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EFFECT OF TOPSOIL STOCKPILING ON THE VIABILITY OF SEED BANK IN FIELD PHYTOPHYSIOGNOMIES CAMPOS DE ALTITUDE

Keywords:
Seed longevity
Bauxite
Degraded areas
Mining
Herbaceous species

ABSTRACT: The viability of propagules during topsoil stockpiling is a limiting factor in ecological restoration projects and little is known about the species distributed in the campos de altitude. This work was carried out to investigate the viability of propagules present in the topsoil under campos de altitude vegetation, stockpiled for up to 12 months after the stripping of areas to be mined. In the south of Minas Gerais, Brazil, between November 2011 and November 2012, four collections of the seed bank were carried out, considering three depths (0 to 10, 90 to 100, and 190 to 200 cm) of the plot of stockpiled topsoil. Using the multivariate analysis, it was verified that the depth factor does not statistically affect the abundance of emerged individuals, while the factor time of stockpiling negatively affects the viability of the seeds. Some species were affected by the stockpiling conditions, only emerging in some collections, while others (*Achyrocline satureioides*, *Ageratum fastigiatum*, *Baccharis dracunculifolia*, *Borreria capitata, Echinolaena inflexa* and *Melinis minutiflora*) had individuals emerged in all collection periods. This study points out the need for the return of the topsoil until the fourth month of stocking, under the risk of monodominance, with a prevalence of species more adapted to predominant conditions of campos de altitude.

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EFEITO DA ESTOCAGEM DE TOPSOIL NA VIABILIDADE DO BANCO DE SEMENTES EM FITOFISIONOMIAS DE CAMPOS DE ALTITUDE

Palavras chave:
Longevidade de sementes
Bauxita
Áreas degradadas
Mineração
Especies herbáceas

RESUMO: A viabilidade dos propágulos durante a estocagem de topsoil é um fator limitante em projetos de restauração ecológica, e pouco se sabe sobre as espécies distribuídas nos campos de altitude. Este trabalho foi realizado com o objetivo de investigar a viabilidade de propágulos presente no topsoil sob vegetação de campos de altitude, estocado por até 12 meses após o decapeamento de áreas a serem mineradas. Entre o período de novembro de 2011 a novembro de 2012 foram realizadas quatro coletas do banco de sementes, sendo consideradas três profundidades (0 a 10, 90 a 100 e 190 a 200 cm) da leira de topsoil estocada. Utilizando a análise multivariada, verificou-se que o fator profundidade não afeta estatisticamente a abundância de indivíduos emergidos, enquanto o fator tempo de estocagem afeta negativamente a viabilidade das sementes. Algumas espécies foram afetadas pelas condições de estoque do topsoil, emergindo somente em algumas coletas, enquanto outras (Achyrocline satureioides, Ageratum fastigiatum, Baccharis dracunculifolia, Borreria capitata, Echinolaena inflexa e Melinis minutiflora) tiveram indivíduos emergidos em todos os períodos de coleta. Este estudo aponta a necessidade do retorno do topsoil até o quarto mês de armazenamento, sob o risco de ocorrer monodominância, com predominância de espécies mais adaptadas as condições campestres.

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INTRODUCTION

The campos de altitude are environments characterized as mountain tops of southern and southeastern Brazil (SAFFORD, 1999; COSTA et al., 2011), with recognized biological, geological, and hydric importance (SAFFORD, 1999; SCHEER et al., 2011), belonging to the domain of the Atlantic Forest. They are characterized by their high ecological fragility, constituted by a rare Brazilian vegetation, with predominance of grasses, herbs, and some pteridophytes (SAFFORD, 1999; CAIAFA; SILVA, 2005; MOCOCHINSKI; SCHEER, 2008). Because they are small species with little commercial interest, most of them from the Asteraceae, Fabaceae, Cyperaceae, and Melastomataceae families (CAIAFA; SILVA, 2005; MOCOCHINSKI; SCHEER, 2008; MEIRELES et al., 2014), the vegetation has been little studied and is among the most unknown (MOCOCHINSKI; SCHEER 2008; MORAS FILHO et al, 2017). It should be noted that these areas are not restricted to Brazil, occurring in regions with high altitudes, such as the "páramos" in the Andes (SAFFORD, 2007).

However, in view of the need to recover areas of altitude fields after mining activities (BRASIL, 1988; SÁNCHEZ, 2011), among other environmental degradation situations, it is necessary to investigate techniques to make environmental recovery plans more efficient. The use of topsoil (RIVERA et al., 2012; GOLOS; DIXON, 2014) is a widespread technique in revegetation activities after bauxite mining (Barros, 2013). The seed bank, contained in the stockpiled topsoil, has favorable implications for vegetation succession and restoration (FERREIRA et al., 2015; SHANGA et al., 2016). Nevertheless, the composition, richness, and density of species present in the soil seed bank are influenced by the storage environment (SNYMAN, 2013; SANTOS et al., 2016), which may be a limiting factor for recovery activities in areas of recognized fragility in the floristic composition.

Seed production often occurs under a narrow range of environmental conditions (OTT; HARTNETT, 2015) and can be abundant in the seed bank only for a given period or year (SNYMAN, 2013). Seeds may also synchronize their germination and dormancy cycles to regular seasonal environmental changes (FENNER; THOMPSON, 2005; GARCIA et al., 2014), increasing their longevity under local conditions (SNYMAN, 2013). Le Stradic et al. (2015), studying 15 species of rupestrian fields, affirmed that the environment presents a great diversity of strategies for germination of its seeds, in which this difference can be seen among species and among families.

Seed bank storage conditions may reduce seeds viability (HU et al., 2013, GOLOS; DIXON, 2014) and lead to a new plant community, unlike the one that provided the material (VAN ETTEN et al., 2014). The current studies have focused the caracterization of environmental conditions that minimize the decline of seed bank viability (PAKEMAN et al., 2012; GOLOS; DIXON, 2014; NASCIMENTO et al., 2016); however, it is necessary to understand the influence of factors intrinsic to the stockpile, such as stockpile height and vegetal composition in different periods, considering that the topsoil can be stored or partially used. (BARROS et al., 2012). Thus, innovative surveys in altitude fields become important tools for the development of strategies of seed bank management (BORGY et al., 2015), since these are highly variable or unpredictable environments, with few studies (BARROS et al., 2013; FERREIRA et al., 2015).

The objective of the present work was to evaluate the viability of the regenerating material present in the topsoil, analyzing the emergence of propagules during one year and in three depths in stocked plots at the time of stripping, before the mining activity.

MATERIAL AND METHODS

Characterization of the study area

The topsoil was collected in a bauxite mining area in the Plateau Region of Poços de Caldas, around 21°52'38" S and 46°27'48" W. The region is inserted in the Atlantic Forest domain (IBGE, 2012) mainly occupied by vegetation associated with Campos de altitude. The climate is mesothermic, Cwb type, according to Köppen classification (Moraes and Jiménez-Rueda, 2008), with a rainfall index of 1,706.00 mm and an average annual temperature of 17.7°C (OLIVEIRA-FILHO, 2014). There are two well-defined seasons: a rainy season, which begins in September and goes through March, and a dry period, which begins in April and lasts until August (PEREIRA; FONTES, 2009). The average precipitation and minimum and maximum temperatures during the study period are presented in Figure 1.

Sampling procedure

To evaluate the viability of stocked topsoil, the soil was sampled from three stocked piles, kept uncovered, downstream of the interference area of the mining activity after the stripping. There were four sample collections over a year (BASKIN; BASKIN, 2014), in different seasons (MARTINS, 2014). The collection of the stocked material started in November 2011, when stripping was performed with a bulldozer, and finished in November 2012 (Figure 2), with the use of topsoil in the mined area.

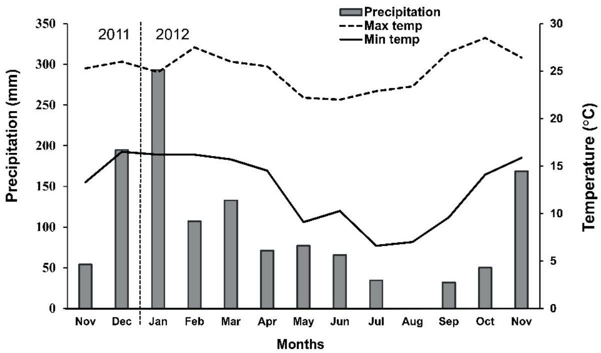

FIGURE 1 Climatic data of monthly precipitation and maximum and minimum temperature of the air in the Poços de Caldas plateau, Brazil. Source: Instituto Nacional de Meteorologia, 2016.

In each collection, three depths (0 to 10, 90 to 100, and 190 to 200 cm) of soil were sampled, taken from the central area, and repeated in three different piles. A clamshell digger was used as the instrument of collection.

Evaluations of the emergence of seedlings from stockpiled topsoil

The collected material was taken to the forest nursery of the Federal University of Lavras and kept in a greenhouse, glass-covered to ensure high luminosity. For the tests, a 2-cm layer of soil was spread over a 1.50-cm bed of sterilized sand in an autoclave arranged in plastic trays. The seedlings that emerged were quantified and identified for the database.

The absolute density (individual·m^{-2}) was calculated considering the dimensions 33 × 44 cm, equivalent to each of the 36 trays. The diversity indexes were calculated according to the values found for species abundance present in the samples. Therefore, the Shannon (H') and Pielou Equability (J') indices were calculated.

The matrices of similarities used were based on the Bray-Curtis index (Magurran, 1988). Differences of species abundance found in the seed bank in each collection were

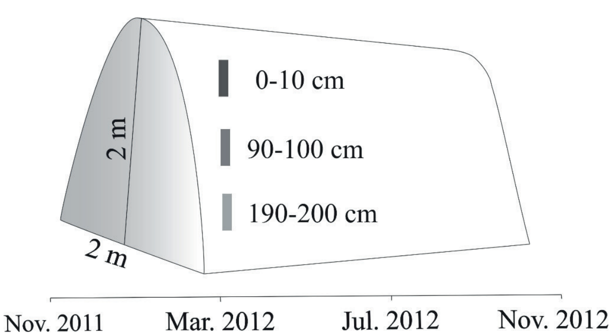

FIGURE 2 Sampling scheme of topsoil stocked during bauxite mining under altitude fields at three depths and four periods in the Poços de Caldas plateau region, MG.

tested by a two-way PERMANOVA, with four periods and at three collection depths. A PERMDISP was also calculated to verify the homogeneity in the dispersion of the points in the multivariate space, and thus, find differences between the levels, considering the distance between the centroids (ANDERSON, 2006). These analyzes were performed by the statistical program PRIMER (version 6.0) with the PERMANOVA update package (CLARKE; GORLEY, 2006; ANDERSON et al., 2008).

Similarity percentage analyzes (SIMPER) were used to estimate the contribution of each species to the difference observed in the composition of seedlings between each collection period. For this analysis, the software Past, version 2.14, was used (HAMMER et al., 2001).

RESULTS

Botanical composition of the vegetation in the field

Forty-eight species, out of 536 emerged seedlings, were identified and distributed into six botanical families. The most representative families were Asteraceae (306 individuals and 11 species), Poaceae (144 individuals and 21 species), Rubiaceae (49 individuals and 3 species), and Cyperaceae (16 individuals and 8 species). Among the identified species, the *Ageratum fastigiatum* (Gardner) (24.44%), *Gamochaeta americana* (13.43%), *Melinis minutiflora* (11.75%), and *Achyrocline satureioides* (8.95%) were predominant, being responsible for 58.58% of the seedlings emerged in the seed bank samplings.

From the information about seeds emerged from different depths, it was possible to visualize the oscillations of the parameters during the evaluation period (Figure 3). The tendency of decrease in the diversity of species throughout the storage period was observed (Figure 3d). However, the number of individuals and the relative density (Figure 3a, b) tended to remain stable until the 4th month, with an increase in the density for the collections of higher depths (90-100 and 190-200 cm). Analyzing the Pielou equability (Figure 3c), a decrease of this index was observed, which was higher from the 4th month.

Abundance of seedlings in relation to the stocking period and storage depth in the pile

It was possible to verify that the species abundance in the three layers studied did not differ significantly from each other (P>0.05) and that the variations occurred were due to chance, however, presenting a significant difference between the collection times of the seed bank (P>0.05) (Table 1).

TABLE 1 Permutational multivariate analysis of variance (PERMANOVA), for abundance of seedlings emerged in the seed bank of altitude fields in three depths and four periods in the Poços de Caldas plateau region, MG.

Source	df	SS	MS	Pseudo-F	P (perm)
Period	3	24060	8019.9	3.04	0.001*
Depth	2	3949.5	1974.8	0.74854	0.808
Interaction	6	13124	2187.4	0.82913	0.825
Residue	23	60678	2638.2	-	-
Total	34	1.01×10^5	-	-	-

df - degree of freedom; SS - sum of squares; MS - mean square; P (perm) - permutation of the P-value.

The PERMDISP (Table 2) analyzes provided information on changes in the variability of the communities present in the seed bank that could be used to infer changes in seed bank diversity (GIORIA et al., 2014).

Despite the statistical difference among the sampled periods, the groups were not distinctly separated by the influence of some species common to all groups (Figure 4). The collections performed at time 1 presented greater similarity, that is, the emerged species resembled among the repetitions; however, over the storage time, greater species dissimilarity occurred (time 4). The greatest dissimilarity, found from the 8th month on, is due to the presence of species only in the initial months of storage (*Achyrocline satureioides* and *Paspalum pilosum*), while others emerged only from this period (*Andropogum bicornis* and *Baccharis dracunculifolia*) (Table 3).

According to the analysis of the contribution percentages of taxa in the similarity (SIMPER) applied to the four sampling times (Table 3), it was verified that the similarity decreased with the storage time. The specie *Ageratum fastigiatum* contributed to the dissimilarity of the treatments due to its distinct behavior in the collection periods, because over the months (Table 3), the number of seedlings decreased. At 12 months, there was a low similarity (9.54), as four species are exclusive representatives of dissimilarity in this collection. By the 8th month, only four species contributed to an average similarity of 24.46.

The present species are colonizers, tending to be repeated in the different periods of storage, however with different abundances in the periods studied. Among

TABLE 2 Average distance to the centroid and standard errors of the PERMIDISP analysis, comparing abundance of emerged seedlings in the seed bank of altitude fields in four collection periods, in soils on bauxite in the Poços de Caldas plateau region, MG.

Period (months)	Average and standard errors
0 (9 samples)	39.218 ± 2.3819 a
4 (9 samples)	40.111 ± 2.2256 a
8 (8 samples)	50.261 ± 2.7641 b
12 (9 samples)	60.649 ± 2.5369 c

The superscript letters represent paired tests, indicating significant differences between samples.

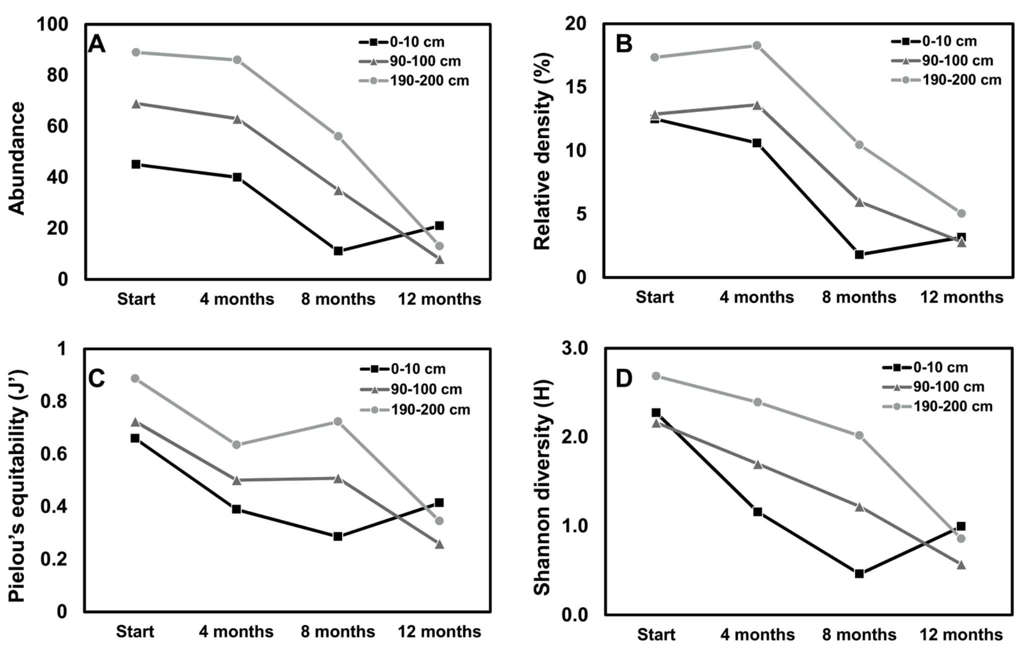

FIGURE 3 Analysis of abundance (A), relative density (B), Pielou's equation (C), and Shannon index (D) of the seed bank in the four collections carried out from November 2011 to November 2012, at three depths (0 to 10, 90 to 100, and 190 to 200 cm) of topsoil stocked during the bauxite mining process under altitude fields at three depths and four periods in the Poços de Caldas plateau region, MG.

TABLE 3 Contributions of the species to the similarities in the collections obtained through the SIMPER routine (90% similarity).

Period	Start		4 months		8 months		12 months	
Average similarity	41.22		39.83		24.46		9.54	
Species	AM	C %	AM	C (%)	AM	C (%)	AM	C (%)
Achyrocline satureioides	0.79	6.89	1.48	17.85	0.62	4.96	-	-
Ageratum fastigiatum	2.27	37.32	1.99	32.52	1.63	43.61	0.44	35.66
Andropogum bicornis	-	-	-	-	-	-	0.22	8.32
Baccharis dracunculifolia	-	-	-	-	-	-	0.22	5.82
Borreria capitata	0.49	3.47	-	-	-	-	-	-
Borreria latifolia	0.89	7.99	0.67	9.84	0.95	37.59	-	-
Cyperus aggregatus	-	-	-	-	-	-	0.22	11.65
Echinolaena inflexa	-	-	0.49	3.91	-	-	-	-
Eragrostis rufescens	-	-	-	-	0.38	4.85	-	-
Gamochaeta americana	1.68	17.13	1.14	7.67	-	-	-	-
Melinis minutiflora	1.4	13.81	1.09	9.01	-	-	0.38	6.16
Panicum pilosum	-	-	-	-	-	-	0.22	6.47
Paspalum pilosum	0.57	4.09	0.99	12.79	-	-	-	-
Schizachyrium tenerum	-	-	-	-	-	-	0.44	20.61

AM - Average Abundance; C% - contribution (%).

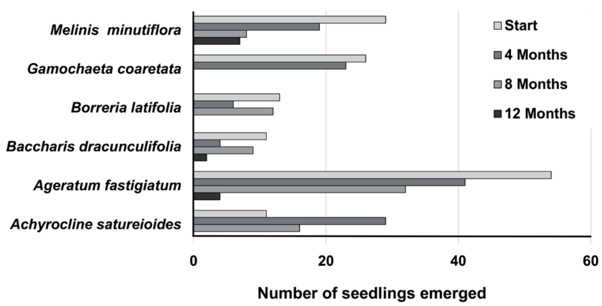

FIGURE 4 Number of seedlings emerged in the seed bank in soils on bauxite in the Poços de Caldas plateau region, MG, by species, in the periods studied.

the species that contributed most to the dissimilarity in the evaluated periods, we highlight *Ageratum fastigiatum*, which contributed with 23% to the dissimilarity between the first and last collection. For these same collections, it was noted that six species contributed with 64% of dissimilarity. Meanwhile, for the species *Achyrocline satureisodes*, the emergence observed at the beginning of the trial (four months) presented low dissimilarity when compared with the final period analyzed (12 months).

DISCUSSION

Composition of species and influences on soil seed bank

The seed bank have 44 species distributed into six botanical families, sufficient to make up the initial vegetation. However, it showed low richness when compared with botanical surveys in campos de altitude. Evaluating nine surveys, carried out in different areas of altitude fields in southeastern Brazil, Ribeiro et al. (2007) verified that the number of species in the seed bank varied between 57 and 127 and between 17 and 49 for families. In this region, the phytosociological survey of the shrubby herbaceous stratum performed by Barros (2014) showed a Shannon diversity index (H ') of 3.10, while in the present study, the highest value for the Shannon diversity index of the seeds was 2.68. This lower diversity is due to the smaller extension of the sampling area for the study of the composition of seed banks (VANDVIK et al., 2016). Although it is not possible to make the selection or predict the diversity of species that will make up the seed bank (NORMAN et al., 2006), it is important to consider environmental factors that may affect the emergence of seedlings, such as seed dormancy mechanisms and species phenology.

Environmental conditions can represent chemical and physical filters (e.g. water and nutritional deficiencies), which induce or limit the establishment of some species (GILARDELLI et al., 2015), making them dominant or rare, able or not to colonize the substrate under the established conditions. Sprengelmeyer and Rebertus (2015) suggest that the determining factors for the presence and/or abundance of plant species in the seed bank are the distance of rock outcrops, soil depth, and altitude. From a different perspective, under storage conditions, seeds of some species are more susceptible to deterioration by the attack of fungi and predators and climatic adversities (SHANGA et al., 2016). The strategy of species propagation, such as number and dispersion of seeds (HORÁČKOVÁ et al., 2015; SHANGA et al., 2016), seed viability, and vegetative propagation (LE STRADIC et al., 2015), and dormant seeds (GARCIA et al., 2014) are also conditioning factors for the species abundance in the recovered areas.

The results of the present study suggest the recognition of the importance of the seed bank in the ecological and functional recovery of mined areas. For van Etten et al. (2014) even with low seed density in the soil seed bank, it is still valuable for post-mining restoration purposes, because in addition to seed supplying, it provides soil microorganisms and suitable means for plant germination and growth . Moreover, some devices can be adopted to improve the dispersion and the capacity of the species for their complete establishment and good development (GILARDELLI et al., 2015). Other complementary interventions, such as planting of seedlings, direct sowing of native plant species, and/or artificial perches, can be considered important to accelerate plant succession (MARTINS, 2014).

Influence of depth on seed bank storage

Although, the significant influence was expected at the depths of the stocked pile, the abundance of plant species of the seed bank did not show significant differences at the various depths (Table 1). In most soils, seed density decreases rapidly with soil depth (FENNER; THOMPSON, 2005). In soil layers with more than 10 cm, the seed abundance is lower when compared with the more superficial layers (OLIVEIRA et al., 2015). However, during the stripping process, the layers are mixed and homogenized (in relation to the diversity of propagules), resulting in non-statistical differentiation in the studied depths.

Greater abundance and density were found in the deeper layer (Figures 3a, b). Possibly, in the upper layers, the seeds found favorable conditions and eventually germinated, while the inner ones maintained the seeds in the absence of light and low moisture (RIVERA et al., 2012; OLIVEIRA et al., 2015). In the 0 to 10 cm layer, the species abundance was more dispersed than in the inner layers of the stocked pile (PERMIDISP). Although, it is a routine in mining to avoid stocking in stacks of more than 2.00 m high, the results of this test showed that the lower layers presented smaller losses of viable seeds than the upper layers. However, further studies should be performed comparing such layers under cover conditions, i.e., excluding the influence of light and moisture.

Effect of storage time on the soil seed bank

Seed bank diversity declined sharply from the fourth month of storage (Figure 3c) and from the eighth month, the individuals present were distributed into a few species (Figure 3d). Evaluating seed viability under controlled conditions, Rivera et al. (2012) found that the percentage of seed survival decreased by around 40% after six months of storage. Thus, in recovery projects, it should be considered that if the topsoil is stocked for more than four months, there may be a significant reduction of plant diversity, as well as the formation of monodominance of some species.

The abundance differed statistically in the sampled times (Table 1) and, according to the PERMIDISP analysis (Table 2), it was verified that until the fourth month the abundance was statistically the same, thus determining a period of up to four months for the use of the topsoil in recovery projects. For Salazar et al. (2011), the seed bank formed in savannas is transient and seasonal, explaining the decreasing viability of the propagules in the bank. These results should not be taken as the unique response of the storage time. Seed bank composition and longevity of species under local conditions (SNYMAN, 2013), besides the interaction between spatial variation and precipitation, may play an important role in the spatial and temporal heterogeneity of seed bank richness and density (SANTOS et al., 2013).

In areas of Australia, with average annual precipitation around 369.00 mm, the decline in viability of the seed bank was observed only in the second year of storage (GOLOS; DIXON, 2014). Since the average annual precipitation of the study area is 1,695.00 mm, it is believed that the moisture favors the loss of viability in seed banks in the altitude fields. Thus, as a measure to maximize the viability of the seeds present in the topsoil, it is recommended to adopt adequate measures for the storage of the material with humidity insulation mainly in rainy seasons.

Behavior of seed bank species throughout storage

The families that contributed the most to dissimilarity were Asteraceae and Poaceae. According to Salazar et al. (2011), the seed banks of savanna environments have more herbaceous species and very few woody species. However, all species presented a unique pattern of emergence over time. The species that contributed the most to the dissimilarity among the collection times studied were almost always the same, varying only the percentage of specific contribution (Table 3).

Species such as *Ageratum fastigiatum*, *Baccharis dracunculifolia*, *Echinolaena inflexa*, and *Melinis minutiflora* had individuals emerged at all times, even if the number of emerged individuals was not constant. These may be persistent species of the seed bank or may still present seasonal dormancy (WALCK et al., 2005). The SIMPER analysis also indicates that *Melinis minutiflora*, an exotic, aggressive, and difficult-to-eradicate species, may become part of the post-recovery community (HOFFMANN; HARIDASSAN, 2008). Therefore, measures must be taken to favor the regeneration of native grasses and to prevent its planting of exotic species.

Seeds of the aforementioned species should be better studied, considering mechanisms that suggest their respective longevity. The same can be recommended for others that, even with less abundance, emerged only in the last collection, such as *Andropogum bicornis*, *Cyperus aggregatus*, *Panicum campestre*, *Panicum pilosum*, and *Paspalum plicatulum*. As recommended by Gilardelli et al. (2000), in stony soils, ecological recovery must be carried out with species that are able to tolerate a longer period of burial, seasonal dormancy, and germination (WALCK et al., 2005).

CONCLUSION

Some species were affected by the stockpiling conditions, only emerging in some collections, while others (*Achyrocline satureioides*, *Ageratum fastigiatum*, *Baccharis dracunculifolia*, *Borreria capitata, Echinolaena inflexa* and *Melinis minutiflora*) had individuals emerged in all collection periods.

This study points that species in the altitude fields present a differentiated behavior regarding the germination and the temporal patterns of seed dispersion. Such facts may lead to monodominance if the topsoil replenishment exceeds four months of storage.

As a measure to maximize the viability of the seeds present in topsoil, it is recommended to adopt adequate stocking practices of the material, isolating it from excess moisture, especially in rainy seasons.

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