Wetland Conservation and the Impact of Invasive Alien Mammals in Northwest Patagonia

Exclusion for herbivores in Estancia Fortin Chacabuco

NATIONAL PARKS RESEARCH PERMIT: Nº 1862

PRINCIPAL RESEARCHER: Dr. Sebastián A. Ballari

SITE: Estancia Fortín Chacabuco, Nahuel Huapi National Park, Argentina.

C·E·N·A·C Parque Nacional Nahuel Huapi

PROGRAMA DE ESTUDIOS APLICADOS A LA CONSERVACION DEL PARQUE NACIONAL NAHUEL HUAPI

Abstract

Wetlands possess significant ecosystem, economic, social, and cultural value. They support a vast biodiversity of species and play a crucial role in providing ecosystem services essential to human societies and their productive activities. Globally, many wetlands have been lost or are undergoing degradation due to land-use changes (e.g., dam construction, agriculture), climate change, pollution, and the impact of invasive alien species. In Patagonia, wetlands—locally known as "mallines"—are highly productive ecosystems, characterized by a notable presence of endemic plants and animals, and are critically important for sustainable livestock farming. However, these wetlands, even those located within protected areas, are primarily threatened by the direct and indirect impacts of multiple invasive alien mammals, such as red deer, fallow deer and wild boar. This project aims to assess the negative impacts of invasive mammal species on the wetlands of Estancia Fortín Chacabuco (EFC) within Nahuel Huapi National Park. These wetlands not only constitute a key ecosystem for sustainable grassland management but also serve as vital refuges for the conservation of native biodiversity and the maintenance of ecosystem services in northwestern Patagonia.

Study site

Six mallines with red deer, deer, wild boar, and cattle were selected for this project at Estancia Fortin Chacabuco, Neuquén Province, Argentina. The approximate location of each site is detailed below (Fig. 1):

I. Repunte Bajo: 41º 0' 45.9066"S - 71º 10' 49.029"O

II. Repunte Alto: 41° 00' 20.6"S - 71° 11' 13.1"O

III. Potrero: 41º 00' 29"S - 71º 10' 04.57"O

IV. Side Sur: 41° 0'22.70"S - 71° 9'35.02"O

V. Tortuga: 40° 59' 23.4"S - 71° 09' 30.2"O

VI. Lagunita: 40°59'9.80"S - 71° 8'58.90"O

Figure 1. Selected wetlands for the project at Estancia Fortín Chacabuco, Nahuel Huapi National Park.

The dominant vegetation in these environments are grasses, *Pappostipa speciosa* in the lower areas and *Festuca pallescens* in the higher areas, with scattered shrubs of *Senecio bracteolatus* and *Mulinum spinosum*, while in the most humid and flooded areas species such as *Juncus balticus*, *Poa pratensis* and *F. pallescens* predominate associated with shrubs of *Ochetophila trinervis*, *Discaria chacaye*, *Berberis microphylla* and *Maytenus boaria*.

Monitoring Habitat Use and Abundance

Wildlife monitoring was conducted using one or two camera traps per wetland (Fig. 2) to assess habitat use. Cameras were installed at a height of 60 cm above ground level, fixed to wooden posts, and operated continuously, recording 10-second videos and/or three photographs at 5-minute intervals. Throughout the sampling period, approximately 1,508 camera-trap days were accumulated across the six sites. Due to weather conditions and logistical constraints, sampling during this year was intermittent; therefore, additional data collection during the project's second year is expected to yield more robust results. The monitoring recorded the use of these wetlands by livestock (cattle and sheep), working animals (horses and dogs), native herbivores (such as guanacos *Lama guanicoe*), and introduced species (such as hares, fallow deer, red deer, and wild boar).

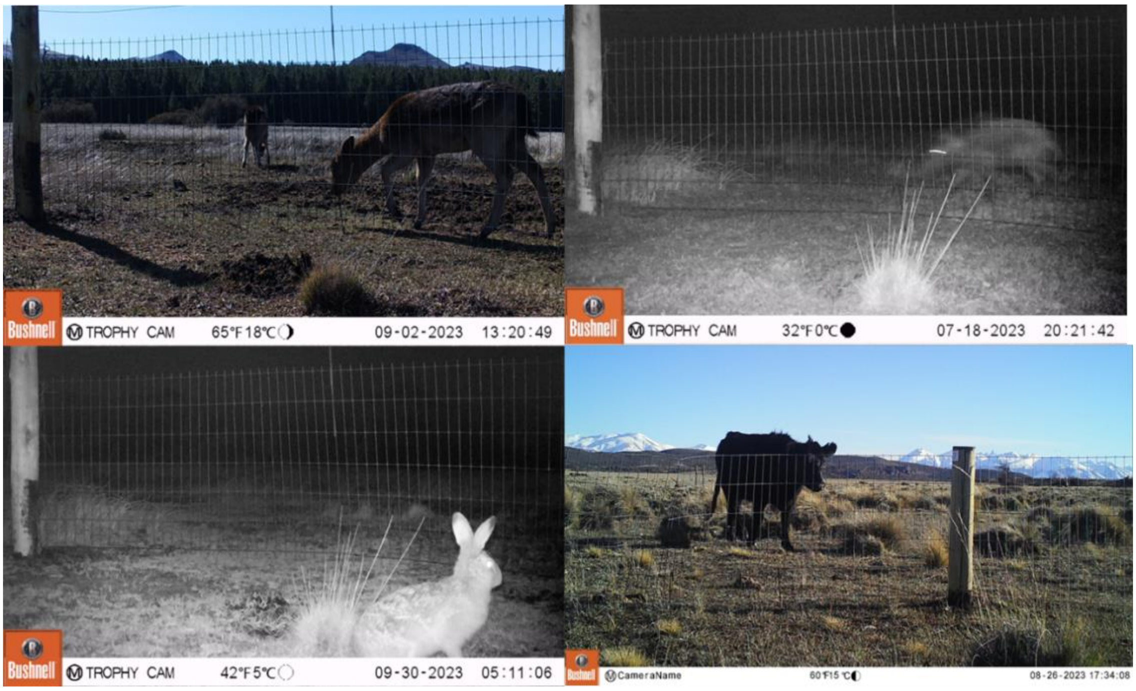

Figure 2. Camera trap records of different herbivores using the mallines of Estancia Fortín Chacabuco: dama deer (top left), wild boar (top right), cattle (bottom right) and hare (bottom left).

Impact of the wild boar rooting.

Exploratory flights were conducted to evaluate the use of UAV equipment (DJI Mini SE drone) at one of the study sites (Repunte Alto; Fig. 3 and 4). However, the available equipment presented limitations (e.g., low image quality, inability to operate in strong winds), making it difficult to complete the survey of rooted surfaces within the wetlands. Arrangements have been made with certificate UAV pilots, who have previously collaborated with Nahuel Huapi National Park and possess professional UAV systems (Fig. 5), to carry out the necessary flights over Fortín Chacabuco. These flights aim to support the annual monitoring of wetlands for this project and enable medium- and long-term analysis of rooting surface dynamics. This activity is expected to advance during the next stage of the project. In parallel, soil samples were collected from areas with and without rooting activity in wetlands where disturbance by wild boar was observed (three sites to date: Repunte Alto, Repunte Bajo, and Tortuga). These samples were deposited at the "Dr. Grenville Morris" Agricultural Experiment Station, Instituto Nacional de Tecnología Agropecuaria (INTA), Bariloche. Currently, the samples are being processed and analyzed to determine their physical and chemical properties, including pH, electrical conductivity, carbon, nitrogen, and phosphorus content, as well as microbial enzyme activity. These analyses are being conducted in collaboration with the hydrology group led by Agricultural Engineer María Victoria Cremona and Dr. Valeria Aramayo from the Natural Resources Research Area of EEA, INTA Bariloche.

Figure 3. Aerial image taken with a DJI Mini SE drone where the sites browsed by wild boar are clearly visible (dark areas).

Figure 4. Aerial image taken with a DJI Mini SE drone, of a sector of the Repunte Alto with signs of damage to the ground due to rooting by wild boar.

Figure 5. Survey test with Nicolas Kruljac, a professional drone pilot, who worked linked to the Nahuel Huapi National Park.

<u>Impact of Deer and Wild Boar on Soil and Vegetation</u>

To quantify herbivory by deer and wild boar in relation to the reduction of biomass, composition, and vegetation richness in wetlands, and to identify their impacts, an exclusion experiment was set up consisting of four treatments (Fig. 6, 7):

1. Wild Boar Exclusion Treatment (EJA): a 16 m² circular plot where eight 0.8 m high wooden posts were used, with a 0.8 m high galvanized steel mesh, allowing entry to deer that can easily jump that height, but not to wild boar.

2. Deer Exclusion Treatment (ECV): a 16 m² circular plot where eight 1.8 m high wooden posts were used, with a 1 m high galvanized steel mesh, and using three strands of galvanized wire to raise the fence height to 1.5 m, preventing deer entry. In this treatment, 0.6 m x 0.6 m spaces were created in the fence to allow wild boar entry.

3. Deer and wild boar exclusion treatment (ETT): A 16 m2 circular plot was used, where eight 1.8-meter-high wooden posts were used, with a 1-meter-high galvanized steel mesh. Three strands of galvanized wire were used to raise the fence height to 1.50 m, preventing deer entry. This also meant no openings for medium and large herbivores. This prevented deer and wild boar from entering.

4. Control treatment, no exclusion (TRL): The center and perimeter were marked on the ground with eight stakes to delimit the treatment.

A replica of each treatment was constructed in each mesh (N=24). Each exclusion is 5 meters in diameter (~16m^2) and was constructed with galvanized steel mesh (section 15cm x 5cm), high-strength galvanized field wire, and eight eucalyptus-wood-impregnated posts. Initially, the exclusion experiment was built with four wooden posts, but due to the entry and damage caused mainly by cows, they were reinforced with four additional posts. Small 20cm x 20cm spaces were provided for the EJA, ECV, and ETT treatments to allow entry of small herbivores (e.g., hare *Lepus europaeus*). Camera traps were used to monitor the treatments and verify their proper functioning.

To evaluate the effects of herbivory, the following measurements were made within each exclusion: 1) maximum vegetation height, 2) plant species richness and cover (native and exotic), 3) soil cover, 4) plant biomass, and 5) soil hardness. Soil hardness was measured using a handheld penetometer at five random points within each enclosure to obtain an average hardness. Plant biomass was obtained by cutting aboveground vegetation into a 50cm x 50cm subplot, which was then oven-dried for 48hs at 60°C to obtain dry weight. To assess changes in plant community composition in each treatment, a 2m x 2m subplot was used to record plant species and their cover.

Figure 6. Exclusion (ETT) in Estancia Fortín Chacabuco.

Figure 7. Exclusion (EJA) in Estancia Fortín Chacabuco.

Three soil samples were collected at random points within each treatment using a 40-inch stainless steel soil sampler probe. Each sample measured 15 cm × 5 cm (Fig. 8). The three samples from each treatment were combined and homogenized into a single composite sample to obtain a representative sample for each treatment within each mallín (N = 24). The samples were deposited at the "Dr. Grenville Morris" Agricultural Experiment Station, Instituto Nacional de Tecnología Agropecuaria (INTA), Bariloche (Fig. 9), where they have been processed to determine their physical and chemical properties, including pH, electrical conductivity, carbon, nitrogen, and phosphorus content, as well as microbial enzyme activity. As with the rooting and non-rooting samples, these analyses are being carried out in collaboration with Agricultural Engineer María Victoria Cremona and Dr. Valeria Aramayo from the Natural Resources Research Area of EEA, INTA Bariloche.

Figure 8. Soil sample 0-15. The volcanic ash horizon is clearly visible in light gray of the last major volcanic event in the region in 2011.

Figure 9. Processing of soil samples at INTA Bariloche.

<u>Impact of deer and wild Boar on wetland microarthropods.</u>

To assess changes in soil microarthropods three soil subsamples measuring 15 cm in height x 5 cm in diameter were collected from each treatment in each wetland. The samples from each treatment were homogenized into a single sample before processing (N = 24). To obtain the microarthropods present in these samples, the CENAC laboratory (PNNH-CONICET) used a Berlese-Tullgren funnel experiment. This instrument is used to capture insects from soil samples (Fig. 10). The device uses a heat source (in this case, a heat-emitting bulb) to dry the sample, forcing the insects to pass through a mesh placed at the bottom of a funnel, where they subsequently fall and are preserved in a jar with 70% alcohol. The samples are stored in the CENAC laboratories for analysis in the next stage of the project (Fig. 11).

Figure 10. Experiment with Berlese-Tullgren funnels.

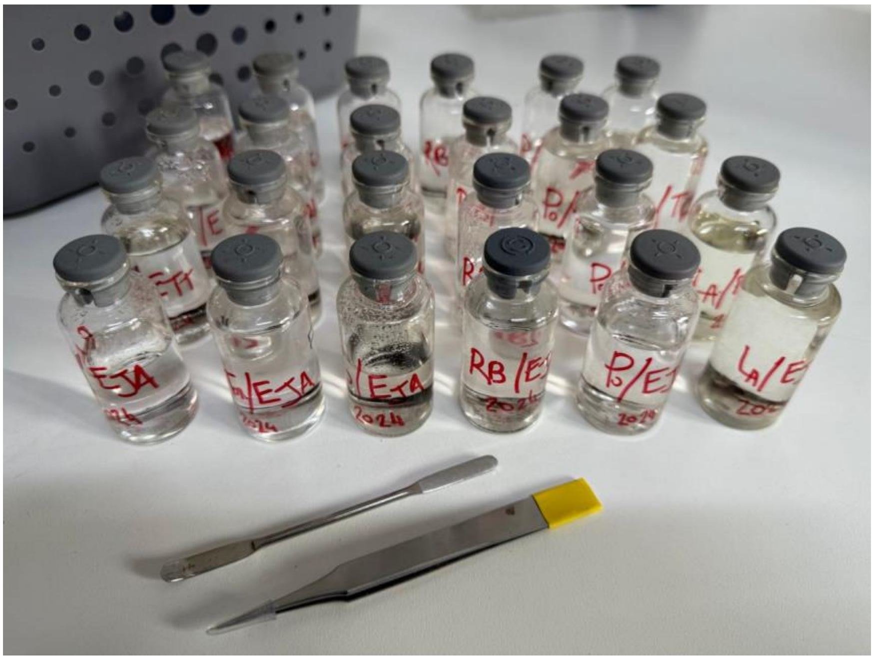

Figure 11. Samples with microarthropods.

<u>Impact of deer and wild boar on wetland macroarthropods.</u>

To assess changes in the ground-walking arthropod community, pitfall traps (Fig. 12) were installed within each treatment. Five plastic containers filled with a propylene glycol solution (to prevent escape and preserve specimens) were buried at ground level in each of the four treatments across the six wetlands. The pitfall trapping experiment was conducted over a five-day period. Arthropods collected from the five containers were pooled into a single composite sample per treatment (N = 23). In one treatment (ETT–Pasture), the experiment could not be conducted due to soil saturation caused by the diversion of an internal water channel.

All collected arthropods were preserved in 70% ethanol for subsequent identification. The samples are currently stored at the CENAC laboratories within Nahuel Huapi National Park and will be analyzed during the next stage of the project (Fig. 13).

Figure 12. Pitfall trap used wetlands.

Figure 13. Samples with macroarthropods.

Results

The results from the first year of the project "Wetland Conservation and the Impact of Invasive Alien Mammals in Northwest Patagonia" are presented below, highlighting the most important and significant findings.

Biomass and vegetation height were statistically analyzed using linear mixed models (REML), comparing the response variables across treatments, with treatments nested within wetlands and wetlands included as a random effect. Here, we report the results for the two variables that exhibited statistically significant differences between treatments.

Biomass (REML, F= 6.57, p= 0.0029)

ETT	***A***
EJA	***A***
ECV	***AB***
TRL	***B***

Maximum height of grasses (REML, F=4.95 p=0.0099)

EJA	***A***
ETT	***A***
ECV	***AB***
TRL	***B***

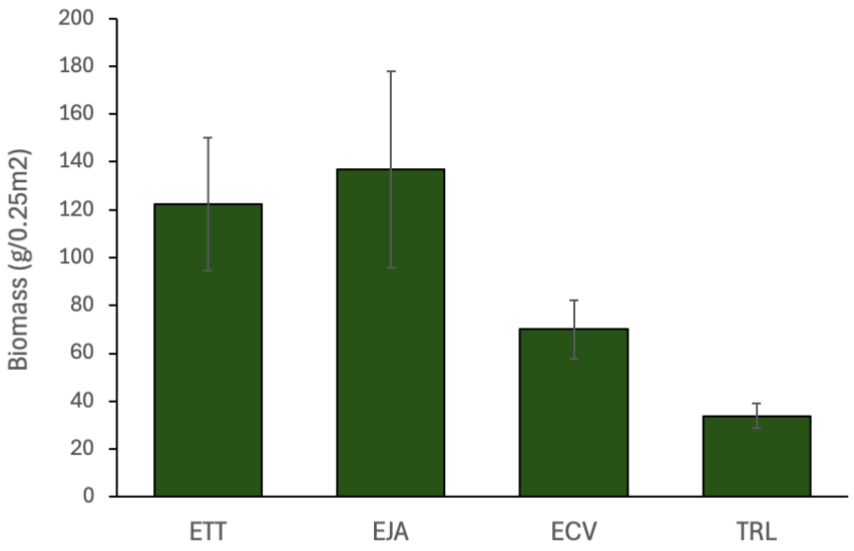

Figure 14. Dry plant biomass compared by treatment. ETT (total exclusion of medium and large herbivores), EJA (excluding wild boar), ECV (excluding deer), TRL (control, no exclusion).

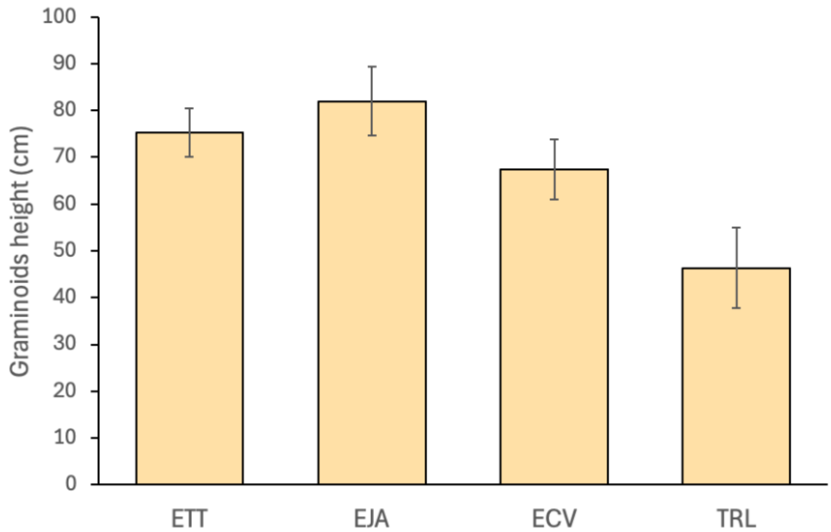

Figure 14. Maximum height of grassess compared by treatment. ETT (total exclusion of medium and large herbivores), EJA (excluding wild boar), ECV (excluding deer), TRL (control, no exclusion).

The response of the marshes to differential herbivore exclusion was very significant and visible in the field (e.g., Fig. 15, 16). Dry biomass (Fig. 14) was significantly higher in all exclusions compared to the control treatment. In particular, the EJA and ETT treatments had higher dry biomass values than the ECV deer exclusion treatment. Dry biomass values among the six marshes showed great variability among treatments, and this may be due to multiple intrinsic factors. These may include not only the variability in soil physical and chemical properties between marshes, but also the variability in conditions that may exist within each wetland at the microsite level. Extrinsic factors for these differences are determined using these environments and the intensity of herbivory by livestock and introduced ungulates present on the ranch, primarily deer.

The effectiveness of the different treatments may also be an important factor influencing the results. For example, in some wild boar exclusion treatments (EJA), where deer are permitted to enter due to the fence height (80 cm), deer presence has been infrequent (e.g., Lagunita). This limited presence may be attributed to various factors, including the specific characteristics of each wetland, such as food availability, local deer abundance, or habitat features surrounding each exclusion area (e.g., presence of shrub cover).

Figure 15. Vegetation response to herbivory exclusion.

Figure 16. Another example of the vegetation response to herbivory exclusion.

Figure 17. Biomass collected before being introduced into the stove.

Considering the total biomass of the four treatments in each wetland, Figure 18 shows that the Tortuga and Lagunita mallines exhibited the highest plant production.

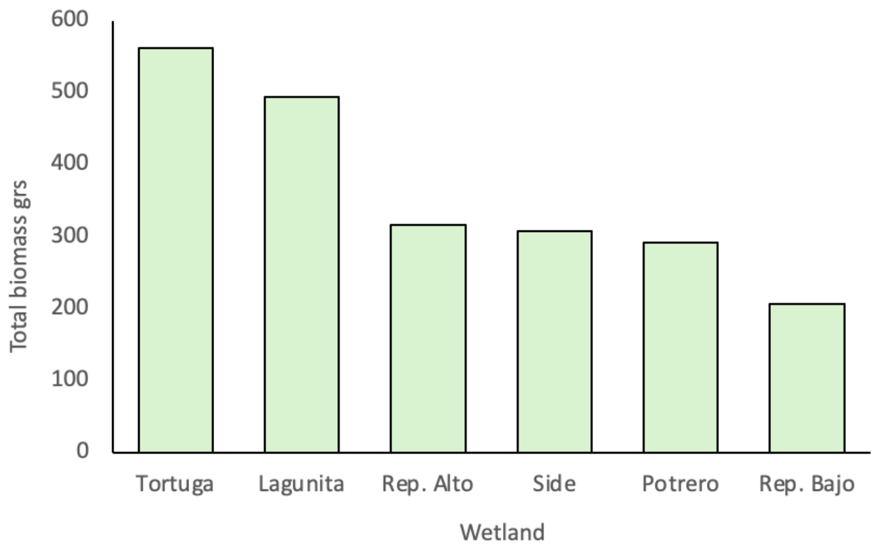

Figure 18. Total biomass (sum of treatments) in each study site.

An interesting variable that did not exhibit statistically significant differences, but does show a clear trend, is the presence of woody plants within the most restrictive herbivore exclusion zones. The development of woody vegetation is a slow process; however, as shown in Figure 19, there is a tendency for an increase over time.

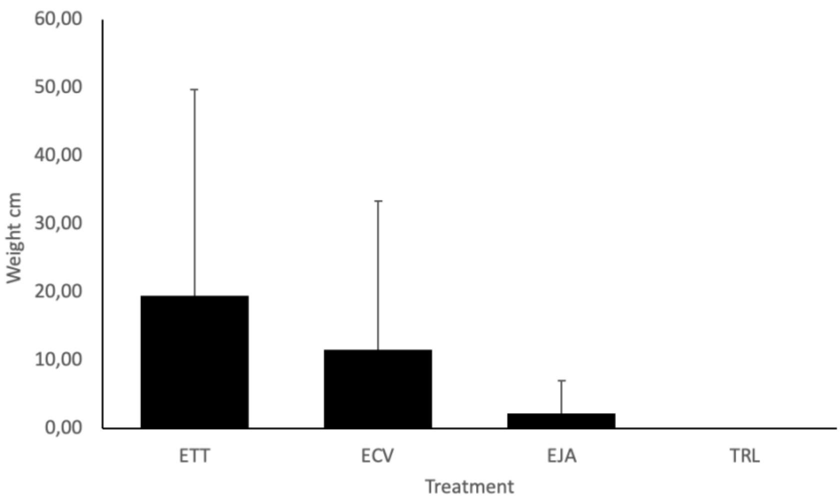

Figure 19. Average height of woody plants per treatment

To assess the effect of trampling by herbivores, soil hardness was measured within each treatment. Although soil hardness did not exhibit statistically significant differences, it was, on average, higher in the control treatments (Fig. 20). Only the wild boar exclusion treatment (EJA) appeared to show a notable difference compared to the control (TRL), with softer soil conditions.

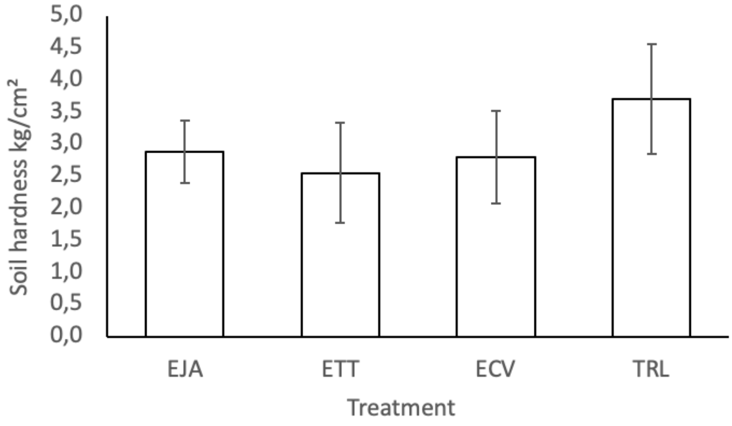

Figure 20. Average soil hardness per treatment.

Subsequently, the following variables were analyzed using GLMs for each treatment: total number of native species, total number of exotic species, and level of herbivory (Poisson distribution), % native species, % vegetation, and % bare soil (Binomial distribution). Significant differences were found in the level of herbivory, with evidence that the control treatment suffered from intense herbivory compared to the exclusion treatments (Fig. 21).

<u>Nivel de herbivoría (GLM, F: 10.34, p=0.0003)</u>

TRL	***A***
EJA	***B***
ECV	***B***
ETT	***B***

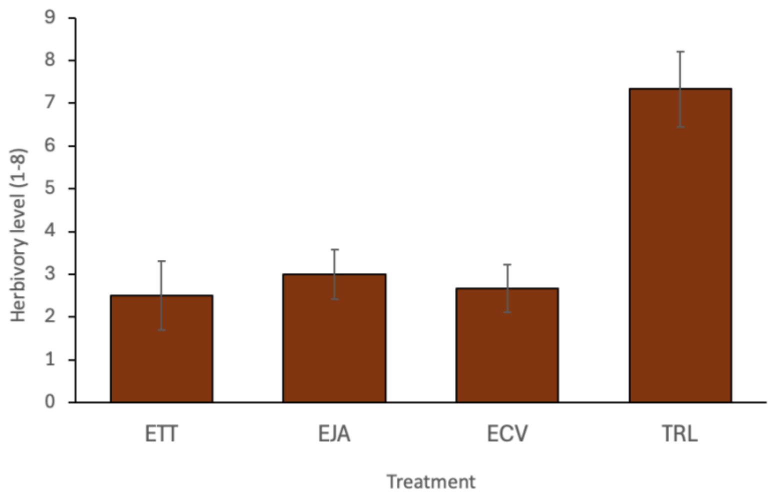

Figure 21. Level of herbivory per treatment.

Regarding soil analysis, pH and electrical conductivity analyses of the EFC samples were performed, evaluating soil samples at time zero, (before the exclusions were placed) and in 2024, after one year of animal exclusion (Figs. 22, 23). For pH, no statistically significant differences were found between the years 2023 and 2024 ($t = -1.37$, $p = 0.184$), indicating that there were no substantial changes in the evaluated treatments. For EC, there are differences between 2023 and 2024 ($t = 2.38$, $p = 0.026$); however, there are no statistically significant differences in electrical conductivity between the different treatments within each year. Thus, the differences observed in electrical conductivity between years are likely due to general variations that affected all treatments similarly.

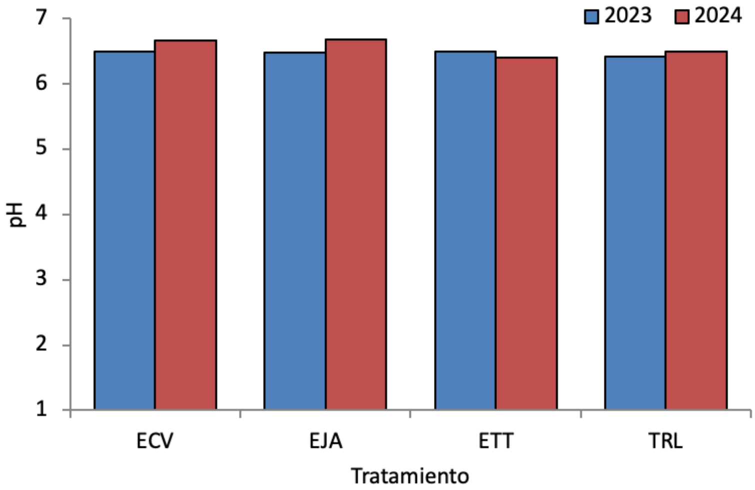

Figure 22. pH per treatment.

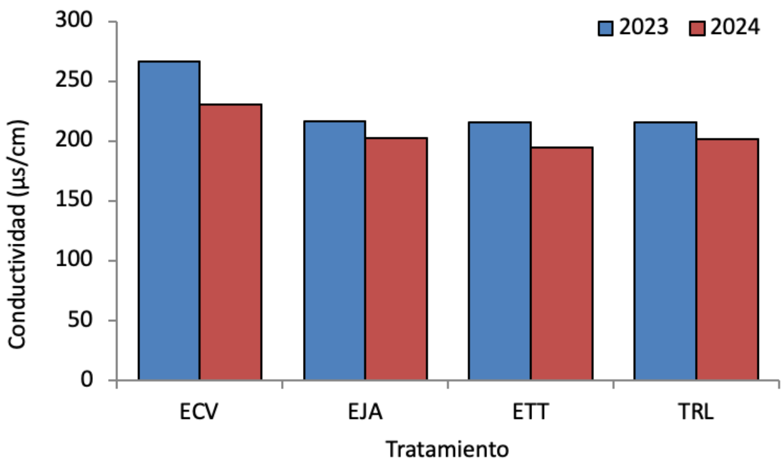

Figure 23. Electrical conductivity by treatment

During the event, the most outstanding research conducted in wetland environments at the ranch was presented and discussed.

Figure 24. Wetland Workshop at Estancia Fortín Chacabuco

Discussion and future perspectives

The results obtained thus far within the framework of this project demonstrate encouraging evidence of the potential impact that introduced herbivores—particularly invasive mammals such as red deer, fallow deer, and wild boar—may have on the wetlands of Estancia Fortín Chacabuco (EFC). Although the behavior and responses of wetlands may vary, the reaction of these highly productive ecosystems to the suppression of trampling and herbivory is clear, as evidenced not only by increased vegetation growth (e.g., grass height) but also by structural changes within these environments (e.g., the emergence of woody plants).

During the next phase of the project, we aim to further advance the objective of monitoring rooted surface areas using drones, analyzing both micro- and macroarthropod communities, and evaluating soil samples to better characterize the differences among treatments. In addition, we plan to incorporate new parameters (e.g., root biomass) and apply new methodologies (e.g., use of devices to assess soil composition and hardness at various depths). We also expect to complete a more robust dataset from camera trap surveys, allowing us to relate wildlife abundance and habitat use indices to the environmental parameters already measured.

Overall, we believe this is a highly comprehensive project with a holistic approach, and we anticipate that differences between treatments will become increasingly evident over time.

Difficulties Encountered

A significant amount of time was invested at the beginning of the project to select the study sites and construct the exclusion experiment in the wetlands of Estancia Fortín Chacabuco. This delayed the start of other project activities.

Rainfall at certain times of the year (i.e., fall and winter) complicated access to some areas of the ranch. Furthermore, the level and seasonality of this rainfall sometimes made it difficult to access some wetlands for surveying. Relatedly, the diversion of irrigation canals on the ranch has also complicated some survey tasks due to soil flooding (e.g., Potrero).

Due to recent job opportunities, two of the researchers collaborating on this project have traveled abroad. Therefore, a doctoral fellow will be sought in the next CONICET doctoral scholarship call to join the team. This has undoubtedly been one of the most significant limitations due to the amount of information available to process. In this regard, for example, processing of samples such as camera trap data and micro- and macro-arthropod samples have been delayed.

Changes in the global economy, and specifically in Argentina, including inflation and the devaluation of the Argentine peso, have led to changes and limitations in the budget. Beyond this, we have worked hard to ensure that the budget provided by Rufford and the contributions from counterparts are strategically used to ensure the project is completed and progress is made within the established timeframe. This situation is evident, for example, in the delay in soil sample analysis, where an agreement was reached with the INTA, which resulted in a reduction in the budget.

Final Comments

Although the differences between treatments do not appear to be decisive so far, it is important to highlight that many of the processes of change being evaluated are slow and require time to show significant changes, if any. For this reason, we hope that the experiment set up at EFC will show more marked changes over time. In this regard, we hope to complete at least the three years of sampling initially planned to prepare a final report and scientific publication showing the results obtained over time. We believe that this type of experiment can provide very important information in the medium and long term and can serve as a basis for generating new research questions and managing mallines as a sustainable resource with priority for conservation. Finally, the results of this project are expected to be used as success indicators for the "Wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) control plan at Estancia Fortín Chacabuco, Nahuel Huapi National Park" (DI-2020-44-APN-DRPN#APNAC). The annual measurement of soil physical-chemical parameters and plant community elements at sites of high relevance due to their biodiversity, grazing quality, and importance in water cycles, such as Patagonian wetlands, is key to monitoring and evaluating the control process of invasive exotic mammals present at Estancia Fortín Chacabuco.

This has become my main project in relation to applied fieldwork, and I sincerely believe it has great potential and applicability in multiple aspects, contributing to the conservation and sustainable development of the northwest Patagonia.

Dr. Sebastián A. Ballari

Grupo Interdisciplinario de Investigaciones Aplicadas al Estudio de la Biodiversidad (GIABI)

CENAC, Parque Nacional Nahuel Huapi-CONICET