A breath of fresh air: comparative evaluation of passive versus active airborne eDNA sampling strategies

Hugo Jager[1], Krijn B. Trimbos[1], Jan-Maarten Luursema[2], Adrianus G.C.L. Speksnijder[3,4], Kathryn A. Stewart[1*]

[1]Institute of Environmental Sciences, Leiden University, The Netherlands

[2] Department of Surgery, Radboud University Medical Center, Nijmegen, the Netherlands

[3] Leiden University of Applied Sciences, Leiden, the Netherlands

[4] Naturalis Biodiversity Center, Leiden, the Netherlands

***** Corresponding author: k.a.stewart@cml.leidenuniv.nl

Abstract

Terrestrial biodiversity's rapid decline worldwide demands for expansion of high-resolution biomonitoring to support science-based policy. Environmental DNA (eDNA) analysis has proven effective at biomonitoring aquatic systems but remains underexplored for terrestrial habitats, partly due to point-source sampling bias and difficulty in upscaling. Air has recently emerged as a promising substrate for terrestrial biomonitoring yet most studies use active samplers which tend to be expensive, bulky, and require power. We used a newly developed, inexpensive, reusable, and easy-to-use passive airborne eDNA sampler (DNAir Sampler) to capture eDNA suspended in air over 96 hours within Rotterdam Zoo, the Netherlands. Its performance was compared to two commonly used active airborne eDNA samplers for vertebrate diversity detection. In total, 88 species were detected, including 24 zoo residents. The DNAir Sampler was the most effective at detecting zoo residents, surpassing active samplers in species richness within 48 hours and continuing to accumulate new species beyond 96 hours. It also detected the furthest species signal (515m). Zoo airborne eDNA further demonstrated a positive correlation to species total biomass. Our findings indicate that for long, unsupervised biomonitoring, passive airborne eDNA sampling outperforms active

sampling for assessing vertebrate communities and potentially reduces detection noise in stochastic air eDNA signals.

Keywords

biomonitoring, environmental DNA, airborne DNA, passive samplers, DNAir Sampler, vertebrate biodiversity, bioaerosols

Synopsis

Passive airborne eDNA sampling outperforms active methods, accumulates species over time, correlates with biomass, and enables scalable biodiversity monitoring across landscapes.

Introduction

Global biodiversity is essential for ecosystem resilience and critical services such as food security and medicine [1,2]. However, anthropogenic pressures, including climate change, habitat destruction, and globalisation threaten biodiversity, necessitating high-resolution biomonitoring to guide conservation efforts [3,4].

Traditional biomonitoring methods are often time-consuming, require expert taxonomic knowledge, can be invasive to the species and fail to document rare species [5]. Environmental DNA (eDNA) approaches, which collect genetic material directly from the environment rather than individuals [5-7], have gained widespread use [4]. These approaches are non-invasive, highly sensitive, cost effective, and capable of detecting taxa across the tree of life while reducing observer bias and enabling large-scale monitoring [8,9]. In many cases, eDNA has outperformed traditional methods [10,11]. While eDNA sampling from water is well-established [12-14], terrestrial applications remain underdeveloped partly due to challenges in identifying optimal substrates [15]. Various terrestrial substrates, including soil, plants and spider webs (among others) have been explored but often yield limited taxonomic coverage and rely on point-source samples that may fail to give a comprehensive and representative estimate of biodiversity [16-19].

Airborne eDNA sampling offers a novel approach for terrestrial biodiversity monitoring. Air contains bioaerosols—genetic material from microorganisms, pollen, and animal tissue fragments [20]. [21] first demonstrated animal DNA detection in air using active (powered) air pumps, with subsequent studies confirming its efficacy across various environments, including zoos and natural habitats [22-24]. Recent research has detected insects, birds, frogs, and mammals in field conditions, highlighting the potential of airborne eDNA for large-scale biodiversity monitoring [7,20,25].

Most air eDNA studies rely on active samplers, which, despite their effectiveness, are costly, bulky, and require power sources, limiting their use in remote areas [26]. Passive air eDNA sampling provides a cheaper and more mobile alternative, with promising results for

detecting diverse taxa [27-29]. However, questions remain regarding its sensitivity and spatial resolution compared to active methods, particularly given evidence of long-range airborne eDNA dispersal [22] and continuous accumulation over time [29]. Understanding airborne eDNA's spatial and temporal dynamics is crucial for optimizing sampling strategies [30].

Our study evaluated the efficacy and sensitivity of a newly-designed passive airborne eDNA sampler (the DNAir Sampler) against two, commonly used, active air eDNA samplers (Pollensniffer and Coriolus Micro) in Rotterdam Zoo, Netherlands. The DNAir sampler, designed to enhance DNA capture efficiency by directing airflow over a protected DNA-binding filter, aims to mitigate the limitations of previous passive methods that rely on surface settlement [27, 29, 31]. We assessed species detection rates, expected zoo vertebrate recovery, species dispersion distances, accuracy in detecting known zoo residents, and the correlation between species biomass and airborne eDNA abundance. Additionally, we compared species accumulation curves to determine optimal sampling strategies across the three eDNA samplers.

MATERIALS AND METHODS

Study site

We collected air samples at Rotterdam Zoo (Diergaarde Blijdorp, Netherlands; Figure 1), a 32-acre zoo wherein sampling took place at five outdoor points and one indoor point. Surrounded by residential areas and water bodies, the zoo houses diverse avian and mammalian species in spacious outdoor enclosures. Besides listed zoo animals, expected eDNA sources include food, visitors, their pets, and nearby wildlife.

FIGURE 1 Map of Rotterdam Zoo (the Netherlands) including the 6 sampling locations (numbered circles). Location 6 represents an indoor site. Photo insert depicts sampling setup of the Pollensniffer (left bottom), Coriolis Micro (left top), and the passive DNAir Samplers (right).

Airborne eDNA sampling

Between March 25 and April 12 2024, we collected air samples at six locations over three 5-day sampling campaigns (Table S1). Locations were selected based on 1) secure placement of passive airborne eDNA samplers out of visitor's reach, 2) sufficient distance to avoid redundant detections, and 3) proximity to at least four enclosures.

Three air sampling devices were used, each varying in mobility, cost and flow-rate: two active samplers - the Pollensniffer (~8 L/min) [32] and the Coriolis Micro (~100 L/min) [33], and the passive DNAir sampler [34] which relied on external air movement. On the first day of each sampling campaign, we deployed 5 passive samplers at ~2.5m height in trees at each location (including the indoor location) and took 1 m-high air samples with both active samplers. To minimize disruption (in consultation with zoo staff) and in accordance with sug-

gested sampling practice by the manufacturers, both active samplers were operated simultaneously for 10 minutes per sampling event. Passive samplers were collected at 6, 24, 48, 72, and 96 hours after deployment. All equipment was sterilized between uses with 30% bleach solution followed by ultrapure water rinsing. Passive DNAir samples were collected on 0.7µm glass fibre filters, stored in 2 ml Eppendorf tubes. The Coriolis Micro samples were collected in 4 mL PBS-filled cones, sealed immediately after sampling. Samples were handled with facemasks and nitrile gloves and transported on ice before storage the same day at -20°C until DNA extraction.

For each campaign, field negative controls were collected: glass fibre filters placed inside without being hung (DNAir Sampler), PBS solution placed within the sampling cup (Coriolis Micro), and a Vaseline-coated slide (Pollensniffer). In total, 90 DNAir samples, 18 Pollensniffer samples, 18 Coriolis Micro samples, and three field negatives per device across the three weeks were obtained.

DNA extraction

For extraction, we fit each sample in a 2mL Eppendorf tube: DNAir filters were cut into small pieces, the Pollensniffer slides were removed from the cassettes and folded with the exposed side facing inwards, the 4mL Coriolis Micro PBL samples were transferred to an Eppendorf tube, centrifuged for two minutes at 10,000rpm, thereafter supernatant removed and pellets retained. We extracted DNA for all samples following the protocol of [35]. Samples were incubated overnight in a 56°C lysis buffer for 14.5 hours. QIAshredder (QIAGEN) was then used to release lysed cellular content from the sample substrates, which after centrifuging for three minutes at 11,000 rpm resulted in two 390 µL tubes per sample. We vortexed samples and added 700µL AL:EtOH (1:1) to each tube. Finally, we eluted DNA in 100µL AE buffer. All samples were stored at -20°C until metabarcoding.

DNA metabarcoding

We used two vertebrate primer sets for metabarcoding: 16S rRNA primer (16Smam1/16Smam2) targeting mammals (~95bp) [36], and 12S rRNA (12S05 forward /12S05 reverse) targeting vertebrates (~97bp) [37]. All primers had 5'-overhang adapters for Nextera XT (Illumina) indexing. A 1:100 dilution of harbor porpoise (*Phocoena phocoena*) DNA (Naturalis Biodiversity Center, Netherlands) served as a positive PCR control, with multiple negative controls to detect contamination.

For the 16S primer set, 20µL reactions consisted of 4µL DNA template, 10µL TaqMan™ Environmental Master Mix 2.0, 1µL of each primer (10µM), 3µL RNase-free water, and 1µL human blocker (60µM) [38]. The thermal cycling profile was 95°C for 10 minutes, followed by 40 cycles of 95°C for 12 s, 59°C for 30 s, 70°C for 25 s, and a final extension time of 72°C for 7 minutes. The thermal cycling profile was 95°C for 10 min, followed by 40 cycles of 95°C for 12 s, 59°C for 30 s, 70°C for 25 s, and a final extension at 72°C for 7 min. The 12S reactions followed the same protocol but omitted the human blocker [39] and had a cycling profile of 94°C for 30 s, 59°C for 45 s, and 72°C for 60 s. Three PCR replicates were performed for all 131 DNA extracts, controls, and both primer sets.

PCR products were visualized on 2% agarose gels (Invitrogen) with SYBR™ Safe alongside a low-range quantitative DNA ladder. Three technical replicates (10µL each) were pooled per DNA extract. Amplicon pools were purified using NucleoMag NGS Clean-up and Size Select beads (Mackerey-Nagel) at a 1.6× bead-to-amplicon ratio. Nextera XT (Illumina) indices were ligated in a second PCR (20µL), consisting of 2µL amplicon pool, 10µL TaqMan™ Environmental Master Mix 2.0, 1.5µL of both N- and S-indices, and 5µL RNase-free water. The thermal profile was 95°C for 10 min, 10 cycles of 95°C for 15 s, 55°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 5 min.

Amplicon pool concentrations were measured using the 4200 Tapestation System (Agilent Technologies) and pooled equimolarly using the OT-2 Robot (Opentrons). The final

pool was purified using a 1.2× bead-to-amplicon ratio and quantified on the 4200 Tapestation. Libraries were sequenced at Macrogen Europe (Netherlands) on an Illumina MiSeq (v3 chemistry, 150bp paired-end), targeting 50,000 reads per sample.

Data preparation

Quality assurance was performed separately for each primer set. Illumina adaptors and primers were trimmed using Cutadapt v4.9 [40], and reads were processed into amplicon sequence variants (ASVs) using DADA2 [41] in R [42]. Filtering parameters included maxEE = 2 and minLen = 50, removing reads with >2 expected errors or <50 bp. Dereplicated and error estimation followed, using DADA2 to calculate true ASV counts. We merged reads, removed chimeras, retaining only ASVs between 80 -106 bp for 12S, and 83 - 118 bp for 16S.

Taxonomic identification was performed using BLASTn against GenBank 12S and 16S databases via a custom pipeline on Naturalis Biodiversity Center's OpenStack GALAXY platform [43]. A weighted LCA algorithm applied cutoffs of 85% query coverage, 98% identity, and a minimum score of 170 [44]. ASVs identified as human, unidentified, or detected in controls were removed. To minimize contamination, negative control read counts were subtracted from DNAir sampler reads for their respective weeks and from all reads for Coriolis Micro and Pollensniffer. ASVs of the same species were grouped for both primer sets.

Afterward, we manually validated all species-level identifications and removed ASVs that were identified as human, could not be identified, and/or were detected in the positive and negative PCR controls. To control for potential contamination, we removed the number of reads present in our negative controls from the read counts in their respective weeks for the DNAir sampler, and from all read counts for both the Coriolis Micro and Pollensniffer All ASVs belonging to the same species were grouped together for both primer sets.

Read counts from both primers were combined into an ASV table summarizing total species read counts per sample. Separate datasets were created for each eDNA sampler, summing species-specific reads across all samples for each method. Identified species were

cross-referenced with a detailed Rotterdam Zoo inventory (N=266), including those housed off-exhibit.

Quantification and statistical analyses

We assessed normality using Shapiro-Wilk and KS tests in R 4.4.0, finding all data normal except zoo species sequencing read and total biomass ($p>0.05$), which were log-transformed. To compare species richness detected by the three airborne eDNA samplers, we performed ANOVA and Tukey's HSD tests.

Species richness accumulation over time was visualized using *vegan* v.2.6.6.1 and *ggplot2* [45,46]. DNAir samples were analyzed across 75 outdoor sites (three replicate campaigns in five locations, each with five samplers collected at 6, 24, 48, 72, and 96 hours). Two accumulation curves were generated: one for all species and one for listed zoo species. These were averaged per campaign and modeled using a Generalized Linear Mixed Model using time as a fixed effect and location as a random effect. For active samplers (Pollensniffer and Coriolis Micro), total and average detected species were plotted per replication. We report static values instead of curves for the active samplers since the active samplers were only used once for each location/replication.

Zoo species detection distances were estimated using Google Earth, calculating the mean detection distance per sampling method. Using these radii, we determined the percentage of expected zoo species detected per sampler over three weeks. Maximum detection distances were also averaged, omitting zeros to prevent overdispersion.

To examine the relationship between species biomass and detection probability (sensu [22,24], we multiplied published mean biomass values (Table S4) by the number of individuals housed in the zoo. A Pearson correlation tested the relationship between total biomass and read counts.

RESULTS

Sequencing results

To detect airborne eDNA from vertebrates and mammals, we targeted mitochondrial regions using 12S and 16S markers. For the 12S dataset, we reduced our original 3,862,876 sequencing reads to 783,731 reads across 293 ASVs after filtering, removing unidentifiable ASVs and correcting for negative controls. Subsequently, questionable species identifications were adjusted using the weighted LCA algorithm in Galaxy. Despite our use of human blocking primers, 299,733 reads were identified as human (*Homo sapiens*), which we subsequently removed. This resulted in 483,998 reads across 161 ASVs, which we identified to 65 taxa,15 of which were expected resident species of the Rotterdam Zoo (total read count of 48,735 across 26 ASVs).

For 16S, we filtered 5,871,156 sequencing reads down to 651,690 reads after filtering, removing unidentifiable ASVs and correcting for negative controls. Of the remaining reads, 293,267 were identified as *Homo sapiens*. After removal, the 16S dataset consisted of 358,249 reads across 92 ASVs that we identified to 47 taxa. Of these taxa, 23 were not identified using the 12S primer set, and 15 ASVs were identified as expected resident species of Rotterdam Zoo (total read count of 146,019 across 23 ASVs).

Cumulatively, we detected airborne eDNA from 88 distinct species, of which 24 were found in both primer sets (Table S2). Of these 24 species, six were expected zoo species: the Bactrian camel (*Camelus bactrianus*), the black rhinoceros (*Diceros bicornis*), the giraffe (*Giraffa camelopardalis*), the European otter (*Lutra lutra*), the Indian rhinoceros (*Rhinoceros unicornis*), and the Arctic fox (*Vulpes lagopus*).

Species detection

Across all sampling weeks at the Rotterdam Zoo, we detected a total of 88 species: 34 mammals, 29 birds, 15 fish, and 10 amphibians (Figure 2). The passive DNAir Sampler accounted for 62.17% of all reads and detected 76 species, whereas the active samplers, the

Pollensniffer and Coriolis Micro, detected 50 species (21.66% of the reads) and 25 species (16.17% of reads), respectively. Of the species diversity detected, the majority (30) were exclusively collected by the DNAir Sampler, and 17 were detected by all three samplers (Figure 2a). This pattern was similar when looking specifically at zoo residents (Fig. 3a). Overall, 24 of the total 88 species detected using these eDNA samplers were listed as Rotterdam Zoo residents: 15 mammals and 9 birds (Figure 3), accounting for 9% of the total inventory at Rotterdam Zoo (including those not on exhibit). Of these zoo species, the DNAir Sampler detected 20 species, 9 of which were exclusive to this passive sampler (Fig. 2a; Fig. 2b), whereas the Pollensniffer collected 14 zoo species, of which 4 were exclusive to the Pollensniffer, and the Coriolis Micro detected 4 zoo species.

a)

b)

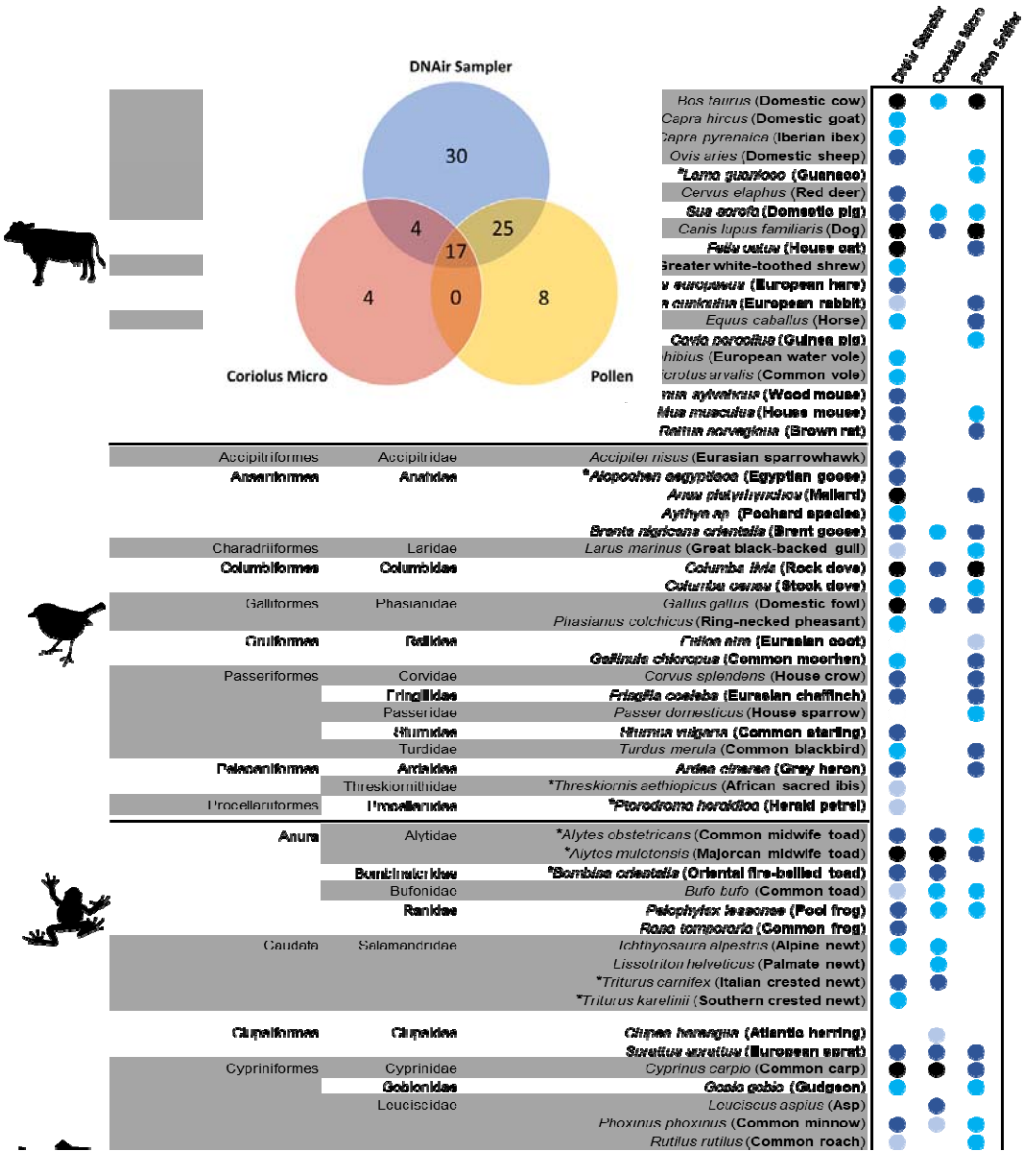

FIGURE 2 a) Venn diagram of total species detection across different air eDNA samplers. b) Non-target species detected (icons per family) across three weeks sampling in Rotterdam Zoo using different air eDNA samplers: DNAir Sampler (passive sampler), Coriolis Micro, and Pollensniffer. Asterisk denotes Netherlands non-native species. Total sequencing reads across all samples per sampler indicated for each species by the corresponding circle colour (Legend bottom right).

a)

b)

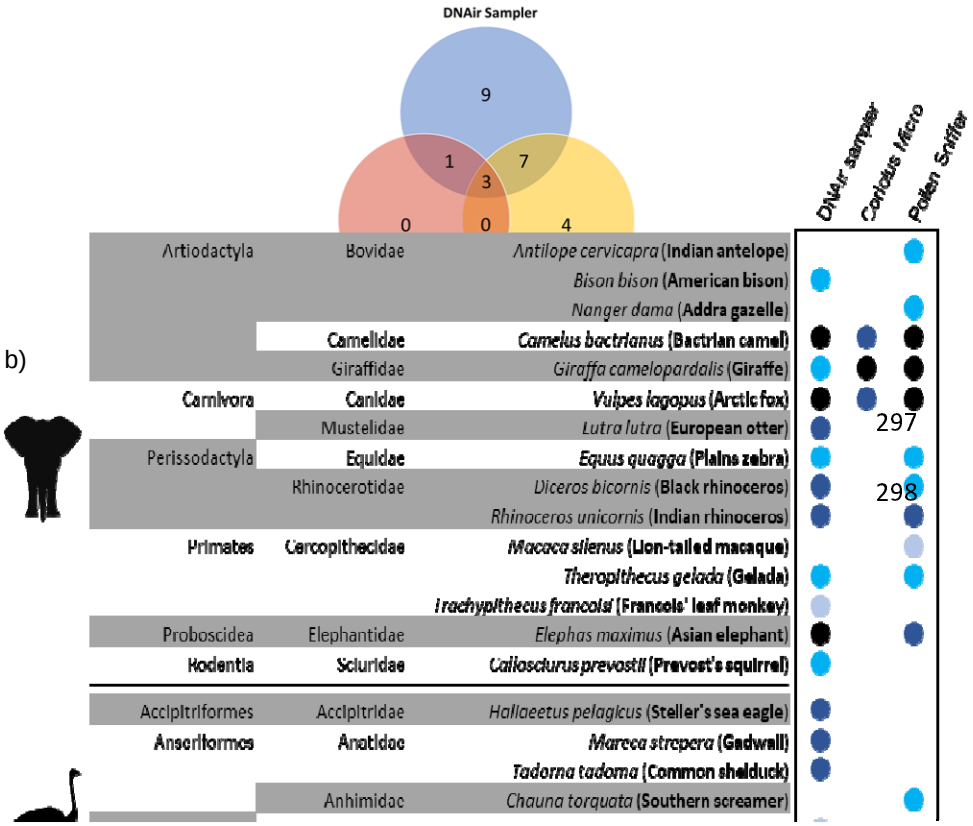

FIGURE 3 a) Venn diagram of total species detection distribution across different air eDNA samplers b) Rotterdam Zoo resident species (icons per family) detected across three weeks of airborne eDNA sampling using different samplers: DNAir Sampler (passive sampler), Coriolis Micro, and Pollensniffer. total sequencing reads across all samples per sampler indicated for each species by the corresponding circle colour (Legend bottom right).

While the majority of the non-target species (N=64) were those native to the Netherlands, including mammals, birds, amphibians and fish, 10 non-native species were also identified (Fig. 2b). At the indoor sampling site, four species were detected that were not detected outdoors.

Accumulating species richness detection over time

Comparing the three air eDNA samplers, the average total detected species richness by the DNAir Sampler per outdoor sampling location exceeded that of the Coriolis Micro (100L/min) after only 6 hours (Fig. 4a), whereas the Pollensniffer at 8L/min was exceeded by the DNAir Sampler between 24 and 48 hours of passive sampling. The DNAir Sampler detected on average 15.07 (SD ± 2.62) species per outdoor site after 96 hours of sampling (Fig. 4a), with site 2 detecting the most species diversity, on average 19 species (SD ± 5.29), and site 3 detecting the least number of species (11.67 SD ± 3.21), after 96 hours. Comparatively, the Pollensniffer detected an average of 6.6 species (SD ± 1.38), whereas the Coriolis Micro de-

tected an average of 3.47 species (SD ± 1.15) per site after 10 minutes of active sampling

Excluding all non-target species, we detected an average of 2.87 zoo species (SD ± 1.57) per outdoor sampling site after 96 hours of sampling using the DNAir Sampler. By comparison, the Pollensniffer and the Coriolis Micro detected an average of 1.67 (SD ± 0.82) and 0.4 (SD ± 0.43) zoo species at each outdoor sampling site, respectively. The average detected zoo species richness by the Coriolis Micro was exceeded after 24 hours of sampling using the DNAir Sampler, and after approximately 72 hours using the Pollensniffer.

Our indoor sampling site (location 6) demonstrated the mean total species richness and zoo species richness obtained by the Coriolis Micro were both exceeded by the DNAir Sampler within the first 24 hours, similar to the outdoor sampling locations (Figure S2). In contrast, the DNAir Samplers only exceeded the species richness obtained by the Pollensniffer after 72 to 96 hours (Figure S2).

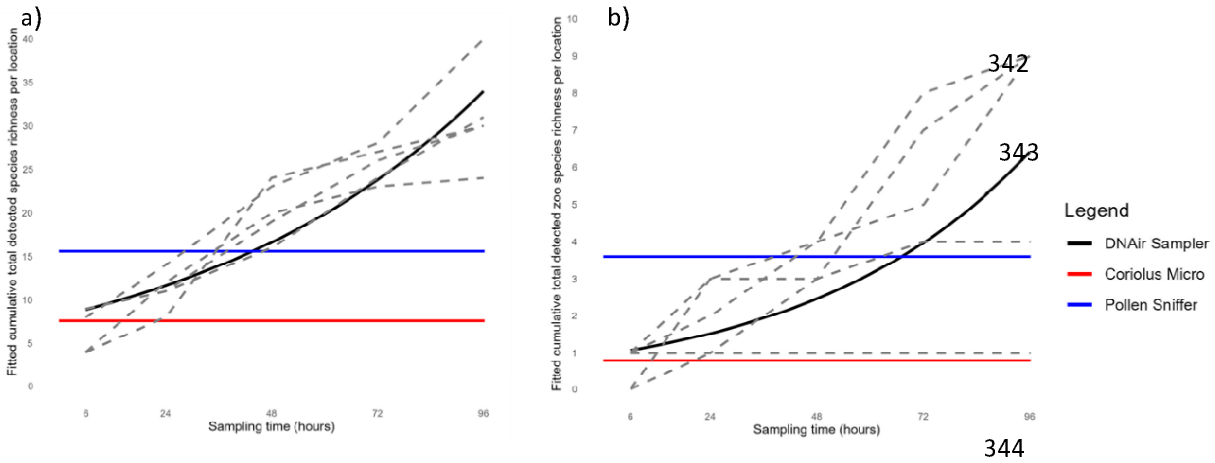

FIGURE 4 Species richness accumulation curves for outdoor sampling locations only. Grey dotted lines represent species richness accumulation curves for the outdoor sampling locations (sites 1-5) as detected by the DNAir Sampler, black line is a GLMM of best fit. (a) accumulation of the total detected species richness by the DNAir Sampler for the outdoor sampling locations over 96 hours across the three weeks plotted against the mean total detected species richness by the Coriolis Micro and Pol-

lensniffer for the outdoor sampling locations across the three weeks, with (b) showing only zoo resident species.

The total detected species richness by the DNAir Sampler per outdoor sampling location exceeded the mean total detected species richness by the Coriolis Micro within the first 24 hours, while it exceeded the mean total detected species richness by the Pollensniffer between 24 and 48 hours of sampling, with the exception of location 3. Our results indicate the DNAir Sampler detected significantly higher total species richness, followed by the Pollensniffer, and the Coriolis Micro detected the least ($F(2,12) = 43.21$, $p < 0.001$) (Figure S3a). The Coriolis Micro detected a total of 0.8 zoo species on average across three measurements per outdoor sampling location (SD ± 0.84), whereas the Pollensniffer detected a total of 3.6 (SD ± 1.67) zoo species on average across three measurements per outdoor sampling location.

We also found total detected zoo species per outdoor sampling location to be significantly different among all three samplers ($F(2,12) = 6.80$, $p = 0.011$), with the highest total species richness detected by the DNAir Sampler (Figure S3b).

Accuracy, dispersion, and biomass

The DNAir Sampler detected zoo species at the greatest average distance (188.67 ± 61.33 m), followed by the Pollensniffer (172.58 ± 91.29 m), and the Coriolis Micro (68 ± 50.21 m), though these differences were not statistically significant ($F(2,10) = 2.8$, $p = 0.11$) (Figure 5a; Table S3). Excluding the indoor site, the DNAir Sampler also recorded the furthest single detection (373 ± 124.37 m), significantly outperforming the Coriolis Micro ($p = 0.036$), with a maximum detection of an American bison (*Bison bison*) at 515 m (location 2) (Figure 5b). The furthest detections by the Pollensniffer and Coriolis Micro were an Arctic fox (*Vulpes lagopus*, 491 m) and a black-crowned night heron (*Nycticorax nycticorax*, 128 m), both at the indoor location (location 6). Outdoors, the farthest detections were the Asian elephant (*Elephas maximus*, 466 m, Pollensniffer) and the night heron again (116 m, Coriolis Micro).

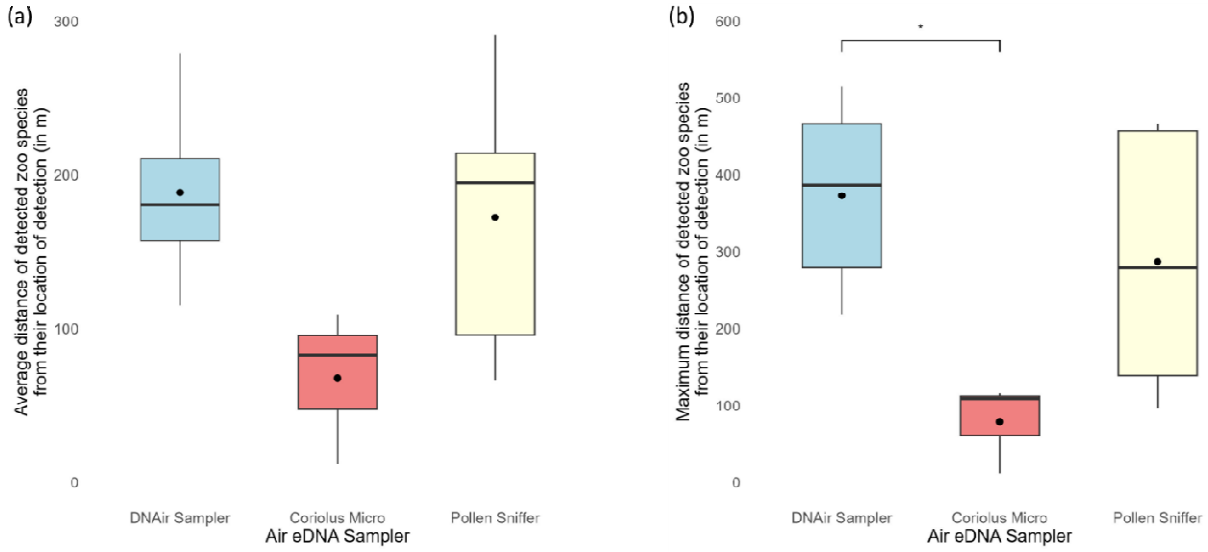

FIGURE 5 Boxplots showing the average (a) and maximum (b) distance in meters between the detected zoo species and the sampling location at which they were detected for the different outdoor sampling locations per air eDNA sampler across three weeks of sampling.

In terms of detection accuracy, the DNAir Sampler was highest, identifying 19.11% (±10.87%) of zoo species within its average range, followed by the Pollensniffer (12.25% ± 5.49%) and the Coriolis Micro (10% ± 13.69%). Using the lowest average detection distance (68 m, Coriolis Micro) as a baseline, both the DNAir Sampler (23.71% ± 22.94%) and the Pollensniffer (21.86% ± 18.31%) showed slightly higher detection accuracy across five outdoor locations, indicating effective identification of nearby zoo species (Fig. 6). Additionally, there was a significant positive correlation between total sequencing reads and the log-transformed biomass of detected zoo species ($r(24) = 0.44$, $p = 0.031$; Fig. 7). This trend remained, though weaker, after removing four large outliers ($r(20) = 0.38$, $p = 0.097$; Fig. S4).

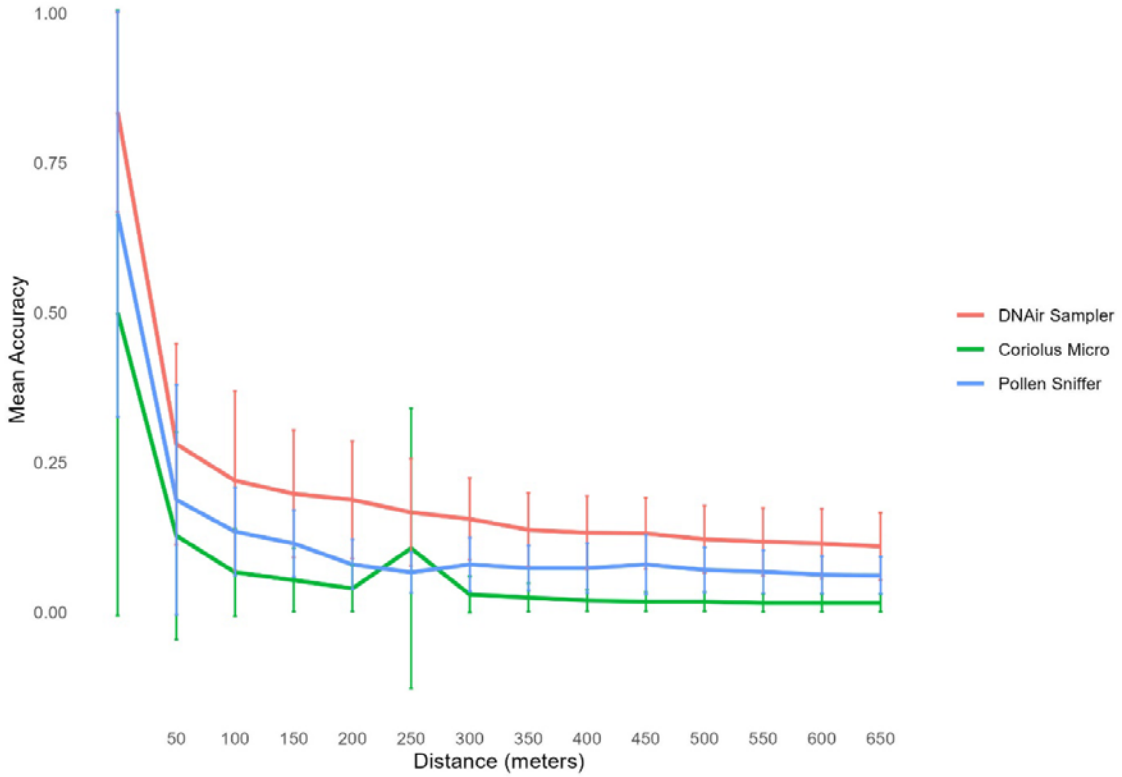

FIGURE 6 Mean % accuracy dispersion curve across distance (meters ± SD) from sampling location. Sampler type is denoted by colour (legend to the right).

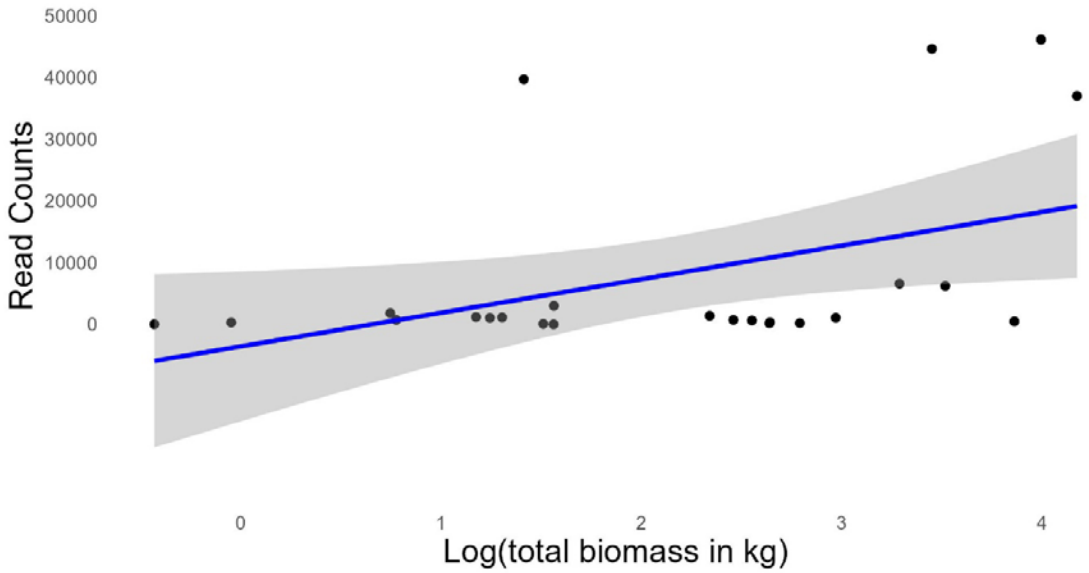

FIGURE 7 Correlation between total sequencing reads (read counts) and Log transformed total biomass (kg) of detected zoo species.

DISCUSSION

This study aimed to assess airborne eDNA sampling strategies by comparing the efficiency of a newly designed passive approach and two commonly used active methods, while exploring the spatial and temporal dynamics of airborne eDNA. Our results show that the DNAir Sampler detected a wide range of zoo and non-target species and was more effective at capturing vertebrate biodiversity over 96 hours than either active sampler. Although mean detection distances did not differ significantly, the DNAir Sampler identified taxa at the furthest range and achieved the highest accuracy within its average detection radius. Additionally, we found a positive correlation between species biomass and total sequencing reads obtained via airborne eDNA.

Species detection within Rotterdam Zoo

We detected airborne eDNA from 88 vertebrate species, spanning both terrestrial (mammals, birds) and aquatic (fish, amphibians) habitats. Of these, 76 species were detected by the passive DNAir Sampler within 96 hours, including 30 not detected by either active sampler—highlighting the superior detection capacity of passive sampling. While the Pollensniffer showed similar diversity to previous active air eDNA studies in zoos [22,24], 64 of the 88 taxa were non-target species, likely local wildlife or food (e.g., livestock, fish).

Currently, little is known about the movement of airborne eDNA, and the impact of sampling strategies. While some species were detected across multiple samples and sampling sites (e.g. *Cyprinus caprio* in 58 samples; *Elephas maximus* in 28 samples), 32 species were present in only a single sample, highlighting the likely stochastic nature in detecting airborne eDNA in general. The greater detection capacity of the DNAir Sampler may stem from factors like sampling height (~2.5 m vs. ≤1 m for active samplers), extended sampling duration (96 hours vs. short active bursts), and a higher cumulative air volume. This longer, pas-

sive sampling may integrate effects tied to eDNA release variability due to species behavior or environmental factors (e.g., wind, rain) increase species detection over other methods.

At the indoor site (location 6), however, active sampling by the Pollensniffer outperformed passive methods possibly due to limited airflow indoors which hampers passive collection but promotes airborne eDNA accumulation [7, 47]. In contrast, the Coriolis Micro detected the lowest diversity despite its higher flow rate. Notably, the Pollensniffer consistently detected more species per location (Fig. S3a), despite sampling less air per minute. This may reflect differences in sampling mechanics—its impingement method might better capture fine aerosols than the vortex mechanism of the Coriolis Micro, where high airflow could reduce DNA particle retention. Further studies using matched air volumes but varying flow rates are needed to test this hypothesis.

Accumulation patterns of airborne eDNA

The DNAir Sampler outperformed the Coriolis Micro in species richness within the first 24 hours, both overall and for zoo species. Compared to the Pollensniffer, it surpassed species richness after 24–48 hours (overall) and 48–72 hours (zoo species). Given that active samplers are noisy, labour-intensive, costly, and require trained personnel, passive sampling—by simply hanging a device and retrieving it after a few days—offers a low-cost, minimally intrusive alternative.

Consistent with [29], who showed airborne eDNA accumulates over 24 hours using water-based passive samplers, we observed no plateau in species detection even after 96 hours. Continuous detection of new species suggests that longer sampling durations could further increase richness. Unlike aquatic eDNA, which may be more homogenous, airborne eDNA might be released in pulses or be influenced by weather, as suggested by [27] and [48]. We observed variation in both species richness and composition over time, highlighting the potential impact of pulsed release, weather, and eDNA persistence on airborne eDNA

signatures. Untangling these effects experimentally and devising optimized sampling strategies is a challenge we look forward to addressing.

Spatial distribution of target species

Out of all airborne eDNA samplers, the DNAir Sampler had the highest average detection distance and accuracy, identifying nearly 1 in 5 target species within its 188 m range. Both the DNAir Sampler and Pollensniffer were more effective at detecting nearby species than the Coriolis Micro, aligning with findings from [22] and [24], who observed stronger detection closer to source animals. While airborne eDNA typically concentrates around its origin, it can also travel considerable distances—such as the American bison (*Bison bison*), detected 515m from its enclosure by the DNAir Sampler within 24 hours. Notably, the furthest detections by the active samplers occurred indoors, underscoring the challenge of tracing eDNA back to its source in natural settings [7]. Similar difficulties have occasionally been reported in aquatic systems for localizing sources of eDNA (e.g., [49]), highlighting challenges regardless of the environmental media sampled.

Conversely, many nearby target species went undetected, with contributing factors likely being heterogeneity, wind patterns, UV degradation, or low species-specific shedding rates [7, 50]. Echoing findings from aquatic studies [51], zoo-based airborne eDNA research has shown a correlation between species biomass and detection likelihood [22,24]. Our results similarly revealed a strong positive relationship between species biomass and sequencing reads, corroborating what is becoming a salient characteristic of eDNA.

Future directions and recommendations

This study is the first to apply the passive DNAir Sampler for airborne eDNA-based vertebrate detection, though its optimal setup remains untested. We used 0.7 µm glass fiber filters, but other materials may enhance detection. For instance, [24] used F8 pleated filters,

and [22] used Sterivex-HV filters (0.22–0.45 µm), finding that pore size did not significantly affect community composition. Since the DNAir Sampler passively collects particles without forcing air through pores, pore size may be less critical than with active samplers.

Several variables remain unexplored—filtering duration, seasonal effects, sampler height, and air movement. Wind direction, in particular, is thought to influence detection more during passive sampling [52], though we did not track it in this study. Despite this, the DNAir Sampler effectively detected both zoo and local species and continued to register new detections over time.

Overall, the DNAir Sampler offers a low-cost, non-invasive, and efficient method for vertebrate biodiversity assessment compared to its active sampler counterparts. Its ease of deployment and wide detection range position it a valuable tool, especially for expanding terrestrial biomonitoring. Future research should refine passive sampling protocols and evaluate their performance in natural habitats. While terrestrial eDNA research lags behind aquatic systems, airborne eDNA has transformative potential for large-scale biodiversity monitoring. Given its strong detection performance across taxa, passive air eDNA is an underappreciated yet powerful method for biodiversity assessments and may emerge as the preferred strategy for future terrestrial biomonitoring, particularly for scaling up efforts.

Resource availability

Upon acceptance, all data will be uploaded to Figshare and raw sequence files will be made available upon request.

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Author contributions

K.A.S., K.T., A.G. C. L. S. and H.J. conceived and planned the experiments, JM.L. built the samplers. H.J. and JM.L. conducted sample collections. H.J. and K.A.S. carried out the laboratory work. H.J., K.A.S. and K.T. planned and carried out the data analysis. All authors contributed to the interpretation of the results. H.J. and K.A.S. took the lead in writing the manuscript but all authors provided feedback, edited, and helped shape the research, analysis and manuscript.

Declaration of interest

The authors declare no competing interests.

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