Vegetation of a hill grassland of the Paititi Natural Reserve (Pampa biome) and early detection of non-native species acting as invasive

Vegetación de un pastizal serrano de la Reserva Natural Paititi (Bioma Pampa) y detección temprana de especies no nativas actuando como invasoras

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Resumen

Introducción y objetivos: Las especies invasoras representan la mayor amenaza para la conservación. Los objetivos de este estudio fueron identificar la flora del pastizal serrano de la Reserva Natural Paititi, realizar la detección temprana de especies vegetales exóticas que pudieran estar actuando como invasoras y establecer la situación actual de aquellas especies nativas consideradas amenazadas.

M&M: Siguiendo el gradiente altitudinal se seleccionaron 14 sitios donde se registraron las características ambientales y la cobertura-abundancia de las especies vasculares. Con los datos recabados se realizó un Análisis de Coordenadas Principales (ACooP) que originó unidades de vegetación que fueron descritas según características ambientales, especies identificadas y tipo de comunidad vegetal. Adicionalmente, se destacaron las especies amenazadas y las exóticas más abundantes, en consecuencia consideradas invasoras.

Resultados: La riqueza total fue de 370 especies, correspondiendo el 26,5% a exóticas. Se identificaron 30 especies amenazadas. El ACooP reconoció ocho unidades de vegetación. Las especies consideradas invasoras fueron *Dactylis glomerata*, *Senecio madagascariensis*, *Holcus lanatus* y *Racosperma melanoxylon*, estas dos últimas con los mayores valores de cobertura.

Conclusiones: Para conservar la biodiversidad y minimizar el proceso de invasión, los esfuerzos de manejo deberían enfocarse en monitorear las especies amenazadas y controlar el avance de las invasoras. La detección temprana de dichas especies en ambientes similares sería fundamental para reducir invasiones vegetales.

Palabras clave

Especies exóticas, pastizales serranos, áreas protegidas, especies amenazadas.

Summary

Background and aims: Invasive species are the greatest threat to conservation. The objectives of this study were to identify the flora that thrive in a hill grassland of the Paititi Natural Reserve, to perform an early detection of non-native plant species that might be acting as invasive, and to establish the current situation of the native species considered threatened.

M&M: Following the altitudinal gradient, 14 sites were selected; the environmental characteristics and cover-abundance of the vascular plant species were recorded in each site. With the collected data, a Principal Coordinate Analysis (PCooA) was performed to group the sites into vegetation units that were later described considering environmental characteristics, identified species and plant community type. Additionally, the threatened species were specified, as well as the most abundant exotic ones, therefore considered invasive.

Results: Total richness reached 370 species, 26.5% corresponding to non-native ones. Thirty threatened species were identified. The PCooA grouped the sites into eight vegetation units. The non-native species considered invasive were *Dactylis glomerata*, *Senecio madagascariensis*, *Holcus lanatus* and *Racosperma melanoxylon*, these last two reached the highest coverage-abundance values.

Conclusions: To conserve biodiversity and minimize the invasion process, management efforts should be focused on monitoring the threatened species and controlling the advance of the non-native species acting as invasive. Early detection of those species in similar environments would be fundamental to facilitate rapid responses towards reducing invasions.

Key words

Exotic species, mountain grasslands, protected areas, threatened species.

Introduction

Biological invasions have been recognized in many parts of the world as the main agents of change in natural environments, generating harmful effects on ecosystems and biodiversity and imposing an enormous cost on human productive activities (Wittenberg & Cock, 2001; Pyšek & Richardson, 2010; van Kleunen *et al.,* 2018). The invasive plants are the non-native plant species –also called introduced, alien, or exotic species– that after their introduction into non-native natural environments, may spread, invading the new territory and generating a high impact on the new ecological niches (Bentivegna & Zalba, 2014). Nevertheless, not all non-native species become invasive, and the ones that do, are invasive only in some places, since the term invasive is associated with specific environmental conditions (Weber & Gut, 2004; van Kleunen *et al.,* 2018). However, species with a history of invasiveness tend to repeat this behavior in the places where they are introduced (Baskin, 2002). According to van Kleunen *et al.* (2018), the definition of an invasive species implicitly assumes that the non-native species should be locally abundant (i.e. produces offspring in large numbers). Therefore, by increasing their abundance in the near future, non-native species can become invasive.

Protected areas are a key component in responding to degradation and environmental changes. However, invasive plant species are a serious problem for protected area managers around the world (Foxcroft *et al.,* 2017; Brancatelli *et al.,* 2020). The management of these areas regarding invasive species involves different strategies: A) prevention and exclusion; B) early detection and rapid assessment; C) control, containment, and eradication (Rejmánek, 2000). Although prevention is the most efficient control strategy, on certain occasions, invasive species are already established in the territory (Anderson *et al.,* 2014; Brancatelli & Zalba, 2018). Since the plants are usually distributed in patches or aggregates according to the environmental characteristics, carrying out floristic surveys in different sites of the area of interest is essential for the early recognition of current or potential invasions and the establishment of management methodologies aimed at minimizing the process of invasion in early stages (Wittenberg & Cock, 2001; Waterhouse, 2003; Brancatelli *et al.,* 2020).

The Pampa biome, an extensive South American grassland region located in Uruguay, southern Brazil, and central Argentina (Scottá & da Fonseca, 2015) (Fig. 1A), has a high degree of landscape fragmentation. It was formerly characterized by grasslands and, to a lesser extent, shrubs (Cabrera, 1968; Cabrera & Zardini, 1978). However, the advance of the agricultural frontier has generated a homogeneous matrix of mainly agricultural lands interspersed with areas of natural or semi-natural vegetation, generally not cultivable (Herrera *et al.,* 2020). As a result, this region faces a serious and growing challenge associated with an increase in invasive non-native species (Frangi, 1975; Bilenca & Miñarro 2004; Echeverría *et al.,* 2017; Brancatelli & Zalba, 2018).

Toward the south of the Pampa biome, the Tandilia Hill System in Buenos Aires Province (Argentina) (Fig. 1A-B) is a discontinuous chain of low hills (called *sierras* in Spanish) and rocky outcrops. The Tandilia Hill System is approximately 350 km in length × 60 km in maximum width and less than 600 m elevation, extending NW to SE (Dalla Salda *et al.,* 2006). In these low mountainous areas, the development of agricultural activities is difficult, which facilitates the conservation of pristine vegetation remnants known as mountain grasslands (Frangi, 1975; Alonso *et al.,* 2009a; Herrera *et al.,* 2017; Kristensen *et al.,* 2014; Echeverría *et al.,* 2017; Brancatelli *et al.,* 2020). These areas are considered biodiversity "hotspots" because they harbor numerous native and endemic species, some of which are threatened (Delucchi, 2006; Herrera & Laterra, 2011; Kristensen *et al.*, 2014; Echeverría *et al*., 2017). Threatened species are considered priority species to preserve, and therefore a fundamental pillar to guide biodiversity conservation and sustainable development policies (IUCN, 2022). At the same time, native species are a very important source of ecosystem services (Barral & Maceira, 2012; Herrera *et al*., 2017). The Strict Nature Reserve of the Paititi Private Natural Reserve (PNR) is the largest nature reserve of the Tandilia Hill System. This reserve is considered a Valuable Grassland Area (Bilenca & Miñarro, 2004) and an Area of Interest for Conservation and Ecotourism (Chebez, 2005). The wide environmental heterogeneity present in the PNR has

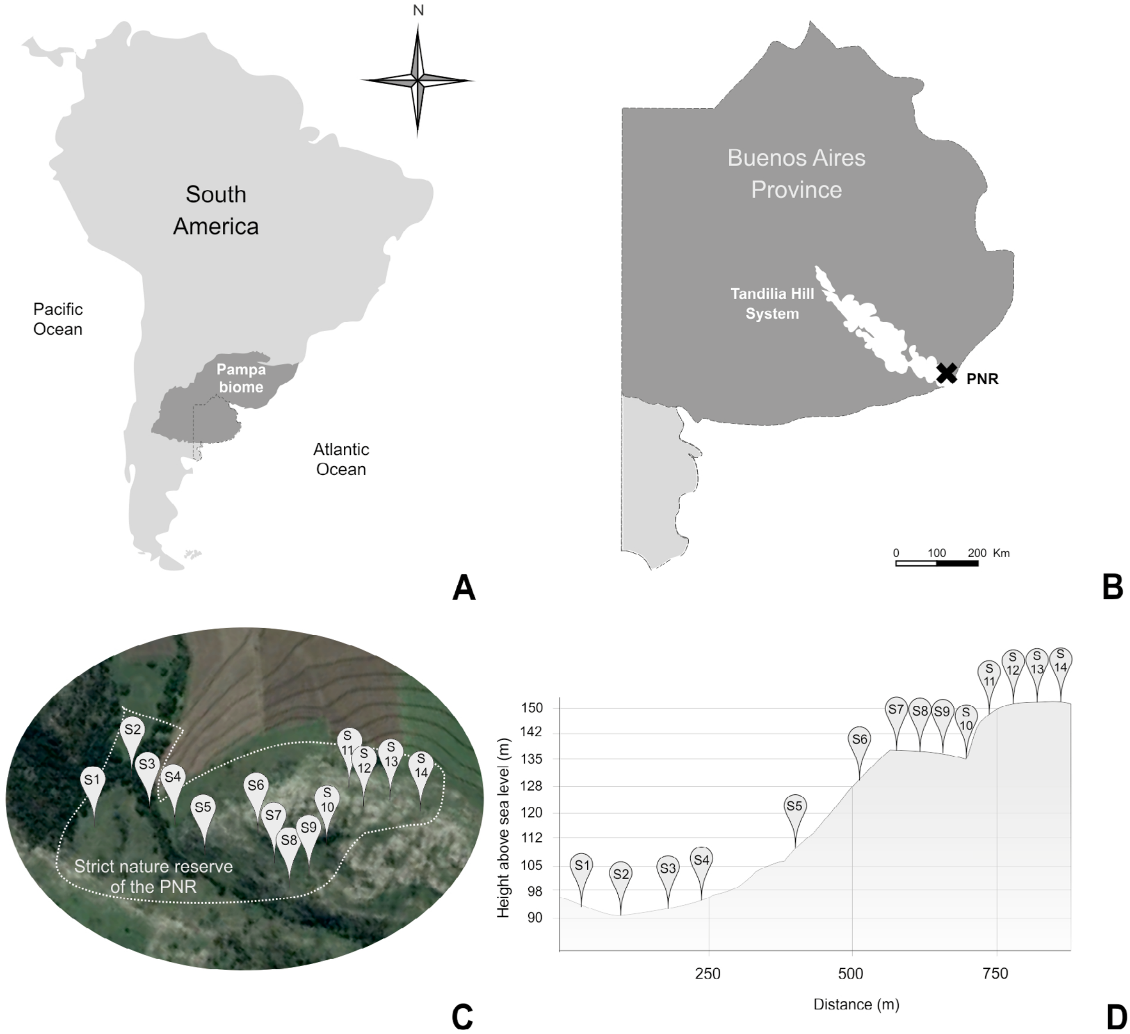

Fig. 1. A: Map of South America (light gray), with delimitation of the Pampa biome (dark gray), modified from Scottá and Da Fonseca (2015), and of Buenos Aires Province (dotted line); **B**: Buenos Aires Province with delimitation of the Tandilia Hill System and Paititi Natural Reserve (PNR); **C**: Delimitation of the Strict Nature Reserve area of the PNR (dotted line). Each marker shows one of the sites (S) selected for the study; **D**: Topographic profile reflecting the altitudinal gradient of the study area and the location of the analyzed sites.

led to the development of a great diversity of native fauna (Cicchino & Farina, 2007; Isacch *et al.*, 2016; Arcusa, 2016; Ferreti *et al.,* 2019; O'Connor *et al.,* 2020). Regarding the plant species, Echeverría *et al.* (2017) carried out a floristic survey in the PNR and identified 360 species, more than 25% being non-native species. These authors alerted that some of those species may become invasive and thus contribute to the deterioration of the natural areas of the reserve.

Considering the value of the PNR as a refuge for the flora and fauna of the grasslands and hill areas of the southeastern extreme of the Tandilia Hill System and the possible threat posed by invasive plants to biological diversity, it is important to know the distribution and representativeness of the plant species that can be found in the reserve. Therefore, the objectives of this work were: 1) to identify all the vascular plants (native and non-native ones) that thrive in sites with different environmental characteristics in the PNR; 2) to quantify their abundance in order to carry out the early detection of non-native species that might be acting as invasive ones; 3) to establish the current situation of those native species that were considered threatened in Buenos Aires Province.

Materials and Methods

Study area

The study was conducted in the hill grassland ecosystem of the PNR located in the southeastern Tandilia Hill System, in 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), in General Pueyrredon district, Buenos Aires Province, Argentina (Fig. 1B). The main vegetation landscape is a large open area covered with grasses (grasslands), although there are also oreophilic steppes, scrublands, and hydrophilic communities (Cabrera, 1968; Cabrera & Zardini, 1978). In particular, in the mountainous areas, different types of grassland and other plant communities can thrive, such as the shrubs and scrubs that are typical of mountain and rocky soils (Frangi, 1975).

The PNR is divided into two areas: one dedicated to educational, recreational, and livestock activities, and the other one categorized as a Strict Nature Reserve so it remains as an area with minimal anthropic interference. The study was carried out in the Strict Nature Reserve, where there is a hill area called *Sierra Chica* and a stream surrounding the base of the west slope of that hill, forming a small pond before continuing its N-E path (Fig. 1C). *Sierra Chica* is oriented N-S, with a maximum elevation of 156 m.a.s.l. in the rocky area at the top, and a minimum of 86 m.a.s.l. in the pond. The hill blocks are mainly composed of a crystalline basement on which Eopaleozoic sediments were deposited. This sediment accumulation gave rise to the development of Mollisol soils of variable depth, loamy texture, high content of organic matter, and slightly acidic pH (Osterrieth & Cabria, 1995; Dalla Salda *et al.,* 2006; Álvarez *et al.,* 2012). 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 also characterized by maritime temperate 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 *et al*., 2000; INTA, 2022).

Sampling and data collection

Following the altitudinal gradient, 14 sites were selected. These sites varied in the physiognomy of the vegetation (grasses, shrubs, or trees), dominant species, and predominant substrate (soil, rock, or water). The sites were georeferenced using a global positioning system (GPS) and digitized on an aerial image of the study area (Fig. 1C-D). A plot was delimited at each site based on the concept of minimum area, with plot size ranging from 4 m^2 to 200 m^2 (Matteucci & Colma, 2002; Escaray, 2007). The soil type was identified. Additionally, electrical conductivity, pH, organic matter content, and available phosphorus were determined in ten soil subsamples of the first 10 cm of the profile by the Soil Analysis Laboratory of Balcarce Agricultural Experimental Station belonging to the Instituto Nacional de Tecnología Agropecuaria.

The floristic composition and the specific cover-abundance in each plot were determined from september 2018 to december 2019. To estimate species coverage, the Braun-Blanquet cover-abundance scale modified by Westhoff and Maarel (1978) was used.

The taxa surveyed were determined at the specific level 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; Zuloaga *et al.,* 2014a, b). For each species, botanical family, origin (non-native, native, or cosmopolitan), and threatened situation in Buenos Aires Province (Delucchi, 2006) were determined.

Analysis of data

Total richness (number of species), as well as richness per site, representativeness of botanical families, and distribution of species by category of origin were determined. Correlations between site altitude and percentage of species according to origin (native versus non-native + cosmopolitan) were calculated using the Pearson correlation (*r*) test (α = 0.1%) through the "cor.test" function of the "corrplot" package of RStudio software Version 1.2.5033 (R Core Team, 2014).

To associate the sites and generate vegetation units with similar characteristics considering their environmental attributes and the species at each site, the qualitative and quantitative variables were jointly analyzed by a Principal Coordinate Analysis (PCooA) using Gower similarity coefficient (Gower, 1971; Cuadras, 2014). The analysis was carried out in the RStudio software Version 1.2.5033 (R Core Team, 2014) using the "cluster" package (Maechler *et al.,* 2016).

The vegetation units were described considering the environmental characteristics, the identified flora and the type of plant community according to Frangi (1975). Additionally, the threatened species were specified, as well as the most abundant non-native ones (≥ 5% cover-abundance values), therefore considered invasive.

Results

In the study area, the total richness reached 370 vascular species, 72.5% being native, 26.5% non-native and 1% cosmopolitan (Appendix S1); ten taxa, *Acmella decumbens* (Sm.) R.K. Jansen, *Aira caryophyllea* L., *Anthoxanthum odoratum* L., *Eryngium ebracteatum* Lam., *Galium richardianum* (Gillies ex Hook. & Arn.) Hicken, *Nassella trichotoma* (Nees) Hack, *Parodia submammulosa* (Lem) R. Kriesli, *Plantago lanceolata* L., *Tripogonella spicata* (Nees) P.M. Peterson & Romasch., and *Wigginsia tephracantha* (Link & Otto) D.M. Porter, had not been previously detected by Echeverría *et al.* (2017) at the study area. The surveyed species belong to 69 families, the most representative ones being Poaceae (78), Asteraceae (72), Fabaceae (21), Cyperaceae (13), Apiaceae (12), Solanaceae (11), and Brassicaceae (10) (Appendix S1). Some taxa were found only in one site and with very low cover values, such as *Pterocaulon cordobense* Kuntze*, Hypericum connatum* Lam*.,* and *Lemna gibba* L., while others were found in most of the sites and in some of them with more than 50% coverage, such as *Racosperma melanoxylon* (R. Br.) Mart. The greatest habitat variation was presented by four species, which were found in nine of the fourteen sites, two non-native species, *Cirsium vulgare* (Savi) Ten, and *Senecio madagascariensis* Poir., and two native ones, *Cypella herbertii* (Lindl.) Herb. and *Vicia nana* Vogel.

The vascular species richness in the plots varied from 6 to 161 species. In the highest sites such as the hillside and summit areas, the percentage of native plants was over 70%, except for site ten (S10) (Fig. 2). This trend was confirmed by the correlation between altitude and percentage of native species, which was significant and positive ($r = 0.67$; p-value = 0.00829); consequently, it was also significant, but negative, between altitude and percentage of non-native + cosmopolitan species.

Relationship between sites

The first three coordinates of the PCooA contribute explaining 45.82, 15.57, and 10.02% of the total variability, respectively. The projection of the study sites in the three-dimensional space showed the formation of eight clusters, which represent the vegetation units (Fig. 3).

Characteristics of the vegetation units

The soils of the units were dark to very dark brown and slightly acid (pH= 5.3–5.8); the superficial horizons had high root density, low electrical conductivity (0.3–0.8 mmhos), low to moderate phosphorus content (3.4–7.6 ppm), and a high percentage of organic matter (6.3–11.4%). The greatest abiotic differences were established in the type and depth of the soil, the slope (Table 1), the degree of solar exposure, and the relief position. For information on the species of each vegetation unit, consult the Appendix S1.

Base Grassland: This unit corresponds to an area located at the base of the hill (Fig. 4A; Table 1). The species richness reached 114 vascular plants, 42% being non-native ones. The area was physiognomically dominated by taxa of the Poaceae family, of the genera *Aristida* L*., Nassella* E. Desv., *Jarava* Ruiz. & Pav., and *Piptochaetium* J. Presl. Floristically, the Base Grassland resembles the typical "Flechillar" grassland community of the Tandil hills dominated by grasses of stabbing caryopses, which look like small arrows (Flechillar derives from the Spanish word 'flecha', which means arrow in English). Six of the species mentioned as threatened in Buenos Aires Province were found, although only two (*Acanthostyles buniifolius* (Hook. & Arn.) R. M. King & H. Rob. and *Senecio selloi* (Spreng.) DC.) had cover-abundance levels greater than 5% (Table 2). Among the non-native species, *Holcus lanatus* L. (Fig. 5A) had the highest level of

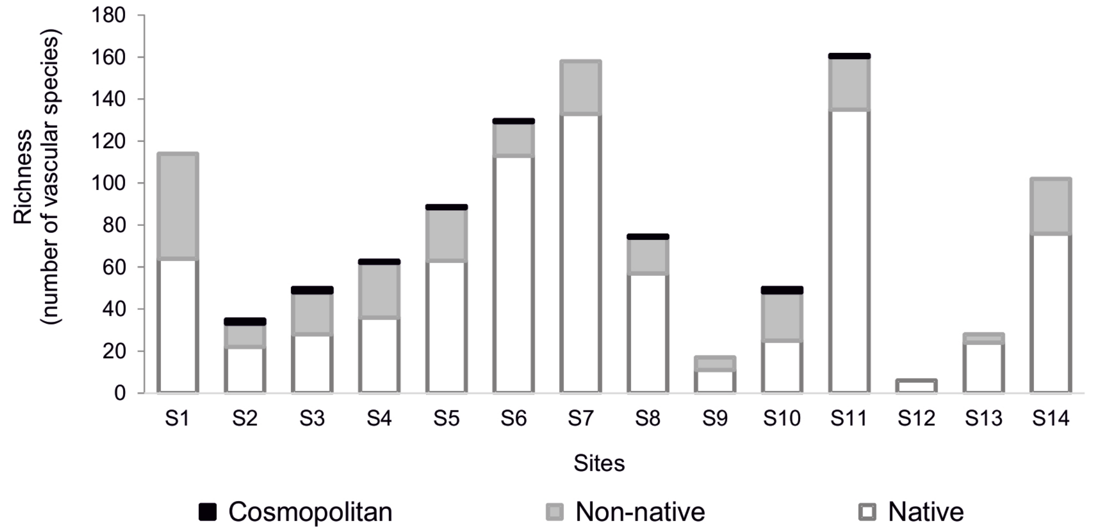

Fig. 2. Vascular species richness in 14 sites of the Strict Nature Reserve area of the Paititi Natural Reserve (Buenos Aires, Argentina) according to their origin category (native, non-native, cosmopolitan).

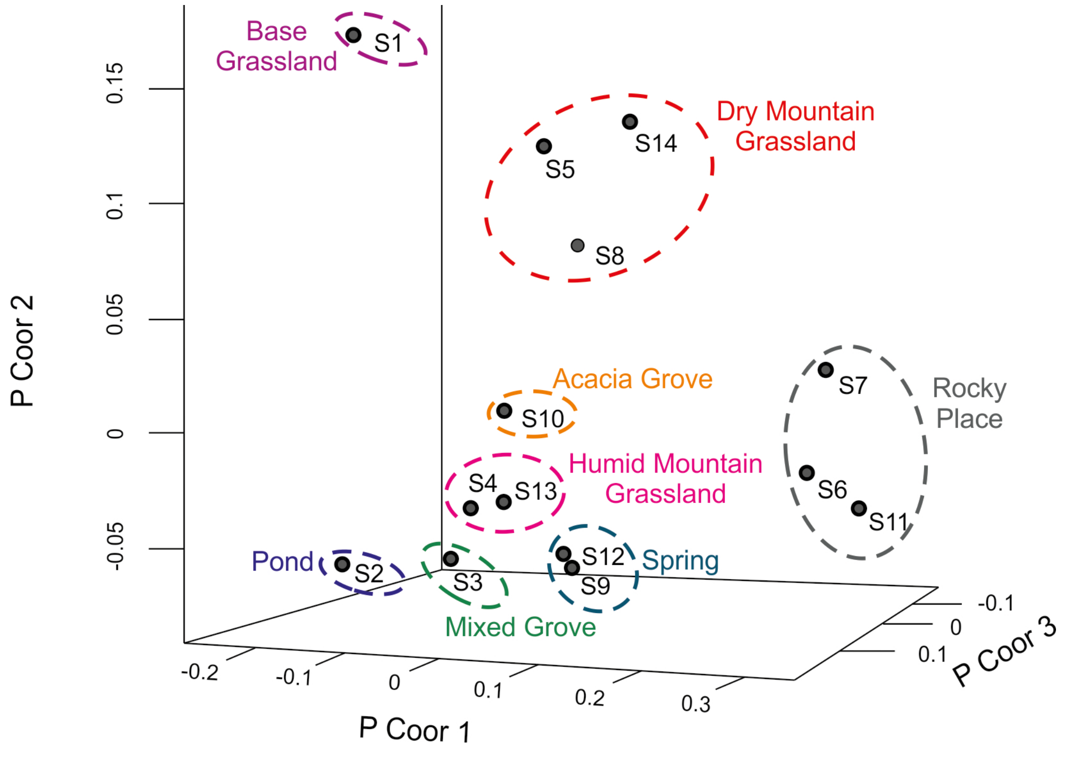

Fig. 3. Principal Coordinates Analysis using combined data of presence/absence of vascular species and environmental characteristics of 14 sites (S) in the Strict Nature Reserve area of the Paititi Natural Reserve (Buenos Aires, Argentina). The clusters (vegetation units) are indicated by dashed lines on the three first principal coordinates.

Table 1. Slope, soil type and soil depth of the vegetation units of the Strict Nature Reserve area of the Paititi Natural Reserve (Buenos Aires, Argentina).

Vegetation Unit	Slope (%)	Soil Type	Soil Depth (cm)
Base Grassland (S1)	7 – 9	Typic Argiudoll	> 100
Pond (S2)	< 2	Typic Argiacuoll	> 100
Mixed Grove (S3)	< 2	Typic Argiudoll	> 100
Humid Mountain Grassland (S4, S13)	< 2	Aquic Argiudolls	50 – 100
Dry Mountain Grassland (S5, S8, S14)	2 – 45	Typic Argiudoll	10 – 80
Rocky Place (S6, S7, S11)	2 – 45	Hapludoll lytic	1 – 40
Spring (S9, S12)	< 2	Hapludoll lytic	1 – 20
Acacia Groove (S10)	3	Typic Argiudoll	> 100

cover-abundance (15-25%), followed by *Dactylis glomerata* L. (Fig. 5B) (5-15%).

Pond: This unit is located in the lowest part of the study area, surrounding a shallow pond (Fig. 4B; Table 1). Thirty-five species were found being 31.4% non-native and 5.7% cosmopolitan. This unit has some floating and marsh plants that are typical of the wetlands and watercourses of Buenos Aires Province (Appendix S1). In this vegetation unit, one native species was categorized as threatened (*Cypella herbertii* ssp. *wolffhuegeli* (Lindl.) Herb. (Hauman) Ravenna) and with very low cover-abundance level (Table 2). The non-native species with the highest cover-abundance level was *Senecio madagascariensis* Poir (5-15%) (Fig. 5C).

Mixed Grove: This unit corresponds to an implanted grove following the eastern course of the stream. It is located at the base of the hill (Fig. 4C; Table 1). The total richness resulted in 50 vascular species, and 36% of the species were non-native ones. The vegetation consisted mainly of perennial species dominated by phanerophytes (34%), *Celtis tala* Gillies ex Planch being the most abundant species. The trees were accompanied by shorter herbaceous plants adapted to the canopy shade. Four threatened species were found in this unit, whose coverage was very low, except for *Colletia paradoxa* (Spreng.) Escal. with 5% (Table 2). No exotic species exceeded 5% cover.

Humid Mountain Grassland: This type of grassland occurs in two sectors of the study area: one at the base, near temporary water courses, and another in depressions or meadows between the rock blocks at the top of the hill (Fig. 4D; Table 1). In this unit, 81 species were identified being 34.5% non-native and 1.2% cosmopolitan. The flora corresponds to the grassland community named "Pajonal (tall grasses) of tussock paspalum", as it is mainly composed of herbaceous plants but physiognomically dominated by *Paspalum quadrifarium* Lam ("tussock paspalum"). In fact, this species showed high cover-abundance levels (50-75%). Three threatened species were found in this vegetation unit, all with very low cover-abundance levels (Table 2). Additionally, *Holcus lanatus* was the non-native species with the highest level of cover-abundance (5-15%).

Dry Mountain Grassland: This unit is found on very steep slopes and at the top of the hill (Fig. 4E; Table 1). The total richness reached 156 species: 25% non-native and 0.6% cosmopolitan. The vegetation was mainly represented by "flechillas" and dicot species, with less than 20% of tree and shrub species. The floristic composition is similar to that found on shrubland, with physiognomic dominance of *Acanthostyles buniifolium, Baccharis coridifolia* DC., *B. dracunculifolia* ssp. *tandilensis* (Speg.) Giuliano and *Colletia paradoxa*, and in a lower stratum, the flora varied according to the position on the ground. The non-native species with the highest level of cover-abundance were *Racosperma melanoxylon* (5-15%), *Dactylis glomerata* (5-15%), and *Holcus lanatus* (15-25%) (Fig. 5). Besides, 19 threatened species were found, three of which had coverage greater than 15% (Table 2).

Table 2. Threatened situation and cover of 30 of the native species considered threatened in Buenos Aires Province according to Delucchi (2006), found in eight vegetation units of the Strict Nature Reserve area of the Paititi Natural Reserve (Buenos Aires, Argentina).

Species	Threatened Situation In Buenos Aires Province	Vegetation Unit							
		Base Grassland	Pond	Mixed Grove	Humid Mountain Grassland	Dry Mountain Grassland	Rocky Place	Spring	Acacia Grove
Adiantum raddianum (L.) Fée	LC			Occasional		Occasional			
Acanthostyles buniifolius (Hook. & Arn.) R. M. King & H. Rob.	CR	15 to 25				15 to 25	15 to 25 %		
Baccharis dracunculifolia DC. ssp. *tandilensis* (Speg.) Giuliano	CR	± 5 %		Less than 5 %		Occasional to 15 %	Less than 5 % to 25		Occasional
Bipinnula pennicillata (Rchb. f.) Cisternas & Salazar	VU						Occasional to less than 5 %		
Colletia paradoxa (Spreng.) Escal.	LC	Less than 5 %		± 5 %		Less than 5 % to 15 %	Less than 5 % to ± 5 %		
Chromolaena squarrulosa (Hook. & Arn.) R. M. King & H. Rob.	DD				Occasional	± 5 %	Occasional		
Cypella herbertii (Lindl.) Herb. ssp. *wolffhuegeli* (Hauman) Ravenna	VU	Occasional	Occasional			Occasional to less than 5 %			
Danthonia montevidensis Hack. & Arechav.	DD					Occasional	Occasional to less than 5 %		
Eryngium serra Cham. & Schltdl.	DD				Occasional	Occasional	Occasional		
Facelis retusa (Lam.) Sch. Bip.	LC						± 5 %		
Galium hypocarpium (L.) Endl.	VU			Occasional		Less than 5 %			Less than 5 %
Gamochaeta pensylvanica (Willd.) Cabrera	VU	Less than 5 %				± 5 %	Occasional		Less than 5 %
Glandularia tenera (Spreng.) Cabrera	CR						Less than 5 %		
Gymnocalycium gibbosum (Haw.) Pfeiff.	V						Less than 5 % to ± 5 %		
Hieracium tandilense Sleumer	CR						Less than 5 %		
Hypochaeris neopinnatifida C.F. Azevedo-Gonçalves & Matzenb.	VU					Occasional			
Hypochaeris pampasica Cabrera	LC					Occasional	Occasional		
Juncus pallescens Lam.	LC				Occasional		Occasional	Occasional	

Species	Threatened Situation In Buenos Aires Province	Vegetation Unit							
		Base Grassland	Pond	Mixed Grove	Humid Mountain Grassland	Dry Mountain Grassland	Rocky Place	Spring	Acacia Grove
Lepidium tandilense Boelcke	VU						± 5 %		
Melica parodiana Torres	VU					Occasional	Occasional to less than 5 %		
Mimosa rocae Lorentz & Niederl.	VU						Occasional		
Plantago tandilensis (Pil.) Rahn	VU					Occasional	Occasional to less than 5 %		
Poa iridifolia Hauman	VU					Occasional	Occasional		
Senecio selloi (Spreng.) DC.	LC	5 to 15 %				± 5 % to 25	Occasional		Occasional
Sommerfeltia spinulosa (Spreng.) Less.	LC					Occasional	Less than 5 %		
Stevia satureiifolia (Lam.) Sch. Bip. ex Klotzsch var. *patagonica*	LC					Occasional	Less than 5 %		
Tillandsia bergeri Mez	VU						Occasional		
Vicia setifolia Kunth. var. *bonariensis* Burkart	VU					± 5 %			
Zephyranthes bifida (Herb.) Nic. García & Meerow	VU						± 5 %	Occasional	
Zephyranthes gracilifolia (Herb.) G. Nicholson	DD						± 5 %		

References. CR: Critically Endangered; DD: Data Deficient; VU: Vulnerable; LC: Least Concern.

Fig. 4. Images of the sites that are part of the vegetation units obtained from the PCooA. **A**: Base Grassland; **B**: Pond; **C**: Mixed Grove; **D**: Humid Mountain Grassland; **E**: Dry Mountain Grassland; **F**: Rocky Place; **G**: Spring; **H**: Acacia Grove.

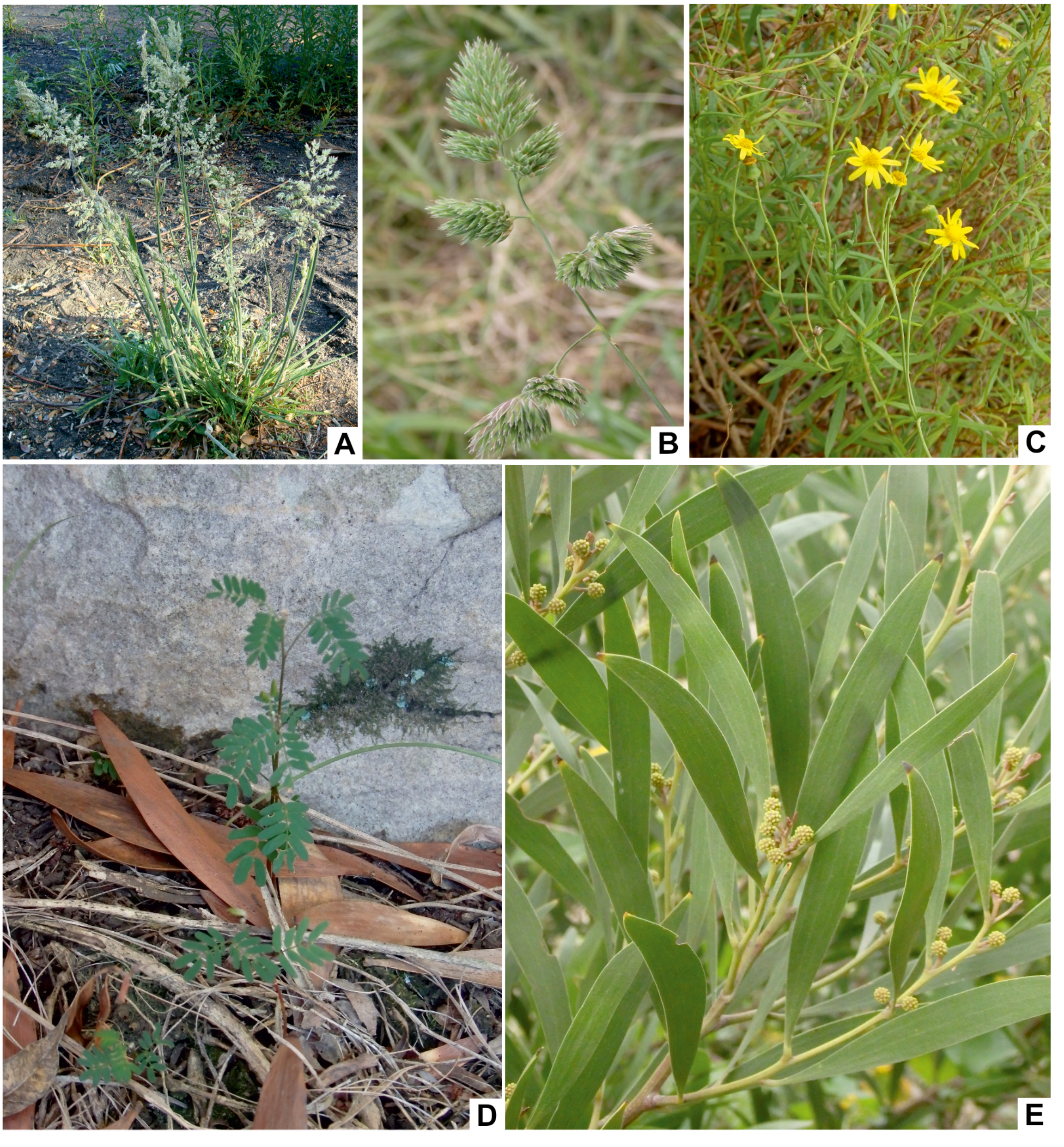

Fig. 5. Most abundant non-native species found in different areas of the Strict Nature Reserve area of the Paititi Natural Reserve (Buenos Aires, Argentina). **A**: *Holcus lanatus;* **B**: *Dactylis glomerata;* **C**: *Senecio madagascariensis;* **D**: seedling of *Racosperma melanoxylon;* **E**: adult of *R. melanoxylon.*

Rocky Place: This unit is located in elevated areas corresponding to the slopes and top of the hill, with abundant rocky outcrops, and stony surfaces (Fig. 4F; Table 1). It is the richest vegetation unit since a total of 239 species were identified: 17% non-native and 0,8% cosmopolitan. The vegetation consisted mainly of herbaceous species, and to a lesser extent, sparse trees and shrubs (38 species). Twenty-five threatened species were found, most of which had low coverage (Table 2). The non-native species with the highest level of cover-abundance was *Racosperma melanoxylon* (5-15%).

Spring: This unit is situated in areas of high to intermediate position in the hill, rocky outcrops of low blocks, which have flat to concave shape and are almost continuous. After the rains, this unit presents temporary springs and small holes with little or no drainage (Fig. 4G; Table 1). The vegetation reached 20 herbaceous species: 30% non-native species. Most of the floristic components are typically found on the water courses of the region, while some are common species of the rocky areas. Two threatened native species were found (*Juncus pallescens* Lam. and *Zephyranthes bifida* (Herb.) Nic. García & Meerow), both with very low coverage (Table 2). No exotic species exceeded 5% cover.

Acacia Grove: This unit occupies areas on the slope of the mountain (Fig. 4H; Table 1). Fifty taxa were identified in this unit, 42% non-native, and 4% cosmopolitan species. The dominant physiognomic species was the non-native species *Racosperma melanoxylon*, which was accompanied in the lower stratum by sparse herbaceous species. In fact, the cover-abundance level of *R. melanoxylon* ranged between 50 and 70%. Four threatened species with very low coverage were found in this unit (Table 2).

Threatened species

In the study area, 30 of the native species considered threatened in Buenos Aires Province (Delucchi, 2006) were identified (Table 2). Regarding their category of threat, 14 are listed as “Vulnerable” and four as “Critically Endangered”. Some of the threatened species were found in two or more vegetation units, but ten of them were only found in a single vegetation unit, in some cases in the Dry Mountain Grassland and in others in the Rocky Place, but always with very low cover-abundance levels. In contrast, *Acanthostyles buniifolius*, *Baccharis dracunculifolia* ssp. *tandilensis*, *Colletia paradoxa,* and *Senecio selloi* exhibited cover-abundance levels ranging from 15 to 25%, and were found in at least three vegetation units.

Discussion

Flora

In a previous survey of the vascular plants of the Strict Nature Reserve area of the PNR, Echeverría *el at.* (2017) identified 360 taxa at the species level. The present study allowed the detection of ten species not previously found in the reserve, possibly due to their low frequency. Therefore, the richness amounted to 370 vascular species. This increase did not affect the predominance of the Poaceae and Asteraceae families, which continued to be the best represented, and neither did it change the proportion of geographic origin of the species since native plants far exceeded the set of non-native and cosmopolitan species by bringing together 72.5% of the species.

The vegetation units varied mainly due to their position in the relief, the slope, the type and depth of the soil, and the established plant community. This coincides with several authors who noted that the floristic composition and distribution of vascular species in hills and mountain areas are associated with the environmental heterogeneity determined mainly by topographic variants, even in short distances (Frangi & Bottino, 1995; Guerrero Campo *et al.,* 1999; Cantero *et al.,* 2014). Among the vegetation units considered in the study area, the flora varied in richness, proportion of species according to their status, and cover-abundance of each species. Some vegetation units were similar to the plant communities described by Frangi (1975) for the Tandil hills of the Albion Group. In some cases, the resemblance was high, as the dominant and main companion species coincided. In others, due to the absence of some species or the replacement of some physiognomic dominants, the flora seemed to be a variant of the community described as “typical”. For example, *Nassella poeppigiana* (Trin. & Rupr.) Barkworth and *Koeleria permollis* Nees, dominant species in the Tandil Flechillar according to Frangi (1975), were not found in the in Base grassland vegetation unit. A similar case was detected in the Rocky place where the floristic composition of the vegetation unit coincides mostly with the community described by Frangi (1975) for Tandil hills named “Roquedal típico” (Typical rocky community), except for several species of physiognomic relevance that were not found in study area, such as *Andropogon ternatus* (Spreng.) Nees, *Arjona tuberosa* Cav. and *Hatschbachiella tweediana* (Hook. & Arn.) R.M. King & H. Rob., among others. Although the detected absent species should be the object of detailed studies, this situation could be due to the location of the PNR, exceeding the distribution limit of these species. This hypothesis would apply to *Hatschbachiella tweediana*, a species for which there are no records in the southern portion of the Tandilia Hill System (Alonso *et al.,* 2009a; Alonso *et al.,* 2009b; Flora Argentina, 2022; Flora del Cono Sur, 2022; GBIF, 2022; iNaturalist, 2022). In turn, some of the absent species, such as *Andropogon ternatus* and *Arjona tuberosa,* have been collected in other hills in General Pueyrredon district, and in Balcarce, a neighboring district (Alonso *et al.,* 2009a; Alonso *et al.,* 2009). In these cases, the action of anthropic alterations (e.g. indiscriminate grazing, urbanization, fire) could have affected the presence of these plants in the study area.

The vegetation units located in high ground positions had the highest total richness and native flora and the dominance of perennial species, in particular geophytes, in agreement with Cantero *et al.* (2017) for rocky mountain areas of the *Sierra de Los Cóndores* in Córdoba Province (Argentina). The sites located in high and intermediate positions of the relief tend to present shallow soils, high exposure to solar radiation, and a large proportion of rocky outcrops and stony surfaces. Therefore, these sites are subjected to greater arid conditions than those located on lower parts of the slopes or in sectors totally or partially shaded by tree canopy (Mazzola *et al.,* 2008; Aguirre Mendoza, 2013; Kristensen & Frangi, 2015). Exotic species are not adapted to these environmental conditions, hence their lower number or representability in those sites.

In the rocky areas and elevated slopes of the study area, the development of phanerophytes is restricted, mainly because of the shallow soils, and hence greater richness of this kind of species is found at the base and on the slopes of the hill. In addition, in the sites located at lower ground positions, a greater number of non-native species were registered, mostly therophytes. The greater abundance of annual cycle species is related to the greater anthropization that characterizes the surrounding rural landscape (Vervoorst, 1967; Zalba & Villamil, 2002; Cantero *et al.,* 2017).

The rural fields close to the PNR would act as plant reproduction reservoirs, and the wind, waterways, birds, mammals, invertebrates, and humans as vectors for their dispersal, introduction, and spread of plant species in a certain area. This situation would promote the colonization and proliferation of non-native species toward the hill grasslands of the PNR, thus affecting the ecosystem.

Threatened species

Thirty vascular species listed as threatened in the Buenos Aires Province (Delucchi, 2006) were found. This result coincides with that found by Echeverría *et al.* (2017) for the same area of the PNR, which indicates that no species have disappeared over the last years when the reserve has been closed to the public. These species are mostly endemic to the mountain grasslands (Echeverría *et al.,* 2017), so they only thrive in mountain environments with little intervention, such as the Strict Nature Reserve area of the PNR. This situation reveals the important role of this reserve in the conservation of mountain grassland flora in general, and of threatened species in particular, as well as warns about the vulnerability of poorly represented taxa and the need to consider them in future management plans.

According to the data collected in the present study, the species *Acanthostyles buniifolius, Baccharis dracunculifolia* ssp. *tandilensis, Colletia paradoxa,* and *Senecio selloii*, which have been cited as threatened in Buenos Aires Province (Delucchi, 2006), were recorded with high levels of cover-abundance and in several sites. This information is encouraging as it indicates that these entities are currently out of danger in the Strict Nature Reserve area of the PNR. In particular, for *Baccharis dracunculifolia* ssp. *tandilensis* and *Senecio selloi*, changes in habitat preference have been reported in the area: *B. dracunculifolia* extending from the hills to the plains near the Atlantic Ocean coast (Scaramuzzino *et al.,* 2015; Manfreda *et al.,* 2020) and *S. selloi* to grasslands dedicated to livestock grazing (Fernández, 2011). Therefore, new surveys in the province could yield information that accounts for the current distribution of native species in ecosystems close to the studied one and thus clarifying the threatened status of the species and eventually incorporating new taxa into the plant red list.

Invasive species

Currently, the most important problems in the PNR associated with invasions involve the non-native species *Dactylis glomerata, Senecio madagascariensis, Holcus lanatus* and *Racosperma melanoxylon* (Fig. 5). These exotic taxa had the greater cover-abundance values and the ability to thrive in different environmental conditions, since they were registered in various vegetation units. All of them have already been cited as invasive species in different locations of Argentina and vary in life forms, morphological attributes, reproduction strategies and invasion capacity (Ahumada *et al.,* 2016). Management efforts on the PNR should be focus on monitoring the threatened species and controlling the non-native with the highest value of cover-abundance in each vegetation unit. The characteristics of each species should be considered in planning the management of the invaded vegetation units, since the integrated control of an invader implies, among other requirements, having complete knowledge of the bioecology of the species (Bentivegna & Zalba, 2014).

Senecio madagascariensis is an herbaceous perennial species known as "fireweed". It belongs to the Asteraceae family and is native to South Africa and Madagascar. It is a toxic species, recognized as invasive in different parts of the world (Dematteis *et al.,* 2020). *S. madagascariensis* has been reported in the Argentinean Pampas as a weed in extensive farmlands in the past (Verona *et al.,* 1982), while it is currently a problem in cattle fields and a growing concern in farms under no-tillage systems and in ruderal areas (Ahumada *et al.,* 2016; Dematteis *et al.,* 2020; Diez de Ulzurrun personal communication, 2022). It is a species with a high production of seeds per plant and easy to disperse thanks to a hairy pappus, characteristics that determine their invasive nature. *Dactylis glomerata* ("orchard grass") and *H. lanatus* ("Yorkshire fog" or "velvet grass") are perennial microthermal species that belong to the Poaceae family. These species are native to Europe and were brought into Argentina as forage. They escaped from cultivation and have become weed species that are considered invasive in different parts of the American continent due to their prolific seed production and their ability to reproduce from tillers (Bastow *et al.,* 2008; Ahumada *et al.,* 2016). *Racosperma melanoxylon* (*Acacia melanoxylon* R. Br.; "Australian blackwood") is an arboreal species native to Australia that belongs to the Fabaceae family. In the southeast of Buenos Aires, it was introduced for its cultivation as ornamental and forestry (Martínez Crovetto, 1947; Carranza, 2007). This evergreen tree causes shade on the lower stratum and has allelopathic compounds that affect the growth and survival of other plants (González *et al.,* 1995; Hussain *et al.,* 2011). The seeds have the ability to remain viable in the seed bank for many years and their germination is stimulated by occasional fires that scarify the seeds (Arán *et al.,* 2017). It also has gemiferous roots and high seed production (Ahumada *et al.,* 2016; Arán *et al.,* 2017). *R. melanoxylon* has shown to be highly invasive in other mountain grasslands of the Tandilia Hill System where it occurs in the wild and it seems to be advancing on adjacent sectors of the hills (Ahumada *et al.,* 2016; Gandini *et al.,* 2019; De Rito *et al.,* 2020).

In relation to the management of the identified invaders in the study area, mechanical controls in *H. lanatus, D. glomerata* and *S. madagascariensis* have not been successful while the use of herbicides has allowed effective chemical controls (Villalba & Fernández, 2005; Ahumada *et al.,* 2016). In the particular case of *R. melanoxylon*, Arán *et al.* (2017) proposed controlling this species by cutting down the plants, both adults and juveniles, as well as uprooting all the roots to prevent new shoots from emerging. They also recommend minimal soil disturbance to prevent mechanical scarification of the seeds in the seed bank. They also suggest favoring the colonization of the land with one or several fast-growing native species to prevent *R. melanoxylon* seedlings from prospering. On the other hand, in a study performed by Campos *et al.* (2002), the combined felling of trees and herbicide application was effective at controlling *R. melanoxylon*. Therefore, a similar strategy could be suitable to control the proliferation of this species in the PNR and in other mountain grasslands. Beyond this, it is important to consider that the control strategy must be adjusted to each situation, and may vary depending on the location of the plant invasion on the ground (top, slope, base of the hill).

It is important to mention that in the study area some non-native species were found that have a history of invasion in mountain grasslands of the Pampa biome but with low representativeness in the PNR. Such is the case of *Eucalyptus camaldulensis* Dehnh., *Gleditsia triacanthos* L., *Ligustrum lucidum* W. T. Aiton, *Rubus ulmifolius* Schott, *Pinus radiata* D. Don and *Prunus mahaleb* L., among others (Ghersa *et al.,* 2002; Zalba & Villamil, 2002; Hoyos *et al.*, 2010; Mazzolari *et al.,* 2011; Ferreras *et al.,* 2014; Mazzolari & Comparatore, 2014; Ahumada *et al.,* 2016).

Therefore, periodic monitoring of the evolution of these species' expansion and rapid actions for their control could prevent future problems of plant invasions and facilitate the protection of hill and mountain grassland biodiversity.

Prevention and early detection are the most efficient methods for dealing with invasive species. When the invasion process advances, the possibility of carrying out successful control measures decreases, management costs and impacts generated by an invasion process increase, and sometimes the problem becomes irreversible (Anderson *et al.,* 2014; Brancatelli & Zalba, 2018). Therefore, to avoid increasing invasions and the appearance of a new invasion focus, vegetation units should be monitored by sporadic surveys. Thus, when an invasive species is detected, rapid action can be taken to restore the affected site or to minimize the invasion process in the early stages, thus preserving the biodiversity.

Conclusions

Although this study was carried out in a small sector of the Tandilia Hill System, the results provide information to understand the capacity of the hill grasslands of the Pampa biome to act as native flora refuges and the importance of maintaining these areas safe from biological invasions in order to conserve the biodiversity.

The scarce resources and differing priorities sometimes make it difficult to respond against plant invasions, a situation that worsens over time. Early detection and notification of new invaders may lead to the implementation of eradication or control programs at the beginning of the invasion process. We believe that gathering and disseminating information about the spread of potentially serious non-native plant species and about problems associated with them will benefit the entire region and prevent invasions in similar areas. We need to emphasize that, although non-native species can generate negative ecological and productive effects, they also perform important ecological functions, such as the provision of food and shelter for insects and small vertebrates. Therefore, the performance of control measures for this group of plants must be carefully evaluated by considering the advantages and disadvantages of making this decision.

Finally, we suggest promoting environmental education and citizen participation activities aimed at valuing and caring for the hill natural environments of the Pampa biome and raising awareness about their importance within the framework of biodiversity conservation policies.

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