POTENTIAL FOR NATURAL REGENERATION IN ECOLOGICAL RESTORATION AREAS IN THE PAMPA: WHAT DOES THE SOIL SEED BANK TELL US?

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ABSTRACT: The objective of the study was to evaluate the soil seed bank as a factor for understanding the natural regeneration potential of Pampa forest formations. We selected four study areas: planting in nuclei, passive restoration, commercial planting of eucalyptus, and reference area. The seed bank was collected from the soil with the aid of a wooden template (0.25 cm x 0.25 cm) at a depth of 5 cm from the soil surface, and we carried out ten random collections per area. We conducted the shade-house study for twelve months and evaluated quarterly, counting and identifying the emerging seedlings. Kruskal-Wallis analysis ($p<0.05$) was used to compare the number of germinated seeds and dispersal syndrome between areas. We calculated the Shannon diversity index, Pielou's evenness, and floristic similarity using the Jaccard index. In total, 1,922 seeds germinated, distributed in 65 botanical families and 10 genera. The families with the highest species richness were Poaceae, Cyperaceae, and Asteraceae. There was a significant difference between the areas analyzed in the number of germinated seeds and in species richness. The herbaceous life form was predominant, as well as the abiotic dispersal syndrome. The areas under planting in nuclei and reference areas showed greater floristic similarity. The areas with application of restorative techniques, demonstrated typical shrub formation of primary succession. The soil seed bank has the potential for natural regeneration and is a good indicator of restoration for a study area.
KEYWORDS: indicator, nucleation, restorative techniques.

INTRODUCTION

Large areas around the world have been degraded and landscapes everywhere are being simplified by different land-use practices. Land degradation by deforestation, biodiversity loss, and the global context of climate change have increased the demand for ecological restoration programs and international commitments in the environmental sector, such as the Bonn Challenge (IUCN, 2011) and the 20x20 initiative (WRI, 2014).

Large-scale programs require restoration approaches with ecological efficiency and a better cost-effectiveness ratio. In this way, passive restoration is one of the lower cost approaches, which uses the local regeneration potential and presents better results in landscapes with a high level of habitat connectivity (CROUZEILLES et al., 2017). It is practiced either without or with reduced active human interventions, once persistent disturbances or stressors are removed (BENAYAS et al., 2009; ZAHAWI et al., 2014). Sites under passive restoration have demonstrated an increase in plant density and species diversity and the ability to provide ecosystem services (CHAZDON, 2008, 2017; CROUZEILLES et al., 2017). Nucleation strategies are an intermediate alternative with less intervention (BECHARA et al., 2016). These strategies are based on the theory proposed by Yarranton and Morrison (1974), in which pioneer shrubs and trees facilitate the recruitment of other species through improved microhabitat and seed dispersal conditions.

The evaluation of ecological processes, which express the natural capacity for regeneration of areas as ecological indicators of restoration, is important to understand the dynamics of the restoration approach, such as applied nucleation and passive restoration, and to improve ecological efficiency. The soil seed bank is an important ecological process which expresses the natural dynamics of vegetation and acts as an indicator of the potential for resilience (TRES et al., 2007). The soil seed bank is a dynamic system (BARBOSA, 2012), as the accumulation of seeds in the soil varies according to the inputs and outputs. Inputs come from dispersion mechanisms (seed rain) and outputs occur through physiological responses related to environmental stimuli (germination), as well as viability and seed predation (GASPARINO et al., 2006). Floristic composition and density of the soil seed bank can be good indicators of the state of conservation (MARTINS et al., 2015) and the potential for restoring the dynamic balance of the area (CALEGARI et al., 2013).

In southern Brazil, the vegetation of the Pampa biome is composed of different phytophysiognomies, with different floristic compositions, which are influenced by geomorphological and climatic characteristics, in addition to those related to soil management and occupation (PILLAR and QUADROS, 1997; BAUERMANN et al., 2008). Grasslands are predominant in the Pampa; however, forest formations also occur (MARCHIORI 2004; VÉLEZ et al., 2009). Forests occurring continuously in the Pampa landscape are restricted to the geomorphological regions of the Encosta do Sudeste, where this study was carried out, and the Central Depression. In the other regions, riparian forests occur in less extensive areas, but with relevant ecosystem services.

In recent decades, natural vegetation sites in the Pampa biome have been replaced by annual crops such as soybeans and rice, or perennials, such as *Eucalyptus* spp., *Acacia mearnsii*, and *Pinus* spp. (OLIVEIRA et al., 2018). Forest restoration projects in the Pampa are scarce and in small numbers, compared to forest formations in other Brazilian regions. There are knowledge gaps concerning the restoration of vegetation of both grassland and forest physiognomies. Therefore, research and monitoring programs are needed to determine natural regeneration potential and the success of restoration projects in this biome (ROVEDDER et. al, 2017).

The current study aims to evaluate the potential for natural regeneration from the soil seed bank in riparian zones. We assessed sites under restoration by applied nucleation and passive restoration, as well as a site with *Eucalyptus* sp. forestry and a Seasonal Semideciduous Forest in the Serra do Sudeste region, Pampa biome.

MATERIAL AND METHODS

Study site

This study was carried out in Rio Grande do Sul State, Southern Brazil. The regional climate type is Cfa according to the Koppen climatic classification, described as subtropical humid with hot summers and without a dry season. The average monthly rainfall varies between 116 mm and 195 mm, and the average altitude is 201 m in relation to sea level

(ALVARES et al., 2013). The predominant soils in the region are Entisols, Inceptisols, and Oxisols (STRECK et al., 2008). The study area is in the Pampa biome, with a vegetation mosaic of grassland, shrubland, and Seasonal Semi-deciduous Forest, with elements of Mixed Ombrophilous Forest in higher altitude zones (ROVEDDER, 2013; OVERBECK et al., 2015; GUARINO et al., 2018). *Eucalyptus* sp. silviculture is the main soil use in the landscape. We assessed four sites in sequence:

Applied nucleation: this is a degraded site (0.5 ha), due to suppression of riparian vegetation and replacement by pasture for livestock. The site was isolated by fencing 12 years ago and applied nucleation was implemented seven years ago. The nuclei were randomly established. Each nucleus was implanted with five seedlings one meter apart, with random distribution of the following species: *Schinus terebinthifolia* Raddi, *Cordia americana* (L.) Gottshling and J.E.Mill, *Enterolobium contortisiliquum* (Vell.) Morong, *Inga vera* Willd., *Vitex megapotamica* (Spreng.) Moldenke, *Luehea divaricata* Mart. and Zucc., *Cedrela fissilis* Vell., *Eugenia uniflora* L., *Psidium cattleianum* Sabine, and *Citharexylum montevidense* (Spreng.) Moldenke.

Passive restoration: degraded site (1.0 ha) due to suppression of riparian vegetation and replacement by pasture for livestock. The site was fenced 12 years ago. The current vegetation cover is composed of shrub-tree phytophysiognomy which is characteristic of secondary succession of the Pampa biome.

Eucalyptus sp. forestry: the area belongs to a private company, and the forest garden is located around the other areas studied. The commercial planting is approximately 8 years old, being in the second rotation, with trees approximately 15 meters high. According to Caumo et al., (2020) information about practices developed before forest planting is scarce, however, it is common in the region to carry out fires to control vegetation. The property is regularly visited by cattle with an indeterminate quantity and length of stay, since they are placed there by neighboring farms who take advantage of the area to feed their animals, even without the consent of the owning company.

Riparian forest: Seasonal Semi-deciduous forest remnant with 24 hectares, typical of the forest formation of riparian zones in the study region. This forest presents an intermediate stage of ecological succession.

Soil seed bank collection and analysis

The soil seed bank was collected in October 2017, with a 25 cm x 25 cm template at a depth of 5 cm from the soil surface. We collected ten random sample repetitions in each site. The samples were placed in plastic trays of 3L each, randomly distributed on benches in greenhouses for germination of the soil seed bank. Ten plastic trays with commercial substrate, randomly distributed among the other trays, formed the control treatment. Irrigation occurred daily. We counted and identified all the emerging seedlings and then removed them from the trays. We considered a seed as germinated when the seedling was emitted, as described by Stockman et al. (2007), who defined the development of essential embryo structures as a normal seedling.

The botany classification followed the APG IV (2016) (Angiosperm Phylogeny Group). The species were classified according to the biotic and abiotic dispersal syndrome (CALLE and HOLL, 2019) and the way of life.

Data analysis

The number of germinated seeds, species richness, and dispersal syndrome of the germinated individuals were submitted to the Shapiro-Wilk and Bartlett tests to verify the assumptions of normality and homogeneity of variance ($p > 0.05$) respectively. The premises were not met, so the data were submitted to the Kruskal-Wallis test ($p<0.05$).

Shannon's diversity index and Pielou's index were analyzed to measure species diversity and uniformity, respectively, of the studied areas.

Floristic similarity was calculated using the Jaccard index (Müeller-Dombois & Ellenberg, 1974), with dendrogram generation.

All statistical analyses were processed in R program (version 4.1) (R Core Team, 2020).

RESULTS

Considering the four areas evaluated, we observed the germination of 1,922 seedlings. Among the germinated seeds, 75 species were identified, 65 at the species level and 10 at the genus level, distributed in 27 botanical families. The total number of unidentified seedlings was 25. The families with the highest species richness were: Poaceae (17 species), Cyperaceae (12 species), and Asteraceae (9 species).

In the nuclei planting, 666 seeds germinated and the most representative species were *Ichnanthus* sp., *Fimbristylis dichotoma* (Retz.) Vahl, *Setaria parviflora* (Poir.) Kerguélen, and *Hydrocotyle* sp., representing 33.78% of germinated individuals. In the passive restoration, 463 seeds germinated and the most representative species were *Centella asiática* (L.) Urb, *Ichnanthus* sp., *Rhynchospora tenuis* Link, and *Hydrocotyle* sp. representing 53% of germinated individuals. In commercial eucalyptus plantations, *Oxalis lasiopetala* Zucc., *Sisyrinchium* sp., *Polygala paniculata* L., and *Hydrocotyle* sp., predominated, representing 44% of the individuals of the total of 257 germinated seeds. In the native forest we observed 536 seedlings, of which the most abundant species were: *Commelina* sp., *Paspalum plicatulum* Michx, *Conyza bonariensis* (L.) Cronquist, *Fimbristylis dichotoma*, and *Cyperus incomtus* Kunth, representing 51.49% of germinated individuals.

The areas of nuclei planting, passive restoration, and native forest differed from the area of commercial eucalyptus planting in the number of germinated seeds. Species richness did not differ significantly for the areas planted in nuclei and native forest by the Kruskal-Wallis test ($p < 0.05$) (Table 1).

For diversity indices (Table 1), applied nucleation showed higher Shannon (H) and Pielou (J) indices. However, the Pielou index was similar to the *Eucalyptus* sp. Forestry area. It is observed that Passive restoration and Riparian forest also presented a similar behavior for the Shannon index.

Areas	**GS**	**R**	**H'**	**J'**
Applied nucleation	66.6 a*	17.6 a	3.39	0.85
Passive restoration	46.3 ab	12.2 b	2.89	0.77
Eucalyptus sp. forestry	25.7 c	8.4 c	3.04	0.84
Riparian forest	53.6 a	13.9 ab	3.14	0.78

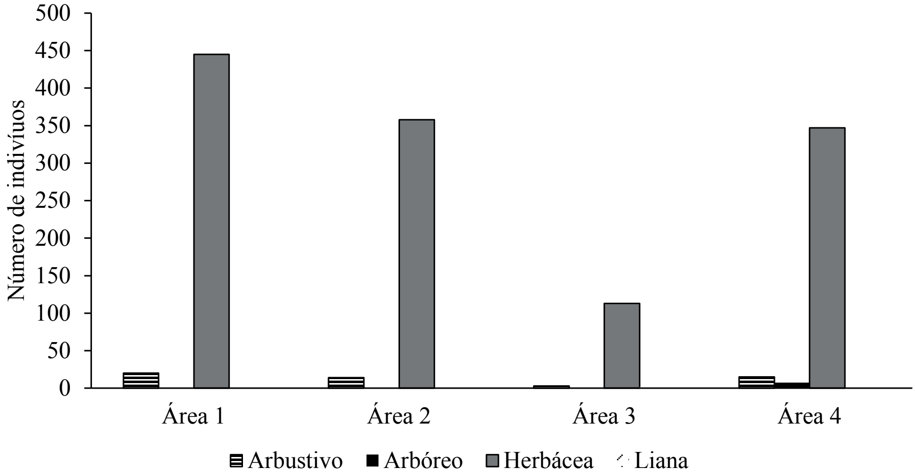
Where: GS = Germinated seeds; R = Richness; H' = Shannon diversity index; J' = Pielou's evenness index.

* Means followed by the same letter, in columns, do not differ from each other, by the Kruskal-Wallis test at 5% error probability.

Table 1 – Mean number of germinated seeds, richness, Shannon diversity index and Pielou evenness for the soil seed bank, in Serra do Sudeste, Pampa biome.

The four areas studied showed a high presence of herbaceous plants (Figure 1). In the nuclei plantation, 65.05% of the individuals were herbaceous, in the passive restoration, 77.48%, in the eucalyptus plantation, 43.96%, and in the native forest, 64.61%.

The presence of lianas was found only in the eucalyptus plantation (1 individual), tree species were identified only in the native forest (7 individuals). Shrub species were found in all areas, with the highest number in nuclei planting (20 individuals), followed by native forest (15 individuals), passive restoration (14 individuals), and eucalyptus plantation (3 individuals) (Figure 1).



Where: (Area 1): planting in nuclei; (Area 2): area under passive restoration; (Area 3): area with eucalyptus plantations; and (Area 4): native forest.

Figure 1 – Number of individuals in the soil seed bank per life form for the four study areas in Serra do Sudeste, Pampa biome.

The abiotic dispersal showed a significant difference by the Kruskal-Wallis test (Table 2) between the studied areas. Nuclei planting did not differ statistically from passive restoration and native forest. Regarding biotic dispersal, there were no significant differences between areas by the same comparative test.

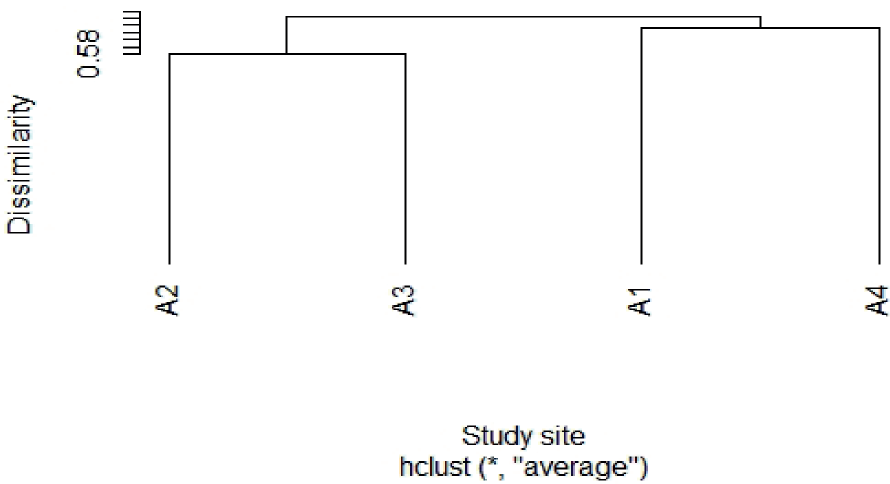
Areas	**Dispersal syndrome**	
	Abiotic[1]	**Biotic[1]**
Applied nucleation	7.5 a*	1.3 a*
Passive restoration	5.2 ab	1.3 a
Eucalyptus sp. forestry	4.0 b	0.8 a
Riparian forest	7.2 a	1.0 a

* Means followed by the same letter, in columns, do not differ from each other by the Kruskal-Wallis test ($p < 0.05$).

[1] Abiotic (wind or gravity), biotic (birds, bats, small mammals).

Table 2 - Average number of seeds germinated by dispersal syndrome in the analyzed areas in the Serra do Sudeste, Pampa biome.

The species composition of the soil seed bank by the Jaccard index ranged from 0.58 to 0.72 between the studied areas (Figure 2), representing high similarity, with values greater than or equal to 0.5 indicating high similarity (KENT and COKER, 1992). Two groups were formed: (1) similarity between the area planted in nuclei and the reference area, and (2): similarity between the passive restoration area and the commercial eucalyptus plantation.



Where: (Area 1): planting in nuclei; (Area 2): area under passive restoration; (Area 3): area with eucalyptus plantations; and (Area 4): native forest.

Figure 2. Floristic similarity dendrogram using the Jaccard index for the soil seed bank in the studied areas in Serra do Sudeste, Pampa biome.

DISCUSSION

The families with the highest species richness were also reported by Caumo et al. (2020) in the same biome. They are considered representative species of anthropic environments (MARTINS et al., 2017), with the expressive number of species of the Poaceae and Asteraceae families being common in soil seed bank studies in the region (NETO et al., 2017; PIAIA et al., 2017). These families are usually composed of pioneer species that produce a high number of seeds during the year, in addition to presenting a long period of dormancy in the soil as a survival strategy (COSTA et al., 2020). Regarding the species of the Cyperaceae family, they are commonly found in wetlands (CAUMO et al., 2020), corroborating the results found, since the study was developed in a Permanent Preservation Area (PPA) with a stream along its entire length.

Among the identified species, four species are exotic, *Centella asiática* (L.) Urban, *Parthenium hysterophorus* L., *Psidium guajava* L., and *Eragrostis plana* Nees, representing 6.4% of the germinated seeds in all the studied areas. The occurrence of these species is possibly a consequence of the proximity between the areas.

Evidently, although the total representation of exotic species is low, the occurrence must be taken into account, mainly because three of the species are considered invasive. (*C. asiática*, *P. guajava*, *E. plana*). The species *E. plana*, has become the most abundant and aggressive invasive species in the Pampa (MALDANER et al., 2019). The occurrence of this species in the studied areas can be explained by the high viability of the seeds, with a long duration as part of the soil seed bank, in addition to the high adaptability to different environmental conditions, capable of developing perfectly in unfavorable environmental conditions (MALDANER et al., 2019).

Among the areas studied, planting in nuclei may favor the increase in the richness and diversity of the soil seed bank due to the diversity indices presented. The same occurred with Piaia et al. (2020) in a study comparing areas under applied nucleation and passive restoration. On the other hand, the indices presented in the area of *Eucalyptus* sp. forestry, are probably related to the lower number of germinated seeds and the lower richness found. Similar results were presented by Procknow et al., (2020) in the same study area. The mathematical ratio of these indices can lead to mistaken interpretations from an ecological point of view, generating an overestimate of diversity (MAGURRAN, 2013).

The predominance of herbaceous species in the pampa biome was also reported by Caumo et al. (2020) and by Piaia et al. (2017) in a transition area between the Atlantic Forest and Pampa biomes. The presence of herbaceous species in disturbed environments is essential for the restoration of plant communities (CAPELLESSO et al., 2015), being a good indicator of resilience (MARTINS et al, 2008). It is common to observe a greater number of herbaceous species at the beginning of population in degraded areas (OLIVEIRA et al., 2018), as they usually have facultative dormancy, in addition to having efficient mechanisms of dispersion (GASPARINO et al., 2006). The absence of individuals of tree species in the areas under restoration can be explained by the initial character of the ecological succession process (OLIVEIRA et al., 2018), demonstrating a characteristic of slow succession in the study area, also observed by Piaia et al. (2020) for the same study site.

The abiotic dispersal syndrome (wind or gravity) was predominant in all areas, mainly in the Riparian forest, indicating that it is a disturbed forest fragment, as according to Venzke et al. (2014) this syndrome is less frequent in conserved forests. Another factor that may be related is the size and shape of the studied fragment, as it is narrow and composed practically of borders. Narrow or small-sized native vegetation fragments have a high edge effect, that is, a high interaction with the surrounding matrix and as a consequence of this phenomenon, individuals become more vulnerable and fragile to environmental fluctuations such as temperature increase, luminosity, winds, and humidity, which promote the death of individuals, the opening of gaps, and the circulation of seeds of abiotic dispersal (CHAZDON, 2016).

The fact that the applied nucleation and passive restoration areas present a significant number of seeds germinated by the abiotic syndrome and do not differ statistically from the Riparian forest, is probably linked to the short distance between these areas, making the seeds easier to disperse.

The low number of individuals with abiotic dispersal syndrome in the *Eucalyptus* sp. Forestry area, is due to the fact that this area can act as a physical barrier, preventing the arrival of propagules, a finding also mentioned by Procknow et al. (2020) in a study on seed rain. The existing vegetation in the area is one of the factors preventing the colonization and establishment of seeds (HOLL et al., 1999), since the presence of extensive areas of Eucalyptus plantation promotes the simplification of the vegetation matrix in the region, resulting in isolation between the areas of natural vegetation and making seed dispersal difficult (BRANCALION, GANDOLFI, RODRIGUES, 2015).

The expressive amount of individuals with abiotic dispersal syndrome in the studied areas is possibly related to the significant amount of herbaceous species present in the areas. Species with autochoric and anemochoric dispersal syndrome are mostly short-cycle herbaceous species (SOUZA et al., 2017; COSTA et al., 2020).

The formation of two groups of floristic similarity between the areas studied, with greater similarity between the areas planted in the nucleus and native forest, corroborate the study by Procknow et al. (2020), where the author concluded that the nuclei were being effective in their processes, mainly by attracting fauna, which are seed dispersers. Consequently, we can attribute this factor to the soil seed bank, since its constitution depends, in most cases, on dispersing agents.

CONCLUSION

The soil seed bank is an efficient indicator to express the potential for natural regeneration in the study area. The regional mosaic of grassland, shrub, and forest species influences the regeneration potential, given the predominance of herbaceous species and the abiotic dispersal syndrome.

The native forest area presents itself as an altered forest, probably due to its elongated shape and remnant area, which favors the edge effect and reduces the expression of species from more advanced successional stages. The *Eucalyptus* sp. forestry area acts as a barrier to propagules. Applied nucleation and passive restoration areas present a typical shrub formation of primary succession that, however, become chronologically stable for the study site.

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