

Floristic Composition Analysis of Soil Transposition in a Seasonal Forest in Rio Grande do Sul, Brazil

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ABSTRACT

This study aimed to identify the floristic composition and density of a seed bank transposed from a Seasonal Forest fragment in Rio Grande do Sul. The seed bank of the (BSI) fragment center and edge (BSII) of another forest fragment was also evaluated, both at medium to advanced successional stages. The seed bank was deposited in Brown-Gray Argisol and Red Argisol using exposed soil plots as control. ANOVA followed by Tukey test ($p < 0.05$) were used to compare treatments for density and species richness. The density of individuals did not differ between treatments. Species richness was higher for BSI and BSII treatments in relation to control. The floristic composition presented different life forms, but was mainly composed of herbaceous species. It was concluded that the seed bank from the two donor areas contributed to the expansion of species richness in two soil types.

Keywords: degraded areas, nucleation, seed bank transposition.

1. INTRODUCTION

Nucleation strategies for ecological restoration are promising because they initiate or facilitate secondary succession processes (Corbin & Holl, 2012). Seed bank transposition is a nucleation technique that consists of removing the superficial soil layer and litter from a conserved donor area for later deposition on a degraded area with the same vegetal typology (Reis et al., 2014). This superficial soil layer deposited on a degraded area serves as seed source, and can introduce abundance and species richness, thus establishing a new successional pattern (Zhang et al., 2001; Hall et al., 2010).

Soil transposition may increase the diversity of regional native species, genetic variability, and the chances of recruiting species adapted to adverse conditions (Reis et al., 2003; He et al., 2016). In this sense, many authors have emphasized the use of the seed bank as an efficient strategy for covering degraded or disturbed areas by including species of different forms of plant life (Tozer et al., 2012; Ferreira et al., 2015; Fowler et al., 2015).

However, there is still lack of scientific information regarding the technique specificities (Corbin & Holl, 2012; Boanares & Azevedo, 2014), mainly in the southern region of Brazil, which is strongly influenced by subtropical climate (Rovedder et al., 2014). There is also a worrying scenario of habitat fragmentation, loss of biological diversity and risk of species extinction due to anthropogenic activities related to poorly managed agriculture, livestock and urbanization (Monteiro & Blauth, 2006). This scenario highlights the increasing need for validating and inserting ecological restoration methodologies for the region.

In this context, the aim of this study was to identify the floristic composition and density of a seed bank transposed from the center and edge of Seasonal Forest fragments deposited in two different soil types in the central region of Rio Grande do Sul in order to generate subsidies to adapt this ecological restoration technique to subtropical conditions.

2. MATERIAL AND METHODS

This study was carried out in Santa Maria (29° 41' 03" S and 53° 48' 25" W), in the central region of Rio Grande do Sul. The average altitude is 103 m above sea level and the climate is Cfa, according to

the Köppen climate classification, being subtropical humid with hot summer and without defined dry season. The average temperature of the coldest month is 13.3°C, and the hottest month is 24.5°C. The average monthly rainfall ranges from 134 mm to 192 mm (Alvares et al., 2013). The relief is smooth-waved and soils are derived from sandstones with siltite and argillite stratification, with Argisols predominating in elevations and middle third of slopes, and Planosols and Gleysols in lower altitudes (Streck et al., 2008). The region is an ecotonal transition zone between *Pampa* and *Mata Atlântica* Biomes. The characteristic forest formation is subtropical Seasonal Forest. Low temperatures in the winter cause physiological rest and partial leaf deciduousness (IBGE, 2012).

We selected two forest fragments for soil collection based on preliminary seed bank analysis under greenhouse conditions (Piaia et al., 2017b). Forest fragments donors are in an agricultural production matrix, showing pastures and annual crops in the surroundings. In order to characterize the floristic composition of each fragment, a floristic assessment of the arboreal stratum was carried out using the quadrant method (Moro & Martins, 2011; Freitas & Magalhães, 2012). Thus, points were systematically distributed in line with 20 m distance between each point. Thus, the seed bank donor forests were described below:

- Seed bank of the forest center (SBI): samples were removed from the center (distance ≥ 50 m from the forest edge) of Seasonal Forest remnant at medium to advanced successional stages, with average canopy height of 8.5 m. The fragment has rectangular shape and 25 ha. Overall, 16 species were observed by the quadrant point method, and the four species with the highest frequency were *Actinostemon concolor* (Spreng.) Müll. Arg., *Eugenia ramboi* D. Legrand, *Plinia rivularis* (Cambess.) Rotman. and *Myrcianthes pungens* (O.Berg) D. Legrand;
- Seed bank of the forest edge (SBII): samples were removed from the edge (distance ≤ 10 m from the forest edge) of a Seasonal Forest remnant at medium to advanced successional stage, with average canopy height of 12 m. The fragment has circular shape and 30 ha. Overall, 10 species were recorded by the quadrant point method. *Actinostemon concolor* (Spreng.) Müll.Arg., *Eugenia ramboi* D. Legrand, *Plinia rivularis* (Cambess.) Rotman. and *Campomanesia xanthocarpa* O. Berg. were the four species with the highest frequencies.

The seed bank was removed with 1 m x 1 m template and 5 cm in depth in 12 points in each area at the beginning of December 2013, corresponding to the end of spring, which is a period of higher seed deposition (Longhi et al., 2005). Collection was performed in a systematized way with 20 m of distance between transect points. Each sample was stored in a plastic bag, where litter was stored separately, receiving the same identification. Subsequently, samples were taken to the experimental area of the Department of Forestry Sciences - Federal University of Santa Maria. Donor forests and seed bank deposition site are approximately 10 km from each other.

Soils of the deposition site were classified as Brown-Grey Argisol (PBAC) and Red Argisol (PV), according to the Brazilian Soil Classification System (EMBRAPA, 2013), consisting of pedsequences typical of the Central Depression of RS, both occurring from flat to soft-wavy relief. PBAC presents hydromorphic character, with formation of water slides during periods of intense rainfall. It originates from siltstones and argillites, and occupies the intermediate portion of the relief. PV occurs in the same toposequence of "Coxilhas", occupying the highest portion of the relief (Streck et al., 2008).

In PBAC, there was predominance of species from the Poaceae botanical family such as *Paspalum* sp. and exotic *Eragrostis plana* Nees (annoni-grass), with presence of species from Apiaceae (*Eryngium* sp.) and Cyperaceae families which are characteristic of humid environments. In PV, there was predominance of species from the Poaceae family such as *Paspalum* sp. and exotic *Eragrostis plana* Nees (annoni-grass). However, the presence of Apiaceae or Cyperaceae was not observed in PV. Both soils presented pasture as land use history, with annual crops such as soybean and eucalyptus plantations in their surroundings.

Treatments consisted of two seed bank donor sites (SBI and SBII) deposited in two soil types (PBAC and PV), plus control treatments (C) on both soils, so there were six treatments (SBI/PBAC, SBII/PBAC, C/PBAC, SBI/PV, SBII/PV, C/PV) with six replicates, each in a completely randomized experimental design.

Seed bank distribution was random in 1 m² plots, with minimum distance of 5 m between each other. Vegetation and superficial soil were previously removed from plots so local regenerating plants would be excluded.

Thus, soil and litter were deposited, corresponding to that sample. Control treatment (C) had only vegetation and surface soil of the 1 m² plots removed, therefore consisting of the exposed soil without receiving the seed bank.

The seed bank transposition was assessed for twelve months from January to December 2014. Seedlings that emerged with serially numbered metal plates were identified. The APG III (APG, 2009) classification system was adopted and the names of species were confirmed through the *Missouri Botanical Garden* website (Tropicos.org, 2016).

Seedlings were classified as life forms in herbaceous, sub-shrubs, shrubs, trees and climbing plants. The diversity index of Shannon (H') and the evenness of Pielou (Magurran, 2013) were then calculated. Subsequently, the calculated H' values were compared by the Hutcheson t-test at 5% probability level. This test verifies the difference between diversity indexes for two samples, therefore comparisons were performed in pairs (Hutcheson, 1970).

Density, species richness and density by life form data were submitted to the Kolmogorov-Smirnov test to verify normality and the Bartlett test for homogeneity of variance, and when assumptions were not recognized, non-parametric statistical analysis was performed.

Differences in treatments for density and species richness were evaluated using ANOVA, and comparisons were performed by the Tukey test (p<0.05). Treatments for density by life form were also compared using the Kruskal-Wallis test (p<0.05). When significant difference between treatments was observed, they were compared by the t-test (p<0.05) (Triola, 1999), according to the *agricolae* package of the R software (Mendiburu, 2015; R Development Core Team, 2013).

Cluster analysis was performed using the Ward linking method with presence and absence of data for the species with the STATISTICA software version 10 to verify the floristic similarity between treatments.

3. RESULTS AND DISCUSSION

Overall, 1,461 individuals from 25 botanical families, 76 genus and 85 species were sampled. Of these 85 species, eight were identified at the botanical family level and 19 were not determined, registered as morphospecies (Table 1). The number of unidentified

Table 1. Number of individuals by species found in transposed seed bank (SBI and SBII) and control (C) in Brown-Grey Argisol (PBAC) and Red Argisol (PV) soil types in Santa Maria, RS.

Family/species	LF	PBAC				PV			
		SBI	SBII	C	Tot	SBI	SBII	C	Tot
Acanthaceae									
Acantaceae sp. 1	her			1	1				
Amaranthaceae									
<i>Amaranthus hybridus</i> L.	her	1			1				
<i>Pfaffia tuberosa</i> (Spreng.) Hicken	her					63	38	50	151
Apiaceae									
<i>Centella asiatica</i> (L.) Urb.	her	3	4		7	1	1		2
<i>Eryngium</i> sp.	her	1		2	3		1	1	2
Araliaceae									
<i>Hydrocotyle</i> sp.	her	5	6		11	1	2	2	5
Asteraceae									
<i>Ageratum conyzoides</i> L.	her	1			1	6	1		7
<i>Baccharis coridifolia</i> DC.	subsh	16	9	1	26				
<i>Baccharis dracunculifolia</i> DC.	sh		1		1				
<i>Baccharis</i> sp.	sh			1	1			1	1
<i>Conyza</i> sp.	her	1		1	2	9	17	2	28
<i>Elephantopus mollis</i> Kunth	her					1	5	2	8
<i>Erechtites hieraciifolius</i> (L.) Raf. ex DC.	her	3	4	2	9	1	3	1	5
<i>Eupatorium ivifolium</i> L.	her	54	82	79	215	12	2	5	19
<i>Eupatorium laevigatum</i> Lam.	sh	8	2	1	11				
<i>Eupatorium macrocephalum</i> Less.	her	2	9	2	13	3			3
<i>Facelis retusa</i> (Lam.) Sch. Bip.	her					2	4	3	9
<i>Gamochoeta pensylvanica</i> (Wild.) Cabrera	her	18	19		37	6	5	3	14
<i>Hypochaeris radicata</i> L.	her		3		3	1	2	5	8
<i>Picrosia longifolia</i> D. Don	her						1		1
<i>Pluchea sagittalis</i> (Lam.) Cabrera	her		1		1				
<i>Senecio brasiliensis</i> (Spreng.) Less.	her					1	2		3
<i>Solidago chilensis</i> Meyen	her		5		5				
<i>Sonchus oleraceus</i> L.	her		3		3				
<i>Vernonanthura tweedieana</i> (Baker) H. Rob.	sh	15	3	2	20	29	2	8	39
<i>Vernonia nudiflora</i> Less.	subsh						5		5
Asteraceae sp. 1	her	9	4	3	16	3	6	3	12
Asteraceae sp. 2	her			3	3				
Asteraceae sp. 3	her		1		1				
Asteraceae sp. 4	her					1	11	5	17
Asteraceae sp. 5	her			1	1				
Asteraceae sp. 6	her			1	1				
Asteraceae sp. 7	her		1		1	1			1
Convolvulaceae									
<i>Ipomoea purpurea</i> (L.) Roth.	cl	25	19	35	79	32	16	65	113
<i>Ipomoea</i> sp.	cl	5	1	3	9	1	1	1	3
Cyperaceae									
<i>Cyperus</i> sp.	her	2	3	3	8				
<i>Kyllinga brevifolia</i> Rottb.	her	1	1	1	3	1		1	2
Euphorbiaceae									
<i>Actinostemon concolor</i> (Spreng.) Müll.Arg.	tr	10	22		32	3	3		6
<i>Gymnanthes klotzschiana</i> Müll. Arg.	tr		1		1	1			1
<i>Sebastiania brasiliensis</i> Spreng.	tr		2		2				

Which: LF = Life form; her = herbaceous; subsh = sub-shrubs; sh = shrubs; tr = trees; cl = climbing plants; Tot. = Total.

Table 1. Continued...

Family/species	LF	PBAC				PV				
		SBI	SBI I	C	Tot	SBI	SBI I	C	Tot	
Fabaceae										
<i>Desmodium</i> sp.	her	8	11	1	20	3	12	5	20	
Iridaceae										
<i>Sisyrinchium alatum</i> Hook.	her						1		1	
<i>Sisyrinchium micranthum</i> Cav.	her	5	2	1	8					
Lamiaceae										
<i>Hyptis</i> sp.	her					1			1	
Lythraceae										
<i>Cuphea carthagenensis</i> (Jacq.) J. F. Macbr.	her	6	4	10	20	1	1	1	3	
Malvaceae										
<i>Sida</i> sp.	her	10	11	40	61	22	25	127	174	
Melastomataceae										
<i>Tibouchina</i> sp.	her	2	8		10					
Moraceae										
<i>Sorocea bonplandii</i> (Baill.) W.C. Burger oer	tr		1		1		1		1	
Myrtaceae										
<i>Eugenia uniflora</i> L.	tr	1			1					
<i>Psidium guajava</i> L.	tr					1			1	
Onagraceae										
<i>Ludwigia</i> sp.	her	11	6	11	28		1		1	
Oxalidaceae										
<i>Oxalis brasiliensis</i> Lodd.	her	2	2		4					
Phyllanthaceae										
<i>Phyllanthus tenellus</i> Roxb.	her					3	1	1	5	
Plantaginaceae										
<i>Plantago</i> sp.	her	3	2		5		1	1	2	
<i>Scoparia dulcis</i> L.	her					2		1	3	
Rubiaceae										
<i>Borreria eryngioides</i> Cham. & Schtdl.	her					1			1	
<i>Richardia brasiliensis</i> Gomes	her						1		1	
<i>Spermacoce latifolia</i> (Aubl.) K.Schum.	her	5	4		9			1	1	
Rutaceae										
<i>Helietta apiculata</i> Benth.	tr	7	1		8		5		5	
Sapindaceae										
<i>Serjania</i> sp.	cl					2			2	
Solanaceae										
<i>Solanum americanum</i> Mill.	her	4	4		8	2	4	1	7	
<i>Solanum mauritianum</i> Scop.	tr	2	2		4	2	2		4	
<i>Solanum sisymbriifolium</i> Lam.	her	1	1		2			2	2	
<i>Solanum viarum</i> Dunal	her		2		2					
Verbenaceae										
<i>Glandularia aristigera</i> (S. Moore) Tronc.	her					4	3	2	9	
<i>Verbena rigida</i> Spreng.	her			2	2	1	1	1	3	
Undetermined										
Morphospecies 1	her			1	1					
Morphospecies 2	her					1			1	
Morphospecies 3	her	3	2	3	8					
Morphospecies 4	her			1	1					

Which: LF = Life form; her = herbaceous; subsh = sub-shrubs; sh = shrubs; tr = trees; cl = climbing plants; Tot. = Total.

Table 1. Continued...

Family/species	LF	PBAC				PV			
		SBI	SBII	C	Tot	SBI	SBII	C	Tot
Morphospecies 5	her	2		1	3				
Morphospecies 6	tr		1		1				
Morphospecies 7	her	1			1				
Morphospecies 8	her							1	1
Morphospecies 9	her							1	1
Morphospecies 10	her							1	1
Morphospecies 11	her					1			1
Morphospecies 12	her					1			1
Morphospecies 13	her							1	1
Morphospecies 14	her					1			1
Morphospecies 15	her			1	1				
Morphospecies 16	her							1	1
Morphospecies 17	her					1			1
Morphospecies 18	her					1			1
Morphospecies 19	her					1			1
Total		253	270	214	737	231	187	306	724

Which: LF = Life form; her = herbaceous; subsh = sub-shrubs; sh = shrubs; tr = trees; cl = climbing plants; Tot. = Total.

species is related to the similar morphology of the initial development of herbaceous species and to the occurrence of low density of seedlings of the same morphospecies. Other studies also reported the difficulty in identifying seedling species from the seed bank (Scoti et al., 2011; Calegari et al., 2013).

The most representative families were Asteraceae and Solanaceae, common in early succession stages (Miranda et al., 2010), a typical result of species that form the persistent seed bank. The species showing the highest number of individuals in the Brown-Grey Argisol (PBAC) were *Eupatorium ivifolium* L., *Ipomoea purpurea* (L.) Roth. and *Sida* sp.. In Red Argisol (PV), the most representative species were *Pfaffia tuberosa* (Spreng.) Hicken, *Ipomoea purpurea* and *Sida* sp. These species were found in all treatments with high density in the control in both PBAC and PV, which indicates the influence of an autochthonous seed bank.

Eupatorium is one of the main genus of shrub and sub-shrub species occurring throughout the southern portion of Rio Grande do Sul, together with *Baccharis*, *Senecio* and *Vernonia* (Rovedder, 2013), which also were registered in this study. This characterizes the ecotonal transition aspect of the central region of Rio Grande do Sul.

P. tuberosa presents occurrence in the Southern, Southeastern and Midwestern regions of Brazil, mainly inhabiting dry and sandy fields (Marchioretto et al.,

2009). This specie was not found in PBAC treatments of hydromorphic character. In fact, hydromorphy can be pointed out as an important ecological filter for seed bank expression (Piaia et al., 2017a). It could be exemplified by the high density of *P. tuberosa* in PV (densities of 10 ind./m² in SBI, 6 ind./m² in SBII and 8 ind./m² in C were observed), and no individuals of this specie in PBAC.

I. purpurea and *Sida* sp. are ruderal species with large distribution in the Brazilian territory (Ferreira & Miotto, 2009). They are able to grow under adverse conditions and have the ability to produce large numbers of seeds with efficient dispersion (Brighenti & Oliveira, 2011).

The highest diversity was found in SBI/PBAC and SBII/PV, and control (C/PBAC and C/PV) showed the lowest diversity (Table 2). This means that the transposed seed bank increased diversity in both study sites. The absence of a defined pattern for floristic composition and diversity for fragment center and edge (BSI and BSII) may have occurred due to the spatial heterogeneity of the forest seed bank (Baider et al., 1999), and due to the influence of the environmental gradient responsible for the different compositions of species in the same fragment (Ferreira et al., 2012). In the same way, the deposition environment of the seed bank also acts as an ecological filter to express the floristic composition, as previously mentioned

Table 2. Floristic richness, density, diversity and evenness indexes found in the transposed seed bank and control (SBI, SBII and C) in Brown-Grey Argisol (PBAC) and Red Argisol (PV) soil types in Santa Maria, RS.

Variables	PBAC				PV			
	SBI	SBII	C	Total	SBI	SBII	C	Total
Total number of families	19	18	11	22	18	17	14	22
Total number of species	36	41	29	57	42	35	33	59
Total number of seedlings	253	270	214	737	231	187	306	724
Species richness/m ²	14.3a*	14.3a	9.2b	-	13.0a	11.8a	9.7a	-
Density (ind./m ²)	42.2a	45.0a	35.7a	-	38.5ab	31.2b	51.0a	-
Shannon - H'	3.00a**	2.32b	1.78c	-	2.38b	2.88a	1.67c	-
Pielou - J'	0.84	0.62	0.53	-	0.64	0.81	0.48	-

*Means followed by the same letter in the row by soil class do not differ by the Tukey test (p<0.05); **Shannon index followed by the same letter do not differ by the Hutcheson's t-test (p<0.05).

Table 3. Density of individuals by life form (ind./m²) for each treatment (SBI, SBII and C) in Brown-Grey Argisol (PBAC) and Red Argisol (PV) in Santa Maria, RS, Brazil.

Soil	Treatments	Life forms			
		Arboreal	Shrub	Herbaceous	Climbing plants
PBAC	SBI	3.33a*	3.83a	30.00a	5.00a
	SBII	5.00a	1.00a	35.67a	3.33a
	C	0.00b	0.67a	28.67a	6.33a
PV	SBI	1.17a	4.83a	26.67a	5.83ab
	SBII	1.83a	0.33b	26.17a	2.83b
	C	0.00b	1.50ab	38.50a	11.00a

*Means followed by the same letter by life form and soil class do not differ by the Kruskal-wallis test (p<0.05).

with respect to soil hydromorphy. In this sense, it is important to highlight the appropriate choice of the seed bank collection site, where it should be stratified, close together and with similar conditions, for example in relation to the soil hydromorphy, to the site that needs to be restored.

Herbaceous life forms were predominant in all treatments in both soil classes (Table 3). Mechanisms such as capacity to develop under adverse conditions, production of large numbers of seeds, efficient dispersion over long distances, and dormancy and uneven germination are typical of this type of biological form, which again show the expression of the persistent seed bank (Long et al., 2015).

Pioneer herbaceous and shrub species are important in seed bank transposition projects for ecological restoration because they are generally aggressive and colonizing. They proportionate rapid soil cover and proliferate rapidly, attract pollinator fauna and seed dispersers, showing early senescence, preparing the environment for subsequent stages (Bechara et al., 2007).

Species of arboreal life forms were only found in plots where there was seed bank deposition in both soil types (Table 3), which evidences the potential of this

technique to include arboreal life forms in restoration areas. *Actinostemon concolor* (Spreng.) Müll.Arg. presented the largest number of individuals for this life form in both soil types (Table 1). This species is understory, has autocory dispersion, and is usually found in aggregate distribution in the forest (Longhi et al., 2000). We emphasize that this was the most abundant species observed in the floristic survey carried out in forest fragments.

In PBAC, 28 species in SBI and SBII were sampled, which were not found in the control treatment; of these, seven species have arboreal behavior. In PV, there were 26 exclusive species in the deposition seed bank plots (SBI and SBII), five of which were arboreal life forms. This demonstrates and reinforces the effectiveness of transposition to increase the richness and insertion of other life forms in degraded areas, and that the collection in two donor sites, namely fragment center and edge, was important for this increase.

The dendrogram obtained by the cluster analysis demonstrates the heterogeneity of the floristic composition found in the nuclei of seed bank transposition and in the control treatment in both soil types (Figure 1).

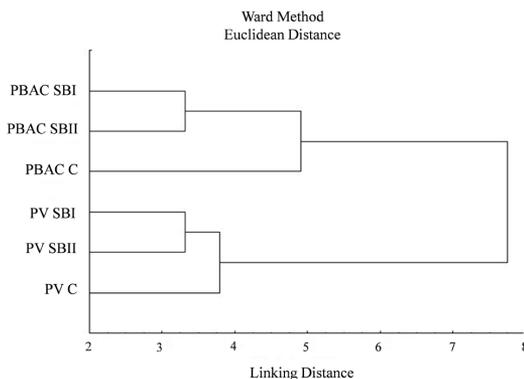


Figure 1. Dendrogram obtained by cluster analysis for the floristic composition of the transposed seed bank and control (SBI, SBII and C) in Brown-Grey Argisol (PBAC) and Red Argisol (PV) soil types in Santa Maria, RS, Brazil.

Closer bonds between SBI and SBII treatments within each deposition area were observed, which demonstrates the transposition effect, showing greater species richness compared to control. The main grouping distinction was observed between soil types (PBAC and PV), evidencing that the environmental characteristics influenced the floristic characteristics of regenerates; and in PBAC, having more hydromorphic character, the occurrence of species such as *Ludwigia* sp., *Centella asiatica* (L.) Urb., *Eryngium* sp., and *Cuphea carthagenensis* (Jacq.) J. F. Macbr. (Table 1) was observed, which are characteristics of humid environments (Boldrini et al., 2008).

4. CONCLUSIONS

- The conditions of the deposition site influenced by the seed bank composition were mainly influenced by the soil hydromorphic characteristics;
- Seed bank removed from the center and edge of the forest fragment at medium to advanced stage was efficient in increasing richness and diversity in both soil types where it was deposited;
- The seed bank transposition started the recovery and initial succession process in Brown-Grey Argisol and Red Argisol soil types.

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