Winds of change: Charting a pathway to ecosystem monitoring using airborne environmental DNA

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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 realise 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 emphasised 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, harmonising 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.

Keywords: airborne eDNA, biodiversity, biosecurity, monitoring, terrestrial, Southern eDNA Society, implementation, aerobiology, aeolian, conservation

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 & Lynggaard, 2023; Johnson & 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 analyse 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 et al., 2023a), 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 et al., 2021a; 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), emphasising the importance of quantifying methodological impacts on data robustness, repeatability, and reliability. Recognising

this momentum, Johnson & 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 are 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 summarise the workshop outputs, provide insights into the advances and future directions of airborne eDNA technology, and offer a workshop statement to summarise current community consensus on the emerging field (see Box 1).

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."

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; Lynggaard et al., 2022; Bohmann & Lynggaard, 2023; Lynggaard et al., 2024). 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 et al., 2021b; Roger et al., 2022) and complementary forms of eDNA (Runnel et al., 2024), airborne eDNA may improve 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 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 & Partridge, 2025), a species native to Japan that has established 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 behaviour, 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.

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, standardised 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 et al., 2023a), 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 durable, low-maintenance, and tamper resistant, with modular or customisable 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 stabilisation, 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 controls 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 standardised 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 minimise 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, standardised 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 optimise 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.

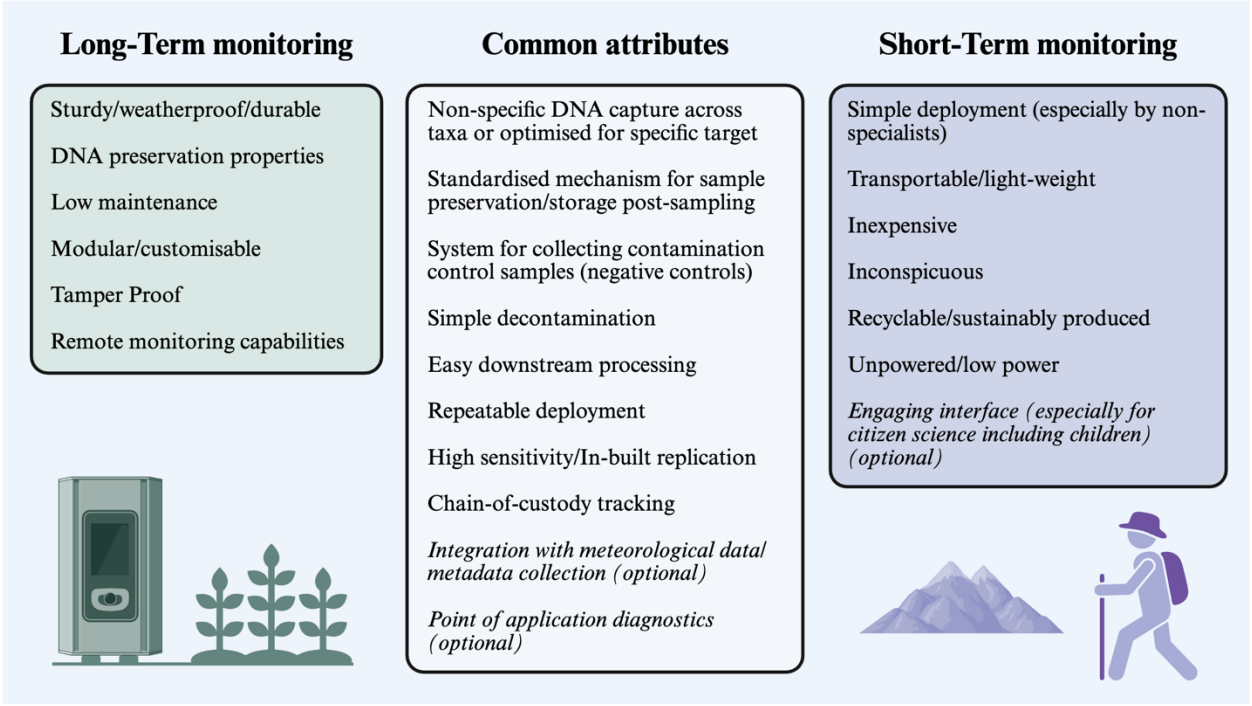

Figure 1. Key attributes of airborne eDNA collection platforms

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 prioritise durability, sample integrity, low maintenance, and modular/customisable 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 emphasise 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, standardised 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.

Advancing understanding of factors influencing airborne eDNA detection

A comprehensive understanding of environmental, ecological, and technical parameters is critical for optimising airborne eDNA monitoring. Table 1 summarises 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; Shogren et al., 2017; Harrison et al., 2019; Barnes et al., 2021; Jo & 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 et al., 2021a; 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 contextualise 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; Deiner et al., 2015; Barnes et al., 2021) 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 uncharacterised detection error. Thus, investigation and validation of a diverse range of parameters will be essential for progressing the utility of airborne eDNA analysis (Atkinson & Roy, 2023; Bohmann & 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.

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

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.

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) [d] Sampling and extraction effects in freshwater systems (Deiner et al., 2015) [d]
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 et al., 2021a) Environmental influence over eDNA particle size in freshwater systems (Barnes et al., 2021) [d] eDNA persistence in controlled freshwater system (Barnes et al., 2014) [d]

Ecology of target species	• Habitat • Behaviour • 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) [d] Improving reliability of eDNA data interpretation using statistical models (Burian et al., 2021)

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 et al., 2023a; Garrett et al., 2023b). 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 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 et al., 2020a), 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 these controls, regional baseline monitoring, through both targeted eDNA surveys and conventional biodiversity assessments, can help contextualise 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 μm). 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 et al., 2023a; Garrett et al., 2023b). In these studies, Garrett et al (2023a/b) 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 artefacts 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-characterised 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 characterised 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 optimisation. 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-characterised systems where reference databases are comprehensive. Initiatives such as the 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 & 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 utilising eDNA data when it offers a valuable, non-invasive tool for biodiversity and biosecurity monitoring (Jerde, 2021).

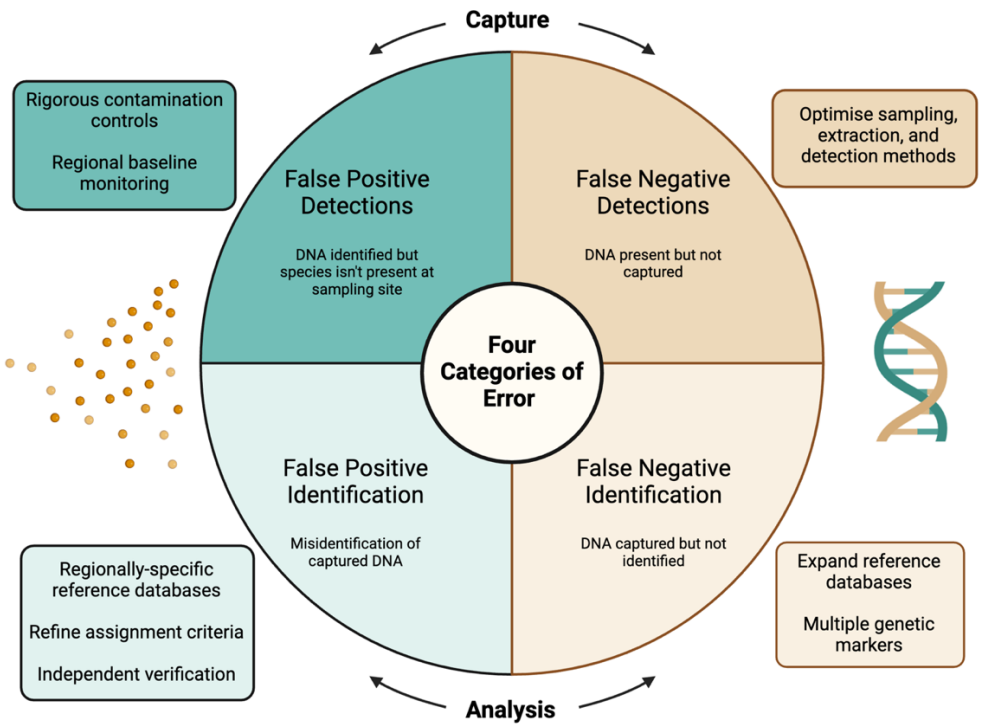

Figure 2. Framework for addressing errors in airborne eDNA analysis

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.

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 ensures 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 prioritise 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 & Black, 2024). 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 et al., 2020b). 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 emphasise 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 et al., 2021b), and acoustic monitoring (Garrett et al., 2023a), 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, modelled on those established in aquatic eDNA studies (Deiner et al., 2015; Goldberg et al., 2016; Minamoto et al., 2016; Deiner et al., 2018), 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 emphasise 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 maximising the long-term impact of airborne eDNA initiatives, fostering a shared commitment to biodiversity monitoring and conservation.

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, realising 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 revolutionise biodiversity monitoring by standardising 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 revolutionise environmental monitoring, offering innovative ways to observe and protect ecosystems. To realise 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.

References

Aalismail, N. A., Díaz-Rúa, R., Geraldi, N., Cusack, M., & Duarte, C. M. (2021). Diversity and Sources of Airborne Eukaryotic Communities (AEC) in the Global Dust Belt over the Red Sea. *Earth Systems and Environment*, *5*(2), 459-471. https://doi.org/10.1007/s41748-021-00219-4

Anees-Hill, S., Douglas, P., Pashley, C. H., Hansell, A., & Marczylo, E. L. (2022). A systematic review of outdoor airborne fungal spore seasonality across Europe and the implications for health. *Science of the Total Environment*, *818*. https://doi.org/10.1016/j.scitotenv.2021.151716

Atkinson, C. T., & Roy, K. (2023). Environmental monitoring for invasive fungal pathogens of ʻŌhiʻa (*Metrosideros polymorpha*) on the Island of Hawaiʻi. *Biological invasions*, *25*(2), 399-410. https://doi.org/10.1007/s10530-022-02922-3

Banchi, E., Ametrano, C. G., Tordoni, E., Stanković, D., Ongaro, S., Tretiach, M., Pallavicini, A., Muggia, L., Verardo, P., Tassan, F., Trobiani, N., Moretti, O., Borney, M. F., & Lazzarin, S. (2020). Environmental DNA assessment of airborne plant and fungal seasonal diversity. *The Science of the Total Environment*, *738*, 140249-140249. https://doi.org/10.1016/j.scitotenv.2020.140249

Barberán, A., Ladau, J., Leff, J. W., Pollard, K. S., Menninger, H. L., Dunn, R. R., & Fierer, N. (2015). Continental-scale distributions of dust-associated bacteria and fungi. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(18), 5756-5761. https://doi.org/10.1073/pnas.1420815112

Barnes, M. A., Chadderton, W. L., Jerde, C. L., Mahon, A. R., Turner, C. R., & Lodge, D. M. (2021). Environmental conditions influence eDNA particle size distribution in aquatic systems. *Environmental DNA*, *3*(3), 643-653. https://doi.org/10.1002/edn3.160

Barnes, M. A., Turner, C. R., Jerde, C. L., Renshaw, M. A., Chadderton, W. L., & Lodge, D. M. (2014). Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science & Technology*, *48*(3), 1819-1827. https://doi.org/10.1021/es404734p

Bell, K. L., Campos, M., Hoffmann, B. D., Encinas-Viso, F., Hunter, G. C., & Webber, B. L. (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., Ewald, N., Valentini, A., Gaboriaud, C., Griffiths, R., Foster, J., Wilkinson, J., Arnett, A., Williams, P., & Dunn, F. (2014). Analytical and methodological development for improved surveillance of the Great Crested Newt. *Defra Project WC1067*.

Bogler, A., Packman, A., Furman, A., Gross, A., Kushmaro, A., Ronen, A., Dagot, C., Hill, C., Vaizel-Ohayon, D., Morgenroth, E., Bertuzzo, E., Wells, G., Kiperwas, H. R., Horn, H., Negev, I., Zucker, I., Bar-Or, I., Moran-Gilad, J., Balcazar, J. L.,…Bar-Zeev, E. (2020). Rethinking wastewater risks and monitoring in light of the COVID-19 pandemic. *Nature Sustainability*, *3*(12), 981-990. https://doi.org/10.1038/s41893-020-00605-2

Bohmann, K., & Lynggaard, C. (2023). Transforming terrestrial biodiversity surveys using airborne eDNA. *Trends in Ecology & Evolution*, *38*(2), 119-121. https://doi.org/10.1016/j.tree.2022.11.006

Bonicalza, S., Valsecchi, E., Coppola, E., Catapano, V., & Thatcher, H. (2024). Citizen science in eDNA monitoring for Mediterranean monk seal conservation. *BMC Ecology and Evolution*, *24*(1), 148. https://doi.org/10.1186/s12862-024-02338-8

Bossers, A., de Rooij, M. M., van Schothorst, I., Velkers, F. C., & Smit, L. A. (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*(40). https://doi.org/10.2807/1560-7917.ES.2024.29.40.2400350

Bowers, R. M., McLetchie, S., Knight, R., & Fierer, N. (2011). Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. *ISME J*, *5*(4), 601-612. https://doi.org/10.1038/ismej.2010.167

Brennan, G. L., Potter, C., de Vere, N., Griffith, G. W., Skjoth, C. A., Osborne, N. J., Wheeler, B. W., McInnes, R. N., Clewlow, Y., Barber, A., Hanlon, H. M., Hegarty, M., Jones, L., Kurganskiy, A., Rowney, F. M., Armitage, C., Adams-Groom, B., Ford, C. R., Petch, G. M.,…Creer, S. (2019). Temperate airborne grass pollen defined by spatio-temporal shifts in community composition. *Nature Ecology & Evolution*, *3*(5), 750-754. https://doi.org/10.1038/s41559-019-0849-7

Burian, A., Mauvisseau, Q., Bulling, M., Domisch, S., Qian, S., & Sweet, M. (2021). Improving the reliability of eDNA data interpretation. *Molecular Ecology Resources*, *21*(5), 1422-1433. https://doi.org/10.1111/1755-0998.13367

Buxton, A. S., Groombridge, J. J., & Griffiths, R. A. (2017). Is the detection of aquatic environmental DNA influenced by substrate type? *PLoS One*, *12*(8), e0183371. https://doi.org/10.1371/journal.pone.0183371

Caliz, J., Triado-Margarit, X., Camarero, L., & Casamayor, E. O. (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 USA*, *115*(48), 12229-12234. https://doi.org/10.1073/pnas.1812826115

573 Chang, Y., Wang, Y., Li, W., Wei, Z., Tang, S., & Chen, R. (2023). Mechanisms, Techniques and
574 Devices of Airborne Virus Detection: A Review. *International Journal of Environmental*
575 *Research and Public Health*, *20*(8). https://doi.org/10.3390/ijerph20085471

576 Chia, P. Y., Coleman, K. K., Tan, Y. K., Ong, S. W. X., Gum, M., Lau, S. K., Lim, X. F., Lim, A. S.,
577 Sutjipto, S., Lee, P. H., Son, T. T., Young, B. E., Milton, D. K., Gray, G. C., Schuster, S.,
578 Barkham, T., De, P. P., Vasoo, S., Chan, M.,…Singapore Novel Coronavirus Outbreak Research,
579 T. (2020). Detection of air and surface contamination by SARS-CoV-2 in hospital rooms of
580 infected patients. *Nature Communications*, *11*(1), 2800. https://doi.org/10.1038/s41467-020-
581 16670-2

582 Childress, J., Faust, C. L., & Deiner, K. (2024). Introduction to special issue: Advancing disease ecology
583 through eDNA monitoring of infectious agents. *Environmental DNA*, *6*(1).
584 https://doi.org/10.1002/edn3.502

585 Clare, E. L., Economou, C. K., Bennett, F. J., Dyer, C. E., Adams, K., McRobie, B., Drinkwater, R., &
586 Littlefair, J. E. (2022). Measuring biodiversity from DNA in the air. *Current Biology*, *32*(3), 693-
587 700.e695. https://doi.org/10.1016/j.cub.2021.11.064

588 Clare, E. L., Economou, C. K., Faulkes, C. G., Gilbert, J. D., Bennett, F., Drinkwater, R., & Littlefair, J.
589 E. (2021). eDNAir: proof of concept that animal DNA can be collected from air sampling. *PeerJ*,
590 *9*, e11030-e11030. https://doi.org/10.7717/peerj.11030

591 Clot, B., Gilge, S., Hajkova, L., Magyar, D., Scheifinger, H., Sofiev, M., Bütler, F., & Tummon, F.
592 (2024). The EUMETNET AutoPollen programme: establishing a prototype automatic pollen
593 monitoring network in Europe. *Aerobiologia*, *40*(1), 3-11. https://doi.org/10.1007/s10453-020-
594 09666-4

595 Deiner, K., Lopez, J., Bourne, S., Holman, L. E., Seymour, M., Grey, E. K., Lacoursière-Roussel, A., Li,
596 Y., Renshaw, M. A., & Pfrender, M. E. (2018). Optimising the detection of marine taxonomic
597 richness using environmental DNA metabarcoding: the effects of filter material, pore size and
598 extraction method. *Metabarcoding and Metagenomics*, *2*, e28963.
599 https://doi.org/10.3897/mbmg.2.28963

600 Deiner, K., Walser, J. C., Mächler, E., & Altermatt, F. (2015). Choice of capture and extraction methods
601 affect detection of freshwater biodiversity from environmental DNA. *Biological Conservation*,
602 *183*, 53-63. https://doi.org/10.1016/j.biocon.2014.11.018

603 Després, V. R., Huffman, J. A., Burrows, S. M., Hoose, C., Safatov, A. S., Buryak, G., Fröhlich-
604 Nowoisky, J., Elbert, W., Andreae, M. O., Pöschl, U., & Jaenicke, R. (2012). Primary biological
605 aerosol particles in the atmosphere: a review. *Tellus Series B-Chemical and Physical*
606 *Meteorology*, *64*. https://doi.org/10.3402/tellusb.v64i0.15598

607 Farrell, J. A., Whitmore, L., & Duffy, D. J. (2021). The Promise and Pitfalls of Environmental DNA and
608 RNA Approaches for the Monitoring of Human and Animal Pathogens from Aquatic Sources.
609 *Bioscience*, *71*(6), 609-625. https://doi.org/10.1093/biosci/biab027

610 Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental
611 DNA from water samples. *Biology Letters*, *4*(4), 423-425. https://doi.org/10.1098/rsbl.2008.0118

612 Foster, N. R., Martin, B., Hoogewerff, J., Aberle, M. G., de Caritat, P., Roffey, P., Edwards, R., Malik,
613 A., Thwaites, P., Waycott, M., & Young, J. (2023). The utility of dust for forensic intelligence:
614 Exploring collection methods and detection limits for environmental DNA, elemental and
615 mineralogical analyses of dust samples. *Forensic Science International*, *344*, 111599-111599.
616 https://doi.org/10.1016/j.forsciint.2023.111599

617 Furlan, E. M., Davis, J., & Duncan, R. P. (2020). Identifying error and accurately interpreting
618 environmental DNA metabarcoding results: A case study to detect vertebrates at arid zone
619 waterholes. *Molecular Ecology Resources*, *20*(5), 1259-1276. https://doi.org/10.1111/1755-
620 0998.13170

621 Garrett, N., Watkins, J., Francis, C. M., Simmons, N. B., Ivanova, N., Naaum, A., Briscoe, A.,
622 Drinkwater, R., & Clare, E. L. (2023a). Out of thin air: surveying tropical bat roosts through air
623 sampling of eDNA. *PeerJ*, *11*, e14772-e14772. https://doi.org/10.7717/peerj.14772

624 Garrett, N., Watkins, J., Simmons, N. B., Fenton, B., Maeda-Obregon, A., Sanchez, D. E., Froehlich, E.
625 M., Walker, F. M., Littlefair, J. E., & Clare, E. L. (2023b). Airborne eDNA documents a diverse
626 and ecologically complex tropical bat and other mammal community. *Environmental DNA*, *5*(2),
627 350-362. https://doi.org/10.1002/edn3.385

628 Geller, K., & Partridge, C. (2025). Evaluation of two environmental DNA (eDNA) approaches for
629 monitoring hemlock woolly adelgid (HWA). *MicroPublication Biology*, *2025*.
630 https://doi.org/10.17912/micropub.biology.001346

631 Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., Spear, S. F.,
632 McKee, A., Oyler-McCance, S. J., Cornman, R. S., Laramie, M. B., Mahon, A. R., Lance, R. F.,
633 Pilliod, D. S., Strickler, K. M., Waits, L. P., Fremier, A. K., Takahara, T., Herder, J. E., &
634 Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to
635 detect aquatic species. *Methods in Ecology and Evolution*, *7*(11), 1299-1307.
636 https://doi.org/10.1111/2041-210x.12595

637 Gonzalez, A., Vihervaara, P., Balvanera, P., Bates, A. E., Bayraktarov, E., Bellingham, P. J., Bruder, A.,
638 Campbell, J., Catchen, M. D., Cavender-Bares, J., Chase, J., Coops, N., Costello, M. J., Czúcz,
639 B., Delavaud, A., Dornelas, M., Dubois, G., Duffy, E. J., Eggermont, H.,…Torrelio, C. Z. (2024).
640 A global biodiversity observing system to unite monitoring and guide action. *Nature Ecology &*
641 *Evolution*, *8*(1), 175-175. https://doi.org/10.1038/s41559-023-02263-x

642 Goodwin, K. D., Aiello, C. M., Weise, M., Edmondson, M., Fillingham, K., Allen, D., Amerson, A.,
643 Barton, M. L., Benson, A., Canonico, G., Gold, Z., Gumm, J., Hunter, M., Joffe, N., Lance, R.,
644 Larkin, A., Letelier, R., Lipsky, C., McCoskey, D.,…White, T. (2024). *National Aquatic*
645 *Environmental DNA Strategy*. T. White House Office of Science, and Policy (OSTP).

646 Gregoric, M., Kutnjak, D., Bacnik, K., Gostincar, C., Pecman, A., Ravnikar, M., & Kuntner, M. (2022).
647 Spider webs as eDNA samplers: Biodiversity assessment across the tree of life. *Molecular*
648 *Ecology Resources*, *22*(7), 2534-2545. https://doi.org/10.1111/1755-0998.13629

649 Gusareva, E. S., Gaultier, N. E., Uchida, A., Premkrishnan, B. N. V., Heinle, C. E., Phung, W. J., Wong,
650 A., Lau, K. J. X., Yap, Z. H., Koh, Y., Ang, P. N., Putra, A., Panicker, D., Lee, J. G. H., Neves,
651 L. C., Drautz-Moses, D. I., & Schuster, S. C. (2022). Short-range contributions of local sources to
652 ambient air. *Proceedings of the National Academy of Sciences Nexus*, *1*(2).
653 https://doi.org/10.1093/pnasnexus/pgac043

Handsley-Davis, M., Kowal, E., Russell, L., & Weyrich, L. S. (2021). Researchers using environmental DNA must engage ethically with Indigenous communities. *Nature Ecology & Evolution*, *5*(2), 146-148. https://doi.org/10.1038/s41559-020-01351-6

Hanson, M., Petch, G., Adams-Groom, B., Ottosen, T. B., & Skjoth, C. A. (2024). Storms facilitate airborne DNA from leaf fragments outside the main tree pollen season. *Aerobiologia*. https://doi.org/10.1007/s10453-024-09826-w

Harrison, J. B., Sunday, J. M., & Rogers, S. M. (2019). Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proceedings of the Royal Society B*, *286*(1915), 20191409. https://doi.org/10.1098/rspb.2019.1409

Havill, N. P., Shiyake, S., Galloway, A. L., Foottit, R. G., Yu, G. Y., Paradis, A., Elkinton, J., Montgomery, M. E., Sano, M., & Caccone, A. (2016). Ancient and modern colonization of North America by hemlock woolly adelgid, (Hemiptera: Adelgidae), an invasive insect from East Asia. *Molecular Ecology*, *25*(9), 2065-2080. https://doi.org/10.1111/mec.13589

Hudson, M., Thompson, A., Wilcox, P., Mika, J., Battershill, C., Stott, M., Brooks, R. T., & Warbrick, L. (2021). *Te Nohonga Kaitiaki guidelines for genomic research on Taonga species*. Te Kotahi Research Institute.

Hutchins, L., Mc Cartney, A., Graham, N., Gillespie, R., & Guzman, A. (2023). Arthropods are kin: Operationalizing Indigenous data sovereignty to respectfully utilize genomic data from Indigenous lands. *Molecular Ecology Resources*. https://doi.org/10.1111/1755-0998.13822

Isley, C. F., Fry, K. L., Sharp, E. L., & Taylor, M. P. (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., Trimbos, K. B., Luursema, J. M., Speksnijder, A. G. C. L., & Stewart, K. A. (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., Wilcox, T. M., McKelvey, K. S., Young, M. K., Schwartz, M. K., Lowe, W. H., Letcher, B. H., & Whiteley, A. R. (2015). Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. *Molecular Ecology Resources*, *15*(1), 216-227. https://doi.org/10.1111/1755-0998.12285

Jarman, S. N., Berry, O., & Bunce, M. (2018). The value of environmental DNA biobanking for long-term biomonitoring. *Nature Ecology & Evolution*, *2*(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*(2), 341-353. https://doi.org/10.1111/jfb.14218

Jo, T., & Minamoto, T. (2021). Complex interactions between environmental DNA (eDNA) state and water chemistries on eDNA persistence suggested by meta-analyses. *Molecular Ecology Resources*, *21*(5), 1490-1503. https://doi.org/10.1111/1755-0998.13354

691 Johnson, M., & Barnes, M. A. (2024). Macrobial airborne environmental DNA analysis: A review of
692 progress, challenges, and recommendations for an emerging application. *Molecular Ecology*
693 *Resources*, *24*(7), e13998. https://doi.org/10.1111/1755-0998.13998

694 Johnson, M. D., Barnes, M. A., Garrett, N. R., & Clare, E. L. (2023). Answers blowing in the wind:
695 Detection of birds, mammals, and amphibians with airborne environmental DNA in a natural
696 environment over a yearlong survey. *Environmental DNA*, *5*(2), 375-387.
697 https://doi.org/10.1002/edn3.388

698 Johnson, M. D., Cox, R. D., & Barnes, M. A. (2019a). Analyzing airborne environmental DNA: A
699 comparison of extraction methods, primer type, and trap type on the ability to detect airborne
700 eDNA from terrestrial plant communities. *Environmental DNA*, *1*(2), 176-185.
701 https://doi.org/10.1002/edn3.19

702 Johnson, M. D., Cox, R. D., & Barnes, M. A. (2019b). The detection of a non-anemophilous plant species
703 using airborne eDNA. *PLoS One*, *14*(11), e0225262.
704 https://doi.org/10.1371/journal.pone.0225262

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

708 Johnson, M. D., Fokar, M., Cox, R. D., & Barnes, M. A. (2021b). Airborne environmental DNA
709 metabarcoding detects more diversity, with less sampling effort, than a traditional plant
710 community survey. *BMC Ecology and Evolution*, *21*(1), 218. https://doi.org/10.1186/s12862-021-
711 01947-x

712 Kestel, J. H., Field, D. L., Bateman, P. W., White, N. E., Allentoft, M. E., Hopkins, A. J. M., Gibberd,
713 M., & Nevill, P. (2022). Applications of environmental DNA (eDNA) in agricultural systems:
714 Current uses, limitations and future prospects. *Science of the Total Environment*, *847*, 157556.
715 https://doi.org/10.1016/j.scitotenv.2022.157556

716 Kieslinger, B., Schäfer, T., Heigl, F., Dörler, D., Richter, A., & Bonn, A. (2017). The challenge of
717 evaluation: An open framework for evaluating citizen science activities. *SocArXiv*.
718 https://doi.org/10.31235/osf.io/enzc9

719 Klymus, K. E., Merkes, C. M., Allison, M. J., Goldberg, C. S., Helbing, C. C., Hunter, M. E., Jackson, C.
720 A., Lance, R. F., Mangan, A. M., & Monroe, E. M. (2020). Reporting the limits of detection and
721 quantification for environmental DNA assays. *Environmental DNA*, *2*(3), 271-282.
722 https://doi.org/10.1002/edn3.29

723 Kraaijeveld, K., de Weger, L. A., Ventayol García, M., Buermans, H., Frank, J., Hiemstra, P. S., & den
724 Dunnen, J. T. (2015). Efficient and sensitive identification and quantification of airborne pollen
725 using next-generation DNA sequencing. *Molecular Ecology Resources*, *15*(1), 8-16.
726 https://doi.org/10.1111/1755-0998.12288

727 Kukutai, T., & Black, A. (2024). CARE-ing for Indigenous nonhuman genomic data—rethinking our
728 approach. *Science*, *385*(6708), eadr2493. https://doi.org/10.1126/science.adr2493

729 Lennartz, C., Kurucar, J., Coppola, S., Crager, J., Bobrow, J., Bortolin, L., & Comolli, J. (2021).
730 Geographic source estimation using airborne plant environmental DNA in dust. *Scientific*
731 *Reports*, *11*(1), 16238-16212. https://doi.org/10.1038/s41598-021-95702-3

732 Littlefair, J. E., Allerton, J. J., Brown, A. S., Butterfield, D. M., Robins, C., Economou, C. K., Garrett, N.
733 R., & Clare, E. L. (2023). Air-quality networks collect environmental DNA with the potential to
734 measure biodiversity at continental scales. *Current Biology*, *33*(11), R426-R428.
735 https://doi.org/10.1016/j.cub.2023.04.036

736 Lodge, D. M. (2022). Policy action needed to unlock eDNA potential. *Frontiers in Ecology and the*
737 *Environment*, *20*(8), 448-449. https://doi.org/10.1002/fee.2563

738 Lynggaard, C., Bertelsen, M. F., Jensen, C. V., Johnson, M. S., Frøslev, T. G., Olsen, M. T., & Bohmann,
739 K. (2022). Airborne environmental DNA for terrestrial vertebrate community monitoring.
740 *Current Biology*, *32*(3), 701-707.e705. https://doi.org/10.1016/j.cub.2021.12.014

741 Lynggaard, C., Frøslev, T. G., Johnson, M. S., Olsen, M. T., & Bohmann, K. (2024). Airborne
742 environmental DNA captures terrestrial vertebrate diversity in nature. *Molecular Ecology*
743 *Resources*, *24*(1), e13840-n/a. https://doi.org/10.1111/1755-0998.13840

744 Madden, A. A., Barberan, A., Bertone, M. A., Menninger, H. L., Dunn, R. R., & Fierer, N. (2016). The
745 diversity of arthropods in homes across the United States as determined by environmental DNA
746 analyses. *Molecular Ecology*, *25*(24), 6214-6224. https://doi.org/10.1111/mec.13900

747 McClenaghan, B., Compson, Z. G., & Hajibabaei, M. (2020). Validating metabarcoding-based
748 biodiversity assessments with multi-species occupancy models: A case study using coastal marine
749 eDNA. *PLoS One*, *15*(3), e0224119-e0224119. https://doi.org/10.1371/journal.pone.0224119

750 McDevitt, J. J., Lees, P. S. J., Merz, W. G., & Schwab, K. J. (2007). Inhibition of quantitative PCR
751 analysis of fungal conidia associated with indoor air particulate matter. *Aerobiologia*, *23*(1), 35-
752 45. https://doi.org/10.1007/s10453-006-9047-6

753 Minamoto, T., Naka, T., Moji, K., & Maruyama, A. (2016). Techniques for the practical collection of
754 environmental DNA: filter selection, preservation, and extraction. *Limnology*, *17*(1), 23-32.
755 https://doi.org/10.1007/s10201-015-0457-4

756 Morisette, J., Burgiel, S., Brantley, K., Daniel, W. M., Darling, J., Davis, J., Franklin, T., Gaddis, K.,
757 Hunter, M., Lance, R., Leskey, T., Passamaneck, Y., Piaggio, A., Rector, B., Sepulveda, A.,
758 Smith, M., Stepien, C. A., & Wilcox, T. (2021). Strategic considerations for invasive species
759 managers in the utilization of environmental DNA (eDNA): steps for incorporating this powerful
760 surveillance tool. *Management of Biological Invasions*, *12*(3), 747-775.
761 https://doi.org/10.3391/mbi.2021.12.3.15

762 Newton, J. P., Allentoft, M. E., Bateman, P. W., van der Heyde, M., & Nevill, P. (2025). Targeting
763 Terrestrial Vertebrates With eDNA: Trends, Perspectives, and Considerations for Sampling.
764 *Environmental DNA*, *7*(1), e70056. https://doi.org/10.1002/edn3.70056

765 Newton, J. P., Nevill, P., Bateman, P. W., Campbell, M. A., & Allentoft, M. E. (2024). Spider webs
766 capture environmental DNA from terrestrial vertebrates. *Iscience*, *27*(2).
767 https://doi.org/10.1016/j.isci.2024.108904

768 Palmer, J. R. B., Oltra, A., Collantes, F., Delgado, J. A., Lucientes, J., Delacour, S., Bengoa, M., Eritja,
769 R., & Bartumeus, F. (2017). Citizen science provides a reliable and scalable tool to track disease-
770 carrying mosquitoes. *Nature Communications*, *8*. https://doi.org/10.1038/s41467-017-00914-9

771 Pawlowski, J., Apothéloz-Perret-Gentil, L., & Altermatt, F. (2020). Environmental DNA: What's behind
772 the term? Clarifying the terminology and recommendations for its future use in biomonitoring.
773 *Molecular Ecology*, *29*(22), 4258-4264. https://doi.org/10.1111/mec.15643

774 Pepinelli, M., Biganzoli-Rangel, A. J., Lunn, K., Arteaga, P., Borges, D., Zayed, A., & Clare, E. L.
775 (2025). Innovative airborne DNA approach for monitoring honey bee foraging and health.
776 *bioRxiv*. https://doi.org/10.1101/2025.04.17.649222

777 Polling, M., Buij, R., Laros, I., & de Groot, G. A. (2024). Continuous daily sampling of airborne eDNA
778 detects all vertebrate species identified by camera traps. *Environmental DNA*, *6*(4).
779 https://doi.org/10.1002/edn3.591

780 Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The Barcode of Life Data System
781 (www.barcodinglife.org). *Molecular Ecology Notes*, *7*(3), 355-364.
782 https://doi.org/10.1111/j.1471-8286.2007.01678.x

783 Rodriguez-Ezpeleta, N., Morissette, O., Bean, C. W., Manu, S., Banerjee, P., Lacoursière-Roussel, A.,
784 Beng, K. C., Alter, S. E., Roger, F., Holman, L. E., Stewart, K. A., Monaghan, M. T.,
785 Mauvisseau, Q., Mirimin, L., Wangensteen, O. S., Antognazza, C. M., Helyar, S. J., Boer, H.,
786 Monchamp, M. E.,…Deiner, K. (2021). Trade-offs between reducing complex terminology and
787 producing accurate interpretations from environmental DNA: Comment on "Environmental
788 DNA: What's behind the term?" by Pawlowski et al., (2020). *Molecular Ecology*, *30*(19), 4601-
789 4605. https://doi.org/10.1111/mec.15942

790 Roger, F., Ghanavi, H. R., Danielsson, N., Wahlberg, N., Löndahl, J., Pettersson, L. B., Andersson, G. K.
791 S., Boke Olén, N., & Clough, Y. (2022). Airborne environmental DNA metabarcoding for the
792 monitoring of terrestrial insects—A proof of concept from the field. *Environmental DNA*, *4*(4),
793 790-807. https://doi.org/10.1002/edn3.290

794 Rowney, F. M., Brennan, G. L., Skjøth, C. A., Griffith, G. W., McInnes, R. N., Clewlow, Y., Adams-
795 Groom, B., Barber, A., de Vere, N., Economou, T., Hegarty, M., Hanlon, H. M., Jones, L.,
796 Kurganskiy, A., Petch, G. M., Potter, C., Rafiq, A. M., Warner, A., Wheeler, B.,…Creer, S.
797 (2021). Environmental DNA reveals links between abundance and composition of airborne grass
798 pollen and respiratory health. *Current Biology*, *31*(9), 1995-2003.e1994.
799 https://doi.org/10.1016/j.cub.2021.02.019

800 Runnel, K., Lohmus, P., Kuengas, K., Marmor-Ohtla, L., Palo, A., Pütsepp, G., Tamm, H., Tammekänd,
801 I., & Lohmus, A. (2024). Aerial eDNA contributes vital information for fungal biodiversity
802 assessment. *Journal of Applied Ecology*. https://doi.org/10.1111/1365-2664.14691

803 Sanders, M., Tardani, R., Locher, A., Geller, K., & Partridge, C. G. (2023). Development of Novel Early
804 Detection Technology for Hemlock Woolly Adelgid, *Adelges tsugae* (Hemiptera: Adelgidae).
805 *Journal of Economic Entomology*, *116*(1), 168-180. https://doi.org/10.1093/jee/toac175

806 Sbrocchi, C. (2015). Building Australia through Citizen Science. *Office of the Chief Scientist Occasional*
807 *Paper Series*.

Schlegel, M., Treindl, A. D., Panziera, J., Zengerer, V., Zani, D., Brannhage, J., & Gross, A. (2024). A case study on the application of spore sampling for the monitoring of macrofungi. *Molecular Ecology Resources*, *24*(4), e13941. https://doi.org/10.1111/1755-0998.13941

Schmid, S., Straube, N., Albouy, C., Delling, B., Maclaine, J., Matschiner, M., Moller, P. R., Nocita, A., Palandacic, A., Ruber, L., Sonnewald, M., Alvarez, N., Manel, S., & Pellissier, L. (2025). Combining eDNA and Museomics to Enhance Biodiversity Monitoring. *EcoEvoRxiv*. https://doi.org/10.32942/X2J05G

Sepulveda, A. J., Hutchins, P. R., Forstchen, M., Mckeefry, M. N., & Swigris, A. M. (2020a). 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., Nelson, N. M., Jerde, C. L., & Luikart, G. (2020b). Are environmental DNA methods ready for aquatic invasive species management? *Trends in Ecology & Evolution*, *35*(8), 668-678. https://doi.org/10.1016/j.tree.2020.03.011

Shogren, A. J., Tank, J. L., Andruszkiewicz, E., Olds, B., Mahon, A. R., Jerde, C. L., & Bolster, D. (2017). Controls on eDNA movement in streams: Transport, Retention, and Resuspension. *Scientific Reports*, *7*(1), 5065. https://doi.org/10.1038/s41598-017-05223-1

Tignat-Perrier, R., Dommergue, A., Thollot, A., Keuschnig, C., Magand, O., Vogel, T. M., & Larose, C. (2019). Global airborne microbial communities controlled by surrounding landscapes and wind conditions. *Scientific Reports*, *9*. https://doi.org/10.1038/s41598-019-51073-4

Tournayre, O., Littlefair, J. E., Garrett, N. R., Allerton, J. J., Brown, A. S., Cristescu, M. E., & Clare, E. L. (2025). First national survey of terrestrial biodiversity using airborne eDNA. *bioRxiv*. https://doi.org/10.1101/2025.04.07.647580

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

Whittington, J., Heuer, K., Hunt, B., Hebblewhite, M., & Lukacs, P. M. (2015). Estimating occupancy using spatially and temporally replicated snow surveys. *Animal Conservation*, *18*(1), 92-101. https://doi.org/10.1111/acv.12140

Wilcox, P. L., Charity, J. A., Roberts, M. R., Tauwhare, S., Tipene-Matua, B., Kereama-Royal, I., Hunter, R., Kani, H. M., & Moke-Delaneyz, P. (2008). A values-based process for cross-cultural dialogue between scientists and Maori. *Journal of the Royal Society of New Zealand*, *38*(3), 215-227. https://doi.org/10.1080/03014220809510555

Xu, C. C. Y., Yen, I. J., Bowman, D., & Turner, C. R. (2015). Spider Web DNA: A New Spin on Noninvasive Genetics of Predator and Prey. *PloS One*, *10*(11). https://doi.org/10.1371/journal.pone.0142503