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Assessment of the Soil Seed Bank Aiming at Transposition to Forest Regeneration in the Western Amazonia

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Abstract

This study evaluated the soil seed bank's germination potential and density in five distinct environmental areas namely: a) regenerated forest, b) secondary forest, c) degraded pasture, d) *Eucalyptus* sp. plantation and e) fallow corn growing area, with a view to regenerate forests in Western Amazonia using tray germination methodology. We assessed floristic similarity and diversity using the Jaccard Similarity Index and the Shannon Diversity Index, respectively. We computed each species' phytosociological parameters: density, frequency and importance value of each species. We recorded a total 3674 individuals from 51 species and 21 families. The families with the highest species richness were Asteraceae, Malvaceae, Cyperaceae and Poaceae that contributed to 43% of the total species observed. The most important species computed in the phytosociological analysis were *Chamaesyce hirta, Corchorus aestuans, Cyperus iria* and *Chamaesyce prostrata.* All species had a herbaceous life form, which in the literature, are considered weeds. We documented the largest number of individuals in the fallow corn growing area that had 3620 plants m^{-2} and the smallest number in the regenerated forest that had 183 plants m^{-2}. We observed the greatest floristic similarity between the secondary forest and *Eucalyptus* sp. plantation (40%), and the greatest floristic diversity in the *Eucalyptus* sp. plantation (H '= 2.59 nats individual^{-1}). In conclusion, the transposition of the soil seed bank is not recommended for forest regeneration and recovery in degraded areas due to massive weed predominance in the soil seed bank.

Keywords: degraded area, ecological succession, nucleation, soil recovery, restoration ecology

1. Introduction

Transposition of soil seed bank (SSB) stands out among techniques used to restore degraded areas. This technique describes the removal topsoil from a conserved area and its deposition in a degraded area with the same plant typology (Boanares & Azevedo, 2014, Reis et al., 2014, Bechara et al. 2016, He et al., 2016, Ribeiro et al., 2017).

The SSB is a collection of viable seeds present on the surface of and inside the soil. The SSB can support vegetation regeneration in degraded areas by introducing an abundance of nutrients, increasing species' diversity, and enhancing conditions that facilitate the establishment of a new successional standard (Martins, 2018).

Using SSB transposition to restore an environment is based on the nucleation concept. Nucleation uses the biotic and abiotic elements in a small diversity nucleus that contains a micro-habitat to generate a new series of random successions. Nucleation promotes ecological propellants and enhances the probability of forming diverse random succession routes (Reis et al., 2014, Martins, 2018).

Nucleation is a relatively quick and low-cost assessment that can provide data on natural regeneration and define strategies to accelerate ecological succession processes in restored areas (Martins, 2018).

However, transposition requires meticulous evaluation of the SSB for its floristic composition and diversity, similarity to other areas, and its phytosiological parameters such as each species' density, frequency and importance value.

Preliminarily evaluating the SSB illustrates its natural regeneration potential since a predominance of weed species' seeds can negatively influence the agroecosystem's future composition and functioning (Skowronek et al., 2014).

The distribution and occurrence of most weeds in degraded areas is dependent on the combined effect of climate, and soil and relief and management practices (Santos et al. 2020).

Weeds interfere in agroecosystems by competing with cultivated crops for nutrients, water, light and space. Moreover, many weed species possess allelopathy mechanisms that hinder or prevent the growth of other plant/weed species.

Several studies have proved that SSB transposition is a viable alternative to forest regeneration in abandoned pastures and other degraded areas (Bechara et al. 2016, Santos et al. 2016, Piaia et al. 2017, Ribeiro et al, 2017, Martins 2018, Oliveira et al., 2018, Santos et al. 2020). However, little is known about the use of seed bank transposition as an environmental restoration tool in western Amazonia.

Therefore, this study evaluated the seed bank's germination and density, potential and its floristic composition, similarity, diversity and performed a phytosociological analysis in five different areas with distinct environmental conditions located in western Amazonia

2. Methods

2.1 Characteristics the Study Area

The research was carried out in in three rural areas located at the following coordinates: 4°57'56.43'' S and 47°20'3.41'' W; 4°56'54.20'' S and 47°22'32.50'' W; and 4°59'51.60'' S and 47°17'59.90'' W, in Açailândia municipality, state of Maranhão, in western Amazonia (Figure 1).

The region presents irregular relief and has an average altitude of 200 m. According to the Köppen classification, the region has an Aw tropical climate with an annual average temperature of 25.9° C and an average annual rainfall of 1334 mm (EDS QGIS, 3.6.1, 2019). Moreover, the region's soils are Red Yellow Latosol and Dystrophic Red Yellow Argisol soils (EMBRAPA, 2018).

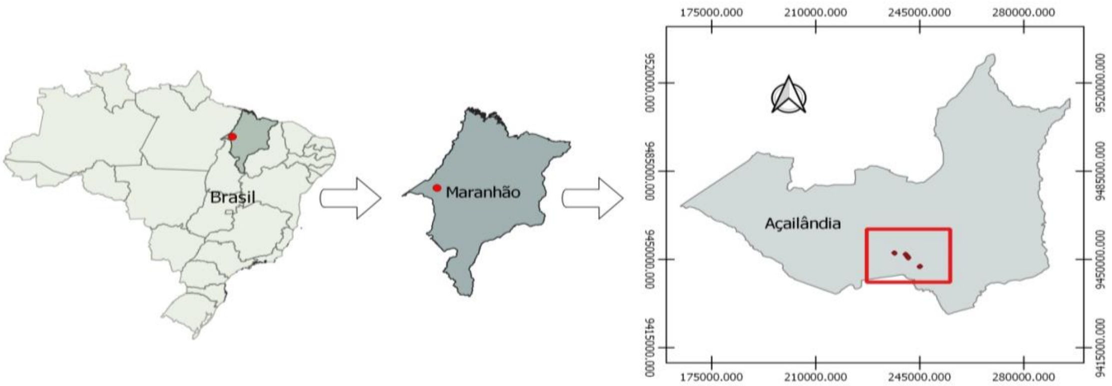

Figure 1. Location map of sample points, municipality of Açailândia, state of Maranhão, western Amazonia, 2019

2.2 Evaluation of the Soil Seed Bank

A transect following the relief slope was marked in each area with a GPS (Garmin Etrex 10®). A soil sample measuring 16 cm x 25 cm x 5 cm was collected using a metallic template at 50 m-intervals. We collected 15 soil samples in each environmental situation. In areas with thick herbaceous vegetation where it was difficult to collect a sample, the ground was manually cut and roots and cuttings removed from the soil's surface.

The soil samples were placed in plastic bags which were then labelled and transported to a plastic greenhouse. In the greenhouse, labelled samples were placed in high density polyethylene trays measuring 30.3 cm long, 22.1 cm wide and 7.5 cm deep. Holes had been drilled into the trays to facilitate drainage.

2.3 Experimental Design

The experiment was laid out in a randomized complete design with five treatments and 15 replications. The treatments were: regenerated forest (T1), secondary vegetation (T2), degraded pasture (T3), *Eucalyptus* sp. plantation (T4) and fallow corn growing area (T5).

The experiment was carried out in a plastic greenhouse with a 150 micra thick polyethylene cover and side shade protection with a 50% shading rate. The greenhouse was located at the Technological Diffusion Center, in the municipality of Imperatriz, State of Maranhão. Irrigation was carried out by means of an automatic micro-sprinkler system, with operating timer at five-minutes intervals at 8:00 am, 12:00 pm and 6:00 pm, during 150 days.

Germination was carried out in trays. Every 15 days, germinated seedlings were identified, counted, and subsequently removed from the trays. The research team made ten evaluations over a period of 150 days.

Species were identified by comparing them with known species in published literature (Lorenzi, 2014). The species that could not be identified were transplanted into tubes and grown until the flowering stage. The flowers were then used to identify the species. We organized the floristic composition according to Angiosperm Phylogeny Group IV' classification system (APG IV, 2016).

2.4 Statistical Analyses

We used the Shapiro-Wilk normality test to assess data for normality and the Bartlett homogeneity tests of variance to compare variances between groups. These tests were carried out at 5% probability before assessing the possibility of performing an Analysis of Variance (ANOVA) test. However, when normality and homogeneity of variance were rejected, we performed the Kruskal-Wallis non-parametric test, followed by Nemenyi's post-hoc test (Emmerling, 2014) for multiple comparisons using the SAS statistical program (SAS, 2000), at 5% probability.

Additionally, we assessed for floristic similarity in the five areas by means of the Jaccard

Similarity Index using the Past 3.25 software (Hamer et al., 2001). Floristic diversity was assessed using the Shannon Diversity Index (Mesquita et al. 2016, Amorim & Mesquita, 2019).

We computed the following phytosociological parameters to analyze the plant community: frequency to describe species' distribution in the plots; density of individuals of each species per unit area and the relative frequency and relative density of each species found in the area. The importance value indicates which species are more important within the studied area (MullerDombois & Ellenberg, 1974).

3. Results and Discussion

3.1 Floristic Composition

In total, there were 3674 individuals belonging to 51 species and 21 families. All species had a herbaceous life form (Table 1).

Herbaceous species predominate in regenerated degraded areas and in recovering forests due to their more open canopy. Such areas include anthropized areas that have a fallow interval, and secondary vegetation (Piaia et al. 2017, Duarte et al. 2019, Santos et al., 2020).

This study areas had numerous anthropization characteristics ranging from cut large arboreal individuals, burnt areas, and no regard to preserve soil fertility before planting pastures, and a greater susceptibility to erosion processes. These characteristics may have facilitated herbaceous species' invasion.

Oliveira et al. (2018) found results similar to those of this study; they had 20 families and 36 species emerging from the same depth used in our study indicating that even in soil profiles from shallow depths have a significant amount of viable seeds (Table 1).

In a different research study carried out with different successional stages in a degraded area, the dominant species in all treatments also had a herbaceous life form. These results corroborate the results of the present study (Bechara, et al. 2016, Piaia et al. 2017, Santos et al., 2020).

In this study, the families with the highest number of species were Asteraceae and Malvaceae with six species each, followed by Cyperaceae and Poaceae with five species each. These four families contributed to 43% of the all the species in this study (Table 1).

Piaia et al. (2017) recorded the predominance of pioneer herbaceous species with greater representation of the Poaceae, Asteraceae and Solanaceae families in soil seed bank fragments from seasonal forests and stabilized gullet. Similar results were reported by Deiss et al. (2018) regarding the *Eucalyptus dunnii* Maiden plantation.

The species *F. dichotoma*, *C. hirta*, *C. prostrata*, *C. mucunoides C. aestuans* and *H. communis* were observed in all the SSB's evaluated environments, showing their great plasticity, that is, their ability to adapt different environments (Table 1).

Table 1. List of species, families, common names and number of individuals, identified in the soil seed bank in areas with five environmental situations: regenerated forest (T1), secondary

vegetation (T2), degraded pasture (T3), *Eucalyptus* sp. plantation (T4) and fallow corn growing area (T5) in the municipality of Açailandia, state of Maranhão, western Amazonia

Species	Family	Common name	Number of individuals				
			T1	T2	T3	T4	T5
Amaranthus deflexus L.	AMARANTHACEAE	Bredo	-	1	-	-	2
Amaranthus hybridus var. *patulus* (Betol.) Thell.	AMARANTHACEAE	Bredo	-	-	-	-	7
Amaranthus sp.	AMARANTHACEAE	Amaranto	12	3	-	-	-
Bidens pilosa L.	ASTERACEAE	Picão Preto	-	4	-	-	-
Eclipta alba (L.) Hassk.	ASTERACEAE	Erva de botão	-	9	9	4	117
Hypochaeris radicata L.	ASTERACEAE	Almeirão do campo	-	1	-	-	-
Parthenium hysterophorus L.	ASTERACEAE	Losna branca	8	-	-	10	-
Synedrellopsis grisebachii Hieron. & Kuntze	ASTERACEAE	Agriãozinho	-	2	2	-	7
Vernonia ferrugínea Less.	ASTERACEAE	Assa peixe	-	-	-	3	-
Commelina benghalensis L.	COMMELINACEAE	Trapoeraba	1	6	-	2	23
Commelina difusa Burm. f.	COMMELINACEAE	Trapoeraba	-	9	28	-	-

Journal of Agricultural Studies
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Ipomoea cairica (L.) Sweet	CONVOLVULACEAE	Jetirana	1	1	-	-	-
Ipomoea purpúrea (L.) Roth	CONVOLVULACEAE	Corda de Viola	1	-	-	-	-
Ipomoea ramosíssima (Poit.) Choisy	CONVOLVULACEAE	Campainha	-	-	-	-	7
Ipomoea sp.	CONVOLVULACEAE	Corda de Viola	2	-	-	3	5
Cyperus difformis L.	CYPERACEAE	Tiririca	-	3	27	-	-
Cyperus hermaphroditus (Jacq.) Standl	CYPERACEAE	Tiririca	-	-	16	1	17
Cyperus iria L.	CYPERACEAE	Titirica	-	32	278	22	340
Fimbristylis dichotoma (L.) Vahl	CYPERACEAE	Grama de oito dias	3	30	48	13	73
Fimbristylis miliacea (L.) Vahl	CYPERACEAE	Cuminho	-	-	-	-	1
Chamaesyce hirta (L.) Millsp.	EUPHORBIACEAE	Erva de Santa Luzia	23	67	38	56	82
Chamaesyce prostrata (Aiton) Small	EUPHORBIACEAE	Baldroega	4	10	31	94	74
Croton lundianus (Didr.) Müll. Arg.	EUPHORBIACEAE	Gervão branco	-	10	-	1	-
Euphorbia sp.	EUPHORBIACEAE	Amendoin bravo	-	2	-	-	

Species	Famíly	Common name	Number of indivíduals				
			T1	T2	T3	T4	T5
Calopogonium mucunoides Desv.	FABACEAE	Calopogonio	1	15	27	6	10
Mimosa pudica L.	FABACEAE	Dormideira	-	-	-	4	2
Corchorus aestuans L.	MALVACEAE	Juta	4	390	149	18	574
Sida glaziovii	MALVACEAE	Guanxuma-branca	-	2	-	17	1
Continuing Table 1...							
Sida rhombifolia L.	MALVACEAE	Vassourinha	-	3	21	14	16
Sida spinosa L.	MALVACEAE	Guaxuma de espinho	-	-	-	8	-
Sida sp.	MALVACEAE	Guaxuma	9	16	1	-	-
Urena lobata L.	MALVACEAE	Caquibosa	-	-	-	-	1
Mollugo verticillata L.	MOLLUGINACEAE	Capim tapete	-	8	9	3	77
Ludwigia leptocarpa (Nutt.) H. Hara	ONAGRACEAE	Cruz de Malta	1	1	-	-	9
Phyllanthus niruri L.	PHYLLANTHACEAE	Quebra pedra	-	14	9	1	69
Lindernia crustacea (L.) F. Muell.	PLANTAGINACEAE	Capim tapete	1	2	6		69

Journal of Agricultural Studies
ISSN 2166-0379
2021, Vol. 9, No. 2

Brachiaria sp.	POACEAE	Brachiaria	-	-	12	10	-
Cynodon dactylon (L.) Pers.	POACEAE	Grama seda	-	-	-	1	1
Digitaria horizontalis Willd.	POACEAE	Capim colchão	-	-	2	4	2
Digitaria sp.	POACEAE	Capim Colchão	-	-	1	1	-
Eleusine indica (L.) Gaertn.	POACEAE	Capim pé de galinha	-	2	21	4	41
Portulaca oleraceae L.	PORTULACACEAE	Baldroega	-	3	4	12	212
Spermacoe verticilata L.	RUBIACEAE	Vassourinha	-	2	-	4	13
Diodia saponariifolia (Cham. & Schltdl.) K. Schum.	RUBIACEAE	Poia do brejo	-	1	-	12	18
Mitracarpus hirtus (L.) DC.	RUBIACEAE	Poia	-	4	2	-	-
Physalis angulata L.	SOLANACEAE	Camapú	-	2	-	-	-
Turnera subulata Sm.	TURNERACEAE	Albina		4	-	-	
Hybanthus communis (A St.-Hil.) Taub.	VIOLACEAE	Bandeira branca	2	4	8	3	3
Unidentified species	-			-	3	18	9

Total of species	15	33	24	28	31
Total of indivíduals	73	663	752	338	1448

The species *F. dichotoma*, *C. hirta*, *C. prostrata*, *C. mucunoides C. aestuans* and *H. communis* were observed in all evaluated environmental situations showing their great plasticity (i.e. their ability to adapt different environments) (Table 1). In contrast, the species *I. purpurea* was only observed in T1 (regenerated forest), the species *Bidens* sp., *H. radiata*, *Euphorbia* sp. and *P. angulata* only found in T2 (secondary vegetation), the species *V. ferruginea* and *S. spinosa* in T4 (*Eucalyptus* sp. plantation), and the species *A. hybridus* in T5 (fallow corn growing area) (Table 1).

The highest seedling emergence flows were observed 15, 45 and 90 days after the start of the study in all treatments except T1 (Figure 2).

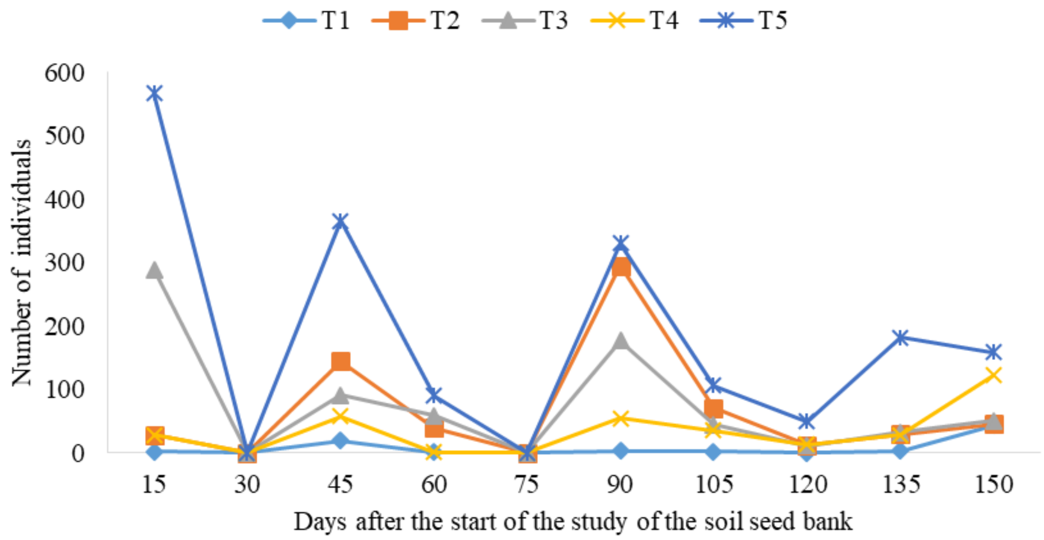

Figure 2. Number of individuals recorded in the soil seed bank during the period of 150 days of evaluation in five environment situations: regenerated forest (T1), secondary vegetation (T2), degraded pasture (T3), *Eucalyptus* sp. plantation (T4) and fallow corn growing area (T5)

Differences in the number of germinated individuals over time are due to the precipitation pulses at the beginning of the rainy season that trigger seed germination in the SSB.

3.2 Floristic Similarity

The Jaccard Similarity Index demonstrated a greatest floristic similarity between T2 and T4 treatments probably due to their geographic proximity and similar climate and soil conditions. The T1 treatment is the most distinct treatment used in this study (Figure 2).

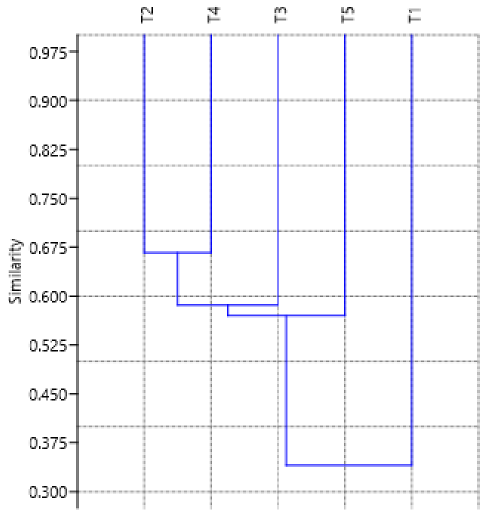

Figure 2. Dendrogram with the Jaccard Similarity Index in five environmental situations: regenerated forest (T1), secondary vegetation (T2), degraded pasture (T3), *Eucalyptus* sp. plantation (T4) and fallow corn growing area (T5)

3.3 Floristic Diversity

Based on the Shannon Diversity Index, floristic diversity was highest in the *Eucalyptus* sp. plantation (H '= 2.59 nats individual^{-1}) followed by the fallow corn growing area (H' = 2.32), degraded pasture (H '= 2.21), forest remnant (H' = 2.16) and secondary vegetation (H '= 1.82 nats individual^{-1}). Similarly, Ribeiro et al. (2017) reported that the Diversity Index in planted areas was higher than the Diversity Index in degraded areas.

Based on results of the Kruskal-Wallis non-parametric test and the Nemenyi's post-hoc test for multiple comparisons, the number of individuals was highest in the fallow corn cultivation area; this difference was statistically significant (Table 2).

Table 2. Comparison between treatments regarding the number of individuals m^{-2}

Treatment	SD	Mean
1	13,76	7,30 b
2	90,77	66,30 a b
3	91,61	75,20 a b
4	37,40	33,80 a b
5	183,38	184,80 a

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Test statistics a,b	Ind
Chi-square	11,770
SD – Standard deviation	4
Significance Sig.	,019

Means with different letters differ by the multiple comparison of Nemenyi test at 5% significance

SD = Standard deviation.

The seed bank in the preserved environment compared to areas in the process of degradation or intensive management are divergent in wealth and abundance.

The statistical analysis also showed a significant difference in the number of individuals p = 0.019 ($p < 0.05$) and confirmed that the total density of viable seeds in the soil seed bank tends to decrease with advances in ecological succession from restoration techniques.

3.4 Phytosociological Analyses

The germination density in the soil seed bank was recorded as 3,620 plants m^{-2} in T5, 1,813 plants m^{-2} in T3, 1,676 plants m^{-2} in T2, 845 plants m^{-2} in T4 and 183 plants m^{-2} in T1.

These results concur with reports by Schorn et al. (2013) that showed 2,846 individuals m^{-2} for remaining environments, 4,292 individuals m^{-2} for fallow areas of forest with eucalyptus plants, and 2,125 individuals m^{-2} for area reforested using *Pinnus* sp.

The result of the phytosociological analysis showed that only six species had a high Importance Value and thus stood out as the dominant species. The importance value (IV) numerically expresses the importance of a given species in a community (Müeller-Dombois & Ellenberg, 1974). (Table 3)

Table 3. Relative density, relative frequency and importance value of the six major species recorded in the soil seedbank in five environmental situations in the western Amazon

Regenerated forest			
Species	RD	RF	IV
Chamaesyce hirta	31.5	33.6	65.1
Sida sp.	12.3	10.5	22.8
Amaranthus sp.	16.4	2.7	19.1
Parthenium hysterophorus	10.9	5.5	16.4
Fimbristylis dichotoma	4.1	11.1	15.3
Chamaesyce prostrata	5.4	5.5	10.9
Secondary forest			

Species	RD	RF	IV
Corchorus aestuans	58.8	26.1	84.9
Chamaesyce hirta	10.1	10.1	20.2
Fimbritylis dichotoma	4.4	6.9	11.3
Cyperus iria	4.8	3.7	8.5
Calopogonium mucunoides	2.2	4.8	7.0
Bidens sp.	0.6	6.4	7.0
Degraded pasture			
Species	RD	RF	IV
Cyperus iria.	36.9	13.7	50.6
Corchorus aestuans	19.3	13.0	32.3
Fimbristylis dichotoma	6.3	13.0	19.4
Chamaesyce hirta	5.0	9.8	14.8
Calopogonium mucunoides	3.5	9.8	13.3
Chamaesyce. Prostrata	4.1	7.1	11.2
Eucalyptus sp. plantation			
Species	RD	RF	IV
Chamaesyce prostrata	27.8	16.0	43.8
Chamaesyce hirta	16.5	14.8	31.3
Cyperus iria	6.5	7.4	13.9
Corchorus aestuans	5.3	7.4	12.7
Fimbristylis dichotoma	3.8	6.1	9.9
Sida glaziovii	5.0	3.7	8.7
Fallow corn growing area			
Species	RD	RF	IV
Corchorus aestuans	31.0	15.1	46.1
Cyperus iria	18.3	7.5	25.8
Portulaca oleracea	11.4	13.9	25.3
Phyllanthus niruri	3.7	9.2	12.9
Lindernia crustacea	2.4	9.2	11.6
Chamaesyce hirta	6.2	5.0	11.2

The species *C. hirta* was among the six dominant species in the seed bank in all areas, while other species portrayed similar behavior in all except one area, for example, *C. aestuans* was nondominant in T1, *F. dichotoma* in T5 and *Cyperus iria* in T1.

All this study's dominant species are exclusively propagated by seeds. However, *F. dichotoma* (Cyperaceae) is also propagate asexually using rhizomes (Lorenzi, 2008).

The species' dominance in soil seedbank is related not only to the area's history, but also to the species' reproductive capacity. For instance, *Fimbristylis dichotoma* (L.) Vahl can produce 6500 seeds per plant, while *Cyperus iria* (L.) can produce 5000 seeds per plant (Thompson et al. 2003).

Weeds characteristically produce a very high number of seeds to ensure species' survival and escape stresses imposed by weed-control methods.

The analyzes carried out show that in a 5 cm profile tray, herbaceous species, considered weeds or invasive plants, prevailed at the end of an emergency. This demonstrates that small seeds, characteristic of weed species, have adaptive advantages such as low predation and ease of incorporation into the soil (Santos et al. 2020).

The fact that trees and shrubs did not germinate can be linked to environmental fragmentation, seasonality (Santos et al. 2020) and floods in riparian forest areas that remove and bury the soil's seed bank (Araújo et al., 2004).

Despite challenges in understanding the entire ecological succession process and how to apply technical knowledge to facilitate recovery processes, further research should focus on the SSB's dynamics over longer germination assessment periods to support planning and executing soil transposition for successful ecological restoration.

4. Conclusions

In a regenerated forest, or degraded pasture, secondary forest, *Eucalyptus* sp. plantation and fallow corn growing areas in Western Amazonia the composition, similarity and floristic diversity of soil seed bank is formed by species of herbaceous life form considered in the literature as weeds with an invasive character, with no more seeds left of shrub-tree species deposited in the seed bank.

Therefore, SSB transposition as a nucleation technique cannot be used to regenerate Western Amazonian forests in areas similar to those we studied.

Alternative complementary interventions, such as planting seedlings and / or direct sowing of native tree species should be used to accelerate plant succession.

Further studies employing alternative nucleation techniques should be conducted to support forest regeneration in Western Amazonia.

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