Plant-herbivore interactions and their influences on the distribution of sub-Saharan antelopes in the Ouadi Rimé Ouadi Achim Wildlife Reserve - Chad.

3rd field mission summary report for the hot and dry season 2025

June 19 – July 22, 2025

Field Team Members:

Caleb NGABA WAYE TAROUM

Conservation Biology Laboratory, University of Neuchâtel (Switzerland)

DJASBEYE Anaclé

Master's Degree in Animal Production, Abéché Institute of Science and Technology (Chad)

YOUSRA Mahamat SABOUN

Master's Degree in Animal Production, Livestock Research Institute for Development (Chad)

TCHAMOGOYE KIDDINDIWA Benjamin

PhD Student in Environmental Sciences, University of N'Djamena

Constant DJEKILLAMBER DJEKADJIM

Junior Vulture Conservation Expert (Chad)

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Introduction

This report follows the last field mission planned as part of Mr. Caleb Ngaba Waye Taroum's doctoral research project, in the Ouadi Rime Ouadi Achim Wildlife Reserve (RFOROA), from June 19 to July 31, 2025. It reports the progress of the mission, the description of the data collection process as well as a descriptive overview of the diversity indices observed at the end of the floristic inventory. This mission was carried out with the authorization of the competent authorities of the Ministry of the Environment and the Ministry of Livestock of Chad (DGRFFP and IRED). The supervision of the work is provided by the Conservation Biology Laboratory of the University of Neuchâtel and the Institute of Science and Technology of Abéché (INSTA). We actively collaborate with the ZODIAC laboratory of IRED, which provides technical support for: the conservation, packaging and shipping of fecal material to the Conservation Sciences laboratory (The Royal (Dick) School of Veterinary Studies) of the University of Edinburgh (United Kingdom). We also benefited from the institutional and logistical support of the NGO Sahara Conservation & its staff in the field, the technical support of Katherine Mertes (SNZCBI), Tim Wacher (ZSL), the Laboratory of Functional Biology of the University of Neuchâtel and Stéphanie Brien (University of Edinburgh). The Swiss Confederation Excellence Scholarship sponsors our training at the Doctoral School of Life Sciences (DSLS) of the University of Neuchâtel. Financially, this mission benefited from the support of The Rufford Foundation, the Segré Foundation - IUCN Save Our Species and the University of Neuchâtel (Switzerland) through the Laboratory of Conservation Biology.

I. Field mission initial objectives

- Vegetation inventory and sampling of woody and herbaceous extracts making up the reserve's plant cover for the hot and dry season, the third seasonal variant after the cold-dry season and the rainy season. The inventory will allow analysis of the floristic diversity indices of the RFOROA. While the plant samples will be used to characterize the biochemical metabolite content of the plants present and those consumed by the animals (cattle and reintroduced antelopes) populating the reserve. The aim of this approach is to deepen factual knowledge on the constitution of ecological niches shared between the reintroduced antelopes (*Oryx dammah* known as "Oryx" and *Addax nasomaculatus* known as "Addax") and the four main domestic species sharing the reserve's habitats (cattle, sheep, goats and camels);
- Collection of fecal samples (fresh primarily) from the above-mentioned mammalian species for metagenomic analyses to identify the plant species consumed. The approach consists of defining the dietary composition of sympatric species sharing the same pastures of the RFOROA, in order to establish a balance sheet of “available fodder resources versus consumed resources”. Secondly, the parasitological analysis of fecal samples will make it possible to identify gastrointestinal parasites common to the target mammals of this study (oryx and addax, cattle, sheep, goats and camels), but also to identify the potential zoonoses for which they are potentially responsible.

Initial mission plan :

- • Carry out an inventory (of woody and herbaceous plants) on twenty-five (25) 50 x 50 m super-plots randomly distributed to cover 95% of the Oryx and Addax distribution area for the period from May 15 to June 15, 2025, a period representative of the high dry season;
- • Collect at least 150 plant samples for metabolomic analyses for all 50 x 50 m super-plots;

- • Collect 15 fecal samples for oryx and addax as well as 10 for each of the four targeted livestock species (cattle, sheep, goats and camels).

II. Details de la mission

19 - 20 June 2025: Preparation of the mission in collaboration with all members of the team from N'Djamena.

20 June 2024 :

- Meeting with the National head of Forestry, Wildlife, and Fisheries Resources Department at the Ministry of the Environment and withdrawal of the official research permit.
- Greetings to the National Director and staff of Sahara Conservation present at the headquarters in N'Djamena. Sharing of the expedition schedule and planned activities upon return from the field.

22 June 2025: Land route N'Djamena – Abéché.

23 June 2025 :

- Trip from Abéché to Oryx Base (RFOROA). The detour via Arada was canceled due to a lack of agreement with the eco-guards who were supposed to participate in the expedition.
- Presentations of the research team and the mission schedule to the Project Oryx staff present at the base. Receipt of operating instructions and preparation of the necessary equipment.

24 - 29 June 2025 :

- Fieldwork begins with the research team, following discussions with the ecological monitoring team and the collection of daily antelope locations to better guide the choice of directions to the super-plots.
- Floral inventories, collection of fecal samples, and interviews with the breeders encountered.

29 June 2025: (afternoon) Direct return to Abéché via Biltine.

30 June 2025: (morning) Trip back from Abéché – N'Djamena.

1st July 2025: (morning) Submission of fecal samples to the Zodiac laboratory of IRED (N'Djamena) for storage at optimal temperature (-15°C) before sterilization waiting the time for the shipment to Edinburgh.

03 July 2025: Report of the expedition to the National Director of Sahara Conservation following a meeting at the NGO's headquarters.

15 – 17 July 2025: Practical training for two members of the mission in encoding floristic inventory data using Kobocollect.

24 July 2025: Scientific communication on the use of remote sensing for the conservation of Sahel-Saharan ecosystems at the Computer Programming Camp initiated by the NGO Action for Education and the Promotion of Women (AEPF-Chad) in partnership with the United States Embassy in Chad and the "Coding 4 Chad" platform.

28 July 2025: Online presentation on the progress of research work to the Director of Operations of the NGO Sahara Conservation.

31 July 2025: Communication on the challenges of the reintroduction of sub-Saharan antelopes in Chad and the usefulness of scientific research for better management of the ecosystems hosting these emblematic species at the "American Corner" (Al-Mouna Center in N'Djamena).

III. Methods and preliminary results

3.1. Herbaceous inventory

3.1.1. Inventory and sampling method

The super-plot implantation and sampling method remained identical to that adopted during the two previous field missions (July and October 2024). It consists of a 50 x 50 m super-plot, in each of which, five 50 x 50 cm quadrats were placed 10 m apart along three 50 m transects. In total, 25 super-plots were inventoried for a total of 375 subplots of 50 x 50 cm, inventoried for the systematic identification of herbaceous plants.

Plate 1. Image A) Quadrat 50 x 50 cm; B) Species coverage count by feet

3.1.2. Population

In total, a population of 927 individuals systematically identified. The inventoried population is divided into: 06 families (not including unassigned families), 10 genera and 17 species including 02 unknown and 02 taxonomically unidentified (Figure 1).

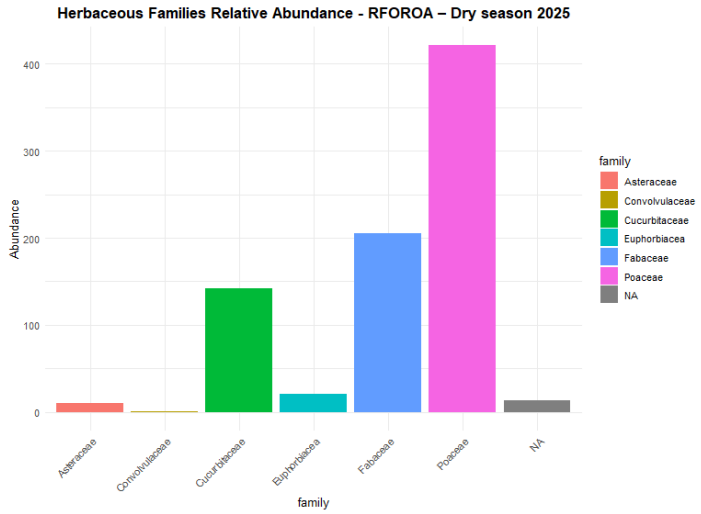

Figure 1 Specific relative abundance of herbaceous plants by family - Dry season - RFOROA, June 2025

The specific distribution by relative abundance shows a predominance of four species, classified in order of magnitude including: *Aristida mutabilis*, *Indigofora colutea*, *Schenofeldia gracilis* and *Citrullus colocynthis.*

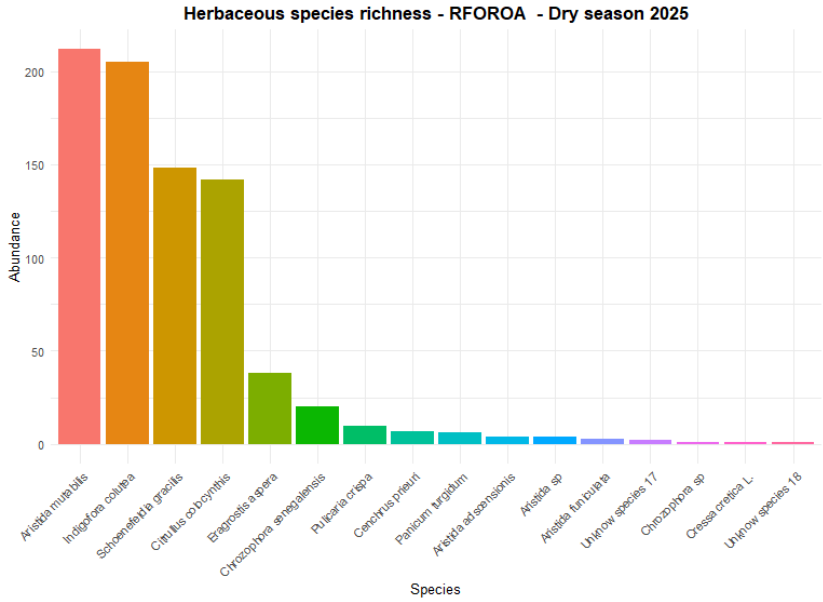

Figure 2 Relative abundance by species – Dry season – RFOROA, June 2025

3.1.3. Diversity indices

The inventoried population has a relative species richness of 17 species. The Shannon index, with a value of 1.84, expresses moderate to relatively high diversity for a fairly uniform species distribution during the dry season. The Simpson index, with a value of 0.8, confirms good regularity in species distribution and low monospecific dominance across the entire inventoried population.

At the scale of the 25 super-plots, the vast majority of them have a diversity of 5 to 7 distinct species, with Shannon values between 1.3 and 1.6 and Simpson values between 0.74 and 0.78. This demonstrates a predominance of stable and balanced herbaceous communities at the individual plot scale (Figure 3).

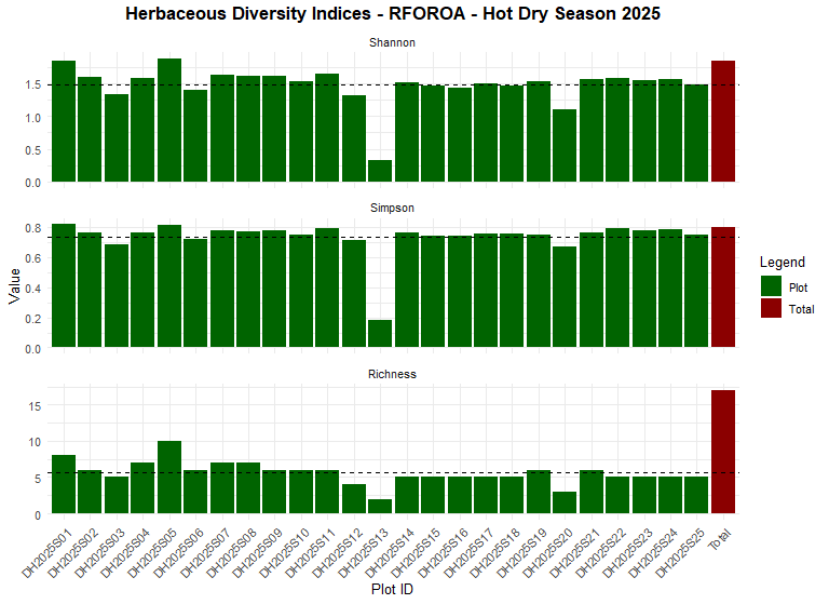

Figure 3 Relative abundance by species – Dry season – RFOROA, June 2025

A closer observation allows us to identify five super-plots that present the highest diversity indices (Shannon ≥ 1.6, Sp. Richness ≥ 7–10): DH2025S01, DH2025S05, DH2025S07, DH2025S08 and DH2025S11. While two super-plots stand out with the lowest diversity indices, including: DH2025S13 (Shannon = 0.33, Simpson = 0.18, Sp. Richness = 2) expressing low diversity with monospecific dominance, and DH2025S20 (Shannon = 1.09, Simpson = 0.67, Sp. Richness = 3) demonstrating both low homogeneity and specific diversity. The general trend is that diversity is relatively homogeneous across most subplots, with only a few plots severely degraded or with low species richness.

Ecologically, these preliminary results from the herbaceous inventory for the hot and dry season show that the herbaceous cover is diverse and proportionally distributed across most of the surveyed areas, ensuring structural resilience during that hot and dry season. However, a small number of subplots show signs of disturbance or reduced community structure. These disturbances are likely linked to burned areas, pasture/penning areas, and the increasingly prevalent bare soils in the reserve.

3.2. Tree inventory

3.2.1. Inventory and sampling method

The woody inventory was conducted on 25 super-plots of 50 x 50 m. Their position was based on the distribution of the reintroduced antelopes (Oryx and Addax) range for the period from May 15 to June 15, 2025. In each super-plot, three 50 m long transects (oriented north-south) and equidistant by 10 m each were used to conduct the inventory. Across 75 transects, all individuals greater than or equal to 1 m in height were identified, over a width of 10 m on either side of each transect.

To estimate the natural regeneration rate, the shoots and live stumps identified within a 10 m radius on either side of each transect were also recorded as natural regeneration. Taxonomic identification and dendrometry parameters (height, crown coverage, circumference at breast height) were systematically recorded for each identified tree base. All-natural shoots as well as trunk stumps were also listed during the inventory.

Plate 2. Balanites aegyptiaca foot height measurement

3.2.2. Population

The inventoried woody stand, composed of 51 individuals, is divided into two families divided into two species and two genera. As in the two previous seasons, the dominant family is the

Zygophyllaceae with *Balanites aegyptiaca* as the dominant species (Figure 4). The least represented family is the Caesalpinaceae with *Boscia senegalensis*.

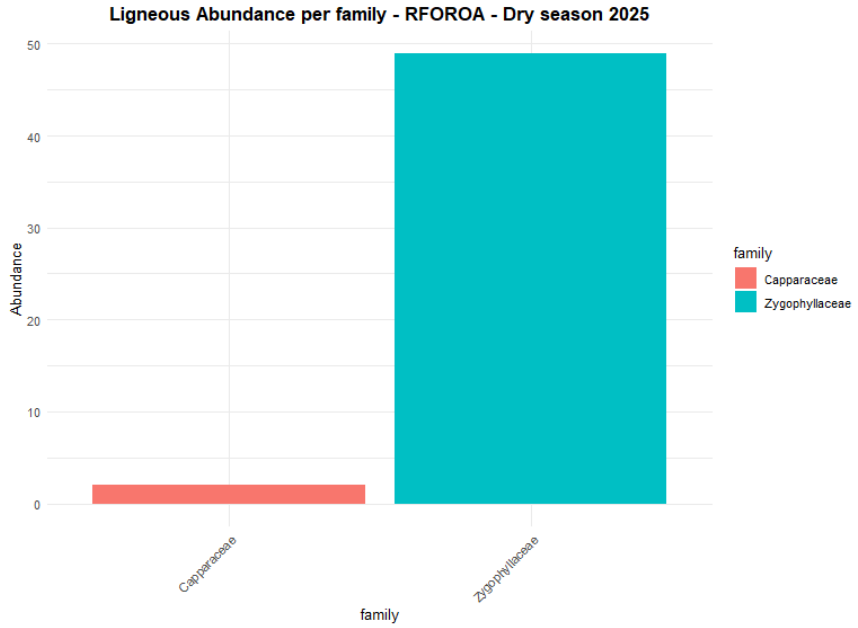

Figure 4. Specific abundance of woody plants by family - Dry season - RFOROA, June 2025

Balanites aegyptiaca stands out with a population of 49 individuals, followed by the species *Boscia senegalensis* with only 02 individuals (Figure 5).

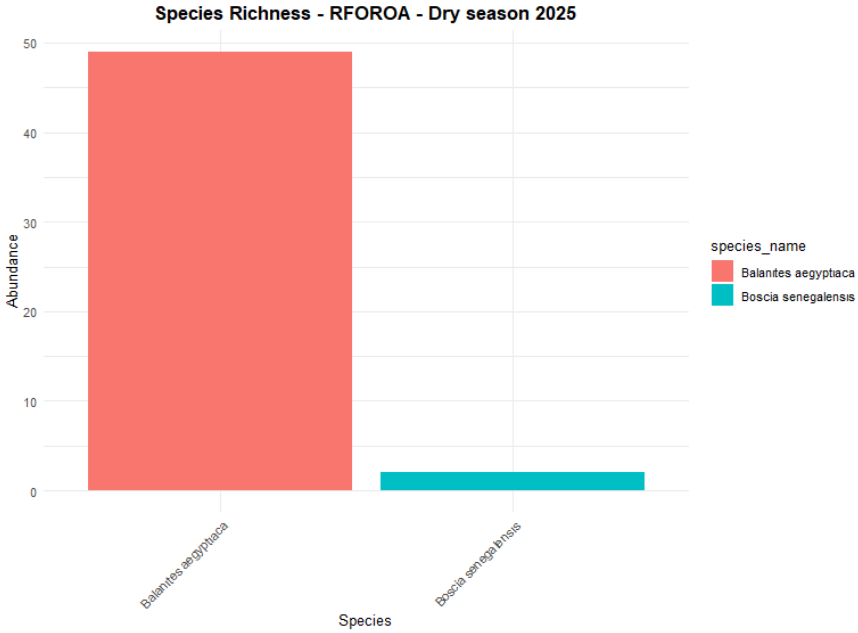

Figure 5. Abundance by species – Dry season – RFOROA, June 2025

3.2.3. Diversity indices

The population exhibits very low species richness across all 25 inventoried super-plots. This is reflected in the diversity index values. Shannon's index (0.165) indicates very low diversity, with a predominance of *Balanites aegyptiaca* followed by a rare species, *Boscia senegalensis*. Simpson's index (0.075), also very low, confirms the strong dominance of the single species mentioned above. Thus, at the landscape level, the values of these indices suggest a community with low diversity, likely simplified due to disturbances (wildfires, overgrazing, bare soil, etc.),

seasonal variability of climatic factors, or natural dominance. At the individual super-plot scale, out of 25 plots surveyed, five have a monospecific population (DH2025S01, DH2025S06, DH2025S07, DH2025S09, DH2025S16), hence a total absence of diversity. While only two (DH2025S08 and DH2025S19) super-plots are populated by two distinct species, thus presenting a slight diversity (Shannon 0.34 for DH2025S08, 0.29 for DH2025S19). The Simpson index (0.20 for DH2025S08, 0.15 for DH2025S19) indicates that *Balanites aegyptiaca* is more abundant than *Boscia senegalensis* in these two plots (Figure 6).

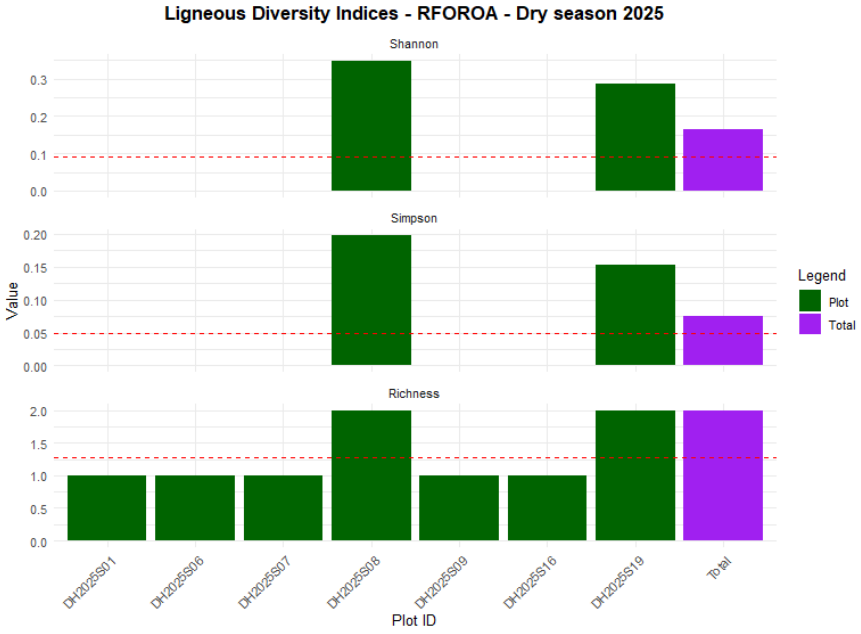

Figure 6. Abundance by species – Dry season – RFOROA, June 2025

The overall community structure is therefore species-poor and heavily dominated by *Balanites aegyptiaca*. Analysis of the diversity of the woody stand across the 25 super-plots reveals very low diversity overall. The overall Shannon index for the dataset and Simpson's index reflect the strong dominance of one species and minimal contribution of others, indicating a highly simplified woody community structure during the hot, dry season of 2025.

3.3. Plant sample extracts

3.3.1. Sampling protocol

Plant extracts (leaves, flowers, or stems for grasses) were sampled within the surveyed super-plots (50 x 50 m). For grasses, sampling was done by elimination from one quadrat (50 x 50 cm) to the next. In the first quadrat (out of 15 quadrats) of each super-plot, an extract from an individual of each identified species was systematically sampled. Then, for the other 14 quadrats, only individuals of the newly identified species were sampled. A total of 375 quadrats measuring 50 x 50 cm were surveyed for the 25 super-plots visited.

All samples were collected using gloves sterilized with ethyl alcohol to avoid any alteration of the genome of the collected species. For trees, extracts were additionally collected using sterilized pruning shears.

3.3.2. Packaging and preparation for metabolic analysis

Each of the plant extract samples was packaged in sterilized paper coffee bags and then dried in 15 ml tubes using silicate gel. A total of 93 plant samples were packaged. All samples collected for the dry season (June 2025) were sent to the functional biology laboratory of the

University of Neuchâtel for biochemical analyses and identification of metabolites characteristic of the identified plants.

Plate 3. Chrozophora senegalensis leaf sample with tube label

3.4. Fecal matter sampling and sample export

3.4.1. Fecal samples

For this mission during the dry and hot season in the reserve, 76 fecal samples were collected for all six-target species. The sampling principle consists of locating a group and collecting the droppings of an individual or individuals of the group (depending on the size of the herd encountered) within a 5 km radius around one of the 25 super-plots. For each sample, we took care to observe and note the visible body condition of the individual.

Species	Sample size
Oryx	16
Addax	8
Cattle	10
Sheep	10
Goat	10
Camel	12
Total	66

Table 1 Fecal samples by species – Dry season – RFOROA – June 2025

3.4.2. Shipping of collected fecal samples

The 66 samples collected for the dry season were conditioned with RNA later in 2 ml cryotubes and then sterilized using a thermoblock at 58 ° C. Each of the samples was subdivided into four aliquots distributed for the benefit of IRED, Sahara Conservation, the University of Neuchâtel and the University of Edinburgh. Administrative procedures are underway for the shipment of the samples via DHL to the Conservation laboratory of the University of Edinburgh directed by Pr. Rob Ogden.

Plate 4. Fecal sampling from an Oryx

Future prospects

- Shipment of the final set of fecal samples to Edinburgh for genomic analysis to identify plant species palatable across the three seasons by the target mammal species in the reserve.
- Finalization of metabolic and biocomputational analysis of plants for all three seasons.
- Consideration of parasitological analysis (qPCR) for the identification of gastrointestinal parasites causing various pathologies common to the six-target species.