Synergies of camera traps and environmental DNA for wildlife surveys in tropical forests

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RÉSUMÉ

Compte tenu de l'urgence de la crise actuelle de la biodiversité, il est de plus en plus crucial de mettre en place des méthodes efficaces de suivi de la faune, en particulier dans les concessions forestières où les impacts combinés de l'exploitation forestière et de la chasse représentent des menaces importantes pour la biodiversité. Les méthodes de suivi de la faune traditionnelles, souvent laborieuses et coûteuses, rendent nécessaire l'exploration de solutions plus efficaces et accessibles. Pour relever ces défis, nous avons comparé trois techniques de suivi alternatives—les pièges photographiques, l'ADN environnemental (ADNe) prélevé sur les feuilles et l'ADNe dans l'eau— dans quatre zones forestières contrastées au sein d'une concession forestière certifiée FSC dans le nord du Congo, chacune ayant des antécédents différents en matière d'exploitation forestière et de pressions de chasse. Nos objectifs étaient de (1) comparer l'efficacité de ces méthodes dans l'évaluation de la richesse spécifique, de l'occupation et de l'abondance relative ; (2) mesurer l'efficacité de chaque méthode en termes de précision et d'exactitude de la richesse spécifique, ainsi que les coûts associés, afin de proposer une méthode de suivi de la faune optimale ; et (3) discuter de l'influence de l'exploitation forestière et de la chasse sur la diversité spécifique. Les pièges photographiques se sont révélés très efficaces pour les mammifères de taille moyenne à grande, tandis que l'ADN environnemental prélevé sur les feuilles s'est révélé précieux pour détecter un plus large éventail d'espèces, y compris les chauves-souris, les petites espèces et les espèces arboricoles. L'ADNe dans l'eau, bien qu'il ait capturé des espèces clés avec un effort d'échantillonnage moindre, a montré une rentabilité limitée en raison de coûts plus élevés par rapport à l'ADNe sur les feuilles. Bien que l'ADNe sur les feuilles soit la méthode la plus rentable, elle nécessitait un échantillonnage plus exhaustif pour égaler la diversité détectée par les pièges photographiques. Cette étude met en lumière les forces et les rôles complémentaires des différentes méthodes de suivi. Le choix de la méthode doit être aligné avec les objectifs de conservation spécifiques : l'ADN environnemental pourrait être utilisée pour identifier les zones de haute valeur de conservation, tandis que les pièges photographiques sont mieux adaptés pour le suivi répété des populations de mammifères de taille moyenne à grande. Nos résultats indiquent que la pression de chasse est davantage influencée par l'accessibilité et la proximité des implantations humaines que par l'historique de l'exploitation forestière. La richesse comparable des espèces à travers les quatre zones forestières suggère que les forêts certifiées bien gérées peuvent servir de refuges critiques pour une faune diversifiée. Les résultats soulignent le potentiel de conservation des forêts certifiées, en mettant en avant l'importance des pratiques de gestion intégrées et durables pour soutenir les populations de faune diversifiées.

Mots-clés : Suivi de la Faune, Pièges Photographes, ADN Environnemental (eDNA), Forêt Tropicale, Pression d'Exploitation Forestière, Pression de Chasse, Stratégie de Conservation

ABSTRACT

Considering the importance of addressing the current biodiversity crisis, effective wildlife monitoring is increasingly essential, particularly in logging concessions where the combined impacts of logging and hunting pose severe threats to wildlife. Traditional monitoring methods can be labor-intensive and costly, necessitating the exploration of more efficient alternatives. To address these challenges, we compared three alternative wildlife monitoring techniques—camera traps (CT), leaf swab environmental DNA (eDNA), and water eDNA—across four contrasting forest areas within an FSC-certified logging concession in northern Congo, each with different logging histories and hunting pressures. Our objectives were to (1) compare the accuracy of these methods in assessing species richness, occupancy, and relative abundance; (2) measure the effectiveness of each method in terms of species richness accuracy and precision, as well as associated costs, to propose an optimal wildlife survey method; and (3) discuss the influence of logging and hunting on species diversity. Camera traps proved highly effective for medium-to-large mammals, while leaf swab eDNA was valuable for detecting a broader range of species including bats, smaller species, and arboreal species. Water eDNA, while capturing key species with relatively less sampling effort, showed limited cost-effectiveness due to higher expenses than leaf swabs. Although leaf swab eDNA was the most cost-efficient, it required more extensive sampling to match the diversity detected by camera traps. This study highlights the strengths and complementary roles of different monitoring methods. The choice of method should align with specific conservation goals: eDNA could be used to identify areas of high conservation value, while camera traps are better suited for repeatedly monitoring medium to large mammal populations. Our findings indicate that hunting pressure was more influenced by accessibility and proximity to human settlements than logging history. The comparable species richness across forest grids suggests that well-managed, certified forests can be critical refuges for diverse wildlife. The results highlight the conservation potential of certified forests, emphasizing the importance of integrated and sustainable management practices to support diverse wildlife populations.

Keywords: Wildlife Monitoring, Camera Traps, Environmental DNA (eDNA), Tropical Rainforest, Logging Pressure, Hunting Pressure, Conservation Strategy

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1. INTRODUCTION

Biodiversity loss and impacts of human activities in the Congo Basin

The ongoing sixth mass extinction, primarily driven by human activities, presents a severe biodiversity crisis (Ceballos et al., 2010, 2015; Pievani, 2014; Ripple et al., 2016; Rull, 2021). Vertebrate extinction rates are 100 times higher than natural rates, highlighting species' rapid and catastrophic loss (Ceballos et al., 2015). Tropical forests, which host more than half of terrestrial biodiversity, are critically threatened by anthropogenic pressures such as overhunting, habitat fragmentation, over-exploitation, changes in land use, invasive species, and the overarching impact of climate change (Morris, 2010; Rull, 2021). These forests face severe challenges, with defaunation rates as high as 20% in African tropical forests (Rull, 2021). The delicate balance of these ecosystems is jeopardized (Tagg et al., 2019), endangering numerous species and essential ecological functions.

African tropical forests occupy approximately 180 million hectares, of which 26% are managed by logging companies for timber production (Doucet et al., 2016). Industrial timber exploitation constitutes an essential economic activity and employment source for all the Congo Basin countries (Haurez et al., 2013). However, the logging industry has wide-ranging impacts on wildlife through direct disturbances, such as the presence of heavy machinery and logging teams, and habitat modifications like canopy damage from tree felling and road construction (Nasi et al., 2009; Billand, 2010). While these direct effects are considered to moderately affect animals, especially large mammals (Clark et al., 2009), the indirect impacts might be more significant (Billand, 2010). Logging often involves building roads and infrastructure, attracting employees and their families to remote forest areas, which increases hunting activities (Nasi et al., 2009; Nasi & van Vliet, 2011). Road network expansion increases hunting pressure, making it challenging to differentiate the effects of logging from those of hunting on wildlife populations (Laurance et al., 2006; Nasi et al., 2009). Poulsen et al. (2011) found that hunting and logging independently affect the densities of various animal guilds in different ways, both positively and negatively.

In recent decades, hunting has become one of the greatest causes of wildlife decline (Fa et al., 2014; Ripple et al., 2016; Tagg et al., 2019). This has led to unsustainable hunting levels that exceed the natural regeneration capacity of wildlife populations (Fa et al., 2014). Mammals, particularly large species, are heavily targeted by hunters, with 91 African species at risk of extinction due to hunting (Ripple et al., 2016). Many of these mammal species, known as "keystone species" or "ecosystem engineers", play vital roles in the ecological functioning of their habitats (Nasi, Taber, et al., 2011; Ripple et al., 2016; Tagg et al., 2019). Despite its negative impacts on wildlife, hunting for bushmeat remains crucial in remote forest areas, serving as a primary source of protein and income for local people (Nasi et al., 2009; Nasi, Taber, et al., 2011; Fa et al., 2014). Additionally, bushmeat holds cultural significance, especially for indigenous communities, being integral to rituals and ceremonies (Nasi, Taber, et al., 2011).

To mitigate their impact on forest ecosystems, logging companies are encouraged to obtain voluntary certifications such as the Forest Stewardship Council (FSC) or Pan African Forest Certification (PAFC), which are the two major responsible management certification schemes in the Congo Basin. These certifications require management plans with strong measures for biodiversity conservation, environmental protection, and social responsibility (Medjibe et al., 2013).

While establishing protected areas has been the predominant strategy to address anthropogenic pressures, it is increasingly evident that these areas alone are insufficient to meet international biodiversity targets (Panlasigui et al., 2018). Nevertheless, there has been a growing acknowledgment

that managed forest concessions can be extensions of protected areas (Chazdon, 1988; Clark et al., 2009; Panlasigui et al., 2018). Recent research highlights significantly higher abundances of large-bodied mammals (over 10 kg) in FSC-certified logging concessions than in non-certified concessions, partly due to anti-poaching measures (Zwerts et al., 2024). Therefore, responsible forest management certification is crucial in promoting biodiversity conservation, particularly in protecting rare and threatened species and their habitats (FSC, 2023).

Wildlife monitoring in tropical forests

Wildlife monitoring is a fundamental requirement for managed logging concessions and has been widely used to assess the impacts of logging and hunting on wildlife populations. Measures of the presence and abundance of certain wildlife species appear to be the most direct indicators of defaunation (Nasi & van Vliet, 2011).

Recces and distance sampling through linear transects have been the two traditionally most used techniques in Central Africa (Maréchal et al., 2014). These techniques, which focus on medium and large mammals (Mena et al., 2021), require a significant investment in time and manpower (Nasi, Taber, et al., 2011). Moreover, distance sampling mainly relies on indirect observations, which require estimating production and degradation rates for dung and nests, which can affect abundance estimations (Buckland et al., 2015).

In recent decades, technological advancements have introduced new wildlife research methods, including acoustic and biologging sensors (Lahoz-Monfort et al., 2021). Acoustic sensors record field sounds and aid in monitoring vocalizing species and detecting poaching activities through gunshots (Lahoz-Monfort et al., 2021). Biologging sensors collect data from sensors placed on or within animals, offering valuable insights into animal health, ecology, and behavior (Lahoz-Monfort et al., 2021).

Camera trapping is also an increasingly used approach (Leempoel et al., 2020). It can be triggered automatically by animal movement to capture videos and pictures even of elusive species (Shrestha et al., 2018), focusing primarily on mid-sized to large terrestrial animals (Lahoz-Monfort et al., 2021; Mena et al., 2021). Camera trap (CT) surveys have been used in conservation to estimate species richness, occupancy, activity patterns, relative abundance, and even density in a non-invasive manner (Fonteyn et al., 2020; Mena et al., 2021). While this approach typically requires long temporal coverage and can be expensive (Leempoel et al., 2020), ongoing technological advancements have facilitated the broader adoption of this technique (Fonteyn et al., 2020). In addition, managing and visualizing a massive number of images can be resource consuming. However, with the emergence of algorithms and artificial intelligence to sort images and identify animals automatically, this burden is progressively alleviated (Lahoz-Monfort et al., 2021).

Species detection through environmental DNA (eDNA) also emerges as a promising alternative to traditional monitoring methods (Leempoel et al., 2020; Mena et al., 2021). eDNA operates on the principle that biological material such as skin fragments, feces, or saliva are shed into the environment, allowing for species identification through environmental sample collection. eDNA sampling from water, soil, or leaf swabs encompasses promising, non-invasive techniques to collect DNA from terrestrial mammals (Ushio et al., 2017; Leempoel et al., 2020; Lynggaard et al., 2023). Vertebrate species can also be indirectly detected through invertebrates such as carrion scavengers or blood-feeding species that have ingested their DNA (invertebrate DNA, iDNA) (Leempoel et al., 2020). However, accurately assessing vertebrate diversity through this approach requires a nuanced understanding of host preferences among blood-feeding species (Calvignac-Spencer et al., 2013).

eDNA analysis allows the estimation of vertebrate species richness in a given environment (Mena et al., 2021) with presence/absence data but provides no information on the population abundance. However, at the landscape level, it can document the species occupancy with the number of sample sites detecting each species. This has been primarily used for water samples (e.g., Lyet et al., 2021).

While eDNA collection offers several advantages for wildlife monitoring, including the detection of small and elusive species, non-invasiveness, and minimal time and manpower requirements (Leempoel et al., 2020; Mena et al., 2021; Van Leeuwen et al., 2023), several challenges persist. The main challenge is the incomplete reference databases leading to identification inconsistencies (Leempoel et al., 2020). Mena et al. (2021) compared camera trapping and eDNA of terrestrial mammals in the Peruvian Amazon forest and determined that about 42% of all recorded species (two methods combined) were absent from the genetic reference database. Another substantial challenge is that despite advancements, few studies have comprehensively assessed mammalian richness using eDNA, especially in tropical environments (Van Leeuwen et al., 2023). This leaves critical questions unanswered, such as the frequency of animal presence required for detectability in eDNA samples, the time during which eDNA stays detectable in the environment before being too degraded for species identification, or the impact of animal size and behavior on the quantity of DNA left behind (Leempoel et al., 2020). Standardized, long-term monitoring protocols are essential for optimizing sampling methods (Leempoel et al., 2020; Van Leeuwen et al., 2023) and facilitating site comparisons (Nasi et al., 2009). Additional challenges include the lack of laboratory access to conduct genetic analyses in many countries and the risk of DNA degradation if not stored under proper conditions (Van Leeuwen et al., 2023).

Comparative studies highlight the unique advantages of eDNA in biodiversity assessment. For instance, eDNA effectively detects species like arboreal mammals that are challenging for camera traps, providing a broader perspective on species diversity that other methods may not capture (Mena et al., 2021). Recent comparisons have demonstrated this complementarity: water revealed more information on mammalian presence and detection rates than camera traps and at a lower cost (Lyet et al., 2021). Conversely, camera trapping often shows a higher detection probability for small mammals than soil eDNA techniques (Verhees et al., 2024). It is also less effective in detecting medium to large mammals than CTs. Therefore, combining multiple methods is essential to effectively survey a wide range of mammalian species (Mena et al., 2021; Van Leeuwen et al., 2023).

Considering the growing necessity for wildlife monitoring, the main objective of this study is to assess the synergies between the use of camera traps and eDNA for wildlife surveys in tropical forests, using data acquired in an FSC-certified logging concession in the north of the Republic of Congo. Specifically, this study aims to (i) compare the accuracy of species richness, relative abundance index (RAI), naive occupancy, and beta diversity resulting from leaf swab eDNA, water eDNA, and camera trap data collection methods, (ii) measure the effectiveness of each method in terms of species richness accuracy and precision, and associated costs to propose an optimal wildlife survey method, and (iii) discuss the combined influence of selective logging and hunting pressure on vertebrate diversity among four contrasted histories of human activities.

2. MATERIAL AND METHODS

2.1. Study area

The northern part of the Republic of Congo is characterized by an equatorial climate with high humidity and rainfall above 1,700 mm, and an average temperature between 24 and 26°C (FAO, 2005). Forests cover about 67% of the Congolese territory, i.e. almost 22 million hectares, and are situated mainly in the North and South-East (FAO, 2020). In the north, 60% of the forest is firm land rainforest. The 40% remaining comprise flooded and swamp forests (CIB-MEFE, 2006).

This study occurred in the logging concessions managed by the company CIB (*Congolaise Industrielle des Bois* – Olam Agri). It is situated in Northern Congo in the department of Sangha, commune of Pokola (Figure 1). CIB was created in 1968 by the fusion of two old forest concessions (CIB-MEFE, 2006). Currently, CIB is awarded four Forest Management Units (FMU) covering 2 million hectares: Pokola, Kabo, Loundoungou-Toukoulaka, and Mimbeli Ibenga. The first three are FSC-certified (ATIBT, 2024). CIB company's headquarters are in Pokola where all management and service operations are centralized together with several industrial wood transformation units. Kabo regroups the living quarter of all the logging operations of the FMU Kabo and one industrial wood transformation unit. CIB manages three other locations regrouping living quarters and logging sites: Ndoki I, Ndoki II, and Loundoungou (CIB-MEFE, 2010).

The forests in this area host a high diversity of plants and animal species (Poulsen et al., 2009). FMUs managed by CIB are adjacent to Nouabalé-Ndoki National Park (NNNP). Because of this proximity, these FMUs could be considered potential extensions of the protected area and crucial to conservation.

Four Forest Production Units (FPU) have been selected for this study: two in the FMU Kabo (FPU 3 and 4), one in the FMU Pokola (FPU 4), and one in the FMU Loundoungou-Toukoulaka (FPU 4; Figure 1). These FPUs have been selected as four sub-areas in our sampling strategy based on their Annual Logging Stands (ALS) operating history constituting a logging and hunting pressure gradient (Table 1). The FPU 4 in the FMU Loundoungou-Toukoulaka (Grid C), has never been logged by CIB (CIB-MEFE, 2010) and has therefore been selected as a control area. Conversely, the FMUs Kabo and Pokola have been logged since the end of World War II, spanning over 70 years (CIB-MEFE, 2007). In Pokola's FPU 4 (Grid B), the selected ALS represents the extreme end of the spectrum, with a significant impact from ongoing logging operations and the creation of transects facilitating hunter movements. Meanwhile, the two other ALS selected in FMU Kabo (Grid A and D) represent intermediate cases. Although logging has ceased and roads are closed in both areas, they remain accessible by foot, particularly in FPU 4, providing potential access for hunters. In the rest of the study, the different ALS selected will be called by their grid name (Table 1).
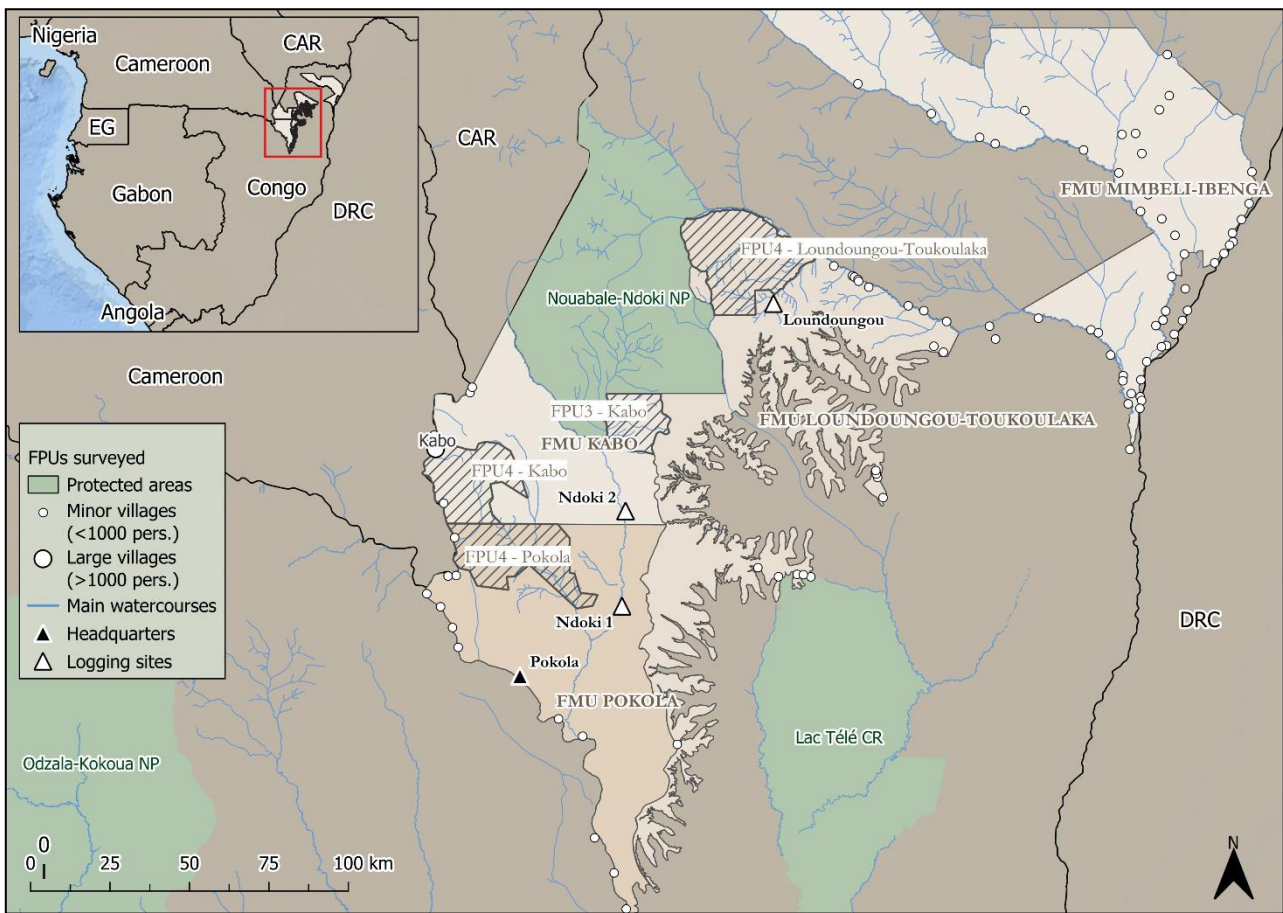
Figure 1: Location of the study area in the north of the Republic of Congo.

Table 1: Characteristics of the four sampled Annual Logging Stands (ALS) selected in each Forest Production Unit (FPU)

	GRID NAME	LAST OPERATING YEAR OF THE ALS SELECTED	HUNTING AND OTHER FEATURES
FPU 4 - Kabo	Grid A	2020 - 2021	• Significant hunting pressure, according to the Peripheral Park Ecosystem Management Project • Proximity of an eco-guard base located in Kabo
FPU 4 - Pokola	Grid B	Prospection in progress	• Right next to the logging area 2024 and prospecting underway • Hunting pressure is assumed to be moderate
FPU 4 - Loundoungou-Toukoulaka	Grid C	Never logged	• Low hunting pressure assumed
FPU 3 - Kabo	Grid D	2014 - 2017	• Hunting pressure is unknown but assumed to be moderate

2.2. Data collection

2.2.1. Sampling design

One sampling grid created with QGIS 3.22.11 has been placed in each of the four selected ALS. Therefore, four 8 x 8 km grids were installed (Figure 2), serving as a basis for the sampling plan of the three survey methods deployed for this study. In each grid, 25 sampling stations were determined with QGIS with a space of 2 km between each site (TEAM Network, 2011). Each grid has been placed based on the logging history, as well as the accessibility and the presence of water (Figure 2). Two teams worked simultaneously with standardized protocols to optimize field deployment time. The total duration of the survey with the three methods lasted from February 2024 to April 2024.
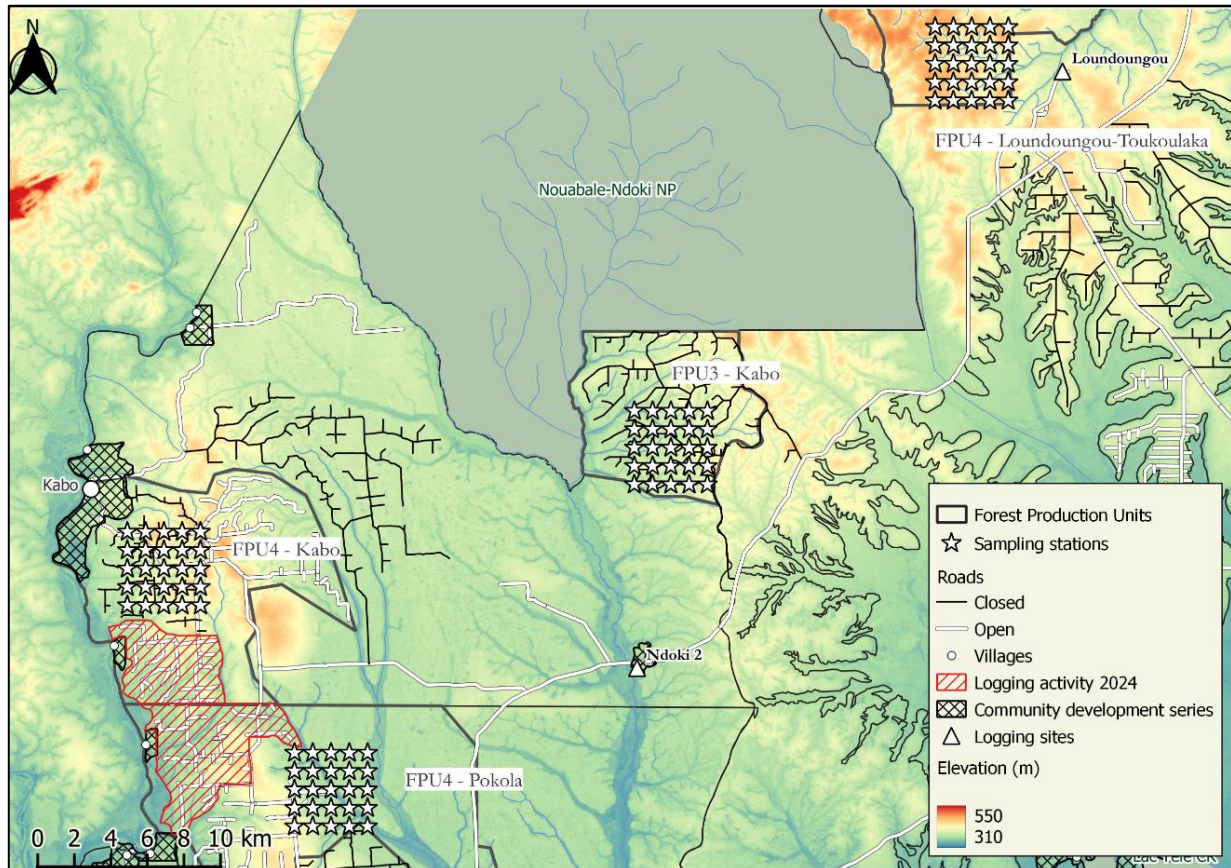
Figure 2: Topography of the study area and sampling grid position.

2.2.2. Camera traps

One camera was placed at each station (25 cameras per grid, total of 100) and left in the field for 40 days to reach a sampling effort of 1000 cameras.days per grid.

The CTs "ScoutGuard, BODYGUARD SG2060-T, Boly Media Communication" were programmed to record a burst of 3 photos at maximum resolution for each detection. A minimum trigger delay of 0 s was set between detection events. Based on the materials available, two types of cameras were used for the project: 53 equipped with LED flash and 47 with infrared light. The Passive Infrared (PIR) sensor was set to high sensitivity for the infrared cameras.

Once in the field and the theoretical geo-referenced point of the station reached, different steps were followed (Meek et al., 2014; Fonteyn et al., 2021):

1) The CT was positioned in a wildlife-friendly site within 100 meters of the camera's theoretical coordinates, avoiding swamps and selecting a location along a wildlife trail.
2) Each CT was placed between 30 and 50 cm from the ground (at knee level). To maximize the detection of animals, the camera was preferably oriented diagonally to the trail, parallel to the ground, and at 2 to 4 meters from the trail. The actual GPS coordinates of the CT location were recorded.
3) To reduce false triggers, the vegetation was slightly cleared within a radius of 3 m in front of the camera. All cut plant parts were discarded more than 20 meters from the camera's location.
4) Several tests and a check of the parameter configuration were carried out before activating and sealing the device.

2.2.3. Environmental DNA

In this study, two complementary eDNA collection methods were employed, namely water samples and leaf swabs.

First, 40 water samples were collected, with 10 samples gathered per grid. The sampling approach was opportunistic, leveraging the Digital Elevation Model (DEM) to identify watercourses inside each grid. Samples were also collected from water points encountered in the field. The collection focused on the upper 10 cm of the water column following established protocols (Miaud et al., 2019; Mena et al., 2021). The samples were procured using a peristaltic pump, a 0.45 µm filtration capsule, a silicone tube connected to a strainer, and a plastic evacuation tube (Appendix 2, Photo 2), following a series of steps (GeCoLAB, 2020):

1) Latex gloves were worn throughout the process to prevent DNA contamination.
2) After activating the pump trigger, water entered the filtration system through the strainer, traversed the filter capsule, and exited through the drainpipe. The filtration process was limited to a maximum duration of 30 minutes.
3) After filtration, water was drained from the capsule, and the two pipes were removed. The capsule's outlet was then sealed with parafilm and a cap.
4) Subsequently, 70 to 75 ml of ethanol was introduced into the capsule using a syringe.
5) The capsule's inlet was sealed using the same approach as its outlet, and the capsule was shaken to ensure thorough soaking of the filter.

Following this process, a field sheet was completed, encompassing information such as grid and sample identifier, collection date, collector's name, GPS coordinates, filtration duration, and the nature of the source (water point or watercourse). The position of each water sample collection point is presented in Appendix 1 (Figure 9).

Leaf swabs were used as a second method to sample eDNA. Sampling was performed at each station on the grid, totaling 100 collection sites. Samples were extracted from plant organs situated at a height of less than 1 meter, chosen due to a higher probability of animal contact. The plants selected for DNA collection were those located along animal trails previously identified for installing camera traps. Three vegetation strata were defined to ensure maximum diversity: ground level, from the ground to 50 cm, and from 50 cm to 1 m. The sampling process was standardized to use a single cotton swab per stratum, swabbing a total surface area of 300 cm². Within each stratum, the method involved swabbing three separate 100 cm² surfaces from different leaves for 10 seconds each (Appendix 2, Photo 2). This ensured the 300 cm² area was swabbed from at least three leaves per stratum. The selection of leaves was guided by the consideration that leaf roughness could influence

the quantity of retained DNA. Leaves with a significant layer of residues (dust) or those showing signs of fauna damage nearby were given preference.

The protocol, inspired by Lynggaard et al. (2023), followed the subsequent steps:

1) Nitrile gloves were worn throughout the process to prevent DNA contamination (Kolby et al., 2015).
2) In each sampling instance, a swab per stratum (i.e., three swabs per site) was immersed in an ethanol solution to preserve nucleic acids.
3) The 300 cm² leaf surface was swabbed with cotton, rubbing the upper surfaces of the leaves with one cotton swab (Appendix 4).
4) After each rubbing, the stems were cut to preserve only the cotton, and the three swabs from the same site were directly consolidated in a 20 ml bottle filled with 96% ethanol.

Comprehensive data were recorded for each sampling site, including grid and sample identifiers, collection date, collector's name, GPS coordinates of the collection point, the number of swabbed leaves per stratum, type of animal trail (tracks, nests, feces), and prevailing weather conditions (rain, dry, recent rain).

To ensure traceability, all eDNA samples (both water and leaves) were labeled with their identifier, including the grid name and sample/station number. They were all stored at ambient temperature on the field and then promptly transferred to a freezer at -20°C (Lynggaard et al., 2023). Because of the duration of the field trips, the first samples collected in each trip stayed at ambient temperature for a maximum of 7 days before being at -20°.

2.2.4. Hunting data

In addition to wildlife survey data, all signs of hunting activities have been recorded as GPS points in the field. These signs comprised fires, camps, sockets, traps, cables, or carcasses, for example.
The presence of humans in CT images has also been recorded. Several cameras were stolen during the experiment, which is also considered as a hunting sign.

2.3. eDNA lab analysis

Metabarcoding was conducted on all DNA samples. This technique uses universal primers to PCR-amplify short DNA markers and identify the target species (Lynggaard et al., 2022). The analysis followed the steps bellow:

1. **DNA sample collection**: Water samples were concentrated via centrifugation, and leaf swabs were processed after ethanol evaporation.
2. **DNA extraction**: QIAGEN QIAamp® DNA Mini and Investigator kits were used for water and leaf swab samples, respectively, following the manufacturer's protocols.
3. **PCR amplification and sequencing**: Two sets of primers coding for 133 bp of mitochondrial CO1 gene (Galan et al., 2018) and a 170 bp amplicon of 12S rRNA (MiMammal; Ushio et al., 2017) were used to amplify vertebrate and mammalian DNA respectively, followed by a purification step. A second PCR allowing the addition of specific tags for sample identification was then performed. These PCR products underwent a second purification step. Following DNA purification, quantification, and pooling at equimolarity, DNA library construction and sequencing were conducted at the University of Liège GIGA Genomics platform using an Illumina NovaSeq® flow cell.
4. **Sequence analysis**: We controlled the quality of demultiplexed reads with FastQC (Andrews, 2010) and trimmed the raw paired-end sequences, removing adapters with Cutadapt v2.9

(Martin, 2011). Using an in-house bioinformatic pipeline and USEARCH (Edgar, 2010), we detected, clustered, and chimera-checked amplicon sequences, which were then assigned to reference databases to determine their read abundance in eDNA samples. Species identification was done via the BOLD database for CO1 and an NCBI GENBANK for 12S with a percentage of matching identity with the closest reference sequences.

The following method generated a table of presence for each taxon per sample. For comprehensive details of the procedure, refer to Appendix 3.

2.4. Sampling costs

To facilitate cost comparison across methods, we calculated five main cost components: material costs, field expenses, transportation, lab costs, and data processing. Material costs encompassed all the materials needed for the experiment (camera traps, batteries, SD cards, silicone, swabs, ethanol tubes, water eDNA kits, and peristaltic pump). Field expenses included the salaries of researchers and workers involved in fieldwork activities and food supplies. Transportation encompassed expenses related to transporting field teams. Lab costs covered genetic analyses for species identification. Finally, data processing covered the species identification and data preparation for CT, sorting the sequence identifications for eDNA, and data analysis for both CT and eDNA. Costs were computed assuming pre-acquired basic equipment (GPS, camping gear, work computer, field boots) and operational data processing software codes.

2.5. CT species identification

Every image captured by CTs was processed using the online tool TrapTagger (traptagger.co.uk), an open-source Artificial Intelligence (AI) platform developed by Wildeye. It has been designed to process and classify CT images rapidly according to species. Unfortunately, Trap Tagger's AI lacks sufficient training data from Central African forests, leading to less effective automatic recognition. Consequently, images with animal occurrences needed manual processing, despite the system's ability to detect false positives, humans, and some top predators. Animals were identified to the highest achievable precision, typically at the species or genus level. All vertebrates were considered for the identification. Species nomenclature followed the IUCN Red List of Threatened Species (IUCN, 2024). The species identification was made with the help of the FauneFAC tool (Fonteyn et al., 2021) and "The Kingdon Pocket Guide to African Mammals"(Kingdon, 2016). Several species were impossible to differentiate on camera trap pictures because they share similar anatomical characteristics (Fonteyn et al., 2021), so we clustered them in groups: the "forest squirrels" group encompasses species of the Sciuridae family, the complex "mongooses'' regroup the marsh mongoose *(Atilax paludinosus)* and the long-nosed mongoose *(Herpestes naso),* the large spotted genet *(Genetta maculata)* and the servaline genet *(Genetta servalina)* are clustered in the "genet" complex, and the "small pangolins'' include the black-bellied pangolin *(Phataginus tetradactyla)* and the white-bellied pangolin *(Phataginus tricuspis)*.

2.6. Data processing

After species identification, all the CT data were retrieved in a CSV file. Observations of the same species were considered independent if the interval between two detections was superior to 30 min (Fonteyn et al., 2020). The data were standardized by removing cameras that operated for less than 38 days. As a result, 16 cameras were excluded, leaving 84 cameras (Grid A: 23, Grid B: 19, Grid C: 19, Grid D: 23). Additionally, all images captured after the 38-day period were deleted from the dataset. All the images without any animals have been removed from the dataset. Uncertain

identifications were excluded from richness and abundance analyses, including images labeled as red duikers, great apes, small predators, small primates, and unknown species. Additionally, human detections were removed from the dataset used for these analyses.

After genetic analysis, we obtained a list of potential identifications. We followed a rigorous method to filter and classify the sequences, removing Operational Taxonomic Units (OTUs) from PCR controls and discarding low-quality sequences. After filtering, we checked the geographical distribution of the remaining species, classifying them as "certain" or "potential" based on their presence in the area or related genus. For detailed criteria and steps, refer to the Appendix 3 - Table 5. Only the "certain" list was used for the following analysis to guarantee accurate results.

2.7. Statistical analysis

The statistical analysis was conducted using Microsoft Excel (version 2406) and R Studio 4.2.2. Three rarefaction curves were calculated for each grid using the `specaccum` package and the `rarefaction` function to compare the species richness among the methods and the grids. A richness value was extracted from each rarefaction curve at the maximum common camera-days value shared by all four grids (i.e., 722 camera-days for CT, 24 samples for Leaf swab eDNA, and 9 samples for water eDNA).

To compare β-diversity across methods, a Venn diagram was constructed to illustrate the shared and unshared species among the three methods. To visualize differences in species composition across survey methods, we performed a Principal Coordinates Analysis (PCoA) using Bray-Curtis dissimilarity distances on a presence/absence matrix incorporating all methods. Subsequently, a PERMANOVA test was conducted on the same Bray-Curtis dissimilarity matrix, to assess the statistical significance across the differences between methods. Additionally, a separate CA was conducted on a species contingency matrix derived from CT data to compare species composition across different grids. In this analysis, species recorded in fewer than two sites were excluded to prevent rare species from disproportionately influencing the ordination and potentially distorting the interpretation of results.

To evaluate the effectiveness of the methods deployed, we conducted a resampling with 10, 15, and 19 cameras per grid. Each resampling scenario involved 1000 repetitions per grid to extract species richness from the rarefaction curve using the same functions as previously described. Subsequently, we calculated the mean richness from the 4000 values generated (1000 per grid scenario). To evaluate the variability introduced by the resampling process, we utilized ANOVA on the aggregated 4000 richness values and extracted the Mean Square Residuals (MSR) root. The resampling was performed without replacement using the `sample` function in R.

For the naive occupancy, we calculated a Proportion of Area Occupied (PAO) for each detected species (or higher-level taxa when species level identification was not possible) in each grid:

$$\frac{Number\ of\ sites\ where\ the\ species\ is\ detected}{Total\ of\ surveyed\ sites}\ \text{(MacKenzie et al., 2018)}$$

This index ranges from 0 to 1 for each species and each grid, and from 0 to 4 for the overall survey. The Relative Abundance Index (RAI) for 100 camera.days of each detected species with CT, was calculated for each grid:

$$\frac{Number\ of\ independant\ detection\ event}{Number\ of\ considered\ camera.days}\ x100\ \text{(O'Brien, 2011)}$$

In order to compare PAO and RAI values, identifications were standardized. Instances where identifications reached the species level in one method but only the genus level in another were standardized to the genus level (e.g., *Genetta servalina* identified by eDNA was standardized to *Genetta* sp. to be compared with CT data).

To assess the relationships between different abundance and occupancy indicators, linear regressions were performed between each pair of indicators (RAI and PAO) derived from the three methods. For each pair of indices, linear regression was also performed for each grid. This was achieved using the `lm` function for linear modeling and the `ggpairs` function for creating matrix plots.

To evaluate the impacts of logging and hunting, RAI values were compared across the four grids using a linear mixed model implemented with the `lmer` function. In this model, the grid was included as a fixed factor to test for differences between areas and species as a random factor to account for variability among species. Similarly, the same linear mixed model was constructed using PAO values derived from camera traps (CT), water eDNA, and leaf swab eDNA. A Shapiro-Wilk test was conducted to check the normality of the linear mixed model residuals. Then, an analysis of variance (ANOVA) was conducted on the linear mixed models to determine the significance of the observed differences. Finally, a pairwise comparison was made using `emmeans` for each pair of means.

To objectify the hunting pressure in each grid, we used six indicators: (i) the number of hunting indices per grid, (ii) the number of stolen cameras, (iii) the human RAI, (iv) the distance to the nearest open road, (v) the distance to the closest human settlement, and (vi) the estimated number of people living within a radius of 15 km from the center of the grid. For the number of hunting indices and stolen cameras per grid, we counted the number of hunting indices systematically collected by GPS during field setup and the number of unfound cameras. For the human RAI, all independent detection events of humans have been considered in this category except the ones of our team and the prospection team of the logging company in Grid B (currently in prospection). The distances to the nearest road and human settlement were calculated from the center of each of the four grids using the `NNJoin` tool in QGIS. The number of local people living within 15 km of the center of the grid was estimated using QGIS with data provided by CIB. This distance was chosen as it represents the average distance that can be feasibly traveled on foot within a day, including the return journey.

3. RESULTS

3.1. Comparison of monitoring methods

3.1.1. Species richness

Using a combination of camera trapping, leaf swab eDNA, and water eDNA, we identified a total of 98 vertebrate species, including critically endangered species such as the African forest elephant (*Loxodonta cyclotis*) and Western gorilla (*Gorilla gorilla*), and endangered species such as the grey parrot (*Psittacus erithacus*), the chimpanzee (*Pan troglodytes*), or the giant ground pangolin (*Smutsia gigantea*) (IUCN, 2024)*. The sampling effort, however, was insufficient to fully survey the species richness in the area (Figure 3). Camera trapping detected 53 species with rarefaction curves approaching an asymptote (Figure 3A), indicating that the sampling effort was nearly sufficient to capture most species present. Leaf swab eDNA identified the highest number of species (66) (Figure 3B). The species accumulation curves for leaf swab eDNA do not approach an asymptote, suggesting that additional sampling could reveal even more species. Water eDNA, detecting 39 species, showed a curve far from an asymptote (Figure 3C), implying that more extensive sampling is necessary to assess species richness accurately.
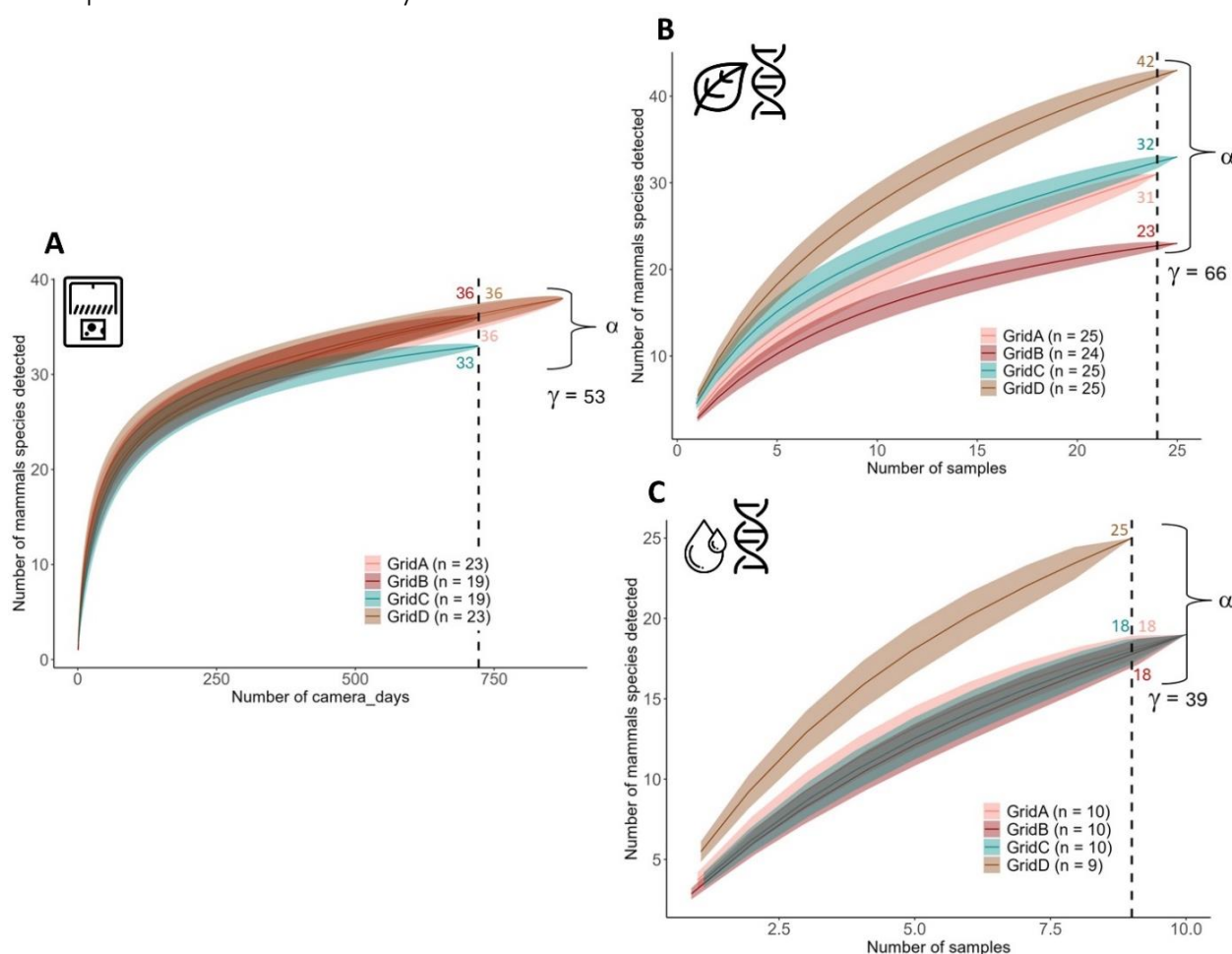
Figure 3 : Rarefaction curves of the three methods. The rarefaction curves of leaf swab (C) and water (B) eDNA are sample-based, and the CT (A) has camera-days on the horizontal axis. The alpha diversity at the scale of each grid is provided for 722 camera-days (A), 24 leaf swabs (B), and 9 water samples (C). Gamma diversity comprises the total variety of inventoried species by each method. The colored shaded areas on rarefaction curves correspond to the rarefied species richness ± its standard deviation.

3.1.2. Species composition

At least one eDNA method detected species most frequently recorded by camera traps. The three methods detected some of the emblematic species of the Congolese tropical rainforest *(Loxodonta cyclotis, Cephalophus* spp*., Cercopithecus* spp*., Potamocherus porcus, Panthera pardus, Gorilla gorilla, Pan troglodytes, Tragelaphus* spp.; Figure 4). Each method revealed unique species not identified by the others. Leaf swab eDNA detected seven bat species that were not found using other methods. Both eDNA techniques identified fish species as well as three arboreal primates (*Lophocebus albigena*, *Colobus guereza*, *Cercopithecus pongonias*) that camera traps missed. Moreover, both eDNA methods combined detected seven species of small rodents that could not be differentiated with CT. Additionally, eDNA provided species-level identification for seven taxa—small rodents, mongooses, *Nectariniidae* sp*., Galago* sp., forest squirrels, *Phataginus* sp., and *Genetta* sp.— while camera traps only identified these taxa at the genus level or higher. Regarding bird detection, our results showed that CT alone detected 12 bird species/taxa, leaf swabs identified 18 species, and water samples identified 6 species.
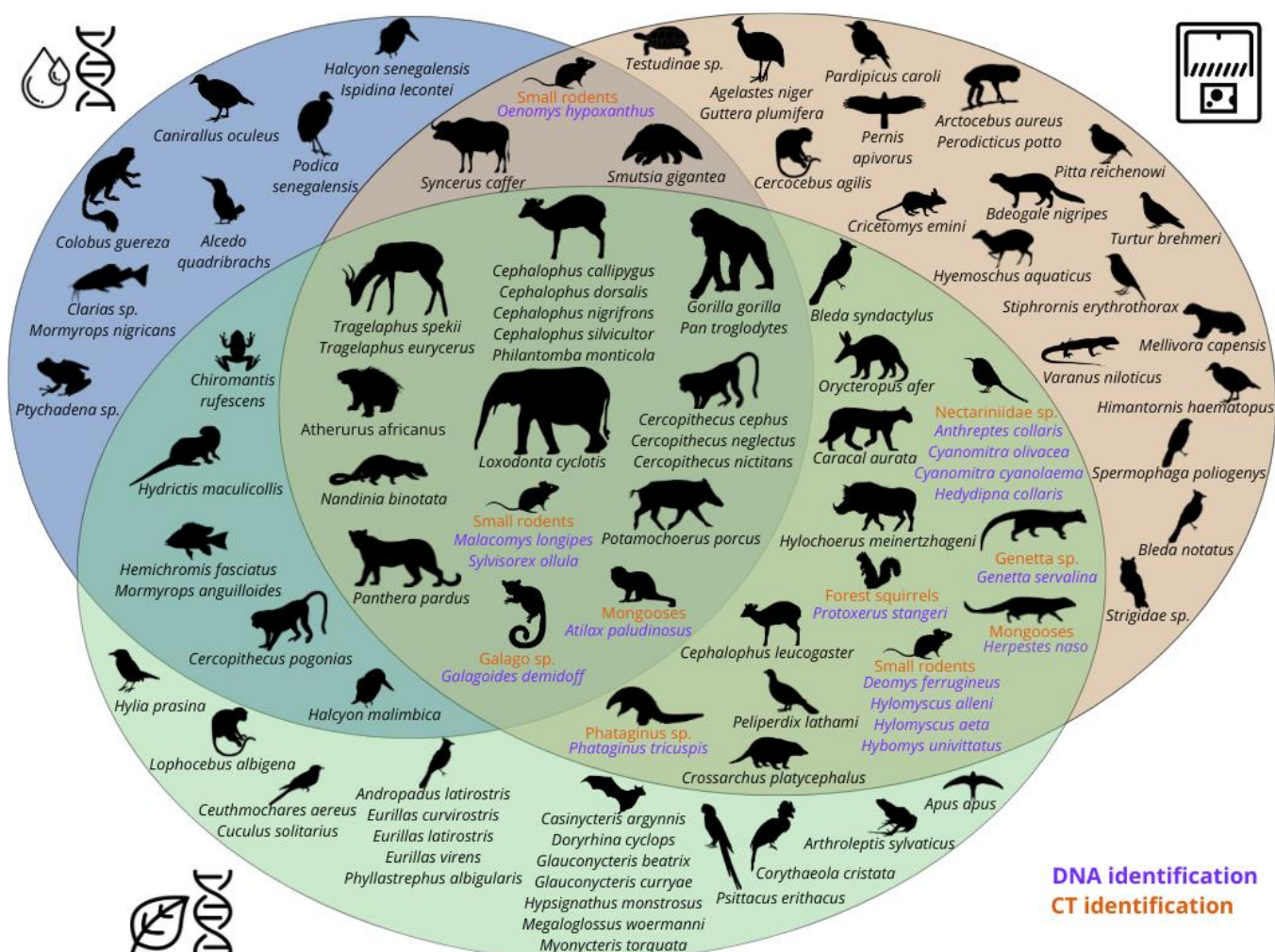
Figure 4: Venn diagram of species detected by CT, water eDNA, and leaf swab eDNA. The names in orange are identifications obtained with CT, and the names in purple were obtained with eDNA methods.

Principal Coordinates Analysis (PCoA) revealed distinct clustering of sampling units by method, supported by PERMANOVA, which indicated significant differences in species composition among the methods (F = 32.189, p***, Figure 5). The clustering between CT and eDNA is even clearer. Water eDNA and leaf swab eDNA are overlapping.
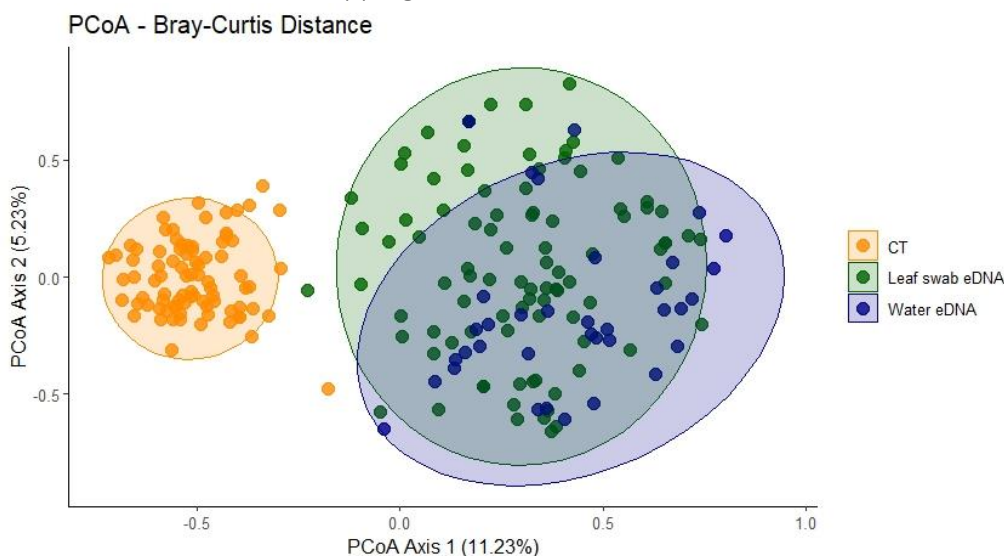
Figure 5: Principal Coordinates Analysis (PCoA) of sampling units based on species composition across survey methods. Points represent sampling units, namely individual camera traps and eDNA samples. The plot shows the ordination of sampling units using Bray-Curtis dissimilarity, with the first two axes explaining 16,46 % of the variance. Points are color-coded by survey method, with ellipses indicating 95% confidence intervals for the dispersion of sampling units within each method.

3.1.3. Species' relative abundance and naive occupancy

The seven taxa identified at different levels by camera traps and eDNA are standardized to the level of identification provided by camera traps to allow comparison of indices between CT and eDNA (see Figure 4). This includes small rodents, mongooses, nectariniids, galagos, forest squirrels, *Phataginus* species, and *Genetta* species.

For each group and species, we calculated the Relative Abundance Index (RAI) and Presence and Absence Occurrence (PAO) for each survey method (Appendix 4, Table 6). Camera traps generally showed higher PAO than eDNA for birds, *Cephalophus* spp., Felidae, great apes, rodents, reptiles, small carnivores, and Suidae compared to eDNA. For species detected by all three methods, camera traps yielded higher PAO values for 13 of the 20 taxa, with exceptions for four small primates *(C. cephus, C. neglectus, C. nictitans,* and *Galago* sp.*)* and three Bovidae *(T. spekii, T. eurycerus* and *C. nigrifrons;* Figure 6). Notably, the sitatunga (*T. spekii*), Brazza's monkey (*C. neglectus*), and black-fronted duiker (*C. nigrifrons*) were better detected by water eDNA.

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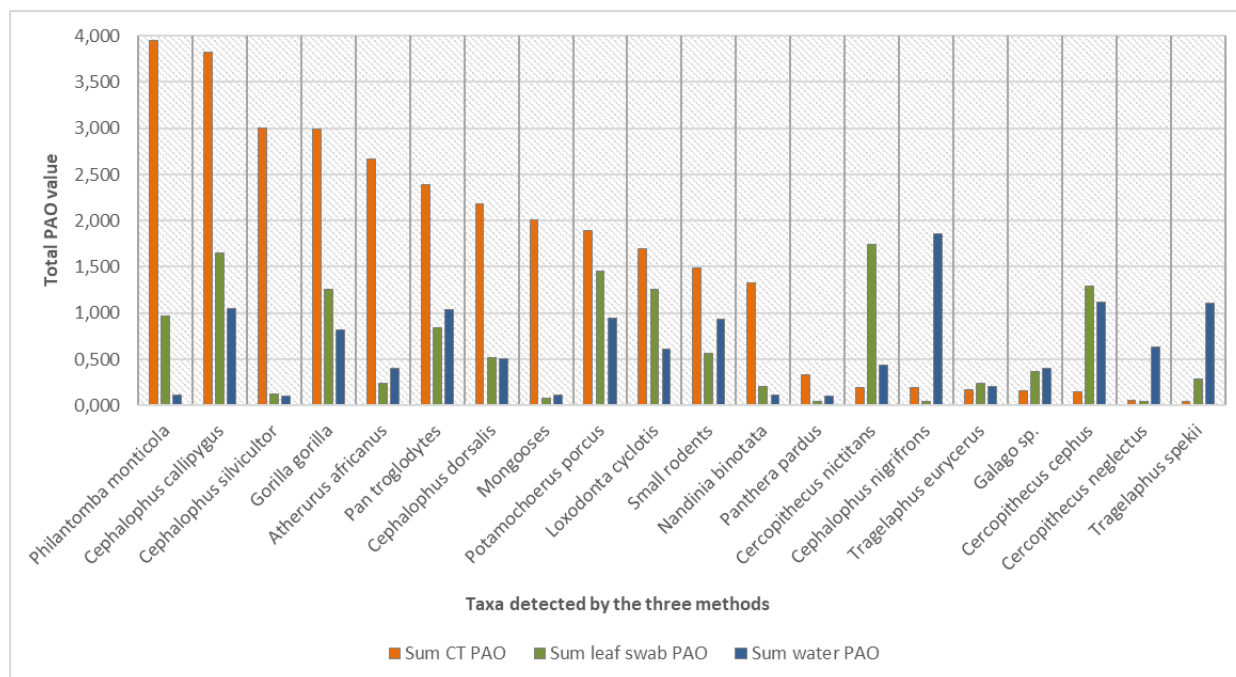
Figure 6: Total PAO values of each survey method for each species detected. Calculated by summing the PAO values from the four grids, resulting in a PAO value from 0 to 4 for each species at the entire sampling area scale.

Linear regressions revealed no strong correlations between indicators from different methods (Figure 7). The highest correlation was between PAO and RAI for camera traps, though it did not exceed R²=0,61. There were negative and statistically insignificant correlations between water eDNA PAO and camera trap abundance estimates. Leaf swab eDNA PAO and camera trap PAO showed slight correlations in some grids (D***, A, and C, p = 0,08), while correlations between leaf swab PAO and camera trap RAI were less evident. However, significant correlations were observed between PAOs of water and leaf swab eDNA in grids C** and D***, indicating that despite differences in sampling effort, these methods reflected similar occupancy patterns.
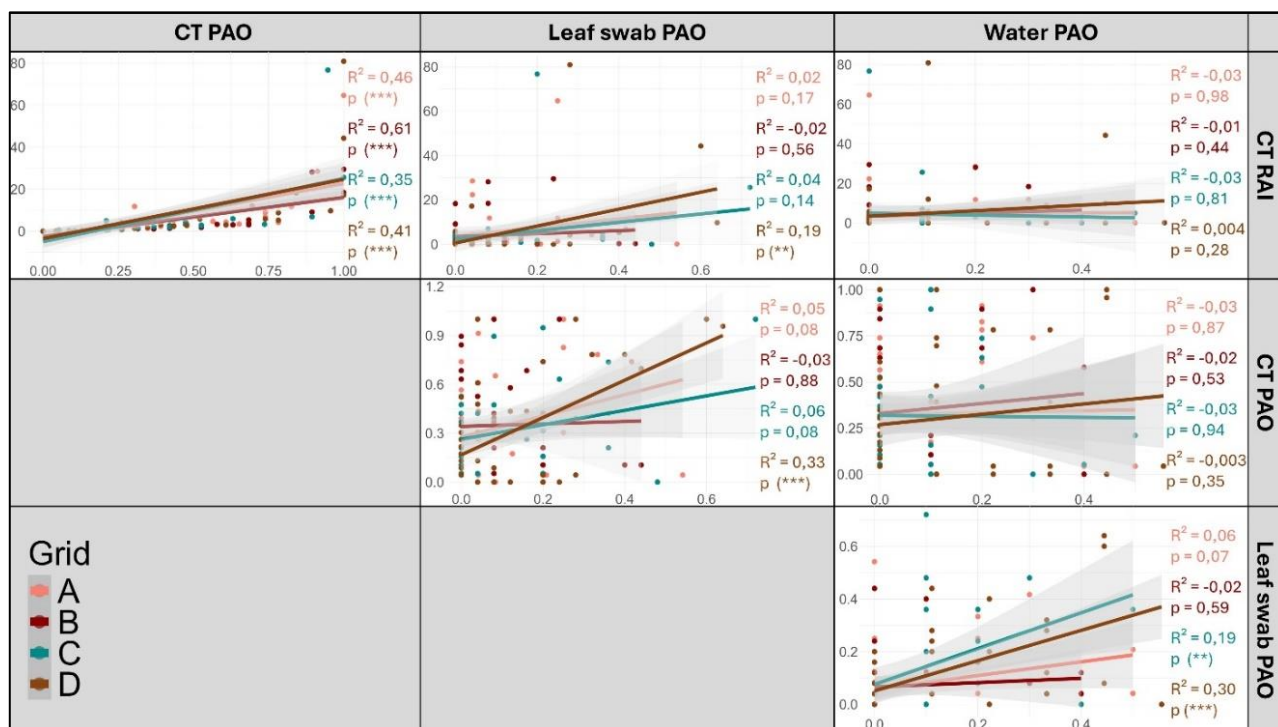
Figure 7: Matrix plot of linear regressions between species abundance and occupancy indicators. The indicators include PAO for camera traps (CT), water eDNA, and leaf swab eDNA, as well as RAI for CT Each cell shows a linear regression between row and column variables, with lines for each grid (A, B, C, D), adjusted R² values, and p-values. Each point represents a species, and the grey shaded areas around the regression lines indicate ± standard error.

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3.1.4. Resampling and cost comparison

The maximum sample size was n = 19 due to two CT grids having only 19 functioning cameras (see data processing). Nevertheless, for n = 19, mean richness was calculated based on resampling, as grids A and D had 23 cameras.

Resampling estimates indicate that with 10 cameras per grid, the expected species richness is 31 ± 1.8, increasing to 33 ± 1.5 with 15 cameras (Table 2). These estimates are close to the mean richness of 35 ± 0,8 observed with 19 cameras. In contrast, with leaf swab eDNA, 10 samples per grid provide a much lower richness estimate (21 ± 2.5) compared to the richness obtained with 19 samples (29 ± 1.7) and the mean richness of 32 observed with 24 samples per grid.

Considering the costs associated with each method (Table 3), leaf swab eDNA is more economical than both CT and water eDNA. The primary expense for CTs is fieldwork, including the installation and retrieval of cameras. However, the 17 000 € (n = 100) paid for CT is a one-time expense (minus the price of batteries and silicone grease) and is considered as an investment. So, for a second survey, the total costs are 29 230 € for 100 cameras, competing with the leaf swab price. Leaf swab eDNA remains approximately 1.7 times cheaper for all simulations (n = 10, 15 and total) than CT (Appendix 5, Table 7, and Table 8). However, with a sample size of only 10 cameras, CT captured more species than 19 leaf swab samples. Remarkably, both methods exhibited the same standard deviation, indicating that cameras achieved higher species richness with the same precision despite the large difference in sample size.

By dividing the cost by the total number of species detected per method (γ), we obtained the following costs per species: 872 € for camera traps (CT), 420 € for leaf swab eDNA, and 860 € for water eDNA. Therefore, CT and water eDNA are the more expensive methods.

Table 2: Resampling results with 10, 15, and 19 cameras per grid and leaf swab samples per grid. Mean richness was calculated as the average of 4000 species richness values obtained from the resampling. Standard deviation was calculated as the square root of the mean squared residuals (MSR) from ANOVA.

Camera traps	N = 10	N= 15	N= 19
Mean species richness	31	33	35
Standard deviation	1,8	1,5	0,8
Leaf swab			
Mean species richness	21	26	29
Standard deviation	2,5	2,1	1,7

Table 3: Costs of survey techniques for 100 CT, 100 eDNA leaf swabs, and 40 eDNA water samples.

Method	Units	Material costs	Field expenses	Transportation	Lab costs	Data processing	TOTAL
CT	100	17.000 €	20.910 €	1.440 €	/	6.880 €	**46.230 €**
Leaf swab eDNA	100	860 €	12.343 €	720 €	11.700 €	2.150 €	**27.773 €**
Water eDNA	40	6.056 €	10.286 €	720 €	15.200 €	1.290 €	**33.552 €**

3.2. Impacts of logging and hunting on species diversity

3.2.1. Species richness

In this analysis, hunting indices are considered the primary indicator of hunting activity because they provide a direct measure, unlike other indirect signs. Rarefaction curves (Figure 2) show that species richness varies across grids. Crossed with hunting signs (Table 4), it indicated that grid D, logged 10 years ago, exhibited the highest species richness and the lowest hunting pressure. Conversely, despite never having been logged, grid C showed lower species richness and was significantly impacted by hunting.

Species richness in grids A and B varies across survey methods. Grid A shows the same richness as grid D with CT but lower richness than grids D and C with eDNA. Despite being near a human settlement, an open road, and having a higher population within a 15 km radius, grid A has a surprisingly low number of hunting indices, five times fewer than grid C. Grid B, currently in prospection, recorded 42 hunting indices and two stolen cameras, indicating significant hunting activity. Despite this high hunting pressure, grid B exhibits similar species richness to grids A and D with CT, but the lowest richness of all grids with eDNA leaf swabs.

Table 4: Hunting signs retrieved per grid.

Grid	Number of hunting indices	Number of stolen cameras	Human RAI on CT	Distance to the nearest open road (km)	Distance to the closest human settlement (km)	Amount of people living within a radius of 15km
A	10	0	0,8	0,3	10,0	2217
B	42	2	0	0,2	20,8	0
C	51	0	0	8,6	10,0	476
D	3	0	0	13,4	23,9	0

3.2.2. Species composition

Correspondence Analysis (CA) on camera trap data revealed that species composition and abundance were generally similar across the grids, with grid A showing the highest variability (Figure 8). Two groups emerged, based on their proximity and overlap: grids A and B versus grids C and D. Grid C, which has never been logged, exhibited a species composition and abundance similar to grid D, which was logged 10 years earlier. Grid D displays higher homogeneity in species composition and abundance than grid A. Some species, such as *Atherurus africanus* and *Cricetomys emini*, are more closely associated with grids A and B.
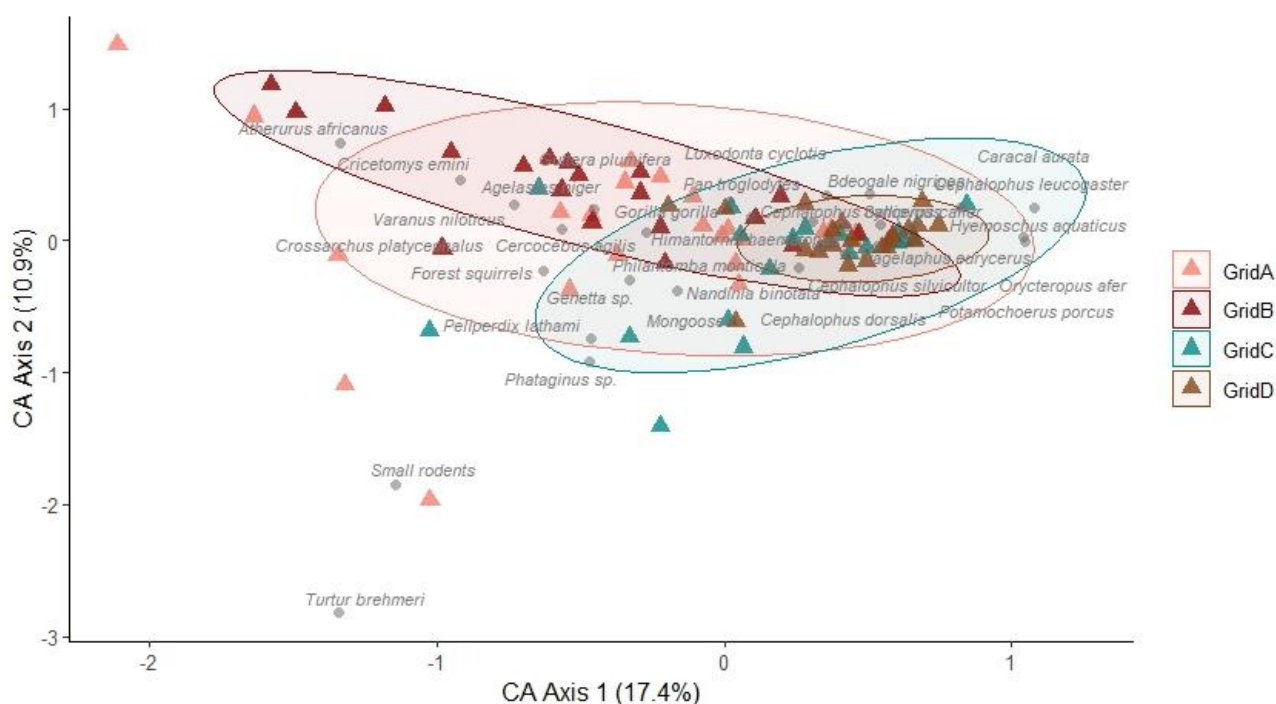
Figure 8: Correspondence Analysis (CA) of species and sites on CT data. The CA was conducted on the filtered dataset (without species with occurrence ≤ 2). The plot shows the ordination of species and sites along the first two CA axes, explaining 28.3% of total variance. Triangles represent sites, color-coded by grid. Ellipses show 95% confidence intervals for site dispersion within each grid. Grey points represent individual species, with labels indicating their names.

3.2.3. Species' relative abundance and naive occupancy

The comparison of RAI and PAO values across the four grids using ANOVA on linear mixed models (LMM) showed no significant differences, indicating that species relative abundance and naive occupancy were similar across all grid areas. Only the PAO for leaf swab eDNA approached marginal significance (p = 0.04). Due to non-normal residuals, these results should be interpreted cautiously. Moreover, further pairwise comparisons did not reveal significant differences between grid means. Additional examinations were conducted on RAI and PAO values across species groups (Appendix 4, Table 6 and Appendix 6, Figure 10).

Grid D exhibited the highest relative abundance of *Suidae*, forest elephants, great apes, blue duikers, *Cephalophus* spp., *Felidae*, and *Orycteropus afer* (Appendix 6, Figure 10). Rodents occupied a greater area in logged or prospected forests. For *Cephalophus* spp., abundance and occupancy were higher in grid D. However, some species within this genus showed higher values in grid C. Birds, small primates, and small carnivores did not show a clear preference for any grid.

The bongo (*Tragelaphus eurycerus*) was exclusively observed in grid A, with eDNA showing higher PAO for this species in the same grid. The leopard (*Panthera pardus*) was detected in all grids except grid B, showing higher naive occupancy in grids C and D. Forest elephants and forest buffalo (*Syncerus caffer*) were more abundant in grids A and D.

4. DISCUSSION

4.1. Comparison of monitoring method accuracy

4.1.1. Species richness

In our comprehensive vertebrate survey of the tropical rainforest in North Congo, we identified 98 vertebrate species. Among the methods used, leaf swab eDNA was the most effective, detecting 67% of the total recorded species, followed by camera traps, which recorded 54%. Water eDNA, despite having a sampling effort 2.5 times smaller than leaf swabs and camera traps, detected 40% of the total species, demonstrating its potential for broader application with increased sampling effort. This finding aligns with Mena et al. (2021), showing that eDNA can achieve high species richness even with limited sampling effort. However, the full potential of eDNA techniques has yet to be fully realized, as indicated by the rarefaction curves (Figure 3). This is expected, given eDNA's capacity to detect a broader range of species (Mena et al., 2021). Furthermore, our results highlight the importance of using different markers to achieve comprehensive wildlife surveys and improve species detection. For instance, the CO1 marker notably detected the forest elephant, a species not included in the 12S database.

4.1.2. Species composition

Our CT survey provided a reliable description of the large to medium-sized mammals in the northern Congolese rainforest, achieving results comparable to those of Morgan et al. (2023), despite our study being only two months long compared to their three-year study. While camera traps are highly effective at detecting medium to large-sized mammals, eDNA also identified several common species in the Congolese tropical forest. These included duikers, forest elephants, red river hogs, Cercopithecus species, and western gorillas *(Gorilla gorilla)*. This supports the findings of Mena et al. (2021) in the Amazon forest.

For birds, leaf swab eDNA proved to be the most efficient, detecting 12 species not identified by camera traps (CT). In contrast, Morgan et al. (2023) recorded 16 bird species using camera traps placed in a specific grid at termite mounds (Appendix 7). Our studies identified different bird species probably because we used distinct monitoring methods. Nevertheless, leaf swabs remain a powerful tool, and our results demonstrate that eDNA serves as a valuable, non-invasive complement to other survey techniques for comprehensive wildlife monitoring (Leempoel et al., 2020; Mena et al., 2021).

We also compare our results with indicator species, which provide essential information on ecosystem health (Carignan et al., 2001; Zhao et al., 2019). Fonteyn et al. (2023), have compiled hundreds of lists of mammal species across Central Africa to identify zoogeographical districts in the region. They list indicator species for artiodactyls and primates estimating their uniqueness across the zoogeographical districts. In the 'Inland' district, where our study was conducted, 8 primate species and 9 artiodactyl species were retained as indicator of the area. Most of them were detected by the three methods except *Cephalophus leucogaster, Cercocebus agilis* and *Lophocebus albigena.* The first was not detected with water eDNA, *C. agilis* was only detected with CT and *L. albigena* only with leaf swab eDNA, highlighting the complementarity of the methods.

The distinct clustering observed between camera traps (CT) and eDNA methods in our PCoA analysis highlights that these methods capture different sets of species. Camera traps excel at detecting medium to large mammals (Lahoz-Monfort et al., 2021), while eDNA methods are effective for capturing species that CT might miss, such as bats, amphibians, small mammals, and arboreal species

(Leempoel et al., 2020; Mena et al., 2021). Our study supports this finding, as eDNA methods, particularly leaf swabs, identified two amphibian species, seven bat species, and seven small rodent species. We could not identify these small rodent species using CT, likely due to their small size and rapid movements (Verhees et al., 2024). This limitation was not confined to small mammals; medium-sized mammals such as *Herpestes naso* (identified as mongooses with CT) and *Genetta servalina* (*Genetta* sp.) were also accurately identified using eDNA. Contrastingly, certain species frequently detected by CT, such as *Cercocebus agilis* and *Cricetomys emini*, were not identified by eDNA, potentially due to behavioral and ecological factors affecting eDNA detectability (Leempoel et al., 2020). These discrepancies emphasize again the need for combining methods to achieve a comprehensive species survey (Leempoel et al., 2020; Mena et al., 2021).

4.1.3. Species' relative abundance and naive occupancy

Camera traps (CT) generally provided a higher proportion of area occupancy (PAO) compared to eDNA methods. This is probably because CT are more capable of detecting these species, which were mostly medium to large-sized mammals. Conversely, the seven species that were better detected by eDNA were mainly arboreal (*C. cephus, C. neglectus, C. nictitans, and Galago* sp.) or typical of wetlands and swamp forests (*T. spekii, C. nigrifrons*; Kingdon, 2016; IUCN, 2024) so better detected with water eDNA.

A correlation was observed between RAI and PAO from CT (Figure 7; R^2 = 0.35 to 0.61), suggesting that CT PAO partially reflects the relative abundance estimated by RAI, aligning with recent findings (Soto-Werschitz et al., 2023). However, RAI and PAO have limitations due to variability among species, home range sizes, and sampling designs (Mackenzie et al., 2005; Efford et al., 2012; Sollmann et al., 2013; Reece et al., 2021). Therefore, both RAI and PAO are influenced by detection probability and should be used cautiously. Despite these issues, RAI is cost-effective and can provide valuable insights into species abundance, showing positive correlations with strong density estimates (Rovero et al., 2009; Reece et al., 2021). PAO, though not a reliable surrogate for abundance, remains useful for monitoring species occurrence (Hedwig et al., 2018).

We did not observe strong linear correlations between CT abundance and occupancy estimates and eDNA occupancy estimates. This contrasts with the findings of Leempoel et al. (2020) in California (USA), who identified a strong relationship between CT PAO and soil eDNA PAO when camera trapping was conducted 30 to 150 days before soil sampling. For leaf swabs, the lack of strong correlation with CT in our study could be due to swabs being collected before CT data, resulting in a mismatch in the periods covered. However, it is assumed that the same individuals visit the same areas over time. Moreover, the results of Leempoel et al. (2020) raise questions about the duration of DNA persistence in the environment, showing that time since camera trapping influence the detection of species.

Several studies have also accounted for detection probability and yielded interesting results. Lyet et al. (2021) found a positive linear correlation between water eDNA detection probability and CT detection rates of terrestrial species. Additionally, Lugg et al. (2018), using a site occupancy detection model, showed a higher detection probability for a freshwater mammal with water eDNA than with classic trapping methods. This shows the importance of taking the detection probability into account.

Our findings suggest that detection effectiveness can vary significantly based on species rarity and how species release DNA into the environment (Ishige et al., 2017; Mena et al., 2021). For example, felids, which occur at lower densities and are at the top of the food chain, are more challenging to detect (Lyet et al., 2021). This is particularly true when using water eDNA, as their presence in water

is infrequent (Ushio et al., 2017; Lyet et al., 2021). Our results confirmed this, with smaller PAO in water for felids. Interestingly, Leempoel et al. (2020) found that felids were over-represented in soil eDNA samples, likely due to their marking behaviors, indicating that soil eDNA might be more effective for detecting such species. These findings emphasize the need to consider species-specific behaviors and ecological traits when choosing the survey method (Leempoel et al., 2020).

4.2. Developing an optimal wildlife monitoring approach

4.2.1. Effectiveness and costs

Management certification schemes, such as FSC and PAFC, necessitate comprehensive fauna monitoring to protect rare and threatened species and identify High Conservation Values (HCV) within logging concessions (Daïnou et al., 2016). Camera traps (CTs) and environmental DNA (eDNA) methods both provide valuable data but have distinct advantages and limitations.

Camera traps offer detailed insights into terrestrial mammal populations and have seen advancements in AI-driven species identification, which enhances their efficiency. Despite the high initial costs and the field expenses, CTs are cost-effective in the long term due to their reusability. Ten cameras per grid give an appreciable idea of the area's richness. Despite deploying 25 cameras per grid, only 19 (on two grids) operated for 38 days due to harsh tropical conditions and potential damages or theft. To improve reliability, installing extra cameras to ensure at least 10 functional units per grid is recommended (Zwerts et al., 2021). Indeed, the more cameras are placed, the better the chance to capture rare and elusive species. It, therefore, depends on the research objective.

On the other hand, eDNA methods are efficient in fieldwork because they involve a single collection phase. With a sampling of 19 leaf swabs, twice as big as the CT sampling with 10 cameras, we capture fewer species with the same precision than the CTs. We would, therefore, advise having between 20 and 25 samples to capture a similar diversity as with camera trapping. While leaf swabs is the cheapest method the high laboratory costs and the need for a larger sampling effort to achieve similar species coverage make them less cost-effective compared to camera traps overall.

Several studies claimed water eDNA can be more cost-efficient than classic survey methods or camera traps. Lyet et al. (2021) demonstrated that a sampling design filtering large volumes of water from a few large streams yielded more species detections per dollar than traditional methods, including trapping or sampling multiple smaller streams in the same area. Water eDNA requires less effort and incurs lower total survey costs than classic techniques for determining species occupancy (Lugg et al., 2018). Our results support these findings, showing that water eDNA, with a reduced sampling effort, effectively captured the main species richness differences across grids and still detected key species of the Congolese tropical forest. However, due to the limited sampling effort, we do not have enough information to determine the efficiency of water eDNA conclusively. Despite a sampling effort 2.5 times smaller than camera traps (CT) or leaf swabs, water eDNA remained more expensive than leaf swabs. The materials for water eDNA are mostly non-reusable, except for the two peristaltic pumps and their batteries (2 800 €). Another constraint is the dependency on water sources, which limits the flexibility of the sampling design. When comparing the cost per species, considering that cameras and peristaltic pumps are reusable, water eDNA still emerges as the more expensive method. Therefore, based on our results, we recommend the use of leaf swabs, which seem more cost-efficient.

For camera trapping several techniques exist to approximate at best abundance without individual recognition such as the Random Encounter Model (REM) (Rowcliffe et al., 2008), The Random Encounter and staying Time (REST) (Nakashima et al., 2017), the distance sampling (Howe et al.,

2017), the Time to Event and the Space to Event model (Moeller et al., 2018). In contrast, eDNA provides only presence data, limiting the information on species abundance. eDNA can, however, give insights into the occupancy of each species in the area. In the context of logging concessions, even if the PAO needs to be used cautiously, it can be a useful indicator of several species' local distribution and occurrence. To access more relevant information, we advise using occupancy models considering the detection probability (Mackenzie et al., 2005). Moreover, for a more precise evaluation, a cost analysis can also be associated with occupancy modeling to assess the most efficient and cost-effective methods to detect changes in occupancy (Beranek et al., 2024).

4.2.2. eDNA obstacles

A major challenge with eDNA is the incomplete reference database, which affects species identification accuracy. Our "potential" list illustrates that many more species (especially birds and fish) could be identified. Still, other closely related species not resident in the study area (according to the IUCN, 2024) were identified instead. This illustrates the limitations of the current databases. As Leempoel et al. (2020) stated, another consequence of incomplete databases is that many sequence identifications with a query coverage or a pairwise identity below the defined filtering threshold are discarded. As a result, we may overlook potential species, including those present in the area or closely related to well-known local species.

Moreover, cross-contaminations during DNA extraction and PCR and working with degraded environmental DNA pose significant obstacles, leading to potential misidentifications (Ushio et al., 2017). Despite robust filtering efforts, inconsistencies in species identification, such as misidentifications of *Felis catus* and *Mammuthus primigenius*, highlight the need for the development of more comprehensive reference database and a careful review by experts familiar with local wildlife (Mena et al., 2021).

Access to genetic laboratories also remains a challenge for eDNA analysis in many regions, necessitating collaboration with countries that have lab facilities (Van Leeuwen et al., 2023).

4.2.3. Recommendations

The choice of method should align with the specific objectives of the monitoring effort. For wildlife monitoring, information on abundance is considered essential (Lyet et al., 2021). Therefore, we recommend using CT for fauna monitoring instead of recces or distance sampling, which require a significant investment in time and manpower (Nasi, Taber, et al., 2011). Regarding the FSC and PAFC requirements implying monitoring animal populations of threatened species, CTs procure useful insights into species relative abundance, community composition, and occupancy (Mena et al., 2021; Reece et al., 2021). When precise abundance estimates are needed, CTs combined with semi-automated distance estimation using deep learning offers an accurate and efficient solution, significantly reducing data processing time (Henrich et al., 2024). Additionally, eDNA combined with occupancy models can provide cost-effective and complementary data on species presence and distribution.

In logging concessions, eDNA can be utilized to identify High Conservation Value (HCV) areas by providing insights on a wide range of species, including endangered ones. More broadly, eDNA can be employed to evaluate the conservation value of specific areas, potentially guiding the delimitation of new protected zones and the selection of high-priority conservation areas. This approach is vital for meeting the global commitment to protect 30% of terrestrial and marine areas by 2030. Historically, protected areas have been designated with a focus on specific taxa (Eckert et al., 2023). eDNA offers an opportunity to select protected areas based on a broader spectrum of biodiversity,

including arboreal primates, birds, bats, amphibians, and even fish. This is particularly important given that currently protected areas represent only 15% of terrestrial vertebrates, plants, and butterflies, encompassing just 6.6% of threatened species (Eckert et al., 2023). Expanding the scope of protected areas using eDNA can significantly enhance biodiversity conservation efforts. We also recommend designating protected areas according to lists of "indicator species" in the survey area, like the one made by Fonteyn et al. (2023) for artiodactyl and primate zoogeographical districts in Central Africa.

4.3. The influence of selective logging and hunting on vertebrate diversity

Our study revealed complex interactions between hunting pressure, logging history, and vertebrate diversity across different grids. Contrary to our initial assumptions, hunting pressure was not more substantial in recently exploited areas but was more influenced by accessibility and proximity to human settlements. For example, grid C had many hunting signs due to a path created by locals from Loundoungou. Conversely, grid D, exploited 10 years ago, had the lowest hunting pressure, likely due to its distance from roads and human settlement and the proximity of Nouabale Ndoki National Park and its anti-poaching patrols. This suggests that accessibility and proximity to human settlements are more critical than logging history in determining hunting pressure (Lhoest et al., 2020).

Grid D, exploited 10 years ago, exhibits the highest species richness and the lowest hunting indices, showing that the biodiversity of logged areas can recover over time, especially with low hunting pressure. With high hunting pressure and lower species richness, grid C also highlighted that human access affects wildlife populations. However, these two grids contain all the indicator species established by Fonteyn et al. (2023) except for the water chevrotain (*Hyemoschus aquaticus*) in grid D and Colobus guereza in grid C, indicating the overall health of these areas. Grid A, logged three years ago, showed more variable species composition and abundance, likely due to recent disturbances. Notably, several indicator species like the white-bellied duiker, the water chevrotain, *Colobus guereza*, and *Lophocebus albigena* were absent in this recently logged area, while the bongo was mainly present in the recently logged forest compared to the other grids. This aligns with previous findings that bongos thrive in disturbed forests (Morgan et al., 2023; IUCN, 2024). The forest elephant's higher occupancy and relative abundance in recently logged areas (grids A and D) are probably due to a higher abundance of tree species typical of secondary forests (Poulsen et al., 2011). The high number of hunting signs in grid B confirms our hypothesis that the paths opened for the logging inventory facilitate access for hunters. Moreover, this area also lacks three indicator species (water chevrotain, *C. guereza,* and *L. albigena*) and shows a significantly lower relative abundance of blue duiker, likely indicating the impact of hunting and prospection activities. However, it is important to note that *L. albigena* and *C. guereza* have only been detected with very low naive occupancy by one of the two eDNA methods, reflecting either their rarity or a very low detection probability with the methods deployed. Additionally, species may alter their behavior in response to human disturbance, making them more difficult to detect (Tagg et al., 2019).

Our study also found that small rodent abundance increased in logged forests, consistent with Gutiérrez-Granados et al. (2021), who observed similar trends in Mexico. This supports the broader observation that while the abundance of medium to large mammals decreases in logged forests, small mammal populations tend to increase (Poulsen et al., 2011; Gutiérrez-Granados et al., 2021). Poulsen et al. (2011) noted that for medium- and large-bodied species, the species richness recovers more quickly than species abundance, which can take longer than the typical 30-year logging rotation. Conversely, the area logged 10 years ago (grid D) exhibited high species richness and relative abundance of taxa, comparable to the never-logged grid C. This similarity may be attributed to the high hunting pressure in the never-logged area, which reduces the densities of frequently hunted species such as pigs, monkeys, and duikers (Poulsen et al., 2011). As a result, grid C cannot serve as

a control area for assessing the impact of logging. Although the least hunted area showed greater abundance and naive occupancy of duikers (*P. monticola and Cephalophus* spp.) and pigs, our study did not find a higher proportion of birds or small mammals in hunted forests like supported by Poulsen et al. (2011).

Among the most hunted species in Gabon, including several duiker's species, the red river hog and the brushed-tailed porcupine (*Atherurus africanus*) (Fonteyn et al., 2024), only the red river hog and the blue duiker showed slightly higher relative abundance and occupancy in the least hunted forest. This suggests they may be subject to more severe hunting pressure in other areas. Moreover, the differences in species responses to hunting pressure indicate that large-bodied mammals such as duikers are more vulnerable compared to some large-bodied rodents (Tagg et al., 2019). However, the minimal differences in the abundance of these species across our hunting gradient suggest that the study area as a whole is not significantly affected by hunting practices.

The presence of top predators like Felidae (*Caracal aurata* and *Panthera pardus*) in logged areas demonstrates that selective logging can be compatible with the conservation of top predators, contrary to some opinions (e.g., Jamhuri et al., 2018). The absence of leopards in the grid under prospection might be attributed to high human presence, hunting pressure, or low detection probability for rare species.

Despite variations, species composition across the four grids remained similar, indicating that logged forests can provide refuge for many species, especially when the hunting pressure is low (e.g., grid D) (Ewers et al., 2024). The impacts of logging and hunting on wildlife are dependent on logging intensity, with lightly logged forests (< 29% biomass removal) retaining high conservation value and contributing significantly to conservation efforts (Ewers et al., 2024). The FSC promotes sustainable logging practices that minimize environmental impact (FSC, 2023), making certified forests ideal for biodiversity conservation.

5. STUDY LIMITATIONS AND PERSPECTIVES

The first limit of this research is that our sampling design involved spatial clustering, with camera traps and eDNA samples concentrated in specific grids. This spatial aggregation may not fully represent the spatial diversity of the Congolese tropical forest, potentially leading to skewed results. However, a more dispersed sampling approach was impractical, given the technical, logistical, and financial constraints of working in such extensive and remote areas. Adapting to these limitations is a common challenge in large-scale ecological research.

Furthermore, financial constraints also restricted us to conducting only 10 water eDNA collections per grid. This limited effort affects the comparability of water eDNA results with other methods, potentially leading to an underestimation of species diversity and occupancy. To properly assess the effectiveness of water eDNA, the same sample size as leaf swab eDNA should be used or a larger amount of water should be filtered (Lyet et al., 2021).

The stringent criteria we applied for sorting eDNA sequence identifications (see Appendix 3) excluded several species that could have enhanced the assessed richness of the studied areas. Given that reference databases are particularly incomplete for under-studied regions like Central Africa, it would have been beneficial to use less stringent thresholds and consider species from the "potential" list in our analysis. However, it is even more crucial to enrich genetic databases to build the most accurate inventories possible (Mena et al., 2021).

Additionally, we had to exclude the data from 16 cameras out of 100 because they ceased to function before the initial 38 days of the survey, diminishing the power of our study. This was likely due to the harsh conditions in the tropical forest, combined with the fact that these cameras had been previously used multiple times. Therefore, it is crucial to consider the wear and tear of equipment in future surveys. This also raises the question about the lifetime of a CT, which should be considered in the cost analysis.

We also acknowledge that our results must be interpreted with caution, as measures of species relative abundance, naive occupancy, and diversity alone may not fully capture the complex impacts of logging and hunting on biodiversity and wildlife communities (Tagg et al., 2019). To properly assess the effects of logging and hunting, conducting studies across various sites managed by different logging companies would be interesting. Another solution would be to undertake a long-term study to observe how biodiversity changes, particularly in forests logged at various periods. This would provide valuable insights into biodiversity recovery dynamics. This approach could refine recommendations for sustainable forest management. Moreover, the intensity of logging should be incorporated as an additional disturbance factor to understand its effects on biodiversity better.

6. CONCLUSION

This study underscores the diverse capabilities and complementary roles of different wildlife monitoring methods in the northern Congo rainforest. Our findings reveal that camera traps are highly effective for identifying medium-to-large mammal species and assessing relative abundance. Even if relative abundance need to be interpreted with caution, it appears to be an effective tool for the rapid assessment of animal relative abundance (Rovero et al., 2009) useful for conservation estate. However, eDNA methods—particularly leaf swabs—offer valuable insights into species richness and occupancy for a broader range of species including arboreal mammals, bats and smaller species. Although combining methods is often costly, it is recommended for a more comprehensive assessment (Zwerts et al., 2021). Ultimately, the choice of monitoring method should be guided by specific research objectives. Environmental DNA can identify areas of high conservation value, whereas camera traps are better suited for monitoring medium to large mammals.

Additionally, our study highlights that hunting pressure is influenced not only by logging history but also by human settlements and accessibility (Lhoest et al., 2020). Importantly, our findings demonstrate that FSC-certified logged forests can still provide refuge for many species. This underscores that when hunting practices are maintained low, certified forests are highly beneficial for biodiversity conservation.

7. PERSONAL CONTRIBUTION

My personal contributions to this master's thesis began with the development of the research protocol and the selection of eDNA methods in collaboration with Simon Lhoest, Barbara Haurez, Guillaume Baltus, and Johan Michaux. In the field, I worked with Guillaume to manage the implementation of the activities in collaboration with the CIB team. This involved selecting grid placements, planning activities, recruiting the field team, installing camera traps, and collecting all DNA samples. I identified the species captured by the camera traps and performed the DNA extraction from the leaf swab samples. Finally, I conducted all statistical analyses, interpreted the results, and developed the discussion with valuable guidance from my supervisor.

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APPENDIXES

Appendix 1: Sampling grids and water collection points positions
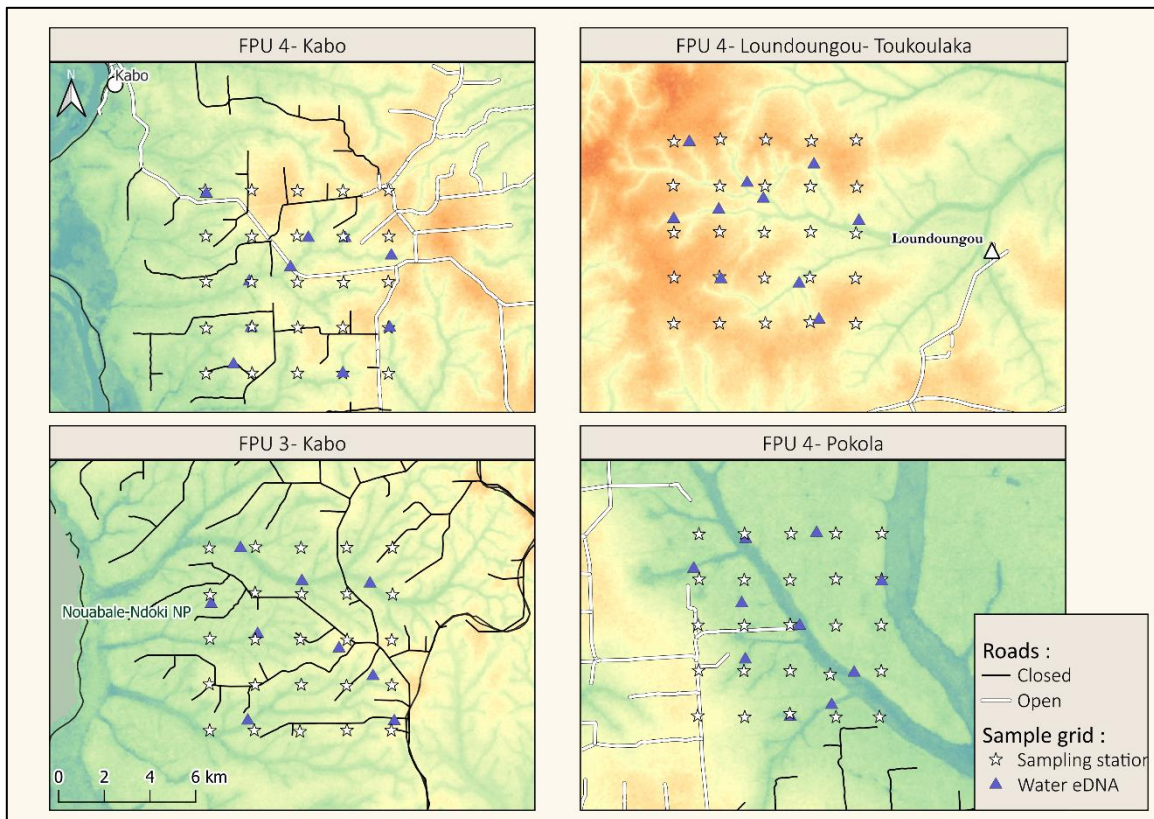
Figure 9: Water collection points position within each grid

Appendix 2: Photos illustrating field sampling methods for eDNA from water and leaf swabs.

Photo 2: Water eDNA - Filtration system

Photo 2: Leaf eDNA – Sampling on the field

Appendix 3: Details of the genetic analysis and "potential" list of identifications

For the water samples, the preservation liquid (ethanol) was retrieved from a sample by flipping the capsule upside down. Suspended particles were then concentrated through multiple centrifugations. This concentrated material was subsequently used as the starting material for the DNA extraction. The extraction of vertebrate DNA has been conducted with the QIAGEN QIAamp® DNA Mini Kit following manufacturer's instructions (QIAamp® DNA Mini and Blood Mini Handbook, 2023).

For leaf swab samples, after ethanol evaporation, each cotton swab was extracted, removing the remaining stems, and placed in a tube containing a buffer solution. The extraction was conducted using the QIAGEN QIAamp® DNA Investigator Kit (QIAamp® DNA Investigator Handbook, 2020). Cell lysis in the buffer solution was performed on a heating plate for 1.5 to 2 hours. Following lysis, cleaning steps were carried out using a column binding system following the manufacturer's protocol. To ensure the absence of contamination, DNA extraction was conducted in a dedicated "cleanroom" and the personnel performing the extraction were wearing nitrile gloves.

Then, vertebrate DNA was PCR-amplified using two sets of primers. Basically, two PCR's (Polymerase Chain Reaction) each followed by a purification were conducted. The markers used in the first PCR were the Cytochrome C oxidase Subunit I (CO1) and the mammalian MiMammal primers located on the 12S gene (Ushio et al., 2017). The utility of these markers is to amplify only vertebrate DNA using primers universal for vertebrate species. The first one has been chosen because of the variety of species it covers and because of its extensive reference database such as Barcode of Life Data Systems (BOLD) (Othman et al., 2021). The second marker typically provides greater taxonomic resolution for mammals, often up to the genus or family level. Indeed, the limit of DNA analysis is the database available on species that can be identified. The 12S database is more specific and therefore more precise than the CO1 that sometimes stops at the genus of the species. Some reference sequences can also be strictly available for one or the other marker. Another argument is that CTs mainly focus on mammals so, in order to identify the synergies between the two survey methods, the mammalian 12S marker was considered interesting. The second PCR assigns a specific nucleotide tag to each sample from a particular station, allowing them to tell them apart after sequencing. We used magnetic beads to purify the samples, removing excess reagents and potential contaminants. To ensure equimolar DNA concentrations between samples, we performed DNA quantification using the fluorometric tool PicoGreen (SigmaAeldrich), followed by necessary dilutions.

Once the DNA of interest had been amplified, a DNA sequencing was conducted. All the samples were placed in a high-throughput sequencer, the Illumina Novaseq® following the manufacturer's instructions. A number of reads was attributed to each sample. For water capsules, 1 to 2 million of reads were attributed to the 12S marker and 2 to 4 million for the CO1 marker. For swabs, 100 000 to 200 000 reads were attributed.

We controlled the quality of these demultiplexed reads with the FastQC software (Andrews, 2010). The raw paired-end sequence reads were quality trimmed, and the adapter sequences removed using Cutadapt v2.9 (Martin, 2011). Sequence analyses were performed using an in-house bioinformatic pipeline. Amplicon sequences were detected, clustered, and checked for chimeras using the VSEARCH software (Rognes et al., 2016). This step provided us with a set of independent sequences and their read abundance in each of the eDNA samples. These sequences were then assigned to reference databases using the USEARCH algorithm (Edgar, 2010). The taxonomic assignment step was conducted against NCBI databases for 12S rRNA, as well as BOLD for CO1, with a percentage of matching identity with the closest reference sequences. The BOLD database was selected for its extensive and controlled dataset. In contrast, the NCBI database is a public repository where any

researcher can submit sequences. While this inclusivity allows for a greater representation of lesser-known species, it also increases the potential for errors due to the lack of stringent oversight.

Once we obtained a list of potential identifications for each sequence, we first removed OTUs found in PCR controls to avoid identifications from contamination. We discarded sequences with query coverage below 96% and/or pairwise identity below 97% for 12S and 98% for CO1. These high thresholds were necessary because lower percentages meant unreliable species identification and genus-level identification was considered insufficient for our purposes. We also removed sequences with fewer than 10 reads or less than 0.01% of total sequence reads. After sorting, we checked the geographical distribution of all remaining species using the IUCN website (IUCN, 2024) and historical/personal site data. We created two identification categories: "certain" and "potential." Species confirmed in the area were classified as "certain." For species not confirmed geographically, we looked at the genus level to see if related species were present in the area. If another species of the same genus or the genus itself was present, the identification was classified as "potential" (see Table 5). This strict classification ensures we have a reliable list of correctly identified species. If more than one database sequences matched with the dataset sequence, both distribution range the higher query coverage and percentage pairwise identity was selected. The resulting output was a table of presence/absence for each taxon per sample.

Table 5: List of "Potential" Identifications from Water eDNA and Leaf Swab eDNA.

Water eDNA		Leaf swab eDNA	
Grid	Species	Grid	Species
A	Aphyosemion elegans	A	Dendrohyrax sp.
	Aphyosemion sp		Genetta sp.
	Aphyosemion sp		Gymnobucco sp.
	Channallabes sp.		Lonchura sp.
	Clarias sp.		Lophocebus sp.
	Enteromius sp.		Praomys sp.
	Marcusenius sp.		
	Mastacembelus niger		
	Polypterus sp.		
	Ptychadena mascareniensis		
B	Aphyosemion elegans	B	Bycanistes sp.
	Aphyosemion sp		Guttera plumifera
	Bathyaethiops caudomaculatus		Lophocebus sp.
	Channallabes sp.		Manis sp.
	Clarias sp.		Pipistrellus sp.
	Distichodus sp.		Polypterus sp.
	Epiplatys chevalieri		Praomys sp.
	Hemichromis sp.		Tauraco persa
	Marcusenius		Zenkerella insignis
	Marcusenius moorii		
	Marcusenius sp.		
	Mesoborus crocodilus		
	Mormyrops sp.		
	Pantodon buchholzi		
	Parauchenoglanis sp.		
	Petrocephalus sp.		
	Polypterus sp.		
	Schilbe sp.		
	Xenopus sp.		

Water eDNA		Leaf swab eDNA	
Grid	Species	Grid	Species
C	Aphyosemion sp	C	Casinycteris sp.
	Aphyosemion sp		Guttera plumifera
	Channallabes sp.		Hipposideros sp.
	Clarias sp.		Lophocebus sp.
	Cypriniformes sp.		Praomys sp.
	Enteromius sp.		
	Genetta sp.		
	Lonchura sp.		
	Marcusenius moorii		
	Marcusenius sp.		
	Pantodon buchholzi		
	Polypterus sp.		
D	Aphyosemion sp	D	Clarias sp.
	Bathyaethiops caudomaculatus		Enteromius sp.
	Clarias sp.		Lophocebus sp.
	Enteromius sp.		Lophoceros fasciatus
	Genetta sp.		Marcusenius sp.
	Hemichromis sp.		Polypterus sp.
	Marcusenius moorii		Praomys sp.
	Marcusenius sp.		
	Mormyrops sp.		
	Pantodon buchholzi		
	Polypterus sp.		

Appendix 4: Relative abundance index (RAI) and proportion of area occupied (PAO) of species detected by the three different methods across the four grids

Table 6: Relative abundance index (RAI) and proportion of area occupied (PAO) of species detected by the different methods across the four grids. Each species' PAO values within each grid range from 0 to 1. The values for each group represent the sum of the species' values comprising the group.

Group	Species	Comparing RAI CT					Comparing PAO CT					Comparing PAO Water					Comparing PAO Leaf swab				
		GRID					GRID					GRID					GRID				
		A	B	C	D	TOT	A	B	C	D	TOT	A	B	C	D	TOT	A	B	C	D	TOT
Amphibian		**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,20**	**0,20**	**0,00**	**0,00**	**0,40**	**0,00**	**0,00**	**0,04**	**0,04**	**0,08**
	Arthroleptis sylvaticus	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,04	0,00	**0,04**
	Chiromantis rufescens	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,10	0,10	0,00	0,00	**0,20**	0,00	0,00	0,00	0,04	**0,04**
	Ptychadena sp.	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,10	0,10	0,00	0,00	**0,20**	0,00	0,00	0,00	0,00	**0,00**
Bats		**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,13**	**0,08**	**0,04**	**0,08**	**0,33**
	Casinycteris argynnis	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,04	0,00	0,00	**0,04**
	Doryrhina cyclops	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,08	**0,08**
	Glauconycteris beatrix	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,04	0,00	0,00	**0,04**
	Glauconycteris curryae	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,04	0,00	0,00	0,00	**0,04**
	Hypsignathus monstrosus	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,04	0,00	0,00	0,00	**0,04**
	Megaloglossus woermanni	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,04	0,00	0,00	0,00	**0,04**
	Myonycteris torquata	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,04	0,00	**0,04**
Bird		**9,84**	**8,86**	**6,65**	**6,41**	**31,76**	**1,48**	**1,42**	**1,37**	**1,39**	**5,66**	**0,10**	**0,10**	**0,20**	**0,22**	**0,62**	**0,42**	**0,04**	**0,48**	**0,48**	**1,42**
	Agelastes niger	0,57	1,39	1,25	0,80	4,00	0,13	0,21	0,26	0,17	**0,78**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
	Alcedo quadribrachys	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,11	**0,11**	0,00	0,00	0,00	0,00	**0,00**
	Andropadus latirostris	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,04	0,00	**0,04**
	Apus apus	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,04	0,00	0,00	0,00	**0,04**
	Bleda notatus	0,00	0,14	0,14	0,00	0,28	0,00	0,05	0,05	0,00	**0,11**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
	Bleda syndactylus	0,00	0,14	0,00	0,00	0,14	0,00	0,05	0,00	0,00	**0,05**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,08	0,04	**0,12**
	Canirallus oculeus	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,20	0,00	**0,20**	0,00	0,00	0,00	0,00	**0,00**
	Ceuthmochares aereus	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,04	0,00	0,04	0,00	**0,08**
	Corythaeola cristata	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,04	0,04	0,00	0,04	**0,12**
	Cuculus solitarius	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,04	0,00	**0,04**
	Eurillas curvirostris	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,08	0,04	**0,12**
	Eurillas latirostris	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,04	0,00	0,00	0,00	**0,04**
	Eurillas virens	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,04	**0,04**
	Guttera plumifera	1,37	3,74	2,91	1,83	9,85	0,35	0,68	0,53	0,35	**1,91**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**

Group	Species	Comparing RAI CT					Comparing PAO CT					Comparing PAO Water					Comparing PAO Leaf swab				
		GRID					GRID					GRID					GRID				
		A	B	C	D	TOT	A	B	C	D	TOT	A	B	C	D	TOT	A	B	C	D	TOT
	Halcyon malimbica	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,11	**0,11**	0,00	0,00	0,04	0,00	**0,04**
	Halcyon senegalensis	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,10	0,10	0,00	0,00	**0,20**	0,00	0,00	0,00	0,00	**0,00**
	Himantornis haematopus	0,69	0,28	0,28	1,37	2,61	0,22	0,05	0,11	0,30	**0,68**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
	Hylia prasina	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,04	0,00	**0,04**
	Nectariniidae sp.	0,00	0,00	0,00	0,11	0,11	0,00	0,00	0,00	0,04	**0,04**	0,00	0,00	0,00	0,00	**0,00**	0,17	0,00	0,08	0,20	**0,45**
	Pardipicus caroli	0,00	0,00	0,00	0,11	0,11	0,00	0,00	0,00	0,04	**0,04**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
	Peliperdix lathami	2,63	2,63	0,83	1,49	7,58	0,43	0,21	0,21	0,22	**1,07**	0,00	0,00	0,00	0,00	**0,00**	0,04	0,00	0,00	0,00	**0,04**
	Pernis apivorus	0,00	0,00	0,00	0,11	0,11	0,00	0,00	0,00	0,04	**0,04**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
	Phyllastrephus albigularis	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,04	**0,04**
	Pitta reichenowi	0,11	0,00	0,00	0,00	0,11	0,04	0,00	0,00	0,00	**0,04**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
	Psittacus erithacus	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,04	0,00	0,04	0,08	**0,16**
	Spermophaga poliogenys	0,00	0,14	0,00	0,00	0,14	0,00	0,05	0,00	0,00	**0,05**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
	Stiphrornis erythrothorax	0,11	0,14	0,00	0,00	0,25	0,04	0,05	0,00	0,00	**0,10**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
	Strigidae sp.	0,00	0,00	0,00	0,11	0,11	0,00	0,00	0,00	0,04	**0,04**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
	Turtur brehmeri	4,35	0,28	1,25	0,46	6,33	0,26	0,05	0,21	0,17	**0,70**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
Cephalophus spp.		**17,73**	**24,10**	**40,30**	**69,79**	**151,93**	**1,74**	**2,16**	**2,89**	**3,22**	**10,01**	**0,80**	**0,80**	**0,80**	**1,11**	**3,51**	**0,29**	**0,24**	**1,24**	**1,00**	**2,77**
	Cephalophus callipygus	11,78	18,42	25,62	44,28	100,11	0,83	1,00	1,00	1,00	**3,83**	0,20	0,30	0,10	0,44	**1,04**	0,25	0,08	0,72	0,60	**1,65**
	Cephalophus dorsalis	2,29	2,35	5,96	5,84	16,43	0,39	0,42	0,63	0,74	**2,18**	0,10	0,10	0,20	0,11	**0,51**	0,00	0,08	0,24	0,20	**0,52**
	Cephalophus leucogaster	0,00	0,14	1,66	2,40	4,20	0,00	0,05	0,32	0,43	**0,80**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,08	0,20	0,16	**0,44**
	Cephalophus nigrifrons	0,11	0,14	0,14	0,11	0,51	0,04	0,05	0,05	0,04	**0,19**	0,50	0,40	0,40	0,56	**1,86**	0,04	0,00	0,00	0,00	**0,04**
	Cephalophus silvicultor	3,55	3,05	6,93	17,16	30,68	0,48	0,63	0,89	1,00	**3,00**	0,00	0,00	0,10	0,00	**0,10**	0,00	0,00	0,08	0,04	**0,12**
Felidae		**0,23**	**0,28**	**1,25**	**2,06**	**3,81**	**0,09**	**0,11**	**0,42**	**0,43**	**1,05**	**0,00**	**0,00**	**0,10**	**0,00**	**0,10**	**0,00**	**0,20**	**0,16**	**0,08**	**0,44**
	Caracal aurata	0,11	0,28	0,83	1,72	2,94	0,04	0,11	0,26	0,30	**0,72**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,20	0,16	0,04	**0,40**
	Panthera pardus	0,11	0,00	0,42	0,34	0,87	0,04	0,00	0,16	0,13	**0,33**	0,00	0,00	0,10	0,00	**0,10**	0,00	0,00	0,00	0,04	**0,04**
Fish		**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,50**	**0,30**	**0,60**	**0,89**	**2,29**	**0,00**	**0,00**	**0,00**	**0,20**	**0,20**
	Clarias sp.	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,11	**0,11**	0,00	0,00	0,00	0,00	**0,00**
	Hemichromis fasciatus	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,20	0,10	0,10	0,33	**0,73**	0,00	0,00	0,00	0,12	**0,12**
	Mormyrops anguilloides	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,20	0,10	0,40	0,44	**1,14**	0,00	0,00	0,00	0,08	**0,08**
	Mormyrops nigricans	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,10	0,00	**0,10**	0,00	0,00	0,00	0,00	**0,00**
	Podica senegalensis	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,10	0,10	0,00	0,00	**0,20**	0,00	0,00	0,00	0,00	**0,00**
Great apes		**8,58**	**6,37**	**5,54**	**14,07**	**34,57**	**1,39**	**1,26**	**1,16**	**1,57**	**5,38**	**0,40**	**0,60**	**0,30**	**0,56**	**1,86**	**0,38**	**0,28**	**0,72**	**0,72**	**2,10**

Group	Species	Comparing RAI CT					Comparing PAO CT					Comparing PAO Water					Comparing PAO Leaf swab				
		GRID					GRID					GRID					GRID				
		A	B	C	D	TOT	A	B	C	D	TOT	A	B	C	D	TOT	A	B	C	D	TOT
	Gorilla gorilla	4,23	3,05	3,32	5,26	15,87	0,78	0,68	0,74	0,78	**2,99**	0,20	0,20	0,20	0,22	**0,82**	0,33	0,16	0,36	0,40	**1,25**
	Pan troglodytes	4,35	3,32	2,22	8,81	18,70	0,61	0,58	0,42	0,78	**2,39**	0,20	0,40	0,10	0,33	**1,03**	0,04	0,12	0,36	0,32	**0,84**
Human		**0,80**	**0,00**	**0,00**	**0,00**	**0,80**	**0,22**	**0,00**	**0,00**	**0,00**	**0,22**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**
	Homo sapiens	0,80	0,00	0,00	0,00	0,80	0,22	0,00	0,00	0,00	**0,22**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
Hyemoschus aquaticus		**0,00**	**0,00**	**0,28**	**0,00**	**0,28**	**0,00**	**0,00**	**0,05**	**0,00**	**0,05**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**
	Hyemoschus aquaticus	0,00	0,00	0,28	0,00	0,28	0,00	0,00	0,05	0,00	**0,05**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
Loxodonta cyclotis		**6,86**	**1,80**	**0,55**	**12,01**	**21,23**	**0,74**	**0,21**	**0,05**	**0,70**	**1,70**	**0,30**	**0,10**	**0,10**	**0,11**	**0,61**	**0,42**	**0,20**	**0,20**	**0,44**	**1,26**
	Loxodonta cyclotis	6,86	1,80	0,55	12,01	21,23	0,74	0,21	0,05	0,70	**1,70**	0,30	0,10	0,10	0,11	**0,61**	0,42	0,20	0,20	0,44	**1,26**
Orycteropus afer		**0,00**	**0,00**	**0,14**	**0,69**	**0,83**	**0,00**	**0,00**	**0,05**	**0,22**	**0,27**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,04**	**0,00**	**0,04**
	Orycteropus afer	0,00	0,00	0,14	0,69	0,83	0,00	0,00	0,05	0,22	**0,27**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,04	0,00	**0,04**
Phataginus spp.		**1,83**	**0,55**	**0,00**	**0,23**	**2,61**	**0,30**	**0,21**	**0,00**	**0,09**	**0,60**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,13**	**0,08**	**0,00**	**0,00**	**0,21**
	Phataginus sp.	1,83	0,55	0,00	0,23	2,61	0,30	0,21	0,00	0,09	**0,60**	0,00	0,00	0,00	0,00	**0,00**	0,13	0,08	0,00	0,00	**0,21**
Philantomba monticola		**64,65**	**29,50**	**76,73**	**80,89**	**251,77**	**1,00**	**1,00**	**0,95**	**1,00**	**3,95**	**0,00**	**0,00**	**0,00**	**0,11**	**0,11**	**0,25**	**0,24**	**0,20**	**0,28**	**0,97**
	Philantomba monticola	64,65	29,50	76,73	80,89	251,77	1,00	1,00	0,95	1,00	**3,95**	0,00	0,00	0,00	0,11	**0,11**	0,25	0,24	0,20	0,28	**0,97**
Reptile		**0,11**	**0,28**	**0,14**	**0,11**	**0,64**	**0,04**	**0,05**	**0,05**	**0,04**	**0,19**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**
	Testudinae sp.	0,11	0,00	0,00	0,00	0,11	0,04	0,00	0,00	0,00	**0,04**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
	Varanus niloticus	0,00	0,28	0,14	0,11	0,53	0,00	0,05	0,05	0,04	**0,15**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
Rodents		**71,51**	**56,79**	**15,51**	**16,82**	**160,63**	**2,87**	**2,95**	**1,89**	**1,65**	**9,36**	**0,50**	**0,30**	**0,20**	**0,44**	**1,44**	**0,17**	**0,24**	**0,08**	**0,40**	**0,89**
	Atherurus africanus	28,49	28,12	5,12	5,26	66,99	0,91	0,89	0,42	0,43	**2,66**	0,20	0,20	0,00	0,00	**0,40**	0,04	0,08	0,00	0,12	**0,24**
	Cricetomys emini	8,92	18,28	2,63	1,60	31,44	0,74	0,84	0,53	0,30	**2,41**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
	Forest squirrels	22,31	9,14	4,43	5,84	41,72	0,91	0,89	0,47	0,52	**2,80**	0,00	0,00	0,00	0,00	**0,00**	0,04	0,00	0,00	0,00	**0,04**
	Small rodents	11,78	1,25	3,32	4,12	20,47	0,30	0,32	0,47	0,39	**1,49**	0,30	0,10	0,20	0,44	**1,04**	0,08	0,16	0,08	0,28	**0,56**
Small carnivores		**10,30**	**11,63**	**6,79**	**10,30**	**39,02**	**1,96**	**2,37**	**1,58**	**1,87**	**7,77**	**0,00**	**0,00**	**0,00**	**0,33**	**0,33**	**0,13**	**0,12**	**0,12**	**0,24**	**0,61**
	Bdeogale nigripes	0,69	1,39	0,97	1,14	4,19	0,26	0,42	0,26	0,26	**1,21**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
	Crossarchus platycephalus	0,80	1,52	0,14	0,00	2,46	0,26	0,21	0,05	0,00	**0,52**	0,00	0,00	0,00	0,00	**0,00**	0,04	0,00	0,00	0,00	**0,04**
	Genetta sp.	3,43	1,80	1,94	2,97	10,15	0,65	0,53	0,42	0,61	**2,21**	0,00	0,00	0,00	0,00	**0,00**	0,08	0,04	0,04	0,04	**0,20**
	Hydrictis maculicollis	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,11	**0,11**	0,00	0,00	0,00	0,08	**0,08**
	Mellivora capensis	0,00	0,42	0,55	0,34	1,31	0,00	0,16	0,21	0,13	**0,50**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**

Group	Species	Comparing RAI CT					Comparing PAO CT					Comparing PAO Water					Comparing PAO Leaf swab				
		GRID					GRID					GRID					GRID				
		A	B	C	D	TOT	A	B	C	D	TOT	A	B	C	D	TOT	A	B	C	D	TOT
	Mongooses	4,69	5,40	2,22	3,09	15,40	0,57	0,68	0,37	0,39	**2,01**	0,00	0,00	0,00	0,11	**0,11**	0,00	0,00	0,00	0,08	**0,08**
	Nandinia binotata	0,69	1,11	0,97	2,75	5,51	0,22	0,37	0,26	0,48	**1,33**	0,00	0,00	0,00	0,11	**0,11**	0,00	0,08	0,08	0,04	**0,20**
Small primates		**1,60**	**3,32**	**1,66**	**1,03**	**7,62**	**0,48**	**0,68**	**0,42**	**0,22**	**1,80**	**0,70**	**0,50**	**0,50**	**1,11**	**2,81**	**1,04**	**1,00**	**1,52**	**0,88**	**4,44**
	Arctocebus aureus	0,11	0,00	0,00	0,00	0,11	0,04	0,00	0,00	0,00	**0,04**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
	Cercocebus agilis	1,03	2,63	1,25	0,92	5,82	0,26	0,42	0,26	0,17	**1,12**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
	Cercopithecus cephus	0,11	0,28	0,00	0,00	0,39	0,04	0,11	0,00	0,00	**0,15**	0,50	0,10	0,30	0,22	**1,12**	0,21	0,40	0,48	0,20	**1,29**
	Cercopithecus neglectus	0,00	0,14	0,00	0,00	0,14	0,00	0,05	0,00	0,00	**0,05**	0,10	0,10	0,10	0,33	**0,63**	0,00	0,00	0,00	0,04	**0,04**
	Cercopithecus nictitans	0,11	0,28	0,00	0,11	0,51	0,04	0,11	0,00	0,04	**0,19**	0,00	0,00	0,10	0,33	**0,43**	0,54	0,44	0,48	0,28	**1,74**
	Cercopithecus pogonias	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,11	**0,11**	0,21	0,16	0,28	0,24	**0,89**
	Colobus guereza	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,11	**0,11**	0,00	0,00	0,00	0,00	**0,00**
	Galago sp.	0,00	0,00	0,42	0,00	0,42	0,00	0,00	0,16	0,00	**0,16**	0,10	0,30	0,00	0,00	**0,40**	0,08	0,00	0,20	0,08	**0,36**
	Lophocebus albigena	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,08	0,04	**0,12**
	Perodicticus potto	0,23	0,00	0,00	0,00	0,23	0,09	0,00	0,00	0,00	**0,09**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,00	**0,00**
Smutsia gigantea		**0,11**	**0,00**	**0,00**	**0,11**	**0,23**	**0,04**	**0,00**	**0,00**	**0,04**	**0,09**	**0,00**	**0,00**	**0,10**	**0,00**	**0,10**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**
	Smutsia gigantea	0,11	0,00	0,00	0,11	0,23	0,04	0,00	0,00	0,04	**0,09**	0,00	0,00	0,10	0,00	**0,10**	0,00	0,00	0,00	0,00	**0,00**
Suidae		**1,37**	**1,80**	**4,99**	**9,95**	**18,11**	**0,30**	**0,42**	**0,21**	**1,04**	**1,98**	**0,00**	**0,00**	**0,50**	**0,44**	**0,94**	**0,25**	**0,20**	**0,36**	**0,72**	**1,53**
	Hylochoerus meinertzhageni	0,00	0,00	0,00	0,23	0,23	0,00	0,00	0,00	0,09	**0,09**	0,00	0,00	0,00	0,00	**0,00**	0,00	0,00	0,00	0,08	**0,08**
	Potamochoerus porcus	1,37	1,80	4,99	9,73	17,89	0,30	0,42	0,21	0,96	**1,89**	0,00	0,00	0,50	0,44	**0,94**	0,25	0,20	0,36	0,64	**1,45**
Syncerus caffer		**0,34**	**0,00**	**0,00**	**0,11**	**0,46**	**0,09**	**0,00**	**0,00**	**0,04**	**0,13**	**0,00**	**0,00**	**0,00**	**0,22**	**0,22**	**0,00**	**0,00**	**0,00**	**0,00**	**0,00**
	Syncerus caffer	0,34	0,00	0,00	0,11	0,46	0,09	0,00	0,00	0,04	**0,13**	0,00	0,00	0,00	0,22	**0,22**	0,00	0,00	0,00	0,00	**0,00**
Tragelaphus spp.		**0,92**	**0,00**	**0,00**	**0,00**	**0,92**	**0,22**	**0,00**	**0,00**	**0,00**	**0,22**	**0,50**	**0,50**	**0,20**	**0,11**	**1,31**	**0,17**	**0,04**	**0,04**	**0,28**	**0,53**
	Tragelaphus eurycerus	0,80	0,00	0,00	0,00	0,80	0,17	0,00	0,00	0,00	**0,17**	0,10	0,10	0,00	0,00	**0,20**	0,13	0,00	0,04	0,08	**0,25**
	Tragelaphus spekii	0,11	0,00	0,00	0,00	0,11	0,04	0,00	0,00	0,00	**0,04**	0,40	0,40	0,20	0,11	**1,11**	0,04	0,04	0,00	0,20	**0,28**

Appendix 5: Costs of the survey methods

Table 7: Costs of camera trapping and leaf swab eDNA for 40 CT and 40 Swab eDNA (resampling = 10)

Method	Units	Material costs	Field expenses	Transportation	Lab costs	Data processing	TOTAL
CT	40	6.800 €	8.364 €	1.440 €	/	2.752 €	**19.356 €**
Leaf swab eDNA	40	344 €	4.937 €	720 €	4.680 €	860 €	**11.541 €**

Table 8: Costs of survey techniques for 60 CT and 60 Swab eDNA (resampling = 15)

Method	Units	Material costs	Field expenses	Transportation	Lab costs	Data processing	TOTAL
CT	60	10.241 €	12.596 €	1.440 €	/	4.145 €	**28.422 €**
Leaf swab eDNA	60	518 €	7.436 €	720 €	7.048 €	1.295 €	**17.017 €**

Table 9: Detailed cost analysis of the three survey techniques for 100 camera traps, 100 leaf swabs, and 40 water samples. The costs encompass materials, transportation, field expenses, data processing, and laboratory analysis. The estimates assume that basic equipment (GPS, camping gear, work computer, field boots) and the necessary data processing software are already available and operational.

Cost estimation for the installation and recovery of 100 cameras in 4 grids with 2 teams

CT - Material costs			
	Cost unit	Amount	Total
Camera traps	150 €	100	15.000 €
Consumables (batteries, SD cards, silicone)	20 €	100	2.000 €
TOTAL			17.000 €

CT- Field expenses				
	Number	Daily cost	Num days	Total
Labourers	5	16 €	41 €	3.280 €
Engineer	2	430 €	41 €	17.630 €
Food supplies	7	4 €	41 €	176 €
TOTAL				20.910 €

CT- Transportation			
Number of trips	Distance (km)	Cost per km	Total
8	360	0,5 €	1.440 €

Cost estimation for data processing of CT

CT - DATA PROCESSING			
Unit	Daily cost	Num days	Total
Species identification	430 €	12	5.160 €
Data preparation for analysis	430 €	2	860 €
Data analysis	430 €	2	860 €
TOTAL			6.880 €

Cost estimation for the collection of 100 eDNA leaf swabs + 40 eDNA water in 4 grids with 2 teams

SWABS- Material costs

Unit	Cost unit	Amount	Total
Swabs	2 €	300 €	660 €
Ethanol tubes	2 €	100 €	200 €
TOTAL			860 €

WATER - Materiel costs

Unit	Cost unit	Amount	Total
Extraction kit	82 €	40 €	3.280 €
Peristaltic pumps	1.200 €	2 €	2.400 €
Pump batteries	63 €	6 €	376 €
TOTAL			6.056 €

SWAB - Field expenses

	Number	Daily cost	Num days	Total
Labourers	5 €	16 €	24 €	1.920 €
Engineer	2 €	430 €	24 €	10.320 €
Food supplies	7 €	4 €	24 €	103 €
TOTAL				12.343 €

WATER- Field expenses

	Number	Daily cost	Num days	Total
Labourers	5 €	16 €	20 €	1.600 €
Engineer	2 €	430 €	20 €	8.600 €
Food supplies	7 €	4 €	20 €	86 €
TOTAL				10.286 €

SWAB - Transportation

Number of trips	Distance (km)	Cost per km	Total
4	360 €	0,5 €	720 €

WATER - Transportation

Number of trips	Distance (km)	Cost per km	Total
4	360 €	0,5 €	720 €

Cost estimation for data processing and lab work for eDNA

SWAB – Data processing

Unit	Daily cost	Num days	Total
Sequences ID sorting	430 €	2	860 €
Data analysis	430 €	3	1.290 €
TOTAL			2.150 €

Lab analysis

Unit	Cost unit	Amount	Total
Genetic analysis swab	117 €	100	11.700 €
Genetic analysis water	380 €	40	15.200 €
TOTAL			26.900 €

WATER – Data processing

Unit	Daily cost	Num days	Total
Sequences ID sorting	430 €	1	430 €
Data analysis	430 €	2	860 €
TOTAL			1.290 €

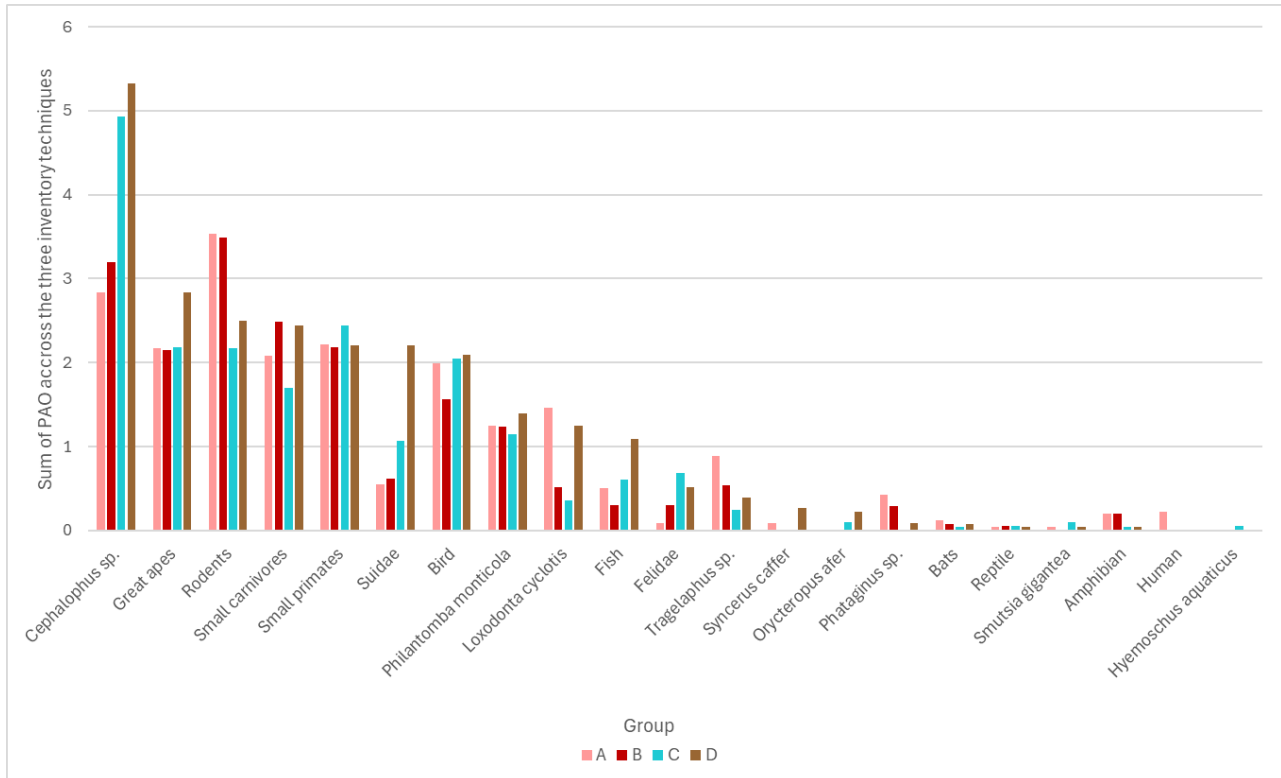## Appendix 6: Bar chart of the sum of PAO per group and grid.

Figure 10: Bar chart of PAO value per group and grid. For a group, the PAO value of each survey technique has been summed. Details of the composition of each group is presented in Appendix 4. Each colored bar corresponds to a grid.

Appendix 7: List of Birds and Mammals Detected by Morgan et al. (2023) in the forests adjacent to and within the Nouabale Ndoki National Park

Table 10: Table listing bird and mammal species detected by Morgan et al. (2023) in the forests adjacent to and withing the Nouabale Ndoki National Park. Species highlighted in pink are those not found in our combined survey (using three methods). Species highlighted in blue were not detected in our survey, but we identified their family or genus.

BIRDS		MAMMALS	
Order	Species	Order	Species
Accipitriformes	*Stephanoaetus coronatus*	Carnivora	*Caracal aurata*
Bucerotiformes	*Horizocerus cassini*		*Panthera pardus*
Columbiformes	*Turtur brehmeri*		*Atilax paludinosus*
Cuculiformes	*Centropus anselli*		*Bdeogale nigripes*
Galliformes	*Agelastes niger*		*Crossarchus platycephalus*
	Guttera plumifera		*Herpestes naso*
	Peliperdix lathami		*Mellivora capensis*
Gruiformes	*Himantornis haematopus*		*Nandinia binotata*
Passeriformes	*Alethe castanea*		*Genetta servalina*
	Illadopsis cleaveri		*Poiana richardsonii*
	Baeopogon indicator	Cetartiodactyla	*Cephalophus callipygus*
	Bleda notatus		*Cephalophus dorsalis*
	Geokichla princei		*Cephalophus leucogaster*
	Neocossyphus poensis		*Cephalophus silvicultor*
Pelecaniformes	*Bostrychia olivacea*		*Philantomba monticola*
Strigiformes	*Strix woodfordii*		*Syncerus caffer*
			Tragelaphus eurycerus
			Tragelaphus spekii
			Hylochoerus meinertzhageni
			Potamochoerus porcus
			Hyemoschus aquaticus
		Chiroptera	*Chiroptera sp.*
		Eulipotyphla	*Eulipotyphla sp.*
		Pholidota	*Phataginus tricuspis*
			Smutsia gigantean
		Primates	*Cercocebus agilis*
			Cercopithecus cephus
			Cercopithecus neglectus
			Cercopithecus nictitans
			Lophocebus albigena
			Galagoides demidoff
			Gorilla gorilla
			Pan troglodytes
		Proboscidea	*Loxodonta cyclotis*
		Rodentia	*Atherurus africanus*
		Tubulidentata	*Orycteropus afer*