



Early Detection of Invasive Species on Roadside Slopes in An-Dean Patagonian Forests of *Austrocedrus chilensis* (Argentina) [†]

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Abstract: The invasion of exotic plants threatens biodiversity, affecting ecosystem services and ecological processes in native ecosystems. Road construction creates new environments and contributes to the introduction and spread of exotic and invasive plants. This study aimed to evaluate the representation of different functional groups (annual/biannual herbs and grasses, perennial herbs and grasses, shrubs, trees) in the invasion of exotic species within the assemblage on roadside slopes in the *Austrocedrus chilensis* forest in northwest Andean Patagonia, Argentina. Roadside slopes (RS) and nearby reference areas (RA) were selected, and the cover of native, exotic, and invasive species from the functional groups was evaluated in 1 m² plots using the Braun-Blanquet method. It was found that invasive perennial herbs and grasses predominated on RS, with a cover ($19.6 \pm 3.0\%$) higher than that of RA ($8.9 \pm 1.5\%$). *Agrostis capillaris* and *Rumex acetosella* were the most abundant invasive species. Native shrubs and perennial herbs and grasses were predominant on RS, with *Baccharis rhomboidalis* and *Acaena pinnatifida* being the most abundant. This study demonstrates that the roadside slopes of the *Austrocedrus chilensis* forest harbor invasive exotic species that can invade nearby natural areas. Early detection of these species is important for proper management and control, thus promoting the conservation of biodiversity in forest environments.

Keywords: biological invasions; conservation; diversity; functional groups; native species



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1. Introduction

The invasion of exotic plants threatens biodiversity, affecting ecosystem services and ecological processes in native ecosystems, causing extinctions of indigenous fauna and flora [1,2]. It is an important driver of environmental change and poses a significant threat to native biodiversity due to the ability of exotic plants to invade and establish in natural or semi-natural areas [3]. The potential for exotic invasive species to displace native plant species remains high unless appropriate invasive species management strategies are implemented [4]. Moreover, some species continue to dominate anthropized landscapes even after the implementation of control strategies [5].

Road construction creates new environments by fragmenting and altering the landscape, leading to vegetation loss and soil degradation [6]. Since their construction, roadside slopes have been influenced by environmental factors (greater exposure to light and increased space due to vegetation loss), which affect vegetation establishment and lead to an assemblage of species distinct from the surrounding environment [6,7]. The disturbances

caused on the roadsides alter species composition, richness, abundance, the assembly of functional groups, and contribute to the introduction and spread of exotic and invasive plants [8–10]. Roadside slopes are sloping surfaces formed by soil movement during road construction [6]. These areas become more homogeneous habitats, where resource availability increases and plant competition decreases, allowing the establishment of ruderal and stress-tolerant species characterized by rapid growth and high seed production [11].

The evaluation of the functional composition of communities related to life cycle and life form is useful to study the colonization and establishment of species on roadside slopes, allowing the comparison of assemblage processes between studies at different scales and regions [9,12]. Typically, the colonization of roadside slopes is dominated by short-lived, monocarpic annual or perennial species, which are often invasive exotic species [1,13]. Certain functional traits within a group of exotic plants can promote invasion success [14], and their analysis allows for more general conclusions about assembly processes compared to species-specific analyses.

Early identification of invasive exotic species, along with their distribution and abundance, provides valuable information on their current status and potential future trajectories, facilitating the development of management and restoration strategies [15,16]. Understanding the abundance of native and invasive species at different scales is essential for effectively planning and executing control and restoration efforts [17]. The study of native vegetation in nearby non-degraded communities provides insights for the recovery of degraded sites, as they serve as sources of propagules for colonization [7]. Exotic species have a strong influence on the current composition of the flora in the temperate forests of Argentinian Patagonia, and their presence and distribution are influenced by changing land uses such as tourism, forestry, livestock, housing developments, road constructions, and the occurrence of fires [18,19]. The influence of exotic plants in *Austrocedrus chilensis* forests due to fires, livestock, and forestry has been previously investigated [20,21]. However, the composition and abundance of functional groups of invasive plants along roadsides in these forests have been little studied. This study aimed to evaluate the representation of different functional groups (annual/biannual herbs and grasses, perennial herbs and grasses, shrubs, trees) in the invasion of exotic species within the assemblage on roadside slopes in the *Austrocedrus chilensis* forest in northwest Andean Patagonia, Argentina. Our hypothesis is that disturbances caused by the presence of roads favor the invasion of exotic species on roadside slopes, with traits related to rapid growth, short life cycles, and herbaceous or grass life forms.

2. Materials and Methods

Fieldwork was carried out at three sampling locations in the roadside slopes within *Austrocedrus chilensis* (ciprés de la cordillera, hereafter ciprés) forest, located near Bariloche city in northwest Andean Patagonia (Río Negro, Argentina) (Figure 1a). To minimize environmental factors that could influence vegetation composition and cover, three specific sampling sites were deliberately selected in the region to ensure similar conditions, including their position along the moisture gradient, their inclination angle, and their orientation relative to the roadside slopes. The climate is temperate cold and humid with a Mediterranean precipitation regime, characterized by rain and snowfall in winter, with annual precipitation ranging from 800 mm in the east to 1400 mm to the west [18]. The ciprés forest grows on poorly developed soils, rocky outcrops, and slopes with high solar exposure. The accompanying vegetation includes trees such as *Aristotelia chilensis*, *Lomatia hirsuta*, and *Maytenus boaria*; shrubs such as *Nothofagus antarctica*, *M. chubutensis*, and *Berberis microphylla*; and herbs like *Alstroemeria aurea* and *Osmorhiza berteroi* [18,22]. At each sampling location, stratification was first applied to ensure representation of variability within roadside slopes (RS) and reference areas (RA) (Figure 1b,c). Both site types were selected for vegetation assessment, with a rectangular shape and dimensions of 50 m × 5 m (250 m²). RS were located adjacent to the road, while RA were positioned 20 m away in the opposite direction (Figure 1c). Then, within each site, 15 plots of 1 m² were randomly

distributed and the cover of native, exotic, and invasive species of the different functional groups was evaluated using the Braun-Blanquet method (Figure 1c,d) [23].

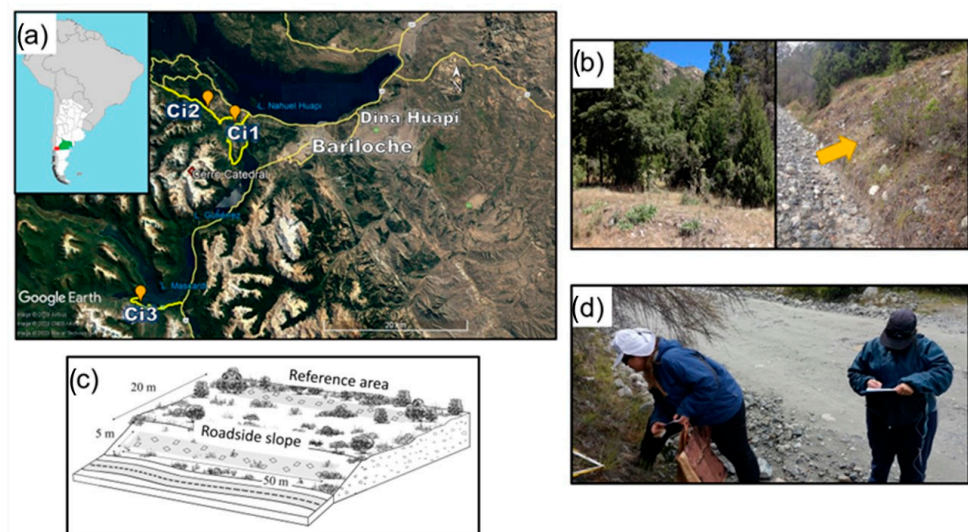


Figure 1. (a) Location of study area, including sampling locations in the *Austrocedrus chilensis* forest (ciprés; Ci1, Ci2, Ci3); (b) Roadside slope (right, indicated by yellow arrow) and reference area (left); (c) Sampling design for vegetation analysis, showing 1 m² plots (square) at each site; (d) Authors conducting the vegetation assessment.

The species recorded in the samples were classified into three categories based on their biogeographic origin and distribution in the Southern Cone: non-invasive exotic, invasive exotic, and native [24]. The invasive exotic species were identified from the list of species included in Resolution 109/21 of the Ministry of Environment and Sustainable Development of Argentina [25]. Additionally, species were grouped into four functional categories based on growth form and life cycle: annual/biannual herbs and grasses (AHG), perennial herbs and grasses (PHG), shrubs (SHR), and trees (TRE) [26]. Species that fell outside the sampling plots were recorded for assessing species richness rather than cover. For the cover analysis, all exotic species were classified as invasive. Only one non-invasive exotic species, *Lupinus arboreus* (Fabaceae), was found at the sites, but it was excluded from the cover analysis since it was located outside the sampling plots. The comparison of cover of functional groups for invasive exotic and native species between sites and among groups was fitted with generalized linear mixed models with a Beta distribution (glmmTMB-R package “glmmTMB”) [27,28]. In these models, the response variable was percent cover (%), while the predictor variables were site type (RS, RA), origin (invasive exotic, native), and functional groups (AHG, PHG, SHR, TRE). The location of the samples (Ci1, Ci2, and Ci3) was considered a random effect influencing the ordinate relative to the origin [29]. This random effect was incorporated into the model because the variability between plots within the same location was less heterogeneous than that between plots from different locations. It is also important to note that introducing this random effect partially reflects the patchy distribution of *A. chilensis* forests, as the Bariloche landscape consists of different Andean Patagonia plant communities [9,22]. The Beta distribution was chosen because of the nature of the response variables, which can be expressed as a proportion (0, 1). The logit link function was used to ensure predictions remained within this range. Other distributions, like the Gaussian distribution, were not suitable as they violated normality and homogeneity of variance assumptions. A hierarchical model selection based on AIC confirmed that the Beta model provided the best fit [27,29]. This selection was performed to identify the best-fitting model by comparing nested models using likelihood ratio tests (LRT) and nonparametric bootstrap [27,30]. The significance of each fixed predictor variable was assessed using likelihood ratio with the Chi-squared statistic, and homogeneity of

variances was visually inspected using residual plots (qq-plot and histogram of residuals) from the mixed models [27]. Multiple post hoc comparisons of origin and functional group type variables were performed using the Benjamini and Hochberg [31] adjustment method (multcomp-R package “glht”) [32]. All analyses were conducted using R software version 4.4.1 [33].

3. Results and Discussion

In the ciprés forest, a total of 65 species were recorded: 45 species in the roadside slope sites (RS) and 55 in the reference area sites (RA). In the RS, 15 species were exotic (34%) (14 invasive), 28 species were native (62%), and two were unidentified species (4%). In the RA, 11 species were exotic and invasive (20%), 43 native species (78%), and one unidentified species (2%).

Exotic invasive perennial herbs and grasses predominated on RS, presenting greater mean cover than exotic invasive annual/biannual herbs and shrubs ($X^2 = 45.6$, $df = 2$, $p < 0.001$), while no differences were found in the mean cover between the other functional groups (annual/biannual herbs and grasses or shrubs) (see Table S1 and Figure 2). The mean cover of exotic invasive perennial herbs and grasses was significantly higher on RS ($19.6 \pm 3.0\%$) compared to RA ($8.9 \pm 1.5\%$) ($X^2 = 5.3$, $df = 1$, $p = 0.021$; Table S1 and Figure 2). The mean cover of invasive shrubs and annual/biannual herbs and grasses were similar at both sites (see Table S1). No invasive trees were found at either site (Figure 2). In RS, exotic invasive species such as *Agrostis capillaris* (perennial grass) with a 6.4% mean cover and *Rumex acetosella* (perennial herb, 5.3% mean cover) were the most abundant; while *Dactylis glomerata* (perennial grass, 4.9%) and *Rosa rubiginosa* (shrub, 3.5%) were the most abundant in RA (Figure 3a).

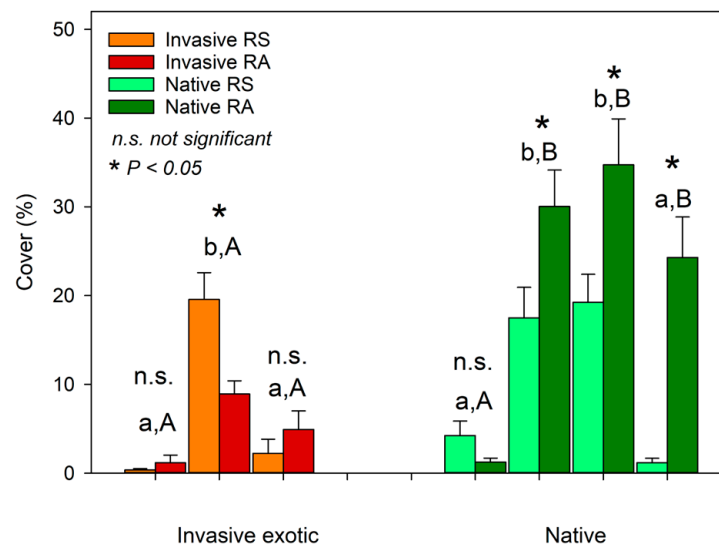


Figure 2. Mean cover (%) $\pm$ (SE) of invasive exotic and native species grouped by functional groups (AHG: annual/biannual herbs and grasses, PHG: perennial herbs and grasses, SHR: shrubs, TRE: trees) on roadside slopes (RS) and reference areas (RA) of *Austrocedrus chilensis* forests. * Indicates significant differences between RS and RA ($p < 0.05$), while “n.s.” denotes non-significant differences. Lowercase letters indicate significant differences ($p < 0.05$) among functional groups within RS, while capital letters indicate significant differences ($p < 0.05$) within RA.

The mean cover of native shrubs ($19.2 \pm 3.2\%$) and native perennial herbs and grasses ($17.5 \pm 3.4\%$) were predominant on RS, with greater mean cover than the other functional groups, annual/biannual herbs and grasses and trees ($X^2 = 45.6$, $df = 2$, $p < 0.001$; Table S1 and Figure 2). Additionally, the mean cover of native perennial herbs and grasses (PHG), shrubs (SHR), and trees (TRE) were significantly lower in RS than in RA (PHG: $X^2 = 6.9$, $p = 0.008$; SHR: $X^2 = 6.4$, $p = 0.012$; TRE: $X^2 = 15.1$, $p < 0.001$; Table S1 and

Figure 2). In RS, native species such as *Baccharis rhomboidalis* (shrub, 10.1% mean cover) and *Acaena pinnatifida* (perennial herb, 5.7%) were the most abundant. In contrast, the native trees *Lomatia hirsuta* (11.7%) and *Aristotelia chilensis* (7.3%) were the most abundant in RA (Figure 3b). In the RS, the mean cover of all native functional groups was lower than in RA, except for annual/biannual herbs and grasses (AHG), which were similar at both sites (AHG: $X^2 = 2.0$, $p = 0.155$; PHG: $X^2 = 6.9$, $p = 0.008$; SHR: $X^2 = 6.4$, $p = 0.012$; TRE: $X^2 = 15.1$, $p < 0.001$; Table S1 and Figure 2).

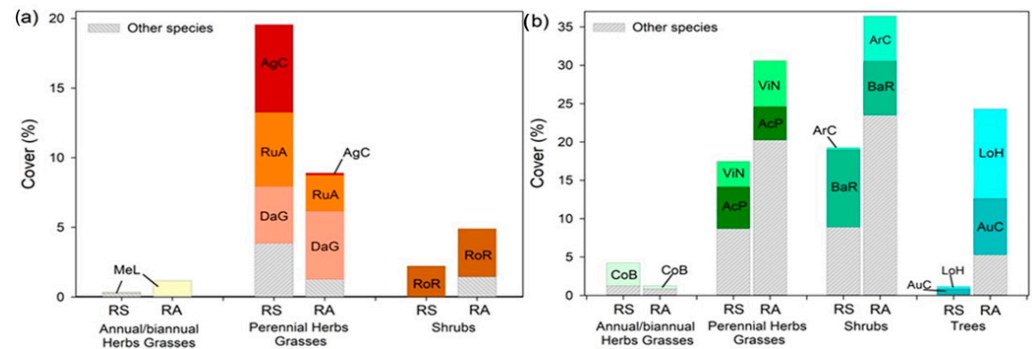


Figure 3. Cover (%) of the most abundant species within each functional group, in the roadside slopes (RS) and reference areas (RA) of *Austrocedrus chilensis* forests. (a) Invasive exotic species: *Agrostis capillaris* (AgC), *Dactylis glomerata* (DaG), *Medicago lupulina* (MeL), *Rosa rubiginosa* (RoR), and *Rumex acetosella* (RuA); (b) Native species: *Acaena pinnatifida* (AcP), *Aristotelia chilensis* (ArC), *Austrocedrus chilensis* (AuC), *Baccharis rhomboidalis* (BaR), *Collomia biflora* (CoB), *Lomatia hirsuta* (LoH), and *Vicia nigricans* (ViN).

The disturbances caused by roadside slopes in ciprés forests alter species composition and promote the presence of invasive exotic species, with perennial herbs and grasses being the most abundant. These results support the proposed hypothesis and are consistent with findings reported by other researchers [34,35]. In Patagonia, a significant presence of invasive annual and perennial exotic herbs and grasses has been recorded in steppe and *Nothofagus antarctica* shrubland road slopes [9,10]. One of the main traits that make these functional groups invasive is the rapid release of a large number of propagules with long-distance dispersal, asexual vegetative reproduction, and a persistent seed bank [36,37]. In our study, *Agrostis capillaris* and *Rumex acetosella* are invasive and ruderal species [sensu [38,39]], with common traits and strategies as previously mentioned. *Agrostis capillaris* has high production of small and light seeds with anemochorous dispersal [19], whereas *R. acetosella* has vegetative reproduction by tillers or rhizomes and a persistent seed bank [40,41], which facilitates their colonization in degraded areas. The habitats in roadside slopes have high light and nutrient availability and reduced competition. But other bad conditions, such as lack of shade, steep slopes, and water deficits, favor the establishment and spread of invasive exotic species [34]. Exotic species exploit microsites where native species struggle to establish, using these disturbed areas as a springboard for invasion [42,43]. Once established, they replicate this mechanism in nearby reference areas, colonizing open gaps, displacing native species, and even posing a threat to native fauna [43,44].

4. Conclusions

The species assemblage differed between roadside slopes and reference areas, and the representation of exotic invasive species among different functional groups (annual/biannual grasses and herbs, perennial grasses and herbs, shrubs, and trees) also varied. The roadside slopes of the *Austrocedrus chilensis* forest harbor invasive exotic species, which can spread into nearby natural areas, particularly those with a perennial life cycle and herbaceous or grass-like growth forms. Functional trait classification can aid in early detection of invasive species near protected areas, as in this study. To address this issue, periodic roadside

monitoring is essential to detect new invasions early and prioritize control of species such as *A. capillaris* and *R. acetosella* through manual removal. Management strategies should also focus on planting disturbance-tolerant native species to prevent exotic plant colonization (e.g., *Baccharis rhomboidalis*, *Acaena pinnatifida*). We recommend: (a) identifying problem species based on criteria such as their threat to natives, red list status, and economic damage; (b) prioritizing actions based on urgency and legislation; (c) planning extractions with clear objectives, timelines, and budgets; (d) using sustainable control techniques; (e) implementing co-management with local communities; and (f) actively restoring key native species through revegetation. Future research should investigate the mechanisms driving roadside invasions and evaluate their long-term impact on forest ecosystems, particularly in the context of climate change and human disturbances. Early detection of these species is important for effective management and control, thereby promoting the conservation of biodiversity in forest environments.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/eesp2024031018/s1>, Table S1: Statistics Chi-square and post hoc contrast statistic of models to contrast the mean cover of functional groups.

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