity assessments and management decisions made from eDNA data; for example, both false positive and false negative identifications can skew biodiversity assessments. The complexity of this problem was illustrated in recent studies surveying bat biodiversity using airborne eDNA (Garrett, Watkins, Francis, et al. 2023; Garrett, Watkins, Simmons, et al. 2023). In these studies, Garrett, Watkins, Francis, et al. (2023) and Garrett, Watkins, Simmons, et al. (2023) worked at a long-term bat monitoring site with 35–40 common species, many of which have undergone multiple recent taxonomic revisions (e.g., *Mimon crenulatum* reclassified to *Gardnerycteris crenulatum* and then to *Gardnerycteris keenani*). Taxonomic flux and ambiguous reference sequences complicated species identification, even with taxon-specific expert input and manual curation. Notably, the study found that closely related species with near-identical barcode sequences simultaneously increased the risk of false positive identifications (misassigning DNA to the wrong species) and false negative identifications (downgrading data to genus level or overlooking valid detections).

To mitigate false positive identifications, independent verification methods such as visual surveys, acoustic monitoring, or

camera trapping are valuable for corroborating eDNA findings, particularly when detections carry management implications. In the bat study, these validation efforts were key to distinguishing genuine detections from artifacts arising from taxonomic ambiguity and regional synonymy, issues that are likely to affect other taxonomic groups, especially when reference databases lack curation. While independent verification remains best practice, there is growing interest in determining when airborne eDNA data, particularly for well-characterized systems, can stand alone as sufficient evidence for community assessments. Ongoing benchmarking and cross-validation efforts will be critical in clarifying where and when this is appropriate.

Continued improvement of bioinformatics pipelines and reference databases will reduce the likelihood of false identifications. Advanced data processing tools can enhance the reliability of eDNA data interpretation, accounting for error which cannot otherwise be eliminated through control of characterized variables (Burian et al. 2021). For false negative identifications, expanding the use of multiple genetic markers (e.g., COI, 12S, 16S) can increase taxonomic coverage and improve resolution. However, this approach introduces additional laboratory complexity, analytical costs, and potential challenges in marker optimization. Marker choice must balance broad taxonomic reach with specificity tailored to monitoring goals. Data processing tools which apply hierarchical occupancy or process-based models have been shown to mitigate the impact of error sources through the estimation of uncertainty related to species detection (McClenaghan et al. 2020).

A major bottleneck remains the availability of reference sequences, particularly for invertebrates and fungi. Workshop participants strongly advocated for coordinated reference sequencing initiatives, ideally in partnership with natural history collections (Schmid et al. 2025) to close these gaps, with a focus on regionally relevant species. Progress on this front will enable greater confidence in using airborne eDNA as a standalone tool, especially in well-characterized systems where reference databases are comprehensive. Initiatives such as Australia's National Biodiversity DNA Library (NBDL; research.csiro.au/dnalibrary), which links whole organellar (mitochondrial and chloroplast) genomes to vouchered specimens and aims to barcode all Australian species, exemplify best practice. Similar large-scale efforts include the Barcode of Life Data System (BOLD; boldsystems.org) (Ratnasingham and Hebert 2007) and the International Barcode of Life (iBOL; ibol.org) project, which have made significant advances in building global barcode libraries. As these resources grow and incorporate rigorous taxonomic validation, they will reduce reliance on supplementary verification in many contexts. However, workshop participants cautioned that these goals remain aspirational for many taxa and regions, reinforcing the continued importance of validation and benchmarking in the near term.

While best practices in field and laboratory protocols and data interpretation remain fundamental, they are insufficient on their own to negate all sources of error. Nonetheless, as has been shown in aquatic systems, the presence of some data uncertainty should not deter managers from utilizing eDNA data when it offers a valuable, non-invasive tool for biodiversity and biosecurity monitoring (Jerde 2021).

5 | Building Partnerships and Trust in Airborne eDNA

As airborne eDNA research matures, models of stakeholder engagement used in water and soil eDNA sampling can serve as useful templates to support successful implementation (Morisette et al. 2021). Achieving this will require early and sustained collaboration with agencies, industries, academic institutions, citizen scientists, and Indigenous communities (Bonicalza et al. 2024). Given the complexity of integrating genetic data with climatic and ecological information, engaging stakeholders from the outset helps ensure research approaches are fit-for-purpose and ethically sound.

The use of airborne eDNA raises important ethical concerns, particularly regarding privacy, consent, and potential misuse. These include risks such as unintended disclosure of sensitive species locations and potential impacts on Indigenous communities and landowners if data are collected without consent (Handsley-Davis et al. 2021). Best practices should therefore prioritize co-design with Indigenous communities, respecting local contexts and protocols, and adhering to FAIR (Findable, Accessible, Interoperable, Reusable) and CARE (Collective Benefit, Authority to Control, Responsibility, Ethics) data governance principles (www.gida-global.org/care) (Hutchins et al. 2023; Kukutai and Black 2024; Takahashi et al. 2025). Frameworks such as the Te Mata Ira and Te Nohonga Kaitiaki Guidelines for Genomic Research with Māori and on Taonga Species from Genomics Aotearoa (Hudson et al. 2021) and the United States' National Aquatic eDNA Strategy (Goodwin et al. 2024) provide guidance on ethical Indigenous engagement. Early and intentional collaboration with Indigenous communities and management agencies helps align scientific goals with practical needs, fostering mutually beneficial and culturally respectful outcomes (Wilcox et al. 2008; Handsley-Davis et al. 2021; Newton et al. 2025).

Stakeholders may approach airborne eDNA analysis with cautious optimism, given its relative early stage as a monitoring tool (Polling et al. 2024) and the need to build confidence in the reliability of eDNA data for biosecurity and conservation management (Sepulveda, Nelson, et al. 2020). Researchers must clearly communicate current limitations and set realistic expectations. For example, airborne eDNA is currently best suited for presence/absence detection rather than delivering abundance estimates. Stakeholders should also understand that species detectability can vary depending on environmental conditions, shedding rates, and site-specific factors. Researchers should emphasize that airborne eDNA is a complementary tool rather than a substitute for traditional methods.

Integrating airborne eDNA analysis with established sampling techniques such as camera traps (Polling et al. 2024), visual surveys (Johnson, Fokar, et al. 2021), and acoustic monitoring (Garrett, Watkins, Francis, et al. 2023) offers opportunities to build trust through corroborative evidence. Co-designing protocols with stakeholders to align with regulatory processes and practical applications will be essential. Additionally, developing well-defined sampling protocols and robust controls, modeled on those established in aquatic eDNA studies (Deiner et al. 2015, 2018; Goldberg et al. 2016; Minamoto et al. 2016),

will ultimately contribute to end-user adoption of airborne eDNA methods.

The simplicity and accessibility of air sampling provide a compelling opportunity to engage communities through citizen science initiatives, expanding monitoring capacity (Palmer et al. 2017) while fostering public awareness and education (Sbrocchi 2015; Isley et al. 2022). By involving citizen scientists in data collection, programs can leverage public interest and participation to boost sampling density and broaden geographic coverage. To ensure the success and sustainability of these programs, it is essential to follow established frameworks for citizen-scientist engagement that emphasize clear goals, transparent data management, and adaptable protocols (Kieslinger et al. 2017). An additional benefit of such initiatives is the potential to create biobanking repositories of samples collected by citizen scientists, generating valuable time-series data for future research (Jarman et al. 2018). Ultimately, effective communication and ongoing collaboration between scientists and participants will be crucial for building trust and maximizing the long-term impact of airborne eDNA initiatives, fostering a shared commitment to biodiversity monitoring and conservation.

6 | Clear Skies Ahead?

Advancing airborne eDNA analysis as a monitoring tool may transform biodiversity and biosecurity management by delivering rapid, non-invasive insights into ecosystems at previously unattainable scales. However, realizing this potential depends on overcoming key challenges, particularly those related to refining collection methods, deepening our understanding of airborne eDNA ecology, and managing data uncertainties. Through focused, collaborative research, the field can transition from experimental trials to practical application, bridging the gap between eDNA research and policy (Lodge 2022).

Integrating airborne eDNA with other monitoring methods, such as remote sensing and traditional field surveys, could expand both the scope and resolution of ecosystem assessments, supporting broader 'One Health' frameworks that link environmental, animal, and human health (Farrell et al. 2021; Childress et al. 2024). As a complementary tool, airborne eDNA has the potential to broaden our understanding of ecosystem dynamics and improve early detection of biodiversity loss and biosecurity threats that otherwise go unnoticed. In the future, data generated through airborne eDNA analysis could become a cornerstone of large-scale monitoring networks, similar to wastewater surveillance for tracking disease outbreaks like COVID-19 (Bogler et al. 2020). Integration of this monitoring tool into global initiatives, such as GBiOS, could revolutionize biodiversity monitoring by standardizing data collection to enable rapid, evidence-based management responses (Gonzalez et al. 2024). The method's ability to integrate genetic information from a wide range of taxonomic groups makes it an ideal candidate for inclusion in global monitoring initiatives. In doing so, airborne eDNA can help build comprehensive global datasets that support comparative ecological research and guide policy at an international scale.

If the significant challenges are overcome, airborne eDNA analysis has the potential to revolutionize environmental monitoring, offering innovative ways to observe and protect ecosystems. To realize the potential of this emerging tool, sampling methods should be refined, and robust parameter validation established. With continued innovation and targeted research, airborne eDNA analysis could set new benchmarks in biodiversity, biosecurity, and conservation practices, ultimately becoming a routine component of ecosystem management. As the field matures, airborne eDNA analysis can evolve from an experimental approach to a reliable tool, guiding decision-making at local, national, and global scales and safeguarding natural resources for future generations.

Author Contributions

E.E.H., L.E.N., and D.M.G. conceived of and secured funding for the workshop. R.L.T. and E.E.H. initiated the manuscript outline. All authors contributed to drafting the manuscript. R.L.T. and E.E.H. created the figures and finalized the manuscript.

Acknowledgments

We acknowledge the Ngunnawal People as the Traditional Custodians of the lands on which the Airborne eDNA Workshop was held and pay our respects to Elders past and present. We are grateful to Paula Doyle, whose exceptional organization and skillful MC'ing were integral to the success of the workshop, and to the Southern eDNA Society for their key organizational support. We thank the staff at Wildbark at Mulligans Flat Woodland Sanctuary for their outstanding assistance in hosting the event. We are also grateful to the workshop participants, whose thoughtful contributions and discussions shaped the outcomes of this work. Special thanks to Olly Berry for his leadership within CSIRO's Environomics Future Science Platform, and to Mariana Hopper and Bruce Deagle for their valuable comments on the manuscript. The workshop was supported by a Synthesis Group Grant from the Centre for Biodiversity Analysis. We also thank CSIRO's Environomics Future Science Platform, Illumina, the Australian Genome Research Facility, EnviroDNA, ThermoFisher Scientific, eDNA Frontiers, Sequench, and Wilderlab for their sponsorship, including support of the workshop and Early- and Mid-Career Researcher prizes.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

References

Aalismail, N. A., R. Díaz-Rúa, N. Geraldi, M. Cusack, and C. M. Duarte. 2021. "Diversity and Sources of Airborne Eukaryotic Communities (AEC) in the Global Dust Belt Over the Red Sea." *Earth Systems and Environment* 5, no. 2: 459–471. https://doi.org/10.1007/s41748-021-00219-4.

Anees-Hill, S., P. Douglas, C. H. Pashley, A. Hansell, and E. L. Marczylo. 2022. "A Systematic Review of Outdoor Airborne Fungal Spore Seasonality Across Europe and the Implications for Health." *Science of the Total Environment* 818: 151716. https://doi.org/10.1016/j.scitotenv.2021.151716.

Atkinson, C. T., and K. Roy. 2023. "Environmental Monitoring for Invasive Fungal Pathogens of ʻŌhiʻa (*Metrosideros polymorpha*) on the Island of Hawai'i." *Biological Invasions* 25, no. 2: 399–410. https://doi.org/10.1007/s10530-022-02922-3.

Banchi, E., C. G. Ametrano, E. Tordoni, et al. 2020. "Environmental DNA Assessment of Airborne Plant and Fungal Seasonal Diversity." *Science of the Total Environment* 738: 140249. https://doi.org/10.1016/j.scitotenv.2020.140249.

Barberán, A., J. Ladau, J. W. Leff, et al. 2015. "Continental-Scale Distributions of Dust-Associated Bacteria and Fungi." *Proceedings of the National Academy of Sciences of the United States of America* 112, no. 18: 5756–5761. https://doi.org/10.1073/pnas.1420815112.

Barnes, M. A., W. L. Chadderton, C. L. Jerde, A. R. Mahon, C. R. Turner, and D. M. Lodge. 2021. "Environmental Conditions Influence eDNA Particle Size Distribution in Aquatic Systems." *Environmental DNA* 3, no. 3: 643–653. https://doi.org/10.1002/edn3.160.

Barnes, M. A., C. R. Turner, C. L. Jerde, M. A. Renshaw, W. L. Chadderton, and D. M. Lodge. 2014. "Environmental Conditions Influence eDNA Persistence in Aquatic Systems." *Environmental Science & Technology* 48, no. 3: 1819–1827. https://doi.org/10.1021/es404734p.

Bell, K. L., M. Campos, B. D. Hoffmann, F. Encinas-Viso, G. C. Hunter, and B. L. Webber. 2024. "Environmental DNA Methods for Biosecurity and Invasion Biology in Terrestrial Ecosystems: Progress, Pitfalls, and Prospects." *Science of the Total Environment* 926: 171810. https://doi.org/10.1016/j.scitotenv.2024.171810.

Biggs, J., N. Ewald, A. Valentini, et al. 2014. *Analytical and Methodological Development for Improved Surveillance of the Great Crested Newt. Defra Project WC1067.* Freshwater Habitats Trust.

Bogler, A., A. Packman, A. Furman, et al. 2020. "Rethinking Wastewater Risks and Monitoring in Light of the COVID-19 Pandemic." *Nature Sustainability* 3, no. 12: 981–990. https://doi.org/10.1038/s41893-020-00605-2.

Bohmann, K., and C. Lynggaard. 2023. "Transforming Terrestrial Biodiversity Surveys Using Airborne eDNA." *Trends in Ecology & Evolution* 38, no. 2: 119–121. https://doi.org/10.1016/j.tree.2022.11.006.

Bonicalza, S., E. Valsecchi, E. Coppola, V. Catapano, and H. Thatcher. 2024. "Citizen Science in eDNA Monitoring for Mediterranean Monk Seal Conservation." *BMC Ecology and Evolution* 24, no. 1: 148. https://doi.org/10.1186/s12862-024-02338-8.

Bossers, A., M. M. de Rooij, I. van Schothorst, F. C. Velkers, and L. A. Smit. 2024. "Detection of Airborne Wild Waterbird-Derived DNA Demonstrates Potential for Transmission of Avian Influenza Virus via Air Inlets Into Poultry Houses, the Netherlands, 2021 to 2022." *Eurosurveillance* 29, no. 40: 2400350. https://doi.org/10.2807/1560-7917.ES.2024.29.40.2400350.

Bowers, R. M., S. McLetchie, R. Knight, and N. Fierer. 2011. "Spatial Variability in Airborne Bacterial Communities Across Land-Use Types and Their Relationship to the Bacterial Communities of Potential Source Environments." *ISME Journal* 5, no. 4: 601–612. https://doi.org/10.1038/ismej.2010.167.

Brennan, G. L., C. Potter, N. de Vere, et al. 2019. "Temperate Airborne Grass Pollen Defined by Spatio-Temporal Shifts in Community Composition." *Nature Ecology & Evolution* 3, no. 5: 750–754. https://doi.org/10.1038/s41559-019-0849-7.

Burian, A., Q. Mauvisseau, M. Bulling, S. Domisch, S. Qian, and M. Sweet. 2021. "Improving the Reliability of eDNA Data Interpretation." *Molecular Ecology Resources* 21, no. 5: 1422–1433. https://doi.org/10.1111/1755-0998.13367.

Buxton, A. S., J. J. Groombridge, and R. A. Griffiths. 2017. "Is the Detection of Aquatic Environmental DNA Influenced by Substrate Type?" *PLoS One* 12, no. 8: e0183371. https://doi.org/10.1371/journal.pone.0183371.

Caliz, J., X. Triado-Margarit, L. Camarero, and E. O. Casamayor. 2018. "A Long-Term Survey Unveils Strong Seasonal Patterns in the Airborne Microbiome Coupled to General and Regional Atmospheric Circulations." *Proceedings of the National Academy of Sciences of the United States of America* 115, no. 48: 12229–12234. https://doi.org/10.1073/pnas.1812826115.

Chang, Y., Y. Wang, W. Li, Z. Wei, S. Tang, and R. Chen. 2023. "Mechanisms, Techniques and Devices of Airborne Virus Detection: A Review." *International Journal of Environmental Research and Public Health* 20, no. 8: 5471. https://doi.org/10.3390/ijerph20085471.

Chia, P. Y., K. K. Coleman, Y. K. Tan, et al. 2020. "Detection of Air and Surface Contamination by SARS-CoV-2 in Hospital Rooms of Infected Patients." *Nature Communications* 11, no. 1: 2800. https://doi.org/10.1038/s41467-020-16670-2.

Childress, J., C. L. Faust, and K. Deiner. 2024. "Introduction to Special Issue: Advancing Disease Ecology Through eDNA Monitoring of Infectious Agents." *Environmental DNA* 6, no. 1: e502. https://doi.org/10.1002/edn3.502.

Clare, E. L., C. K. Economou, F. J. Bennett, et al. 2022. "Measuring Biodiversity From DNA in the Air." *Current Biology* 32, no. 3: 693–700. e5. https://doi.org/10.1016/j.cub.2021.11.064.

Clare, E. L., C. K. Economou, C. G. Faulkes, et al. 2021. "eDNAir: Proof of Concept That Animal DNA Can Be Collected From Air Sampling." *PeerJ* 9: e11030. https://doi.org/10.7717/peerj.11030.

Deiner, K., J. Lopez, S. Bourne, et al. 2018. "Optimising the Detection of Marine Taxonomic Richness Using Environmental DNA Metabarcoding: The Effects of Filter Material, Pore Size and Extraction Method." *Metabarcoding and Metagenomics* 2: e28963. https://doi.org/10.3897/mbmg.2.28963.

Deiner, K., J. C. Walser, E. Mächler, and F. Altermatt. 2015. "Choice of Capture and Extraction Methods Affect Detection of Freshwater Biodiversity From Environmental DNA." *Biological Conservation* 183: 53–63. https://doi.org/10.1016/j.biocon.2014.11.018.

Després, V. R., J. A. Huffman, S. M. Burrows, et al. 2012. "Primary Biological Aerosol Particles in the Atmosphere: A Review." *Tellus B: Chemical and Physical Meteorology* 64: 15598. https://doi.org/10.3402/tellusb.v64i0.15598.

Farrell, J. A., L. Whitmore, and D. J. Duffy. 2021. "The Promise and Pitfalls of Environmental DNA and RNA Approaches for the Monitoring of Human and Animal Pathogens From Aquatic Sources." *Bioscience* 71, no. 6: 609–625. https://doi.org/10.1093/biosci/biab027.

Ficetola, G. F., C. Miaud, F. Pompanon, and P. Taberlet. 2008. "Species Detection Using Environmental DNA From Water Samples." *Biology Letters* 4, no. 4: 423–425. https://doi.org/10.1098/rsbl.2008.0118.

Foster, N. R., B. Martin, J. Hoogewerff, et al. 2023. "The Utility of Dust for Forensic Intelligence: Exploring Collection Methods and Detection Limits for Environmental DNA, Elemental and Mineralogical Analyses of Dust Samples." *Forensic Science International* 344: 111599. https://doi.org/10.1016/j.forsciint.2023.111599.

Furlan, E. M., J. Davis, and R. P. Duncan. 2020. "Identifying Error and Accurately Interpreting Environmental DNA Metabarcoding Results: A Case Study to Detect Vertebrates at Arid Zone Waterholes." *Molecular Ecology Resources* 20, no. 5: 1259–1276. https://doi.org/10.1111/1755-0998.13170.

Garrett, N., J. Watkins, C. M. Francis, et al. 2023. "Out of Thin Air: Surveying Tropical Bat Roosts Through Air Sampling of eDNA." *PeerJ* 11: e14772. https://doi.org/10.7717/peerj.14772.

Garrett, N., J. Watkins, N. B. Simmons, et al. 2023. "Airborne eDNA Documents a Diverse and Ecologically Complex Tropical Bat and Other Mammal Community." *Environmental DNA* 5, no. 2: 350–362. https://doi.org/10.1002/edn3.385.

Geller, K., and C. Partridge. 2025. "Evaluation of Two Environmental DNA (eDNA) Approaches for Monitoring Hemlock Woolly Adelgid (HWA)." *MicroPublication Biology* 2025: 10–17912. https://doi.org/10.17912/micropub.biology.001346.

Goldberg, C. S., C. R. Turner, K. Deiner, et al. 2016. "Critical Considerations for the Application of Environmental DNA Methods to Detect Aquatic Species." *Methods in Ecology and Evolution* 7, no. 11: 1299–1307. https://doi.org/10.1111/2041-210x.12595.

Gonzalez, A., P. Vihervaara, P. Balvanera, et al. 2024. "A Global Biodiversity Observing System to Unite Monitoring and Guide Action." *Nature Ecology & Evolution* 8, no. 1: 175. https://doi.org/10.1038/s41559-023-02263-x.

Goodwin, K. D., C. M. Aiello, M. Weise, et al. 2024. *National Aquatic Environmental DNA Strategy*. T. White House Office of Science, and Policy (OSTP).

Gregoric, M., D. Kutnjak, K. Bacnik, et al. 2022. "Spider Webs as eDNA Samplers: Biodiversity Assessment Across the Tree of Life." *Molecular Ecology Resources* 22, no. 7: 2534–2545. https://doi.org/10.1111/1755-0998.13629.

Gusareva, E. S., N. E. Gaultier, A. Uchida, et al. 2022. "Short-Range Contributions of Local Sources to Ambient Air." *PNAS Nexus* 1, no. 2: pgac043. https://doi.org/10.1093/pnasnexus/pgac043.

Handsley-Davis, M., E. Kowal, L. Russell, and L. S. Weyrich. 2021. "Researchers Using Environmental DNA Must Engage Ethically With Indigenous Communities." *Nature Ecology & Evolution* 5, no. 2: 146–148. https://doi.org/10.1038/s41559-020-01351-6.

Hanson, M., G. Petch, B. Adams-Groom, T. B. Ottosen, and C. A. Skjoth. 2024. "Storms Facilitate Airborne DNA From Leaf Fragments Outside the Main Tree Pollen Season." *Aerobiologia* 40: 415–423. https://doi.org/10.1007/s10453-024-09826-w.

Harrison, J. B., J. M. Sunday, and S. M. Rogers. 2019. "Predicting the Fate of eDNA in the Environment and Implications for Studying Biodiversity." *Proceedings of the Royal Society B* 286, no. 1915: 20191409. https://doi.org/10.1098/rspb.2019.1409.

Havill, N. P., S. Shiyake, A. L. Galloway, et al. 2016. "Ancient and Modern Colonization of North America by Hemlock Woolly Adelgid, (Hemiptera: Adelgidae), an Invasive Insect From East Asia." *Molecular Ecology* 25, no. 9: 2065–2080. https://doi.org/10.1111/mec.13589.

Hudson, M., A. Thompson, P. Wilcox, et al. 2021. *Te Nohonga Kaitiaki Guidelines for Genomic Research on Taonga Species*. Te Kotahi Research Institute.

Hutchins, L., A. Mc Cartney, N. Graham, R. Gillespie, and A. Guzman. 2023. "Arthropods Are Kin: Operationalizing Indigenous Data Sovereignty to Respectfully Utilize Genomic Data From Indigenous Lands." *Molecular Ecology Resources* 25: e13822. https://doi.org/10.1111/1755-0998.13822.

Isley, C. F., K. L. Fry, E. L. Sharp, and M. P. Taylor. 2022. "Bringing Citizen Science to Life: Evaluation of a National Citizen Science Program for Public Benefit." *Environmental Science & Policy* 134: 23–33. https://doi.org/10.1016/j.envsci.2022.03.015.

Jager, H., K. B. Trimbos, J. M. Luursema, A. G. C. L. Speksnijder, and K. A. Stewart. 2025. "A Breath of Fresh Air: Comparative Evaluation of Passive Versus Active Airborne eDNA Sampling Strategies. bioRxiv." https://doi.org/10.1101/2025.03.26.645491.

Jane, S. F., T. M. Wilcox, K. S. McKelvey, et al. 2015. "Distance, Flow and PCR Inhibition: eDNA Dynamics in Two Headwater Streams." *Molecular Ecology Resources* 15, no. 1: 216–227. https://doi.org/10.1111/1755-0998.12285.

Jarman, S. N., O. Berry, and M. Bunce. 2018. "The Value of Environmental DNA Biobanking for Long-Term Biomonitoring." *Nature Ecology & Evolution* 2, no. 8: 1192–1193. https://doi.org/10.1038/s41559-018-0614-3.

Jerde, C. L. 2021. "Can We Manage Fisheries With the Inherent Uncertainty From eDNA?" *Journal of Fish Biology* 98, no. 2: 341–353. https://doi.org/10.1111/jfb.14218.

Jo, T., and T. Minamoto. 2021. "Complex Interactions Between Environmental DNA (eDNA) State and Water Chemistries on eDNA Persistence Suggested by Meta-Analyses." *Molecular Ecology Resources* 21, no. 5: 1490–1503. https://doi.org/10.1111/1755-0998.13354.

Johnson, M., and M. A. Barnes. 2024. "Macrobial Airborne Environmental DNA Analysis: A Review of Progress, Challenges, and Recommendations for an Emerging Application." *Molecular Ecology Resources* 24, no. 7: e13998. https://doi.org/10.1111/1755-0998.13998.

Johnson, M. D., M. A. Barnes, N. R. Garrett, and E. L. Clare. 2023. "Answers Blowing in the Wind: Detection of Birds, Mammals, and Amphibians With Airborne Environmental DNA in a Natural Environment Over a Yearlong Survey." *Environmental DNA* 5, no. 2: 375–387. https://doi.org/10.1002/edn3.388.

Johnson, M. D., R. D. Cox, and M. A. Barnes. 2019a. "Analyzing Airborne Environmental DNA: A Comparison of Extraction Methods, Primer Type, and Trap Type on the Ability to Detect Airborne eDNA From Terrestrial Plant Communities." *Environmental DNA* 1, no. 2: 176–185. https://doi.org/10.1002/edn3.19.

Johnson, M. D., R. D. Cox, and M. A. Barnes. 2019b. "The Detection of a Non-Anemophilous Plant Species Using Airborne eDNA." *PLoS One* 14, no. 11: e0225262. https://doi.org/10.1371/journal.pone.0225262.

Johnson, M. D., R. D. Cox, B. A. Grisham, D. Lucia, and M. A. Barnes. 2021. "Airborne eDNA Reflects Human Activity and Seasonal Changes on a Landscape Scale." *Frontiers in Environmental Science* 8: 563431. https://doi.org/10.3389/fenvs.2020.563431.

Johnson, M. D., M. Fokar, R. D. Cox, and M. A. Barnes. 2021. "Airborne Environmental DNA Metabarcoding Detects More Diversity, With Less Sampling Effort, Than a Traditional Plant Community Survey." *BMC Ecology and Evolution* 21, no. 1: 218. https://doi.org/10.1186/s12862-021-01947-x.

Kestel, J. H., D. L. Field, P. W. Bateman, et al. 2022. "Applications of Environmental DNA (eDNA) in Agricultural Systems: Current Uses, Limitations and Future Prospects." *Science of the Total Environment* 847: 157556. https://doi.org/10.1016/j.scitotenv.2022.157556.

Kieslinger, B., T. Schäfer, F. Heigl, D. Dörler, A. Richter, and A. Bonn. 2017. "The Challenge of Evaluation: An Open Framework for Evaluating Citizen Science Activities. SocArXiv." https://doi.org/10.31235/osf.io/enzc9.

Klymus, K. E., C. M. Merkes, M. J. Allison, et al. 2020. "Reporting the Limits of Detection and Quantification for Environmental DNA Assays." *Environmental DNA* 2, no. 3: 271–282. https://doi.org/10.1002/edn3.29.

Kraaijeveld, K., L. A. de Weger, M. Ventayol García, et al. 2015. "Efficient and Sensitive Identification and Quantification of Airborne Pollen Using Next-Generation DNA Sequencing." *Molecular Ecology Resources* 15, no. 1: 8–16. https://doi.org/10.1111/1755-0998.12288.

Kukutai, T., and A. Black. 2024. "CARE-Ing for Indigenous Nonhuman Genomic Data—Rethinking Our Approach." *Science* 385, no. 6708: eadr2493. https://doi.org/10.1126/science.adr2493.

Lennartz, C., J. Kurucar, S. Coppola, et al. 2021. "Geographic Source Estimation Using Airborne Plant Environmental DNA in Dust." *Scientific Reports* 11, no. 1: 16238. https://doi.org/10.1038/s41598-021-95702-3.

Littlefair, J. E., J. J. Allerton, A. S. Brown, et al. 2023. "Air-Quality Networks Collect Environmental DNA With the Potential to Measure Biodiversity at Continental Scales." *Current Biology* 33, no. 11: R426–R428. https://doi.org/10.1016/j.cub.2023.04.036.

Lodge, D. M. 2022. "Policy Action Needed to Unlock eDNA Potential." *Frontiers in Ecology and the Environment* 20, no. 8: 448–449. https://doi.org/10.1002/fee.2563.

Lynggaard, C., M. F. Bertelsen, C. V. Jensen, et al. 2022. "Airborne Environmental DNA for Terrestrial Vertebrate Community Monitoring." *Current Biology* 32, no. 3: 701–707.e5. https://doi.org/10.1016/j.cub.2021.12.014.

Lynggaard, C., T. G. Frøslev, M. S. Johnson, M. T. Olsen, and K. Bohmann. 2024. "Airborne Environmental DNA Captures Terrestrial Vertebrate Diversity in Nature." *Molecular Ecology Resources* 24, no. 1: e13840. https://doi.org/10.1111/1755-0998.13840.

Madden, A. A., A. Barberan, M. A. Bertone, H. L. Menninger, R. R. Dunn, and N. Fierer. 2016. "The Diversity of Arthropods in Homes Across the United States as Determined by Environmental DNA Analyses." *Molecular Ecology* 25, no. 24: 6214–6224. https://doi.org/10.1111/mec.13900.

McClenaghan, B., Z. G. Compson, and M. Hajibabaei. 2020. "Validating Metabarcoding-Based Biodiversity Assessments With Multi-Species Occupancy Models: A Case Study Using Coastal Marine eDNA." *PLoS One* 15, no. 3: e0224119. https://doi.org/10.1371/journal.pone.0224119.

McDevitt, J. J., P. S. J. Lees, W. G. Merz, and K. J. Schwab. 2007. "Inhibition of Quantitative PCR Analysis of Fungal Conidia Associated With Indoor Air Particulate Matter." *Aerobiologia* 23, no. 1: 35–45. https://doi.org/10.1007/s10453-006-9047-6.

Minamoto, T., T. Naka, K. Moji, and A. Maruyama. 2016. "Techniques for the Practical Collection of Environmental DNA: Filter Selection, Preservation, and Extraction." *Limnology* 17, no. 1: 23–32. https://doi.org/10.1007/s10201-015-0457-4.

Morisette, J., S. Burgiel, K. Brantley, et al. 2021. "Strategic Considerations for Invasive Species Managers in the Utilization of Environmental DNA (eDNA): Steps for Incorporating This Powerful Surveillance Tool." *Management of Biological Invasions* 12, no. 3: 747–775. https://doi.org/10.3391/mbi.2021.12.3.15.

Newton, J. P., M. E. Allentoft, P. W. Bateman, M. van der Heyde, and P. Nevill. 2025. "Targeting Terrestrial Vertebrates With eDNA: Trends, Perspectives, and Considerations for Sampling." *Environmental DNA* 7, no. 1: e70056. https://doi.org/10.1002/edn3.70056.

Newton, J. P., P. Nevill, P. W. Bateman, M. A. Campbell, and M. E. Allentoft. 2024. "Spider Webs Capture Environmental DNA From Terrestrial Vertebrates." *Iscience* 27, no. 2: 108904. https://doi.org/10.1016/j.isci.2024.108904.

Palmer, J. R. B., A. Oltra, F. Collantes, et al. 2017. "Citizen Science Provides a Reliable and Scalable Tool to Track Disease-Carrying Mosquitoes." *Nature Communications* 8: 916. https://doi.org/10.1038/s41467-017-00914-9.

Pawlowski, J., L. Apothéloz-Perret-Gentil, and F. Altermatt. 2020. "Environmental DNA: What's Behind the Term? Clarifying the Terminology and Recommendations for Its Future Use in Biomonitoring." *Molecular Ecology* 29, no. 22: 4258–4264. https://doi.org/10.1111/mec.15643.

Pepinelli, M., A. J. Biganzoli-Rangel, K. Lunn, et al. 2025. "Innovative Airborne DNA Approach for Monitoring Honey Bee Foraging and Health. bioRxiv." https://doi.org/10.1101/2025.04.17.649222.

Polling, M., R. Buij, I. Laros, and G. A. de Groot. 2024. "Continuous Daily Sampling of Airborne eDNA Detects All Vertebrate Species Identified by Camera Traps." *Environmental DNA* 6, no. 4: e591. https://doi.org/10.1002/edn3.591.

Ratnasingham, S., and P. D. N. Hebert. 2007. "BOLD: The Barcode of Life Data System." *Molecular Ecology Notes* 7, no. 3: 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x. https://www.barcodinglife.org.

Rodriguez-Ezpeleta, N., O. Morissette, C. W. Bean, et al. 2021. "Trade-Offs Between Reducing Complex Terminology and Producing Accurate Interpretations From Environmental DNA: Comment on "Environmental DNA: What's Behind the Term?" By Pawlowski et al. (2020)." *Molecular Ecology* 30, no. 19: 4601–4605. https://doi.org/10.1111/mec.15942.

Roger, F., H. R. Ghanavi, N. Danielsson, et al. 2022. "Airborne Environmental DNA Metabarcoding for the Monitoring of Terrestrial Insects—A Proof of Concept From the Field." *Environmental DNA* 4, no. 4: 790–807. https://doi.org/10.1002/edn3.290.

Rowney, F. M., G. L. Brennan, C. A. Skjøth, et al. 2021. "Environmental DNA Reveals Links Between Abundance and Composition of Airborne Grass Pollen and Respiratory Health." *Current Biology* 31, no. 9: 1995–2003.e4. https://doi.org/10.1016/j.cub.2021.02.019.

Runnel, K., P. Lohmus, K. Kuengas, et al. 2024. "Aerial eDNA Contributes Vital Information for Fungal Biodiversity Assessment." *Journal of Applied Ecology* 61: 2418–2429. https://doi.org/10.1111/1365-2664.14691.

Sanders, M., R. Tardani, A. Locher, K. Geller, and C. G. Partridge. 2023. "Development of Novel Early Detection Technology for Hemlock Woolly Adelgid, *Adelges tsugae* (Hemiptera: Adelgidae)." *Journal of Economic Entomology* 116, no. 1: 168–180. https://doi.org/10.1093/jee/toac175.

Sbrocchi, C. 2015. *Building Australia Through Citizen Science*. Office of the Chief Scientist Occasional Paper Series.

Schlegel, M., A. D. Treindl, J. Panziera, et al. 2024. "A Case Study on the Application of Spore Sampling for the Monitoring of Macrofungi." *Molecular Ecology Resources* 24, no. 4: e13941. https://doi.org/10.1111/1755-0998.13941.

Schmid, S., N. Straube, C. Albouy, et al. 2025. "Combining eDNA and Museomics to Enhance Biodiversity Monitoring. EcoEvoRxiv." https://doi.org/10.32942/X2J05G.

Sepulveda, A. J., P. R. Hutchins, M. Forstchen, M. N. Mckeefry, and A. M. Swigris. 2020. "The Elephant in the Lab (And Field): Contamination in Aquatic Environmental DNA Studies." *Frontiers in Ecology and Evolution* 8: 609973. https://doi.org/10.3389/fevo.2020.609973.

Sepulveda, A. J., N. M. Nelson, C. L. Jerde, and G. Luikart. 2020. "Are Environmental DNA Methods Ready for Aquatic Invasive Species Management?" *Trends in Ecology & Evolution* 35, no. 8: 668–678. https://doi.org/10.1016/j.tree.2020.03.011.

Shogren, A. J., J. L. Tank, E. Andruszkiewicz, et al. 2017. "Controls on eDNA Movement in Streams: Transport, Retention, and Resuspension." *Scientific Reports* 7, no. 1: 5065. https://doi.org/10.1038/s41598-017-05223-1.

Takahashi, M., T. G. Frøslev, J. Paupério, et al. 2025. "A Metadata Checklist and Data Formatting Guidelines to Make eDNA FAIR (Findable, Accessible, Interoperable, and Reusable)." Environmental DNA 7, NO. 3. Portico. https://doi.org/10.1002/edn3.70100.

Tignat-Perrier, R., A. Dommergue, A. Thollot, et al. 2019. "Global Airborne Microbial Communities Controlled by Surrounding Landscapes and Wind Conditions." *Scientific Reports* 9: 14441. https://doi.org/10.1038/s41598-019-51073-4.

Tournayre, O., J. E. Littlefair, N. R. Garrett, et al. 2025. "First National Survey of Terrestrial Biodiversity Using Airborne eDNA." *Scientific Reports* 15, no. 1: 19247. https://doi.org/10.1038/s41598-025-03650-z.

Trujillo-González, A., D. N. Thuo, U. Divi, K. Sparks, T. Wallenius, and D. Gleeson. 2022. "Detection of Khapra Beetle Environmental DNA Using Portable Technologies in Australian Biosecurity." *Frontiers in Insect Science* 2: 795379. https://doi.org/10.3389/finsc.2022.795379.

Whittington, J., K. Heuer, B. Hunt, M. Hebblewhite, and P. M. Lukacs. 2015. "Estimating Occupancy Using Spatially and Temporally Replicated Snow Surveys." *Animal Conservation* 18, no. 1: 92–101. https://doi.org/10.1111/acv.12140.

Wilcox, P. L., J. A. Charity, M. R. Roberts, et al. 2008. "A Values-Based Process for Cross-Cultural Dialogue Between Scientists and Maori." *Journal of the Royal Society of New Zealand* 38, no. 3: 215–227. https://doi.org/10.1080/03014220809510555.

Xu, C. C. Y., I. J. Yen, D. Bowman, and C. R. Turner. 2015. "Spider Web DNA: A New Spin on Noninvasive Genetics of Predator and Prey." *PLoS One* 10, no. 11: e0142503. https://doi.org/10.1371/journal.pone.0142503.