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Running title: Seed dispersability in an OCBIL grassland

Limited seed dispersability in a megadiverse OCBIL grassland

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Abstract

Some tropical grasslands can be referred as OCBILs (old climatically-buffered, infertile landscapes). OCBIL theory predicts that species should exhibit reduced dispersability, linked with long-term stable environmental conditions. Seed rain, i.e. the number of seeds reaching a given area, is poorly known in OCBIL grasslands despite its role in modulating resilience. We aim to test the prediction of limited dispersability as one cause of slow recovery capacity in *campo rupestre*, a megadiverse OCBIL grassland, southeastern Brazil. We ran a field experiment to compare annual seed rain dynamics between preserved sites and sites disturbed by gravel exploitation. We chose three paired preserved and disturbed plots, placing six sampling blocks in each plot. We installed one sticky and one funnel trap per block. We collected the samples monthly for 12 months to estimate seed density and richness. We provide the first community-level study on seed rain in *campo rupestre* which suggests widespread seed limitation in both preserved and disturbed areas, expressed by the low seed density in traps (average of 2.6 seeds/m²/day) and a remarkable low seed density found for 90% of the taxa. Although seed density was much lower in relation to other seed rain studies in grasslands, species richness (92 seed morphospecies) is the highest reported in the literature. Funnel traps were the most efficient trap type in catching seeds. We found a significant increase in the number of seeds and species richness captured in funnel traps during the rainy season. Disturbance seems to reduce seed arrival dynamics in sticky traps and increase seed arrival dynamics in funnel traps. Approximately 32% of morphospecies were registered exclusively in disturbed plots and 25% exclusively in preserved plots. The limited seed dispersability found is of high relevance for a better understanding of seed ecology and resilience in *campo rupestre* and may partially explain the poor recovery of these disturbed areas after soil removal.

Keywords: seed rain, seed trap, tropical grassland, resilience, seed limitation

56

57 **Introduction**

58 Some tropical grasslands can be referred as OCBILs (old climatically-buffered, infertile
 59 landscapes). OCBILs host disproportionally high levels of terrestrial global biodiversity
 60 hotspots, where edaphic conditions play a key role in structuring plant communities and
 61 shaping plant populations (Hopper, 2009; Hopper et al., 2016). OCBIL theory predicts that
 62 species should exhibit reduced dispersability, linked with long-term stable environmental
 63 conditions (Hopper, 2009). Indeed, dispersal away from maternal plants is associated with high
 64 costs of dispersal (Bonte et al., 2012), and high mortality risks as it increases the likelihood of
 65 seed arrival on a different soil type therefore creating a competitive disadvantage (Hopper,
 66 2009; Rajakaruna, 2018; Corlett & Tomlinson, 2020). Indirect evidence of reduced
 67 dispersability includes: i) the absence of obvious means of seed dispersal and ii) high local
 68 endemism across many phylogenetically-distant lineages. This phenomenon has been well
 69 demonstrated for the Southwest Australian Floristic Region, where many native plant species
 70 do not display dispersal structures, and some display phylogenetic clustering (Hopper et al.,
 71 2016). Such landscapes are also highly vulnerable to many kinds of soil disturbances (e.g.
 72 cultivation or plantation after ploughing, topsoil removal, quarrying, etc.) (Hopper et al., 2016;
 73 Buisson et al., 2019), and the precise mechanism driving poor recovery is still a matter of
 74 controversy (Standish & Hobbs, 2010; Dayrell et al., 2016; Arruda et al. 2020), and warrants
 75 further investigation.

76 The megadiverse and nutrient-poor Brazilian *campo rupestre* vegetation is an OCBIL
 77 presenting extremely high levels of plant endemism (Echternacht et al., 2011; Colli-Silva et al.,
 78 2019). The lack of dispersal mechanism has been reported for several dominant families in this
 79 ecosystem such as Poaceae, Cyperaceae, Xyridaceae, Eriocaulaceae and Velloziaceae (e.g.
 80 Silveira et al., 2016; Morellato & Silveira, 2018). While local endemism and unassisted

dispersal constitute evidence of reduced seed dispersability, there is still no direct, empirical evidence that seed rain is limited. The lack of knowledge on dispersal limitation prevents us assessing how endemic species and plant communities respond to changing climate conditions and land uses (Corlett & Tomlinson, 2020).

Generally speaking for grasslands, major anthropogenic soil disturbances have widespread and long-lasting effects on ecosystem resilience (*sensu* Hodgson et al., 2015). Natural recovery, and thus plant community assembly, after drastic soil disturbances is mainly based on seed dispersal from surrounding sites, and not on the internal species pool (i.e. remaining vegetation, seed bank or bud bank) which is often reduced or sometimes absent (Bakker et al., 1996; Poschlod et al., 1998; Campbell et al., 2003; Shu et al., 2005; Buisson et al., 2006; Le Stradic et al., 2018b; Torök et al., 2018). Anthropogenic soil disturbances are common in *campo rupestre* as they are highly threatened by iron ore mining, sandstone and gravel quarrying, which have intensified over the past decades (Silveira et al., 2016; Fernandes et al., 2018). Plant communities from *campo rupestre*, although highly resilient to natural endogenous disturbances, such as fires (Le Stradic et al., 2018a), seem extremely vulnerable to human-caused exogenous soil disturbances (Le Stradic et al., 2018b). The latter study has shown that disturbed plant communities in *campo rupestre* remained very different from reference sites even eight years after disturbance, with almost no recovery of the natural vegetation (Fig. 1).

Seed dispersal is the first and a critical step in the hierarchical filter model proposed to summarize processes shaping plant community composition and assembly (Lortie et al., 2004; Poschlod et al., 2013; Török et al., 2018). It is a complex, multi-step ecological phenomenon (Schupp et al., 2010). Seed rain, the quantity and diversity of seeds reaching a given area (Baskin & Baskin, 2014), is a crucial part of the seed dispersal process. Studying seed rain can provide key information on regeneration and vegetation dynamics (e.g. which species, how many seeds, where and when seeds are arriving at a given location), thereby it is a useful tool

to assess recovery (*sensu* Hodgson et al., 2015) potential in disturbed areas (Turnbull et al., 2000). Unfortunately, only less than 10% of the seed rain studies in grasslands were carried out in the tropics (Arruda et al., 2018), therefore preventing us from understanding recovery potential in some of the world's most biodiverse and impacted areas (Parr et al., 2014).

Seed rain is usually quantified and qualified by placing traps in the plant community to catch seeds that are then counted and identified (Arruda et al., 2018), and the use of different seed trap types has been shown to contribute to a more reliable estimate of the seed rain (Chabrierie & Alard, 2005). The time of year at which seeds integrate the seed bank has a bearing on the establishment of vegetation after disturbance (Roberts, 1986; Torök et al. 2018). Additionally, seasonality can influence abiotic-related secondary dispersal processes, such as water runoff during the rainy season (de Rouw et al., 2018). *Campo rupestre* is characterized by distinct seasonal climatic patterns and for the total annual fruit production in plant communities in sandy soils, with significantly more fruits being produced during the dry season (Le Stradic, 2018c).

Here, we aim to test the prediction of OCBIL theory (Hopper, 2009) of limited dispersability of species in *campo rupestre* vegetation as one cause of this slow recovery. The underlying assumption is that vegetation and soil patchiness on OCBIL create non-linear relationships between distance from the parental plants and suitable conditions for seedling establishment. In most cases, the optimum conditions for establishment occur near the surroundings of the parental plant. Our specific goals were: (1) to characterize the annual seed rain dynamics in preserved areas and disturbed areas in *campo rupestre*; (2) to compare seed rain in disturbed areas and preserved areas to examine whether seed limitation is linked to low recovery and whether it is intrinsic to the ecosystem. We predict that a pronounced dispersal limitation is intrinsic to the *campo rupestre* vegetation, in both disturbed and preserved habitats. Nevertheless, we expect to find important differences in seed rain composition

between preserved and disturbed areas, due to the predominance of short distance dispersal on OCBIL species. Additionally, considering that *campo rupestre* is characterized by distinct seasonal patterns for the total annual fruit production in the plant community in its sandy soils, we expect to find higher densities of seed rain during the dry season.

Material and methods

Study region

We conducted this study at the vicinity of the Serra do Cipó National Park, in the southern portion of the Espinhaço Range, south-eastern Brazil (43° 35'W, 19° 17'S). The annual precipitation averages around 1,400 mm and climate is markedly seasonal with dry months from April to middle September, especially during the winter, and rainy months from October to March, with most rainfall occurring in the hot summers (Silveira et al., 2019). We considered the dry season from April to September and the rainy season from October to March (Silveira et al., 2019). Altitude at the study site ranges between 1,150 and 1,300 m a.s.l.. The main vegetation comprises the *campo rupestre*, a megadiverse, fire-prone montane grassland establishing on quartzite-derived rocks, with shallow and severely nutrient-impovertished sandy soils (Silveira et al., 2016).

The landscape encompasses a mosaic formed by patches of rocky outcrops and boulders where sclerophyllous small trees grow and patches of grasslands, dominated by monocots and sparsely distributed shrubs and forbs (Le Stradic et al., 2015). Small quarries were exploited for soil extraction during the paving of the MG-010 road in 2002. As a result of this exogenous disturbance, the aboveground vegetation and upper soil horizons were totally destroyed (Le Stradic et al., 2018b). We chose these small quarries as the target disturbed plots for the present study as they have slow recovery and very little vegetation cover (Appendix S1).

Sampling design

We performed a factorial experiment involving the assessment of seed rain on three paired disturbed and preserved plots of 100 m² each (20 m × 5 m). In all sites, the paired disturbed and preserved plots were adjacent at approximately 30 meters away from each other (i.e. thus away from site margins) (Fig. 2). Each pair is composed by a preserved area of *campo rupestre* with white sandy soils where no soil disturbances occurred, and an area disturbed by soil removal for gravel extraction which resulted in drastic or even complete removal of both bud and seed banks (Appendix S1). The three disturbed plots were placed inside small scale disturbed areas (<500m²) surrounded by a vast matrix of preserved areas of *campo rupestre*. Sites were distant at least 2 km from each other to ensure spatial independence. In April 2016, we placed six sample blocks in each plot (Fig. 2).

We used sticky and funnel traps (Fig. 2; Appendix S2), which allow capturing complementary information on seed rain (Chabrierie & Alard, 2005), and are the most common traps used to estimate seed rain in grasslands (Arruda et al., 2018). Sticky traps allow seed catch from primary seed dispersal by wind, and funnel traps allow seed catch from seeds dispersed by wind and secondary dispersal by wind on the ground and by water run-off. To assess seed rain, we used a total of 72 seed traps (36 sticky traps and 36 funnel traps) placed in pairs inside each block on the six plots (three disturbed and three preserved) (Fig. 2).

Funnel traps presented a total area of 0.0176 m² / trap and were buried in the soil so their opening was levelled with the soil surface. The sticky traps presented a total area of 0.0225 m² / trap and were situated 25 cm above ground and at a 45° sloping angle, to feature the optimum design to capture seed rain (Appendix S2). We also chose this experimental setting to make our data comparable to previous seed rain studies in grasslands (Chabrierie & Alard, 2005). We placed all the sticky traps facing the main wind direction registered for the year 2014 in the

closest meteorological station, which it is located approximately 30 km from the study area (INMET Instituto Nacional de Meteorologia, 2015).

We collected the samples on all seed traps monthly over one year (from April 2016 to March 2017), and immediately prepared them for a subsequent period of sampling. For the sticky trap samples, we examined the material retained under a magnifying glass to count and identify seeds. For the funnel trap samples, we washed the material retained inside the bags in a 250- μ m sieve to reduce the amount of fine soil particles before examining them under a stereoscope. Species could be either identified to the genus or family level. In most cases, they could not clearly be assigned to a genus or family. Therefore, we morphotyped the seeds. For our questions, morphotyping is acceptable (see also Jara-Guerrero et al., 2020), since we were interested in seed rain quantity, density and seed species richness.

We reported our data as seeds/m²/day in order to make our data comparable with other seed rain studies (Arruda et al., 2018). To evaluate if our sampling area was adequate to sample the communities, we built rarefaction curves for each trap type and plot type based on the methods developed by Chao et al. (2014), using an R package for rarefaction and extrapolation of species diversity (iNEXT package) on presence/absence data of each species (Appendix S3). First, the data from each trap was summed over the year. Then, we calculated seed density using cross-multiplication to transform seed density/trap/year to seed density/m²/year. Finally, we divided values by 365 days to obtain seed density/m²/day. Considering that using the same protocol for seed species richness would have led to overestimation, we 1) used the function *estimateD()* to compute species richness estimates for a 1 m² level of sample size (44 sticky traps and 57 funnel traps in order to reach 1 m²) using the extrapolated data generated by rarefaction curves (Hsieh et al., 2016), and then 2) re-sampled species richness from the confidence intervals supplied by *estimateD()* in order to estimate species richness in m²/day.

Finally, we conducted a floristic survey (species presence/absence) to determine species richness and composition for the standing vegetation (Jara-Guerrero et al., 2020) for each plot in both preserved and disturbed sites during the rainy season (plot size = 20 m × 5 m = 100 m²). The total area covered by vegetation in all degraded plots was less than 5% and in preserved ones higher than 70%. We identified the plant species by consulting herbarium collections, literature and taxonomists. The nomenclature of the floristic checklist follows APG IV (2016). For taxa at the seedling stage, species-level identification was not possible, but it was not essential as our interest was mature plants potentially producing seeds found in the seed rain. All permits to visit and collect biological data were authorized by ICMBio of the Brazilian Ministry of Environment. Data collection in sites located on private lands was authorized by the owners and ICMBio.

Statistical analyses

We performed all analyses assuming a Gamma error distribution of the two response variables (seed density and species richness) as there were count data transformed to m²/day, thus continuous variables. We employed generalized linear mixed effects models (GLMM, glmer, in lme4 package in R) with fixed and random effects (Crawley, 2013). In order to show the effects of plot types on response variables, each of the four models (one for each trap type and response variable) included “plot type” as the fixed effect and “plots” nested in “sites” as a random effect (Bates et al., 2014).

In order to show the effects of temporal patterns on seed rain, each of the four models (one for each trap type and response variable) included “seasons” × “plot type” as the fixed effects and “plots” nested in “sites” as a random effect. We also performed post-hoc Tukey comparisons among treatments (Crawley, 2013). For all analyses, we established α values of 0.05.

Differences between plant community composition in the standing vegetation and in the seed rain at each site were analyzed using two Correspondence Analyses, designed to explore categorical variables, such as the presence / absence data (Garson, 2012; with *ade4* package in R). We compared the similarity for seed rain data for sticky and funnel traps between plot types using the Sørensen index, based on presence/absence of the species (Kent & Coker 1992; Sørensen, 1948). We present the results for Sørensen index as values from zero to one in a similarity index, where a value of 1 means that the two subsets compared share all their species, while a value of 0 means they share none. We used the floristic survey data, as well as with the seed rain data (in funnel traps and sticky traps separately and in both traps together), from species or morphospecies lists compiled for each plot type.

Results

Seed rain richness and abundance

For the overall data for all seed traps over one year, we found 92 seed morphospecies. From the 92 seed morphospecies, we were able to identify 29% at family-level and 14% at genus-level. From the 10 more common morphospecies, we identified 70% at family-level and 40% at genus-level (Appendix S4). We found a total of 1,378 seeds over one year in a total area of 1.45 m² covered by all seed traps, representing an average of 2.6 seeds/m²/day. We also found less than 20 seeds in the annual accumulated seed rain for 90% of the morphospecies and that 75% of the seeds belonged to only 10 morphospecies, with only four morphospecies presenting more than 100 seeds in the annual accumulated seed rain (Fig. 3). *Lychnophora* sp1 (Asteraceae) was the most common species retrieved with 331 seeds, followed by *Poaceae* sp2 with 300 seeds, *Rhynchospora* sp3 (Cyperaceae) sp. with 108 seeds, and Morphospecies 1 with 102 seeds (Fig. 3).

Seed rain between plot types and seed trap types

We found a significant difference in seed density and species richness/m²/day between plot types. We found that 33% of the morphospecies were exclusively registered in preserved plots and 26% exclusively in disturbed plots considering the data for both trap types. From all seeds (i.e. 1,378 seeds) in the seed rain, 77% were exclusively registered in funnel traps, 10% only in sticky traps and 13% in both trap types. When we consider only the data for sticky traps, 52% of the morphospecies occurred exclusively in disturbed plots and 26% exclusively in preserved plots. For funnel trap data, we found that 24% of the morphospecies were exclusively registered in disturbed plots and 34% exclusively in preserved plots. Overall, the Sørensen similarity indices for the presence-absence of species in the seed rain between plot types was low for sticky (0.32) and high for funnel (0.6) traps (Table 1). Sørensen similarity indices between trap types on similar plots were low (disturbed = 0.17; preserved = 0.16; Table 1).

Disturbed plots had a lower average seed density in sticky traps ($0.4 \pm \text{SE } 0.1$ seeds/m²/day) than preserved plots ($1.9 \pm \text{SE } 0.4$ seeds/m²/day) but higher average in funnel traps ($5.4 \pm \text{SE } 0.9$ seeds/m²/day) than in preserved plots ($3.5 \pm \text{SE } 0.5$ seeds/m²/day; Fig. 4). We found the same patterns for species richness. Funnel traps allowed catching the highest species richness, first in disturbed and then in preserved plots although these were not significantly different (respectively $0.24 \pm \text{SE } 0.02$ and $0.011 \pm \text{SE } 0.01$ species/m²/day; $\chi^2=1.60$, $p=0.206$; Fig. 4). Sticky traps on disturbed plots caught fewer species, and significantly the fewest on disturbed plots ($0.14 \pm \text{SE } 0.001$ species/m²/day; $\chi^2=22.32$, $p<0.001$; Fig. 4).

Temporal patterns of seed rain

We found significant differences in the seed density and species richness in both plot types between the dry and rainy seasons (Fig. 5). The significantly higher seed density during the

rainy season collected in funnel traps ($\chi^2=6.69$, $p=0.010$; Fig. 5), was driven by a larger arrival of seeds from the three most common morphospecies [*Lychnophora* sp1 (Asteraceae); Poaceae sp2; *Rhynchospora* sp3 (Cyperaceae)] in December and January. This higher seed density was coupled with a higher species richness, although this was only marginally significant ($\chi^2=3.30$, $p=0.069$; Fig. 5). In sticky traps, we found a higher species richness during the dry season ($\chi^2=16.30$, $p<0.001$) and a higher species richness on preserved areas ($\chi^2=4.88$, $p=0.027$; Fig. 4).

Seed rain and floristic similarity between plot types

The Correspondence Analysis on the seed rain showed strong dissimilarities in species composition between disturbed plots (Fig. 6). One disturbed plot had 22 species in common with its preserved plot (e.g. *Rhynchospora* sp., while only 10 and five species in common for other sites; Fig. 6). While preserved sites had some species in common on the seed rain, such as Poaceae sp1, *Perama* sp1 (Rubiaceae), *Xyris* sp1 (Xyridaceae), Asteraceae sp4, they also had species characteristic of each one of them, such as Asteraceae sp3, Poaceae sp4 and Poaceae sp6, and *Rhynchospora* sp1 (Cyperaceae) and *Rhynchospora* sp4 (Cyperaceae). The only species common to all three sites was *Lychnophora* sp1 (Asteraceae).

The total species richness in the standing vegetation was 74 species, distributed in 48 genera and 26 families (Appendix S5). Poaceae, Cyperaceae and Asteraceae were the most representative families in both plot types. We found 39 species occurring exclusively in preserved plots, 18 exclusively in disturbed plots and 17 in both plot types. Preserved plots shared 15 species, like *Bulbostylis paradoxa*, *Homolepis longispicula* (Cyperaceae), *Lychnophora ericoides* (Asteraceae), *Gomphrena* sp. (Amaranthaceae), *Vellozia albiflora* and *Vellozia variabilis* (Velloziaceae), which were exclusive from preserved plots. Axis 1 of the Correspondence Analysis run on vegetation data separated the three preserved plots from the

three disturbed plots (Fig. 6). All disturbed plots were characterized by the presence of *Rhynchospora riedeliana* (Cyperaceae) and *Mesosetum loliiforme* (Poaceae), but these species were also found on most preserved plots (Appendix S6). Axis 2 separated two disturbed plots characterized with *Andropogon* sp., *Croton* sp. and *Rhynchospora brasiliensis* from the other disturbed plots characterized by *Polygala paniculata* and *Schwenckia americana*.

Discussion

Campo rupestre has limited seed dispersability

The significant differences in seed arrival across time and space found here provide valuable information about seed rain dynamics and show an intrinsic seed dispersal limitation within preserved disturbed areas of *campo rupestre*. Our data suggests widespread seed rain limitation, expressed by the low seed density in traps, supporting the prediction of OCIBLs theory of limited dispersability (Hopper, 2009), and suggest that seed limitation may strongly influence plant community re-assembly and limits plant recovery after soil disturbance.

The overall averages of seed density per day registered here are much lower than those reported in other grasslands, despite the fact that the species richness found here is among the highest already reported in the literature (Jackel & Poschlod, 1994; Urbanska et al., 1998; Lyaruu, 1999; Chabrierie & Alard, 2005; Jakobsson et al., 2006; Shang et al., 2013; Fibich et al., 2013; Marteinsdottir, 2014). For example, Jackel & Poschlod (1994) found 11786 seeds/m²/year and 9634 seeds/m²/year respectively in two calcareous grasslands; and Chabrierie & Alard (2005) found 22.13 seeds/m²/day (8,079 seeds/m²/year) in funnel traps and 4.84 seeds/m²/day (1,766 seeds/m²/year) in sticky traps in a chalk temperate grassland. These values are almost ten times higher those found here. As another example, Buisson et al., (2006) found 153.2 seeds/m²/day in sticky traps in a study over six months in a degraded grassland composed

mostly by annuals species. The low density in the seed rain found here also support the idea of dispersal limitation in *campo rupestre*.

The remarkable low seed density found for 90% of the morphospecies corroborates the predictions of OCBIL theory of accentuated dispersal limitation on this ancient ecosystem (Hopper et al., 2016; Silveira et al., 2016). In young, often disturbed, fertile landscapes (YODFELs), large-scale disturbances create vast areas free of vegetation, which offer opportunities for recolonization and succession. As a result, plant species with strategies for long distance dispersal are successful in YODFELs (Hopper, 2009). In contrast, the combination of higher climatic stability with lower soil fertility in OCBILs results in conditions that select for short distance seed dispersal, probably because the benefits of staying close to the parental plant has been higher than the benefits of dispersing seeds away from the parental plants for millions of years (Hopper, 2009; Bonte et al., 2012). Unassisted seed dispersal is common in many lineages of dominant species in *campo rupestre* (Morellato & Silveira, 2018), suggesting that community dynamics and resilience may be hampered by dispersal limitation, as in other OCBIL grasslands. Additionally, factors related to low seed set, such as the predominance of small and isolated populations (Morgan, 1999), may be related to the important seed dispersal limitation registered in our study and deserve further investigations in *campo rupestre*.

Seed rain is different in preserved and disturbed plots

The ability of plant communities to recover from exogenous disturbances from both internal and external species pool is a key driver of vegetation dynamics and resilience. The intense disturbances linked to soil removal by gravel exploitation in *campo rupestre* result in a drastic or even complete removal of both bud and seed banks (Le Stradic et al., 2018; Buisson et al., 2019; Arruda et al., 2020). Thus the natural recovery success in these disturbed areas relies on

the arrival of new diaspores. Our data is in accordance with studies that place seed limitation as an important driver of low resilience in disturbed grasslands (Urbanska & Fattorini, 2000; Lehouck et al., 2009). The limited seed arrival in both preserved and disturbed plots, particularly in sticky traps, can be explained by a widespread lack of obvious seed dispersal mechanisms in the majority of species in *campo rupestre* (Morellato & Silveira, 2018). The higher average seed density in sticky traps in preserved plots shows the importance of short distance dispersal events from plants inside the plots (Chabrierie & Alard, 2005). Some seeds retrieved in sticky traps did not represent any classical adaptation for wind dispersal, such as wings or plumes, and may not accurately describe all components of the dispersal processes for many species (Howe & Smallwood 1982). For example, some light seeds may disperse from tall mother plants by wind over several meters and may be caught in sticky traps (Chabrierie & Alard, 2005). This may partially explain why disturbance seems to affect seed arrival dynamics in sticky traps, due to the absence of parental plants or to the small height of the mother plants on these plots.

Only 17 species from the floristic survey occur in both plot types, indicating a strong dissimilarity between preserved and disturbed areas even 15 years after the disturbance. The strong dissimilarity within disturbed plots and the fact that 57% from all plant genera from the floristic survey were only recorded in preserved plots sustain the poor and unpredictable recovery trajectory in these plots (Leps et al., 2000; 2007), which is likely caused by a high spatial species turnover and dispersal and/or establishment limitation. Poaceae and Cyperaceae were the most representative families in the standing vegetation in both plot types and presented together with Asteraceae the high abundance in the seed rain in both plot types, suggesting that these plant families could be prioritized in restoration projects in *campo rupestre*. On the other hand, *Vellozia* (Velloziaceae), one of the richest genera in the standing vegetation in preserved

plots, was absent in disturbed plots both in the standing vegetation and in the seed rain, which place them as challenging key plant groups for assisted reintroduction in disturbed areas.

Seed rain varies with trap types

Funnel traps were the most efficient trap type in catching seeds, supporting the idea that this trap type is the most effectiveness in estimating seed rain in grasslands (Page et al., 2002; Chabrierie & Alard, 2005). Although we retrieved only a small portion of the annual accumulated seed rain in sticky traps, 10% of the morphospecies were exclusively found in this trap type, including two out of the ten more common ones. These findings indicate that the combination of different seed trap types increases the accuracy and sampling of seed rain studies (Chabrierie & Alard, 2005). The higher average of seeds/m²/day found for funnel traps in disturbed plots can be related to a reduced activity of ground-dwelling animals in these areas (Brandão et al., 2011), relaxing the strength of seed predation, and thus increasing the chances of seed arrival in funnel traps *via* secondary wind dispersal or runoff water (Chambers & MacMahon, 1994; de Rouw et al., 2018).

The total species richness in the seed rain was higher than that in the floristic survey, implying a species influx from seeds coming from outside the plots. However, this influx may be more related to plant megadiversity and high spatial species turnover in *campo rupestre* than to high dispersal potential by the plant community (Silveira et al., 2016). Approximately 32% of morphospecies were registered exclusively in disturbed plots and 25% exclusively in preserved plots, suggesting differences in the seed rain composition between disturbed and preserved areas. The Correspondence Analysis for the morphospecies occurrence indicates a certain similarity of seed rain patterns among preserved plots and a marked difference in seed rain composition between disturbed plots, which together with seed rain limitation may partially explain the poor recovery of these disturbed areas after soil removal.

Seed rain varies with seasons

Campo rupestre is characterized by distinct seasonal patterns for the total annual fruit production in the plant community in its sandy soils, with significantly more fruits being produced in the dry season (Le Stradic, 2018c), which may partially explain the significant increase in the number of barochoric seeds (e.g. Poaceae) captured in sticky traps during the dry season. On the other hand, the significant increase in the number of seeds and species richness captured in funnel traps during the rainy season, may indicate a strong influence of seasonal abiotic-related secondary dispersal processes, such as water runoff during the rainy season (e.g. Cyperaceae seeds, de Rouw et al., 2018) and may explain the relative lower seed density in funnel traps during the dry season. The ability of rainfall to disperse seeds depends on local surface cover (de Rouw et al., 2018). Bare or sparsely covered soils in disturbed plots can favor seed dispersal by runoff water and wind along the ground, whereas dense ground cover in preserved plots represents barriers to secondary dispersal by these abiotic factors (Schurr et al., 2005). Cyperaceae are characterized by a high proportion of species with buoyant, water-dispersed seeds (Leck & Schütz, 2005), which may partially explain the equivalent seed arrival rates between disturbed and preserved plots for species in this family.

Conclusion

This study brings the first community-level information about seed dispersal in *campo rupestre* and represents a novel contribution for seed rain studies on tropical grasslands. Our data suggest widespread seed limitation in both preserved and disturbed areas and a remarkable low seed density found for 90% of the taxa captured on the seed traps. As a consequence, the recovery success in these disturbed areas of *campo rupestre* cannot only rely on the natural arrival of new diaspores. Active restoration techniques, such as direct seeding, should be

considered in future restoration practices in *campo rupestre*. Approximately 1/3 of seeds of morphospecies were registered exclusively in disturbed plots and 1/4 exclusively in preserved plots, proving that the collection of data on both degraded and preserved areas is crucial for a better understanding of the seed rain in a restoration context. Despite the fact that funnel traps were the most efficient trap type in catching seeds, the use of more than one type of seed trap proved to be crucial for a more reliable and complete measurement of the seed rain and its ecological aspects. The significant difference in the number of seeds and species richness captured within trap types between seasons, shows the importance of seasonal comparisons in tropical environments when studying seed rain.

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Authors' contributions

A.J.A, F.A.O.S., P.P. and E.B conceived the ideas and designed methodology; A.J.A., P.A.J., H.T.S.R., F.Y and E.B. collected the data; A.J.A. and E.B. analyzed the data; A.J.A. led the

writing of the manuscript with inputs from F.A.O.S, P.P. and E.B. All authors contributed critically to the drafts and gave final approval for publication.

Supporting Information

Appendix S1: Picture of the disturbed site

Appendix S2: Seed trap types design

Appendix S3: Rarefaction curves

Appendix S4: Seed image bank

Appendix S5: Plant species occurrence according to plot type considering all sites.

Appendix S6: Multidimensional representation of the composition of plant species

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