Journal for Nature Conservation 86 (2025) 126931

Contents lists available at ScienceDirect

Journal for Nature Conservation

journal homepage: www.elsevier.com/locate/jnc

Chemical control of the invasive exotic *Acacia melanoxylon* R. Br. and plant succession in the Pampa Biome (Argentina)

Sofía Rojas [a], María L. Echeverría [b,c,*], Tomás O'Connor [d], Viviana M. Comparatore [a,e]

[a] Universidad Nacional de Mar del Plata, Facultad de Ciencias Exactas y Naturales, Funes 3250, CC 7600, Mar del Plata, Buenos Aires, Argentina
[b] Universidad Nacional de Mar del Plata, Facultad de Ciencias Agrarias, Ruta 226, km 73,5, CC 273, 7620, Balcarce, Buenos Aires, Argentina
[c] Instituto Nacional de Tecnología Agropecuaria, Centro Regional Buenos Aires Sur, Estación Experimental Agropecuaria Balcarce, Argentina
[d] Instituto de Investigaciones Marinas y Costeras (IIMyC), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata-CONICET, Mar del Plata, Argentina
[e] Instituto de Investigaciones Marinas y Costeras (IIMyC), CONICET, Argentina

A R T I C L E I N F O

Keywords:
Biological invasions
Exotic plants
Grasslands
Racosperma melanoxylon
Native plants
Tandilia

A B S T R A C T

Acacia melanoxylon is considered an invasive tree species in Argentina, particularly within the Tandilia Hill System, where it has rapidly expanded, affecting native grassland ecosystems. The aim of this study was to evaluate the chemical control of this invasive species within a Private Natural Reserve located in the Tandilia Hill System and to assess the natural succession of vegetation after tree removal. To achieve this, an experiment was conducted using a Randomized Complete Block Design with four replications and four treatments. In three treatments, different herbicides were applied, while one treatment served as a control. From November 2016 to October 2018, vegetation species were recorded. The variables analyzed included green foliar coverage of the acacias, green foliar coverage of the Poaceae assemblage, and vascular species richness. Significant differences in green foliar coverage of acacias were detected between treatments on all sampling dates. An increase in the average green foliar coverage of the Poaceae assemblage was observed in the Picloram + Triclopyr treatment. No significant differences in species richness were detected between treatments at the final sampling date. This study demonstrated the effectiveness of the chemical treatments in controlling young *A. melanoxylon* trees. Furthermore, after a considerable time had passed since the application of the treatments, the Poaceae group and the percentage of native species were not negatively affected. This suggests that chemical control can not only be useful for managing this invasive tree species but can also contribute to the recovery of native ecosystems in the Tandilia Hill System.

1. Introduction

Biological invasions, along with other drivers of ecosystem degradation (e.g., habitat exploitation, environmental pollution, climate change, loss of pollinators and other species), constitute the central component of global change and a major threat to biodiversity, human health, and food security (Pysek & Richardson, 2010; Jeschke et al., 2014; Zalba & Amadeo, 2017; Van Kleunen, 2018; Pyšek, 2020; Liu, 2021). These invasions cause changes in terrestrial biota, modifying the roles of native species, altering evolutionary processes, and leading to changes in the abundance and richness of native species, including their extinctions (Vitousek, D́Antonio, Loope, & Westbrooks, 1996; Mack et al., 2000; Beaury, 2023; Carneiro, 2024).

The Pampa Biome (Fig. 1A-B), historically characterized by natural grassland communities (Cabrera, 1968; Cabrera & Zardini, 1978; Baldi 2006; Scottá & Da Fonseca, 2015), has experienced fragmentation and replacement over the last century due to the expansion of agriculture, livestock farming, and forestry, among other anthropogenic activities (Baldi 2006; Isacch et al., 2017; Yezzi et al., 2020). In this sense, the original grasslands have suffered the introduction of exotic species, such as the invasive *Gleditsia triacanthos, Pinus halepensis, Rubus ulmifolius* and *Spartium junceum*, among others, which has led to the disappearance of original plant communities (Zalba & Villamil, 2002; Zalba et al., 2008; Mazzolari et al., 2011; Sanhueza & Zalba, 2012; Fernandez et al., 2017). In particular, in Buenos Aires Province (Argentina), the Ventania and Tandilia hill systems (Fig. 1B) act as biodiversity refuges (Echeverría et al., 2017), providing shelter for native flora and fauna species (Echeverría et al., 2017; Isacch et al., 2017; Vera et al., 2020; Aranguren

* Corresponding author at: Ruta 226 Km 73.5, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata, Argentina.
E-mail address: mlecheverria@mdp.edu.ar (M.L. Echeverría).

https://doi.org/10.1016/j.jnc.2025.126931
Received 8 October 2024; Received in revised form 31 March 2025; Accepted 31 March 2025
Available online 3 April 2025
1617-1381/

et al., 2023). However, these hill areas are threatened by the presence of invasive woody plants that replace native grasslands and homogenize the landscape (Zalba &Villamil, 2002; De Rito et al., 2020; Echeverría et al., 2023).

Acacia melanoxylon R. Br. (*Racosperma melanoxylon*), known as "Blackwood," is a perennial tree species belonging to the Fabaceae family, native to Australia and Tasmania (Dodet & Collet 2012; Richardson, 2015; Hussain et al., 2020). Invasions caused by this species have been reported in Spain, Portugal, New Zealand, Australia, South Africa, North America, and Argentina (Ghersa et al., 2002; Richardson & Rejmanek, 2011; De Rito et al., 2020). This evergreen species reduces light availability for understory plants and releases allelopathic substances from its flowers and phyllodes, which alter the soil environment. Consequently, this significantly reduces the germination and growth of native seedlings (Arán et al., 2013; 2017; Kiptoo & Kiyapi, 2022). Additionally, like other species in the *Acacia* Mill. genus, its seeds can remain viable in the soil seed bank for many years, and their germination is stimulated by occasional fires that scarify the seed coat and significantly increase the germination rate (Arán et al., 2013). Another feature that enhances its invasiveness is its ability to reproduce asexually through gemmiferous roots (ĎAlfonso, 2026) and the presence of shoots that facilitate the establishment of clonal populations (Souza-Alonso et al., 2017). Additionally, this species can impact the entire ecosystem due to its high water consumption, which can disrupt the hydrological balance (Jiménez et al., 2010). In Argentina, the distribution of *A. melanoxylon* was promoted by humans in forestry plantings (Martínez Crovetto, 1947; Carranza, 2007). Currently, this species is found in the wild in grasslands of the Tandilia system, having successfully invaded various parts of the hills (De Rito et al., 2020; Echeverría et al., 2023; Gandini et al., 2019; Wraage, 2023; Zaninovich et al., 2023; ĎAlfonso, 2026).

Research conducted in various parts of the world have demonstrated effectiveness of certain chemical products in combating and eradicating invasive tree species of the genus *Acacia* (Campos et al., 2002; Richardson et al., 2006; Rodrigues Santos & Monteiro, 2007; Souza-Alonso, 2013). Rodrigues Santos & Monteiro, 2007 indicated that the application of Glyphosate on the stumps of specimens of *Acacia melanoxylon* and *A. dealbata* showed effectiveness in the control of these species, followed by Triclopyr and Metsulfuron-methyl, also applied on stumps. On the other hand, Campos, et al. (2002) determined that using Triclopyr on the stumps immediately after cutting is extremely effective for the control of *A. melanoxylon*. The effectiveness of Triclopyr was also proven in the invasive species *A. dealbata* by Souza-Alonso (2013). On the other hand, the combination of Glyphosate and Metsulfuron-methyl turned out to be effective in controlling *Acacia mearnsi* and *A. melanoxylon*, reducing and eradicating the cover of these species

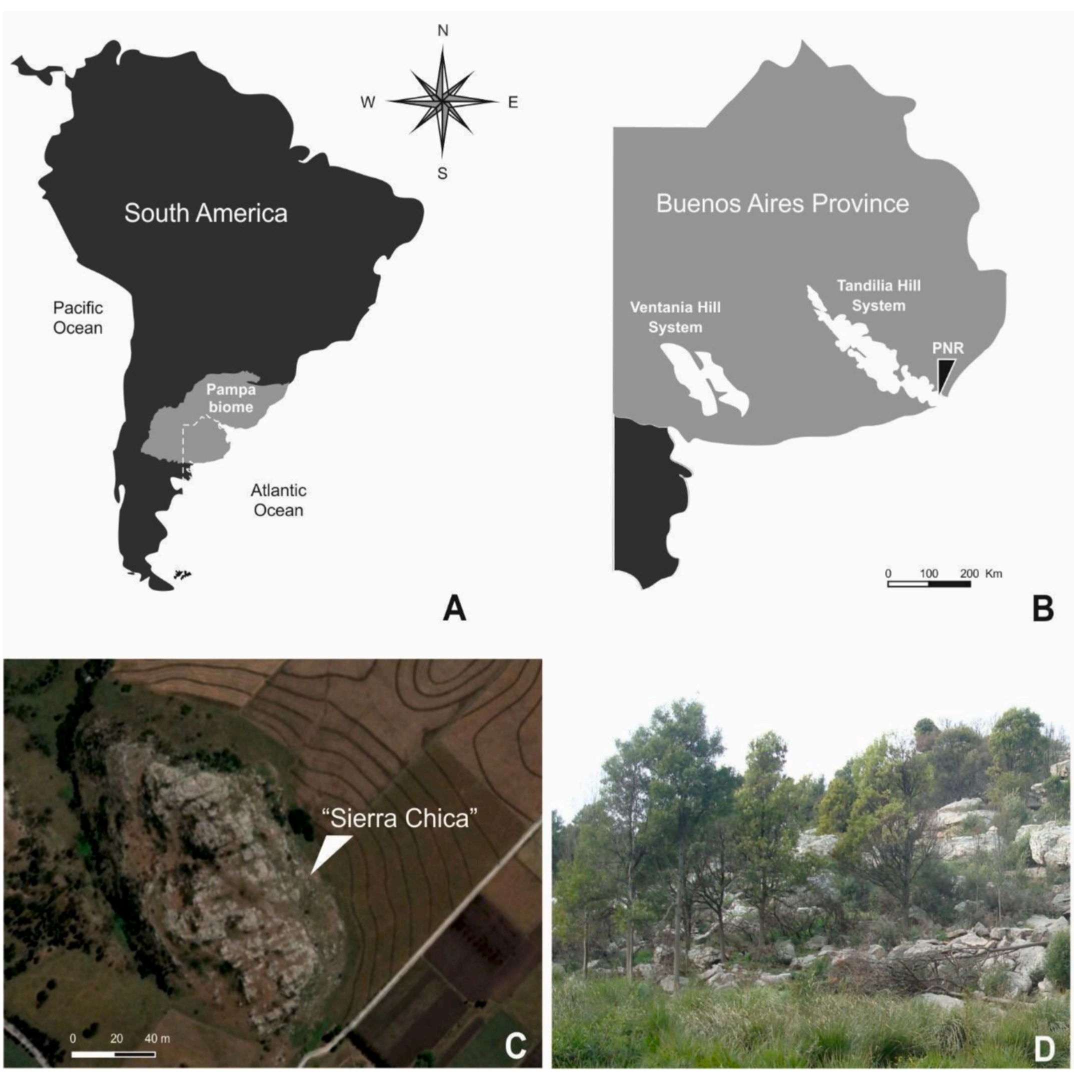

Fig. 1. A: Map of South America (dark gray), showing the delimitation of the Pampa biome (light gray), modified from Scottá and Da Fonseca (2015), and Buenos Aires Province (dotted line); B: Buenos Aires Province with the delimitation of the Ventania and Tandilia Hill Systems and Paititi Natural Reserve (PNR); C: Aerial photo of the "Sierra Chica"; D: Photo of *Acacia melanoxylon* trees invading a hill grassland of the PNR.

(Richardson et al., 2006).

Considering that the invasion by *A. melanoxylon* threatens the biodiversity of the grasslands in the Pampa Biome and the effectiveness that certain chemical products have shown in controlling other woody species of the Fabaceae family, the objectives of the present work were: 1- to analyze the effectiveness of different herbicides for the control of *A. melanoxylon* in a hill ecosystem of the Tandilia System; 2- to examine the different stages of vegetative succession following the application of these treatments.

2. Materials and methods

2.1. Study Area

The study was conducted in a hill grassland ecosystem of the Paititi Natural Reserve (PNR), named "Sierra Chica", which has an area of 26 ha (Fig. 1 A-C). The PNR is located in the southeastern Tandilia Hill System, on the southeast flank of "Sierra De Difuntos" (37° 54′ S – 57° 49′ W; geodetic datum WGS84), belonging to the orographic group of "Sierras de Mar del Plata" (Guazzelli, 1999). Sierra Chica is oriented N-S, with a maximum elevation of 156 m and a minimum of 86 m above sea level. The hill blocks are mainly composed of a crystalline basement on which Eopaleozoic sediments were deposited, allowing the development of Molisol soils (Osterrieth & Cabria, 1995; Dalla Salda et al., 2006).

The climate of the region is humid-subhumid, mesothermal, with low water deficiency, noticeable seasonal variation in temperature, and a short cold period. It is characterized by maritime temperate conditions ranging between 32 °C and below 0 °C, with a mean annual temperature of 14 °C. Rainfall is usually distributed throughout the year with an average of 850 mm per year (Falasca, 2000; INTA, 2020).

The flora of the study area is represented by herbs, mainly from the Poaceae family –*Paspalum quadrifarium* and species from the genera *Poa, Chascolytrum, Melica, Piptochaetum* and *Nassella*. Thus, the characteristic vegetation forms grasslands. However, shrubs are also found forming shrubland communities with *Baccharis dracunculifolia* ssp. *tandilensis, B. articulata*, *B. coridifolia, Coletia paradoxa* and *Dodonaea viscosa* (Echeverría et al. 2017; 2023; 2024). Additionally, some sectors of the northern slope are invaded by *Acacia melanoxylon* (Fig. 1 C-D) (Echeverría et al., 2023; Zaninovich et al., 2023).

2.2. Experimental design and data collection

In November 2016, an experiment was set up following a Randomized Complete Block Design (RCBD) with four replications and four treatments. The blocking criterion used was the altitudinal gradient of the north slope, and the blocks were named Base, Slope, Pampa, and Top (Fig. 2A).

In each block, four plots of 9 m^2 were delineated (Fig. 2B), to which four treatments were assigned: in three of them, different herbicides were applied to *A. melanoxylon* young plants, while one remained as a control treatment. In each plot, the coverage of *A. melanoxylon* was similar (approximately 30 %), and the specimens of this species had a total height less than 1.5 m and a diameter at breast height (DBH) less than 6 cm. The chemical treatments consisted on the application of the following herbicides to the *A. melanoxylon* plants:

- 0.007 % Metsulfuron (60 % a.i. Metsulfuron-methyl, *Metsulfuron 60 WG-InsuAgro*; 1.05 g *Metsulfuron 60 WG-InsuAgro* in 15 l of water), treatment named "Metsulfuron";
- a combination of 0.005 % Metsulfuron with 2 % Glyphosate (60 % a.i. Metsulfuron-methyl, *Metsulfuron 60 WG-InsuAgro*, and 48 % a.i. Glyphosate, *Glifoglex-Gleba*; 0.75 g of *Metsulfuron 60 WG-InsuAgro* and 0.3 kg of *Glifoglex-Gleba* in 15 l of water), named "Metsulfuron + Glyphosate";
- 6 % Picloram and Triclopyr (3 % a.i. Picloram and 6 % a.i. Triclopyr, *Togar ® BT- Dow Agro Science*; 0.9 l of *Togar® BT Dow Agro Science* in 15 l of diesel oil), named "Picloram + Triclopyr".

No adjuvants were added to the application solutions in any of the treatments. The herbicides were applied using 15 l capacity backpack sprayers that had a lance and at the end a hollow cone nozzle. The applications were made until the plants reached the "point of runoff" to maximize contact of herbicides with plants foliage.

From the application of the products, in November 2016, until mid-October 2018, records were taken on nine dates (November and December 2016, January, February, May, July, September 2017, May and October 2018), corresponding to 0, 31, 61, 95, 154, 224, 288, 542, and 699 days after treatment application (DAA). The variables recorded in each plot were green foliar coverage of the acacias (GCA) and green foliar coverage of Poaceae assemble (GCP), which were determined by visual assessment. Additionally, the richness of vascular species was recorded. The species surveyed were determined based on the following flora book collections: Flora of the Province of Buenos Aires (Cabrera, 1963, 1965a, 1965b, 1967, 1968, and 1970) and Flora Argentina (Zuloaga et al., 2012a; b; 2014a; b). Scientific names were updated based on the Flora Argentina database (https://www.floraargentina.edu.ar/). For each species, the botanical family, life cycle (annual or perennial), and origin (exotic or native) were determined.

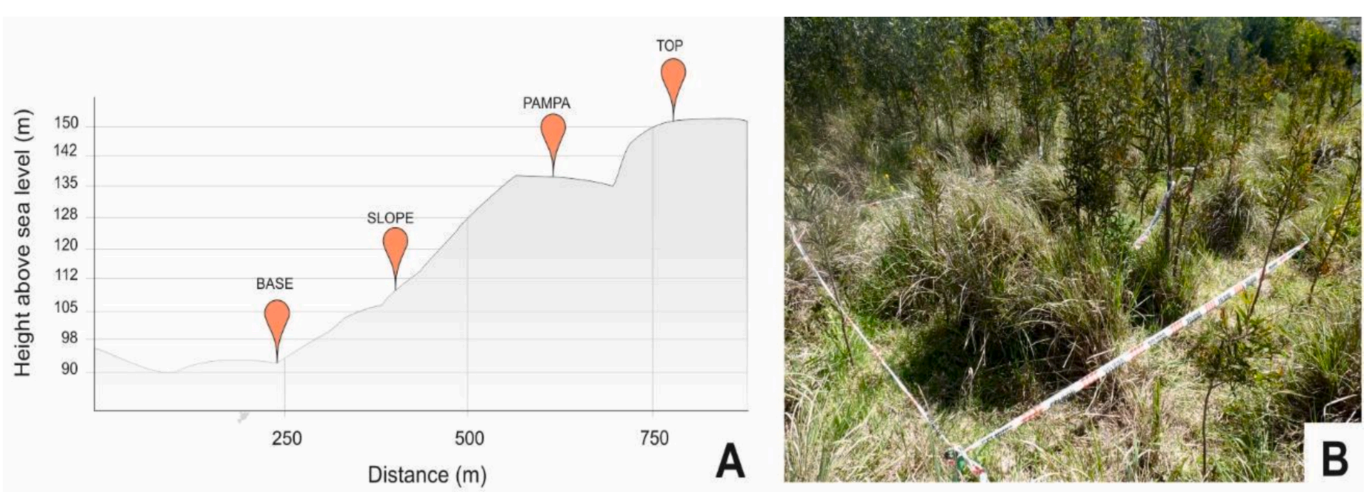

Fig. 2. A: Orographic profile reflecting the altitudinal gradient of the study area and the location of the four blocks ("Base", "Slope", "Pampa", and "Top"); B: Photo of a replicate (plot) before applying the treatments.

2.3. Data Analysis

With the data from each variable (GCA, GCP and vascular species richness), the equality of variance was corroborated using Levene's test ($p > 0.05$) and a residuals vs. fitted plot. The normal distribution of data was also corroborated using Shapiro-Wilk normality test ($p > 0.05$) and a normal Q-Q plot. After corroborating the homogeneity of variances and the normal distribution of the data, univariate analyses were performed using a linear model with repeated measures over time for the variables GCA, GCP, and vascular species richness. Analyses of Variance (ANOVA) were performed, and when the interaction between treatment and observation date (or days after treatment application) was significant ($\alpha = 0.05$), further analysis was conducted with an ANOVA by date. Treatment means were compared using the Tukey test for a significance level of $\alpha = 0.05$ at each date.

To determine the relationship between the plots under study based on the species surveyed at the initial (0 DAA) and final (699 DAA) sampling dates, multivariate analyses were performed. For this, Principal Coordinates Analyses (PCoA) were conducted on the presence/absence records of each species in the plots using the Jaccard similarity coefficient to develop the distance matrices (Cuadras, 2014).

All analyses were performed using the InfoStat software (Di Rienzo et al. 2016).

3. Results

3.1. Green foliar coverage of the acacias (GCA)

The effect of the herbicides on the acacias became evident at 31 DAA and continued to be evident during the subsequent sampling dates (Figs. 3 and 4). The treatment × date interaction for the GCA variable was significant ($p < 0.001$). Upon detecting interaction, the behavior of the treatments was analyzed at each sampling date. Except for the first sampling date (0 DAA), significant differences in GCA between the treatments were detected on all dates.

From 31 DAA, the highest GCA values were recorded in the control treatment, reaching an average GCA of approximately 89 % at the end of the sampling period (699 DAA) (Figs. 3, 4, and 5). It is important to note that in the final sampling date, the GCA percentage varied from 80 to 100 %, with the soil surface of the control plot in the slope block covered only by a layer of dry phyllodes of *A. melanoxylon*. Additionally, from 31 DAA until the end of the sampling period, the GCA value in the Metsulfuron + Glyphosate treatment plots was 0 %, and this treatment differed significantly from the control (Figs. 4 and 5). The Picloram + Triclopyr treatment exhibited a similar behavior to Metsulfuron + Glyphosate, except on the 95 DAA sampling date, where a mean GCA of 3.4 % was recorded, and thus it did not differ significantly from the other treatments (Fig. 4). Regarding the Metsulfuron treatment, it is important to clarify that the application of this treatment was inadequate. This situation was evident in the successive post-application dates as some branches in the Base and Slope blocks showed no signs of herbicide

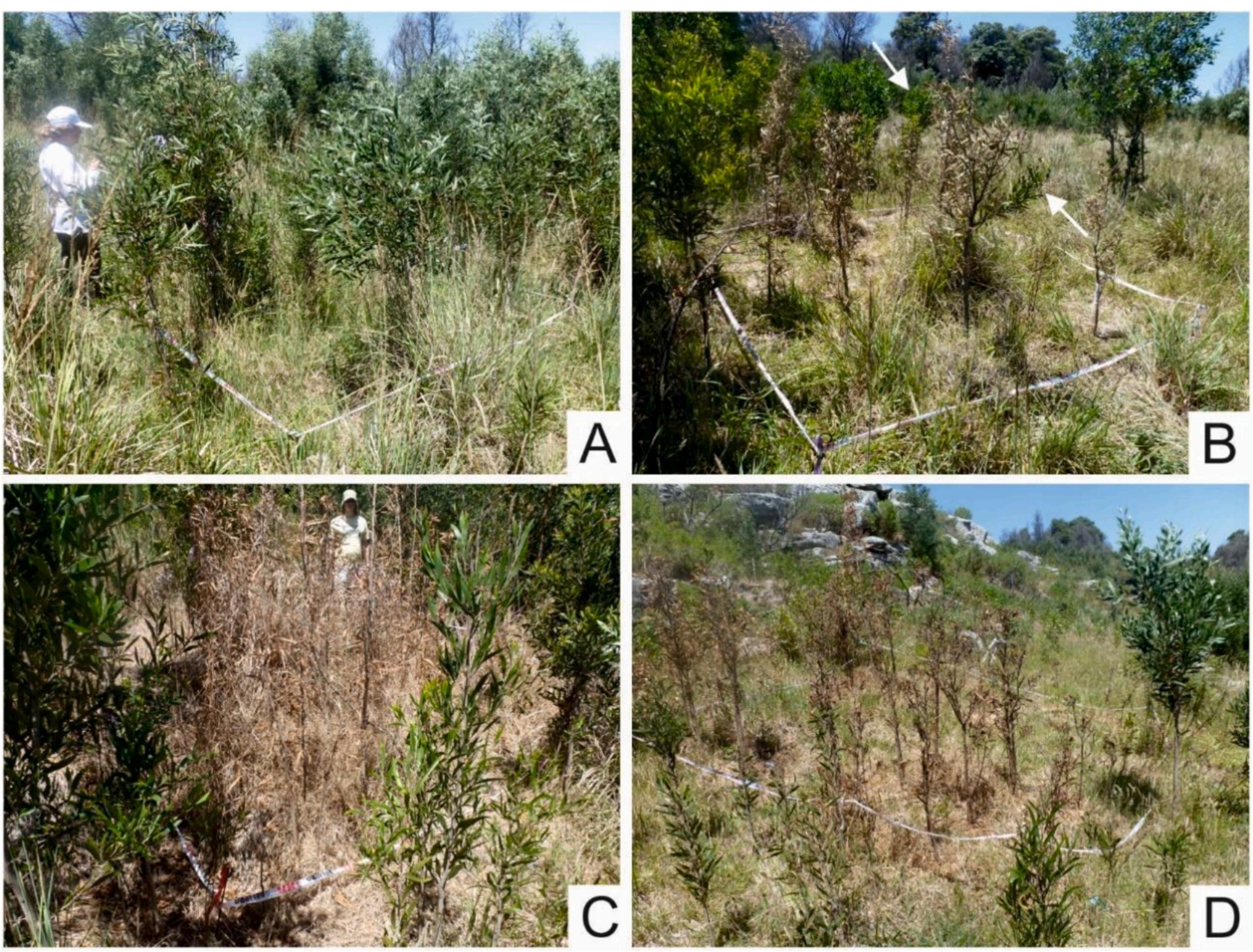

Fig. 3. Appearance of experimental plots on December 20th, 2016 (31 DAA). A: Control; B: Metsulfuron (white arrows indicate unintended herbicide application errors); C: Metsulfuron + Glyphosate; D: Picloram + Triclopyr.

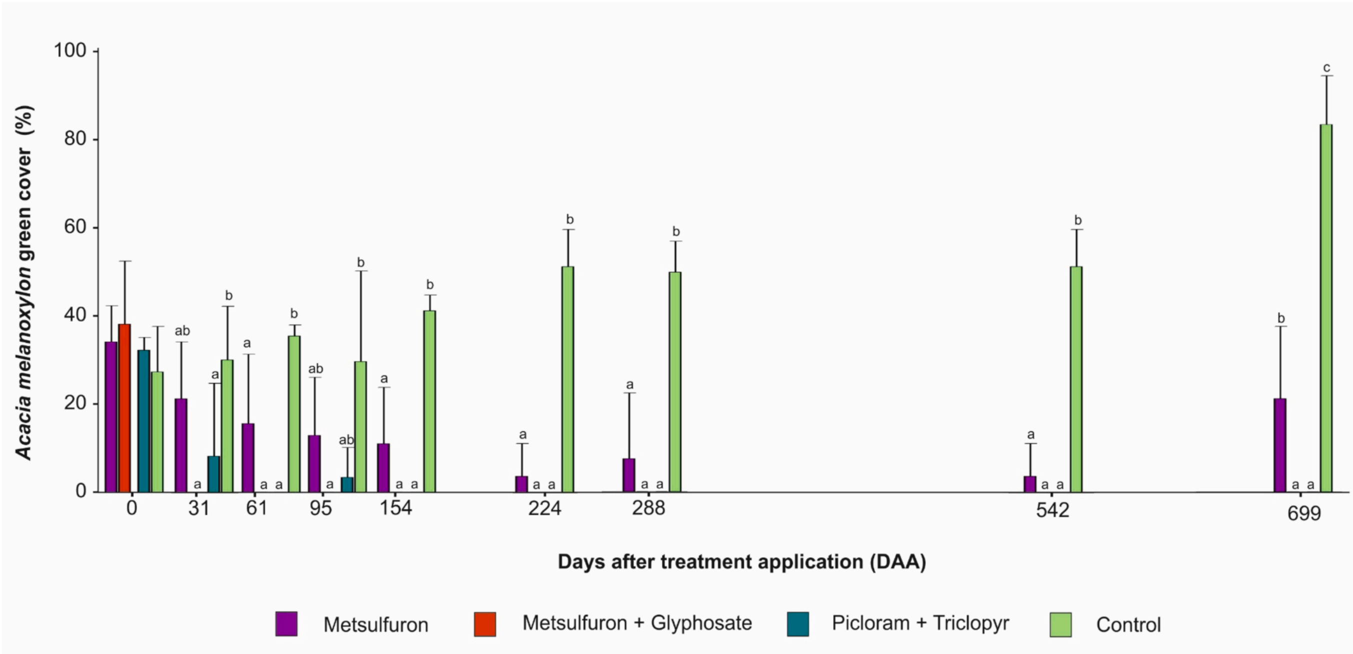

Fig. 4. Mean percentage of green foliar coverage of *Acacia melanoxylon* per treatment on nine sampling dates expressed in days after treatment application (DAA). The vertical bars indicate standard deviation. Equal letters within the same date indicate non-significant differences, p-value > 0.05.

damage (Fig. 3B). Thus, this treatment showed intermediate behavior between the control and the other chemical treatments; from 154 DAA it differed significantly from the control and, on the last sampling date, with an average GCA value of 26.3 %, it differed significantly from the other chemical treatments (Fig. 4).

3.2. Green foliar coverage of Poaceae (GCP)

The treatment × date interaction for the GCP variable was significant ($p < 0.001$). When performing an ANOVA by date, significant differences were detected on all dates except for date 1 (Fig. 6).

After the application of herbicides, a periodic reduction in the average GCP over time was observed in the control treatment, reaching the lowest value recorded in the study on the last sampling date (699 DAA). Comparing the average GCP of this treatment at the beginning and end of the trial, it was reduced by approximately 90 % (from 62.5 % at 0 DAA to 6.3 % at 699 DAA). From 31 DAA to 95 DAA, the Metsulfuron + Glyphosate treatment differed significantly from the other treatments, exhibiting the lowest average GCP values, ranging from 6.3 % GCP at 31 DAA to 14.3 % at 95 DAA. Additionally, in this treatment, the recorded GCP was less than half of what was recorded in the other treatments and days after considered application. From 224 DAA until the end of the trial, the GCP of the Metsulfuron + Glyphosate treatment did not differ significantly from the control or the Metsulfuron treatment. After 61 DAA, a progressive increase in the average GCP over time was recorded in the Picloram + Triclopyr treatment, reaching the highest value of the study on the last sampling date (81.3 %). Thus, on the last two sampling dates, it differed significantly from the control treatment. For the Metsulfuron treatment, intermediate average GCP values were recorded throughout the study compared to the other treatments. This situation was clearer from 224 DAA onwards, where it did not differ significantly from the other treatments (Fig. 6).

3.3. Identified vascular flora and its evolution over time after the application of treatments

3.3.1. Overview of vascular flora

During the surveys conducted, a total of 50 vascular plant species were recorded in the experimental plots (Table 1). The identified species belong to 24 botanical families, with the most represented being Asteraceae (26 %) and Poaceae (18 %), followed by Fabaceae (8 %) and Solanaceae (6 %), and to a lesser extent by Amaryllidaceae (4 %). The species *Blechnum auriculatum* Cav. and *Pteridium esculentum* (G. Fost.) Cockayne var. *arachnoideum* were the only ones belonging to the fern group.

The overall percentage of native species considerably exceeded that of exotic species (72 % natives vs. 28 % exotics). The higher representation of native species was maintained throughout all the sampling dates (Fig. 7A). Comparing the average number of native species at the beginning and end of the trial, the average remained similar (six species vs. five species), while the average number of exotic species slightly decreased (three species vs. one species), although on both dates there were notable deviations from the mean (Fig. 7A), revealing variability among the plots. Additionally, the overall percentage of perennial species was significantly higher than that of annual species (78 % perennials vs. 22 % annuals). The higher representation of perennials was maintained throughout all the sampling dates, although it decreased in the later dates as 7.8 species were recorded at 0 DAA and 4.5 species at 699 DAA; on both dates, there were notable deviations from the mean, revealing variability among the plots. Moreover, comparing the average number of annual species on the first and last sampling dates, there were no notable changes, with 1.5 species and 1.9 species, respectively (Fig. 7B). It is worth noting that due to the high GCA recorded in the control treatment plots on the last sampling dates, much lower native species richness values were recorded than at the beginning of the trial, even recording only *A. melanoxylon* in one of the plots, as previously mentioned.

The PCoA performed to analyze the relationship between the study plots based on the species surveyed at 0 DAA is presented in Fig. 8A. The first two coordinates explained 25.3 % of the total variance. In this figure, the plots of the same block are very close to each other, reflecting the similarity between them, particularly the low similarity between the plots of the Base (right) and Pampa (left) blocks. In contrast, the plots corresponding to the Top and Slope blocks, which had intermediate similarity, generally resembled those of the Pampa and Base blocks, respectively.

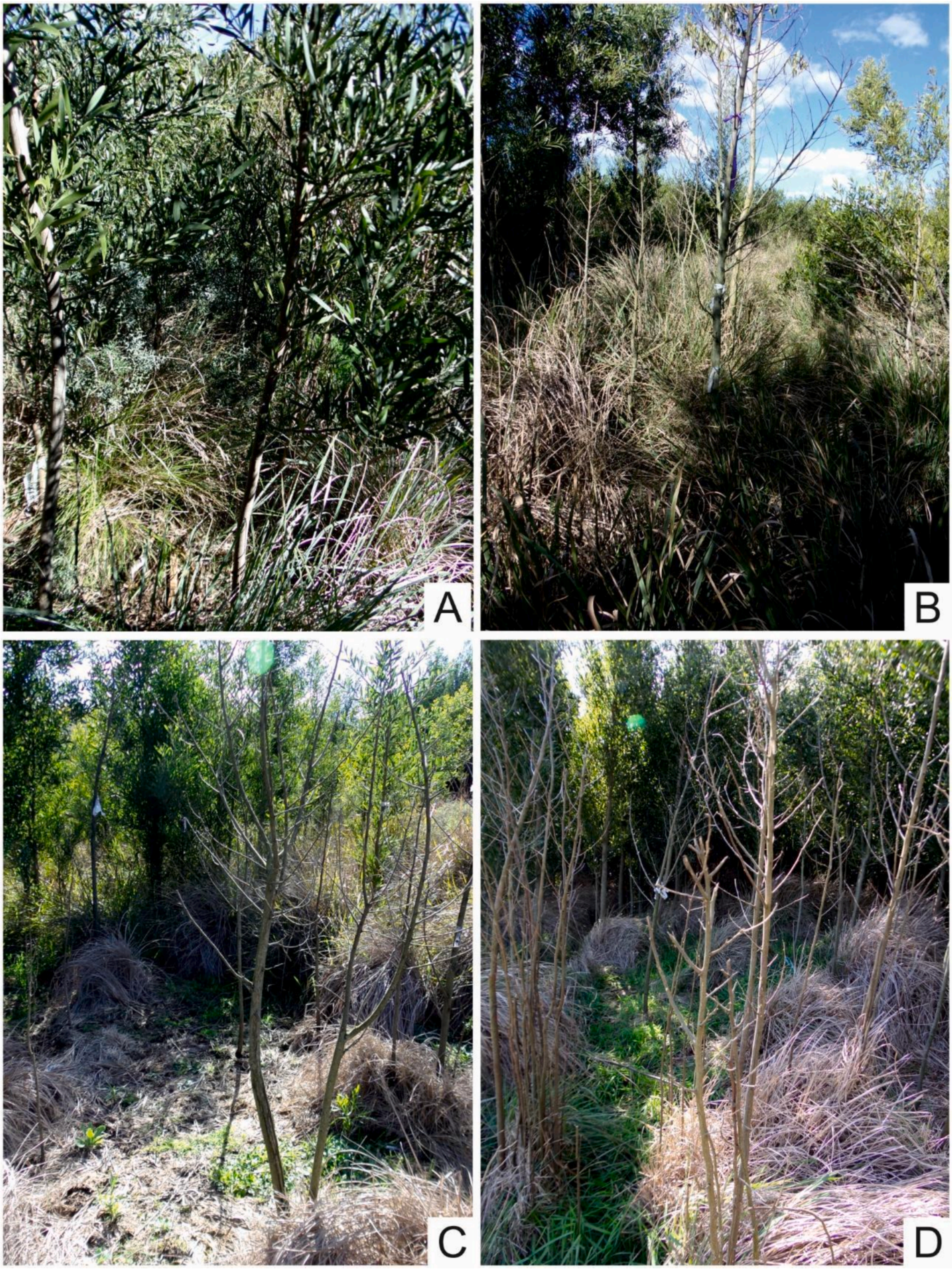

Fig. 5. Appearance of experimental plots on October 19th, 2018 (699 DAA). A: Control; B: Metsulfuron; C: Metsulfuron + Glyphosate; D: Picloram + Triclopyr.

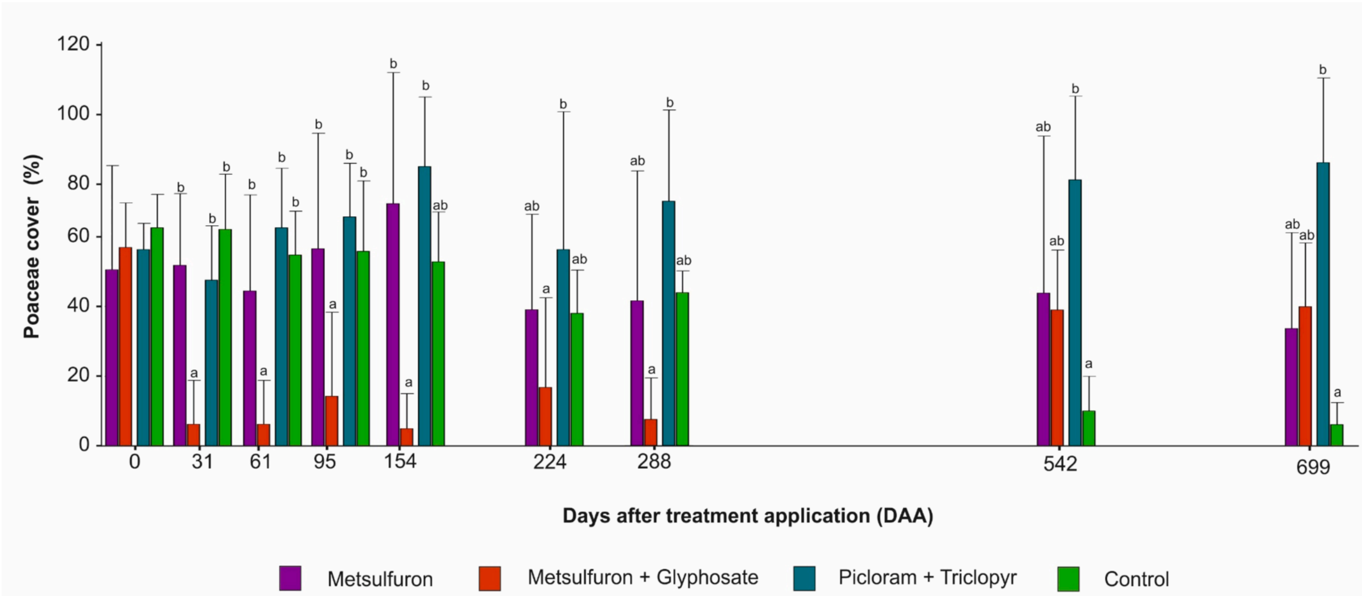

Fig. 6. Mean green foliar coverage of Poaceae per treatment on nine sampling dates expressed in days after the application of the treatments (DAA). Vertical bars indicate standard deviation. Equal letters within the same date indicate non-significant differences, p-value > 0.05.

In Fig. 8, the resulting graph from the PCoA performed to analyze the relationship between the study plots based on the species surveyed corresponding to the final sampling date (699 DAA) is also observed. The first two coordinates explain 28.5 % of the total variance. Unlike what was observed for 0 DAA, not all plots of the same block are close to each other, reflecting less similarity between them compared to the initial sampling date. What can be observed is a marked separation of the control treatment plots (towards the left side of the graph) compared to the plots treated with Metsulfuron + Glyphosate (located towards the right side of the graph).

3.3.2. *Richness Analysis after the application of treatments*

The treatment × date interaction for the richness variable was significant ($p < 0.001$), and when performing an ANOVA by date, significant differences were detected on two of them: 31 and 61 DAA (Fig. 9). In this way, at 31 DAA, the richness of the Metsulfuron + Glyphosate treatment differed significantly from the other treatments and presented the lowest average species richness records (0.75 species vs. 5–6 species); even the plots at the base and the top showed an absolute absence of living plant species. Meanwhile, at 61 DAA, the Metsulfuron + Glyphosate and Picloram + Triclopyr treatments exhibited the lowest average species richness records and differed significantly from the Metsulfuron and control treatments (1.75 species Metsulfuron + Glyphosate and 4.75 species Picloram + Triclopyr vs. 5.75 species Metsulfuron and 7 species control) (Fig. 9). For the remaining dates, no significant differences were detected between the means of the different treatments for this variable, although significant variation within each treatment was recorded, as can be seen from the estimated standard deviations for each treatment (Fig. 9). After 23 months, the average number of species, regardless of the treatment considered, decreased by 40 %, with an average of 9.4 species for the initial date and 5.7 species for the final date (Fig. 9).

Although no significant differences between treatments were detected for the final sampling date (699 DAA) for the species richness variable, it is worth mentioning that regardless of the herbicide, the percentage of native species exceeded that of exotic species by more than 60 % (data not shown), and the control plot of the Slope block was monospecific, presenting only *A. melanoxylon*.

4. Discussion

4.1. *Effectiveness of treatments for controlling Acacia melanoxylon*

Due to the threat posed by biological invasions, understanding the biology of invasive woody species, their impact on native communities, their control, and eradication in invaded areas is crucial for biodiversity conservation and ecosystem sustainability worldwide (Mack et al., 2000; Zalba & Villamil, 2002; Van Wilgen & Wilson, 2018; Brancatelli et al., 2020).

In this study, all chemical treatments were observed to be effective in controlling *Acacia melanoxylon*, with Metsulfuron + Glyphosate and Picloram + Triclopyr treatments showing the best results, as no green coverage of acacia was recorded over time in the plots under these treatments. Regarding the Metsulfuron treatment, results reflected a lower average percentage of green acacia cover (GCA) compared to the Control treatment for all study dates, although there was not a complete absence of acacia in all plots. This situation is linked to unintended application errors of the herbicide, more evident in the last sampling date, where correctly treated plots completely eliminated the green cover of acacia. Papa & Massaro (2005) emphasize that small errors in the dosage or application of this herbicide can significantly alter results due to lack or excess of the product. Therefore, it could be inferred that the effectiveness level of Metsulfuron on acacias would have been possibly the same as for the other chemical treatments if the application had been correct.

This trial recorded a significant reduction in the green foliar cover of *A. melanoxylon* plants following the application of the selected chemical treatments. Campos et al. (2002), Rodrigues Santos & Monteiro (2007) and Souza-Alonso (2013) reported declines in growth and, over time, the death of Acacia species specimens after using herbicides, mostly applied on previously cut plants (stumps) and sometimes repeated. The final results obtained in this work for controlling *A. melanoxylon* were similar to those obtained by these researchers, although in this case, the application was done only once and on standing plants. Considering that the environment invaded by *A. melanoxylon* in the PNR is characterized by areas with steep slopes and exposed rock that hinder transit and access to invaded zones, the fact of not cutting the plants and applying the herbicide directly on the standing plant, only once, would represent an advantage as it speeds up their control.

Table 1
Vascular species surveyed in the study area (Tandilia Hill System, Argentina).

Group of vascular plant	Family	Species	Origen	Cycle
Ferns	Blechnaceae	*Blechnum auriculatum* Cav.	Native	Perennial
	Dennstaedtiaceae	*Pteridium esculentum* (G. Forst.) Cockayne var. *aracnoideum*	Native	Perennial
Angiosperms	Apiaceae*	*Eryngium* sp.	Native	Perennial
	Asteraceae*	*Ambrosia tenuifolia* Spreng	Native	Perennial
		Baccharis dracunculifolia DC.	Native	Perennial
		Carduus acanthoides L.	Exotic	Annual
		Chrysolaena flexuosa (Sims) H. Rob.	Native	Perennial
		Chromolaena sp.	Native	Perennial
		Cirsium vulgare (Savi) Ten.	Exotic	Annual
		Crepis capillaris (L.) Wallr.	Exotic	Annual
		Conyza sp.	Native	Annual
		Helminthotheca echioides (L.) Holub	Exotic	Perennial
		Hypochaeris chillensis (Kunth) Hieron.	Native	Perennial
		Senecio madagascariensis Poir.	Exotic	Perennial
		Senecio selloi (Spreng.) DC.	Native	Perennial
	Brassicaceae*	*Nasturtium officinale* W. T. Aiton	Exotic	Perennial
	Caryophyllaceae*	*Spergula* sp.	Native	Annual
	Convolvulaceae*	*Convolvulus hermanniae* Ĺ Hor.	Native	Perennial
	Cucurbitaceae*	*Cucurbitella asperata* (Gillies ex Hook & Am) Walp	Native	Perennial
	Fabaceae*	*Acacia melanoxylon* R. Br. (=*Rancosperma melanoxylon* (R. Br.) Pedley)	Exotic	Perennial
		Lathyrus pubescens Hook. And Arn.	Native	Perennial
		Vicia nana Vogel	Native	Annual
	Geraniaceae*	*Geranium robertianum* L.	Exotic	Annual
	Lamiaceae*	*Ballota nigra L.* subsp. *foetida* Hayek	Exotic	Perennial
	Lythraceae*	*Cuphea glutinosa* Cham. And Schltdl.	Native	Perennial
	Malvaceae*	*Pavonia cymbalaria* A. St. Hil. & Naudin	Native	Perennial
		Sida rhombifolia L.	Native	Perennial
	Passifloraceae*	*Passiflora caerulea* L.	Native	Perennial
	Primulaceae*	*Anagalis arvensis* L.	Exotic	Annual
	Rhamnacea*	*Colletia paradoxa* (Spreng.) Escal.	Native	Perennial
	Sapindaceae*	*Dodonaea viscosa* Jacq.	Exotic	Perennial
	Scrophulariaceae*	*Buddleja thyrsoides* Lam.	Native	Perennial
	Solanaceae*	*Salpichroa organifolia* (Lam.) Baill.	Native	Perennial
		Solanum chenopodioides Lam.	Native	Perennial
		Solanum commersonii Dunal ex Poir.	Native	Perennial
	Verbenaceae*	*Verbena intermedia* Gillies and Hook. Ex Hook. Phil.	Native	Perennial
	Amaryllidaceae **	*Allium triquetrum* L.	Exotic	Perennial
		Zephiranthes bifida (Herb.) Nic. García	Native	Perennial
	Cyperaceae **	*Carex* sp.	Native	Perennial
	Poaceae **	*Briza minor* L.	Exotic	Annual
		Chascolytrum rufum J. Presl	Native	Perennial
		Dichanthelium sabulorum (Lam.) Gould and C. A.	Native	Perennial
		Holcus lanatus L.	Exotic	Perennial
		Nassella megapotamia (Spreng. Ex Trin.) Barkworth	Native	Perennial
		Paspalum quadrifarium Lam.	Native	Perennial
		Piptochaetium montevidense (Spreng.) Parodi	Native	Perennial
		Setaria vaginata Spreng. var. *bonariensis* Nicora.	Native	Annual
		Sorghastrum pellitum (Hack.) Parodi	Native	Perennial
		Vulpia bromoides (L.) Gray	Exotic	Annual

(*dicot species; ** monocot species).

4.2. Effect of treatments on green Poaceae coverage (GCP) over time

Considering the Control treatment, during the early stages of succession, GCP did not differ from the rest of the treatments, except for the Metsulfuron + Glyphosate treatment. In these early stages, the Control treatment had a GCP percentage over 50 %. Over time, the Control showed intermediate behavior compared to other treatments and, towards the end, completely differed from the rest due to a considerable decrease in GCP. This behavior could be explained by the active growth of untreated *A. melanoxylon*, generating shade in the understory and reducing the photosynthetic capacity of grasses (Woledge, 1978; Assuero & Tognetti, 2010). Moreover, the litter of *A. melanoxylon* could have inhibited the growth of Poaceae due to its allelopathic effects (González et al., 1995), contributing to the reduction of GCP.

In the plots under Metsulfuron + Glyphosate treatment, GCP drastically decreased from 31 DDA and remained low during successive dates, except for the last dates where it increased again. The drastic decrease in GCP from 31 DDA is likely due to the effect of these herbicides on the lower stratum vegetation of *A. melanoxylon*. Metsulfuron-methyl, being a systemic herbicide, controls dicot species, known as "broadleaf species" (Table 1), as well as ferns (Arizatela et al., 2008). On the other hand, Glyphosate, being a non-selective systemic herbicide with a broad spectrum, can eliminate herbs, shrubs, and trees (Tu & Randall, 2001). During the application of this treatment, the dripping of these herbicides from the acacia foliage affected the lower stratum flora, causing the death of Poaceae tillers a few days after application. The increase in GCP recorded in plots under this treatment in subsequent samplings could be due to the elimination of *A. melanoxylon* foliage and big Poaceae plants, *Paspalum quadrifarium* in particular. This situation could have created favorable environmental conditions for other species of this family, which were growing under the canopy of *P. quadrifarium* or in the seed bank, enhancing their germination and/or growth and development, thereby increasing the vegetation cover of this species group.

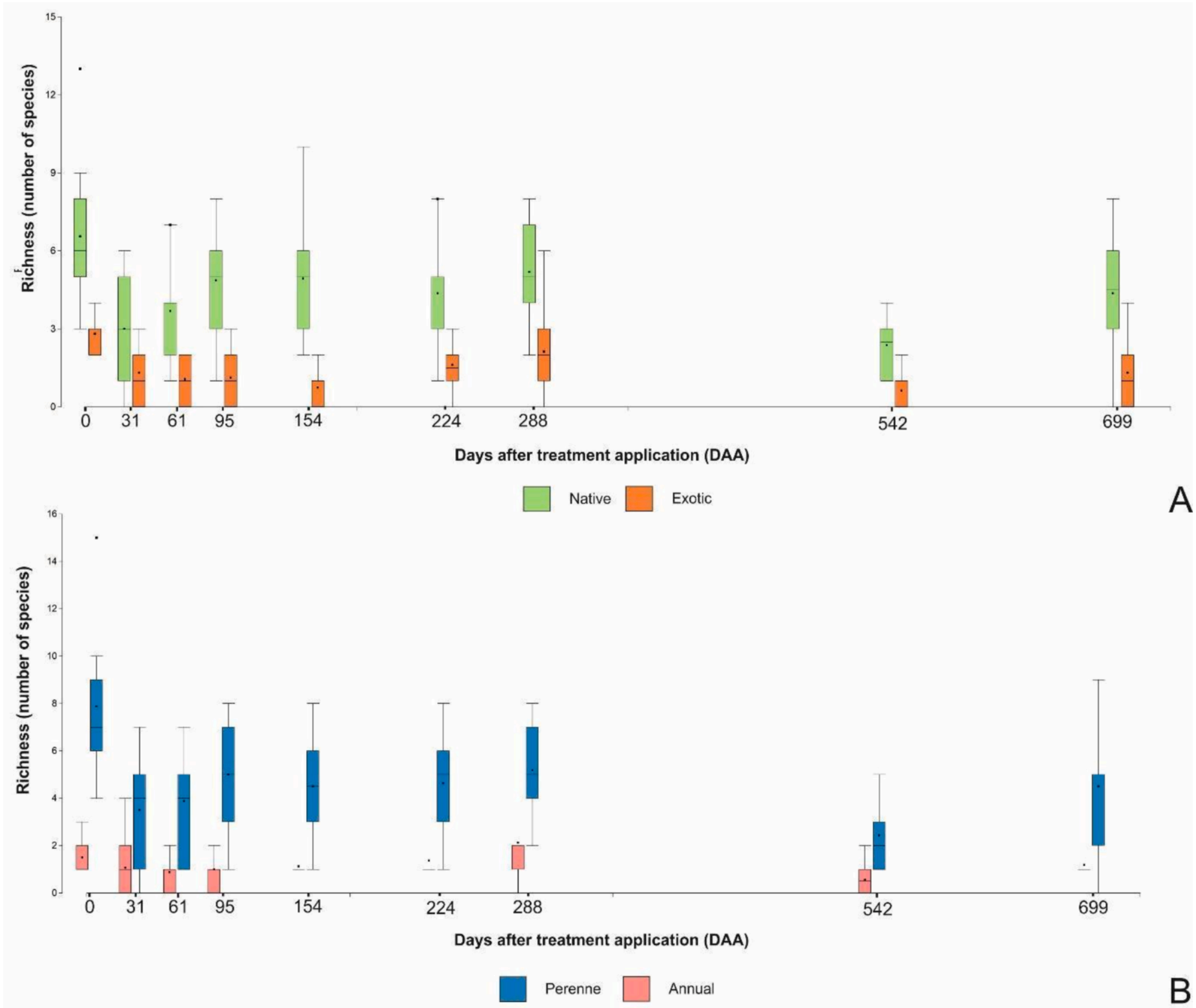

Fig. 7. Evolution of richness on nine sampling dates expressed in days after the application of the treatments (DAA) according to the A) origin of the species (native vs. exotic); B) life cycle of the species (perennial vs. annual). The dots inside the boxes indicate the average. Points outside the boxes indicate outliers or extreme values. *Ref.:* 0 DAA: 11/19/16; 31 DAA: 12/20/16; 61 DAA: 01/19/17; 95 DAA: 02/22/17; 154 DAA: 04/22/17; 224 DAA: 07/1/17; 288 DAA: 09/3/17; 542 DAA: 05/15/18; 699 DAA: 10/19/18.

Regarding the plots that received the Metsulfuron treatment, in the initial and intermediate dates, Poaceae cover was high. This could be due to the fact that, as previously mentioned, it is a selective herbicide for dicot species and ferns (Bedmar et al., 2006; Arizatela et al., 2008), promoting the growth of Monocotyledoneae species, such as Poaceae and Cyperaceae (Arizatela et al., 2008). However, in the last measurements, decreases in GCP were evident, possibly due to unintended errors in herbicide application, allowing some *A. melanoxylon* plants to remain alive and generate shading and allelopathy conditions in those plots, hindering the growth of lower stratum Poaceae species.

In the case of plots that received the Picloram + Triclopyr treatment, an increase in Poaceae cover was observed in the intermediate and final dates. This could be due to both Picloram and Triclopyr being selective herbicides used to control a wide variety of dicot species, especially woody and semi-woody plants (Córdoba, 2007; Tu & Randall, 2001). Picloram can filter through the roots of treated plants and be absorbed by other species, while Triclopyr is specifically used for species that resprout from roots (Tu & Randall, 2001). Due to these characteristics, the combination of these products was effective in controlling the treated *A. melanoxylon* plants, reducing their coverage by killing them and creating better light conditions in the lower levels, favoring the foliar development and coverage of Poaceae, which are immune to the action of these chemicals.

4.2.1. Overview of the identified vascular flora

The three best-represented families during the study were Asteraceae, followed by Poaceae and Fabaceae. These results coincide with those obtained in other studies conducted in the mountainous areas of the Tandilia mountain system in the Balcarce and General Pueyrredón districts (Buenos Aires province, Argentina) (Escaray, 2007; Alonso et al., 2009; Valicenti et al., 2010; Echeverría et al., 2017; 2023). The representation of these families was also similar to findings in the Ventania mountain system (Buenos Aires province, Argentina) by Long (2018), who additionally recorded a high number of species of Caryophyllaceae and Cyperaceae. In other mountainous areas of the country, such as the Sierra de los Comechingones in Córdoba province, the representation of Asteraceae, Poaceae, and Fabaceae families was similar to that obtained in this study (Oggero & Arana, 2012). In a survey conducted in the Upper Atuel River Valley in Mendoza province, Moricii et al. (2010) obtained the same results, with a high representation of the Verbenaceae family as well.

In Argentina, family-level representation is similar to the aforementioned studies and the present work, with the best-represented families being Asteraceae, Poaceae, Fabaceae, Solanaceae, Cyperaceae, Orchidaceae, Cactaceae, Malvaceae, Brassicaceae, and Euphorbiaceae (Zuloaga et al., 2019).

Regarding status and cycle, a higher percentage of native species

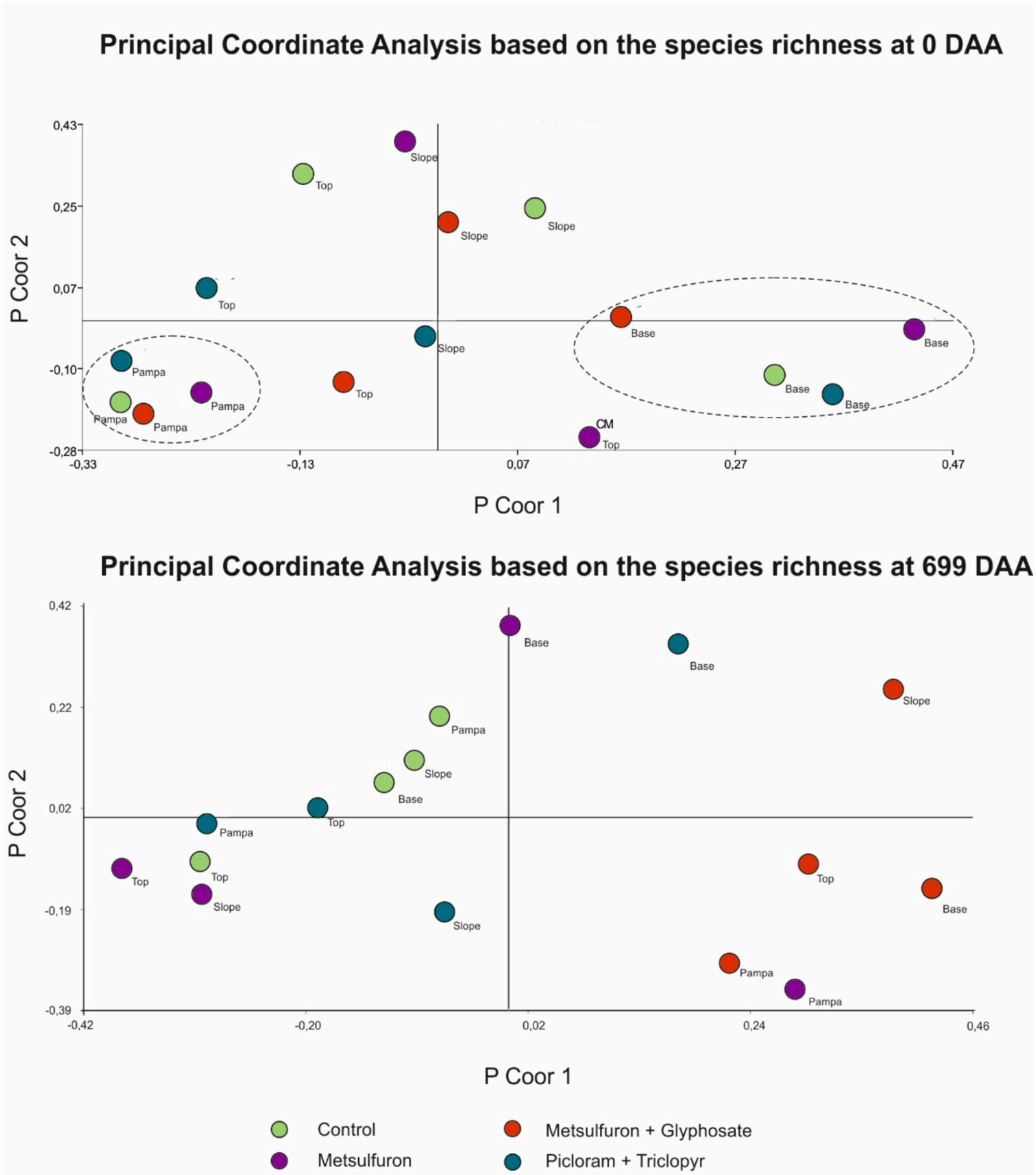

Fig. 8. Principal Coordinate Analysis based on the specific richness registered in 16 plots that belong to the four blocks (Base; Slope; Pampa; Top) at 0 DAA (days after treatment application) and 699 DAA. *Ref.:* The ellipses represent the groupings of the plots according to the similarity between the species.

with perennial cycles were recorded. These results also align with those obtained by other authors in neighboring mountainous areas (Escaray, 2007; Alonso et al., 2009; Valicenti et al., 2010; Echeverría et al., 2017, 2023; Polo et al., 2024). Of the 50 species determined in this study, two species that had not been previously surveyed in the "Sierra Chica" of the PNR by Echeverría et al. (2017; 2023) were identified: *Cuburbitella asperata* and *Allium triquetrum*.

The fact that a higher proportion of native species was observed highlights the importance of these mountainous systems as reservoirs of native species. These environments foster environmental heterogeneity, allowing the existence of high specific diversity (Cantero, 2016). Additionally, these mountainous systems are considered true refuges for species with different ecological requirements, being crucial for biodiversity conservation (Kristensen & Frangi, 1995; Kristensen, 2014). This situation should not be underestimated, as the Austral Pampas region is continuously subjected to agro-industrial practices, causing habitat fragmentation and homogenization, and the replacement of natural ecosystems with agricultural landscapes, such as orchards, farms, crop fields, and pastures (Herrera et al., 2016), promoting the loss or decrease of biodiversity, causing irreversible damage to the planet and the ecosystem services that nature provides to humans (Cardinale et al., 2001).

4.2.2. *Effect of treatments on species richness*

When analyzing species richness in the plots under the Metsulfuron + Glyphosate treatment, it was observed that the date following the application of the products differed significantly from the rest of the treatments and, on the following date, from the Control and Metsulfuron treatments, presenting the lowest number of species. This effect was probably due to the dripping of Glyphosate from the foliage of the acacia plants reaching the lower strata of the plots, and since it is a non-selective herbicide (Tu & Randall, 2001), it controlled most of the species in those plots, in some cases with 100 % efficacy.

In the plots under the Picloram + Triclopyr treatment, in the third sampling date, although it did not differ significantly from the remaining treatments, intermediate levels of richness were recorded, higher

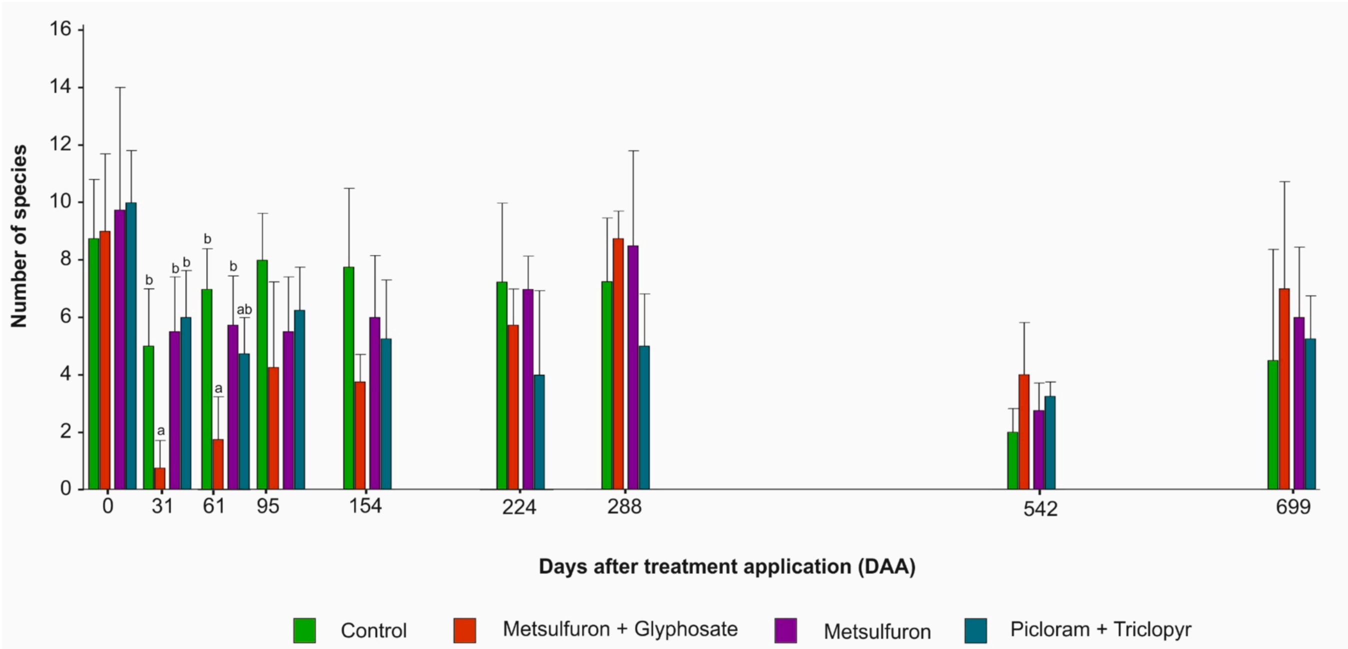

Fig. 9. Mean specific richness per treatment on nine sampling dates expressed in days since the application of the treatments (DDA). Vertical bars indicate standard deviation. Equal letters within the same date indicate non-significant differences, p-value > 0.05.

than those of the Metsulfuron + Glyphosate treatment and lower than those of the Control and Metsulfuron treatments. This situation is possibly due to the plants of the lower stratum being reached by dripping from the acacia foliage by these selective herbicides, causing the observed decline in the richness of dicot and fern species. Since Metsulfuron is a selective herbicide for broadleaf species, a similar result would be expected in the plots under this treatment. However, this did not occur, possibly due to application failures of this treatment in some plots.

Although no significant differences were observed between treatments for the richness variable from the fourth sampling date onward, a progressive decrease in the number of species was observed over time in the Control treatment, along with a strong reduction in vegetation cover given by these species. This could also be due to unintended errors that occurred in some plots during product application, allowing some *A. melanoxylon* plants to remain alive, hindering the growth of Poaceae species in the lower stratum, as reported in other situations with *A. melanoxylon* invasion (Arán, García- Duro, Reyes, & Casal, 2013, 2017; Gandini et al., 2019; ĎAlfonso, 2026). Additionally, in certain plots under this treatment, richness decreased to the point where only *A. melanoxylon* was observed, highlighting the great invasive potential of this species, negatively impacting the growth and development of others.

Therefore, it is important to emphasize that this study has demonstrated that the presence of *A. melanoxylon* plants progressively reduces species richness in the sites where it thrives, reaching extremely low values and even becoming the only species present. This makes *A. melanoxylon* a current and potential threat to the biodiversity of the Tandilia mountain ecosystems.

An important aspect to highlight is that, over time, variations in the number of species were notable at the plot level. As reflected in the Principal Coordinates Analysis conducted at the beginning of the study and considering species richness, the plots were not floristically identical. In other words, the plots were selected ensuring that the coverage of acacias, Poaceae, and total richness were similar, but not the identity of the lower stratum species. Therefore, in this particular case, the effect of the treatments would not be the only factor conditioning the plant species to thrive in the experimental units. The flora present at each site would be more associated with environmental effects, mainly given by the altitudinal gradient capable of generating differences in the study plots.

Mountain ecosystems are environments with high environmental heterogeneity, resulting from their topographical irregularities, which leads to floristic composition varying according to the physical and chemical properties of the soil, availability of sites for seed germination, rock erosion patterns, and the degree of sun exposure (Whittaker et al., 1967; Torres Ribeiro et al., 2007; Kristensen & Frangi, 2015). Based on this, the results of this study align with those obtained by Cavagnaro (1988), Suárez & Vischi (1997) and Mazzola et al. (2008) in other mountainous environments in Argentina, where it was found that floristic composition varies significantly with increasing altitudinal gradient.

It is important to note that over time, in the plots under herbicide treatment, the number of native species was higher than the number of exotic species, mainly perennial, such as *Baccharis dracunculifolia*, *Senecio selloi*, *Lathyrus pubescens*, *Sida rhombifolia*, *Pavonia cymbalaria*, *Solanum chenopodioides*, *S. commersonii*, *Colletia paradoxa*, and Poaceae. This reflects the importance of outcrops as “hotspots” for the conservation of endemic species (Milchunas & Noy-Meir, 2002; Echeverría et al., 2017, 2023), creating microsites that provide protection or refuge for a large number of species and nesting and feeding sites for wildlife (Mares & Seine, 2000).

5. Conclusions

This study confirmed the effectiveness of the chemical treatments used for controlling *Acacia melanoxylon*. Additionally, it allowed the evaluation of plant succession processes following herbicide application, documenting the effects of each treatment on the treated plots, which directly or indirectly affected the species in the lower strata. A noteworthy point is that, even after considerable time had passed since the application of the products, the Poaceae group and the percentage of native species in general were not adversely affected. This is of utmost importance given the significance of grasslands in the region and the large number of native and endemic species that these mountains host. Therefore, it is essential that the control methods used for invasive species do not harm these species.

Based on the above, the results of this study provide a valuable tool for selecting chemical control methods against this tree species, offering useful information for controlling and mitigating its spread not only in this reserve but also in other environments, particularly in mountainous areas where steep slopes and surface rock hinder the use of heavy machinery and limit access to invaded sites, making logging and/or removal of specimens difficult. The results also contribute to understanding how the surrounding environment changes over time in the absence of this invasive species following herbicide application.

Final considerations

Considering the results obtained, the importance of correctly applying the products used to achieve effective control of the target species is highlighted. However, despite the problems that occurred during the application of the herbicides, it is worth mentioning that all the treatments used in this study could be recommended to control *Acacia melanoxylon* under similar situations since they not only hit target plants but in all cases the number of plant species, particularly native ones, increased in relation to the control. Future studies on controlling this species should focus on determining the most effective herbicide concentrations while accounting for tree size, assessing whether external adjuvants enhance herbicide effectiveness, and identifying the optimal growth or developmental stage for application. Additionally, researches should address the challenge of controlling large trees that are not easily affected by chemical application while emphasizing the importance of avoiding excessive herbicide use to minimize environmental impact and reduce costs. In this regard, it is also recommended to consider the negative impacts on soil, water, and human health. For instance, it is well known that some herbicides can negatively affect soil and water microbial activity and composition, leading to consequences for fertility, water retention capacity, and the balance of aquatic ecosystems (Van Bruggen et al., 2018). Furthermore, prolonged exposure to certain herbicides has been linked to health risks such as endocrine disruption and respiratory issues (Rani et al., 2021). Given these concerns, future research should also explore alternative control techniques. In this regard, other removal methods, such as stump applications, could offer potential benefits by preventing herbicide dispersion as they have shown more favorable results in reducing sprouting and regrowth (Knapp et al., 2023).

Finally, in pursuit of conserving the biodiversity of the reserve, threatened by the invasion of *Acacia melanoxylon*, an interdisciplinary approach starting with environmental education is necessary. This should engage educational communities and the broader society, addressing the challenges posed by invasive species and promoting activities for their prevention, mitigation, control, and eradication.

6. Funding Sources

This work was financially supported by Universidad Nacional de Mar del Plata (Argentina) [research projects AGR 557/18, AGR 616/20 and AGR 669/22].

CRediT authorship contribution statement

Sofía Rojas: Writing – original draft, Methodology, Investigation, Data curation. **María L. Echeverría:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Tomás O'Connor:** Writing – original draft, Methodology, Investigation. **Viviana M. Comparatore:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank Esteban González Zugasti, owner of the Paititi Natural Reserve, who gave us permission to carry out this research and provided us logistical support. To Professor Francisco Bedmar from de UNMdP and to the generous editor and reviewers for the guidance and valuable comments.

Data availability

Data will be made available on request.

References

Alonso, S. I., Guma, I. R., Nuciari, M. C., & Van Olphen, A. (2009). Flora de un área de la Sierra La Barrosa (Balcarce) y fenología de especies con potencial ornamental. *Revista de la Facultad de Ciencias Agrarias, 41*, 23–44.

Arán, D., García- Duro, J., Reyes, O., & Casal, M. (2013). Fire and invasive species: Modifications in the germination potential of *Acacia melanoxylon, Conyza canadensis* and *Eucalyptus globulus*. *Forest Ecology and Management, 302*, 7–13. https://doi.org/10.1016/j.foreco.2013.02.030

Arán, D., García- Duro, J., Reyes, O., & Casal, M. (2017). Understanding biological characteristics of *Acacia melanoxylon* in relation to fire to implement control measurements. *Annals of Forest Science, 74*, 61. https://doi.org/10.1007/s13595-017-0661-y

Aranguren, M. F., Velasco, M. A., Trofino-Falasco, C., Pizzarello, M. G., Vera, D. G., & Berkunsky, I. (2023). Mammals of the Tandilia Mountain system, current species inhabiting Pampean highland grasslands. *Neotropical Biology and Conservation, 18*(1), 13–29. https://doi.org/10.3897/neotropical.18.e98374

Arizatela, M., Anzalone, A., & Silva, A. (2008). Efecto del uso del Metsulfurón- metil y Glifosato sobre malezas asociadas a cafetales en Venezuela. *Bioagro, 20*, 79–88.

Assuero, S. G., & Tognetti, J. A. (2010). Tillering regulation by endogenous and environmental factors and its agricultural management. *The Americas Journal of Plant Science and Biotechnology, 4*, 35–48.

Baldi, G., Guershman, J. P., & Paruelo, J. M. (2006). Characterizing fragmentation in temperate South America grasslands. *Agriculture Ecosystem and Environment, 116*, 197–208.

Brancatelli, G. I., Amodeo, M. R., Cuevas, Y. A., & Zalba, S. M. (2020). Invasive pines in Argentinian grasslands: Lessons from control operations. *Biological Invasions, 22*, 473–484. https://doi:10.1007/s10530-019-02103-9.

Beaury, E. M., Sofaer, H. R., Early, R., Beaury, E. M., Pearse, I. S., Blumenthal, D. M., Corbin, J. D., Diez, J., Dukes, J. S., Barnett, D. T., Ibáñez, I., Petri, L., Vilà, M., & Bradley, B. A. (2023). Macroscale analyses suggest invasive plant impacts depend more on the composition of invading plants than on environmental context. *Glob. Ecol. Biogeogr., 32*, 1964–1976. https://doi.org/10.1111/geb.13749

Bedmar, F., Perdigon, J. A., & Monterubbianesi, M. G. (2006). Residual phytotoxicity and persistence of chlorimuron and metsulfuron in soils of Argentina. *Journal of Environmental Biology, 27*, 175–179.

Cabrera, A. L. (1963). *Compuestas. Flora de la Provincia de Buenos Aires. Colección Científica Instituto Nacional de Tecnología Agropecuaria. Tomo 4 (6)*. Buenos Aire: Instituto Nacional de Tecnología Agropecuaria.

Cabrera A. L. (1965a). Oxalidáceas a Umbelíferas. Flora de la Provincia de Buenos Aires. Colección Científica Instituto Nacional de Tecnología Agropecuaria. Tomo 4 (4). Instituto Nacional de Tecnología Agropecuaria, Buenos Aires.

Cabrera, A. L. (1965b). *Ericáceas a Caliceráceas. Flora de la Provincia de Buenos Aires. Colección Científica Instituto Nacional de Tecnología Agropecuaria. Tomo 4 (5)*. Buenos Aires: Instituto Nacional de Tecnología Agropecuaria.

Cabrera A. L. (1967). Piperáceas a Leguminosas. Flora de la Provincia de Buenos Aires. Colección Científica Instituto Nacional de Tecnología Agropecuaria. Tomo 4 (3). Instituto Nacional de Tecnología Agropecuaria, Buenos Aires.

Cabrera A. L. (1968). Gimnospermas y Monocotiledóneas (excepto Gramíneas). Flora de la Provincia de Buenos Aires. Pteridáceas. Colección Científica Instituto Nacional de Tecnología Agropecuaria. Tomo 4 (1). Instituto Nacional de Tecnología Agropecuaria, Buenos Aires.

Cabrera A. L. (1970). Gramíneas. Flora de la Provincia de Buenos Aires. Colección Científica Instituto Nacional de Tecnología Agropecuaria. Tomo 4 (2). Instituto Nacional de Tecnología Agropecuaria, Buenos Aires.

Cabrera, A. L., & Zardini, E. M. (1978). *Manual de la Flora de los alrededores de la Provincia de Buenos Aires* (2nd ed.). Buenos Aires: ACME.

Campos, J., Rocha, M., & Tavares, M. (2002). Controlo de Acácias com Fitocidas nas Dunas do Litoral. *Silva Lusitana, 10*, 201–206.

Cantero, J. J., Núñez, C., Mulko, J., Brandolín, P., & Amuchastegui, A. (2016). Vegetación y flora de afloramientos basálticos del centro de Argentina. *Arnaldoa, 23* (1), 185–218.

Carneiro, L., Miiller, N. O., Cuthbert, R. N., & Vitule, J. R. (2024). Biological invasions negatively impact global protected areas. *Sci Total Environ., 948*, Article 174823. https://doi.org/10.1016/j.scitotenv.2024.174823

Carranza, S. L. (2007). Revisión bibliográfica sobre *Acacia melanoxylon*, su silvicultura y su madera. *Revista de la Facultad Agronomía, 106*, 145–154.
Cavagnaro, J. B. (1988). Distribution of C3 and C4 grasses at different altitudes in a temperate arid region of Argentina. *Oecologia, 76*, 273–277.
Córdoba, L. G. (2007). Control químico de arbustos sobre alambrados para evitar su destrucción por incendios. *Dissertation thesis. Facultad de Ciencias Exactas y Naturales. Universidad Nacional de La Pampa. Argentina., 61*, pp.
Cuadras, C. W. (2014). *Nuevos métodos de análisis multivariante*. Barcelona: CMC Editions.
Cardinale, B.J. Matulich K.L., Hooper D.U., Byrnes J.E., Duffy E., Gamfeldt L., Balvanera P., Ó Connor M.I. & Gonzalez A. (2001). The functional role of producer diversity in ecosystems American Journal of Botany, 98, 572 - 592 10.3732/ajb.1000364.
Dalla Salda, L., Spalletti, L., Poire, D., De Barrio, R., Echeveste, H., & Benialgo, A. (2006). Tandilia. *Instituto Superior de Correlación Geológica: Serie Correlación Geológica, 21*(1), 17–46. https://www.scielo.org.ar/scielo.php?script=sci_arttext&pid=S 1666-94792006000100002&lng=es&tlng=es.
De Rito, M. V., Fernández Honaine, M., & Herrera, L. P. (2020). Aplicación De Un Índice De Naturalidad Para Las Sierras Del Sistema De Tandilia. Revista Mus. Argent. Ci. Nat. *N. S., 22*, 75–90. https://doi.org/10.22179/revmacn.22.672.
ĎAlfonso A. Scaramuzzino R. (2026) *Acacia melanoxylon* R. Brown. In: O. A. Fernández, E.S. Leguizamón & H.A. Acciaresi. (Eds). Malezas e invasoras de la Argentina. Tomo II. Descripción y reconocimiento. (pp. 605). Editorial de la Universidad Nacional del Sur. EdiUNS. Bahía Blanca.
Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., & Robledo C.W. (2016) InfoStat (versión 2016). [computer software] http://www.infostat.com.ar.
Dodet, M., & Collet, C. (2012). When should exotic forest plantation tree species be considered as an invasive threat and how should we treat them? *Biological Invasions, 14*, 1765–1778.
Echeverría, M. L., Alonso, S. I., & Comparatore, V. M. (2017). Survey of the vascular plants of Sierra Chica, the untouched area of the Paititi Natural Reserve (southeastern Tandilia mountain range, Buenos Aires province, Argentina). Check List. *The Journal of Biodiversity data., 13*, 1003–1036. https://doi.org/10.15560/13.6.1003.
Echeverría, M. L., Alonso, S. I., & Comparatore, V. M. (2023). Vegetation of a hill grassland of the Paititi Natural Reserve (Pampa biome) and early detection of nonnative species acting as invasive. *Boletín de la Sociedad Argentina de Botánica, 58*, 71–90. https://doi.org/10.31055/1851.2372.v58.n1.38512
Echeverría, M. L., Alonso, S. I., & Comparatore, V. M. (2024). Flora nativa de valor ornamental potencial del extremo sudoriental del sistema serrano de Tandilia (Argentina): Una alternativa de uso y conservación. *Boletín de la Sociedad Argentina de Botánica, 59*(2), 221–238. https://doi.org/10.31055/1851.2372.v59.n2.44399.
Escaray, F. J. (2007). Estudio Florístico de una Ladera de la Sierra del Volcán (Sistema de Tandilia). Agricultural Engineering Thesis, Facultad de Ciencias Agrarias. *Universidad Nacional de Mar del Plata, Buenos Aires, 98*.
Falasca, S., Ulberich, A., Bernabé, N., & Mordenti, S. (2000). Principales características agroclimáticas del sudeste bonaerense, República Argentina. *Revista Geográfica, 127*, 91–102.
Fernandez, R. D., Ceballos, S. J., Malizia, A., & Aragón, R. (2017). *Gleditsia triacanthos* (Fabaceae) in Argentina: A review of its invasion. *Australian Journal of Botany, 65*, 203–213. https://doi.org/10.1071/BT16147
Gandini, M., Bardi, J., Scaramuzzino, R., D'Alfonso, C., & Lara, B. (2019). *Racosperma melanoxylon* (Fabaceae) en las Sierras de Azul, Sistema de Tandilia (Buenos Aires, Argentina): Distribución en las últimas décadas. *Boletín de la Sociedad Argentina de Botánica, 54*, 142–143.
Ghersa, C. M., de la Fuente, E., Suarez, S., & Leon, R. J. (2002). Woody species invasión in the Rolling Pampa grasslands, Argentina. *Agriculture, ecosystems & environment, 88*, 271–278. https://doi:10.1016/S0167-8809(01)00209-2.
González, L., Souto, X. C., & Reigosa, M. J. (1995). Allelophatic effects of *Acacia melanoxylon* R.Br. phyllodes during their decomposition. *Forest Ecology and Management, 77*, 53–63.
Guazzelli, M. A. (1999). *Efectos del fuego sobre la fauna y los caracteres fisicoquímicos del suelo en las Sierras Septentrionales de la Provincia de Buenos Aires. Thesis Dissertation, Facultad de Ciencias Exactas y Naturales*. Buenos Aires, Argentina: Universidad Nacional de Mar del Plata.
Herrera, L., Sabatino, M., Gastón, A., & Saura, S. (2016). Grassland connectivity explains entomophilous plant species assemblages in an agricultural landscape of the Pampa Region, Argentina. *Austral Ecology, 42*, 486–496. https://doi:10.1111/aec.12468.
Hussain, M. I., El-Sheikh, M. A., & Reigosa, M. J. (2020). Allelopathic potential of aqueous extract from *Acacia melanoxylon* R. *Br. on Lactuca sativa. Plants, 9*(9), 1228. https://doi:10.3390/plants9091228.
Isacch, J. P., Bó, M. S., Vega, L. E., Favero, M., Baladrón, A. V., Petrelli, M. G., Stellatelli, O. A., Cardoni, D. A., Copello, S., Block, C., Cavalli, M., Comparatore, V. M., Jelicich, M. R., Biondi, L. M., García, G. O., & Seco Pon, J. P. (2016). Diversidad de Tetrápodos en un mosaico de ambientes del sudeste de la ecorregión Pampeana como herramienta para planificar en conservación. *Revista Museo Argentino Ciencias Naturales, 18*, 211–233. https://doi.org/10.22179/REVMACN.18.463.
INTA. Instituto Nacional de Tecnología Agropecuaria. Instituto de Clima y Agua. (2023). Retrieved from http://inta.gob.ar/instdeclimayagua . Accessed September 12, 2024.
Jeschke, J. M., Bacher, S., Blackburn, T. M., Dick, J. T. A., Essl, F., Evans, T., Gaertner, M., Hulme, P. E., Kühn, I., Mrugała, A., Pergl, J., Pyšek, P., Rabitsch, W., Ricciardi, A., Richardson, D. M., Sendek, A., Vilà, M., Winter, M., & Kumschick, S. (2014). Defining the Impact of Non-Native Species. *Conservation Biology, 28*(5), 1188–1194. https://doi:10.1111/cobi.12299.
Jiménez, E., Vega, J. A., Pérez-Gorostiaga, P., Fonturbel, T., & Fernández, C. (2010). Evaluation of sap flow density of *Acacia melanoxylon* R. Br. (blackwood) trees in overstocked stands in north-western Iberian Peninsula. *European Journal of Forest Research, 129*, 61–72.
Knapp, L. S. P., Coyle, D. R., Dey, D. C., Fraser, J. S., Hutchinson, T., Jenkins, M. A., Kern, C. C., Kanpp, B. O., Mddox, D., Pinchot, C., & Wang, G. G. (2023). Invasive plant management in eastern North American Forests: A systematic review. *Forest Ecology and Management, 550*, Article 121517.
Kiptoo, T. K., & Ole Kiyapi, J. L. (2022). Ecological Impacts and Management Strategies of *Acacia melanoxylon* Species in the African Ecosystems. *Africa Environmental Review Journal, 5*(2), 114–127. https://doi.org/10.2200/aerj.v5i2.256
Kristensen, M., & Frangi, J. L. (1995). La Sierra de la Ventana: Una Isla de Biodiversidad. *Ciencia Hoy, 5*, 25–34.
Kristensen, M. J., Lavornia, J., Leber, V., Pose, M. P., Dellapé, P., Salle, A., Braccalente, L., Giarratano, L., & Higuera, M. (2014). Estudios para la conservación de la Pampa austral. I. Diagnóstico de la biodiversidad local. *Revista estudios ambientales., 2*, 105–117. https://doi.org/10.47069/estudios-ambientales.v2i1.1061.
Kristensen, M. J., & Frangi, J. L. (2015). Vegetación casmofítica y mesoclimas de afloramientos rocosos en Ventania (Buenos Aires, Argentina). *Boletín Sociedad Argentina de Botánica, 50*, 35–46. https://doi.org/10.31055/1851.2372.v50.n1.11018.
Liu, C., Diagne, C., Angulo, E., Banerjee, A. K., Chen, Y., Cuthbert, R. N., Haubrock, P. J., Kirichenko, N., Pattison, Z., Watari, Y., Xiong, W., & Courchamp, F. (2021). Economic costs of biological invasions in Asia. *NeoBiota, 67*, 53–78. https://doi.org/10.3897/neobiota.67.58147
Long, M. A. (2018). *Especies comunes y raras en la flora de las Sierras Australes Bonaerenses: Causas históricas, ecológicas y ambientales*. Argentina: Universidad Nacional del Sur. Bahía Blanca. Buenos Aires. Thesis Dissertation.
Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M., & Bazzaz, F. A. (2000). Biotic Invasions: Causes, epidemiology, global consequences and control. *Ecological Applications, 10*, 689–710. https://doi:10.2307/2641039.
Mares, M. A., & Seine, R. H. (2000). The fauna of inselbergs. In S. Porembski, & S. Barthlott (Eds.), *Inselbergs: biotic diversity of isolated rock outcrops in tropical and temperate regions* (pp. 483–491). Springer. Berlin.
Martínez Crovetto, R. (1947). La naturalización de *Acacia melanoxylon* en Balcarce (Provincia de Buenos Aires). *RIA, 1*, 101–102.
Mazzola, M. B., Kin, A. G., Morici, E. F., Babinec, F. J., & Tamborini, G. (2008). Efecto del gradiente altitudinal sobre la vegetación de las sierras de Lihue Calel (La Pampa, Argentina). *Boletín Sociedad Argentina de Botánica, 43*, 103–119.
Mazzolari, A. C., Comparatore, V. M., & Bedmar, F. (2011). Control of elmleaf blackberry invasion in a natural reserve in Argentina. *Journal for Nature Conservation, 19*, 185–191. https://doi.org/10.1016/j.jnc.2010.12.002
Milchunas, D., & Noy-Meir, I. (2002). Grazing refuges external avoidance of herbivory and plant diversity. *Oikos, 99*, 113.
Moricii, E. F. A., Prina, A., Alfonso, G. L., & Muiño, W. (2010). Flora y vegetación del Valle Superior del Río Atuel (Mendoza-Argentina). *Boletín Sociedad Argentina de Botánica, 45*, 109–118.
Oggero, A. J., & Arana, M. D. (2012). Inventario de las plantas vasculares del sur de la zona serrana de Córdoba, Argentina. *Hoehnea, 39*, 171–199.
Osterrieth, M. L., & Cabria, F. (1995). Mapa de Capacidad de Uso de Suelos. In J. L. Del Río, J. Bó, J. Martínez Arca, & V. Bernasconi (Eds.), *Carta Ambiental del Partido de General Pueyrredón. Tomo 1. Informe Instituto de Geología de Costas y del Cuaternario* (pp. 63–68). Mar del Plata: Universidad Nacional de Mar del Plata.
Papa, J. C., & Massaro, R. (2005). Herbicida Metsulfuron Metil en Barbechos químicos. Información Técnica de Trigo Campaña 2005. INTA. *Estación Experimental Agropecuaria Rafaela. Publicación Miscelánea, 103*, 54–56.
Pysek, P., & Richardson, D. M. (2010). Invasive species, Environmental Change and Management, and Health. *Annual Review of Environment and Resources, 35*, 25–55. https://doi.org/10.1146/annurev-environ-033009-095548
Pyšek, P., Hulme, P. E., Simberloff, D., Bacher, S., Blackburn, T. M., Carlton, J. T., Dawson, W., Essl, F., Foxcroft, L. C., Genovesi, P., Jeschke, J. M., Kühn, I., Liebhold, A. M., Mandrak, N. E., Meyerson, L. A., Pauchard, A., Pergl, J., Roy, H. E., Seebens, H., van Kleunen, M., Vilà, M., Wingfield, M. J., & Richardson, D. M. (2020). Scientists' warning on invasive alien species. *Biol Rev, 95*, 1511–1534. https://doi.org/10.1111/brv.12627
Polo, N., Echeverria, M. L., Palacio, M. A., Varela, S., & Herrera, L. (2024). Flora con potencial valor apícola en pastizales serranos del extremo sudoriental del Sistema serrano de Tandilia con diferente manejo productivo. *Revista del Museo Argentino de Ciencias Naturales nueva serie, 26*(1), 11–24.
Rani, L., Thapa, K., Kanojia, N., Sharma, N., Singh, S., Grewal, A. S., Srivastav, A. L., & Kaushal, J. (2021). An extensive review on the consequences of chemical pesticides on human health and environment. *Journal of cleaner production, 283*, Article 124657.
Richardson, D. M., & Pysek, P. (2006). Plant invasions: Merging the concepts of species invasiveness and community invisibility. *Progress in Physical Geography, 30*, 409–431.
Richardson, D. M., & Rejmanek, M. (2011). Trees and shrubs as invasive alien species- a global review. *Diversity and Distributions, 17*(5), 788–809. https://doi:10.1111/j.1472-4642.2011.00782.x.
Richardson, D. M., Le Roux, J. J., & Wilson, J. R. (2015). Australian acacias as invasive species: Lessons to be learnt from regions with long planting histories. *Southern Forests: A Journal of Forest Science, 77*(1), 31–39. https://doi.org/10.2989/20702620.2014.999305
Rodrigues Santos, A., & Monteiro, A. (2007). Controlo de Invasoras Lenhosas no Parque Ecológico do Funchal. *Silva Lusitana, 15*, 249–255.

Sanhueza, C., & Zalba, S. M. (2012). Experimental control of Spanish broom (*Spartium junceum*) invading natural grasslands. *Management of Biological Invasions, 3*(2), 97. https://doi.org/10.3391/mbi.2012.3.2.04

Scottá, F. C., & Da Fonseca, E. L. (2015). Multiscale trend analysis for pampa grasslands using ground data and vegetation sensor imagery. *Sensors, 15*, 17666–17692. https://doi.org/10.3390/s150717666

Souza-Alonso, P., Lorenzo, P., Rubido-Bará, M., & González, L. (2013). Effectiveness of management strategies in *Acacia dealbata* Link invasion, native vegetation and soil microbial community responses. *Forest ecology and management, 304*, 464–472. https://doi:10.1016/j.foreco.2013.05.053.

Souza- Alonso, P., Rodriguez, J., Gonzalez, L., & Lorenzo, P. (2017). Here to stay: Recent advances and perspectives about Acacia invasión in Mediterranean áreas. *Annals of Forest Science, 74*, 55. https://doi:10.1007/s13595-017-0651-0.

Suárez, S., & Vischi, N. (1997). Caracterización fisonómico-estructural de la vegetación serrana (Alpa Corral-Córdoba-Argentina). *Multequina, 6*, 21–32.

Torres Ribero, K., Opazo Medina, B. M., & Rubio Scarano, F. (2007). Species composition and biogeography relations of the rock outcrop flora on the high plateau of Itatiaia, SE-Brazil. *Brazilian Journal of Botany, 30*, 623–639.

Valicenti, R., Farina, E., Scaramuzzino, R., & D́ Alfonso, C.. (2010). Ordenación de la vegetación en el paisaje Boca de la Sierras (Azul, Sistema de Tandilia). *RASADEP., 1*, 111–122.

Van Bruggen, A. H., He, M. M., Shin, K., Mai, V., Jeong, K. C., Finckh, M. R., & Morris, J. G., Jr (2018). Environmental and health effects of the herbicide glyphosate. *Science of the total environment, 616*, 255–268.

Van Kleunen, M., Bossdorf, O., & Dawson, W. (2018). The ecology and evolution of alien plants. Annual Review of Ecology. *Evolution and Systematics, 49*, 25–47.

Van Wilgen, B. W., & Wilson, J. R. (2018). *The status of biological invasions and their management in South Africa in 2017. South African National Biodiversity Institute Kirstenbosch and DST- NRF Centre of*. Stellenbosch: Excellence for Invasion Biology.

Vera, D. G., Di Pietro, D. O., Tettamanti, G., Eirin, M., Trofino-Falasco, C., Aranguren, M. F., Williams, J., & D., Kacoliris, F.P., & Berkunsky, I.. (2021). An annotated list of the reptiles of the highland grassland of Tandilia Mountains, Argentina. *Neotropical Biology and Conservation, 16*, 185–204. https://doi:10.3897/neotropical.16.e60629.

Tu M. Hurd C. & Randall J.M. (2001). Weed control methods handbook: tools & techniques for use in natural areas. The Nature Conservancy Wildland Invasive Species Team. https://www.invasive.org/gist/products/handbook/methods-handbook.pdf.

Vitousek, P. M., D́Antonio, C. M., Loope, L. L., & Westbrooks, R. (1996). Biological Invasions as Global Environmental Change. *American Scientist, 84*, 468–478.

Whittaker, R. H., Buol, S. W., Niering, W. A., & Havens, Y. H. (1967). A soil and vegetation pattern in the Santa Catalina Mountains, Arizona. *Ecology, 48*, 440–450.

Woledge, J. (1978). The effect of shading during vegetative and reproductive growth on the photosynthetic capacity of leaves in a grass sward. *Annals of Botany, 42*, 1085–1089. https://www.jstor.org/stable/42764092.

Wraage, C. P. (2023). *Caracterización y comparación de la composición y diversidad de plantas vasculares de laderas norte y sur, en sierras del sudeste bonaerense invadidas y no invadidas por Acacia melanoxylon Dissertation thesis*. Mar del Plata, Buenos Aires, Argentina: Universidad Nacional de Mar del Plata.

Yezzi, A. L., Nebbia, A. J., & Zalba, S. M. (2020). Synergistic Interactions of Grassland Fragmentation, Fire and Invasive Woody Plants in the Argentine Pampas. *Diversity, 13*(12), 637. https://doi:10.3390/d13120637.

Zalba, S. M., & Villamil, C. B. (2002). Woody plant invasión in relictual grasslands. *Biological Invasions., 4*, 55–72. https://doi.org/10.1023/A:1020532609792

Zalba, S. M., Cuevas, Y. A., & Boó, R. M. (2008). Invasion of *Pinus halepensis* Mill. following a wildfire in an Argentine grassland nature reserve. *Journal of Environmental Management, 88*(3), 539–546. https://doi.org/10.1016/j.jenvman.2007.03.018

Zalba, S. M., & Amodeo, M. R. (2017). Sex morphs and invasiveness of a fleshy- fruited tree in natural grasslands from Argentina. *Botany, 95*, 913–922. https://doi.org/10.1139/cjb-2017-0041

Zaninovich, S. C., Herrera, L., Carro, N. G., González Zugasti, E. A., & Montti, L. (2023). Bases para el manejo adaptativo de la leñosa invasora *Acacia melanoxylon* (Fabaceae) en la Reserva Natural Privada Paititi, sierras del Sistema de Tandilia, Argentina. *Boletín Sociedad Argentina de Botánica, 58*, 37–55. https://doi.org/10.31055/1851.2372.v58.n1.38462.

Zuloaga, F. O., Antón, A. M., & Rúgolo, Z. (2012a). *Flora Argentina. Flora Vascular De La República Argentina 3(1)*. Córdoba: Gráficamente Ediciones.

Zuloaga, F. O., Antón, A. M., & Rúgolo, Z. (2012b). *Flora Argentina. Flora Vascular De La República Argentina 3(2)*. Córdoba: Gráficamente Ediciones.

Zuloaga, F. O., Belgrano, M., & Antón, A. M. (2014a). *Flora Argentina. Flora Vascular De La República Argentina 7 (1)*. Córdoba: Conicet.

Zuloaga, F. O., Belgrano, M., & Antón, A. M. (2014b). *Flora Argentina. Flora Vascular De La República Argentina, 7*. Estudio Sigma S.R.L.

Zuloaga, F., Belgrano, M., & Zanotti, C. (2019). Actualización del Catálogo de las Plantas Vasculares del Cono Sur. *Darwiniana, Nueva Serie., 7*, 208–278. https://doi.org/10.14522/darwiniana.2019.72.861.