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Invasive alien species in protected areas: the dynamics of *Pinus taeda* at Rio Canoas State Park – Brazil

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ABSTRACT

Biological invasion is a growing problem, and species of the genus *Pinus* are known to be a problem in the forests southern Brazil, including Conservation Units (CUs). Here, we studied the ecology of *Pinus taeda* invasion at Rio Canoas State Park (PAERC) based on forest inventory, soil seed bank analysis and seed rain assessment in three distinct successional stages inside the park denoted as “Pinus invasion”, “Old Growth Vegetation” and “Initial Vegetation”. Forest inventory of 33 (20x20m) plots, found *Pinus* in 2 of the 3 evaluated environments. Seed rain was collected bimonthly using 33 (1x1m) seed traps for a period of one year. The major seed distribution periods were in April and June, confirming data found in the literature. The seed bank was analyzed in February (summer) and June (winter) of 2018. Samples were kept in a greenhouse for a period of 120 days each. Summer evaluation showed no emergence of *Pinus taeda* seedlings, but the winter evaluation (June) did show the emergence of seedlings. Results showed that the soil seed bank is not persistent. Accordingly, the *Pinus* invasion reported at PAERC requires a restoration program, as well as one that controls reinfestation.

Keywords: 1. Restoration Ecology 2. Forest Conservation 3. Invasive species. 4. Conservation Unit

Introduction

Biological invasion is the second driver of biodiversity loss and species extinction across the world (Bellard et al. 2016). The Convention on Biological Diversity (CBD

2002) defined invasive alien species (IAS) as “species whose introduction and/or spread outside their natural past or present distribution threatens biological diversity”. IAS can also bring consequences to human well-being and livelihood (Mazza et al. 2014; Shackleton et al., 2018; Kumar and Singh, 2020). As pointed out by Shackleton et al. (2018) in a review of 51 sources, invasive species harm livelihoods and increase vulnerability through encroaching on land and reducing mobility or access. They can also decrease the supply of natural resources used by households and reduce agricultural production of livestock and/or crops, resulting in losses of income and increased vulnerability.

Several tree species are responsible for biological invasions, including those belonging to the genus *Pinus* (Dodet and Collet 2012). This genus plays an important economic role locally, nationally, and worldwide (FAO 2016; Cubbage et al. 2020). However, its forestry over time has resulted in biological invasion in the regions adjacent to plantations, likely promoted by a series of ecological traits, such as easy establishment, fast growth, anemophily, anemochory, high propagule pressure and short generation time (Dodet and Collet 2012; Bechara et al. 2013; Sampaio et al. 2013).

In Brazil, some species of *Pinus* are considered invasive (e.g., *P. caribaea*, *P. elliottii*, *P. oocarpa*, *P. glabra*, *P. patula* and *P. taeda*); therefore, several authors have reported on the process of invasion across the Mata Atlântica (Ziller and Galvão 2002; Falleiros et al. 2011; Zenni and Ziller 2011; Bechara et al. 2013, 2014; Zenni and Simberloff 2013; Bognola et al. 2018) and Cerrado (Conciani et al. 2018; Ramos et al. 2019) biomes. (

Besides the threat to biological diversity on a global scale, the ecological risk of invasive species is even greater in protected areas (Spear et al. 2013). Specifically, invasive species pose a threat to the ecology of these areas by compromising long-term

conservation and associated ecosystem services.. The management of invasive species is considered a priority in Brazilian Conservation Units (CUs) (MMA 2012). Decreasing the threat of invasive species in protected areas is essential. However, IAS management control is chronically lacking, and this opens the door to the advance of biological invasions (Ziller and Dechoum 2013).

Biological invasion of *Pinus* spp., mainly *P. elliottii* and *P. taeda*, in a Brazilian Conservation Unit known as Rio Canoas State Park (PAERC) has been reported (FATMA 2007; Ricardo et al. 2018; Lopes et al. 2019). PAERC is situated at the Brazilian Atlantic Forest plateau, also known as the Araucaria Forest (or Mixed Ombrophilous Forest), an ecosystem which hosts a high degree of species richness and endemism, but troubled by extreme degradation (Ribeiro et al. 2009). PAERC is home to important species of flora and fauna of the Atlantic Forest, including endangered tree species such as Araucaria (*Araucaria angustifolia*), imbuia (*Ocotea porosa*), cedro (*Cedrela fissilis*) and the tree fern Xaxim (*Dicksonia sellowiana*) (FATMA 2007).

Here, we examine the dynamics of biological invasion of *Pinus taeda* inside PAERC, describing its current status in order to understand the processes of invasion, integrating the temporal scales of past (already installed), present (seed rain) and future (soil seed bank), to suggest actions to control *Pinus* and support alternative approaches for restoration and management strategies in CUs.

Material and methods

Study area

The study was carried out at Rio Canoas State Park (PAERC) located in the municipality of Campos Novos, Santa Catarina State, southern Brazil. The Park was created in 2004 as environmental compensation from Campos Novos Hydroelectric

Power Plant and Dam. PAERC consists of 1,133.25 ha and is surrounded by a boundary zone around the park of 3,105.24 ha (Figure 1) (FATMA 2007).

The original vegetation is Mixed Ombrophilous Forest in transition with Deciduous Seasonal Forest, both ecosystems of the Brazilian Atlantic biome (FATMA 2007). The climate is classified as subtropical, superhumid mild mesothermal – Cfa, according to the Köppen Climate Classification System (Alvares et al. 2013). Average annual temperature is 16.5°C, annual rainfall is 1,780 mm. The area experiences 6 to 19 annual frosts (Nogueira et al. 1987). The soil types found are Latosol and Cambisol, alluvial with a humic or prominent surface horizon (Embrapa 2004). The study area is situated on the Geomorphological Unit Rio Iguaçu/Rio Uruguai. It is characterized by an intense dissection of the plateau, along with the main drainage by the Canoas river, and large slopes between valleys (Lopes et al. 2019).

After the creation of PAERC in 2004, evidence of invasion, mainly by *P. taeda*, was soon identified, ushering in the first control actions carried out in 2008. However, with the lack of maintenance, came subsequent invasion outbreaks (FATMA 2007). Recent analysis of the landscape surrounding PAERC indicated 523.2 hectares of *Pinus* spp. forestry at the CU's boundary (Ricardo et al. 2018), as well as more than 5,000 ha within a 10 km radius of the park (Lopes et al. 2019).

Our study area was located at the northern and southern borders, covering approximately 91.3 ha, termed as the “recovery zone” in the management plan (FATMA 2007), where a *Pinus taeda* plantation was removed in 2008. We also included plots along the main northeast-southwest internal road., .

Sampling and assessment

Vegetation in three different successional stages were included in the sampling:

- 1) Old Growth Vegetation (OGV), i.e., native forest at an advanced stage of regeneration with no record of invasive species;
- 2) Pinus invasion (EPI), i.e., areas with *Pinus taeda* invasion after removal (recovery zone);
- and 3) Initial Vegetation (EIV), i.e., areas in the initial stage of regeneration with no previous record of invasion.

However, in the latter case, we assumed a risk of invasion based on such favorable features as open canopy and proximity to *Pinus* spp. plantations located in the boundary zone.

Based on field assessments, sample units of 200 meter linear transects were established with three 20 x 20 m plots (400 m²), maintaining a spacing of 60 m between plots and distance of 10 m from edges, totaling 11 transects and 33 plots surveyed (Figure 1). The sampling intensity covered 1.5 % of the recovery zone (Kersten and Galvão 2011). From the total area sampled (13,200 m²), nine plots (3,600 m²) were in OGV, six plots (2,400 m²) in EIV, and 18 plots (7,200 m²) in EPI.

Each plot (20 x 20 m) was again divided into four 10 x 10 m subplots in which we carried out: I) Total plot (400m²) - forest inventory considering tree individuals with DBH (Diameter at Breast Height at 1.3 m) ≥ 10 cm; II) Subplot 1- inventory of all individuals with DBH < 10 cm (regeneration); III) Subplot 2- sample of the soil seed bank at the site; and IV) Subplot 3: collectors installed to evaluate seed rain.

Density (number of individuals per hectare), mean of DBH, basal area (BA) in meters per hectare (m.ha⁻¹), and ratio in percentage of the basal area, always considering two categories, i.e., *Pinus* spp. and other species, were measured.

To assess germination potential of the seed bank, soil samples of 25 cm x 25 cm x 5 cm (as per Brown 1992) and litter, were taken in summer (February 2018) and winter (June 2018).. After storage in identified plastic bags, samples were placed in perforated plastic trays, and covered with a first layer of commercial forest substrate measuring 2

cm in depth.. A sample from the seed bank was placed above the first layer, and the litter was positioned on the surface. Seed bank samples were maintained in a greenhouse, and irrigated daily for a period of 120 days at an average temperature of 24°C. Seedling growth was measured monthly and separated into: *Pinus* and other species.

To evaluate seed rain, permanent 1 m² seed wood frame collectors covered with screen/shading fabric were placed in each plot at a height of 0.8 m from the ground. Six seed collections were placed in December 2017 and then February, April, June, August and October of 2018. The material was also separated into *Pinus* and other species. Seeds showing no damage were visually evaluated and considered in the counting. The density of *Pinus* seeds, as well as other species, was quantified, along with the seasonal pattern of seed dispersal, and the number of seeds was estimated per hectare.

Confidence intervals were calculated according to the variables analyzed, taking into account the three different environments and using R software (R Core Team 2018).

Results

Considering stem DBH ≥ 10 cm, *Pinus taeda* individuals were found in EPI and EIV, with the highest stem density in EPI where forestry was previously practiced.. *Pinus taeda* occurred in 50% of the plots evaluated at EPI and EIV; however, their density was lower than the other species combined (Figure 2). The average of *P. taeda* DBH ranged from 10,0 cm (EPI) to 13,6 (EIV) in the area assessed. Some pinus individuals measured close to 40 cm of DBH. The dominance of *P. taeda* in EPI and EIV was approximately 30% and 50% of the basal area value, respectively. Concerning regeneration (DBH < 10 cm), seedlings of *P. taeda* were found only in 11% of plots of EPI, representing around 3% of stem density and 6,7% of the basal area .

Seed rain of *Pinus* occurred all year long, except in OGV where no pine seeds were found. Seed rain for EPI and EIV showed both temporal and spatial heterogeneity (Figure 3). The annual volume of *Pinus taeda* seed dispersion found was 125,000 seeds per hectare in EPI and 404,000 seeds/ha/year in EIV. At the peak of seed rain, over 250,000 seeds of pine per hectare (June 2018) were recorded. In EPI, *Pinus taeda* seeds were recorded throughout the year in all measurements, and the highest value was recorded in April. The average seeds from other species were higher in October. For EIV, the averages of *P. taeda* seeds were more abundant in June and less abundant in February. Special attention is needed in EIV because *Pinus* seeds represented more than 50% of total propagule available during the period of this study.

Concerning seed bank evaluation (Table 1), the summer evaluation (February) showed no development of *Pinus taeda* seedlings during the emergence period (120 days), only seedlings of other species. The winter evaluation (June), however, had some emergence of *P. taeda* in EIP and EVI. This result could be associated with seed rain data, considering that the second evaluation followed the seed rain peak, as shown in Figure 2.

Discussion

As expected, *Pinus taeda* was not found in the OGV plots, which are more structured with the highest mean DBH and basal area. In contrast, *P. taeda* individuals were found in EIV where no previous data were recorded, reinforcing the invasive potential of the species and expanding the occurrence in PAERC. These results supported the lower capacity of the genus to colonize structured environments, such as native forest, even young secondary fragments such as those found in EIV.

Density and basal area values found in EPI and EVI were similar to those of other studies that found around 25% of stems as pine and up to 50% of basal area (Menon and

Carvalho 2012; Ramos et al. 2019). This high basal area indicates competition for environmental attributes, such as light and habitat, which may compromise maintenance and survival of native species in the medium and long term (Ramos et al. 2019).

The average of BA (22.19 m²/ha) and DBH (19.4 cm) found in the OGV plots were similar to other old-growth forest sites in the region. According to the official Floristic and Forest Inventory of Santa Catarina, forests have an average BA of 26.76 m²/ha and average DBH of 20.4 cm (Vibrans et al. 2013).

The structural parameters of EIV were higher than those reported by Siminski et al. (2011; 2021) for young secondary forests in the region. The difference could be explained by the presence of individuals of *P. taeda* with high DBH in the area assessed. Some individuals measured close to 40 cm of DBH, indicating that the invasive process in EIV is long-standing and that control in these areas was lacking during the first management intervention in 2008.

The production of seeds found at PAERC can be an important factor for invasive success of *P. taeda*. A similar pattern, in which a peak in seed rain occurs around the months of April/May, with year round seed rain has been found in other studies in the Atlantic Forest (Bechara et al. 2013, 2014; Fockink et al. 2020).

The annual volume of *Pinus taeda* seed dispersion found in this study (125,000 seeds/ha/year in EPI and 404,000 seeds/ha/year in EIV) is lower than that obtained by Bechara et al. (2013; 2014) with 2 million *Pinus elliotti* seeds/ha/year, or that of Fockink et al. (2020) with 750.000 *Pinus taeda* seeds/ha/year. However, studies in the literature have recorded seed rain values in areas adjacent to forestry stands where the density of *P. taeda* is higher than that found in PAERC.

These EPI and EIV areas deserve attention as they are located closer to the park's border, which means that they are more susceptible to invasion from boundary zone

1 plantations, as consequence of the anemochoric dispersion of seeds. The average
2 dispersion distance of *Pinus* seeds is between 50 and 300 meters (Nathan et al. 2002;
3 Williams et al. 2006; Tomazello et al. 2017), but it can reach as far away as 5 km,
4 depending on the direction and speed of the wind (thermal uplift), topography, and
5 vegetation successional stage (Zanchetta and Pinheiro 2007).

6 Anemochoric dispersion increases susceptibility to invasion in locations with
7 higher solar incidence or vegetation in early stages of regeneration, even without trees
8 (Higgins and Richardson 1998; Zenni and Simberloff 2013). Sometimes, a single
9 reproductive individual may become the source of invasion; as estimated by Bechara et
10 al. (2014), a single *P. elliottii* tree (30-40 years old) may disperse 2,500 viable seeds ha-
11 1 per year.

12 The results also suggest that the *Pinus taeda* seed bank is ephemeral. This
13 conclusion is based on the failure of seeds dispersed in the previous year to germinate in
14 the summer season. . Seeds of *P. elliottii* were only viable when stored for 90 days in the
15 soil of an area in the restinga, a sandy coastal ecosystem associated with the Atlantic
16 Forest biome, suggesting a transient seed bank species (Bechara et al. 2013). The low
17 seed viability in the soil may seem like an advantage to control *Pinus* invasion, but
18 continuous seed rain represents a constant threat of reinfestation (Bechara et al. 2014).

19 Seed bank composition depends on the history of vegetation cover and stock age,
20 comprising a dynamic system whereby the accumulated stock varies according to inputs
21 and outputs (Martínez-Ramos and Soto-Castro, 1993). The density of other species in the
22 seed bank was ten times lower than the results of Martins and Engel (2007) who evaluated
23 soil seed banks in tropical forest fragments with different disturbance histories in Brazil.
24 Results indicate that management strategies to restore these forests should take into

account the possibility of recovering soil seed bank processes and dynamics (Martins and Engel 2007).

The impact on forest regeneration caused by the pine's biological invasion, particularly at EIP and EIV, calls for its immediate control and eradication, combined with a restoration strategy to recover native biodiversity and a program to manage re-infestation, including the cutting of isolated reproducing trees (Bechara et al. 2013; Ferracin et al. 2013). Regrowth was not observed in stumps cut at 10 cm above ground (Bognola et al. 2018). Equally important, a prevention system for invasive alien species must be urgently implemented in Brazilian protected areas (Ziller and Dechoum 2013).

All ecosystems have the potential for restoration through natural regeneration; therefore, it appears that an integrative restoration approach, i.e., one that includes passive restoration, nucleation and plantation, would be a suitable strategy by which to achieve a naturally growing forest, considering inputs, costs, and divergence from natural processes (Vogel et al. 2015; Bechara et al. 2016; Meli et al. 2017; Trentin et al., 2018).

The results also suggest the need for the construction of "Pinus-free zone" between the park and forestry stands at the boundary, looking to minimize seed dissemination, as recommend by Zanchetta and Pinheiro (2007). To achieve this goal, it is important to engage in dialogue among stakeholders, such as park management, government, forestry companies and boundary residents to build an alternative for pine forestry activities.

Conclusions

Even after the control of *Pinus taeda* in 2008 at PAERC, a dominant presence of the species persists in former forestry sites and initial regeneration environments, leading to the need for the removal of individuals inside PAERC. The seed bank and seed rain analyses suggest that autumn (April, May and June) is the season that deserves more

attention because that is when the largest *Pinus* seed dispersion occurs. Although the *Pinus* seed bank was not shown to be persistent, in other words ephemeral, continuous seed rain could represent a constant threat of reinfestation

A restoration strategy inside the PAERC is necessary for recovery through natural regeneration, particularly at EIP and EIV. In addition, it is necessary to engage in dialog with stakeholders, including residents of the boundary zone, to control pine forestry activities, looking to minimize seed dissemination

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1 Table 1. Estimated number of seedlings emerged per hectare (with the relevant 95%
2 confidence intervals) of *Pinus taeda* and other species in different successional stage of
3 PAERC based on samplings carried out in summer (February) and winter (June).
4

Greenhouse period (days)	Old Growth Vegetation		<i>Pinus</i> invasion		Initial Vegetation	
	Pinus seedlings (x 10 ⁶)	Other seedlings (x 10 ⁶)	Pinus seedlings (x 10 ⁶)	Other seedlings (x 10 ⁶)	Pinus seedlings (x 10 ⁶)	Other seedlings (x 10 ⁶)
Summer sampling (February 2018)						
30 days	0	4.78 2.91-6.64	0	8.18 5.62-10.74	0	8.96 3.92-13.99
60 days	0	2.41 0.67-4.16	0	6.15 4.6-7.69	0	8.58 4.91-12.26
90 days	0	4.0 1.78-6.39	0	4.18 2.42-5.94	0	5.49 2.34-8.64
120 days	0	4.81 3.27-6.36	0	5.79 4.32-7.26	0	7.28 4.63-9.92
Winter sampling (June 2018)						
30 days	0	6.59 3.64-9.54	0.01 -0.006-0.04	9.40 6.79-12.01	0	19.28 6.43-32.12
60 days	0	2.11 1.38-2.84	0.008 -0.008-0.02	6.16 3.93-8.39	0.02 -0.02-0.07	12.0 -0.03-24.03
90 days	0	8.35 2.31-1.44	0	5.64 2.76-8.52	0	5.92 0.62-11.21
120 days	0	1.49 0.88-2.1	0	3.85 2.90-4.81	0	3.78 0.83-6.73

Figure 1. Location of Rio Canoas Park and its boundary zone (IMA 2020). Source: <http://mapas.sosma.org.br/> (SOS Mata Atlântica 2020); IBGE (2016); Application Area Map of Law n° 11.428/2006 (BRASIL 2006); drawn by Daisy Christiane Zambiasi (2021). Where: 1) Initial Vegetation (EIV); 2) Old Growth Vegetation (OGV); and Pinus invasion (EPI).

Figure 2. Structural parameters of 33 plots surveyed at Rio Canoas State Park (PAERC), taking into account individuals with DBH greater than 10 cm ($DBH \geq 10$ cm) and regeneration ($DBH < 10$ cm). Where: 1) Initial Vegetation (EIV); 2) Old Growth Vegetation (OGV); and Pinus invasion (EPI). The X inside the boxplots are the average.

Figure 3. Estimated number of seeds of *Pinus taeda*. and other species (total) collected in the seed rain at PAERC along one year. The data represent the estimated value of seeds per hectare in each of the successional stage evaluated. Where: 1) Initial Vegetation (EIV); 2) Old Growth Vegetation (OGV); and Pinus invasion (EPI). The X inside the boxplots are the average.

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