**Progress report: Assessing ecological consequences of non-native willow management: Can we restore native riparian forests and shrublands from northwestern Patagonia? (ID 40869-1)**

In this report, we summarize the performed activities and results obtained in these past months with the support of The Rufford Foundation. We also show how we communicated preliminary findings and the planned future activities.

The management and/or eradication of invasive species are widely recommended actions to limit damages and restore native ecosystems. However, these actions could produce undesired or unexpected effects such as re-invasions, secondary invasions, and changes on ungulates herbivory, that threaten the conservation of native ecosystems. Our project focuses on determining the ecological impact of managing the invasion of non-native willows (*Salix* spp.) alongside riparian forests and shrublands in northwestern Patagonia. This study has been carried out since March 2021 in the Chacabuco stream in the Nahuel Huapi National Park Reserve, province of Neuquén, Argentina. Here, we reported the activities carried out since November 2023 when the Rufford grant was received.

**Objective A:** Evaluate the effects of manual removal of non-native willows on attributes of riparian plant communities such as abundance, composition, structure (i.e., passive restoration), use by non-native ungulates, and abiotic characteristics of sites

**Performed activities:** We conducted field samplings since austral autumn (March-June) 2023 to winter (June-September) 2024. We sampled different attributes of plant communities, ungulates use, and abiotic characteristics in ten plots (20 x 10 m along the Chacabuco stream) where non-native willows were removed manually (i.e., removal treatment) and ten plots with willow presence (i.e., control plots).

Attributes of plant communities:

- We measured the tree structure of the 20 x 10 m plots during summer (December-March) 2024. Particularly, we recorded all live or dead individuals from tree species and measured their diameter at breath height (DBH) with a diametric tape in order to calculate tree density and basal area.
- To estimate the age of each non-removed willow, we drilled their trunks with an increment borer during autumn 2024.
- We continued evaluating the passive restoration on two enclosed 2 $m^2$ subplots (i.e., micro-fences) and other two subplots under herbivory pressure (i.e., control subplots) inside each 20 x 10 m plot during summer 2024. Particularly, we estimated the understory and canopy cover of each plant species, measured the woody species height with a wood tape, and counted the number of saplings of non-native willows and woody natives.

Non-native ungulates use:

- We continued recording the number of pellets and their identification and estimating the soil rooting cover (%) by wild boar in each 20 x 10 m plots during autumn and spring 2023, and autumn 2024.
- In the same plots, we installed ten camera traps to record ungulates and wild mammals' activities during autumn-winter 2024.

- We continued estimating ungulates browsing for each woody species in the four subplots of passive regeneration during summer 2024.

Abiotic characteristics:

- We continued estimating bare soil cover (%) in the four subplots of passive regeneration during summer 2024.
- We conducted the final measurements of Photosynthetically active radiation (PAR) and compaction of soil in the 20 x 10 m plots during autumn 2024 and summer 2024, respectively.

**Results obtained:**

Attributes of plant communities:

-Tree structure: The following results represents the tree structure before the application of the willow removal treatment. The total tree density was similar between removal treatment and control. In both treatments, the relative density of willows was between 373 trees/ha - 221 trees/ha (~ 25%). The most abundant native species were *Ochetophila trinervis* (450-600 trees/ha, ~40%) and *Nothofagus antarctica* (300-150 trees/ha, ~20%), followed by *Diostea juncea* (100 trees/ha, ~10%) and *Discaria chacaye* (100 trees/ha, ~10%). We only found dead individuals of native species (75 trees/ha, less than 8%). On the other hand, the total basal area was similar between treatments. In both treatments, the relative basal area of willows was of 15-50 $m^2$/ha (~20-60%). The basal area of dead individuals only belonged to native species.

-Willows age and passive restoration: We will count the tree rings of willows in the next months. As we plan to conduct a last sampling of passive restoration during summer 2025, we will analyze the results when all the data has been collected.

Non-native ungulates use:

-Pellets count and soil rooting by wild boar: Total pellet number of non-native ungulates, mostly sheep and deer, was significantly lower in the removal treatment than in control plots until spring-summer 2023. Moreover, the total pellet number was higher in the spring-summer periods than the autumn-winter periods. The highest number of pellets corresponded to cattle, sheep, and deer. The soil rooting by wild boar was lower in removal treatment than in control plots at different time periods sampled.

-Camera trap: Until now, we detected several wild mammals and ungulates. We found four native species: Cougar (*Puma concolor*), Andean Fox (*Lycalopex culpaeus*), Humboldt's Hog-nosed Skunk (*Conepatus humboldtii*), and Armadillo (*Chaetophractus* sp.) (Figure 1). We detected five wild non-native mammals: Red Deer (*Cervus elaphus*), Fallow Deer (*Dama dama*), European Hare (*Lepus europaeus*), American Mink (*Neovison vison*), and Wild Boar (*Sus scrofa*); and three domestic non-native ungulates: Cattle (*Bos taurus*), Sheep (*Ovis aries*), and Horse (*Equus ferus*) (Figure 2).


Figure 1: Wild native mammals. From left to right and from top to bottom: Cougar (*Puma concolor*), Andean Fox (*Lycalopex culpaeus*), Humboldt's Hog-nosed Skunk (*Conepatus humboldtii*), and Armadillo (*Chaetophractus* sp.)


Figure 2: Non-native wild and domestic mammals. From left to right and from top to bottom: Red Deer (*Cervus elaphus*), Fallow Deer (*Dama dama*), European Hare (*Lepus europaeus*), American Mink (*Neovison vison*), Wild Boar (*Sus scrofa*), Cattle (*Bos taurus*), Sheep (*Ovis aries*), and Horse (*Equus ferus*).

-Ungulate browsing: Removal treatment had significant higher average ungulate browsing than control plots. The native species most browsed were the shrub *Escallonia*

*virgata*, and the small trees *Ochetophila trinervis* and *Discaria chacaye*, while the non-native species most browsed was the shrub *Rosa rubiginosa*. We also observed non-native willows browsed in the control plots and sprouts of the removed non-native willows browsed in the plots with removal treatment (Figure 3).

Figure 3: Deer and cattle browsing non-native willows and other native woody species (above), and cattle browsing sprouts of removed non-native willows (below).

<u>Abiotic characteristics:</u>

-PAR: Before willow removal, the PAR was similar between plots. In spring 2021, after willow removal, the PAR increased twice in plots with removal treatment than control plots. This pattern was repeated during autumn 2022 and after two years during the same season (autumn 2024).

-Soil compaction: At different soil depths, soil compaction was similar between plots before and three years after the willow removal treatment. The compaction was the lowest at 5 cm depth, while compaction was similar between 10 cm and 15 cm depths.

**Objective B:** Evaluate the effects of willow removal on the regeneration of planted native and non-native tree species (i.e., active restoration) and preference of ungulates on these species.

**Performed activities:** We conducted the final samplings during austral autumn 2024. We measured characteristics regarding establishment and growth of saplings of native species (*Ochetophila trinervis* and *Salix humboldtiana*) and non-native willows planted within Objective A plots (i.e., removal treatment and control) (Figure 4). We also determined the ungulates preferences considering these species. At the end of the experiment, we removed non-native willow saplings from the sites in order to avoid their spreading.

Particularly, we measured for each sapling the stem height with metric tape, diameter at root collar with caliper, and count the number of total and browsed branches, and sprouts. In addition, we counted the number of live saplings to calculate the % of survival and mortality, and registered the possible cause of death.

Figure 4: Planted saplings of *O. trinervis* (left), *S. humboldtiana* (center) and non-native willow (right).

**Results obtained:** After two years of the initial plantation (spring 2021)**,** *Ochetophila trinervis* survival was lower in the willow removal treatment than in control plots (30% vs 50% of live saplings). Relative changes in height did not vary between treatments, but did change over two years. After the first growing season (autumn 2022), *O. trinervis* had a reduction in height with a tendency to be greater in the removal treatment (~40-30%). After the second season (autumn 2023), *O. trinervis* grew up to their initial height. However, after the third season (autumn 2024), there was no significant height growth (~5-10%). After three growing seasons, *O. trinervis* had a notable diameter at root collar growth that was similar between the treatments (~40-60%). Ungulate browsing was

moderate to intense (between 25% to 75% browsed branches) and similar between treatments and growing seasons (Figure 5).

After one year since the initial plantation (summer 2023), *S. humboldtiana* had lower survival than non-native willow in both treatments (80% vs 95% of live saplings). After the first growing season (autumn 2023), the survival of both willow species dropped to 6% in both treatments. After the second growing season (autumn 2024), only non-native willows survived (less than 5%). In summer 2023 (mid-experiment measurements), *S. humboldtiana* and non-native willow saplings had a height reduction apparently greater in the removal treatment. Additionally, non-native willow had a less height reduction than *S. humboldtiana* (5% vs 30%). In summer 2023, *S. humboldtiana* browsing was greater in the removal treatment than in control plots (20% vs 10% browsed branches), while non-native willow browsing was greater in the control plots than removal treatment (45% vs 35%). In addition, the non-native willow was more browsed than *S. humboldtiana* (Figure 5).

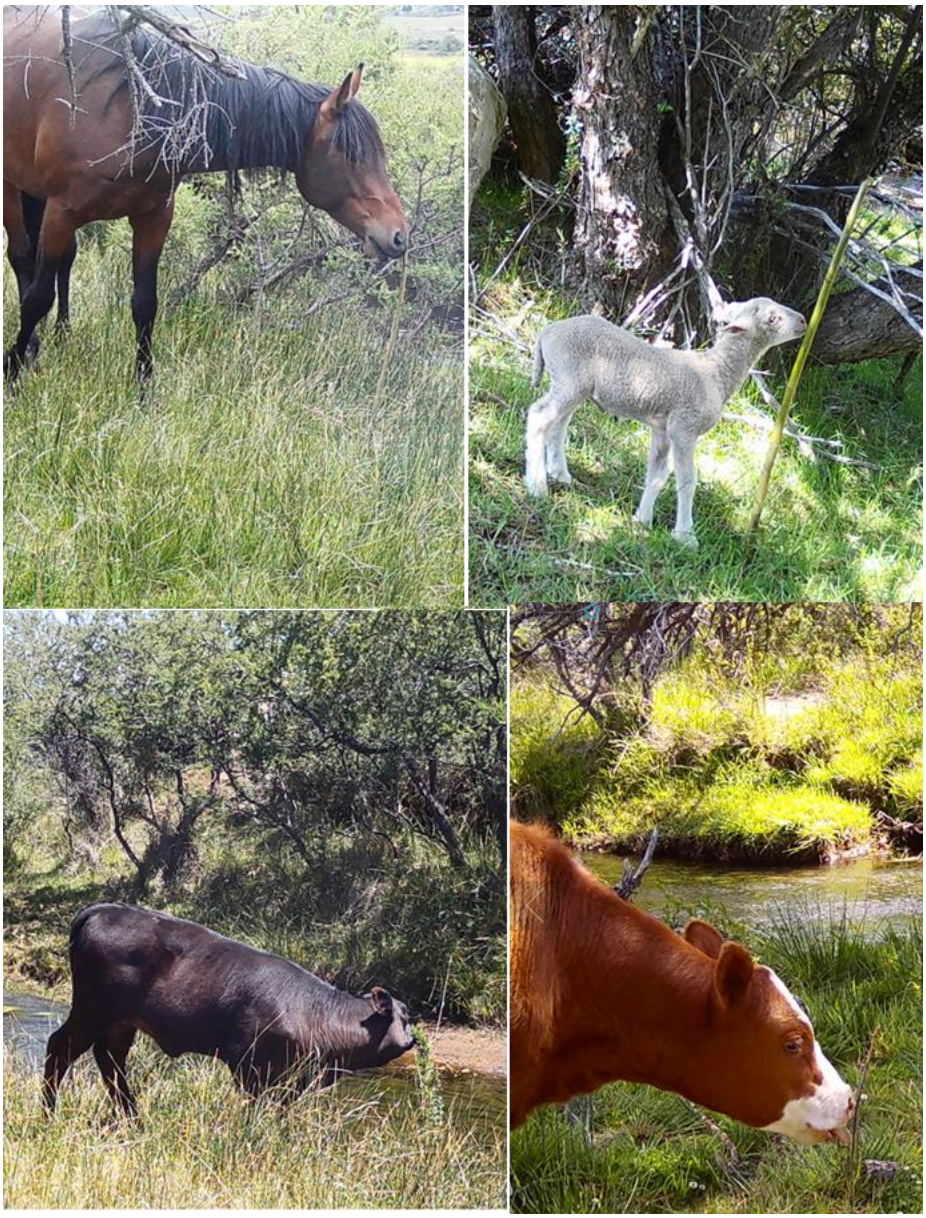

Figure 5: Domestic ungulates (horse, sheep, and cattle) browsing saplings of the different species planted.

## Partial conclusions

- Removal reduces use of these sites by ungulates, mainly wild boar. This is positive considering that non-native ungulates could threaten the passive regeneration of plant communities after willow removal. However, browsing of woody species was greater in sites with willow removal. Therefore, to maximize the regeneration of these species, they must be protected by enclosures.
- Willow removal could affect negatively the performance of native species saplings. *Ochetophila trinervis* had a decrease in survival and *Salix humboldtiana* in height growth. Therefore, planting with these native species should be done trying to maximize the species survival and protecting them from ungulates to enhance their growth.
- Willow saplings were more affected by other external and/or intrinsic conditions than by willow removal, such as summer droughts. Therefore, future trials are needed to improve *S. humboldtiana* survival and the invasion of non-native willow could be stopped in certain regions with drier conditions.

## Communication of findings

We have shown our current findings at the XXX Argentine Meeting of Ecology ("Reunión Argentina de Ecología", RAE, San Carlos de Bariloche, Argentina), III National Meeting of Ecological Restoration of Argentina ("Encuentro Nacional de Restauración Ecológica de la Argentina", ENREA, Neuquén City, Argentina), and IX Ecovalle Forum ("Foro Ecovalle", Neuquén City, Argentina) (Figure 6). At these meetings, we discussed our findings and their implication on willow management with other researchers and the community. Also, we established contacts with researchers who work regionally on willow management and we plan to invite them to the future workshop.


Figure 6: Talks and posters presented at the different meetings.

## Future activities and communication of findings

Most of the proposed activities have been completed. Regarding Objective A, during the next field season (austral spring 2024-summer 2025) we will finish the samplings of attributes of plant communities (passive restoration) and ungulates use (pellet counts, ungulate browsing, and camera traps). Regarding Objective B, the field measurements were finished, and currently we are writing a manuscript that will be submitted to an indexed international journal.

We are constantly in contact with Gwen Hulsegge, Nicolás Rodriguez, and Justo Jones (Fortín Chacabuco and Nahuel Huapi ranches administrators) to organize the field samplings and to share them our findings with technical and evaluation reports.

Currently, we are processing the data of ungulate use (Objective A) to show them at the XXXV Argentine Mammalogy Meeting ("Jornadas Argentinas de Mastozoología", JAM, San Luis City, Argentina) in November 2024. During spring 2024, we will participate in the event "Fortín Chacabuco Tranqueras Abiertas" where Fortín Chacabuco ranch invites schools and the community to participate in guided tours with researchers working within the ranch. Near our study sites, there are interpretation trails which we are planning to use for a guided tour. During autumn 2025, we plan to organize a local-regional workshop to invite the community key stakeholders, share our results, and discuss possible management guidelines for willow invasion and restoration of riparian habitats.