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Seed covering and dry periods in the rainy season interfere with direct seeding success in the restoration of post-mined grasslands

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Abstract. Among the limitations for the use of direct seeding in the ecological restoration of severely degraded areas in tropical grasslands, the association between dry periods and an inhospitable substrate stands out. This work evaluated whether covering seed with a soil layer and the addition of a thin topsoil layer to the degraded substrate interferes with native plant establishment in degraded areas. The effect of rainfall variations on direct seeding results was also measured. The establishment of seven native species was evaluated under four different conditions: 1) seeding on degraded substrate, 2) seeding covered by 1 cm degraded substrate layer, 3) seeding on 1cm topsoil layer, and 4) seeding covered by 1 cm topsoil layer. In general, species with smaller seeds showed higher establishment percentages in treatments in which seeds were deposited on the substrate. Legume species, which have larger seeds, achieved better establishment percentage when seeds were covered by the substrate. The addition of topsoil was beneficial for *Bulbostylis fimbriata* (Cyperaceae), while for the other species, the effect was null or harmful. Data also showed that rainfall amount and distribution affected the establishment rate. Direct seeding is an advantageous alternative for the ecological restoration of tropical grassland degraded by mining. Better knowledge on sowing management and behavior of native species can contribute to improving the efficiency of this technique.

[Keywords: active restoration techniques, germination, mine recovery, seed mass, topsoil]

Resumen. La cobertura de semillas y los períodos de sequía en la temporada de lluvias interfieren con el éxito de la siembra directa en la restauración de áreas mineras en las sabanas tropicales. Entre las limitaciones para el uso de la siembra directa en la restauración ecológica de áreas severamente degradadas en las sabanas tropicales se destaca la asociación entre períodos de sequía y el sustrato inhóspito. En este trabajo se evaluó si la cobertura de las semillas por una capa de suelo y si la adición de una fina capa de tierra vegetal (*topsoil*) al sustrato degradado interfieren con el establecimiento de las plantas nativas en un área degradada. También se midió cómo las variaciones en las precipitaciones afectan los resultados de la siembra directa durante el período de estudio. Se evaluó el establecimiento de plántulas de siete especies nativas bajo cuatro condiciones diferentes: 1) sembradas sobre sustrato degradado, 2) cubiertas por una capa de 1 cm del sustrato degradado, 3) sembradas sobre una capa de tierra vegetal (*topsoil*) de 1 cm, y 4) cubiertas por una capa de 1 cm de tierra vegetal. En general, las especies con semillas más pequeñas mostraron mayores porcentajes de establecimiento en los tratamientos en que las semillas se depositaron sobre el sustrato. Las leguminosas, que tienen semillas más grandes, lograron mejores porcentajes de establecimiento cuando sus semillas estaban cubiertas por el sustrato. La adición de tierra vegetal fue beneficiosa para *Bulbostylis fimbriata* (Cyperaceae), mientras que para las otras especies no tuvo ningún efecto o fue perjudicial. Los datos también mostraron que la cantidad y distribución de la lluvia afecta los porcentajes de establecimiento. La siembra directa es una alternativa ventajosa para la restauración ecológica de áreas degradadas en las sabanas tropicales. Incrementar el conocimiento del manejo de la siembra y el comportamiento de las especies nativas puede contribuir a mejorar la eficiencia de esta técnica.

[Palabras clave: técnicas de restauración activa, germinación, recuperación de minas, masa de semillas, tierra vegetal]

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Introduction

In recent years, direct seeding has gained prominence in the ecological restoration of degraded areas (Grossnickle and Ivetić 2017) due to its low cost, ease of use and promotion of great diversity of species and functional groups (Cole et al. 2011; Palma and Laurance 2015; Grossnickle and Ivetić 2017; Raupp et al. 2020). The direct seeding is more feasible to manage herbaceous-shrub species than seedling planting techniques. Thus, direct seeding contributes to greater and faster soil coverage in degraded areas (Grossnickle and Ivetić 2017; Sampaio et al. 2019). Therefore, this technique favors the succession process, promoting the restoration of ecological functions and ecosystem services in recovery areas (Kirmer et al. 2012; Coutinho et al. 2019). Although there is an increasing number of direct seeding studies in different ecosystems (Palma and Laurance 2015; Ceccon et al. 2016; Grossnickle and Ivetić 2017), investigations on direct seeding or similar techniques for the restoration of severely degraded areas, such as post-mined areas on tropical grassland environments, are rare and normally show low plant establishment rates (Le Stradic et al. 2014; Figueiredo et al. 2021).

The substrate conditions are one of the main limitations for the direct seeding success in post-mined areas. In these areas, the total loss of the superficial soil layers, gives rise to a substrate with harsh chemical, physical and biological characteristics, making the plant establishment extremely difficult (Figueiredo et al. 2016; Le Stradic et al. 2018). In addition, plant establishment in severely degraded areas in grasslands faces dry periods with high solar radiation and temperature, even during the rainy season (dry spells) (Assad et al. 1993), which further accentuate some of the harsh substrate characteristics. One of the most relevant ways to improve plant establishment and survival rates using direct seeding in severely degraded lands is to invest in improving substrate conditions, developing seeding techniques and seed technology (Madsen et al. 2016; Grossnickle and Ivetić 2017), which alleviate harsh substrate and environmental conditions. Thus, it is necessary to evaluate alternative methods able to facilitate seed germination, plant emergence and establishment in post-mined areas.

Seed covering in direct seeding could be an important way to alleviate harsh environmental conditions in order to facilitate plant establishment. Seed covering assists plant establishment by protecting them from desiccation, predators and transportation by rainwater and wind (Woods and Elliott 2004; Doust et al. 2006; Garcia-Orth and Martinez-Ramos 2008; Sovu et al. 2010; Doust 2011; Wang et al. 2014). On the other hand, seed covering could hamper germination and emergence due to the absence of light and may act as a physical barrier preventing the seedling from reaching the soil surface, especially small-seeded species (Bond et al. 1999; Milberg et al. 2000). Thus, understanding how species with different morphological and ecophysiological features respond to seed covering after direct seeding could be an important tool to increase the technique's efficiency, reduce costs and optimize the use of seeds in the application of direct seeding in the restoration of post-mined areas.

Another factor that could contribute to plant establishment in severely degraded areas is the introduction of microorganisms able to associate with plants, facilitating germination and helping them to tolerate and overcome the harsh substrate conditions such as water and nutrient deficit (Wubs et al. 2016). An efficient and low-cost way of introducing great diversity of microorganisms in degraded areas is through the addition of small portions of superficial soil (topsoil) from preserved areas (Figueiredo et al. 2018). The addition of topsoil also contributes to increased fertility in the microenvironment, which can also help plant establishment. Although, some studies indicate a positive effect of topsoil on plant growth on degraded substrates (Machado et al. 2013; Figueiredo et al. 2018), they are limited to a few species. Thus, it is important to evaluate the technique with greater diversity of species under field conditions.

Several studies have shown that natural variations in environmental conditions interfere with results of experiments on the restoration of degraded areas such as plant diversity and density (Stuble et al. 2017; Manning and Baer 2018; Groves and Brudvig 2019). Considering the low water retention in substrates of post-mined areas (Figueiredo et al. 2016), the common occurrence of dry periods during the rainy season (Assad et al. 1993) and the dependence on environmental conditions, especially water availability for seeds to germinate and establish (Grossnickle and Ivetić 2017), it is important to quantify how rainfall intensity and distribution in different years can interfere with plant establishment.

The quantification of environmental conditions on plant establishment using direct seeding can provide data and information to support the adoption or not of measures to reduce plant death due the hydric deficit, as well as reducing costs and also contributing to optimize seed use.

Considering the relevant constraints of the degraded substrate and the restrictive environmental conditions of tropical grasslands for the use of direct seeding in the restoration of post-mined areas and the need for increasing seed germination and plant establishment rates, the objectives of this study are: 1) to verify in a post-mined area if seed cover with local substrate (1 cm) interferes with the establishment rates of seven native species; 2) to measure whether planting seeds over thin topsoil layer (1 cm) or covered by this layer affect the plant establishment rates compared to seeding on degraded substrate, and 3) to estimate to what extent rainfall intensity and distribution affect establishment rates in direct seeding without seed cover.

For the reasons presented previously, we expect that seed covering would increase germination and seedling establishment percentage. In addition, we expect that the species would respond differently to this management, depending on the morphological and ecophysiological seed characteristics. We predict that the addition of topsoil would help the germination and establishment of species, since this management is able to increase soil fertility and the diversity and abundance of soil microorganisms, which can prompt species germination and establishment. We also expect that dry periods during the rainy season would reduce seed germination and/or seedling establishment.

MATERIALS AND METHODS

Study area

The rupestrian grassland area in which the seeds were collected and the area degraded by bauxite mining used in field experiments are located at the Municipal Natural Park of Andorinhas (20°21′ S - 43°30′ W) in the municipality of Ouro Preto, Minas Gerais Brazil. The rupestrian grassland is one of the ecosystems of the Cerrado biome (Brazilian savanna) and the study area is characterized by a shrub-herbaceous vegetation on iron duricrust outcrops, locally known as ‘cangas’. The degraded area was mostly without vegetation and with dystrophic and compacted lateritic substrate (Machado et al. 2013). According to the Köppen classification (Álvares et al. 2014), the climate of the region is described as Cwb, humid mesotherm with dry, mild winters and rainy summers. The average annual rainfall in the municipality is 1610 mm with more than 90% of the rainfall concentrated between the months of October and April (Castro et al. 2012).

Tested species and seed collection

The seven rupestrian grassland species evaluated in this study were: *Chromolaena squalida* (DC.) R. M. King and H. Rob. (Asteraceae), *Eremanthus erythropappus* (DC.) MacLeish (Asteraceae), *Senna reniformes* (G. Don) H. S. Irwin and Barneby (Fabaceae), *Centrosema coriaceum* Benth (Fabaceae), *Bulbostylis cf. fimbriata* (Nees) C.B. Clarke (Cyperaceae), *Diplusodon microphyllus* Pohl (Lythraceae) and *Sporobolus metallicolus* Longhi-Wagner and Boechat (Poaceae). We collected the seeds from at least 10 individuals per species, when the species exhibit mature fruit and seed dispersal. The collection dates are shown in Table 1.

From the total of the seeds harvested of each species, we selected three random samples to determine the relationship between seed lot mass and the number of seeds. For this purpose, in each sample we weighed and counted the number of seeds. In the case of *S. reniformes* and *C. coriaceum*, we distinguished, visually, empty and damaged seeds from full and perfect seeds, counting only full seeds. We selected full *S. metallicolus* and *B. fimbriata* seeds by difference in density in aqueous medium, as proposed by Figueiredo et al. (2012). In the other species, it was not possible to separate full seeds from empty and damaged ones, thus we counted all seeds. For all species, we estimated the mass of one thousand seeds according to guidelines of Brasil (2009).

Germinability

We performed the germination experiments under controlled conditions on lateritic substrate to determine the proportion of germinable seeds, that is, part of the total number of seeds (i.e., full, empty and dormant seeds) able to germinate at the time of setting up field experiments. For this purpose, we collected the substrate at depths greater than one meter from the degraded area where field experiments were carried out. We characterized

Table 1. Characteristics of seeds of each species used in the experiment. Weight seed mix: weight of seeds plus impurities of each species added to the seed mix; Nº seeds per gram of mix: number of seeds per gram of mix; Nº germinable seeds/m²: number of germinable seeds sown per square meter; Germinability: germinability rate in the lateritic substrate under controlled conditions.

Tabla 1. Características de las semillas de cada especie utilizada en el experimento. Weight seed mix: masa de semillas más impurezas en la mezcla de semillas; Nº seed per gram of mix: número de semillas por gramo de semillas más impurezas; Nº germinable seeds m²: número de semillas viables sembradas por metro cuadrado; Germinability: porcentaje de germinabilidad en el sustrato laterítico en condiciones controladas.

Family	Species	Herbarium registration number	Seed collection date	Weight of 1000 seeds (g)	Weight seed mix (g)	Nº seed per gram of mix	Nº germinable seed m^{-2}	Germinability (%)
Cyperaceae	*Bulbostylis fimbriata*	31505	jul-18	0.09	4	19540	24699	31.6
Poaceae	*Sporobolus metallicolus*	29150	ago-19	0.10	6	750	3340	74.2
Asteraceae	*Eremanthus erythropappus*	31513	out-19	0.25	28	749	5492	26.2
Asteraceae	*Chromolaena squalida*	31503	ago-19	0.27	12	519	1495	24.0
Lytraceae	*Diplusodon microphyllus*	31502	ago-19	0.69	26	138	1678	46.7
Fabaceae	*Senna reniformes*	31515	ago-19	18.56	10	44	85	19.5
Fabaceae	*Centrosema coriaceum*	31795	abr-19	27.5	6	34	106	51.7

the granulometry and fertility parameters of this, following recommendations by Teixeira et al. (2017). Two dm³ of this material were placed in pots of 30 cm in diameter and 10 cm in height, using four pots with 100 seeds each per specie. We distributed the seeds, randomly, over the substrate surface without covering.

The germination experiment started in December 2019 and pots were kept in greenhouse under natural light, controlled temperature (25 °C) and sufficient irrigation to keep the substrate constantly moist. To measure germinability, we counted the seedlings with at least one pair of leaves. We assessed germination weekly and the experiment was completed 30 days after the last germination. To overcome *S. reniformes* and *C. coriaceum* seed dormancy, we submitted the seeds evaluated in germination experiments under controlled and field conditions to immersion in concentrated sulfuric acid for 20 minutes and then washed in running water for five minutes. This procedure increased the germinability rates of *S. reniformes* and *C. coriaceum* (personal observation).

Seed mix preparation

We mixed seeds containing impurities (small leaf and fruit fragments) of the seven species, making up a mix that we used in field experiments. The sum of the weight of seeds of all species in the mix was 92 g. The mass of seeds of each species added to the mix and the number of germinable seeds of each species sown per square meter are shown in Table 1. The number of germinable seeds corresponds to part of the total number of seeds (full, empty and dormant seeds) equivalent to the germinability percentage obtained under controlled conditions. The number of seeds of each species added to the seed mix was determined based on establishment rates obtained in similar study started in 2018 (Figueiredo et al. 2021).

Topsoil and plant biomass collection

The topsoil used in this study was collected from a preserved rupestrian grassland area around the degraded area. For topsoil collection, we delimited, randomly, four plots of 0.25 m² in which we collected the first 10 centimeters of soil, excluding litter. After collection, we homogenized and distributed the topsoil in the plots that received this treatment. We collected three topsoil samples after topsoil homogenization and one laterite composite sample per treatment. We analyzed the composite topsoil and lateritic substrate samples to determine physical and chemical characteristics, such as grain size and fertility indicators, according to Teixeira et al. (2017). As the lateritic substrate used in the germination experiment under controlled conditions and the substrate from experimental plots presented similar values for fertility parameters, we decided to present in the results only the average of values of all lateritic substrate samples (Table S1, Supplementary Material).

Plant biomass was used during soil preparation (see below) and was composed of litter collected from the same topsoil collection area. After collection and plant biomass homogenization, we collected three samples to perform chemical characterization (Table S1, Supplementary Material). We perform these chemical analyses according to methodology proposed by Carmo et al. (2000).

Experimental design

In the degraded bauxite mine we used a backhoe machine to turn the substrate to depth of approximately 50 cm in 18 plots with 1 m^2. After turning, we incorporated 30 L (2 kg of dry mass) of plant biomass (litter) per plot into the first 20 cm of substrate in all experimental plots. In this experimental area we evaluated the seeding establishment rates of the seven species under four different conditions with three replicates each, randomly arranged. We evaluated the following treatments: Laterite covered seed (LC; in this treatment, we deposited the seed mix on the degraded substrate and covering later with 1cm laterite layer [10 liters per square meter]); Laterite uncovered seed (LU; we deposited the seed mix on the laterite substrate without covering); Topsoil covered seed (TC; we deposited the seed mix on the lateritic substrate and then covering with 1 cm topsoil layer); Topsoil uncovered seed (TU; we added 1 cm of topsoil layer on the lateritic substrate, after which we added the seed mix without covering). Additionally, to make sure that the number of seedlings recruited from seeds present in the litter and in the topsoil seed bank did not significantly interfere with the number of seedlings from the seed mix, we installed two control treatments, in which seed mix was not added. The two control treatments were the following: Control (substrate turning and addition and incorporation of plant biomass); Topsoil (substrate turning, addition and incorporation of plant biomass and addition of 1-cm topsoil layer [10 L/m^2]). Considering the objective of control treatments (to show the number of seedlings recruited from the seed bank), we did not use the results of these treatments in the statistical analyses and considered them in the discussion section.

We installed the field experiments in November 2019, at the beginning of the rainy season. We assessed the number and density of individuals of each species present in each plot and calculated the establishment rates 120 days after planting. For this, we divided the plot into four quadrants, and in each one of them, we collected, identified and counted all seedlings contained in two 78.5 cm^2 circles randomly arranged in the quadrants. Thus, in each plot we sampled 628 cm^2 (i.e., 6.3% of the plot area). Due to the easier counting of species that presented small density and taller individuals, we decided to count all the individuals of *S. reniformes* and *C. coriaceum* present in the entire plot. By knowing the seedling density of each species in the sampled area, we estimated the number of individuals of each species per plot. Subsequently, we estimated the establishment rates of each species by the ratio between the number of individuals per plot in relation to the number of germinable seeds sown per plot.

In order to evaluate the effects of environmental conditions on plant establishment we compared pairwise the establishment rates of the laterite uncovered seeding treatment carried out in two different years (2018 and 2019). We compared the establishment rate of five of the seven studied species (except *C. coriaceum* and *S. reniformes*), conducted in the treatment with laterite without cover (LU), observed in two different years: 2019 (present study) and 2018 (Figueiredo et al. 2021). The previous study (Figueiredo et al. 2021) also used the same methods as the present one. For this aim, in the first 18 weeks of both studies, we monitored rainfall amount and distribution in the region through data collected by a pluviometric station (www.snirh.gov.br/hidrotelemetria/Mapa.aspx).

Statistical analyses

For statistical procedures, we evaluated the establishment data (response variable) of each species. Initially, we tested if data were parametric, by checking their normality requirements (Kolmogorov- Smirnov test) and variance homoscedasticity (Bartlett test). Since the establishment data of *B. fimbriata* and *S. reniformis* did not meet normality requirements and/or variance homoscedasticity, they were transformed by Box-Cox transformation and those tests were repeated to check both normality and homoscedasticity.

The effect of substrate (topsoil, laterite) and seeding technique (covered, uncovered), and the interaction between them were evaluated by a two-factor Analysis of Variance (two-way ANOVA), followed by a Tukey test, if significant differences were found. In order to assess the effects of environmental conditions of the different years (2018 and 2019) on establishment rates under the seeding on laterite uncovered treatment (LU) we performed the Student's t-test. All statistical tests were performed with 5% significance, and using MINITAB 18® statistical software.

Results

All species in this study showed differences in establishment rates, in at least one of the evaluated substrates, when comparing the sowing of seeds with and without the substrate cover. The difference between the two conditions varied between 18 and 98% (Figure 1). Species with smaller seeds (weight of 1000 seeds <0.7g) established better when sown on the substrate. On the other hand, species with larger seeds, such as legumes, with one thousand seed weight between 17 and 30 g established better when they were covered with the substrate (Figure 2). The only species that responded positively to the use of topsoil was *Bulbostylis fimbriata* (Cyperaceae) (Figure 1). Although no statistical differences were observed, in some cases, the covering of the smaller seeds with topsoil showed higher establishment rates than the covering with laterite. Conversely, the species with larger seeds presented lower establishment rates when covered with topsoil than when covered with laterite (Figure 1). In control and topsoil treatments, *E. erythropappus* was the only established species with average density of 0.5 individuals/m².

The rainfall regime showed differences between the two years (2018, 2019) in which the experiment was carried out. Cumulative rainfall in the first 120 days of experiment was 28% higher in the second year (911 mm in 2018 and 1168 in 2019). Another striking difference in the rainfall levels between the two years is that in 2018, there was a 12-day sequence with only 7 mm of rain from the seventh week and another 30-day sequence with only 21 mm of rain from the tenth week of experiment (Figure 3). The establishment percentage found in laterite uncovered seed treatment performed in 2019 was 3 to 63 times higher than values found in experiment carried out in the previous year (Figure 4).

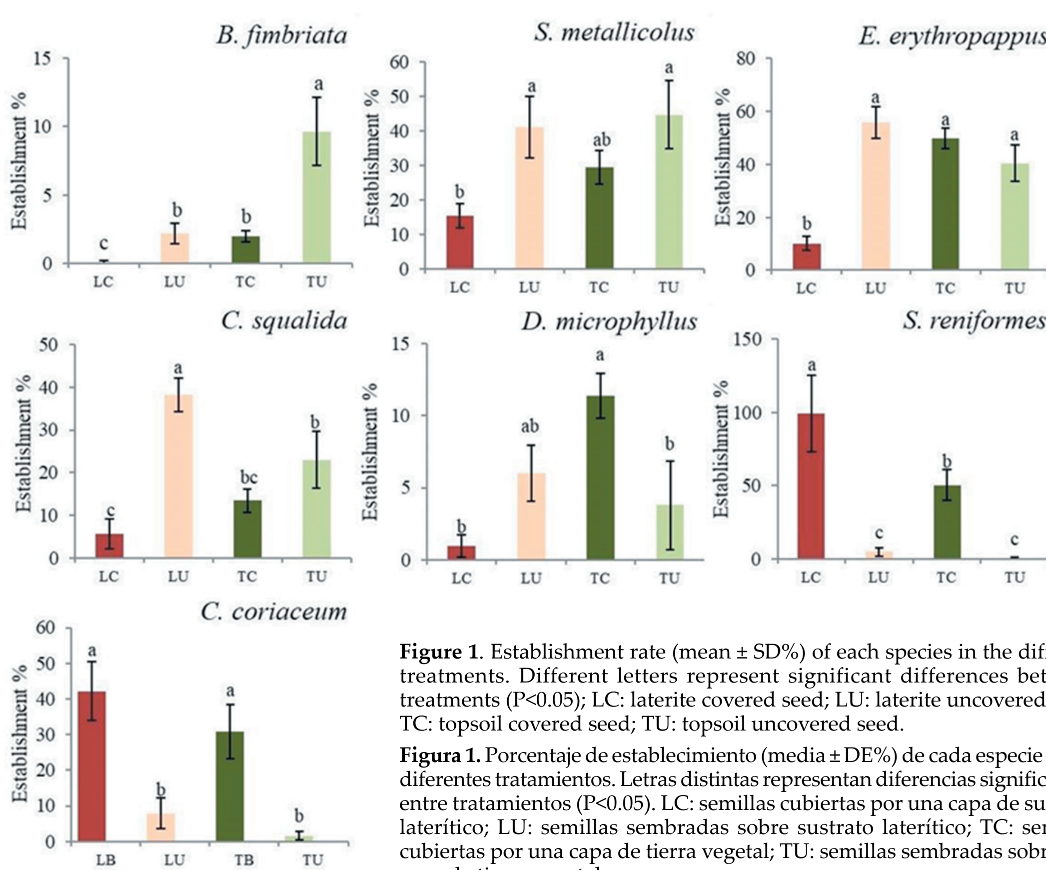

Figure 1. Establishment rate (mean ± SD%) of each species in the different treatments. Different letters represent significant differences between treatments (P<0.05); LC: laterite covered seed; LU: laterite uncovered seed; TC: topsoil covered seed; TU: topsoil uncovered seed.

Figura 1. Porcentaje de establecimiento (media ± DE%) de cada especie en los diferentes tratamientos. Letras distintas representan diferencias significativas entre tratamientos (P<0.05). LC: semillas cubiertas por una capa de sustrato laterítico; LU: semillas sembradas sobre sustrato laterítico; TC: semillas cubiertas por una capa de tierra vegetal; TU: semillas sembradas sobre una capa de tierra vegetal.

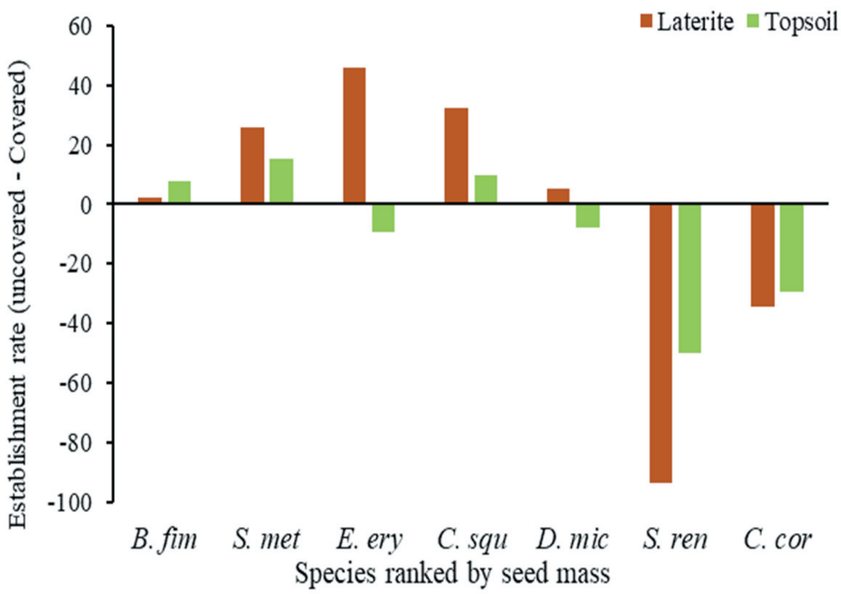

Figure 2. Difference between the establishment rates in treatments of the same substrate without seed covering and treatments with seed covering (uncovered seed establishment rate minus covered seed establishment rate). The species are ranked in ascending order of weight of one thousand seeds: *B. fimbriata* (B. fim), *S. metallicolus* (S. met), *E. Erythropappus* (E. ery), *C. squalida* (C. squ) and *D. microphyllus* (D. mic), *S. reniformis* (S. ren) and *C. coriaceum* (C. cor).

Figura 2. Diferencia entre las tasas de establecimiento en tratamientos del mismo sustrato sin cobertura de semillas y tratamientos con cobertura de semillas (tasa de establecimiento de semillas sembradas sobre sustrato menos tasa de establecimiento de semillas cubiertas). Las especies están clasificadas en orden ascendente por peso de mil semillas: *B. fimbriata* (B. fim), *S. metallicolus* (S. met), *E. Erythropappus* (E. ery), *C. squalida* (C. squ) y *D. microphyllus* (D. mic), *S. reniformis* (S. ren) y *C. coriaceum* (C. cor).

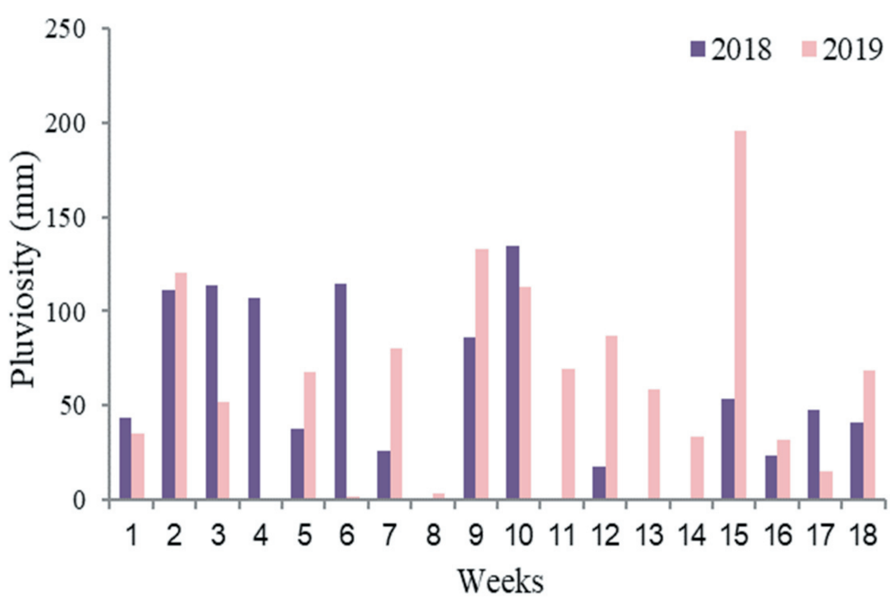

Figure 3. Rainfall in the experimental area in the first 18 weeks of studies started on October 21, 2018 (2018) and November 25, 2019 (2019). Source: 'Cachoeira dos Prazeres' Meteorological Station (20°46'78'' S - 43°47'03'' W). Agência Nacional das Águas. URL: www.snirh.gov.br/hidrotelemetria/Mapa.aspx.

Figura 3. Precipitaciones en la región del área experimental en las primeras 18 semanas del estudio iniciado el 21 de octubre de 2018 (2018) y el 25 de noviembre de 2019 (2019). Fuente: Estación Meteorológica "Cachoeira dos Prazeres" (20°46'78''S, 43°47'03''W). Agência Nacional das Águas. URL: www.snirh.gov.br/hidrotelemetria/Mapa.aspx.

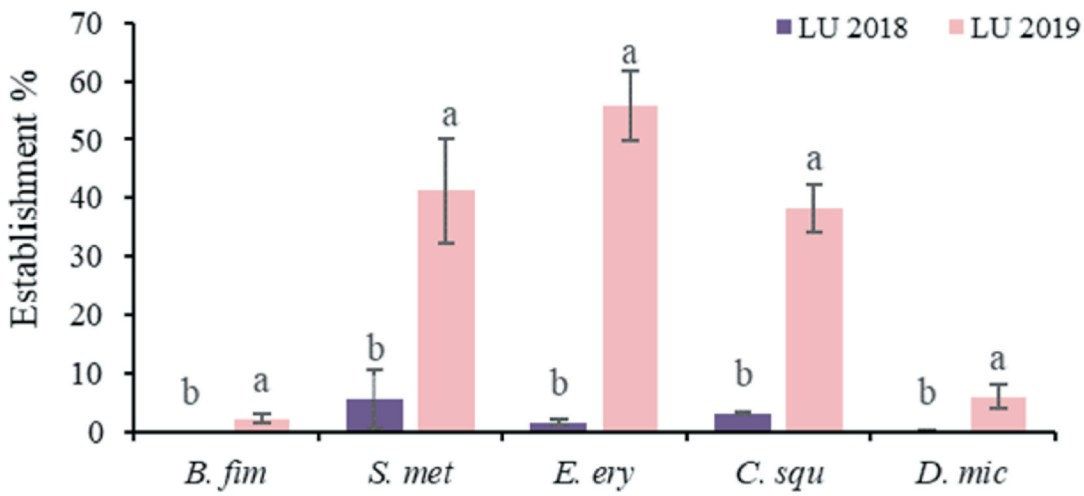

Figure 4. Establishment rate (mean ± SD%) of laterite uncovered seed 2018 (LU 2018) and laterite uncovered seed 2019 (LU 2019). Different letters represent significant differences between treatments (P<0.05). *B. fimbriata* (B. fim), *S. metallicolus* (S. met), *E. erythropappus* (E. ery), *C. squalida* (C. squ) and *D. microphyllus* (D. mic).

Figura 4. Porcentaje de establecimiento (media ± DE%) de semillas sembradas sobre sustrato laterítico 2018 (LU 2018) y semillas sembradas sobre sustrato laterítico 2019 (LU 2019). Letras distintas representan diferencias significativas entre tratamientos (P<0.05). *B. fimbriata* (B. fim), *S. metallicolus* (S. met), *E. erythropappus* (E. ery), *C. squalida* (C. squ) and *D. microphyllus* (D. mic).

Discussion

Seed covering after direct seeding significantly affected establishment rates. We observed a tendency for species with larger seeds (such as legume species) to be favored with covering, which contributed to a greater establishment, while small-seeded species tend to be impaired with this management.

The results agree with evaluations carried out with grasses of the Brazilian grasslands (Cerrado) (Fontenele et al. 2020) and with Fynbos species (Bond et al. 1999). Both studies show that covering of small seeds, depending on depth, could harm seedling emergence. Our results also corroborated several studies on the germination of rupestrian grassland species under laboratory conditions, indicating the light-dependence or stimulation of germination. The only families in rupestrian grasslands where non-photoblastic species were found were Fabaceae, Verbenaceae and Bromeliaceae (Nunes et al. 2016). Germination of Fabaceae species from other environments, like Brazilian Caatinga and Argentinean Chaco Seco, were also indifferent to light (Araújo et al. 2007; Funes et al. 2009).

The covering of small seeds usually restricts germination (Traba et al. 2004; Limón and Peco 2017), as observed for many species in this study with mass of one thousand seeds below 0.7 g and covered with laterite. The reduction in germination and emergence is usually due to the absence of light or because the substrate layer acts as a barrier that prevent the seedling from reaching the surface (Bond et al. 1999; Milberg et al. 2000). On the other hand, larger seeds with more reserves such as found in the legume species evaluated in this study, can germinate in the dark and, when covered, may benefit from protection against desiccation (Doust et al. 2006; Vieira and Scariot 2006; Sovu et al. 2010). In addition, covering protects seeds from pathogens and predators, major limitations to plant establishment using the direct seeding technique (Woods and Elliott 2004; Doust et al. 2006; Garcia-Orth and Martinez-Ramos 2008; Sovu et al. 2010; Doust 2011). Most studies evaluating the effects of seed covering with soil after direct seeding indicate that it promotes better establishment rates, compared to the absence of covering (Negreiros Castilho et al. 2003; Woods and Elliott 2004; Doust et al. 2006; Vieira and Scariot 2006; Garcia-Orth and Martinez-Ramos 2008; Sovu et al. 2010; Alem 2018). Although these studies do not show a relationship between seed mass and covering response, most evaluated species presented seeds larger than those in the present study. On the other hand, other studies in different biomes have shown that the absence of covering after seeding may not interfere or promote better emergence rates of some species, regardless of seed size (Vieira and Scariot 2014; Silva and Vieira 2017; Alem 2018). Despite the relationship between the seed mass and the response to covering observed in this study, it is important to consider that the study was carried out with a small number of species of an ecosystem with a great flora diversity and that the species with larger seeds in this study belonged to a single family.

The knowledge of the best seeding method for each plant group can help in the optimization of the seed volume used in direct seeding operations (Kildisheva et al. 2020). It is important, especially in rupestrian grasslands, where seed availability and quality are limited (Silveira et al. 2016; Dayrell et al. 2017) and the costs of seeds can represent a significant part of the total direct seeding costs (Figueiredo et al. 2021).

The use of topsoil, compared with treatments without topsoil, only proved to be advantageous for *B. fimbriata* in both conditions evaluated (covered and uncovered) and in *D. microphyllus* and *E. erythropappus*, when seeds were covered (Figure 1). In the case of *D. microphyllus* and *E. erythropappus*, the increase in establishment rates observed when covering seeds with topsoil is probably more associated with the fact that the topsoil has less efficiency in acting as a barrier when covering seeds than differences in microbiota or in substrate fertility, since this increase was not observed when comparing topsoil uncovered seed in relation to laterite uncovered seed treatments. In fact, in the other species in which seed covering impaired establishment, it was observed that topsoil covering was, even without statistical significance, less harmful. The opposite was observed in species with better results when seeds were covered, especially in *S. reniformis* (Figure 1). Topsoil has much higher organic matter content than laterite substrate (Table S1, Supplementary Material), which makes it less dense and compacted. These topsoil characteristics may have mitigated the negative effects of covering of small seeds. Differences of a few millimeters in covering thickness are significant for the germination rates of some species with small seeds (Fontenele et al. 2020). Similarly, it is believed that differences in soil density can also be significant.

We expected that the small increase in fertility in the microenvironment and the possibly greater diversity and abundance of microorganisms promoted by the addition of topsoil would facilitate germination and establishment (Oki et al. 2016; Figueiredo et al. 2018). However, establishment rates were higher, even without statistical significance, for laterite in some of the evaluated species. A possible explanation for the lower establishment found in topsoil treatments is the fact that this substrate possibly has higher amounts of pathogens and predators that can affect plants, as proposed by Voorde et al. (2012) and Bertacchi et al. (2016). Although topsoil did not contribute to the establishment of many species in this study, Figueiredo et al. (2018) observed that even in small portions, it promoted plant growth. Thus, this subject would be better evaluated in other long-term direct seeding field experiments.

In view of the similarity in the methodology used in laterite uncovered treatment started in 2018 to the same treatment started in 2019, the most likely cause of the strikingly smaller establishment rates in 2018 (Figure 4) is the distinct rainfall amount and distribution in the first 120 days of experiment (Figure 3). Small seeds have few reserves and seedling survival and growth strongly depend on environmental conditions and resources. These species have low initial investment in deep roots, leaving them restricted to the exploitation of resources in the surface layer of the substrate (Westoby et al. 1992) and making them more susceptible to the effects of the absence of rain (Passaretti et al. 2020). In addition to these plant characteristics, substrates of degraded rupestrian grassland areas usually have low water retention capacity (Figueiredo et al. 2016). Thus, more continuous water supply would improve seedling establishment.

Although the average rainfall in periods of both experiments is above the average for the region (Castro et al. 2012), the reason for the reduction of the establishment observed in the 2018 experiment is probably attributed to the periods of drought that occurred from the seventh and tenth weeks, in December and January, respectively (Figure 3). Although these periods of water scarcity are short compared to the dry season, damage to plant establishment may be relevant (Engelbrecht et al. 2006; Vieira and Scariot 2006; Pellizzaro et al. 2017). The small size of seedlings sown at the beginning of the rainy season and the fact that these events coincide with periods with higher average temperatures and number of solar radiation hours, make the dry period even more severe.

The understanding of the impact of environmental conditions in results of ecological restoration projects of degraded areas in tropical grasslands, especially its more detailed quantification in future studies, has great relevance. This information can support the cost-benefit assessment of adopting practices that mitigate water scarcity in the first months after planting, such as irrigation. In some cases, investing in measures to reduce harsh environmental conditions in order to increase plant establishment and survival rates can reduce the cost of plants per hectare by up to five times (Madsen et al. 2016).

Direct seedling experiments in different biomes, normally using only full and healthy seeds and considering all sowed seeds, have presented establishment rates between 18 and 21% (Palma and Laurance 2015; Grossnickle and Ivetić 2017). In this way, the results obtained in the present study can be considered promising, since for six of the seven species evaluated, between 26 and 52% of germinable seeds were able to establish in a dystrophic substrate. Considering the establishment percentages obtained in the first 120 days of experiment, the results of this study show that direct seeding, even of species with small seeds, can be an alternative for the restoration of mined grassland areas. The cover of seed with substrate during restoration of post-mined areas has to be evaluated, take into consideration the seed traits of the species utilized. Since planting management and environmental conditions can significantly interfere with the success of this technique, further studies aimed at searching for ways to improve substrate conditions and the direct seeding management are necessary. This study also presents establishment rate of species in the field, which can serve as a reference for defining the amount of seeds per square meter to be used in future experiments or projects for the recovery of degraded areas.

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