Environmental DNA

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Environmental DNA Traveling by Air

Simplified, Portable and Cost-Efficient Airborne eDNA Metabarcoding to Expand Tropical Biodiversity Assessments

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ABSTRACT

Aquatic environmental DNA (eDNA) analysis has been widely established as a sensitive and non-invasive species detection tool for global biodiversity monitoring. In contrast, the uptake of airborne eDNA has been constrained by the absence of robust, high-throughput samplers suited to humid tropical settings. We present a simplified, portable, and cost-efficient active air sampler that injects air into a liquid collection medium, shifting airborne eDNA from stationary, resource-heavy set-ups to field-deployable, scalable workflows. Using a greenhouse vertebrate mock community to test the sensitivity of our approach, airborne eDNA sampling recovered most taxa present, with high detection rates for birds, mammals, and reptiles. An optimized collection buffer increased sequencing yield by about one fourth over sterile water. To subsequently assess its application in biodiversity research and conservation science, we tested its functionality in the Chocó-Andean montane forests of northern South America, using a tree-of-life metabarcoding approach as part of ongoing biodiversity surveys to inform the establishment of a protected area network. While aquatic eDNA detected hundreds of vertebrates and thousands of arthropods and embryophytes, airborne eDNA recovered slightly lower OTU richness. Airborne sampling was particularly effective for mammals, whereas aquatic sampling recovered more actinopterid, amphibian, and avian OTUs. Overlap in species detection between methods was low, highlighting the complementarity of airborne and aquatic eDNA-based species assessments. By integrating accessibility, portability, and scalability, our approach broadens the applicability of airborne eDNA, where combining airborne and aquatic eDNA metabarcoding can rapidly deliver broad-scale biodiversity data to support conservation planning in megadiverse yet resource-limited regions.

1 | Introduction

Environmental DNA (eDNA) has provided researchers with a broad and powerful toolkit, transforming our ability to detect, monitor, and protect global biodiversity (Altermatt et al. 2025; Iacaruso et al. 2025). As a non-invasive, yet highly sensitive and scalable method, expanding and refining this toolkit is critical to assess ecological change and underpin conservation decision-making in the face of globally accelerating biodiversity loss (Deiner et al. 2017; Bálint et al. 2018; Stephenson 2020). Currently, eDNA analyses are used for a wide spectrum of applications ranging from targeted species-specific detection to enrichment-based metagenomic sequencing using both DNA and RNA-based workflows (Ficetola et al. 2008; Shokralla et al. 2012; Ruppert et al. 2019; Yates et al. 2021; Manel et al. 2025). These methods have been applied across all types of ecosystems, including aquatic, terrestrial, and organism-associated sampling (Taberlet et al. 2012; Lyman et al. 2022; Newton et al. 2025) and, more recently, in airborne media (Aalismail et al. 2021; Clare et al. 2022; Lynggaard et al. 2022). Airborne eDNA analysis has consequently been highlighted as a major emerging trend in global biomonitoring (Sutherland et al. 2022; Lodge 2024).

Airborne eDNA has rapidly moved from proof-of-concept to a potential complementary method to terrestrial biodiversity monitoring. Indeed, independent studies from confined zoo mock communities have shown that vertebrate eDNA can be captured from air and identified through metabarcoding (Clare et al. 2021, 2022; Lynggaard et al. 2022), stimulating applications for various taxonomic groups in the wild (Roger et al. 2022; Ip et al. 2026; Tulloch et al. 2025), and leading to the detection of hundreds of species over large spatial or temporal scales from air (Aalismail et al. 2021; Polling et al. 2024; Tournayre et al. 2025). At the same time, the aerobiology literature has a longer history in metabarcoding plants and fungi from air, demonstrating robust detection and quantification of pollen and spores (Kraaijeveld et al. 2015; Bell et al. 2016; Johnson et al. 2019a, 2021). However, recent syntheses have highlighted several substantial challenges in the application and interpretability of airborne eDNA metabarcoding. These include understanding particle generation/transport and the spatial scale of inference, specificities regarding sampler design and nucleic acid capture efficiency, as well as marker choice (e.g., 12S, COI, ITS, trnL) in relation to bioinformatic biases, and limited reference availability (Johnson et al. 2019b; Lodge 2024; Johnson and Barnes 2024; Lynggaard et al. 2024; Roger et al. 2022). Thus, airborne eDNA methods are still poorly standardized, and may be more stochastic in the detection of species occupancy or abundance compared to aquatic or terrestrial eDNA analysis (Johnson and Barnes 2024; Johnson et al. 2019a; Lynggaard et al. 2024). Sampler design remains one of the central technical challenges currently limiting airborne eDNA research. Existing approaches vary widely in airflow efficiency, consistency of sampled air volume, and suitability across environmental conditions, contributing to substantial methodological heterogeneity and constraining reproducibility across studies (Bøifot et al. 2020; Jager et al. 2026; Lynggaard et al. 2022; Roger et al. 2022; Littlefair et al. 2023). Passive samplers can increase yield only by extending exposure durations which in turn could result in degradation of the collected nucleic acids. Compared to passive airborne eDNA samplers, active samplers can process large volumes of air within short time intervals, enabling targeted, high-resolution sampling, whereas passive filters excel through their minimal logistical requirements and low resource demands during deployment (Jager et al. 2026). Membrane-based active systems are limited by reduced airflow when small pore sizes are used to maximize particle binding or when water clogs the pores in humid environments, thereby decreasing differential pressure through the membrane (Soo et al. 2016). Commercial alternatives such as cyclonic separators, electret filters, water-based vacuums, or particle filtration units mitigate some of these issues but require vast equipment or infrastructure and involve high acquisition costs. Furthermore, eDNA applications outside a purely academic context—such as in applied conservation—are still largely restricted to aquatic ecosystems and particular taxonomic groups, such as fish and amphibians (Ruppert et al. 2019; Zhang et al. 2023; Plewnia et al. 2025). Particularly megadiverse yet understudied tropical regions lack behind in deploying molecular monitoring approaches to curb biodiversity loss (Erens et al. 2025; Lopes et al. 2021, 2025; Plewnia, Hildwein, et al. 2026). This is reflected in existing airborne eDNA studies which are often confined to controlled environments such as caves or greenhouses (e.g., Garrett, Watkins, Francis, et al. 2023, Johnson and Barnes 2024), where variables can be managed and species assemblages are known. These settings are valuable for methodological validation, but much of earth's biodiversity resides in complex, dynamic environments where sampling conditions are harsher and species distributions are less predictable. Tropical rain forests, especially montane cloud forests, pose specific challenges for airborne sampling, with high humidity, dense vegetation, and variable airflow between understory and canopy coupled with highly diverse and complex communities and incomplete reference data resulting in limited application of airborne eDNA studies (Garrett, Watkins, Francis, et al. 2023).

The goal of this paper is to present a simplified, cost-efficient, and field-deployable airborne eDNA sampling and metabarcoding approach. Other than previous simplified approaches (Lynggaard et al. 2022; Garrett, Watkins, Francis, et al. 2023), our method uses direct air injection into a liquid collection medium for active filtration, implemented via a compact sampler that can be manufactured locally at minimal cost. Using a tropical greenhouse vertebrate assemblage as a mock community, we demonstrate the sensitivity of our novel aerial eDNA sampling approach. We subsequently use our approach for integrative airborne and aquatic "tree-of-life" metabarcoding across the Chocó-Andean biodiversity hotspot of Colombia and Ecuador to show its versatility in a challenging field setting, with the aim of performing a rapid biodiversity assessment to support the science-based creation of a protected area network. By combining accessibility, portability, and scalability, this approach lowers the technical and financial barriers to airborne eDNA monitoring, broadening its application from experimental studies to real-world conservation practice.

2 | Material and Methods

2.1 | Air Sampler Design

We designed a simplified sampling device for collecting airborne eDNA through particle precipitation in a collection liquid (Figure 1B). The system is based on a membrane pump with a fully 3D-printable and miniaturized housing design, powered by three rechargeable 18,650 lithium-ion batteries connected in series or an USB to 12 V adapter (Figure S1). The sampler costs less than 30 Euros per unit (Table S1). Using negative pressure, air is constantly drawn through an opening at the top of a 1 L collection vial (Nalgene bottles) filled with 700 mL collection liquid, allowing a throughput of ~20 L min^{-1} (Supplementary Methods). Injected air is released via a tubing with a heptagonal nozzle in finely dispersed bubbles at the bottom of the collection vial, generating a constant air stream ascending through the collection liquid column. Particles

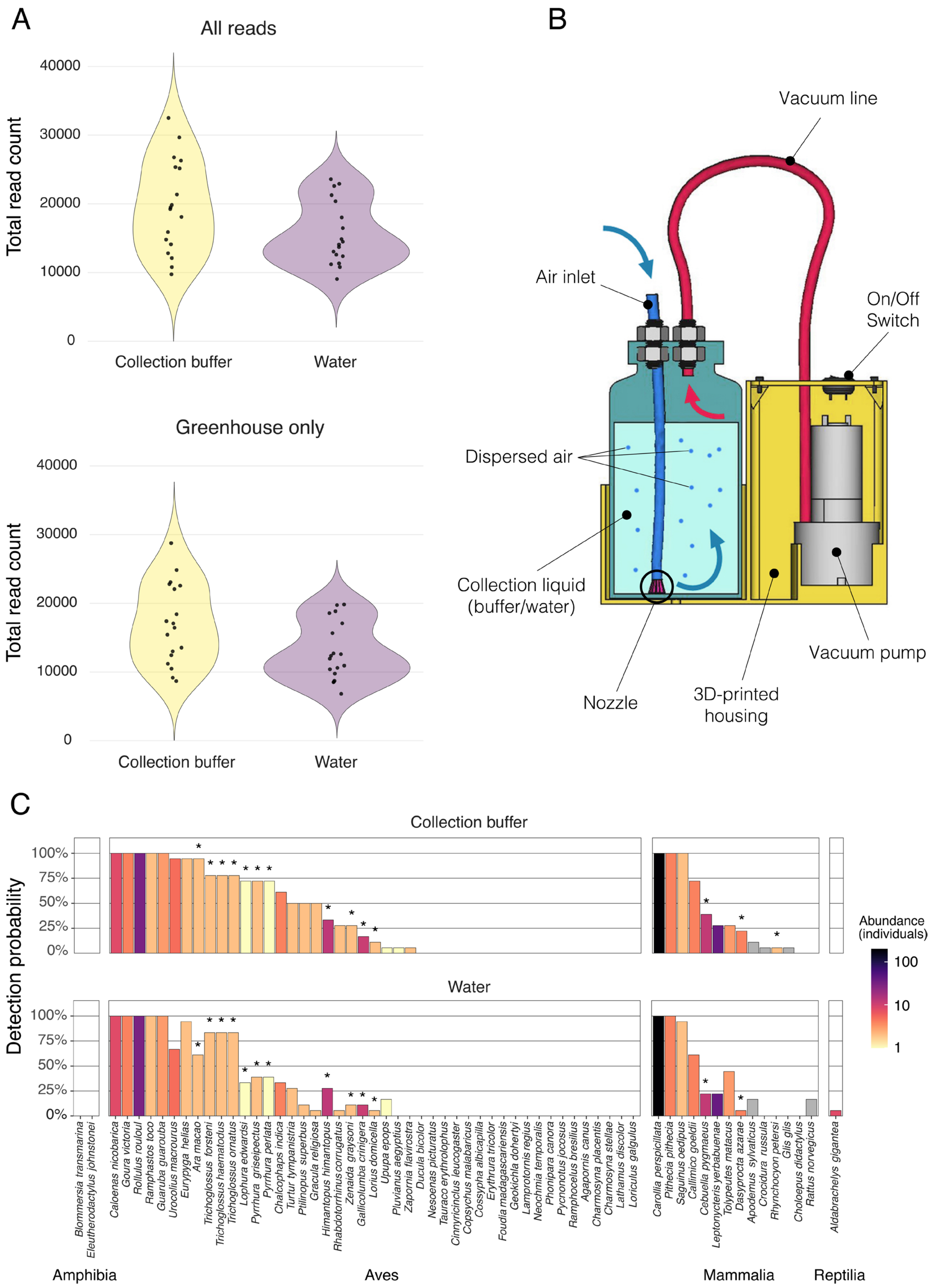

FIGURE 1 | Legend on next page.

FIGURE 1 | (A) Read numbers recovered from sampling using collection buffer vs. sterile distilled water. Above: Read count of all OTUs, below: Read count of OTUs from species known to occur in the greenhouse only. Displayed are all 18 replicates per sampling medium derived from 9 filter samples per medium. (B) Schematic overview of the airborne eDNA sampler. The 3D-printed housing is shown in yellow and encloses the pump (light gray). The inlet hose is depicted in blue, the outlet hose in red, collection liquid in light blue and the air-dispersing nozzle in purple. A more detailed description of the system components and their arrangement is provided in Figure S1. (C) Detection probabilities among 18 replicates per collection medium for all species occurring in the greenhouse mock community for the two collection liquids. Asterisk indicates BLASTn assignments that were only correct on genus-level likely due to missing reference barcodes on GenBank. Color scale denotes the number of individuals housed in the greenhouse. Bars in gray depict pest species without known number of individuals.

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such as dust or cellular debris are precipitated on the air-liquid interface into the liquid. We deployed and compared two collection liquids: (i) sterile distilled water and (ii) "collection buffer" consisting of sterile distilled water with 10% absolute Ethanol, 1% Polyethylene glycol, 0.001% Polysorbate 20 (Tween 20), 10 mM Tris–HCl and 5 mM KCl. With this, the collection buffer was optimized to reduce surface tension (i.e., increasing dispersion of air in smaller bubbles) and increase polarity of the liquid with the intention to augment DNA precipitation capacity. While the sampler housing was 3D printed from ABS, the heptagonal injector nozzle was printed from UV-curing resin to improve chemical resistance against decontaminants such as bleach. Detailed instructions and specifications as well as STL files for 3D printing and technical drawings are available in the Supplementary Methods.

2.2 | Validation and Optimization of Airborne eDNA Sampling in a Greenhouse

To validate the functionality of our sampler, we filtered air from a greenhouse at Karlsruhe Zoological Garden, Germany (~15,000 m^3), which is home to a diverse known community of tropical vertebrates including 14 mammals, 45 birds, two amphibians, and one reptile species occurring in unequal abundance (i.e., "complex" community structure; Figure 1C). Some of the animals were kept in enclosures in confined but openly ventilated areas of the greenhouse. In the greenhouse, three samplers were placed in an elevated gallery about 3 m above ground without direct visitor access. Collection liquid was assigned randomly to samplers after each liquid change. Permanent active ventilation systems in the greenhouse likely contribute to a more evenly distribution of airborne particles compared to forest ecosystems. Throughout the greenhouse trial, a researcher always stayed in proximity to prevent animals from directly interacting with the sampling devices. We collected nine samples with sterile distilled water and nine with the collection buffer by injecting air through suction of the pump as described above. After aerial sampling for 30 min each, collection liquids were filtered with cellulose nitrate filters (47 mm diameter, 0.45 μm pore size; Nalgene) using the self-manufacturable portable aquatic eDNA pump of Plewnia et al. (2025). All 18 samples were collected on a single day with negative controls processed alongside samples during sampling and in the lab. Field negative controls included 700 mL of sterile distilled water or collection buffer (two negative controls each), filtered simultaneously on site without being used for aerial sampling. Lab negative controls comprised sterile cellulose nitrate filters as extraction controls and nuclease-free water as PCR controls (two negative controls each). Sequencing libraries were prepared in duplicates per sample as described below, resulting in 36 replicates for the greenhouse experiment.

2.3 | Sample Processing and Sequencing

We extracted DNA from cellulose nitrate filters using the Blood & Tissue kit (Qiagen) with deviations from the manufacturer's protocol as described in Plewnia et al. (2025). DNA quantity was measured from isolates using the high sensitivity assay for dsDNA on a Qubit fluorometer. We amplified a~110 bp long fragment of the 12S rRNA in duplicates per isolate using the 12S-V5 primer pair specific to vertebrates (Table 1). Initial PCRs were run in 10 μL volumes using the Multiplex kit (Qiagen) and 2 μL of DNA isolate with 35 cycles. We prepared libraries with dual indexing in a subsequent PCR with 6 cycles using the primer combinations described in Lange et al. (2014), pooled samples to equal amounts and purified pools with magnetic beads (1:1 ratio, NucleoMag, Macherey-Nagel) following manufacturer's instructions. We sequenced pools on a MiSeq platform (Illumina) targeting ~20,000 reads coverage for each PCR replicate using v2 reagent kits following the manufacturer's instructions.

2.4 | Case Study in a Tropical Cloud Forest

To evaluate the functionality of the novel sampling design for applied conservation assessments, we collected a total of 36 unique air samples at three pristine cloudforest sites in the Chocó-Andean biodiversity hotspot in northernmost Ecuador (two sites with 4 and 8 air samples respectively in Provincia Carchi, Figure 2A) and adjacent Colombia (one site with 24 air samples in Departamento Nariño, Figure 2A) in April to July 2025. Here, collated distribution data on species richness and threatened species presence is informing conservation planning for the creation of a new network of protected areas across Carchi executed by Habitats Conservation (www.habitatsconservation.org) and Centro Jambatu de Investigación y Conservación de Anfibios (www.anfibiosecuador.ec). Sampling followed an opportunistic scheme and was constrained by the extreme remoteness, lack of scientific infrastructure, lack of constant electricity and heavy rains. This necessitated an opportunistic sampling duration (Figure S1) and the use of bottled mineral water as collection liquid. We conducted tree-of-life metabarcoding targeting vertebrates (primer pair 12S-V5), embryophytes (primer pair UniPlant) and arthropods (primer pair NoPlant) (Table 1) with the latter marker being known to also provide ample fungal metabarcodes as "bycatch". We deployed two air filters placed on

TABLE 1 | Overview of primer pairs used in this study.

Gene	Primer	Sequence (5′-3′)	Amplicon length	Annealing temperature	Target	References
12S rRNA	12SV05F	TAG AAC AGG CTC CTC TAG	~110	50°C	Vertebrates	Riaz et al. (2011)
	12SV05R	TTA GAT ACC CCA CTA TGC				
COI	NoPlantF_270	RGC HTT YCC HCG WAT AAA YAA YAT AAG	~116	46°C	Arthropods	Krehenwinkel et al. (2022)
	mlCOIintR_W	GRG GRT AWA CWG TTC AWC CWG TNC C				
ITS2	UniPlantF	TGT GAA TTG CAR RAT YCM G	~339	48°C	Embryophytes	Moorhouse-Gann et al. (2018)
	UniPlantR	CCC GHY TGA YYT GRG GTC DC				

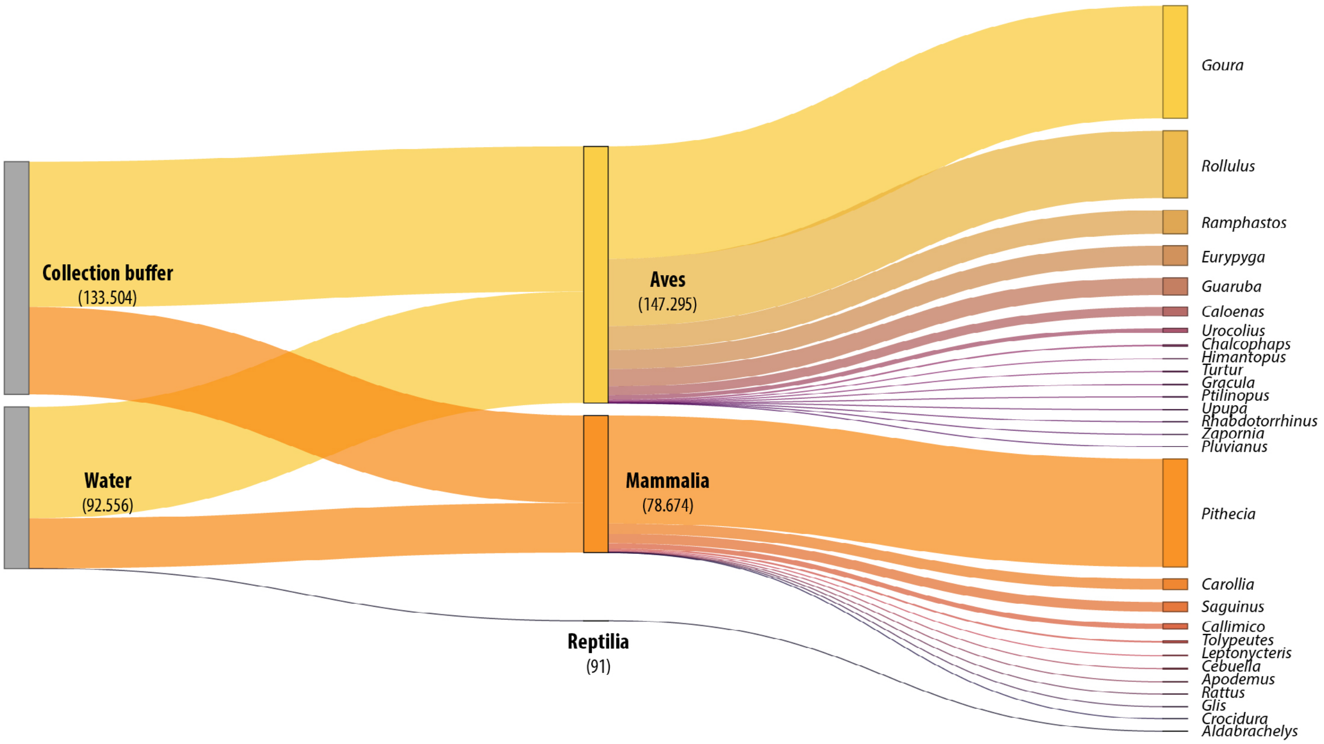

FIGURE 2 | Sankey network displaying the distribution of vertebrate reads across taxonomic groups for both collection liquids from the greenhouse mock community. Number of reads shown in brackets. An interactive HTML document with detailed information about the read count per group is available in the figshare folder.

stream banks in montane rainforest. Samplers were placed 1–2 m apart from each other on the ground with no dense vegetation covering the samplers on the stream banks. Besides air sampling, we simultaneously collected a total of 48 aquatic eDNA samples from the respective (0.5–2 m wide) streams for comparison, with water sampling < 2 m away from air sampling sites on the stream banks (Figure S1). Water was filtered on site as described above and filters preserved in 450 μL ATL buffer (Qiagen) or 300 μL DNA/RNA Shield (Zymo Research) to prevent DNA degradation (Plewnia et al. 2025). One mineral water sample was processed as negative control alongside in the field totaling four samples over the study period. Additional information on sampling sites is detailed in (Figure S1). Samples were processed as described in the "Sample processing and sequencing" section of the greenhouse experiment. In brief, DNA was extracted from filter membranes using the Blood & Tissue kit. Samples were amplified in duplicate with the 12S-V5, NoPlant, and UniPlant primer pairs (Table 1). Initial PCRs were performed in 10 μL reactions using the Multiplex kit with 2 μL DNA template (35 cycles). Dual indexing was performed in a second PCR (6 cycles). Libraries were pooled with equal volumes, purified with magnetic beads, and sequenced on two partial Illumina MiSeq runs targeting ~20,000 reads per sample replicate.

2.5 | Bioinformatics

Processing and filtering of demultiplexed paired-end MiSeq reads was done as detailed in previous work (Plewnia, Hildwein, et al. 2026). In brief, we built 3% radius Operational Taxonomic Units (OTUs) only maintaining clusters with at least 5 contributing sequences using VSEARCH (Rognes et al. 2016) and mapped OTUs on a local subset of GenBank's nucleotide database containing all unique sequences of the respective marker (unique sequence databases of MIDORI2; downloaded on 14. December 2024, Leray et al. 2022; in the case of the UniPlant marker, we mapped sequences to the curated ITS database PLANiTS, Banchi et al. 2020) using BLASTn 2.5.0 (Camacho et al. 2009). We filtered OTUs for having ≥ 5 reads in a respective sample due to the common erroneous assignment of few reads through index-hopping (Plewnia, Hildwein, et al. 2026) and based on the remaining dataset, removed OTUs that were present in at least one negative control. Reads of PCR duplicates were combined samplewise after filtering.

Downstream analysis and visualization were conducted in R 4.2.2 (R Core Team 2022) using the packages ape, dplyr, ggnewscale, ggplot2, ggtree, ggtreeExtra, grid, phangorn, phyloseq, picante, tidyr, treeio, vegan, and viridisLite. In the case of greenhouse samples, a 99% sequence similarity cutoff was applied to all samples for accepting taxonomic annotations. In addition, OTUs assigned to humans in the 12S-V5 marker (48.15% of all reads in greenhouse trial and 59.97% in case study) were excluded from read count comparisons in the greenhouse experiment to eliminate effects of potential contamination of the sampling material. The same was done with OTUs corresponding to the genus *Pan*, as these are not distinguished from human reads with the 12S-V5 marker. For species accumulation and detection probability curves, we merged OTUs in the OTU table that were assigned to the same species in their best BLASTn hit. We used Welch *t*-tests to compare read counts and DNA quantity measurements between groups as the data did not conform to normality. For OTU-based analysis of our case study, no sequence similarity cutoff was applied as taxonomic annotation is often hampered by the incomplete reference barcode availability for tropical species. We aligned OTUs using mafft (Katoh et al. 2013) with the – auto flag, removed divergent sequences and insertions with CIAlign (Tumescheit et al. 2022) and inferred maximum likelihood (ML) phylogenetic trees using IQ-TREE2 (Minh et al. 2020) with ModelFinderPlus (Kalyaanamoorthy et al. 2017) and 1000 replicates each of the ultrafast bootstrap (Hoang et al. 2018) and the SH-aLRT test (Guindon et al. 2010). We calculated three metrics to examine alpha diversity of OTU assemblages: number of detected OTUs (OTU richness), Shannon diversity and Faith's phylogenetic diversity (FPD). We tested normality with Shapiro tests. Because the data was not normally distributed, we used Wilcoxon tests to determine differences in alpha diversity between methods and sites, Kruskal–Wallis tests to assess differences among sites, and Dunn's tests for post hoc pairwise comparisons. To assess differences in community structure between sampling methods across sites, we calculated unweighted and weighted UniFrac distance matrices and visualized results with NMDS plots. Beta diversity between methods, sites and markers was compared using the adonis2 function (vegan) to perform a permutational analysis of variance (PERMANOVA) with 999 permutations. We used the formula *distance ~ sampling method + site* for each marker separately. We also tested for homogeneity of multivariate dispersions (PERMDISP) among groups with the function betadisper (vegan).

3 | Results

3.1 | Liquid-Based Precipitation of Airborne Particles Allows the Detection of Vertebrate Communities

We explored capture of airborne eDNA particles using a novel collection liquid-based precipitation approach in a greenhouse mock community. Isolates obtained from this experiment were quantified with a Qubit fluorometer, but no significant difference in DNA concentration was found between the two collection liquids ($t_{(15.56)} = -1.32$, $p = 0.207$; Figure S2). After sequencing, a total of 350,275 reads were retrieved and assigned to 111 OTUs. The collection buffer samples yielded significantly more reads compared to those obtained with sterile, distilled water as collection liquid (197,889 reads vs. 152,386 reads), both when considering all obtained reads ($t_{(30.08)} = 2.05$, $p = 0.049$) as well as those of species only occurring in the greenhouse mock community ($t_{(30.94)} = 2.40$, $p = 0.023$), resulting in a 22.99% increase in overall yield when using the collection buffer (Figure 1). Of all species known to occur in the greenhouse, we detected 26 bird species (57.78% detection rate), 13 mammal species (92.86% detection rate), and no amphibian species and one reptile species (100% detection rate) (Figure 1 and Figure 3). However, 11 of the detected bird species and 3 of the detected mammal species were correctly identified at the genus level only, as for some species no 12S reference barcodes were available on GenBank. Of the species inhabiting the greenhouse, 5 were uniquely detected by the collection buffer-based sampling and 2 by sterile, distilled water only (Figure 1). Read numbers were heavily skewed toward few taxa, with the birds *Goura victoria* and *Rollulus rouloul* constituting more than half of all avian reads, and the primate *Pithecia pithecia* constituting more than half of all mammalian reads (Figure 3). In addition, airborne sampling detected and correctly assigned 13 taxa on species-level and an additional eight on genus-level known to occur in the zoo but not in the greenhouse. Other detected taxa included common dietary ingredients fed to animals in the greenhouse or wider area of the zoo (15 species), animals natively occurring around the zoo (7 species), and common pets with DNA most plausibly introduced by visitors (3 species) (Supporting Information). Species accumulation rates varied greatly between these species groups but approached saturation in all cases at the given sampling depth (Figure S3).

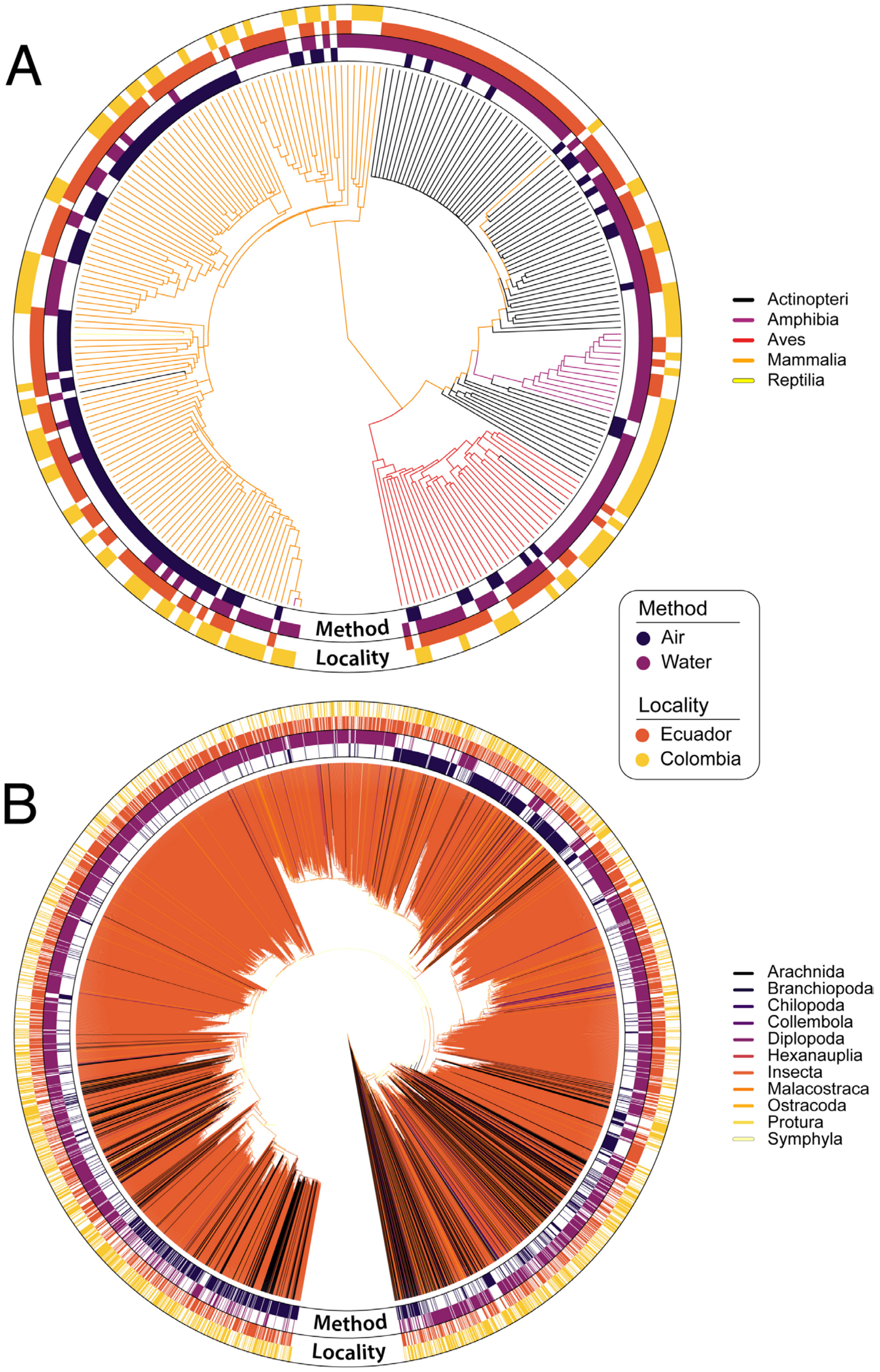

FIGURE 3 | Tree-of-life metabarcoding for both airborne and aquatic eDNA samples in the Chocó-Andean biodiversity hotspot based on sites in Colombia and Ecuador. Phylogenetic tree topology inferred with maximum likelihood (see Methods for phylogenetic reconstruction) displaying the detected (A) vertebrate community from the 12S-V5 marker, and (B) invertebrate community from the NoPlant marker.

3.2 | Aquatic and Airborne eDNA Metabarcoding Recover Complementary Species Assemblages for Biodiversity Assessments

Using airborne and aquatic eDNA sampling in tandem in two neighboring regions of the Chocó-Andean biodiversity hotspot, we conducted tree-of-life metabarcoding using three markers (Figures 4 and 5). In total, after quality filtering, we retrieved 946,203 reads from the NoPlant (arthropods and fungal "bycatch"), 944,045 from the 12S-V5 (vertebrates), and 532,251 from the UniPlant (embryophytes) marker. Sequencing showed saturation at the given coverage (Figure S4). We detected 970 arthropod OTUs from air and 2836 from water, 125 vertebrate OTUs from air and 169 from water, as well as 137

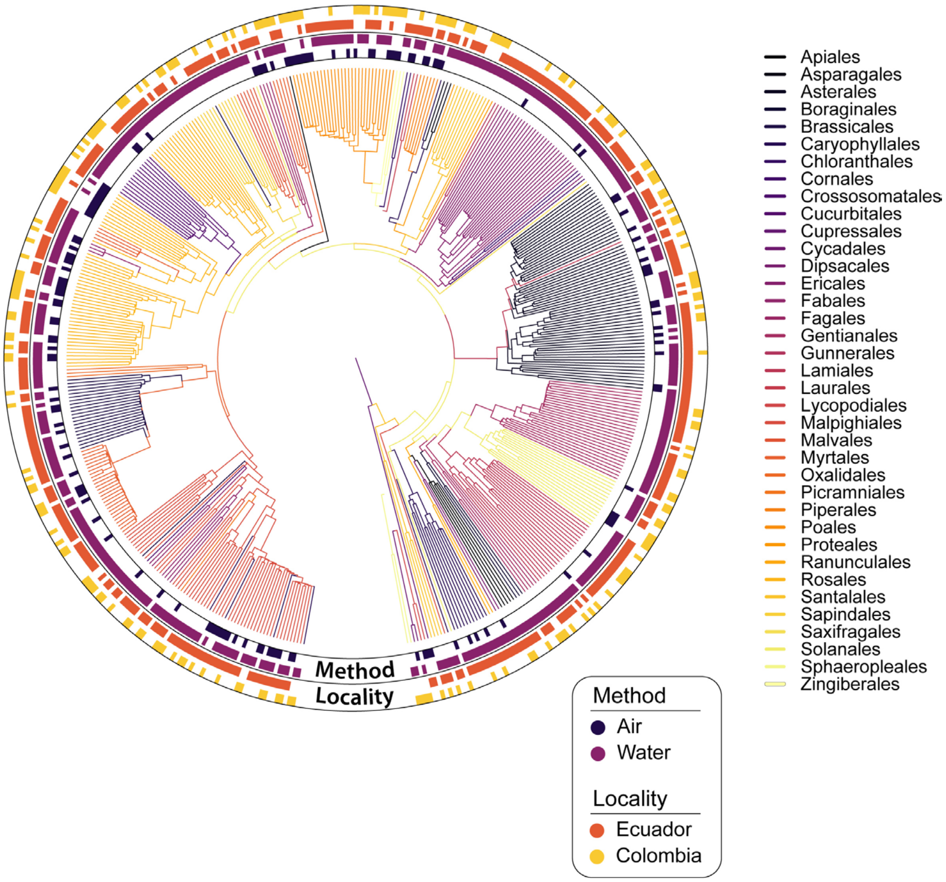

FIGURE 4 | Tree-of-life metabarcoding for both airborne and aquatic eDNA samples in the Chocó-Andean biodiversity hotspot based on sites in Colombia and Ecuador. Phylogenetic tree topology inferred with maximum likelihood (see Methods for phylogenetic reconstruction) displaying the detected embryophyte community from the UniPlant marker.

embryophyte OTUs from air and 425 from water (Figure S5). Most OTUs were detected uniquely either through airborne or aquatic sampling (Figure 2 to Figure 4), with only 11.15% of OTUs from 12S-V5, 1.14% of OTUs from NoPlant, and 12.85% from UniPlant being recovered by both methods. Airborne sampling was the most effective method for detecting mammalian OTUs (62.58% airborne OTUs), whereas aquatic sampling recovered more actinopteri, amphibian, and avian OTUs (79.86% aquatic OTUs; Figures 4, 5, and S5). We found significant differences between sampling methods in vertebrate alpha diversity (OTU richness, Shannon, FPD), with aquatic eDNA recovering higher diversity than airborne eDNA for all three markers (12SV5: OTU richness: Wilcoxon, $W = 195.5$, $p = 0.007$; Shannon: $W = 78$, $p < 0.001$; FPD: $W = 105$, $p < 0.001$; NoPlant: OTU richness: $W = 169$, $p < 0.001$; Shannon: $W = 68$, $p < 0.001$; FPD: $W = 14$, $p = < 0.001$; UniPlant: OTU richness: Wilcoxon, $W = 2$, $p < 0.001$; Shannon: $W = 1$, $p < 0.001$; FPD: $W = 0$; $p < 0.001$) (Figures S6 and S7). In the case of 12SV5, the cloud forest of Chinambí showed significantly higher alpha diversity than La Planada and El Ángel (Kruskall–Wallis, OTU richness: $H = 15.1$, $p < 0.001$; Shannon: $H = 6.3$, $p = 0.04$; FPD: $H = 11.6$, $p = 0.003$). But post hoc comparisons revealed that Shannon diversity differed only between La Planada and El Ángel (Dunn's test, LP<PEA, $z = -1.94$, $p = 0.039$). In contrast, with the NoPlant marker, La Planada exhibited the lowest OTU richness ($H = 17.3$, $p < 0.001$) and FPD ($H = 8.9$, $p = 0.011$), while Shannon diversity did not differ significantly among sites ($H = 2.3$, $p = 0.32$) (Figure S7). With the UniPlant marker, Chinambí also showed significantly higher alpha diversity for both OTU richness ($H = 9.1$, $p = 0.01$) and FPD ($H = 11$, $p = 0.004$), yet Shannon diversity did not differ between sites ($H = 1.1$, $p = 0.6$). Post hoc comparisons confirmed that Chinambí harbored the greatest diversity (OTU richness, CH>LP, $z = 2.90$, $p = 0.006$; FPD, CH>LP, $z = 1.92$, $p = 0.005$).

Further, community structures differed significantly between sampling methods as shown by both PERMANOVA analyses (12SV5: Unweighted UniFrac $R^2 = 0.21$, $p = 0.001$, weighted UniFrac $R^2 = 0.58$, $p = 0.01$; NoPlant: $R^2 = 0.19$, $p = 0.001$, $R^2 = 0.23$, $p = 0.001$; UniPlant: $R^2 = 0.26$, $p = 0.001$, $R^2 = 0.27$, $p = 0.001$) and by a clear group separation in the NMDS (Figure 2), yet with a slight overlap between clusters. We detected significant differences in community structure and diversity between Colombian and Ecuadorian sites (12SV5: Unweighted UniFrac, $R^2 = 0.11$, $p = 0.001$; weighted UniFrac, $R^2 = 0.1$, $p = 0.01$; NoPlant: Unweighted UniFrac, $R^2 = 0.09$, $p = 0.001$; weighted UniFrac, $R^2 = 0.07$, $p = 0.003$; UniPlant: Unweighted UniFrac, $R^2 = 0.20$, $p = 0.001$; weighted UniFrac, $R^2 = 0.32$, $p = 0.001$) (Figure 2). Group dispersion was significantly different between sampling methods only when using the NoPlant marker for the weighted UniFrac ($p = 0.001$); in all other cases, the assumption of homogeneity of variance was met (Figure 2).

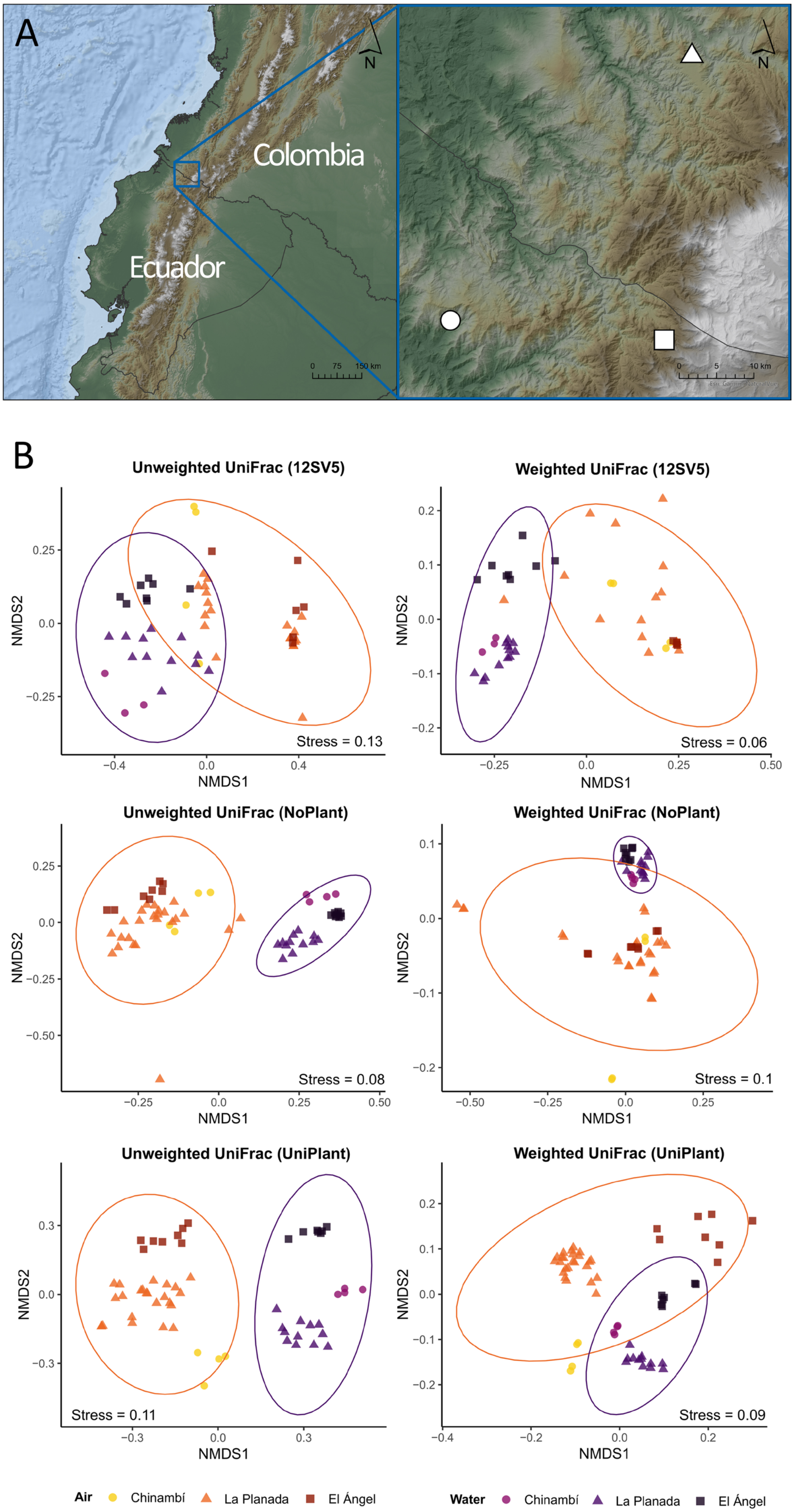

FIGURE 5 | Legend on next page.

FIGURE 5 | (A) Sampling in the Chocó-Andean biodiversity hotspot of Colombia and Ecuador. (B) In all three markersets, the sampling method revealed significantly different species assemblages (12SV5: Unweighted UniFrac R2 = 0.21, $p = 0.001$, weighted UniFrac R2 = 0.58, $p = 0.01$; NoPlant: Unweighted UniFrac R2 = 0.19, $p = 0.001$, weighted UniFrac R2 = 0.23, $p = 0.001$; UniPlant: Unweighted UniFrac R2 = 0.26, $p = 0.001$, weighted UniFrac R2 = 0.27, $p = 0.001$) with spatial structure of the sampled sites revealed by both methods.

4 | Discussion

The rapid expansion of environmental DNA (eDNA) techniques into terrestrial and aerial systems offers unprecedented opportunities to monitor biodiversity beyond aquatic habitats (Newton et al. 2025). However, the practical uptake of airborne eDNA metabarcoding has so far been limited by the lack of portable, affordable, and standardized samplers that can operate in resource-limited settings (cf. Johnson and Barnes 2024). We here introduce a miniaturized, cost-efficient, and field-deployable 3D-printable sampling device for airborne eDNA that is based on air injection into a liquid collection medium. Our study is a proof-of-concept for the novel sampling technique and requires direct comparison with other active and passive sampling approaches in future studies.

Validation in a greenhouse mock community showed that the majority of present vertebrate taxa could be recovered from airborne DNA, with partial OTU saturation being reached already with less than ten sequencing replicates, but single sampling replicates recovering only a fraction of the total community (Fig. S3). However, the relative distribution of sequence reads did not reflect the abundance of our greenhouse mock community, and even a high number of replicates did not detect all species. Instead, detection probability and read numbers were skewed toward a few species, such as the primate taxa in the greenhouse, all of which were represented. In contrast, Azara's agouti (*Dasyprocta azarae*) and Linnaeus' two-toed sloth (*Choloepus didactylus*) were not detected, perhaps reflecting species-specific differences in particle shedding predicted by microhabitat (arboreal primates vs. ground dwellers) or activity (vast movement of primates vs. limited activity of sloths). Bird species that were presumed to show high particle shedding rates due to their possession of pulviplume feathers (Delhey et al. 2007) were well represented in the samples: Five out of nine Columbiformes species, as well as the sunbittern (*Eurypyga helias*), were among the most abundant OTUs when considering read numbers, while Passeriformes that shed little feather dust (Delhey et al. 2007) were barely detected. This reflects species-specific differences in particle shedding rates—an aspect that needs to be considered when using airborne eDNA sampling for abundance estimates. As such, species-specific morphological traits, movement patterns, and overall activity likely influence nucleic acid density at different sampling heights. However, these aspects remain unknown, and differences in nucleic acid dispersal and precipitation likely differ between greenhouse settings and natural settings.

Compared to water, the optimized collection buffer increased the number of metabarcoding reads obtained from samples but failed to increase DNA concentrations. As we pooled equal volumes and no normalization was considered, differences in read counts likely reflect direct differences in amplification success, which in turn is to large parts determined by template concentration when using extraction techniques that remove inhibitors as in our study (Cruz et al. 2021). With the short sampling duration, degradation is an unlikely cause of the differences, though for future environmental RNA (eRNA) applications, further tests on nucleic acid stability in different collection liquids may be required (Yates et al. 2021, 2023). Future work will be needed to outline DNA binding mechanisms and further optimize collection liquids for improved nucleic acid binding and preservation. Quantification of the sampled air volume will aid comparative studies. Increasing the effective interaction surface between air and liquid (i.e., through increased air dispersion or buffer volume) may enhance particle capture efficiency.

Using combined airborne and aquatic eDNA metabarcoding to conduct rapid biodiversity assessments in the Chocó-Andean montane forests of northern South America, our study recovered hundreds of vertebrate OTUs and thousands of arthropod OTUs over three sampling areas with little sampling effort. Our field trials furthermore demonstrated that sampling can be performed under challenging field settings and was highly effective using bottled mineral water only, an important consideration when operating without access to lab-grade reagents. Collating such tree-of-life biodiversity occurrence data would traditionally require extensive efforts from multiple taxonomic specialists over prolonged sampling periods (Johnson et al. 2021). The combination of airborne and aquatic eDNA sampling recovered distinct communities with little overlap between methods. Detection probability with either method is likely mediated by the natural history of the species, with airborne eDNA mostly recovering additional arboreal and canopy-dwelling species rarely detected from water (cf. Aalismail et al. 2021; Garrett, Watkins, Simmons, et al. 2023; Newton et al. 2025; Tournayre et al. 2025). Shared detections might either reflect truly biphasic organisms (aquatic insect larvae with terrestrial or flying adult forms) or could be derived from cross-medium transfer of DNA particles, for instance through aerosolized water releasing aquatic particles into the air (Ip et al. 2025) or through nucleic acid raindrop wash-off entering streams. The latter is highlighted by the UniPlant data, where numerous species were only detected in water from montane streams that do not harbor aquatic macrophytes and hence are derived from leaf litter or rain wash. However, as there are only a small number of shared reads, a combined sampling strategy can be considered complementary when assessing species richness (Johnson and Barnes 2024; Newton et al. 2025), which is in line with recent work (Ip, et al. 2026). Further research is needed to better understand and predict the mechanisms of nucleic acid transfer between environmental media.

Several inherent limitations remain for large scale application of airborne DNA (e.g., Lodge 2024; Johnson and Barnes 2024; Lynggaard et al. 2024). Airborne particles are subject to undirected and often unpredictable dispersal (Tournayre et al. 2025), potentially leading to detections from non-local sources as shown with our greenhouse trial, while waterborne eDNA may better reflect spatially connected communities inhabiting ecosystems in a confined catchment area (Ushio et al. 2017; Sales et al. 2020). Further, stochasticity in detection appears higher than in aquatic eDNA, making the number of replicates a key factor for successful detection (Johnson and Barnes 2024). Numerous unanswered questions remain regarding the dispersal, stability and precipitation of DNA and RNA in air as well as the influence of weather on aerial sampling, for instance through UV-dependent degradation or rain wash-out (Johnson and Barnes 2024; Berelson et al. 2025).

In contrast to our sampling design, previously available field-deployable sampling devices rely either on passive accumulation of airborne particles on a collection medium (Ip et al. 2026; Lin et al. 2025; Jager et al. 2026), or are powered by small axial or radial 5–24 V fans for active particle collection (Lynggaard et al. 2022; Jager et al. 2026; Garrett, Watkins, Francis, et al. 2023). However, in these systems, the efficiency of particle accumulation is constrained by the pore size of the filter membrane (Soo et al. 2016). Using filters with small pore sizes (i.e., with high particle binding capacity) limits airflow through the membrane, in turn decreasing differential air pressure between membrane and fan (Soo et al. 2016). In addition, membrane-based collection is prone to fail in humid environments where the surface tension of a wet membrane rapidly clogs airflow through the pores. Commercially available samplers such as cyclonic separators, electret filters, water-based vacuums and particle filters avoid the problems mentioned above but are constrained by their high energy requirement and relatively high initial expenses (Bøifot et al. 2020; Jager et al. 2026; Lynggaard et al. 2022; Roger et al. 2022; Littlefair et al. 2023). Our cost-efficient approach circumvents these issues by using direct air injection into a liquid collection medium.

Future development in airborne eDNA research is required to improve efficiency of DNA capture and spatial resolution. First, electrostatic collectors or particle size-selective inlets could enhance capture rates, potentially allowing for targeted enrichment of aerosols carrying organismal cells or spores (Bøifot et al. 2020; Lin et al. 2025). Targeting eRNA could provide a more accurate and time-sensitive snapshot of local communities by excluding genetic material introduced over long distances due to very fast decay rates of some RNA types compared to DNA (Yates et al. 2021, 2023). As a result, only local, recent RNA may be sufficiently intact for sequencing, likely increasing both temporal and spatial resolution of airborne nucleic acid sampling. eRNA has so far only been explored in aquatic systems and further research to explore decay rates, transport patterns and suitability for airborne applications is needed. Third, integration with species-specific or multiplexed CRISPR-based assays could enable rapid detection of priority taxa on site (Williams et al. 2019; Yang et al. 2024; Plewnia, Hoenig, et al. 2026). This would allow complete field-deployable species detection in real time, having great promise for monitoring of invasive species or pathogens in early introduction stages where timely responses are key to mitigation (Plewnia, Hoenig, et al. 2026). Advances in sampling and processing, such as automated airborne eDNA capture coupled with timely on-site sequencing, may further reduce the gap between organismal presence and detection. These innovations, combined with the portability, modularity, and affordability of the present design, could accelerate the transition of airborne eDNA from an emerging research tool to a mainstream component of applied conservation and ecological monitoring (Ip et al. 2026).

By making airborne eDNA metabarcoding more accessible for conservationists working in some of the world's most biodiverse yet under-monitored regions, our approach complements existing aquatic and terrestrial eDNA methods and broadens the molecular toolkit available for global biodiversity assessment. We expect that the adaptable, cost-efficient, and field-deployable system will help ensure that airborne eDNA transforms from experimental deployments to a standard component of biodiversity monitoring programs.

Author Contributions

C.H. and A.P. conceptualized the study. C.H., J.E., L.R., C.L., and A.P. collected and analyzed data. H.K. and S.L. supervised the study. A.P., C.H., and J.E. wrote the first draft with contributions from all authors. All authors revised the manuscript and agreed on the final version.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in figshare at https://doi.org/10.6084/m9.figshare.30225943.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Schematic of the novel sampling device (left). 3D-printed ABS housing = yellow, membrane pump with motor = gray (within yellow housing), bottle = turquoise, collection buffer = light blue, ambient air inlet = dark blue, 3D-printed resin heptagonal nozzle = purple, vacuum hose to pump = red, miscellaneous accessories (pneumatic bulkhead fitting, screws and switch) = gray or black. Airflow indicated by arrows. Picture of the assembled sampler (right). In the assembled configuration, the blue hose is used both as the air inlet and as the vacuum line connected to the pump. The air inlet line is visible through the semi-transparent bottle and extends into the collection buffer. **Figure S2:**. DNA concentrations (measured with the Qubit dsDNA High Sensitivity assay) as obtained from collection buffer and sterile distilled water, as collected in the greenhouse test community. **Figure S3:**. Cumulative species richness for taxa detected with airborne eDNA metabarcoding in the greenhouse test community. Species are grouped according to their known occurrence in the greenhouse (1 = species present in the greenhouse, 2 = genus present in the greenhouse, 3 = species somewhere else in the zoo, 4 = genus somewhere else in the zoo, 5 = not native in the region and not home in the zoo, 6 = potential feed ingredients, 7 = common pets, 8 = species somewhere in the zoo and feeder animals, 9 = native species in the region). Replicates displayed on X axis contain samples from both collection liquids. **Figure S4:** Rarefaction curves showing OTU accumulation over sequencing depth for airborne DNA (air) and eDNA (water) samples for all markers. Each line represents one sequencing replicate (A and B). Vertical line at 20,000 reads. **Figure S5:** Number of OTUs recovered with each method per phylum for both markers used in the case study. Chordates are further resolved on class-level. UniPlant not displayed as no overlap is found with other markers and all OTUs fall into the phylum Streptophyta. **Figure S6:**. Alpha diversity approached with three methods (OTU richness, Shannon diversity, Faith's phylogenetic diversity) for airborne versus aquatic eDNA metabarcoding. **Figure S7:**. Alpha diversity approached with three methods (OTU richness, Shannon diversity, Faith's phylogenetic diversity) for the three sampling locations (Colombia: La Planada, Ecuador: Chinambí and El Ángel). **Table S1:** Material and price list for assembly of one sampling device.