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, ACT 2601, Australia
[2] Coastal People, Southern Skies Centre of Research Excellence, Department of Mathematics and Statistics, University of Otago, Dunedin, Otago 9016, New Zealand.
[3] Department of Natural Resources Management, Texas Tech University, Lubbock, TX 79409 USA
[4] Department of Biology, York University, Toronto, Ontario, Canada
[5] Netherlands Organization for Applied Scientific Research (TNO), Princetonlaan 8, 3584 CB Utrecht, Netherlands
[6] Scion (New Zealand Forest Research Institute), Titokorangi Drive, Rotorua 3046, New Zealand
[7] Better Border Security (B3, B3nz.org.nz), New Zealand
[8] Biodiversity and Conservation Science, Department of Biodiversity, Conservation and Attractions, Kensington 6151, Western Australia, Australia.
[9] Faculty of Science and Technology, University of Canberra, Canberra, ACT 2617, Australia
[10] Health & Biosecurity, Commonwealth Scientific Industrial Research Organisation, Canberra, ACT 2601, Australia
[11] Molecular Ecology and Evolution Group (MEEG), School of Science, Edith Cowan University, Joondalup 6027, Australia
[12] Department of Anatomy, University of Otago, Dunedin 9016, New Zealand
[13] Australian Antarctic Division, Department of Climate Change, Energy, the Environment and Water, Kingston, Tasmania, 7050, Australia
[14] Agriculture Victoria Research, AgriBio Centre, Bundoora, VIC 3083, Australia.
[15] Te Aka Mātuatua - School of Science, University of Waikato, Private Bag 3105, Hamilton 3240, New Zealand
[16] Fenner School of Environment and Society, The Australian National Univ., Canberra, ACT 2601, 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 6102, Australia
[18] Manufacturing, Commonwealth Scientific Industrial Research Organisation, Melbourne, VIC 3168, Australia
[19] Institute of Biogeochemistry and Pollutant Dynamics (IBP), ETH Zurich, Zurich, Switzerland
[20] DNAir, Winterthur, Switzerland
[21] Health & Biosecurity, Commonwealth Scientific Industrial Research Organisation, Brisbane, QLD 4001, 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 4556, Australia

* Corresponding author email: erin.hahn@csiro.au

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 actionable recommendations from the workshop, such as addressing outstanding questions about airborne eDNA ecology, refining sampling strategies, 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 required across different bioaerosol sources. Given its ability to capture DNA from diverse sources, airborne eDNA analysis has been applied across multiple fields, including biodiversity assessments (Clare et al., 2022), detection of rare or elusive species (N. Garrett et al., 2023b), monitoring of GMOs and invasive species (Trujillo-González et al., 2022), tracking of allergenic pollen (Kraaijeveld et al., 2015), and pathogen surveillance (Sanders et al., 2023). Together, these applications facilitate cross-disciplinary ecological and evolutionary research enabling comprehensive ecosystem health monitoring.

Airborne eDNA analysis holds immense promise for monitoring applications across diverse ecosystems, capturing genetic material from air to complement substrate-restricted eDNA methods. This unique potential could enable broad-scale biodiversity assessments in locations where other 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 (Mark D Johnson et al., 2019), detection limits (Foster et al., 2023), and source estimation for airborne eDNA (Gusareva et al., 2022; Lennartz et al., 2021), 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 (Johnson & Barnes, 2024).

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 focussed on the use of airborne eDNA for detecting macro-organisms. The workshop revealed that many challenges faced by airborne eDNA analysis are shared with other forms of eDNA, such as aquatic or soil-based methods, but a subset of challenges – such as accounting for exceptionally low DNA concentrations and establishing appropriate field controls – are unique to the medium of air. Key discussions at 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 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 (M. D. Johnson et al., 2019). These studies paved the way for early applications of airborne eDNA analysis in terrestrial biodiversity assessments (Bohmann & Lynggaard, 2023; Clare et al., 2022; Lynggaard et al., 2022; 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 enables a broader understanding of ecosystem dynamics and facilitates the development of comprehensive biodiversity baselines. In addition, its potential for broad-scale taxonomic monitoring 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), as well as 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; Sanders et al., 2023; Trujillo-González et al., 2022). 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 the invasive hemlock woolly adelgid in North America, a species native to Japan (Geller & Partridge, 2025).

Airborne eDNA collection offers an opportunity to sample in inaccessible regions and at previously unattainable scales. Not unlike other eDNA approaches, airborne eDNA analysis may allow for 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).

Airborne eDNA collection

Platforms used to collect airborne eDNA vary widely in design and material composition, generally falling into two categories: passive or active samplers. The choice between these

methods depends equally on monitoring goal and project resources, as passive samplers are typically simpler and more cost-effective, while active samplers tend to be more complex and expensive.

Passive samplers rely on natural air movement to collect eDNA. With simpler designs requiring lower maintenance, passive sampling can be deployed at higher density to increase temporal and spatial replication, leading to more precise detection probability and occupancy estimates, and reducing the effect of random variation due to fluctuating environmental conditions (Burian et al., 2021; Whittington et al., 2015). Examples of passive samplers include Big Spring Number Eight dust traps (Johnson et al., 2023; Mark D Johnson et al., 2019), modified Wilson and Cooke towers, marble-filled pan traps (Mark D Johnson et al., 2019), filter and funnel sedimentation traps (Schlegel et al., 2024), and sticky traps (Runnel et al., 2024). Some studies have also explored opportunistic passive sampling methods, such as collecting spiderwebs to capture airborne eDNA (Gregoric et al., 2022; Newton et al., 2024; Xu et al., 2015).

In contrast, active samplers use powered equipment, such as fans, to intentionally draw air through or onto a particle collection system, including filters, impingers, or cyclonic separators. This method may increase the volume of air sampled over a given time period, which would impact the effective test area and detection probability, though further research is needed to quantify this effect. 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 (N. Garrett et al., 2023b; Lynggaard et al., 2022), and repurposed pollution monitoring stations (Littlefair et al., 2023).

As new systems are developed and tested, variation in platform design 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 scale and longevity of the monitoring objectives. Broadly, we have classified collection platforms into those suitable for long-term and short-term monitoring. Long-term monitoring platforms would be designed to deliver repeated sampling tailored to a target site or application (e.g., pest monitoring in agricultural systems or biodiversity assessments at long-term research sites). Conversely, short-term monitoring platforms would be designed to deliver mobile and flexible sampling appropriate for a wider variety of research questions (e.g., establishing invasion fronts in biosecurity controls efforts or supporting citizen science initiatives). Long-term monitoring platforms might emphasise durability, automation, and robust data management while short-term platforms might prioritise portability, simplicity, and affordability to enhance accessibility. Regardless of the approach, attendees underscored that before any method or device can be widely adopted for monitoring purposes, critical sampling parameters must be validated to ensure reliable and accurate data generation.

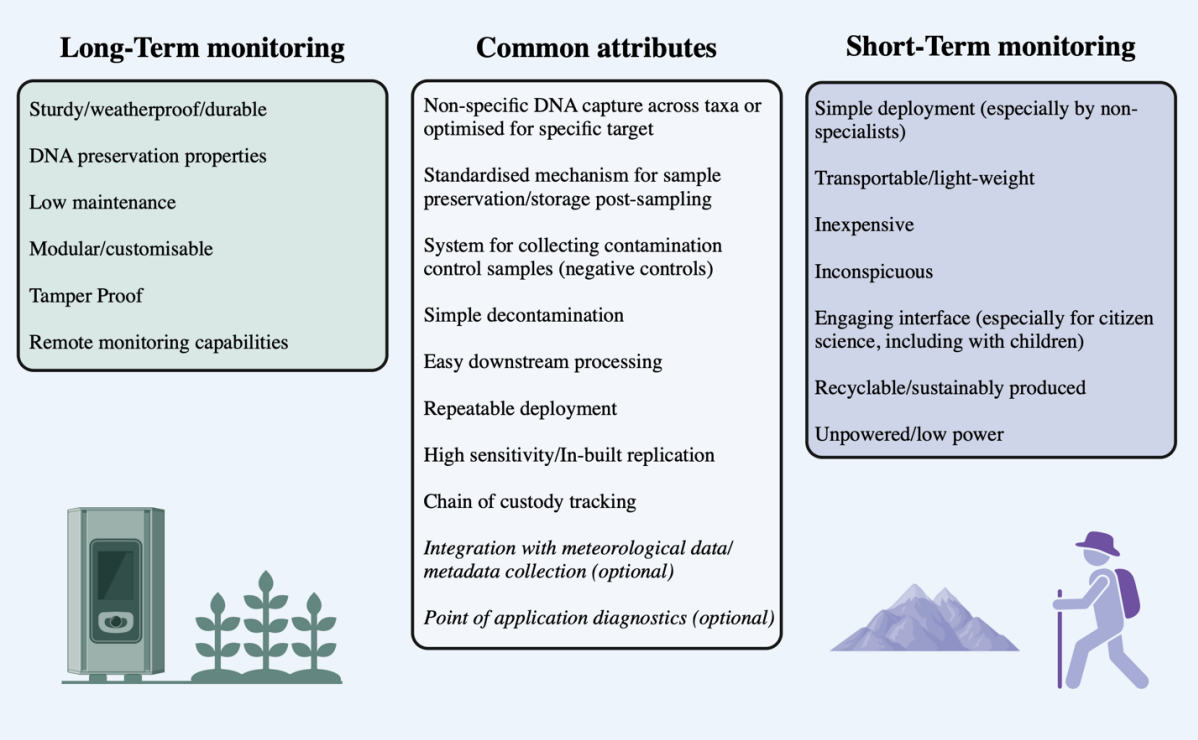

Figure 1. Key attributes of airborne eDNA collection platforms

Ideal airborne eDNA collection devices will balance critical common attributes with fit-for-purpose design. For long-term monitoring, platforms should prioritise durability, sample integrity, and seamless integration into existing infrastructure while minimising maintenance. Short-term monitoring platforms, particularly for citizen science initiatives, should emphasise simplicity, cost-effectiveness, and user-friendly interfaces. Both types of devices should incorporate features that ensure reliable sampling and downstream processing.

Advancing understanding of factors influencing airborne eDNA detection

A comprehensive understanding of environmental and technical parameters is critical for effective airborne eDNA monitoring. It is broadly understood that eDNA generation, persistence and degradation (i.e., eDNA ecology) can be impacted by temperature, humidity, and other environmental factors and introduces variability in species detection (Barnes et al., 2021; Barnes et al., 2014; Harrison et al., 2019; Jo & Minamoto, 2021; Shogren et al., 2017). Additionally, a broad range of technical factors, such as sampling and analytical methodology, influence data composition. While we cannot eliminate all sources of variability in airborne eDNA datasets, we can characterise and account for them. Insights into factors influencing detection variability from other substrates, such as soil and water, may be applicable to DNA carried in the air, however, not all features may be mirrored across eDNA sources. Airborne eDNA also presents unique challenges, such as potentially very low DNA concentrations, rapid particle sedimentation, and the influence of complex air currents, all of which necessitate specific investigation. Without insight

into these factors, conservation or biosecurity actions informed by airborne eDNA data may risk misinterpretation and inefficiency due to the introduciton of 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).

Recognising the significance of factors impacting species detection, workshop attendees compiled a list of critical parameters that may require validation (Table 1), noting that the need for validation will depend on study objectives. While the field works toward understanding these factors, it will be important to communicate study limitations when reporting results to account for uncharacterised sources of detection variability. Importantly, airborne eDNA studies should clearly articulate their experimental design, use of controls, and data analysis approach to facilitate identification of potential sources of detection error.

While not unique to air sampling, eDNA-derived biodiversity data is prone to error from several major sources, including, contamination of DNA in the workflow, inefficient DNA capture, misidentification of DNA, and changing taxonomies (Burian et al., 2021; Furlan et al., 2020; N. Garrett et al., 2023a; N. R. Garrett et al., 2023). Based on discussions at the Workshop, we have developed a four-part framework for articulating sources of error in eDNA datasets (Figure 2). In this framework, errors are divided into four categories: (1) false negative detections, where DNA present in the environment is not captured; (2) false negative identifications, where captured DNA cannot be accurately identified; (3) false positive detections, where DNA is correctly identified but should not be present in the sample; and (4) false positive identifications, where DNA is misidentified. Each of these errors stems from different sources, including data collection and assignment, and therefore 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 conducted in the presence of potentially contaminating air sources. For this reason, it is critical to establish appropriate controls for field sampling and laboratory processing as current methodologies may not adequately address contamination issues specific to airborne eDNA. While most studies include 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). By using controls to remove background signals, airborne eDNA datasets will be more informative and reliable for management questions.

Sources of error stemming from DNA identification can have broad-reaching impacts on management decisions made from eDNA data. For example, biodiversity estimates can be skewed by both forms of false identification. The complexity of this problem was illustrated in a recent study surveying bat biodiversity using airborne eDNA (N. Garrett et al., 2023b; N. R. Garrett et al., 2023). In this study, Garrett et al (2023a/b) attempted to trace reference sequence provenance and manually curate their identifications using taxon-specific expert advice to confirm species identification but still could not conclusively confirm all detections. 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). 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).

While best practices in field and laboratory protocols and data interpretation remain important, they are insufficient on their own to negate all sources of error. However, as has been observed in aquatic ecosystem monitoring, data error should not deter managers from utilising eDNA data when eDNA monitoring stands to deliver a means of cost-effective non-invasive species detection (Jerde, 2021).

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 • Replication • Sample preservation • Bioinformatics • 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 cutoffs. Identification of appropriate controls.	Sampling and processing effects on terrestrial plant detection (Mark D Johnson et al., 2019) Sampling impacts on airborne viral detection (Chang et al., 2023) Aquatic study recommendations (Goldberg et al., 2016) δ Sampling and extraction effects in freshwater systems (Deiner et al., 2015) δ
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) δ eDNA persistence in controlled freshwater system (Barnes et al., 2014) δ
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 (M. D. Johnson et al., 2019) Influence of land-use type and seasonality on airborne bacterial and fungal community composition (Anees-Hill et al., 2022; Bowers et al., 2011; Caliz et al., 2018)
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 (Buxton et al., 2017; Jane et al., 2015) δ Improving reliability of eDNA data interpretation using statistical models (Burian et al., 2021)

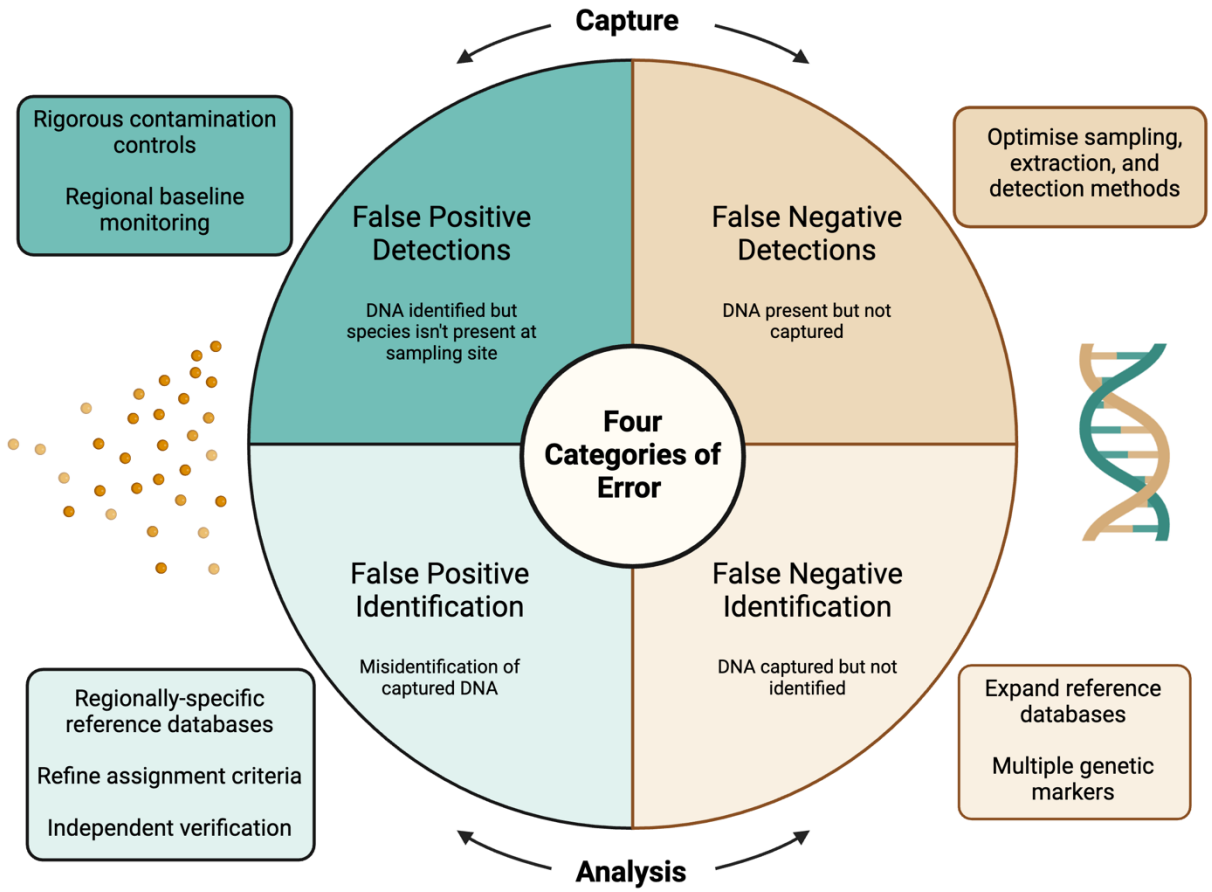

Figure 2. Framework for addressing errors in airborne eDNA analysis

Framework for understanding error in eDNA analysis, highlighting four distinct error categories and distinct mitigation strategies. Green and tan indicate positive and negative conclusions, respectively, with the upper and lower halves describing detection and identification errors, respectively.

Building partnerships and trust in airborne eDNA

As airborne eDNA research matures, models of stakeholder engagement used in water and soil eDNA sampling can be adopted to encourage successful implementation (Morisette et al., 2021). To facilitate stakeholder engagement, early and sustained collaboration with agencies, industries, academic institutions, citizen scientists, and Indigenous communities will be required (Bonicalza et al., 2024). Given the complexity of integrating genetic data with climatic and ecological information, engaging stakeholders from the outset ensures that research approaches are fit-for-

purpose and ethically sound. Best practices should prioritise co-designing studies with Indigenous communities, respecting local contexts and protocols, and adhering to FAIR and CARE 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 Indigenous engagement and should inform study development. Early and intentional engagement with Indigenous communities and management agencies can align scientific goals with practical needs, fostering mutually beneficial and culturally respectful outcomes (Handsley-Davis et al., 2021; Newton et al., 2025; Wilcox et al., 2008).

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). Effective stakeholder engagement requires researchers to clearly communicate current limitations, set realistic expectations, and emphasise the technology's role as 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 (N. Garrett et al., 2023b), provides an opportunity to build confidence through corroborative evidence. To facilitate adoption, it is essential to co-design protocols with stakeholders, ensuring alignment with regulatory processes and practical applications. Developing well-defined sampling protocols and robust controls, modelled on those established in aquatic eDNA studies (Deiner et al., 2018; Deiner et al., 2015; 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 provides a powerful opportunity to engage communities in citizen science initiatives, expanding monitoring capabilities (Palmer et al., 2017) while fostering environmental awareness and education (Isley et al., 2022; Sbrocchi, 2015). By involving citizen science in data collection, programs can leverage public interest and participation to enhance sampling density and geographic coverage. To ensure the success and sustainability of these program, 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 pivotal in building trust and maximising the 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 through the provision of rapid, non-invasive insights into ecosystems at previously unattainable scales. Realising its potential is reliant on overcoming numerous challenges, particularly those concerned with evaluating collection methods, addressing the complexities of airborne eDNA ecology, and accounting for data error. Through focused collaborative research, the field will transition from experimental research to practical application – which is required to bridge 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 the scope and resolution of ecosystem assessments, aligning with broader 'one health' approaches that link environmental, animal, and human health (Childress et al., 2024; Farrell et al., 2021). Using airborne eDNA analysis as a complementary tool broadens understanding of ecosystems, improving our capacity to detect biodiversity loss and biosecurity threats that otherwise go unnoticed. In the future, data generated through airborne eDNA analysis has the potential to 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 like GBiOS could revolutionise how biodiversity is monitored through the provision of standardised data to inform rapid, evidence-based management decisions (Gonzalez et al., 2024). The capacity of airborne eDNA to integrate genetic information from a wide range of taxonomic groups makes it an ideal candidate for such global initiatives. By providing a unified framework for biodiversity monitoring, airborne eDNA can facilitate the development of 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. Insights gained using airborne eDNA analysis could set new benchmarks in biodiversity, biosecurity, and conservation practices, seeing the technology integrated as a routine part of ecosystem management. As the field matures, airborne eDNA analysis has the potential to transition from an experimental approach to a reliable tool, guiding decision-making at local, national, and global scales and safeguarding natural resources for future generations.

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