

Seed ecology and grassland resilience : the case of campo rupestre

André Jardim Arruda

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Programa de Pós Graduação em Biologia Vegetal

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SEED ECOLOGY AND GRASSLAND RESILIENCE: THE CASE STUDY OF CAMPO RUPESTRE

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Área de Concentração Ecologia Vegetal

BELO HORIZONTE – MG

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‘’What didn’t you do to bury us / but you forgot that we were seeds”

Dinos Christianopoulos

To my parents,

ABSTRACT

Our knowledge of tropical old-growth grassland resilience (i.e. resistance to degradation and capacity for recovery) remains limited relative to our knowledge of temperate grasslands. Although highly resilient to endogenous disturbances such fires, the vegetation dynamics in old-growth fire-prone tropical grasslands seems hampered by anthropogenic disturbances, especially topsoil degradation. After topsoil disturbance, the internal species pool (i.e. remaining vegetation and the seed bank) is often depleted or even absent, and natural regeneration depends mainly on seed dispersal from surrounding sites via the seed rain. However, plant communities on areas disturbed by soil removal can remain very different from preserved sites many years after degradation, with almost no recovery of the natural vegetation. Despite extremely relevant, seed dispersal dynamics and resilience of tropical old-growth grasslands after soil disturbance remains much overlooked. In this thesis, I carried out a review and three experimental studies about seed rain and diaspore removal dynamics in grasslands. In CHAPTER1, I did a systematic literature survey about seed rain studies in global grasslands. I (1) assessed where, how and why research on seed rain has been carried out; (2) examined how methodological design and results have been reported; and (3) provided guidelines for future research on seed rain in grasslands. I found a remarkable unbalance in the numbers of studies between grassland types, which becomes even more dissimilar across global climatic ranges when the area covered by each grassland type is take into consideration. I identified significant knowledge gaps in grassland seed rain research. I also found a great disparity of methods and data being reported across studies. Additionally, I found that only a few attempts have been made to assess the seed trap efficiency and no studies to date have proposed any protocol to evaluate the effectiveness of seed sorting methods. Then, in CHAPTER 2, I ran a field experiment to propose a simple standard protocol to evaluate the efficiency of two seed trap types (sticky and funnel traps) and of a seed sorting method to assess the efficient of the methods prior to seed rain studies. As a case of study, in CHAPTER 3 and 4, I studied seed rain and secondary diaspore removal dynamics in preserved areas and in areas degraded by gravel exploitation for road construction in *campo rupestre* vegetation, a megadiverse edaphic grassland in southeastern Brazil. The small number of seeds captured in the seed rain, indicates seed limitation and suggests a close causal relationship between seed dispersal limitation and the low resilience after soil disturbance. The identification of some relatively abundant species in the seed rain places these species as good targets for reintroduction in future restoration projects. Topsoil removal changed the identity of ants interacting with diaspores, resulting in contrasting outcomes from ant-diaspore interactions. The lack of diaspore removal towards

degraded areas indicates that establishment limitation is a factor hampering natural regeneration. Our findings help to explain, at least partially, why natural regeneration is compromised after soil removal, which strongly influences diaspore fate and interactions with potential ground-dwelling dispersers, resulting in different ecological outcomes and strong influencing vegetation dynamics and regeneration. I expect that these results will guide future research on seed dispersal and resilience in grasslands, underpinning decisions on restoration and conservation practices on these threatened environments.

RÉSUMÉ

Nos connaissances sur la résilience (résistance à la dégradation et capacité de rétablissement) des pelouses tropicales anciennes restent limitées par rapport à nos connaissances sur les pelouses tempérées. Bien que très résilientes aux perturbations endogènes telles que le feu, la dynamique de la végétation dans les pelouses tropicales sujette au feu semble être freinée par les perturbations anthropiques, en particulier la dégradation du sol. Après la perturbation du sol, le pool interne d'espèces (i.e. la végétation restante et la banque de graines) est souvent réduit et parfois absent, et le rétablissement naturel dépend principalement de la dispersion des graines à partir des sites alentours via la pluie de graines. Cependant, les communautés végétales des zones dont le sol a été perturbé peuvent rester très différentes des sites de référence de nombreuses années après la dégradation, avec pratiquement aucun rétablissement de la végétation naturelle. Bien qu'extrêmement pertinente, la dynamique de dispersion des graines et la résilience des pelouses tropicales anciennes après la perturbation du sol restent très peu étudiées. Dans cette thèse, j'ai effectué une synthèse bibliographique et trois études expérimentales sur la dynamique de la pluie de graines et sur l'enlèvement des diaspores dans les pelouses. J'ai d'abord effectué une revue systématique de la littérature sur les études de pluie de graines dans les pelouses du monde. J'ai (1) évalué où, comment et pourquoi des recherches sur la pluie de graines ont été menées ; (2) examiné la manière dont la conception méthodologique et les résultats ont été rapportés ; et (3) fourni des lignes directrices pour les recherches futures sur la pluie de graines dans les pelouses. J'ai trouvé un déséquilibre remarquable dans le nombre d'études entre les types de pelouses, qui devient encore plus dissemblable d'une plage climatique à l'autre lorsque le domaine couvert par chaque type de pelouse est pris en compte. J'ai identifié d'importantes lacunes dans les connaissances dans la recherche sur la pluie de graines des pelouses. J'ai également constaté une grande disparité entre les méthodes et les données présentées dans les différentes études. De plus, j'ai constaté que seules quelques tentatives avaient été faites pour évaluer l'efficacité des pièges à graines et qu'aucune étude à ce jour n'a proposé de protocole pour évaluer l'efficacité des méthodes de tri des graines. Ensuite, je mène une expérience sur le terrain pour proposer un protocole standard simple permettant d'évaluer l'efficacité de deux types de pièges à graines (pièges collants et entonnoirs) et une méthode de tri des graines permettant d'évaluer l'efficacité des méthodes avant les études de pluie de graines. Les deux derniers chapitres concernent la dynamique de la pluie de graines et de l'enlèvement secondaire des diaspores dans des zones préservées et dégradées par l'exploitation de gravier pour la construction de routes dans la végétation de *campo rupestre*, une pelouse édaphique mégadiverse du sud-est du Brésil. Le petit nombre de graines récoltées à partir de la pluie de graines indique la limitation des graines et suggère une relation de cause à effet étroite entre la limitation de la dispersion des graines et la faible résilience après perturbation du sol. L'identification de quelques espèces relativement abondantes dans la pluie de graines place ces espèces en tant que bonnes cibles pour la réintroduction dans futurs projets de restauration. Les perturbations ont modifié l'identité des fourmis en interaction avec les diaspores, ce qui a entraîné des résultats contrastés des interactions fourmis - diaspores. L'absence de déplacement des diaspores vers les zones dégradées indique que la limitation en graines est un facteur entravant la régénération naturelle. Mes résultats aident à expliquer, du moins en partie, pourquoi la régénération naturelle est compromise après perturbation du sol, ce qui influe fortement sur le destin des diaspores et sur les interactions avec les potentiels disperseurs, influençant la dynamique et le rétablissement de la végétation. Je propose en conclusion des orientations pour les futures recherches sur la dispersion des graines et la résilience des pelouses, qui sont fondamentales pour faciliter les décisions sur les pratiques de restauration et de conservation de ces environnements menacés.

RESUMO

O nosso conhecimento sobre a resiliência (isto é, resistência à degradação e capacidade de recuperação) de savanas tropicais permanece muito aquém do nosso conhecimento já adquirido sobre as savanas temperadas. Savanas tropicais, embora altamente resilientes à perturbações endógenas, como o fogo, se mostram extremamente vulneráveis à certas perturbações antrópicas como a degradação do solo, a quais podem ocasionar drástica alteração da dinâmica da vegetação. Após a perturbação da camada superficial do solo, o conjunto interno de espécies (isto é, a vegetação remanescente e o banco de sementes) são frequentemente reduzidos ou até mesmo completamente eliminados. Nestes casos, a regeneração natural depende principalmente da dispersão de sementes da vegetação do entorno através da chuva de sementes. No entanto, comunidades vegetais em áreas perturbadas pela remoção do solo podem permanecer, mesmo após vários anos ao distúrbio original, muito diferentes do ecossistema de referência, apresentando baixíssima capacidade de regeneração natural. A dinâmica de dispersão de sementes em savanas tropicais, apesar de extremamente relevantes para uma melhor compreensão sobre a resiliência desses ecossistemas frente a ações antrópicas, permanece pouco estudada. Nesta tese, realizei uma revisão e três estudos experimentais sobre a dinâmica da chuva de sementes e da remoção de diásporos no *campo rupestre*, uma savana tropical natural, megadiversa e muito antiga localizada no sudeste do Brasil. No primeiro capítulo, realizei uma pesquisa bibliográfica sistemática sobre estudos de chuva de sementes em áreas não florestais no mundo com os seguintes objetivos: (1) avaliar onde, como e por que as pesquisas sobre chuva de sementes foram realizadas; (2) examinar como o desenho metodológico e os resultados foram relatados; (3) fornecer diretrizes para futuras pesquisas sobre chuva de sementes em áreas não florestais. Nesta revisão, eu encontrei um notável desequilíbrio no número de estudos entre os tipos diferentes tipos de áreas não florestais, o qual se torna ainda mais notável em relação à distribuição dos estudos entre as faixas climáticas globais e em relação à área total recoberta por cada ecossistema (ex. savanas tropicais). Neste estudo, foram identificadas algumas importantes lacunas de conhecimento relativas a estudos sobre a chuva de sementes em áreas não florestais. Foi evidenciado também uma grande disparidade entre os métodos de estudo de chuva de semente e sobre a maneira de reportar os dados observados, o que dificulta comparações entre estudos. Verificamos que apenas algumas tentativas foram feitas para avaliar a eficiência das armadilhas de captura de sementes e que nenhum estudo até o momento propôs algum protocolo para avaliar a eficácia dos métodos de captura de sementes utilizando armadilhas de sementes para áreas não florestais. No segundo capítulo, foi proposto e testado um protocolo simples para avaliar a eficiência de dois tipos de armadilhas de sementes (armadilhas pegajosa e de funil) e a eficácia de um método de busca e triagem de sementes coletas em armadilhas de funil, os quais podem ser realizados previamente aos estudos de chuva de semente. Como casos de estudo, no terceiro e quarto capítulos, pesquisei a dinâmica da chuva de sementes e de remoção secundária de diásporos em áreas preservadas e em áreas degradadas pela exploração de cascalho para a construção de estradas na vegetação do *campo rupestre*. O pequeno número de sementes capturadas na chuva de sementes indica limitação na dispersão de sementes e sugere uma estreita relação causal entre a limitação de dispersão de sementes e a baixa resiliência observada nestas áreas degradadas após a perturbação do solo. A identificação de espécies relativamente abundantes na chuva de sementes coloca essas espécies como potenciais alvos para futuros projetos de restauração visando a reintrodução de espécies. A perturbação do solo alterou a identidade dos animais forrageadores interagindo com diásporos, influenciando na qualidade e na quantidade das interações entre áreas preservadas e degradadas. A ausência de remoção secundária de diásporos direcionada para áreas degradadas e a saída de diásporos de área degradadas para

preservadas, indica que a remoção secundária de sementes pode ser um fator contribuindo para a limitação de estabelecimento, dificultando assim a regeneração natural. Nossos resultados ajudam a explicar, pelo menos parcialmente, por que a regeneração natural é comprometida após a remoção do solo no *campo rupestre*, a qual tem uma forte influência na dinâmica de dispersão de sementes e consequentemente na capacidade de regeneração da vegetação. Espero que estes resultados orientem futuras pesquisas sobre dispersão de sementes e resiliência de savanas tropicais frente a ações antrópicas, podendo assim embasar decisões sobre práticas de restauração e conservação nesses ambientes ameaçados.

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And, please forgive me if I forgot someone.

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INTRODUCTION

1. **Grassland resilience**

1.1 Resilience

In an era overwhelmed by anthropogenic disturbances and environmental disasters (Cooke et al. 2018), resilience might be crucial to natural ecosystem persistence. Resilience can be defined as the capacity of systems to resist and recover from disturbances (Hodgson et al. 2015). Grime (1979) defined disturbance as "any factor that removes biomass, as distinguished from environmental stress, which relates to factor that limit biomass production." The most common forms of disturbance include herbivory, natural fires, and activities resulting from human-driven land-use change (Lessard 2019).

When exposed to disturbance, ecosystems vary in the nature and intensity of changes, which depend on ecosystem resistance and recovery capacity to disturbance over time (Ingrisch & Bahn 2018; Hoover et al. 2014) (Fig. 1). Resistance is a measure of the system ability to persist during a disturbance, which is measured through the concurrent impact of the disturbance on response parameters, such as plant survival (Walker et al. 2004; Ingrisch & Bahn 2018). On the other hand, recovery captures the endogenous processes that pull disturbed systems back towards a reference state and functioning over time (Mumby & Steneck 2008; Hodgson et al. 2015).

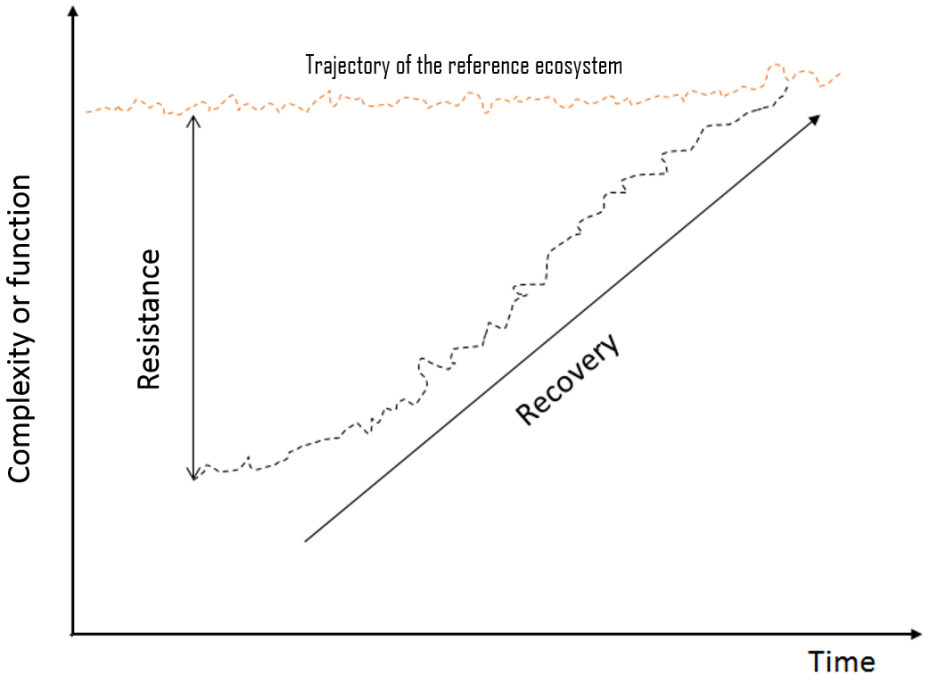


Figure 1: Representation of resilience, which can be decomposed on the resistance and recovery capacity of the system to a disturbance (loss of complexity and function) over time (modified from Hobbs & Norton 1996; Clewell & Aronson 2007).

Quantifying ecosystem responses to disturbance usually required measurements of a large number of 'indicators' or 'metrics' of resilience (Ingrisch & Bahn 2018), demanding an integrative scientific effort in diverse knowledge areas. Such knowledge about ecosystem resilience is fundamental to guide the management of natural systems as well as restoration and conservation efforts (Buisson et al. 2019).

1.2 *Old-growth grassland resilience*

Grasslands cover approximately 40% of global land surface and are ecosystems dominated by graminoids (i.e. grasses and grass-like plants) and forbs (Gibson, 2009; Parr et

al., 2014). Natural grasslands are thought to have had a global distribution for at least 15 million years (Jacobs et al., 1999; Dixon et al., 2014), and are maintained by frequent fires, megafaunal herbivores, and edaphic factors that limit tree growth and forest expansion (White et al. 2000; Dantas et al. 2016).

When ancient, grasslands have been recently classified by Veldman et al. (2015) as old-growth grasslands, which are composed of plant communities that require centuries or millennia to assemble, and that exhibit exceptional biodiversity, including high plant species richness and endemism (Echternacht et. al. 2011; Buisson et al. 2019; Colli-Silva et al. 2019). Nevertheless, along the past decades, such grasslands are declining at alarming rates mainly due to agricultural conversion, mining and urban sprawl (Dixon et al. 2014; Parr et al. 2014; Buisson et al. 2019). Therefore, initiatives to conserve and restore old-growth grasslands are urgently needed (Veldman et al. 2015; Bond 2016; Buisson et al. 2019).

Old-growth grasslands, although highly resilient to endogenous disturbances, such as fires (Bond & Keeley 2005), are extremely vulnerable to human-caused exogenous disturbance (Le Stradic et al. 2018, Buisson et al. 2019). Topsoil removal, mainly associated to urban sprawl, road construction, quarrying, and mining activities, is known to strongly affect the vegetation dynamics in old-growth grasslands (Silveira et al. 2016; Fernandes et al. 2018). However, our knowledge of tropical old-growth grassland recovery capacity after exogenous disturbances, remains limited relative to our knowledge of temperate grasslands (Buisson et al. 2019).

For example, in an old-grassland in Brazil, Le Stradic et al. (2018) found that even almost one-decade after excavation for gravel, quarry sites had almost no grassland species. However, the precise mechanisms driving poor recovery after top soil disturbance in old-grow grasslands (e.g. plant recruitment limitation) still a matter of controversy (Standish & Hobbs 2010; Veldman et al. 2015) and warrants further investigation (Dayrell et al. 2016; Buisson et

al. 2019). A series of hypotheses explaining the evolution and ecology on very old, climatically buffered, infertile landscapes (OCBILs) were proposed by Hopper (2009). The great unusual susceptibility to major soil disturbances among OCBILs may be explained by seed limitation, slow plant growth and extremely-impoverished soils (Hopper 2009; Hopper et al. 2016).

2. **Vegetation dynamics**

2.1 *Plant community assembly*

A community can be defined as a group of organisms representing multiple species living in a specified place and time (Vellend 2010). Despite simple to define, understanding or predicting local conditions and processes that shape biological communities remain one of the main challenges in ecology (Weiher et al. 2011, Götzenberger et al 2012). The composition of communities depends on a grate range of processes related to abiotic and biotic conditions that influence important ecological process, such as plant community assembly (Weiher & Keddy 2001; Kraft & Ackerly 2014).

Plant community assembly theory investigates the mechanisms that structure plant communities, considering processes that shape the identity and abundance of species from its species pool (Götzenberger et al. 2012). The regional species pool contains potential colonists of the plant community that are "filtered" to the final community by regional processes (e.g. seed dispersal), and environmental and biotic filters (e.g. seed predation) (White & Jentsch 2004). Biotic, abiotic and evolutionary aspects represent important filters to successful establishment of plant species to the local species pool (Kraft & Ackerly 2014), but the relative strength of each factor varies across ecosystems (Kraft et al. 2015).

A crucial step on community assembly research is better understand why only a fraction of the total species pool is able to reach a particular site, unveiling dispersal patterns, and its

responses to abiotic and biotic filters (Prach & Pyšek 2001; Kraft & Ackerly 2014). Additionally, it is important to consider not only ecological and biogeographic aspects but also evolutionary aspects on plant community assembly, which may vary greatly across ecosystems and strongly influence dispersal dynamics (Hopper et al. 2009).

A clear understanding of the governing forces and rules that shape species assembly in a particular community is vital for successful ecological restoration by providing a better understanding of how plant communities behave in different environmental scenarios (Török et al. 2018). The approaches to determine processes filtering species to communities can be applied either from species pool to community or from community to species pool (Temperton & Hobbs 2004), and provide valuable information about the mechanisms that structure biological communities.

2.2 Dispersal filter

Dispersal filter is an important concept in plant community assembly, in which species occurrence can be partially explained by differences in dispersal success (Fig. 2) (Burns 2005; Fraaije et al. 2015). These filters are composed by factors that serves as a barrier during the dispersal process controlling the colonization potential of the plant community (Kraft & Ackerly 2014). Plant communities constrained by seed dispersal processes are referred to as ‘‘seed limited’’ (Turnbull et al. 2000, Foster & Tilman 2003). To understand the process related to seed limitation we need to determine the relative importance and the possible variations between each dispersal filter in space and time (Lortie et al. 2004).

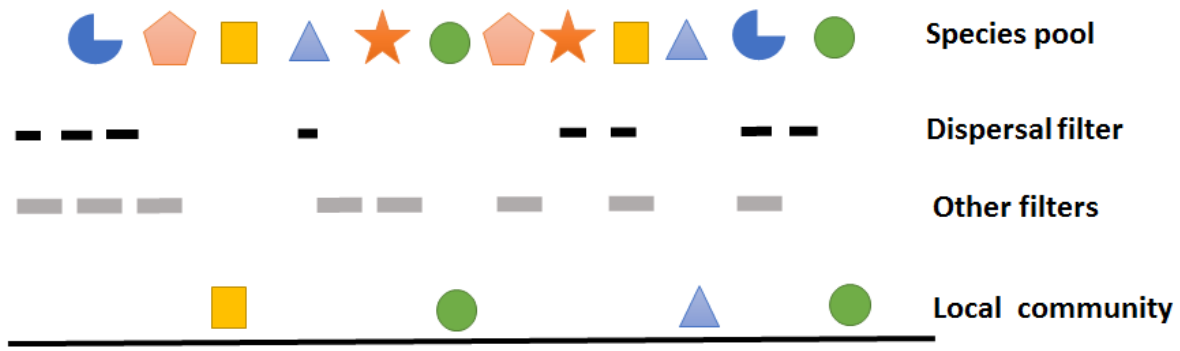


Figure 2: Basic conceptual model of how dispersal filter may control plant community assembly from a regional species pool. Each object represents a plant species. The species present in the local community are those that have been able to successfully disperse and succeed through the other environmental filters.

Biotic and abiotic factors can influence seed dispersal limitation and are crucial in plant community assembly (Kraft & Ackerly 2014). Biotic factors are those related to associations between seeds and other organisms (e.g. seed predators, facilitation, competition), which may have important consequences for community assembly (Götzenberger et al. 2012, Kraft & Ackerly 2014). On the other hand, abiotic factors are interactions between seeds and non-living physical and chemical elements of the environment (e.g. wind and water run-off), that may influence the dispersal of a species (Morin 1999; Vellend 2010). Seed limitation is thus a complex process, controlled not only by biotic and abiotic factors but also by evolutionary aspects from the plant community that should be taken into account (Weiher & Keddy 2001; Lortie et al. 2004). Differences in dispersal abilities (e.g. quantity and quality of seeds) are common between different plant community species, and is a critical factor structuring dispersal filters (Hubbell 2001; Myers & Harms 2009; Hooper 2009; Fraaije et al. 2015).

The dispersal filter is of extreme relevance in restoration ecology (Török et al. 2018) and was first described in a biological succession context after disturbance (Nobel & Slatyer 1977). However, the assessment of the relative importance of seed limitation across time, space and ecosystem types remains challenging (Oster et al. 2009). Thus, a better understanding to what extent dispersal filter determines community assembly and what factors are involved in this process remain critical for the design of effective restoration strategies (Brederveld et al. 2011; Török et al. 2018).

3. **Ecology of seed dispersal**

3.1. *Seed dispersal*

Plants are sessile organisms, and apart from a small number of species that are able to disperse via vegetative fragmentation (particularly aquatic species), plants depend mostly on seeds to disperse (Kraft & Ackerly 2014). Seeds are the products of sexual reproduction in most vascular plants and are the means by which plants produce offspring capable of disperse (Vander Wall et al. 2005). We can simply define seed dispersal as movement of seeds with potential consequences for gene flow across space (Vellend 2010; Kraft & Ackerly 2014). On the other hand, seed dispersal can be seen as a complex process, represented by multiple stages (departure, transfer and settlement) and influenced by a wide variety of mechanisms and factors (Burgess et al. 2016). Consequently, distinguishing the effects of diverse mechanisms and factors influencing seed dispersal in plant communities, and analyzing their implications in plant assembly and recruitment, is a challenging task in seed ecology (Schupp et al. 2010; Arnan et al. 2012; Török et al. 2018).

Seed dispersal represents the first step for recruitment in plant communities, influencing plant demography and spatial distribution (Jordano et al. 2007; Schupp et al. 2010). A

successful seed dispersal event consists of a displacement from a source into a site (the seed shadow) where a seed can than germinate and establish (Jordano et al. 2007). On the other hand, dispersal may be a risky choice for the plants considering that the elevated investment in reproduction may not always result in dispersal success (Ronce 2007). Seed mortality may be high in some plant communities due to non-favorable habitat conditions and to predation (Ims & Andreassen 2000; Hanski et al. 2000), and may greatly vary between species, ecosystems and habitats (Matter 2006; Schtickzelle et al. 2006). Because seed fate is potentially influenced by many factors and mechanisms that shape plant community assembly, methods to quantify and qualify the spatial and temporal seed dispersal dynamics are of extreme relevance (Russo et al. 2006).

3.2. *Studying seed dispersal*

To quantify the spatial distribution of seed dispersal (seed shadow) and summarize the consequences of dispersal movements two metrics are often used: dispersal rate and dispersal kernel (Bowler & Benton 2005; Ronce 2007). Dispersal rate describes the expected proportion of seed arrival in an area over the time (Robledo-Arnuncio et al. 2014). On the other hand, dispersal kernel represents the probability for a seed to disperse to any position relative to the maternal plant and its consequences on seed fate (Klein et al. 2013). We can estimate these dispersal metrics by direct observation of diaspores deposition or by tracking individual diaspores (Cousens et al. 2008; Jones & Muller-Landau 2008). However, as seed dispersal is expected to be influenced by a vast diversity intrinsic (e.g. phenotype) and extrinsic variables (e.g. human disturbance), direct measurements is notoriously challenging (Russo et al. 2006; Ronce 2007).

Studying seed dispersal dynamics in plant communities does not simply imply quantifying dispersal rates and kernels of single species, but also assessing how seed dispersal

metrics can vary across plant species, environmental conditions and time (Ronce 2007). Consequently, the measurement of dispersal dynamics in plant communities can demand great methodological efforts and be challenging to interpret (Clobert et al. 2001; Ronce 2007). For example, diaspore-animal interactions (e.g. secondary seed removal and seed predation) and abiotic factors (e.g. wind and water dynamics in the landscape), may greatly vary across time and space between and within ecosystems and plant species (Levin & Muller-Landau 2000; Westcott et al. 2005; Nathan 2007; Jordano et al. 2007; Burgess et al. 2016), challenging our ability to measure, estimate and compare seed dispersal dynamics (Robledo-Arnuncio et al. 2014).

3.3. *Dispersal over time and space*

Dispersal can greatly vary over time and space due to variation in mechanism and factors influencing seed dispersal across these scales (Robledo-Arnuncio et al. 2014). Thus, assimilate comprehensive temporal and spatial scales, may be a critical step to obtain accurately estimates and ecological inferences about seed dispersal in plant communities (Kraft & Ackerly 2014). Temporal issues, such as dispersal fluctuations across seasons, can be crucial in predicting plant community assembly and recruitment success, but have received much less attention than spatial aspects in seed dispersal (Robledo-Arnuncio et al. 2014).

The spatial pattern of seed deposition may mediate the probability of success of dispersal through its outcomes on deposition in a favorable site (e.g. germination gaps) and on post-dispersal interactions with ground foraging animals (Rico-Gray & Oliveira 2007; Christianini & Oliveira 2009). The predominance of short distance dispersal events in plant communities may affect plant persistence, migration and seedling recruitment in disturbed areas (Thomson et al. 2011; Török et al 2018). On the other hand, despite the fact that most seeds travel only a short distance, some seeds can present remarkable ability to achieve long-distance

dispersal events (Kraft & Ackerly 2014). Spatial and temporal aspects, such as environmental conditions (e.g. disturbance) and seasons (e.g. rainy season) may greatly influence qualitative and quantitative aspects of seed dispersal (Robledo-Arnuncio et al. 2014). Hence, taking into account both spatial and temporal dispersal patterns between plant species can be a crucial step on studies about seed dispersal (Thomson et al. 2011; Tamme et al. 2014).

3.4 *Dispersal across plant species*

Variation in dispersal potential across plant species can be substantial (Dalling et al. 2002; McEuen & Curran 2004), resulting in potentially large differences in recruitment capacity (Vittoz & Engler 2007; Thomson et al. 2010; Tamme et al. 2014). Variation in dispersal potential may be a result of how physical (e.g. ballistic mechanisms and floatability) and biological (e.g. diaspore-animal interactions) components of seed dispersal may vary between plant species (Burgess et al. 2016). For example, wind-dispersed species normally presents small seeds and/or dispersal structures (e.g. wings) to travel long distances in the wind (Ganeshaiah & Shaanker, 1991). Additionally, diaspores from different species may greatly differ in their interactions dynamics with animals, directly influencing the quantitative and qualitative outcomes for seed dispersal success (Jordano et al. 2007; Schupp et al. 2010).

In the case of animal-diaspore interactions, dispersal dynamics may be a result of animals' preferences for habitats and diaspores (Vander Wall 1997; Rodríguez-Pérez et al. 2012). Seed predators may target specific species (Roselli 2014), influencing in different ways the role of seed limitation in natural recovery across species. On the other hand, seed dispersers may increase seed survival and germination by foraging on fruit and cleaning seeds (Christianini et al. 2007) or depositing seeds in favorable locations (Sternberg et al. 2007, Arnan et al. 2012)

Directed dispersal represents the arrival of seeds in a particular location and can direct influence seed dispersal success (Wenny 2001; Christianini et al. 2007). Directed dispersal can be partially explained by the way in which seed movement is affected by disperser behavior (e.g. ants carrying seeds to dump-piles) and habitat conditions (e.g. wind dynamics) (Schurr et al. 2005; Trakhtenbrot et al. 2014) and is a common process in seeds dispersed by wind, water run-off or animals (Robledo-Arnuncio et al. 2014; Chabrerie & Alard, 2005; de Rouw et al. 2018). However, less evident is the relative influence of habitat conditions (e.g. disturbance) on directed dispersal by wind and water run-off (Chabrerie & Alard, 2005; de Rouw et al. 2018).

3.5. *Seed rain*

Seed rain is the number of seeds reaching an area, and it usually is quantified and qualified by placing traps in the plant community to catch seeds that then are identified and counted (Baskin & Baskin 2014). Seed rain is thus a measurement of seed dispersal rates, representing species dispersal potential in time and space (Page et al. 2002). As an important component of seed dispersal, seed rain measurements can provide crucial information on successional trajectories, thereby being a useful tool to assess recovery potential in disturbed areas (Turnbull et al. 2000; Török et al. 2018). Seed rain can been analyzed both (1) indirectly, by studies of plant reproductive potential (Boughton et al. 2016) and seed bank dynamics (Bertiller & Aloia 1997), and (2) directly, by collecting either seeds visible on the ground, by observing the movements of granivore animals (Izhaki et al. 1991), or from diaspore traps (Kollmann & Goetze 1998).

3.6. *Secondary seed dispersal*

Secondary seed dispersal can be defined as the relocation of a diaspore dispersed to a given area by a different factor responsible for the primary seed dispersal and which may reshape seed shadows and strongly influence plant community assembly (Christianini & Oliveira 2009; Robledo-Arnuncio et al. 2014). Empirical studies examining secondary seed dispersal showed important variability among diaspores and sites (Schupp et al. 2010). Abiotic (e.g. water run-off) and biotic (animal-diaspore removal) factors may provide by secondary dispersal an increase in seed dispersal success expanding seed shadow and survival or hampering seed dispersal success (e.g. seed predation) (Rico-Gray & Oliveira 2007; Sternberg et al. 2007, Arnan et al. 2012).

As diaspores constitute a highly nutritive food resource for animals (Thorsen at al. 2011; Schowalter 2016), secondary dispersal by animals is of great relevance on plant community assembly and plant recruitment in disturbed systems (Martinson & Fagan 2014). The ways in which vertebrates and invertebrates interact with diaspores have been crucial to the development of theoretical models about seed dispersal and predation (Nathan & Casagrandi 2004). By moving seeds, ground foraging animal seed predators can accidentally work as seed dispersers and even facilitate seed germination (Rico-Gray & Oliveira 2007; Gómez et al. 2019), thus promoting regeneration (Schupp 1988).

3.7. *Seed dispersal and restoration*

Dispersion is the first mechanism to act when colonizing a new biotope (Török et al. 2018). From the moment that only a fraction of the total species pool is able to reach a particular site (i.e. available species), a better understanding about the biotic and abiotic drivers and filters that are governing or limiting plant recovery is crucial (Prach & Pyšek 2001; Török et al. 2018). Understanding to what extent anthropogenic modifications are relevant to seed limitation is fundamental to predict the capacity of ecosystems to respond to anthropic changes (Török et al.

2018). For example, prior to restoration, seed rain and secondary seed dispersal can be evaluated in order i) to assess the potential for regeneration or passive restoration and ii) to plan restoration actions (Jacquemyn et al. 2011; Pardini et al. 2017; Török et al. 2017), because they allow i) better understanding of ecological processes and ii) therefore adjustment of management.

The effects of dispersal on vegetation recovery dynamics still need to be better explored (Török et al. 2018). For that, the development of studies about seed rain dynamics, dispersal vectors and seed disperser networks are crucial to a better understand of the consequences of human disturbance on dispersal dynamics (Robledo-Arnuncio et al. 2014). In seed-limited ecosystems, any reduction in seed quantity (e.g., seed predation) may compromises plant recruitment, while processes that increase seed dispersal success may prompt plant recruitment (Calviño-Cancela 2007). Thus, better understanding the mechanisms controlling seed dispersal (e.g. dispersal agents) and those outcomes (e.g. benefits and costs) is crucial for restoration practices in seed-limited ecosystems (Arnan et al. 2012; Dayrell et al. 2016; Török et al. 2018).

4. **Seed dispersal in campo rupestre**

4.1. *Campo rupestre*

The Brazilian *campo rupestre* is an OCBIL - Old Climatically-Buffered Infertile Landscape (*sensu* Hopper et al. 2016), that encompasses old-growth fire-prone tropical grasslands associated to extremely poor soils on ancient mountaintops (Fig. 3) (Silveira et al. 2016). *Campo rupestre* vegetation harbors a highly diversified flora with remarkable levels of plant endemism (Echternacht et. al. 2011; Colli-Silva et al. 2019) and is characterized by a predominantly herbaceous stratum, with shrubs and herbs associated to rocky outcrops and shallow soils with low nutrient contents (Giulietti et al. 1997; Oliveira et al. 2015). Despite

c*ampo rupestre* be geologically and floristically associated to the Cerrado and Caatinga biomes (Moro et al., 2015; Neves et al., 2018), several authors highlight the singularities of *campo rupestre* vegetation and indicate it as a unique bioregion (Prance 1994; Zappi et al., 2017; Colli-Silva et al. 2019).



Figure 3: *Campo rupestre* is an old-growth fire-prone tropical grasslands associated to extremely poor soils on ancient mountaintops and is characterized by a predominantly herbaceous stratum, with shrubs and herbs associated to rocky outcrops and shallow soils with low nutrient contents (Photo: Arruda, A.J.)

Campo rupestre vegetation occurs between 800 and 2,000m altitude and is especially found along the Espinhaço Range (Fernandes 2016), a mountain range that extends almost continuously for over 1,200 km^2 from southeast to northeast Brazil and represents an enclave between the Atlantic Forest, Cerrado and Caatinga biomes (Conceição et al. 2016). The Espinhaço Range is mostly composed by Precambrian quartzite outcrops originating from ancient sea floor and desert deposits, and that evolved under tectonic and climatic stability,

representing one of the most ancient landscapes on earth (Conceição et al. 2007; Barbosa et al. 2015).

The extreme weathering dynamics combined with the nature of the nutrient-poor parent rocks from the Espinhaço Range, results in shallow, acidic, excessively drained and nutrient impoverished soils (Benites et al. 2007; Oliveira et al. 2015). In this bioregion, the combination of ample altitudinal and latitudinal ranges, topographic aspects (e.g. isolation among vegetation islands), historical climatic and biogeographic stability, high habitat heterogeneity and strong soil nutrient limitations are recognized as the main reason for the extraordinary floristic richness and endemism of *campo rupestre* (Silveira et al. 2016; Colli-Silva et al. 2019).

Campo rupestre vegetation is amongst the most biologically diverse and unique in the world, harbouring more than 6,000 plant species with some families reaching up to 80−90 % of endemism (Echternacht et al. 2011; Silveira et al. 2016). Despite its high richness and plant heterogeneity, some plant families and genera confer a certain unicity to this bioregion, such as: Velloziaceae (e.g. *Vellozia*), Xyridaceae (*Xyris*) and Asteracae (*Lychnophora*) (Mello-Silva et al. 2011; Colli-Silva et al. 2019). While some plant families in *campo rupestre* are extremely rich (e.g. the ten richest families account for more than a half of the flora in *campo rupestre*), almost 1/4 of the plant families are represented by a single species (Colli-Silva et al. 2019). The families Eriocaulaceae, Velloziaceae and Xyridaceae have their centre of diversity in the *campo rupestre*, presenting high levels of endemism and many narrowly endemic species (e.g. species occurring in single location or population) extremely threatened by human-caused disturbance (Costa et al. 2008; Echternacht et al. 2011).

4.2. *Campo rupestre resilience to human-caused disturbance*

The vulnerability of mountain ecosystems to human-caused disturbance is well recognized (Jacobi et al. 2007; Foggin 2016), posing great challenges for conservation and restoration attempts (Buisson et al. 2019; Le Stradic et al. 2018b). The Espinhaço Range harbors

not only a biological treasure but huge reserves of gold, diamonds and iron (Fernandes et al. 2016), which are the main reasons for the fact that more than 20% of *campo rupestre* natural areas have been impacted by human activities since the 18th century (Magnanini, 1961; Fernandes et al. 2016). Human-caused disturbances in *campo rupestre* have intensified along the past decades, especially by urban expansion, quarrying and mining activities, and drastically hampering plant communities' dynamics in these disturbed sites (Barbosa et al. 2010; Fernandes et al. 2018).

Plant communities from *campo rupestre,* although highly resilient to endogenous disturbances (e.g. fire), are extremely vulnerable to human-caused exogenous soil disturbances (Le Stradic et al. 2018b; Buisson et al. 2019). Le Stradic et al. (2018b) showed that plant communities colonizing quarrying (inducing the destruction of vegetation and upper soil horizons) remained very different from reference sites even eight years after degradation, with almost no recovery of the natural vegetation. Generally speaking, for grasslands, after topsoil disturbance, the internal species pool (i.e. remaining vegetation, seed bank) is often reduced or even absent and natural recovery depends mainly on seed dispersal from surrounding sites via the seed rain (Bakker et al. 1996; Campbell et al. 2003; Shu et al. 2005; Buisson et al. 2006; Török et al. 2018).

Growing evidences suggest that the low resilience of *campo rupestre* vegetation to soil disturbance can be closely linked to seed dispersal limitation and environmental filter related to the extreme harshness in disturbed sites, which may hinder seed germination and plant establishment (Coelho et al. 2008; Garcia et al. 2012, 2014; Silveira et al. 2012a). However, we still have a lot to advance to understand what mechanisms and factors hamper recovery in *campo rupestre,* especially those related to seed dispersal limitation (Le Stradic et al. 2018b).

4.3. *Seed dispersal dynamics in campo rupestre*

Despite the marked seasonal climate in *campo rupestre*, distinct seasonal patterns for the annual fruit production are not clear in the plant community (Buisson et al. 2017; Le Stradic 2018c). Many families typical from *campo rupestre* exhibit no obvious mechanisms for seed dispersal by animals (Silveira et al. 2016), and unassisted and anemochoric seed dispersal mechanisms seems to be dominant (Conceição et al. 2007a; Jacobi and Carmo 2011). Unassisted dispersal seems to be the prevalent dispersal mode among dominant plant families such as Poaceae, Cyperaceae, Xyridaceae, Eriocaulaceae, and Velloziaceae (Silveira et al. 2016). Anemochoric seeds are expected to be common in species-rich families, such as Orchidaceae, Asteraceae, Apocynaceae, Bignoniaceae and Bromeliaceae (Silveira et al. 2016). On the other hand, the production of fleshy fruits compose an important part of the known animal-dispore interactions in *campo rupestre* (Fernandes et al. 2016). Despite the production of fleshy fruits in *campo rupestre* is attributed to a few Neotropical clades typically vertebrate-dispersed (e.g. Melastomataceae, Myrtaceae and Cactaceae) (Fernandes et al. 2016), it is not restricted to these clades (e.g. *Coccoloba cereifera*) (Faustino & Machado 2006; Guerra & Pizo 2014), and diaspore-animal interactions still need to be better studied for the *campo rupestre* plant communities.

Diaspore-animal interactions in tropical ecosystems are often composed of a great diversity of processes that often involves different agents of dispersal in subsequent steps (Vander Wall & Longland, 2004; Camargo et al. 2019). Most seed dispersal studies in Neotropical savannas have focused on primary seed dispersal in few plant species typically vertebrate-dispersed (Faustino & Machado 2006; Guerra & Pizo 2014, Guerra et al. 2017), and diaspore interactions with invertebrates still need to be better explored to better characterize their potential in modulating natural regeneration in disturbed sites. While most diaspores in Neotropical savannas do not present any apparent characteristics that promote ant dispersal (Christianini & Oliveira 2010), some studies have shown ants as important seed dispersers of

several non-myrmecochorous plant species (Christianini et al. 2007; Christianini & Oliveira 2009, 2010; Lima et al. 2013; Guerra et al. 2018). However, the role of ants as diaspore dispersers and the consequences for plant recruitment across different habitats are still poorly understood (Camargo et al. 2019).

4.4. *Seed dispersal limitation in campo rupestre*

Seed dispersal limitation is an important driver of plant community assembly in old-grow grasslands, making community re-assembly a lengthy process (Bond & Parr 2010; Parr et al. 2014; Veldman et al. 2015; Buisson et al. 2019). In c*ampo rupestre*, as in others OCBILs (old climatically-buffered infertile landscapes), plant species most probably disperse predominantly poorly and at low rates (Hopper et al. 2016). Additionally, due to the high soil specificity and heterogeneity in OCBILs, it is expected a predominance of short-distance dispersal events, as long-distance dispersal would incur high risks of seeds landing in unsuitable locations (Hopper 2009). However, while the high vulnerability of *campo rupestre* to human-caused exogenous soil is be commonly linked to dispersal limitation (Le Stradic et al. 2018b; Buisson et al. 2019; Morellato & Silveira 2018), only a handful studies have addressed the mechanisms and factors filtering and influencing plant community assembly in *campo rupestre* (Medina & Fernandes 2007; Lima et al. 2013; Fernandes 2016; Dayrell et al. 2016; Guerra et al. 2018; Le Stradic 2018b).

The available data for *campo rupestre* seed dispersal dynamics suggest that poor seed banks and seed quality are major bottleneck for plant recruitment in *campo rupestre* (Dayrell et al. 2017; Le Stradic et al. 2018). It has been shown that several native species in *campo rupestre* produce high percentages of empty or unviable seeds (Dayrell et al. 2017), which may strongly hamper dispersal success in *campo rupestre* (Dayrell et al. 2016). The low richness and diversity reported for soil seed banks in *campo rupestre* (Medina & Fernandes 2007; Le

Stradic et al. 2018b), indicates that regeneration from the seed bank or topsoil transfer seems to be not feasible in *campo rupestre* (Le Stradic 2018b).

The low number of seeds found in the seed banks may be partially explained by the high proportion of perennial species in *campo rupestre* plant community (Le Stradic et al. 2015a), which is in accordance with the reproductive patterns in other OCBILs (Goldblatt & Manning 2002; Mucina et al. 2014). Generally speaking, it is expected a predominance of transient seed-banks in perennial plant communities, as a result of a reduction in seed production investment and of seeds residence period in the soil (Thompson et al. 1998). Even so, soil seed bank still a crucial element for plant maintenance and recovery in perennial grassland communities (Kalamees & Zobel 2002). Thus, understanding mechanisms and factors (e.g. seed rain, fruit production dynamics, dispersal vector outcomes) influencing seed bank dynamics in *campo rupestre*, is crucial for a better understanding of plant community assembly and recovery.

Thus, a huge knowledge gap about other factors and mechanisms that may influence seed dispersal success (e.g. seed rain, seed predation and seed quality) of plants in *campo rupestre* persists (Le Stradic 2018b). Further studies of seed rain dynamics, seed quality and germination (Ferndandes 2016) are thus necessary to shed a light on this matter, which may allow a better understanding of the reliability of residual seed bank and seed rain to recover *campo rupestre* vegetation after human-caused disturbance.

5. **Study area**

We conducted the field experiments at the vicinity of the Serra do Cipó National Park, in the southern portion of the Espinhaço Range, south-eastern Brazil (43º 35'W, 19º 17'S). The annual precipitation in the region averages around 1,400 mm and the climate is markedly seasonal with most rainfall occurring in the hot summers (from October to March; Silveira et al. 2016). Altitude at the study site ranges between 1,150 and 1,300 m a.s.l. The main vegetation

comprises the mountaintop *campo rupestre* (Silveira et al. 2016). During the paving of the MG010 highway in 2002 small quarries were exploited for soil extraction, when upper soil horizons (around 1 m) were destroyed, removing all vegetation and leaving the soils altered (Fig. 4 & 5) (Soizig et al. 2018).

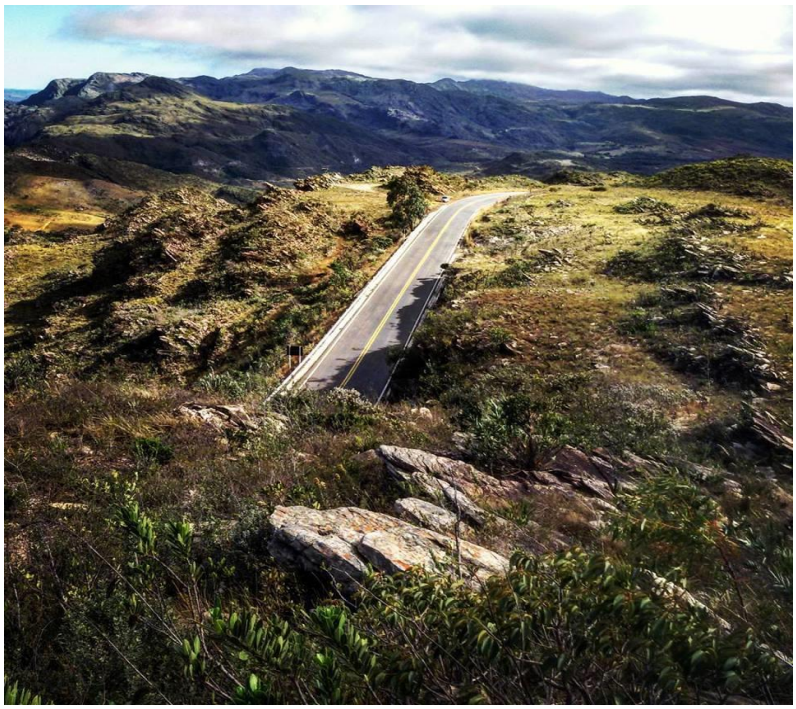


Figure 4: MG010 highway crossing a preserved matrix of *campo rupestre* (Photo Arruda A.J.).



Figure 5: Quarry exploited for soil extraction during the paving of the MG010 highway in 2002 surrounded by a preserved matrix of *campo rupestre* (Photo Arruda A.J.).

These disturbed sites had their plant community composition drastically altered in relation to the surrounding preserved areas and presents low recovery capacity (Fig. 6) (Le Stradic et al. 2018b).



Figure 6: Satellite view of a disturbed site surrounded by preserved *campo rupestre* vegetation. The road in the image is the highway MG-010. (Google earth 7.1 accessed on march 2017).

All permissions 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.

6. Objectives

Despite extremely relevant, seed dispersal dynamics and resilience of grasslands remains much overlooked. The following chapters are aimed to critically evaluate our current knowledge about seed rain in grasslands, propose better practices in seed dispersal studies and contribute to our knowledge about mechanisms and factors that influence the dispersal filter in an old-growth grasslands (Fig. 7).

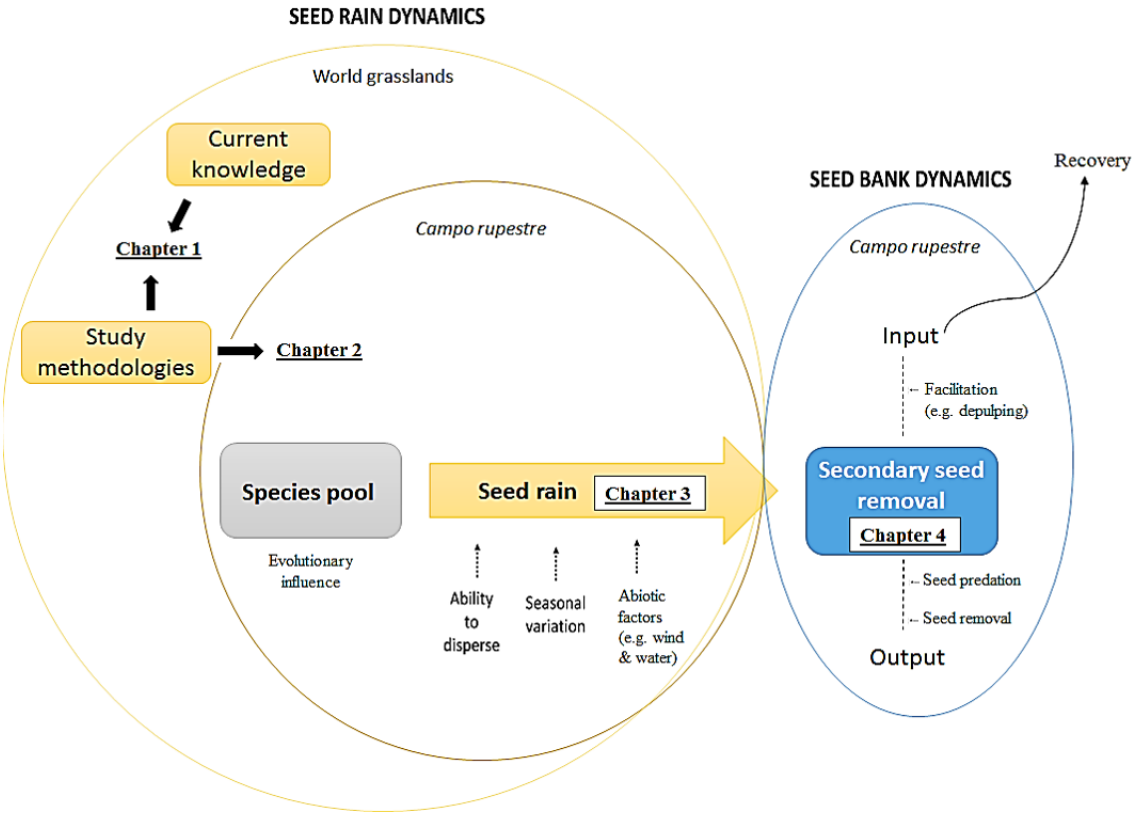


Figure 7: Insertion thesis chapters according to knowledge areas and spatial abrangences. "Input" and "output" represent the outcomes of secondary seed removal on seed bank dynamics according to each site.

First, I carried out a review and then three experimental studies about seed rain and diaspore removal dynamics in grasslands. In CHAPTER1, I did a systematic literature survey about seed rain studies in global grasslands to assess: (1) where, how and why research on seed rain has been carried out; (2) examined how methodological design and results have been reported; and (3) provided guidelines for future research on seed rain in grasslands. Then, in CHAPTER 2, I ran a field experiment to propose a simple standard protocol to evaluate the efficiency of two seed trap types (sticky and funnel traps) and of a seed sorting method to assess the efficient of the methods prior to seed rain studies. As a case of study, in CHAPTER 3 and 4, I studied seed rain and secondary diaspore removal dynamics in preserved areas and in areas disturbed by gravel exploitation for road construction in *campo rupestre*. In this way, we aimed to test the prediction of OCBIL theory (Hopper 2009) of poor dispersability in *campo rupestre* vegetation.

Additionally, we were able to disentangle the relative contributions of two important factors influencing dispersal filter: seed arrival and secondary animal diaspore interactions. By comparing these processes between habitats (e.g. preserved and disturbed sites) and plant species, we aim to find out about the outcomes of plant assembly and recovery in *campo rupestre*.

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CHAPTER 1: How have we studied seed rain in grasslands and what do we need to improve for better restoration?

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1. Abstract

Seed rain, the number of seeds reaching an area, is a process that plays a key role in recruitment and regeneration in plant communities. A better understanding of seed rain dynamics is therefore a critical step for restoration practices. A wide variety of methods to study seed rain in grasslands are available, but there is little agreement to which is the most appropriate one. Here we: 1) assessed where, how and why research on seed rain has been carried out; 2) examined how methodological design and results have been reported and 3) provide guidelines for future research on seed rain in grasslands. We built a database of 185 papers from a systematic literature survey between 1980 and November 2016 and we found a remarkable unbalance of the numbers of studies between grassland types, which becomes even more dissimilar across global climatic ranges when the area covered by each grassland type is addressed. We also found a great disparity of methods and data being reported across studies. Despite recent progress in understanding seed rain dynamics, large knowledge gaps in important issues, such as the role of native dispersers, method efficiency and application of mechanistic models still persist. Finally, we propose guidelines for the implementation of minimum standardized methodology and data reporting, which will foster higher quality, transparency, reproducibility and value of seed rain studies and grassland restoration.

Key-words: meadow, prairie, rangeland, seed limitation, seed trap, steppe

2. Conceptual Implications

• An unbalanced distribution of seed rain studies among grassland types calls for additional research efforts on non-temperate grasslands to better support restoration

• We identified significant knowledge gaps in grassland seed rain research, which should now be tackled: the role of (1) native animals as seed dispersers, (2) pre-dispersal and post-dispersal seed predation, (3) non-native species, (4) method efficiency, (5) application of mechanistic models, (6) active restoration standards

• The lack of standardization of methodology, terminology and data reporting prevents a critical appraisal of the role of seed rain in restoration of grasslands

• We recommend the implementation of guidelines for methodology and data reporting, which will depend on future collaborative efforts

3. Introduction

Seed rain is the number of seeds reaching an area, and it usually is quantified and qualified by placing traps in the plant community to catch seeds that then are identified and counted (Baskin & Baskin 2014). Seed rain is a critical step in plant life cycle, as it represents a demographic bridge between the adult and seedling stage (Harper 1977). Seed rain reflects species dispersal potential, and therefore the persistence or potential for change of the standing vegetation (Page et al. 2002). Additionally, it allows the arrival of seeds into suitable uncolonized microsites (Baker 1974), with major implications for biological invasions and restoration ecology by revealing relevant information about how target species can reach a restored site, and whether seed rain is effective and efficient to restore a given site (Turnbull et al. 2000). Many plant communities are seed limited, meaning that microsites where seeds can arrive and germinate, remain vacant, which can be related to limited seed production and/or limited dispersal of available seeds among sites (Clark et al. 1998). Hence, seed rain estimates

can provide a hint of successional direction by predicting the probabilities of propagule arrival (D'Angela et al. 1988).

Over the last decades, seed rain has been analyzed both i) indirectly, by studies of plant reproductive potential (Boughton et al. 2016) and seed bank dynamics (Bertiller & Aloia 1997), and ii) directly, by collecting either seeds visible on the ground, by observing the movements of granivore animals (Izhaki et al. 1991) or from diaspore traps (Kollmann & Goetze 1998). The great diversity of methodologies reported for seed rain studies may have a close relation with the wide range of biological processes that may be involved, which makes the delimitations of this biological process often unclear (Fig. S1).

In essence, an ideal method for studying seed rain is the one that can assess what seed, how many seeds and when that seed arrives in the seed bank (Schott 1995). Despite this, few seed rain studies use standardized methods that would allow cross-vegetation comparisons. As an example, numerous types of seed traps have been used in ecological studies with different characteristics, such as shape, size, height above soil surface and trap inclination, all of which can affect their effectiveness (Bakker et al. 1996, Chabrerie & Alard 2005). A wide variety of seed rain measuring methods is available in the literature. However, there is little agreement to which is the most appropriate method taking into account a wide range of possible variables. This is especially true for grassy biomes, for which knowledge on natural regeneration following disturbance lags behind that of forest ecosystems (Meli et al. 2017).

Grassy ecosystems cover around 52 million km², ca. 40% of the global land surface (White et al. 2000; Gibson 2009). Over the last decades huge areas of native, old-growth grasslands have been lost to agricultural expansion, desertification, mining, urbanization and other changes or have been degraded by changes in fire regimes, exotic species introduction, fertilization, drainage, liming, overgrazing, etc. (White et al. 2000; Veldman et al 2015). In the face of increasing land-use pressures and increasing biodiversity loss in grasslands, biogeographical studies of spatial and temporal patterns of seed rain are essential to restoration

and management practices (Bakker et al. 1996). While seed rain patterns in grasslands are very difficult to assess and to predict, a better understanding about their influence on the resilience and succession in these environments is urgently needed, aiming to achieve biodiversity conservation goals, secure ecosystem services (Parr et al. 2014) and provide basis for better restoration practices.

Given that seed rain plays a pivotal role in restoration ecology, we explore the interplay between seed rain research and restoration in grasslands 20 years after Bakker et al. (1996) stressed the role of seed rain and seed banks in restoration ecology. Our goals were: 1) to assess where, how and why research on seed rain was carried out; 2) to examine methodological design and results reported; and 3) to provide guidelines for future research in seed rain, aiming to standardize and foster higher quality, transparency, reproducibility and value to seed rain studies in grasslands.

4. Material and Methods

4.1.Literature survey and grassland classification

We surveyed papers in the Web of Science which have been published from 1950 to 30th November 2016, by searching the following terms in the title, abstract, and key words of papers: "seed rain" plus "grassy biome", "grassland", "meadow", "prairie", "rangeland", "savanna", or "steppe". We removed papers dealing only with seed production or seed bank, and included only studies that addressed the releasing process of diaspores from the parent plant and their movements until reaching the soil. After removing redundant papers, our survey comprised 185 papers published between 1980 and 2016. From those, we removed the papers not related to grasslands (those addressing seed rain in forest ecosystems), and papers that did not study seed rain in detail, represented by studies that did not present any qualitative or quantitative data on seed rain over space or time. We were left with 98 papers that matched our criteria for this review.

For each study, we classified the grassland type according to Dixon et al. (2014). We considered seven grassland types: alpine grassland; boreal grassland; cool semi-desert grassland; temperate grassland; Mediterranean grassland; tropical grassland and warm semi-desert grassland. Using ArcGIS software, we built a map plotting the geographic distribution of seed rain studies together with the global grassland distribution through the geographical coordinates available in Dixon et al. (2014).

4.2.Relevance of seed rain studies to restoration ecology

We considered studies with direct relevance to restoration ecology, such as those that addressed any of the following processes: recolonization, succession and predictions after degradation, endangered species conservation, spread of non-native species and natural plant communities or species dynamics. We classified degradation types reported in each study as endogenous or exogenous disturbances (*sensu* McIntyre and Hobbs 1999). The endogenous category refers to disturbances to which ecosystems were repeatedly exposed through evolutionary time and which, with an appropriate regime, allow ecosystems to maintain biodiversity. On the other hand, the exogenous category refers to novel, mainly anthropogenic disturbances, which may be incompatible with the maintenance of grassland biodiversity. We classified the ecological restoration processes, whenever mentioned, as passive or active restoration. Passive restoration stands for cases when only natural regeneration processes were evaluated, whereas active restoration refers to the implementation of restoration techniques, such as hay spreading or seed sowing (Rey Benayas et al. 2008).

4.3. Methodological design

We classified the studies depending on the methodologies used for seed rain studies according to the nature and scope of the experimental design into two categories: i) direct measurements and ii) mathematical or mechanistic models and simulations. The direct

measurements included the use of seed traps (Bakker et al. 1996) and focal or seed tracking observations (e.g. Ferguson & Drake 1999; Piazzon et al. 2012). Mathematical or mechanistic models and simulations used numerical methods for seed rain dynamics predictions and simulations (e.g. Doisy et al. 2014).

In order to obtain additional information about the dispersal dynamics, we searched for studies that used methods to evaluate the effect of abiotic (such as water and wind) and biotic vectors of seed dispersal. Additionally, we recorded seed dispersal distance whenever provided in the original study.

Owing to the fact that seed trap characteristics may strongly influence results (Bakker et al. 1996; Chabrerie & Alard 2005), we evaluated the following aspects for studies that employed seed traps as a method to estimate seed rain: trap type, total number of traps, trap efficiency in collecting seeds, trap protection against granivores, trap position on the ground, height categories for traps lodged above ground, trap position in relation to the main wind direction, and sticky traps slope angle. We classified the traps in the following categories: funnel trap, sticky trap, tray or gap trap and other traps.

4.4.Methodologies and data reporting

To evaluate standardization in methodologies and data reporting, we analyzed seed trap size and total surface measurements for the two most common trap types, funnels and sticky traps. Considering that traps with equivalent areas can have very different formats, which together with the total trap area, can affect the study outcome, we assessed how these measurements were reported in different ways, sorting them into three possible categories: i) trap area only; ii) format measures (width and length or diameter), iii) not informed. Secondly, we evaluated how many studies calculated the total area sampled by the traps. Thirdly, we evaluated how the data collected or estimated were reported. We created four categories of data

reporting: seed density only, total number and density, total number only, not informed. Finally, we examined what seed traits (Jiménez-Alfaro et al. 2016) were reported in each study.

5. **Results**

We found an increase in the number of seed rain studies in grasslands over the two last decades, with the maximum number of studies/year of 10 in 2005. We found relative increases in the number of studies coinciding with the publication of review papers (Fig. S2).

We found seed rain studies in grasslands across all vegetated continents, in tropical (N=7), temperate (N=82) and boreal (N=3) ranges (Fig. 1). However, the geographic distribution of studies was strikingly uneven, with most studies concentrated in Europe (N=46) and in North America (N=21). More than half (57%) of the seed rain studies were conducted in temperate grasslands, while the rest was spread among warm semi-desert grasslands (14%), alpine grasslands (10%), Mediterranean grasslands (8%), tropical grasslands (6%), boreal grasslands (3%) and cool semi-desert grasslands (1%). Only three studies were done in flooded grasslands, all belonging to the temperate grassland type.

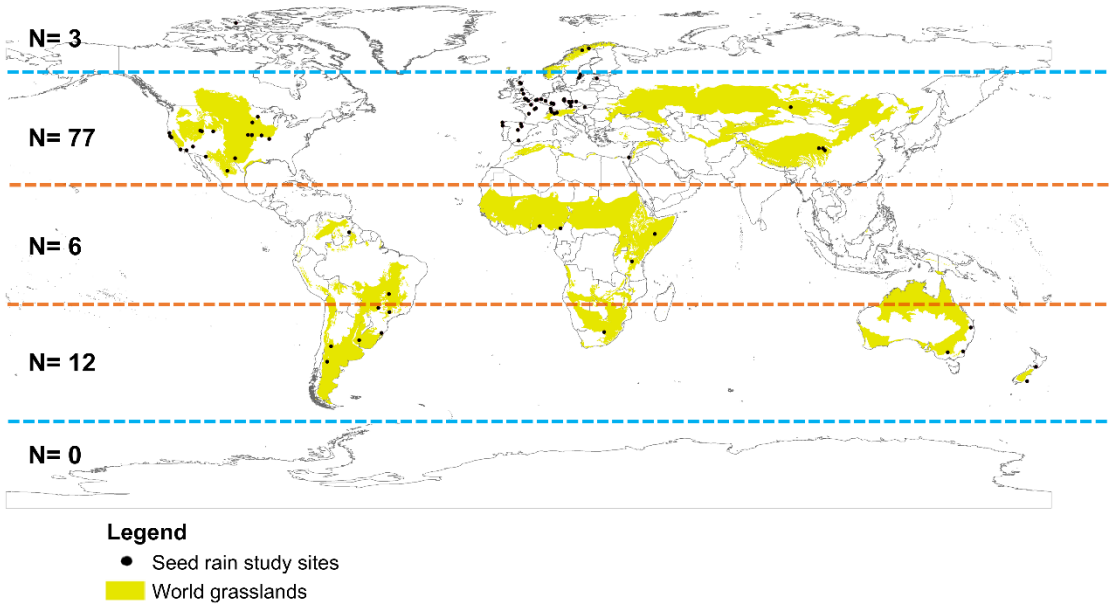


Figure 1: Geographic distribution of seed rain studies (black dots) in grasslands. Red dashed lines depict tropical areas and blue dashed lines depict boreal areas. Native grasslands distribution in yellow follows Dixon et al. (2014).

Eighty-four papers (85%) of the selected studies were directly or indirectly related to ecological restoration. Among the studies related to ecological restoration, 80 papers (95%) mentioned some type of disturbance, from which 73 (91%) reported exogenous disturbances, such as cultivation and mining and only seven (8%) of them addressed endogenous disturbance events. Regarding the latter, five studies addressed fire regimes, one mentioned drought regimes and fire and one simulated small soil disturbances naturally created by a native small mammal from a temperate grassland. Additionally, from the eighty-four studies applied to ecological restoration, 64% addressed passive restoration aspects only and 36% active restoration processes. From the 30 studies that addressed active restoration, only 11% tested seed sowing techniques.

From the ninety-eight selected studies, 97% used direct measurements and 3% mathematical or mechanistic models and simulations. Additionally, across all selected studies, 21 mentioned or surveyed biotic dispersal. Within these 21 studies, birds were the main dispersers studied (42% of the studies), followed by livestock (29%), such as sheep, cattle and donkeys, ants (29%), other mammals not including bats (14%), bats (10%) and lizards (5%). Only 9 studies mentioned the influence of abiotic factors, such as wind (8 studies) and water (1 study). From the eight studies that provide some information about the main wind direction, five only mentioned and three measured this variable. Additionally, we checked how many studies estimated seed dispersal distance and found that only 24 studies took seed dispersal distance into account, from which less than a quarter presented seed dispersal distance estimates or measurements.

Fifty-nine percent of seed rain studies were conducted at the community-level and 37% only at species-level. From the 37 studies that evaluated the seed rain at species-level, 51% included only one species. Additionally, from all studies, 20 mentioned or evaluated the impact of non-native species, of which 70% reported non-woody species and 30% woody species.

Only 46 studies provided information on complementary physical and physiological seed traits, such as size, weight, dormancy and viability. From those, 54% provided information on a single seed trait and less than 5% on more than three traits. Among the seed traits reported in these 46 studies, seed morphological adaptations for dispersal (other than size and weight) were the most common (39% of the studies), followed by seed size (33%), seed weight (30%), seed viability (24%), seed dormancy (15%), releasing height (11%), seed longevity (4%) and moisture content (2%).

Sampling length for the 79 studies that provided this kind of information ranged from less than 1 month up to 60 months. Additionally, 76 studies provided information on sampling frequency, which ranged from weekly to 24 months intervals. Regarding the methods for seed identification, 89 studies dealt with this aspect. From those, 50% used visual identification through reference collections, 33% did not mentioned any seed identification method and 15% used seedlings emerging from seeds to identify species.

From the 76 studies (77%) that used seed traps, 88% used only one type of seed traps, 8% used two types, 3% used three types and only one study used five types. The most common seed trap type was the funnel trap (34%), followed by the sticky trap (18%) and tray or gap trap (17%). Additionally, 18% of the studies used other types of traps. Surprisingly, we found information on the total number of traps only for 56 out of the 76 studies. Among these, the number of traps per study ranged from 14 to 576, with nearly half of the studies using less than 90 traps.

Among the 76 studies that used seed traps, 62 (82%) did not provide any information about the seed trap efficiency in collecting seeds. Only eight studies (10%) mentioned trap

efficiency and only six studies (8%) tested seed traps for efficiency. Only 18 studies (24%) provided information on trap protection against granivores. Information on seed trap position related to the ground was found for 56 studies only. Among these 56 studies, 52% placed traps aboveground, 50% at ground level and only 4% underground (some studies included more than one type). For aboveground traps, we found a minimum height value of 0.5 cm and maximum of 90 cm. We also found that 32% of aboveground traps were positioned at a height of 50 cm or more, followed by those at 15 to less than 30 cm (28%), at one to less than 5 cm (25%) and at less than 1 cm (21%).

Additionally, we analyzed separately some specific aspects of trap types. We evaluated the sloping angle for sticky traps in the 17 studies (22%) that used this kind of trap. From these, 76% did not report this variable. Among those which reported this information, the angle of 0° (parallel to soil surface) was the most common (3 studies), followed by the angles of 45° and 90°, each one present in one study.

We evaluated seed trap measurements for the most common traps, funnel and sticky traps. For the funnel and sticky traps, respectively 30% and 6% of the studies provided the seed trap surface area only. Subsequently, we evaluated trap area separately for each type of traps. For the funnel and sticky traps, most studies used particular trap area sizes. Additionally, only 14% of the studies calculated the total area covered by the funnel or sticky traps.

We also evaluated how the total number of seeds collected or estimated was reported for the 82 studies that reported these data. Half of the studies presented seed density only (50%), followed by total seed number and seed density (28%) and total seed number only (22%).

6. Discussion

Our data clearly shows a lack of standardization in approaches, methods and data being reported in seed rain studies across global grasslands. We found unbalanced distribution of seed rain studies among hemispheres, different grassland types and, more importantly, that even

basic aspects of a study (experimental design and number of species/seeds sampled), were not properly reported in many cases. Seed rain studies have different goals, and one should expect a wide diversity of methods in the literature. However, the lack of justification for the employment of different methods, and incomplete data reporting strongly hamper our ability to compare results among studies, hence preventing a better appreciation of the role played by seed rain in restoration ecology.

6.1. General and biogeographical information

The large proportion of seed rain studies related to ecological restoration underlines the great relevance of seed rain studies in restoration ecology (Bakker et al. 1996; Freund et al. 2015). The small number of studies evaluating the effect of endogenous disturbances points out that this issue still needs to be better explored: endogenous disturbances usually have to be re-established as a means of or after restoration. For example, fire-stimulated flowering (Keeley et al. 2000) and seed release (Holmes & Richardson 1999) may affect natural regeneration patterns. However, natural recovery of some ecosystems may be quite slow if important drivers of recovery, such as the availability of propagules or dispersers are limited (Hubbell et al. 1999; Le Stradic et al. 2014).

The geographical distribution of studies can substantially influence conclusions reached by ecologists, being therefore critical to know which biomes, regions, and landscapes remain understudied and undervalued (Martin et al. 2012). The remarkable unbalance between the numbers of studies worldwide indicates knowledge gaps, especially in old-growth tropical grasslands in the southern hemisphere (Veldman et al. 2015). Considering the total area per grassland type (Dixon et al. 2014), alpine grasslands showed the second lowest area among the formations, but had more seed rain studies than tropical grasslands, which have an approximately 27 times higher total area (Table 1).

6.2. High diversity of estimates of seed rain

The considerable dominance of direct measurements to evaluate seed rain patterns reflects the sampling effort carried out in field experiments with use of seed traps or focal observations over the last few decades. Despite the potential usefulness of mechanistic models in providing reliable estimates of plant dispersal distances, they are still little explored by ecologists (Bullock et al. 2017). The development of dispersal mechanistic models is crucial to improve our understanding about relevant topics on restoration ecology, such as population dynamics in fragmented landscapes (Gilbert et al. 2014) and the arrival of non-native species (Hastings et al. 2005). Meanwhile, there is still a long way to go from mechanistic models to processes applicable to seed dispersal, in order to reduce the effort required to measure dispersal directly in the field (Bullock et al. 2006).

Animals play important roles in dispersing seeds of native and non-native species. Most studies surveyed explored the role of frugivorous passerines or livestock as dispersers of grassland species. However, we found a large knowledge gap on the ecological role played by other native animals, such as lizards (Piazzon et al. 2012), beetles or ants (Nicolai & Boeken 2012; Lima et al. 2013) and non-volant mammals (Genrich et al. 2017) in dispersing seeds of native grassland species. Additionally, future research should focus on the roles of seed dispersers in dispersing seeds of invasive species, which will provide relevant information for restoration actions and post-restoration management (Buisson et al. 2017).

Spatial patterns of seed deposition and issues related to seed sourcing, seed quality, availability and dormancy-breaking are of great interest for evaluating the effectiveness of dispersal and relevant hurdles for plant community reassembly after degradation (Dayrell et al. 2016). However, assessing the contribution of local *versus* distant diaspore sources can be a great challenge (Bullock et al. 2017), which may explain how estimating seed dispersal distance has been mostly overlooked by seed rain studies in grasslands.

Sampling length, sampling frequency and seed identification techniques can have a great influence on the results. Thus, whenever possible, studies should prioritize longer collection periods and short collection frequencies. Short-term studies may imply non-overlapping patterns of dispersal period of some species. Furthermore, results are likely to be affected by the frequency of checking, mainly when traps are insufficiently or not at all protected against seed predation (Gorchov et al. 1993).

6.3. Sampling effort and lack of standardization in the use of seed traps

Measurements of seed rain in the field can be carried out using seed traps with different shapes, sizes, heights and inclinations (Chabrerie & Alard 2005). However, trap effectiveness at capturing seeds can vary not only among trap types and characteristics, but also over different vegetation or plant species and environmental conditions (Kollmann & Goetze 1998). Funnel traps have often been described in the literature as the best trapping method by catching the highest number of seeds (Kollmann & Goetze 1998). However, funnel trap efficiency may also retain diaspores belonging to seed banks through water run-off, and therefore ignoring secondary dispersal may result in overestimates of seed rain. Sticky traps, which are especially suitable for studying seed rain of anemochorous species, can be easily adapted to the local aerodynamic environment by the control of height, orientation and inclination (Chabrerie & Alard 2005). However, sticky traps can catch large amount of insects, drastically reducing their efficiency and making it difficult to identify the sampled seeds (Poschlod 1990). Alternatively, the use of soil gaps or pots with sterile soil have been often attributed as a more realistic method by the integration of seed arrival with other field natural conditions, such as secondary removal and predation (Kollmann and Goetze 1998), despite the recommendation for the avoidance of these variables for other trap types (Debussche & Isenmann 1994). Considering that trapping success can be very variable not only between trap types, but across environments and plant

communities, comparisons between studies should always be made with caution (Kollmann & Goetze 1998).

Despite the fact that effects of seed trap area and height on sampling effectiveness have been debated since the seminal publications of Fischer (1987) and Jackel & Poschlod (1994), we still need to better explore how seed traits, such as size, weight and releasing height (Fischer et al. 1996; Jackel & Poschlod 1994; Tackenberg et al. 2003) can influence seed trap efficiency. Recommendations about the number of traps for seed rain studies have already been reported (Fischer 1987, Kollmann & Goetze 1998), but the number of traps needed greatly vary depending on the goals and background of the studies.

Seed trap effectiveness tests are fundamental in seed rain research and should be conducted prior to field experiments (Debussche & Isenmann 1994), but we found they are rarely carried out. Ignoring such issues may have serious consequences in the reliability of seed rain estimates. Standardization of sampling efforts (Jackel & Poschlod 1994) and in tests of trap efficiency in collecting, storing and protecting seeds against granivores will be fundamental for a better understanding of the operation of different seed traps along distinct plant communities and geoclimatic conditions.

6.4.Methodologies and data reporting

Basic information about methods and results must be thoroughly, clearly and transparently reported to enable comparisons and facilitate inclusion in meta-analyses (Gerstner et al. 2017). Seed trap format measures, area and the total number of seeds collected or estimated reported by the studies evaluated here are remarkably unstandardized, sometimes unclear or sometimes data were even not provided. We also found a great variety of sizes for both sticky and funnel traps, which were rarely accompanied by a clear methodological justification. Finally, only few studies provided information on the proportional seed trap area (seed trap area over total plot area). Altogether, the lack of standardized methodologies in seed

rain studies undermine the reliability of studies and prevent comparisons among sites, thereby preventing the evolution of the scientific knowledge on a key ecological process underpinning ecological restoration.

6.5.Implications and guidelines for future seed rain studies

When reporting data, researchers should have in mind that their data can be readily used by the scientific community to build-up knowledge from single studies (Gerstner et al. 2017). Therefore, it is imperative that not only experimental designs are clear and justified, but that data reporting adheres to minimum standards. Here, we propose guidelines for future research on seed rain studies in grasslands with the hope that standardized methodology, or at least better justified methods, will provide the scientific community with better conditions for the critical assessment of publications, fostering scientific knowledge.

First, we propose the standardization for the number of seeds collected over time and space, according to the minimum parameters: total number of seeds per species sampled at each sampling interval; total number of species sampled at each interval; total number of seeds/species per trap over the total sampling time and total number of seeds/m² per day. Second, given the disparity of methods and data reporting across studies that used seed traps, we propose that the following minimum standard is reported: trap format measurements (width and length or diameter); total trap area per unit (cm²); number of traps per plot; total area covered by traps per plot (cm²); and percentage (%) of plot area covered by the traps. We recognize that our modest proposal will not be enough to standardize research at global scale, but this is the first step towards common and shared vocabulary, methods and data reporting.

Prior to restoration, seed rain can be evaluated in order to assess the potential for regeneration and passive restoration. Restoration assessments should include seed rain data in post-restoration monitoring programs (Jacquemyn et al. 2011) and increasing knowledge on pre- and post-dispersal seed predation (Pardini et al. 2017) are to play an increasing role in

determining restoration success and should be integrated into seed rain studies whenever possible. Despite considerable progress in recent decades, we still face great challenges to improve our knowledge and increase the use of seed rain studies to better support grassland restoration (Table 2). More research on restoration of tropical and sub-tropical grasslands is required due to the fact that techniques used for temperate grassland restoration are not successful in restoring tropical ones (Le Stradic et al. 2014). Additionally, understanding to what extent anthropogenic modifications are relevant to seed limitation will be fundamental to predict the capacity of grasslands to respond to these changes.

Future successful restoration may also depend on seed addition, on which we still need to advance in the practical and theoretical framework (Bakker et al. 2003). We must also recognize the importance of considering several characteristics of seed rain, besides species composition and seed abundance. Spatial patterns of dispersal and seed traits, such as dormancy, longevity, releasing time and duration are fundamental for a better understanding on the formation of seed banks and the dynamics of community re-assembly following disturbances (Jiménez-Alfaro et al. 2016).

Lastly, we need to critically stress the fact that cross-vegetation comparisons of seed rain data are prevented due to lack of standardized research protocols. We believe that the lack of standardization in methodology and data reporting creates a unique opportunity for the scientific community to put efforts on a global protocol of seed rain methods. An increased implementation of guidelines for methodology and data reporting will foster higher quality, transparency, reproducibility and value to seed rain studies in grasslands. Future collaborative, international efforts are paramount for a global assessment of the role played by seed rain in restoration ecology.

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Table 1. Comparison between the number of seed rain studies with the total area for each grassland type according to Dixon et al. 2014 (=98 studies).

Grassland type	Total area (Km^2)	N° of seed rain studies	Studies/100.000 km^2
Boreal Grassland	246.322	3	1.21
Alpine Grassland	591.357	10	1.69
Mediterranean Grassland	1.594.750	8	0.50
Warm Semi-Desert Grassland	3.030.720	13	0.43
Cool Semi-Desert Grassland	5.661.110	1	0.02
Temperate Grassland	8.104.830	55	0.68
Tropical Grassland	16.156.620	6	0.04

Table 2. Future challenges and proposed solutions to improve our knowledge and increase the use of seed rain studies to support grassland restoration.

Challenges	Solutions
Seed rain spatial patterns, heterogeneity and limitation	Increase periods of observation and reduce sampling intervals
	Predict latitudinal and climate variations effects on seed rain
	Incorporate landscape metrics and estimate the relative importance of different dispersal modes
	Discuss how the number of traps should vary according to study goals
	Consider the main direction of seed import

Seed trap efficiency and lack of standardization in the use of seed traps	Consider vegetation growing height and dispersal syndromes Provide justification for the choice of seed traps (position on the ground, format, area, etc.) Test trap efficiency and protect seeds against granivory Critically evaluate and report negative aspects of each trap
Access seed collection identity and quality	Increment reference seed collections X-ray analyses
Success of restoration practices and monitoring	Reduce the seed supply of undesirable species Introduce target species via seed addition Evaluate the effect of endogenous disturbances Understand the boundaries and interactions between direct and indirect methods Create opportunities to increase seed input from native seed dispersers
Increase practicality and applicability of methods to study seed rain	Development of dispersal mechanistic models for undersampled vegetation Avoid indirect estimates and forecasts by indirect methods Assess the influence of wind, flooding and relief on seed dispersal
Unbalance between the numbers of studies across grasslands types	Direct efforts to overlooked regions and environments
Biotic dispersers	Address the role of overlooked vectors in the dispersal of non-woody species
Hurdles for plant	Understand how issues related to seed sourcing, seed quality, availability and dormancy-breaking mechanisms

community reassembly after degradation	
Unstandardized or unclear methodology and lack of data reporting	Methods and results must be thoroughly, clearly and transparently stated Standardization for the number of seeds collected over time and space Standardization for the trap format measurements and area within the sample area per plot

Supporting information

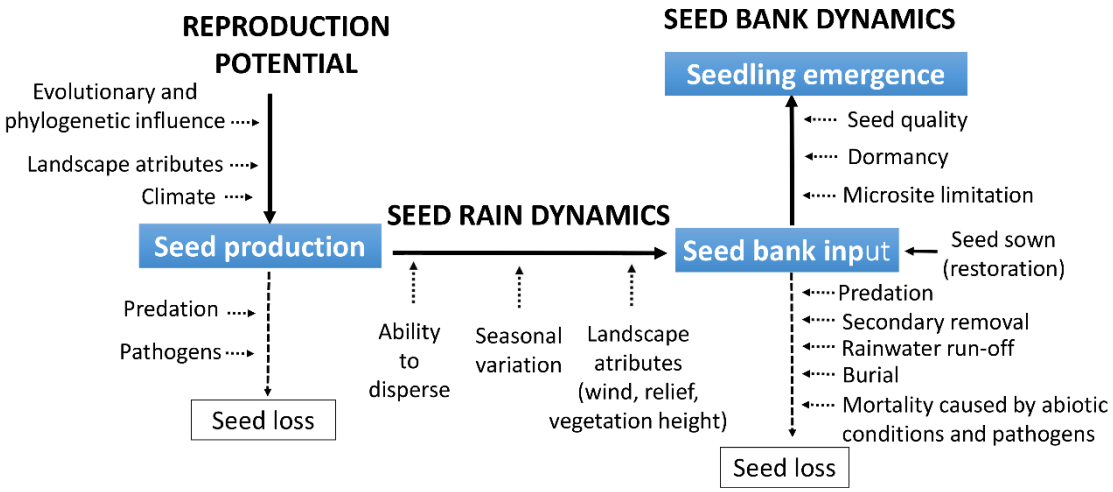


Figure S1: Theoretical framework showing the boundaries between seed rain dynamics (direct measurements) and other indirectly related processes (indirect measurements), represented by aspects of reproductive potential and seed bank dynamics.

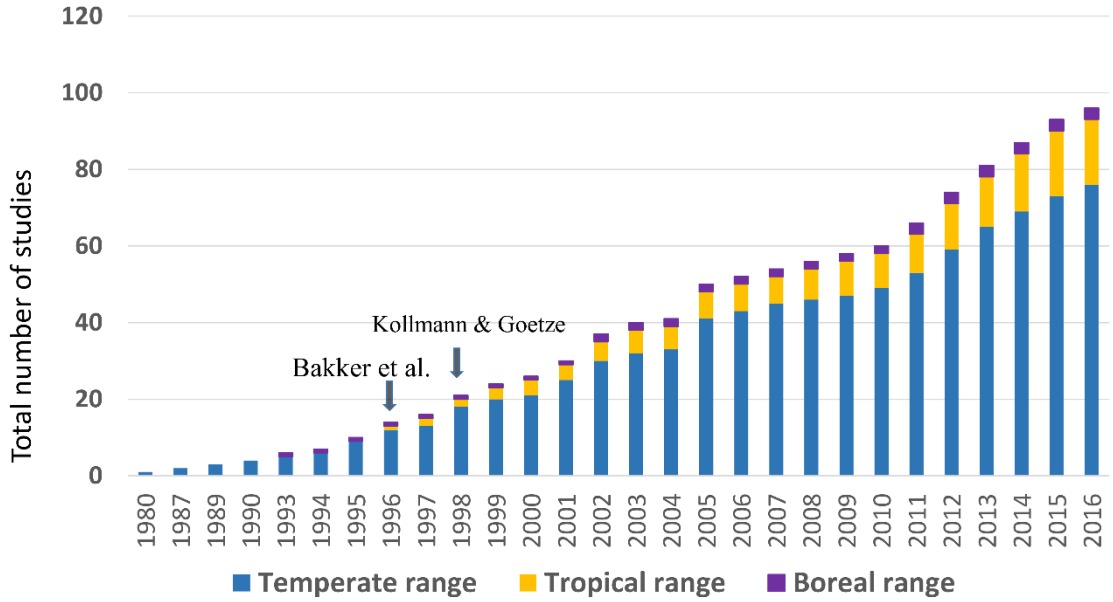


Figure S2: Cumulative number of seed rain studies in grasslands from 1980 to 2016 within climatic global ranges. The arrows indicate when the revisions on the topic of seed rain of Bakker et al. and Kollmann & Goetze were published.

CHAPTER 2: A simple standardized protocol to evaluate the reliability of seed rain estimates

1. Abstract

Seed dispersal has key implications for community dynamics and restoration ecology. However, measuring this process is challenging, and the lack of standardization in measurement prevents accurate estimates of seed rain. Seed trap effectiveness and accuracy of seed sorting methods are vital to obtaining reliable, reproducible and comparable results. We propose and describe a standardized protocol for evaluating the effectiveness of two seed trap types to preserve the seeds captured, and the accuracy of a seed sorting method.

We selected widely available seeds (arugula, quinoa, sesame and sunflower) to produce a gradient of seed size, weight and color. We worked with both sticky and funnel traps, which capture complementary processes of seed rain. This protocol was tested in a tropical grassland, where traps were set for 30 days. We chose three previously trained observers, who received a determined number of seeds of the four species, mixed in an equal amount of soil, to test the accuracy of the seed sorting method. The number of seeds in each sample was previously noted, but not known to each observer.

The standardized protocol, carried out in the tropical grassland, suggests that we underestimate seed dispersal of small, smooth seeds that can be easily mistaken for debris or soil particles. Seeds on sticky traps may be more vulnerable to removal by wind and rain, whereas seeds in funnel traps are more susceptible to decay. We found no evidence of observer effect on seed sorting for funnel trap samples. However, accuracy on seed sorting for funnel trap samples tended to decline as seed size decreased, suggesting a size-dependence in seed retrieval success. Our standardized protocol addressing trap effectiveness and seed sorting methods will increase reliability of data obtained in seed rain studies in grasslands and allow more reliable comparisons between datasets. We provide detailed instructions on how to build the traps with

low-cost, readily available material, and argue that these practicable tests can be adapted to different scenarios.

Keywords: seed loss, seed rain, seeds sorting, seed trap, trap effectiveness

2. Introduction

Seed dispersal studies are vital to understanding plant distribution and community resilience, and they guide conservation and restoration activities (Török et al., 2018). A useful way of studying seed dispersal is to estimate seed rain—*i.e.* the number of new seeds reaching a given area—using seed traps to collect propagules at particular locations, then identifying and counting them. However, measuring seed rain is challenging, and a lack of methodological standardization persists, compromising the accuracy of seed rain estimates and impairing comparison of data between studies (Arruda et al., 2018). Determining the effectiveness of seed traps, and of seed sorting methods for samples, is crucial for improving reproducibility, but it is rarely tested in seed rain studies (Thompson & Mcginnes, 1963; Jackel & Poschlod, 1994; Kollmann & Goetze, 1998).

Evaluation of seed trap effectiveness involves two processes: trap capacity to capture seeds (seed catch), and trap capacity to retain seeds (seed retention) and avoid seed loss (Box 1; Fig. 1). Additionally, the accuracy of seed sorting methods for trap samples depends not only on seed size—small seeds are harder to find—but also on the ability to separate seed material from debris, which can strongly affect seed retrieval rates (Cottrell, 2004). Knowing the seed retrieval rate of seed sorting is important for determining the influence of seed size, of observer effect, and of sample composition, or more precisely, the color and size of soil particles, debris and litter often present in samples (Debussche & Isenmann, 1994).

Ants, among other invertebrates, are known for their ability to collect large amounts of seeds and can have a major impact on seed trap effectiveness (Predavec, 1997). Seed decay can vary greatly between seed types and is also modulated by other biotic and abiotic conditions

such as pathogens, humidity and the amount of litter/soil accumulated within traps (Roberts, 1972; Box 1). Additionally, seed loss by wind or water run-off can vary greatly between trap types, seasons and plant communities, thereby influencing seed retrieval rates. Therefore, to maximize seed catch and minimize seed loss, the use of complementary seed trap types is recommended (Chabrerie & Alard, 2005).

Funnel traps can be used to study local seed rain and the transportation of seeds by water run-off (Jackel & Poschlod, 1994). Funnel traps are effective in seed catch, but the loss of seeds to predation and to decay caused by excessive moisture arise as potential problems (Schott, 1995; Kollmann & Goetze, 1998; Jensen, 1998). Sticky traps, in turn, are more suitable for studying wind-dispersed species (Jefferson & Usher 1989). While sticky traps carry a lower risk of seed predation, checking them is often hindered by trapped insects and debris (Kollmann & Goetze, 1998). Both sticky and funnel traps may also bias the seed catch towards larger seeds that are more easily detected by visual assessment, while soft seeds may easily rot, and smooth seeds may be lost through rain or wind action (Kollmann & Goetze, 1998; Cottrell, 2004). Despite the current state of knowledge, the influence of seed size and weight on the retention rate of seed traps is still not well understood.

Among the possible methods for sorting seeds in funnel-trap samples, the most effective is direct seed inspection after sieving to separate seeds from debris (Kollmann & Goetze, 1998), (Cottrel, 2004). Seed identification requires training; however, one's capacity to find and sort seeds also depends on seed traits such as size and color (Martin & Barkley, 1961; Cottrell, 2004). Considering that results can be biased by differences in one's capacity to find and sort seeds from the samples, it is important to obtain, prior to data interpretation in seed rain studies, an estimate of how many seeds are missed in the sorting procedure. Our goal was to propose and describe a simple standardized protocol to evaluate the effectiveness in seed retention of two types of seed trap (Box 1, Fig. 1), and the accuracy of a seed sorting method in assessing seed rain. We tested these standardized protocols in a tropical grassland as a proof-of-concept.

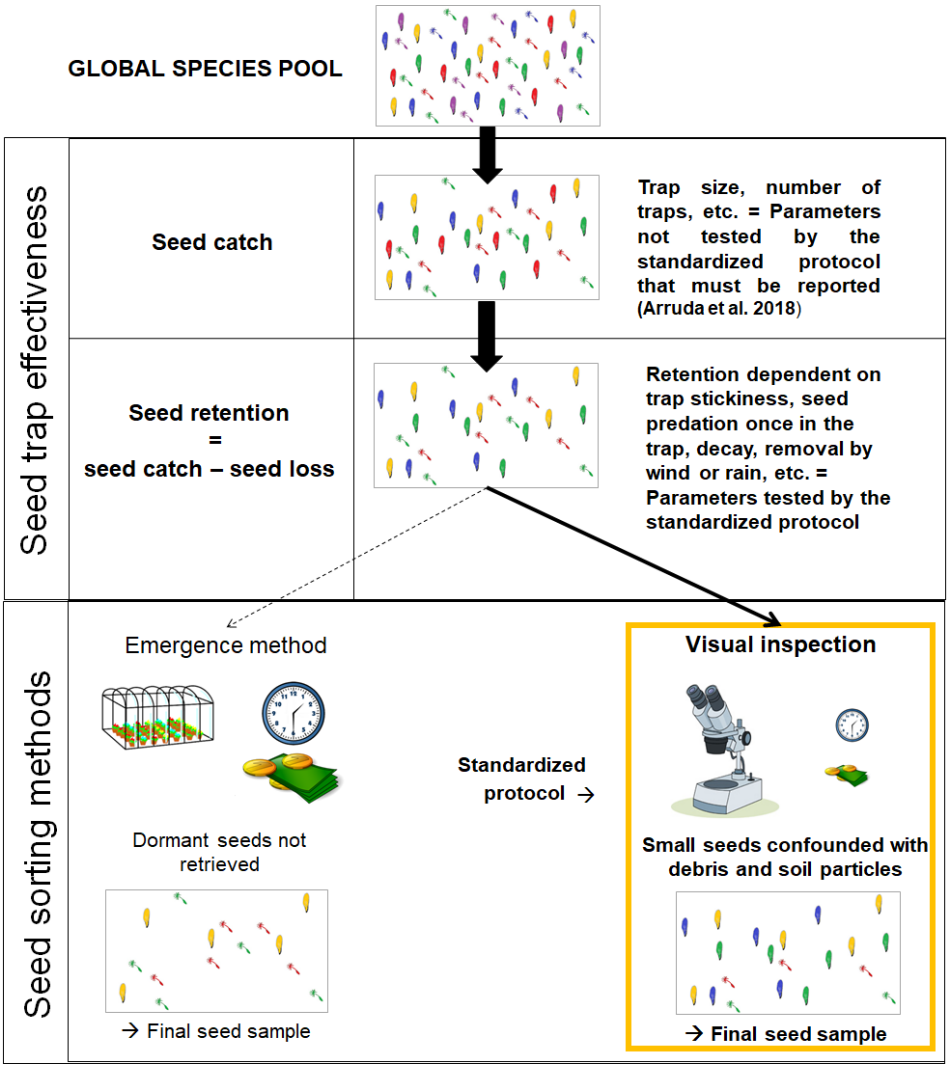


Figure 1. Conceptual framework showing two key stages of seed rain measurements needing standardization. Each stage is composed of sequential steps in which the number and richness of seeds is potentially decreased (the direction of the black arrow, Box 1). There are two possible methods for seed sorting in trap samples. The grow-out method involves transferring the collected material to trays in greenhouses and identifying species from growing seedlings. This method is time- and labor-consuming, and underestimates dormant seeds. In the direct seed inspection method, each sample is processed for seed separation, and identification using a magnifying glass. Standardizing seed counting at the seed sorting stage is essential to decrease the likelihood of scoring bias, which potentially decreases the number of seeds even further.

3. Description and implementation

3.1. *Seed traps*

We tested sticky and funnel traps (Fig. 2), which capture complementary processes of seed rain (Chabrerie & Alard, 2005) and are the most common traps used to estimate seed rain in grasslands (Arruda et al., 2018). We provide detailed instructions on how these two seed traps can be built using low-cost and readily available material (Appendices S1–S2).

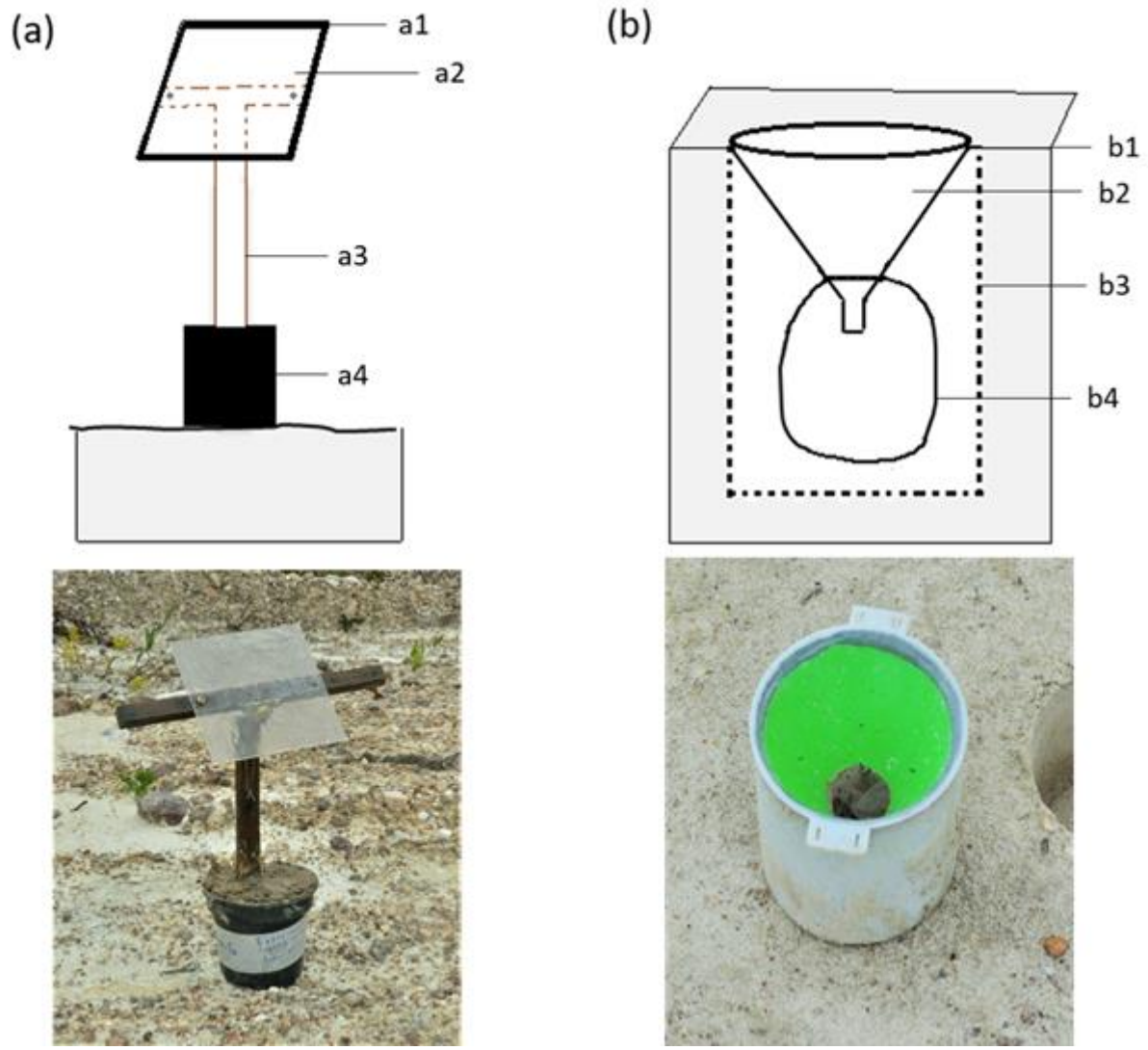


Figure 2. (a) Sticky trap. a1: clear Plexiglas® plate (0.0225 m²); a2: sticky gel over a thin plastic film placed over the Plexiglas plate; a3: wooden pole; a4: plastic pot filled with concrete and (b) Funnel trap. b1: ground level; b2: PVC plastic funnel section (0.01 m²); b3: white PVC pipe with bore holes 1 cm diameter; b4: bag of < 0.1mm mesh size.

3.2. *Standardized protocol*

Four species should be used: arugula (*Eruca sativa*), quinoa (*Chenopodium quinoa*), sesame (*Sesamum indicum*) and sunflower (*Helianthus annuus*). These seeds are available in any market or garden center around the world and provide variation in seed size, weight and colors (Table 1). To assess seed retention, the number of traps and the length of time that traps should be left in the field can be adapted to each study and grassland type. On each trap, 10 seeds of each species should be gently dropped, totaling 40 seeds per trap (Table 1). On funnel traps, all seeds should be put straight into the bag collectors. The standardized protocol should be run either during the same season(s) as the study or, if run over one year, during the season in which conditions are most challenging for preservation of the seeds on the traps.

For the sticky traps, all samples collected from each trap should be examined under a magnifying glass. For the funnel traps, bags should be collected separately from each trap, and their content should be washed in a 250-µm sieve to reduce the amount of fine soil particles, then examined under a magnifying glass to count and identify seeds. We counted all seeds and recorded any signs of damage. To evaluate seed loss during the seed sorting of funnel traps samples, a second experiment should examine the sorting accuracy with funnel traps samples. We chose to test the seed sorting accuracy with only funnel trap samples because the traps can accumulate much litter and soil in the field, making it difficult to retrieve seeds. For this test, a given number of seeds of the four species should be mixed in soil, in a proportion equal to that commonly found inside the funnel trap bags. The soil used should be taken from the study area to control for color, debris and litter composition. We randomized and noted the number of seeds in each sample (with a minimum of 3 and maximum of 30 seeds per species); this number was not known to the observers. Three previously trained observers sorted the samples, searching for, identifying and counting the seeds.

The proportion of seeds retrieved at the end of the experiments (retrieval success) is obtained by calculating the percentage of seeds retrieved by each observer. Both experiments should be performed using generalized linear models that assume a quasibinomial distribution and use retrieval success as the response variable. For the seed trap effectiveness experiment, trap type and species are the categorical variables (interaction was tested). For the seed sorting experiment, species and experimenters are the categorical variables (interaction was tested). In both cases, post-hoc Tukey tests can be run. We performed these analyses with R (R Core Team, 2018), packages *base* and *emmeans*.

3.3. *Proof of control*

In order to proof control our protocol, we conducted fieldwork in the southern part of the Espinhaço mountain range, southeastern Brazil (43º 35' W, 19º 17' S). The annual precipitation averages around 1,400 mm, and climate is markedly seasonal, with most rainfall occurring in the hot summers (Silveira et al. 2016). We conducted the experiment in March, at the end of the raining season, when high temperatures, strong winds and rainy days prevail, creating the most challenging conditions in the study area. The main vegetation comprises the mountaintop *campo rupestre*, fire-prone grasslands that establish on quartzite-derived rocks, with shallow and severely nutrient-poor sandy soils (Silveira et al., 2016).

We set six of each type of seed trap, placed randomly and left for a period of one month, as this the most common timeframe used to sample seed rain in grasslands (Arruda et al., 2018). For the second experiment, testing seed sorting accuracy with funnel trap samples, we ran the protocol with three previously trained observers.

4. **Results**

In the proof-of-concept experiment, funnel traps performed best in the retention of arugula seeds (41.4% more efficient than sticky traps). We found no significant difference in

the performance between seed traps for sesame seeds. Funnel traps were ineffective in retaining quinoa seeds under the field conditions, while the sticky trap (GLM quasibinomial, $p<0.001$) had a good retention rate for quinoa seeds (88.6%). We found no difference between the traps in sunflower seed retention, with both performing well (98.57% for funnel trap and 100% for sticky trap).

The seed retention test revealed that, for both seed trap types, quinoa and arugula seeds had the lowest retention rates. We found only 1.4% and 42.9% of the arugula seeds on sticky and funnel traps, respectively, after one month in the field. Although we found most of the quinoa seeds (88.6%) in sticky traps, no quinoa seeds were retrieved from funnel traps (Fig. 3). Most sesame and sunflower seeds were retrieved from both sticky (respectively 97.1% and 100%) and funnel traps (respectively 88.6% and 98.6%) (Fig. 3). We retrieved almost half of the arugula seeds (42.9%) and most of the sesame and sunflower seeds (respectively 88.6% and 98.6%) (Fig. 3).

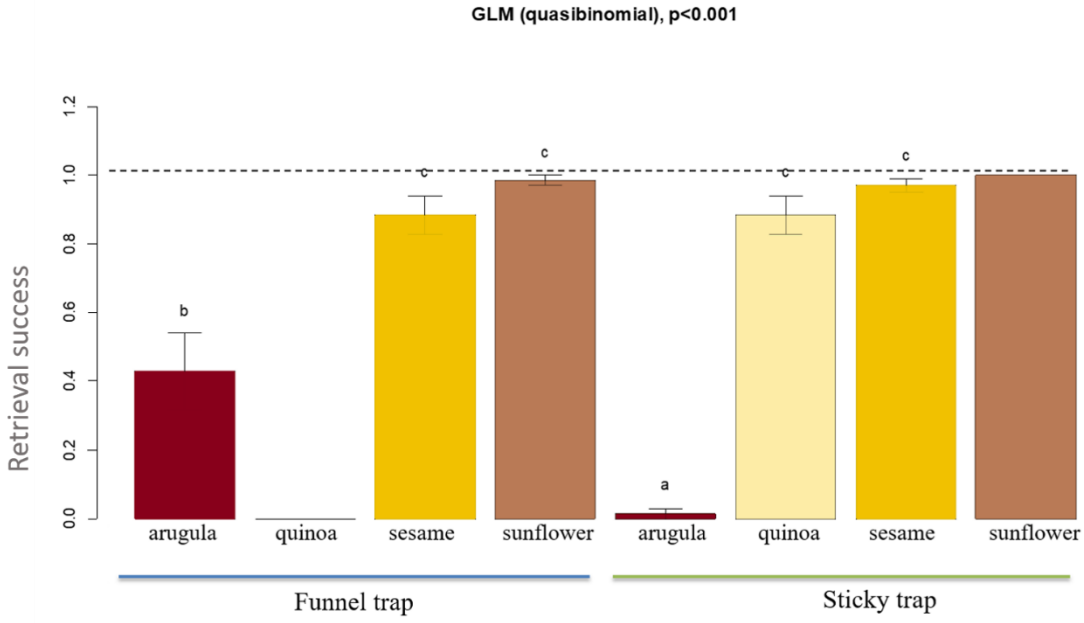


Figure 3. Retrieval success for four species in two seed trap types over a 30-day period (***=$p<0.001$). For each species, 10 seeds were placed in each trap type (indicated by the dashed line).

We found no evidence to support an observer effect (GLM quasibinomial, $p<0.001$), finding most seeds of all species during seed sorting: 88.6% of arugula seeds, 97.9% of quinoa seeds, 96.9% of sesame seeds and 100% of sunflower seeds (Fig. 4).

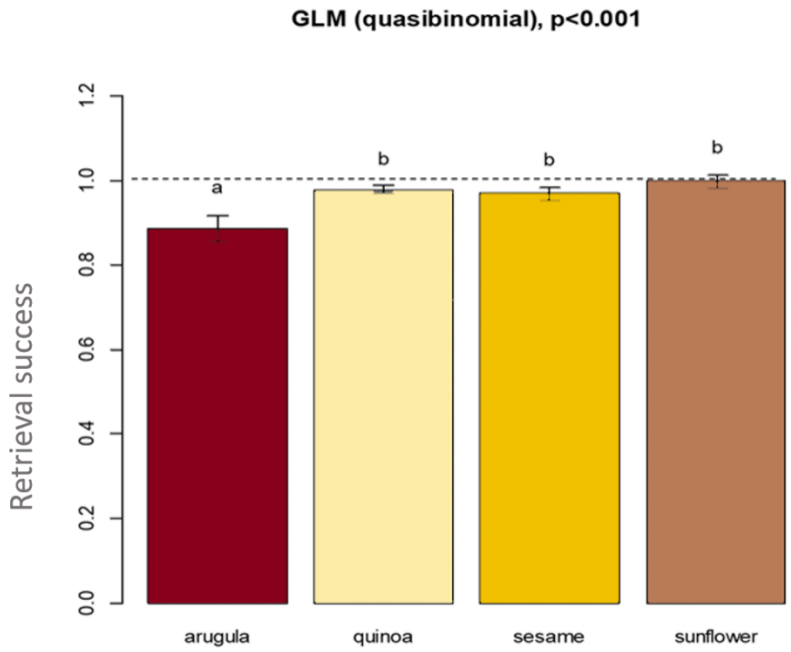


Figure 4. Retrieval success on the sorting accuracy test of the funnel trap samples for the four species.

5. Discussion

Improving the accuracy and precision of seed rain estimates is necessary to further our understanding of both seed dispersal and seed limitation, and to support ecological restoration (Török et al., 2018). However, few studies test seed trap effectiveness (Arruda et al., 2018), thus precluding attempts to understand these processes on a global scale. Our results show that, under the tested field conditions, we are underestimating seed dispersal of small, smooth, dark seeds because they are mistaken for debris deposited in seed traps. Despite finding no observer effect on seed sorting for funnel trap samples, we found that accuracy tended to decline as seed

size decreased, suggesting that size-dependence in seed retrieval success is more common than previously thought (Kollmann & Goetze, 1998).

Despite the lower risk of seed predation with sticky traps, due to the strong glue over the Plexiglas® plate, the seeds on sticky traps are more exposed, and thus more vulnerable to removal by wind and precipitation, than seeds in funnel traps. Sticky traps may also be problematic because insects, dust and litter can easily accumulate on the trap's glue, hindering the visual search for seeds. Traps near the ground are more vulnerable to contaminants, catching large quantities of dust and litter, especially during the rainy season. We believe that the height of our sticky traps (25 cm above the soil) greatly reduced their contamination by soil particles, but it did not prevent contamination by insects. Notably, many of the insects were mere incidental captures rather than active seed predators. In contrast, contamination by insects was negligible for funnel traps.

The high loss rates found for quinoa and arugula seeds in funnel traps indicate alarming losses for small seeds in general, which probably result from water accumulation within the traps. The weak structure of the quinoa seed coat can make these seeds more vulnerable to mechanical stress, fluctuations in humidity and temperature, and growth of microorganisms (Mohamed-Yasseen et al., 1994). Seeds with hard seed coats, such as sesame and arugula, are generally long-lived (Priestley, 1986). Seed decay is likely the main cause of seed loss in funnel traps, as the observers in the sorting accuracy experiment found most of the seeds of all four species tested, and seed removal by animals is unlikely due to the shape of the funnel trap.

Despite finding no observer effect on seed sorting for funnel trap samples, we found that accuracy tended to decline with decreasing seed size and for seeds of darker color. Soils with high content of organic particles may directly impact the accuracy of seed sorting methods because soil particles are similar in color to some seeds; sorting methods should account for this similarity. In a real seed rain study under the tested field conditions, shorter sampling intervals may reduce the contamination of seed samples and mitigate seed losses. Precautionary

devices against resuspension or decay of seeds in funnel traps should also be rigorous to ensure that seeds are optimally stored, since some seeds may soon start germinating or decaying in moist trap samples.

6. Conclusion

This study clearly demonstrates that the traits of seeds influence their retrieval from seed traps used in seed rain studies. We provide a detailed standardized protocol that can be easily implemented in any seed rain study in grasslands using sticky and funnel traps. The use of both trap types maximizes seed capture. When discussing the results of a seed rain study using the proposed protocol, one must offer the caveat that the protocol likely underestimates the seed types (small, smooth and dark) for which we found low retrieval success with the standardized protocol. Under our field conditions, our seed rain study would not allow us to conclude that small, soft seeds cannot be dispersed by water run-off, as they may decay in funnel traps; nor could we infer anything regarding small, dark, smooth seeds, as they can be lost from sticky traps or not found by observers. Finally, we argue that our standardized protocol addressing trap effectiveness and seed sorting methods will increase reliability of data obtained in seed rain studies in grasslands and allow more reliable comparisons between datasets.

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Table 1: Seed average width, length and weight for the four species that should be used in the protocol from measurements made from 15 seeds of each species.

Species and family	Weight (mg)	Width (mm)	Length (mm)	Color
Arugula (*Eruca sativa*, Brassicaceae)	2.2±0.0005	1.3±0.1	1.8±0.2	Dark brown
Quinoa (*Chenopodium quinoa*, Amaranthaceae)	4.6±0.001	2.1±0.001	2.3±0.2	Whitish
Sesame (*Sesamum indicum*, Pedaliaceae)	6.4±0.001	1.9±0.2	5.2±0.3	Pale beige
Sunflower (*Helianthus annuus*, Asteraceae)	51.2±0.01	5.4±0.60	10.5±0.65	Pale grey with black strips

Box 1 – Definitions of terms related to seed rain estimates

Seed dispersal – the horizontal movement of diaspores away from the mother-plant

Seed rain – the number of seeds reaching a given area

Seed trap effectiveness – the ability of seed traps to accurately and precisely estimate seed rain. Seed trap effectiveness is determined by seed catch and seed retention.

Seed catch – a property of seed traps that refers to its ability to trap seeds from the seed rain. The final number of seeds captured by a seed trap is affected by both seed retention and seed loss.

Seed retention – a property of seed traps that refers its capacity to maintain seeds on/in traps after seed catch until seed retrieval.

Seed loss – process caused by seed predators, pathogens and unknown causes that decreases seed trap effectiveness and produce the final trap sample

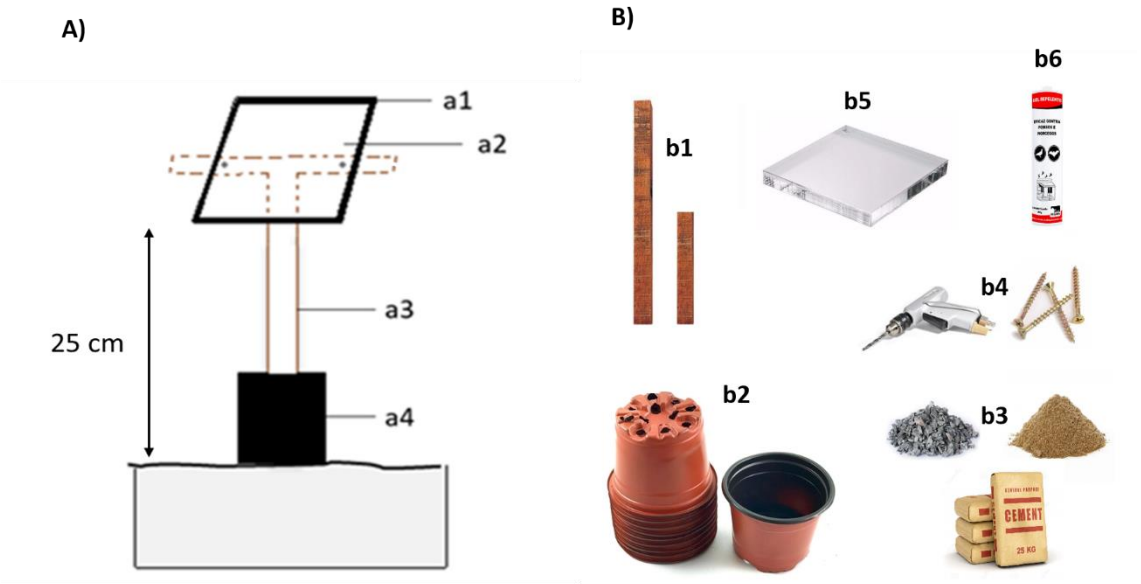
Seed aging - the progressive deterioration of the structures and functions of the seed over time, and which will ultimately leads to seed death

Supporting information

Data S1. Sticky traps construction guidelines

Use a plastic gardening pot of 10.5 cm high, 14.5 cm wide at top, 11 cm wide at base, with a volume of 1,160 liters. Make two wooden poles from weather-resistant wood: one measuring 3 cm wide × 3 cm thick × 20 cm long; and the other, 3 cm wide × 3 cm thick × 32.5 cm long. Place the 32.5-cm wooden pole in the bottom of the plastic pot, and fill the plastic pot with concrete (1/3 of cement, 1/3 of sand and 1/3 of gravel), fixing the wooden pole at the center and aligned at a 90° angle to the bottom of the pot (Fig. supp. 1). The upper end of this wooden pole should be approximately 22 cm above the ground. Next, using a drill and screws, attach the 20-cm wooden pole to the top of the 32.5-cm pole that is fixed inside the plastic pot. The 20-cm pole should be centralized, and its upper face should form a 45° angle with the ground. Then, using a drill and screws, fix the Plexiglas® plate (0.0225 m^2) to the 20-cm pole, in a centralized position. The Plexiglas® plate should be located 25 cm above ground, facing the main wind direction and sloping at a 45°angle (Fig. supp. 1). Cover the Plexiglas® plate with a thin plastic

film to allow sample collection, and recover with a new the plastic film after each collection. Coat the Plexiglas® plate with a clear sticky Isobutene/Butene Polymer gel over the thin plastic film. This Polymer gel is originally produced to deter birds and bats from roofs (ROGAMA, São Paulo, Brazil), and it retains its adhesive qualities over the period of exposure in the field, independent of weather conditions.

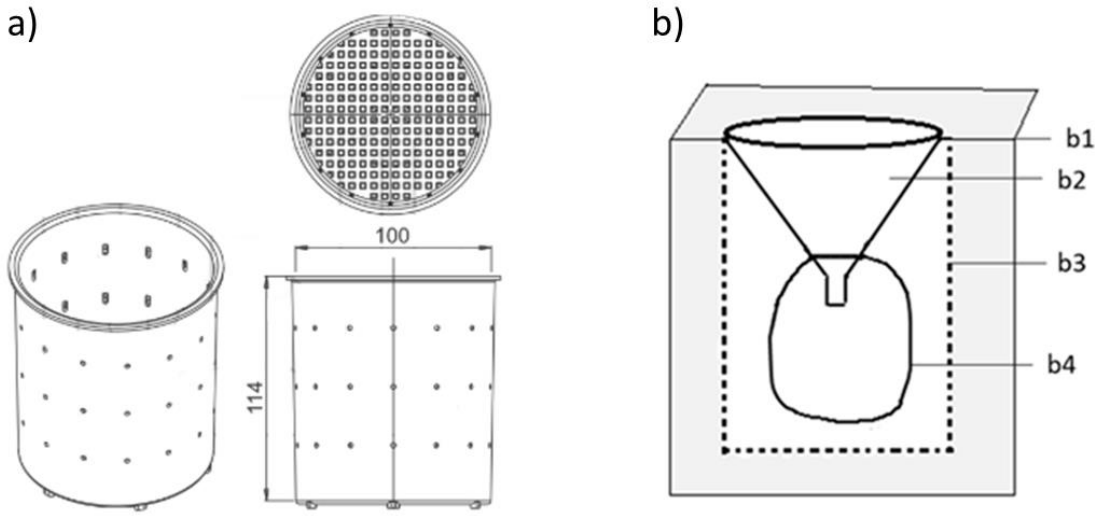


Appendix S1: A) Sticky trap. a1: clear Plexiglas plate (0.0225 m²); a2: sticky gel over a thin plastic film placed over the Plexiglas® plate; a3: wooden pole; a4: plastic pot filled with concrete. B) Material needed to build a Sticky trap. b1: two wooden pole; b2: plastic pot (1,16 liters) ; b3: concrete (cement, sand and gravel) ; b4: drill and screws for wood; b5: Plexiglas plate (0.0225 m²) ; b6: Isobutene/Butene Polymer gel.

Data S2. Funnel traps construction guidelines

Use a PVC pipe measuring 11.4 cm high × 15.0 cm wide (top) × 10 cm long, originally designed for cheese production. This cylindrical plastic structure should have holes, 1 cm in diameter, on the sides and bottom to allow drainage. Attach a conical PVC plastic funnel, 15 cm in

diameter, to the top end of the cylindrical plastic structure (approximately 0.0177 m² in area). At the bottom of the funnel, the seeds are caught in a disposable polypropylene bag, with pores smaller than 0.1 mm, fixed with an elastic band to the base of the funnel. This polypropylene bag is originally produced as a disposable hygienic hair cap, and it retains its integrity over the period of exposure in the field, independent of weather conditions (TALGE, Santa Catarina, Brazil).



Appendix S2: a) External structure of the funnel trap before PVC funnel installation b) Internal structure of the funnel trap installed in the field; b1: ground level; b2: PVC plastic funnel (15 cm² upper end area); b3: PVC pipe with bore holes 1 cm diameter; b4: bag of < 0.1mm mesh size.

CHAPTER 3: How can seed rain dynamics in disturbed and preserved areas help to understand the resilience of a megadiverse tropical grassland?

1. Abstract

Questions

Seed rain, i.e. the number of seeds reaching a given area, is a process that plays a key role in the resilience of plant communities. A better understanding of seed rain dynamics is therefore a critical step for restoration practices. However, only 6% of the seed rain studies on grasslands were carried out in the tropics. We ran a field experiment to compare annual seed rain dynamics between disturbed and preserved sites of a tropical montane grassland with extremely low resilience to soil removal.

Location

Campo rupestre, megadiverse edaphic grassland, southeastern Brazil.

Methods

We studied seed rain dynamics in preserved sites and sites disturbed by gravel exploitation which implied soil removal. We choose three paired preserved and disturbed plots, placing six sampling blocks in each plot. We used two types of seed traps per block (one sticky and one funnel trap / block). We collected the samples monthly for 12 months between May 2016 and April 2017 to estimate seed density and richness. We also conducted a floristic survey in all plots.

Results and Discussion

Despite the fact that the total number of seeds (1408 seeds in a total area of 1.45 m^2 covered by all seed traps) is much lower in relation to other seed rain studies in grasslands, species richness is among the highest reported in the literature. We found a total of 92 seed morphospecies and a significant difference of seed richness and diversity between trap types, area types and over seasons. Funnel traps presented the higher seed density per day in both plot types, with a clear tendency in collecting more seeds during the rainy months. The intrinsic seed dispersal limitation in *campo rupestre* seems to be even stronger in disturbed areas, with a marked reduction of seed density in disturbed plots during the dry season.

Conclusions

The small number of sampled seeds indicates strong seed limitation, especially in disturbed areas, and suggests a close causal relationship between seed dispersal limitation and the low resilience after soil disturbance. The identification of some relatively abundant plant groups in the seed rain can be used to prioritize species for restoration proposes.

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

2. Introduction

OCBILs are old climatically-buffered infertile landscapes, which host a disproportionally high levels of terrestrial global biodiversity hotspots, and where edaphic control plays a key role in structuring plant communities and shaping plant populations (Hooper 2009; Hopper et al. 2016). Such landscapes are highly vulnerable to soil disturbance (Hopper et al. 2016; Buisson et al. 2018), but the precise mechanisms driving poor recovery is still a matter of controversy (Standish & Hobbs 2010; Dayrell et al. 2016), and warrants further investigation. Many species from worldwide-distributed OCBILs lack apparent mechanisms for seed dispersal (Hopper et al. 2016) suggesting that dispersal costs are high (Bonte et al. 2012), and selection should have favored the evolution of seed dispersal close to the parent plant. Therefore, one might expect seed limitation to be an important driver of vegetation dynamics and resilience in such landscapes.

Seed rain (the quantity and diversity of seeds reaching a given area) is a critical process in plant communities and can provide crucial information on successional trajectories, thereby being a useful tool to assess recovery potential in disturbed areas (Turnbull et al. 2000). Seed rain is usually quantified and qualified by placing traps in the plant community to catch seeds that are then counted and identified (Baskin & Baskin 2014). The use of different seed trap types can contribute to a more reliable estimation of the seed rain (Chabrerie & Alard 2005). A recent review found that only 6% of the seed rain studies on grasslands were carried out in the tropics (Arruda et al. 2018), therefore preventing us from having a better knowledge on recovery potential in some of the world's most biodiverse and impacted areas (Parr et al. 2014). Globally, seed limitation is a strong factor shaping plant communities (Turnbull et al. 2000), but unfortunately, available information on seed rain dynamics in tropical grasslands is still scarce (Bond & Parr 2010; Parr et al. 2014; Veldman et al. 2015).

The megadiverse and nutrient-poor *campo rupestre* vegetation is an example of an ecosystem structured by seed limitation (Dayrell et al. 2016). *Campo rupestre* is an OCBIL characterized by a marked seasonal climate, but distinct seasonal patterns are not clear for the annual fruit production in the plant community (Buisson et al. 2017; Le Stradic 2018c). Despite its huge diversity and endemism (Echternacht et. al. 2011; Colli-Silva et al. 2019), *campo rupestre* species and communities are highly threatened by the extraction of iron ore mining and sandstone quarrying (Fernandes et al. 2018). These activities strongly affect the whole ecosystem, posing great challenges for the resilience of such disturbed landscapes and to restoration attempts (Buisson et al. 2018; Le Stradic et al. 2018b). Generally speaking, for grasslands, after topsoil disturbance, the internal species pool (i.e. remaining vegetation, seed bank) is often reduced or even absent and natural recovery depends mainly on seed dispersal from surrounding sites via the seed rain (Bakker et al. 1996; Campbell et al. 2003; Shu et al. 2005; Buisson et al. 2006; Török et al. 2018). Whereas seed dispersal plays a key role in plant succession, little is known about seed bank dynamics for the *campo rupestre* (Medina & Fernandes 2007, Le Stradic et al 2018b), and no information is available about seed rain.

Plant communities from *campo rupestre*, although highly resilient to endogenous disturbances, such as fires (Le Stradic et al. 2018a), are extremely vulnerable to human-caused exogenous soil disturbances (Buisson et al. 2018; Le Stradic et al. 2018b). Nevertheless, the vegetation dynamics in these unique landscapes has been drastically hampered, especially by topsoil degradation associated with urban expansion, quarrying and mining activities, which have intensified along the past decades (Silveira et al. 2016; Fernandes et al. 2018). Le Stradic et al. (2018b) showed that disturbed plant communities in *campo rupestre* after topsoil removal remained very different from reference sites even eight years after degradation, with almost no recovery of the natural vegetation. Here, we aimed to test the prediction of OCBIL theory (Hopper 2009) of poor dispersability in *campo rupestre* vegetation. To ascertain whether site conditions favor or hamper spontaneous recovery of disturbed areas, we compare seed rain

dynamics in disturbed and preserved areas at *campo rupestre*. The specific objectives of this study were to (1) characterize and compare the monthly seed rain dynamics in preserved areas and disturbed areas along one year (2) determine if seed availability is likely limiting plant recovery in disturbed areas (3) identify common target species in the seed rain for restoration practices.

3. Material and methods

3.1.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 (43º 35'W, 19º 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 (Brito et al. 2017, Silveira et al. 2016). We consider the dry season from April to September the rainy season from October to March. with most rainfall occurring in the hot summers (Brito et al. 2017). Altitude at the study site ranges between 1150 and 1300 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-impoverished sandy soils (Silveira et al. 2016).

The landscape encompasses a mosaic formed by patches of rocky outcrops and boulders where sclerophyllous treelets and shrubs grow amongst an herbaceous stratum, dominated by monocots and sparsely distributed shrubs and forbs (Guerra et al. 2017). In 2002, the road MG010 was asphalted, a process during which small quarries were exploited for soil extraction, destroying vegetation and upper soil horizons (Le Stradic et al. 2018b), and leading to biological invasion (Barbosa et al. 2010). We choose these small quarries as the target disturbed areas for the present study, as they are until now significantly altered in relation to the surrounding reference ecosystem and with very little vegetation cover (Appendix S1).

3.2.Sampling design

We performed a factorial experiment involving the assessment of diaspore catching along three paired disturbed and preserved plots of 100 m² each. 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). In April 2016, we placed six sample blocks in each paired plot (Appendix S2). We used sticky and funnel traps (Appendix S3), which allow capturing complementary processes of seed rain (Chabrerie & 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 airborne-dispersed seeds, and funnel traps allow seed catch from seeds dispersed by run-off. We provided detailed instructions on how to build these two seed traps using low-cost and readily available material, and tests on the trap effectiveness in the study area and on sorting methods accuracy (Chapter 2).

To assess seed rain, we thus 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) (Appendix S2). The total area of 0.24 m² covered by both seed traps on each plot was greater than in other studies of seed rain in grasslands (Urbanska & Fattorini 2000; Shang et al. 2013), and thus appropriate to sample the seed rain in *campo rupestre*. We collected the samples on all seed traps monthly over one year (from April 2016 to March 2017).

For the sticky trap samples, we examined the material retained in the plastic film 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-µm sieve to reduce the amount of fine soil particles before examining them under a magnifying glass. Previously trained observers sorted the samples, searching for, identifying and counting the seeds (Chapter 2).

For each plot, we conducted a floristic survey to determine species richness and composition for the standing vegetation in each community. 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 or without reproductive structures, species-level identification was not possible, but not essential as our interest was seed rain, and thus mature plants. To support seed identification, we built a seed image bank from seeds from the traps and those taken from the surrounding vegetation (Appendixes S4 and S5), since there is no literature available to identify species from seeds.

3.3.Statistical analyses

We employed generalized linear mixed effects models (GLMMs, glmer for non-normal datasets, with lme4 package in R) with fixed and random effects to analyze the datasets of seed rain (Crawley 2013). In order to show the effects of trap types and plot types on the number of seeds / trap and on species richness / trap, each of the two models included plot type and trap type as the fixed effects as well as sampling blocks nested within plot types as random effects (Bates et al. 2014). In order to show the effects of temporal patterns on seed rain, four models were run, one for each plot type and response variables, with months as the fixed factor and same as previous models for the random factors. The response variables were seed density (the number of seeds / m^2 considering the annual data and the area of both traps in each block for each plot) and species richness. We performed all analyses assuming a Poisson distribution error of the response variable. We also performed post-hoc Tukey comparisons among treatments (Crawley 2013). For all analyses, we established α values of 0.05.

Regarding the floristic survey data, differences between plant community composition between sites were analyzed using a Correspondence Analysis, which is designed to explore categorical variables, such as the presence / absence data of our floristic survey (Garson 2012; with *ade4* package in R).

Finally, in order to compare the number of species in common between plot types, we draw Venn diagrams with Venny 1.0 (Oliveros 2007-2015) with the floristic survey data, as well as

with the seed rain data (in funnel traps, in sticky traps and in both traps together), from species or morpho-species lists compiled for each plot type.

4. Results

4.1.Seed rain richness and diversity

Considering the data for all seed traps over one year, we found 92 seed morphospecies and a total of 1,408 seeds in an area of 1.44 m^2, which represents an average of 2.7 seeds/m^2 per day (Table 1). Preserved plots had higher average of seeds/m^2 in sticky traps but not in funnel traps (Table 1). From the 92 seed morphospecies, we were able to identify 29% at family-level and 14% at genus-level. For 90% of the morphospecies we found less than 20 seeds in the annual accumulated seed rain. We found that 75% of the seeds belonged to only 10 morphospecies (Fig. 1).

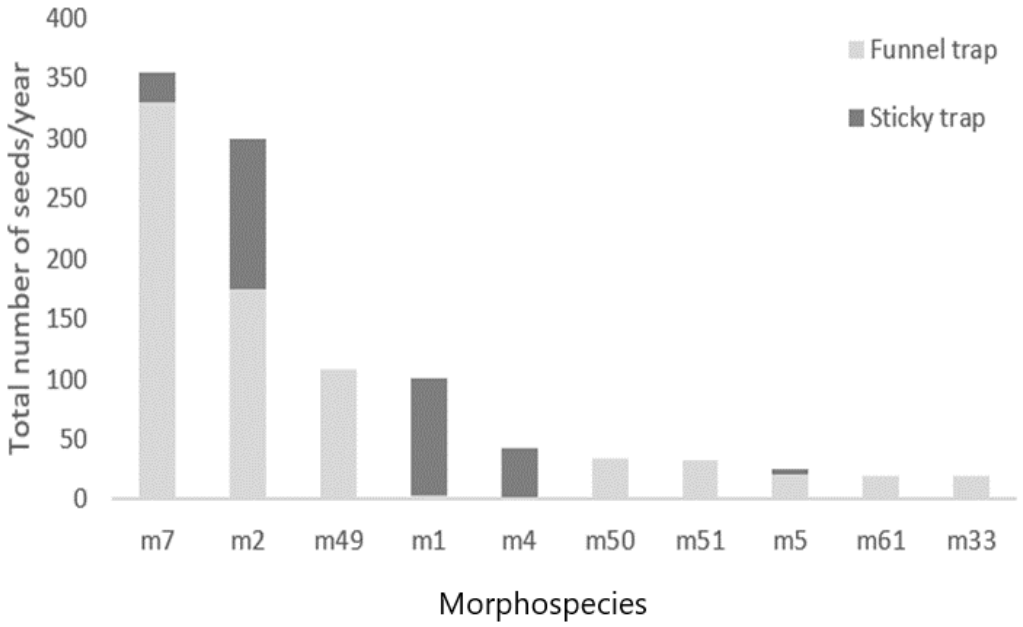


Figure 1: Annual accumulated seed rain for the 10 more common seed morphospecies found in funnel and/or sticky traps considering all plots sampled in *campo rupestre* vegetation, southeastern Brazil.

From the 10 more common seed morphospecies, we identified 70% at family-level and 40% at genus-level (Appendix S5). Only four morphospecies presented more than 100 seeds in the annual accumulated seed rain (Fig. 1). Morphospecies-7 (*Lychnophora*, Asteraceae) was the most common morphospecies retrieved with 331 seeds, followed by morphospecies-2 (Poaceae) with 300 seeds, morphospecies-49 (Cyperaceae) with 108 seeds, and morphospecies-1 with 102 seeds (Fig. 1). Only five species retrieved in the seed rain produce vertebrate-dispersed berries (Table S1), but only one species was caught in the disturbed sites.

4.2.Seed rain between plot and seed trap types

Whether the seed rain data is considered for both trap types together or in funnel traps only, about 30 morphospecies were registered exclusively in disturbed plots and about 20 exclusively in preserved plots (Fig. 5). Indeed, we retrieved most seeds in funnel traps (74%). We found 77% of the seed morphospecies only in funnel traps, 10% only in sticky traps and 13% in both trap types. Funnel traps presented a higher seed density per day than sticky traps in both plot types, and especially in disturbed ones (Table 1). Considering only sticky trap data, 10 morphospecies were registered exclusively in preserved plots and five morphospecies exclusively in disturbed plots. Regarding the 10 more common morphospecies in the annual accumulated seed rain, three morphospecies (number 1, 50 and 51) were exclusively found in disturbed plots.

We found a significant difference in richness and in the number of seeds / trap between trap and plot types (GLMM Poisson: $F= 22.29$, $p<0.001$ and $F=184.75$, $p<0.001$ respectively). The highest number of seeds was caught by funnel traps on disturbed plots followed by funnel traps on preserved plots (GLMM Poisson $F=184.75$, $p<0.001$; Fig. 2). Sticky traps on disturbed plots were the less efficient (Fig. 2). Funnel traps also allowed catching the highest species richness, both on disturbed and preserved plots (GLMM poisson $F= 22.29$, $p<0.001$; Fig. 2). Again, sticky traps on disturbed plots were the less efficient, catching the fewest species (Fig. 2).

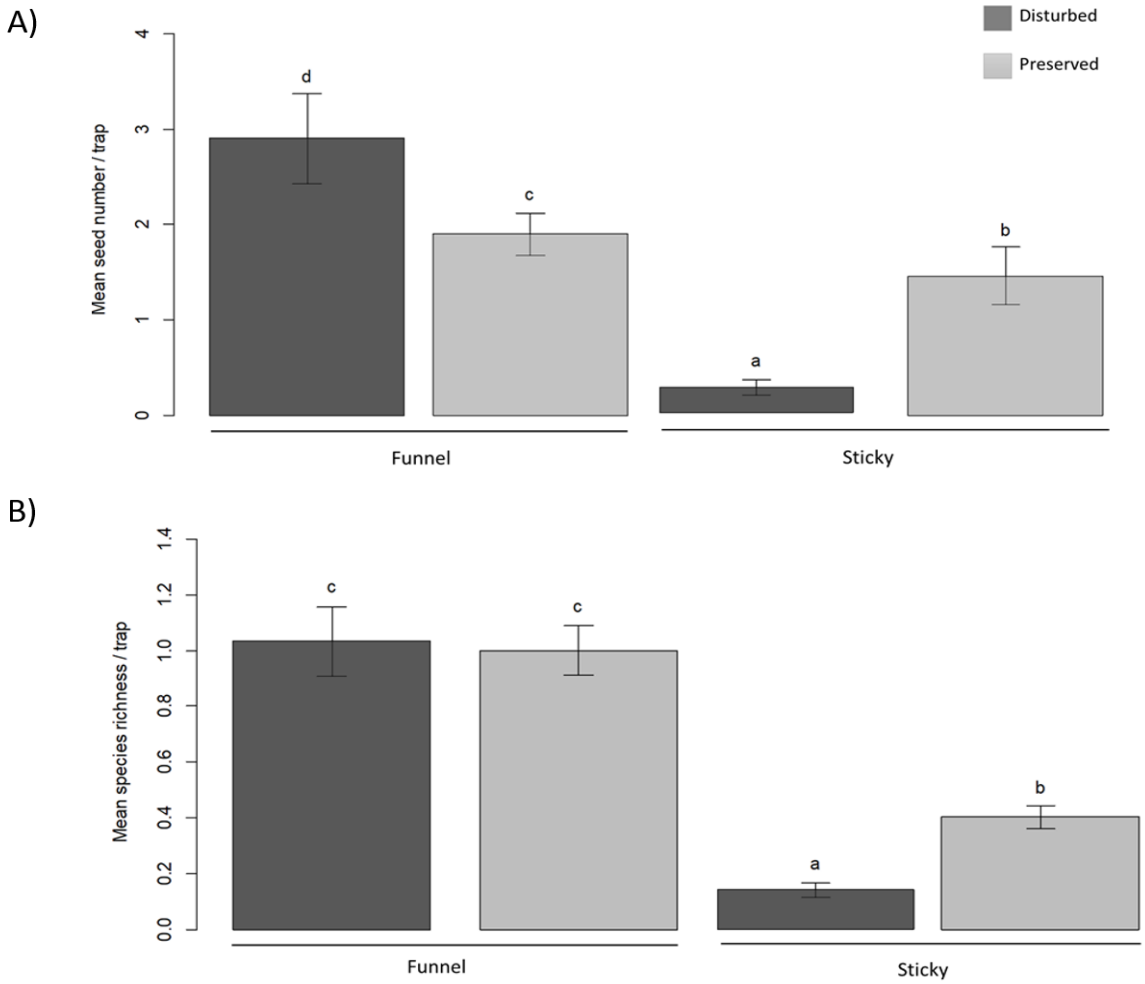


Figure 2: Values per trap in funnel and sticky traps in disturbed and preserved sites in *campo rupestre* vegetation, southeastern Brazil considering: A) mean number of seeds (GLMM Poisson: F= 22.29, p<0.001) B) seed morphospecies richness. Different letters indicate statistically significant means (GLMM Poisson, F=184.75, p<0.001).

Considering the two most common morphospecies, we found that morphospecies-7 was mostly retrieved in funnel traps (GLMM Poisson F=153.84, p<0.001; Appendix S6), and that morphospecies-2 presented a significant distribution difference between trap types within and between both plot types (GLMM Poisson F=64.83, P<0.001; Appendix S7).

4.3.Temporal patterns of seed rain

We found a significant difference for the seed density and species richness in both plot types between dry and rainy season, with a marked reduction of the number of seeds in disturbed plots during the dry season (Fig. 3).

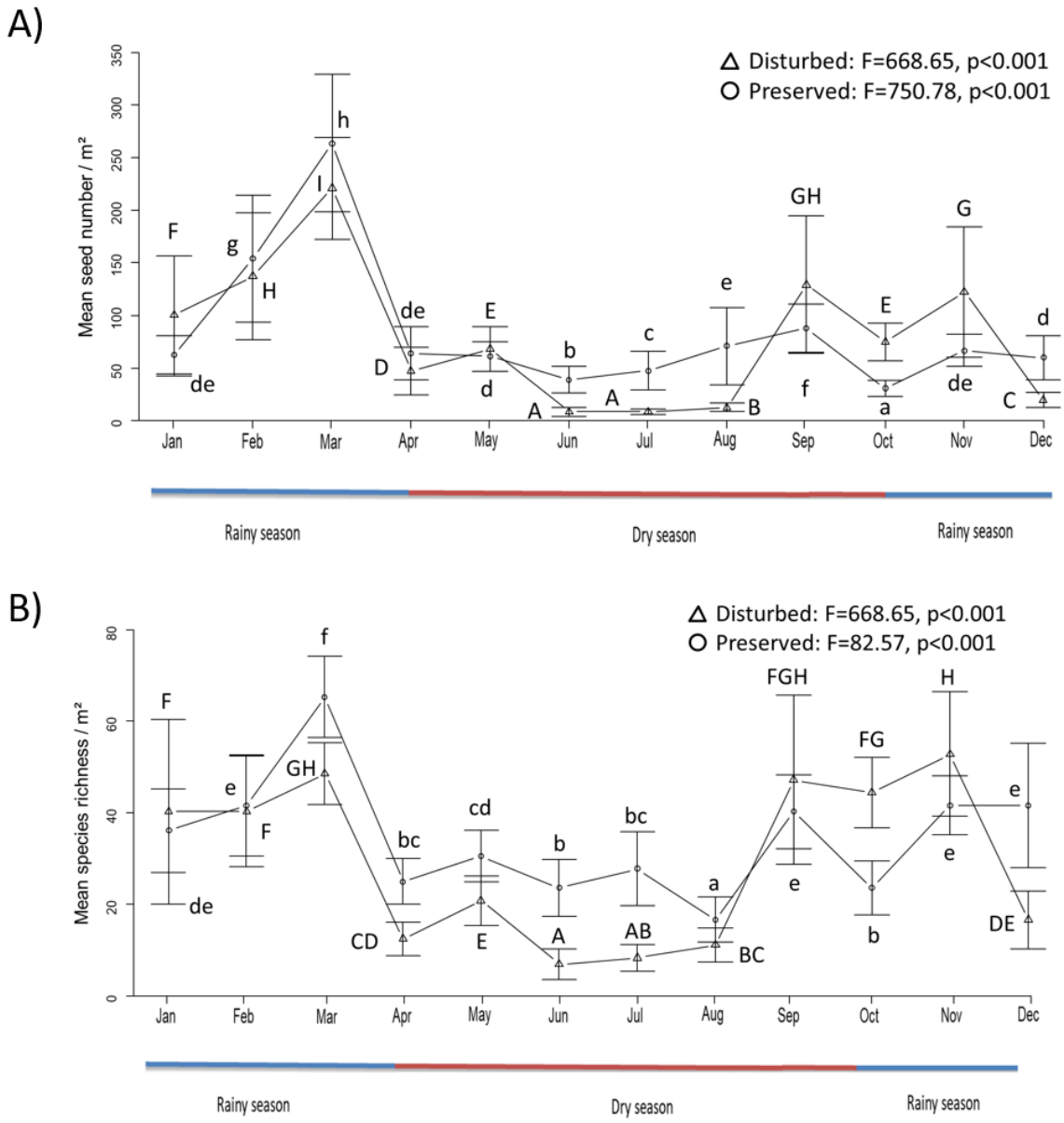


Figure 3: Monthly seed rain over one year (April 2016 to March 2017) in disturbed and preserved sites in *campo rupestre* vegetation, southeastern Brazil considering: A) monthly seed rain density; B) monthly seed morphospecies richness per m². Different letters indicate statistically significant means (GLMM Poisson).

In funnel traps, we found a clear tendency in having more seeds during the rainy months in comparison to the dry season (Appendix S8). Higher seed density during the rainy season was mostly caused by a greater arrival of seeds from the four most common morphospecies in December and January (Fig. 4).

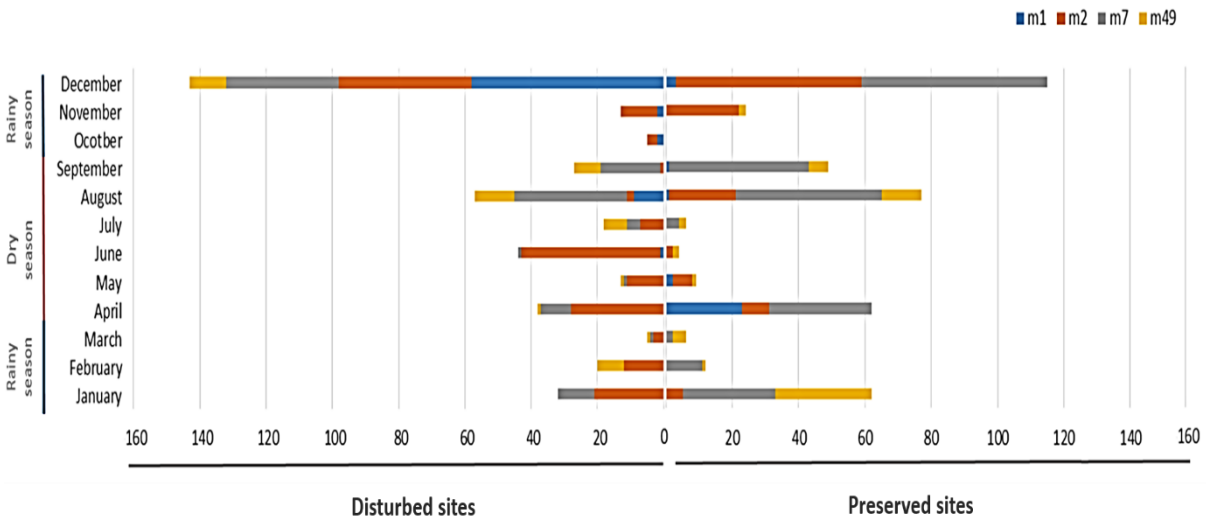


Figure 4: Monthly accumulated seed rain for the 4 more common seed morphospecies collected on both trap types in disturbed and preserved sites (m1=morphospecies-1; m2=morphospecies-2; m7=morphospecies-7; m1=morphospecies-49).

4.4.Seed rain and floristic similarity between plot types

The Correspondence Analysis on the seed rain showed that the three preserved sites are more similar between themselves than disturbed sites, and characterized by many morphospecies, such as morphospecies-13, 21 and 57 (Fig. 5). Each disturbed site had a particular species composition and was characterized by either morphospecies-14, 28 or 83 (Fig. 5).

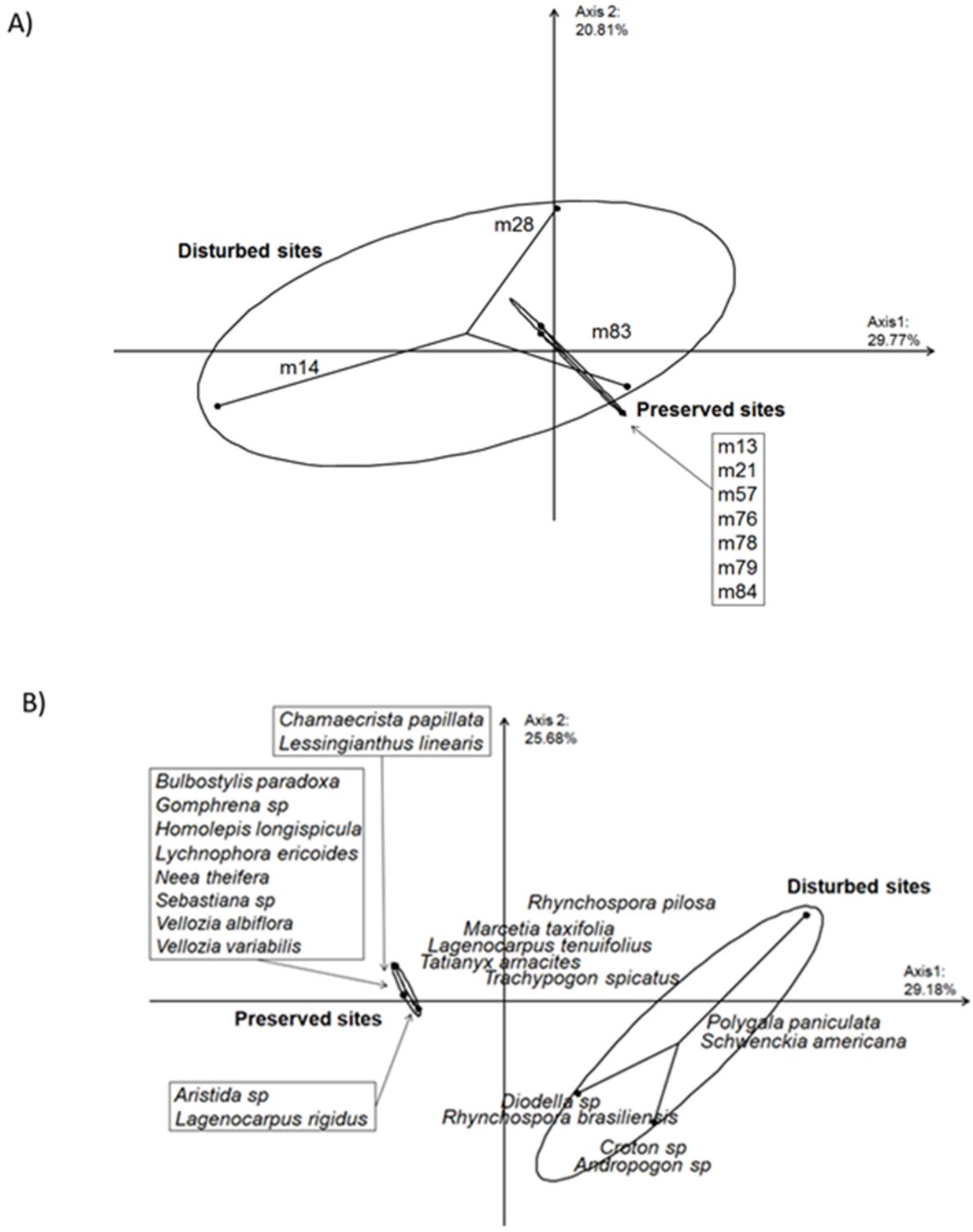


Figure 5: Multidimensional representations of: A) the seed rain composition over one year (m= morphospecies); and B) plant species occurrence in disturbed and preserved sites in *campo rupestre* grassland in southeastern Brazil analyzed with Correspondence Analyses.

The total species richness in the floristic survey considering all plots was of 74 species, distributed in 48 genera and 26 families (Appendix S9). Poaceae, Cyperaceae and Asteraceae were the most representative families in both plot types. We found 33 plant genera occurring exclusively in preserved plots, with *Bulbostylis* (Cyperaceae) and *Vellozia* (Velloziaceae) as the richest ones, with three species each. We found ten plant genera occurring exclusively in disturbed plots, with *Panicum* (Poaceae) and *Polygala* (Polygalaceae) as the richest ones, with two species each. Eleven genera occurred in both plot types, with *Rhynchospora* (Cyperaceae) and *Lagenocarpus* (Cyperaceae) as the richest ones, with six and two species respectively. We found 41 species occurring exclusively in preserved plots, 18 exclusively in disturbed plots and 17 in both plot types (Fig. 6).

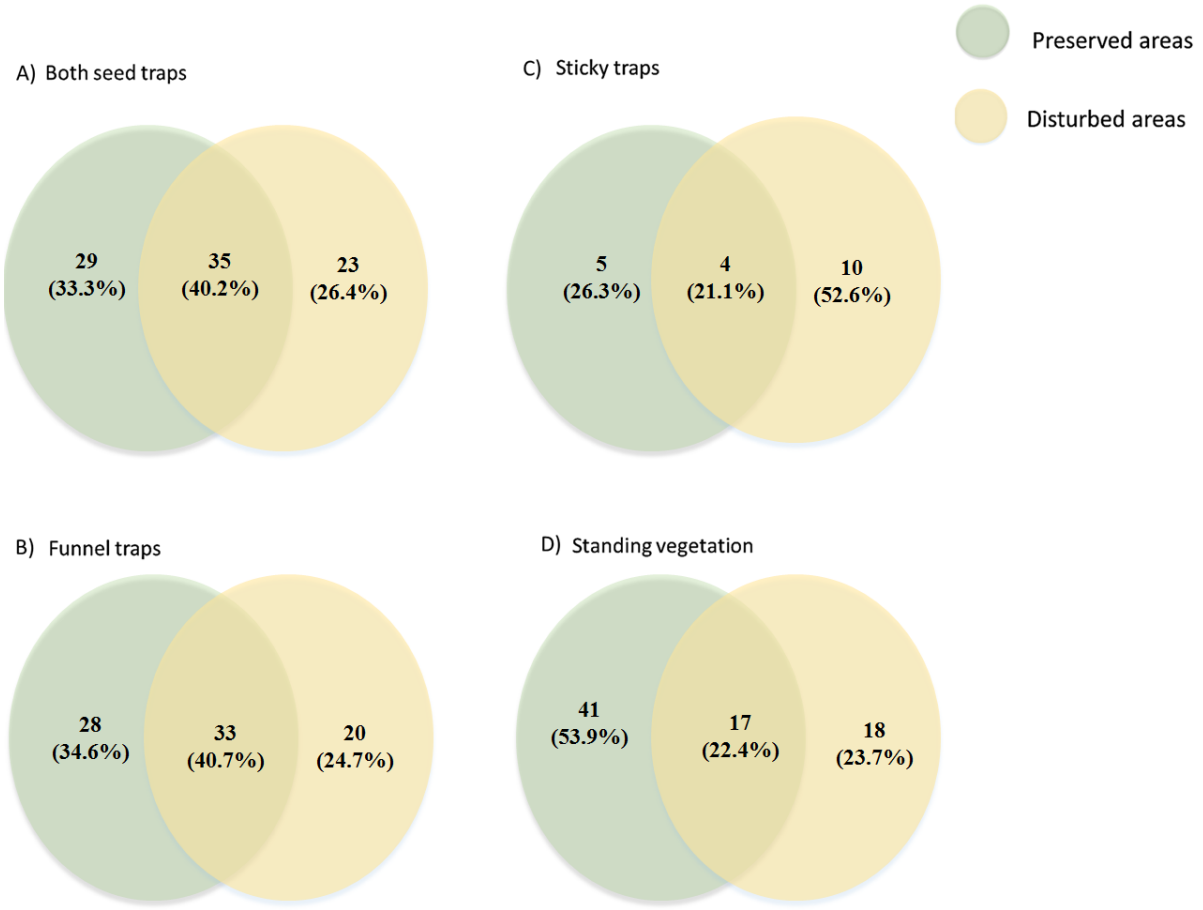


Figure 6: Venn diagrams of the number and proportion of morphospecies (seed rain) or plant species (floristic survey) exclusively registered in preserved or disturbed areas or in both plot types in *campo rupestre* grassland in southeastern Brazil considering the data for A) both trap types; B) only funnel traps; C) only sticky traps; D) the standing vegetation.

Mesosetum loliforme (Poaceae) was the only species recorded in all plots. *Rhynchospora consaguinea* (Cyperaceae), *Rhynchospora riedeliana* (Cyperaceae) and *Trachypogon spicatus* (Poaceae) were the second more common species, occurring in 83% of the plots. Axis 1 of the Correspondence Analysis separated the three preserved plots, very similar to each other and characterized by species like *Bulbostylis paradoxa, Homolepis longispicula* (Cyperaceae) and *Lychnophora ericoides* (Asteraceae), from the three disturbed plots characterized by *Rhynchospora riedeliana* (Cyperaceae) (Fig. 6). Axis 2 separated two disturbed plots characterized with *Rhynchospora brasiliensis* and *Croton* sp. from the last disturbed plots characterized by *Rhynchospora pilosa* and *Marcetia taxifolia*.

5. Discussion

The significant differences in seed arrival patterns between plot types provide valuable information about seed rain dynamics in *campo rupestre*. The intrinsic seed dispersal limitation of *campo rupestre*, inferred from the lack of obvious mechanisms for seed dispersal in the majority of its species (Silveira et al. 2016, Hopper et al. 2016), was shown here, can be even stronger in disturbed areas, and suggests that seed limitation strongly limits resilience in sites with extensive soil disturbance. The fact that only few diaspores of five species producing berries were retrieved in seed traps, for which we expect long-distance dispersal events (Schupp 1993; Guerra et al. 2017; Chen et al. 2019), suggests that even species dispersing seeds for long distances are not present in disturbed sites. This also supports the idea that strong dispersal limitation is linked with low resilience. Our results agree with previous studies about seed rain in disturbed areas (Urbanska & Fattorini 2000; Lehouck et al. 2009), thus suggesting that seed limitation is an important driver of low ecosystem resilience in disturbed areas.

The values of seed density found here are far below that found in tropical pristine and disturbed forests (Holl 1998, Cole et al. 2010). The overall averages of seed density per day

registered is much lower even than those reported in other grasslands, despite the fact that the species richness found is among the highest already reported in the literature (Urbanska et al. 1998; Lyaruu 1999; Urbanska et al. 2000; Chabrerie & Alard, 2005; Jakobsson et al. 2006; Shang et al. 2013; Fibich et al. 2013, Marteinsdottir, 2014). The remarkable low number of seeds found for 90% of the seed morphospecies, corroborates with the predictions of OCBIL theory that of accentuated dispersal limitation on this ancient ecosystem (Hopper et al. 2016; Silveira et al. 2016), suggesting that community dynamics and resilience may be affected by dispersal limitation in other edaphic grasslands.

Funnel traps were the most efficient trap type in catching seeds, endorsing that this type of trap is the most efficient for seed rain studies in grasslands (Kollmann &Goetze 1998; Bullock et al. 2001; Page et al. 2002; Chabrerie & Alard 2005). Although we retrieved only a small portion of the annual accumulated seed rain in sticky traps, one from each ten morphothypes were exclusively found in this trap type, including two of the ten more common morphospecies. These findings are in agreement with the previous experiment of Chabrerie & Alard (2005) reinforcing that the use of different seed trap types increases the accuracy and sampling of all seed rain studies. The higher average of seeds/m² per 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), allowing seeds not to be predated on and thus to end up in higher rates in funnel traps via runoff water (de Rouw et al. 2018). Additionally, the ability of rainfall to disperse seeds depends on local surface cover (de Rouw et al. 2018). Bare or sparsely covered soils in these disturbed plots can favor seed dispersal by runoff water, whereas dense ground cover in preserved plots represent barriers to seed transport and assures water infiltration reducing runoff water.

The higher average seed density in sticky traps in preserved plots shows the importance of short distance dispersal events from plants inside the plots (Chabrerie & Alard, 2005). Despite few seeds recorded in sticky traps did not present any classical adaptation for wind dispersal,

such as wings or plumes, syndromes are useful only as general organizing tools (Howe & Smallwood 1982). Some light seeds may disperse from tall mother plants by the wind over several meters, being often found in sticky traps (Chabrerie & Alard, 2005). This may partially explain why disturbance seems to affect seed arrival dynamics in sticky traps, due to the absence of mother plants or to the small height of the mother plants in these areas.

Even if *campo rupestre* is characterized by a marked seasonal climate, distinct seasonal patterns are not clear for the annual fruit production in the plant community (Buisson et al. 2017; Le Stradic 2018c). Surprisingly, we found a significant increase in the number of seeds and species richness in the rainy season in both plot types. It may indicate the strong influence of secondary dispersal processes related to seasonal abiotic factors, such as water runoff (de Rouw et al. 2018). The relative lower seed density in disturbed plots during the dry season reinforces the need to assess the temporal dynamics of seed limitation and resilience in tropical grasslands.

The total species richness in seed rain was higher than in the floristic survey, implying in a plant richness influx by seeds coming from outside the plots. However, this influx may reflect more the huge plant diversity found in *campo rupestre* than its plant community dispersion hability (Silveira et al. 2016). Approximately 32% and 25% of seeds morphospecies were registered exclusively in disturbed or preserved plots, respectively, signalizing different patterns of seed rain between disturbed and preserved areas. However, as almost all of the morphospecies registered exclusively in disturbed areas were found in funnel traps, it may have a close relation to a higher effectiveness of funnel traps in disturbed areas favored by water runoff seed dispersal. Most of the seed morphospecies collected in sticky are exclusively from preserved plots, reinforcing the importance of short distance dispersal events even for wind dispersed seeds. We found the morphospecies-1, 50 and 51, which are between the 10 more common morphospecies, exclusively in funnel traps in disturbed plots, which indicates the importance of water seed dispersal for this relevant morphospecies. The Correspondence

Analysis for the morphospecies occurrence indicates a strong similarity of seed rain patterns in preserved plots and a marked unpredictability of seed rain richness composition in disturbed sites and is in agreement with the results found in the floristic survey.

All species registered in the floristic survey on the disturbed plots occur naturally in the *campo rupestre*, indicating the presence of effective buffer zones formed by the reference ecosystem that prevent the arrival of unwanted invasive species. Only 17 species from the floristic survey occurs in both plot types, indicating a strong dissimilarity between plot types. Additionally, the strong dissimilarity within disturbed plots reveals a poor recovery trajectory in these plots (Leps et al. 2000; Leps et al. 2007). The fact that 57% from all plant genera from the floristic survey were only recorded in preserved plots, is in accordance with our findings that dispersal limitation is a strong filter limiting plant establishment in disturbed areas. Poaceae and Cyperaceae were the most representative families in both plot types, signalizing them as good targets for research on seed ecology and restoration project in *campo rupestre*. Genera, such as *Bulbostylis* (Cyperaceae) and *Vellozia* (Velloziaceae), that were the richest genera in the standing vegetastion for the preserved plots but were absent disturbed plots, should be priority in future efforts as challenging key groups for assisted reintroduction in disturbed sites. Species, such as *Mesosetum loliforme* (Poaceae), *Rhynchospora consaguinea* (Cyperaceae), *Rhynchospora riedeliana* (Cyperaceae) and *Trachypogon spicatus* (Poaceae), were the recurrent species in both plot types, which may indicate great ability to disperse from the surrounding reference ecosystem and naturally establish in disturbed areas.

The seed arrival patterns for the four more common morphospecies allow some ecological inferences. Despite the fact that morphospecies-1 was mainly found on sticky traps and specially in preserved plots, according to its morphological characteristics it is probably not dispersed by wind. Morphospecies-1 seeds may manage to end up on sticky traps in preserved site if the mother plant is higher than trap height, explaining why this morphospecies was normally not found on sticky traps on disturbed sites. The low retrieval rates of morphospecies-

1 on funnel traps, could be consequence of secondary removal events by ground-dwelling animals, that may not allow these seeds to stay long enough on the ground to be dispersed by rainfall to funnel traps. In the same way, morphospecies-2 that was identified as a Poaceae, despite not being a typically an anemochoric seed, presented significant retrieval rates on sticky traps and with higher arrival rates in preserved plots, which may be explained as well by the absence or the small size of the mother plants in the disturbed plots. Morphospecies-7, identified as a *Lychnophora* (Asteraceae), was significantly found with higher quantity in funnel traps on disturbed sites. *Lychnophora* seeds may be an important resource for ground-dwelling animals, thus maybe being more removed in preserved plots due to more conserved interaction networks with ground-dwelling animals (Chapter 4). Morphospecies-49, identified as a *Rhynchospora* (Cyperaceae), was only found in funnel traps and presented similar arrival rates in both plot types. Cyperaceae is characterized by a high proportion of species with buoyant, water-dispersed seeds (Praeger, 1913; Leck & Schütz 2005), which may partially explain the equivalent seed arrival rates between disturbed and preserved plots for this family. Another possible explanation is that the *Rhynchospora* presented the highest richness of species between the genera that were registered in both plot types, with higher plant density on preserved plots and shorter dispersal distances and lower density on disturbed plots but longer dispersal via water run-off due to higher bare ground.

The scarcity of previous information about seed morphologyfrom *campo rupestre* together with the high richness and heterogeneity of this ecosystem (Silveira et al. 2016), hampered the taxonomic identification of most morphospecies. So far, there are no specific literature, images bank or collections to guide seed identification of *campo rupestre* plant community. Our image bank represents the first effort to build a database of seed images for the *campo rupestre* and even with a small number of species it has already been extremely relevant in the identification of important plant groups on seed rain. We could identify some relatively abundant plant genera

and families in the seed rain, placing them as good targets for future research on seed ecology and restoration projects.

6. Conclusion

The world's tropical old-growth grasslands dramatically declined over the past decades due to land-use changes (Parret al., 2014), demanding an urgent development of efficient restoration practices (Buisson et al. 2018). Therefore, unveiling ecological process linked to ecosystems resilience, such as where, when and why seed limitation can occurs, is extremely important for developing successful restoration strategies. This study brings the first information about the reliability of seed dispersal for natural recovery of *campo rupestre* after anthropogenic disturbance and represents an important contribution for seed rain studies on tropical grasslands. As a consequence of intense disturbances linked to soil removal by gravel exploitation, the natural recovery success in these disturbed areas of *campo rupestre* strongly relies on the arrival of new diaspores. The seed dispersal limitation shown here partially explains to the low resilience observed in these megadiverse montane grasslands (Le Stradic et al. 2018), but establishment limitation should also be investigated.

Measuring seed density and richness is of high relevance for a better understanding of seed ecology and resilience of this ecosystem. That was as well an important step to understand how disturbance affects seed arrival in disturbed areas. Future studies should focus on the fate of seeds that actually have managed to arrive in the disturbed sites, to address dispersal limitation, the next step towards community reassembly (Török et al. 2018). The seed image bank presented in this work is the first effort to build a database for *campo rupestre* plant community. The distinct seasonal patterns of the number of seeds can be of great relevance for future research on seed ecology and for restoration practices in *campo rupestre*.

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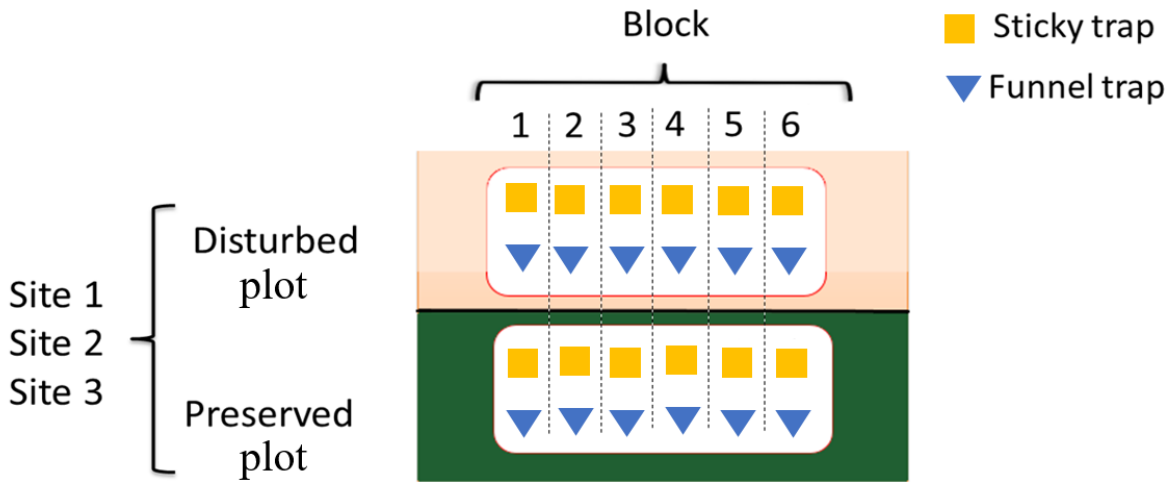
Table 1: Average seed density (seeds/m^2 per day) in seed rain estimated by sticky and funnel traps in *campo rupestre* vegetation, southeastern Brazil in preserved and disturbed sites. Averages are followed by Standart Deviation (+-SD).

	Both plot types	Preserved areas	Disturbed areas
Sticky traps	1.4 ±1.2	2.4 ± 2.1	0.4 ± 0.2
Funnel traps	3.9 ± 3.0	3.1 ± 2.6	4.8 ± 3.4
Both trap types	2.7 ± 2.1	2.8 ± 2.3	2.6 ± 1.8

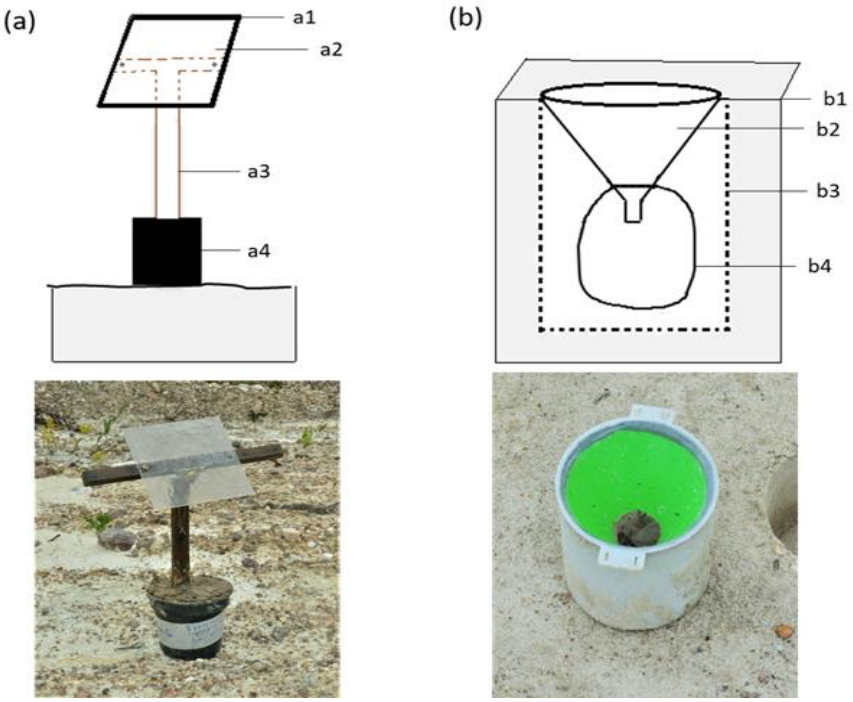
Supporting information



Appendix S1: Small quarry used for soil extraction that destroyed the aboveground vegetation and upper soil horizons in 2002 when the road MG010 was paved. Areas similar to this one remain significantly altered in relation to the surrounding reference ecosystem and with very low vegetation cover (Photo AJ Arruda, taken in 29/09/2015).



Appendix S2: Factorial experiment involving the assessment of diaspores catching in six blocks over three paired disturbed and preserved neighbor plots using two types of seed traps.



Appendix S3: (a) Sticky trap. a1: clear Plexiglas® plate (0.0225 m²); a2: sticky gel over a thin plastic film placed over the Plexiglas plate; a3: wooden pole; a4: plastic pot filled with concrete and (b) Funnel trap. b1: ground level; b2: PVC plastic funnel section (0.01 m²); b3: white PVC pipe with bore holes 1 cm diameter; b4: bag of < 0.1mm mesh size.

Appendix S4: Seed image bank from seeds taken from the surrounding vegetation, organized alphabetically by family and within each family in alphabetical order by genus and by specific epithet when of the same genus. (NI=not identified).





Asteraceae

Bacharis sp.



Asteraceae

Lychnophora sp1



Asteraceae

Lychnophora sp2



Cyperaceae

Lagenocarpus sp1



Cyperaceae

Lagenocarpus sp2



Cyperaceae

Lagenocarpus sp3



Cyperaceae

Rhynchospora brasiliensis **Boeckeler**



Cyperaceae

Rhyncophora sp.



Cyperaceae

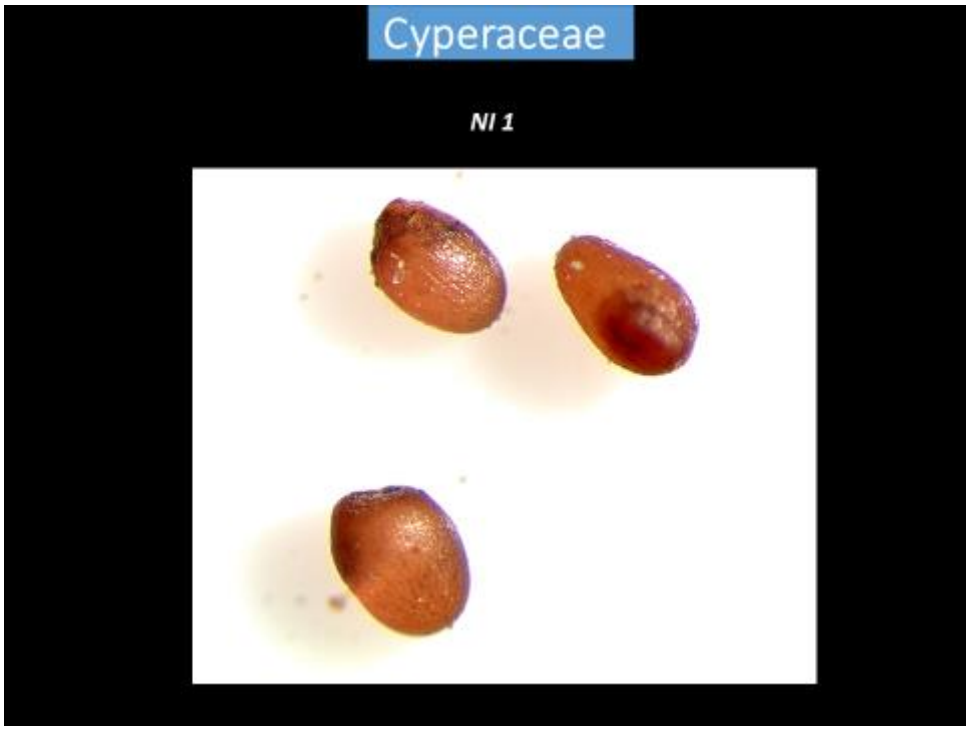
Scleria sp1

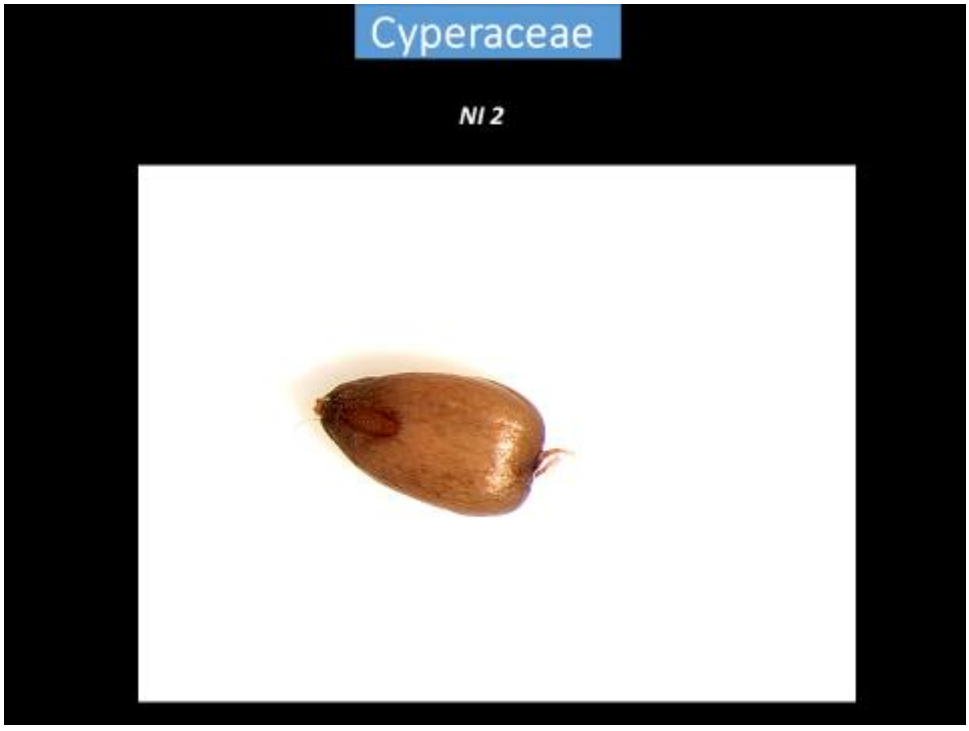


Cyperaceae

Scleria sp2




Cyperaceae
NI 1


Cyperaceae
NI 2

Iridaceae

Trimezia sp.



Malphighiaceae

***Byrsonima cipoensis* Mamede**



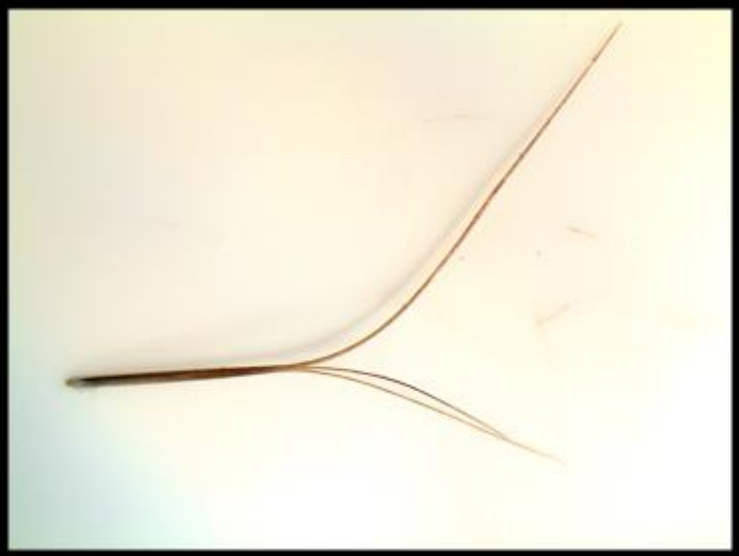
Malphighiaceae

Byrsonima sp.



Poaceae

***Aristida torta* (Nees) Kunth**



Poaceae

***Axonopus* sp.**



Poaceae

***Echinolaena inflexa* (Poir.) Chase**



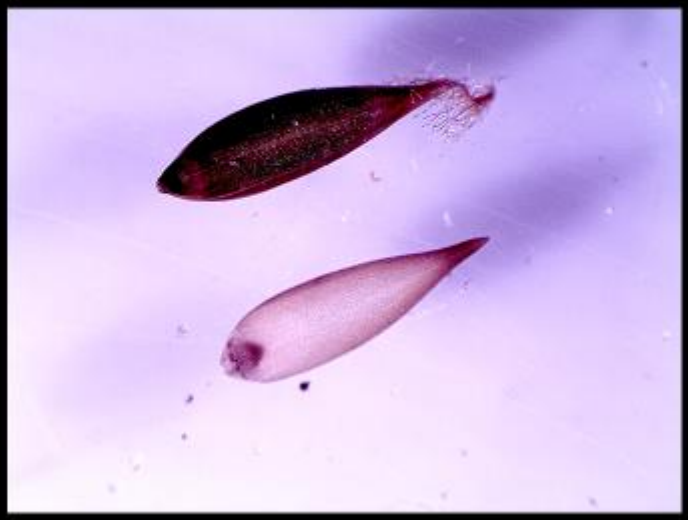
Poaceae

Mesosetum sp.



Poaceae

NI 1



Poaceae

NI 2



Polygalaceae

Polygala glochidata **Kunth**



Fabaceae

NI 1



Lamiaceae

Hyptis sp1



Lamiaceae

Hyptis sp2



Lythraceae

***Diplusodon orbicularis* Koehne**



Rubiaceae

Perama sp.



Rubiaceae

NI 1



Velloziaceae

Barbacenia sp1



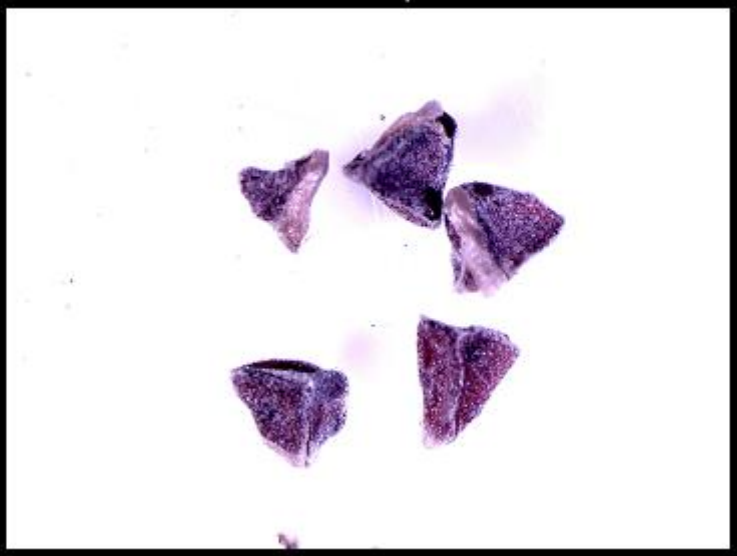
Velloziaceae

Barbacenia sp2



Velloziaceae

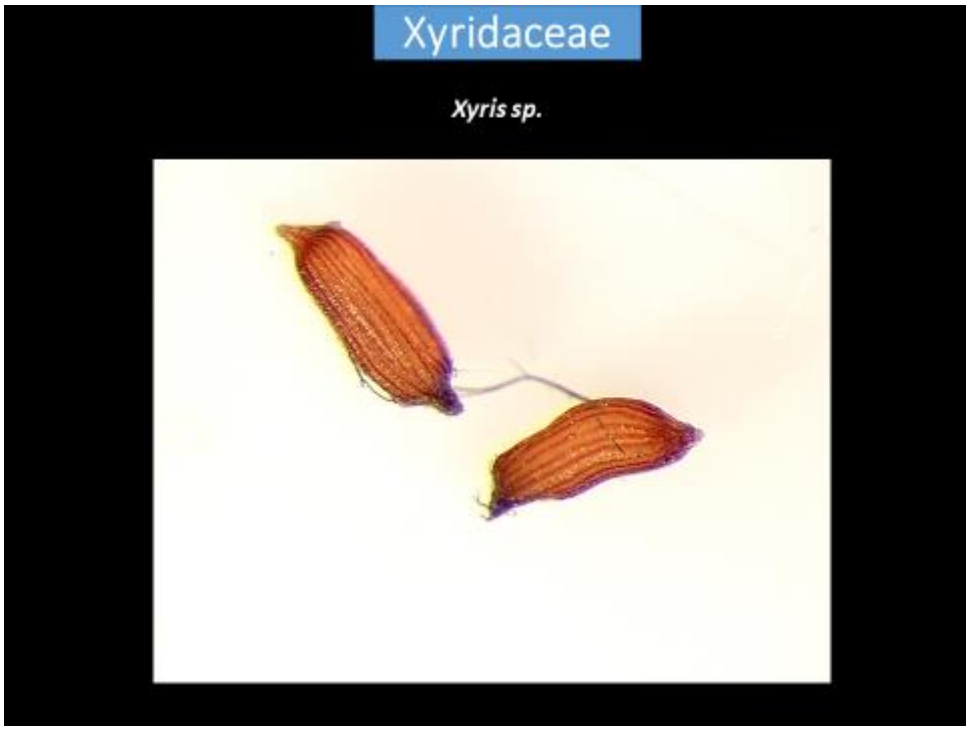
Vellozia sp1



Velloziaceae

Vellozia sp2





Appendix S5: Seed image bank from the 10 more common seed morphospecies in the annual accumulated seed rain estimates. (NI=not identified).



Morphospecie-2



Genus	Family
NI	Poaceae

Morphospecie-4



Genus	Family
Perama	Rubiaceae

Morphospecie-5



Genus	Family
Xyris	Xyridaceae

Morphospecie-7



Genus	Family
Lychnophora	Asteraceae

Morphospecie-33

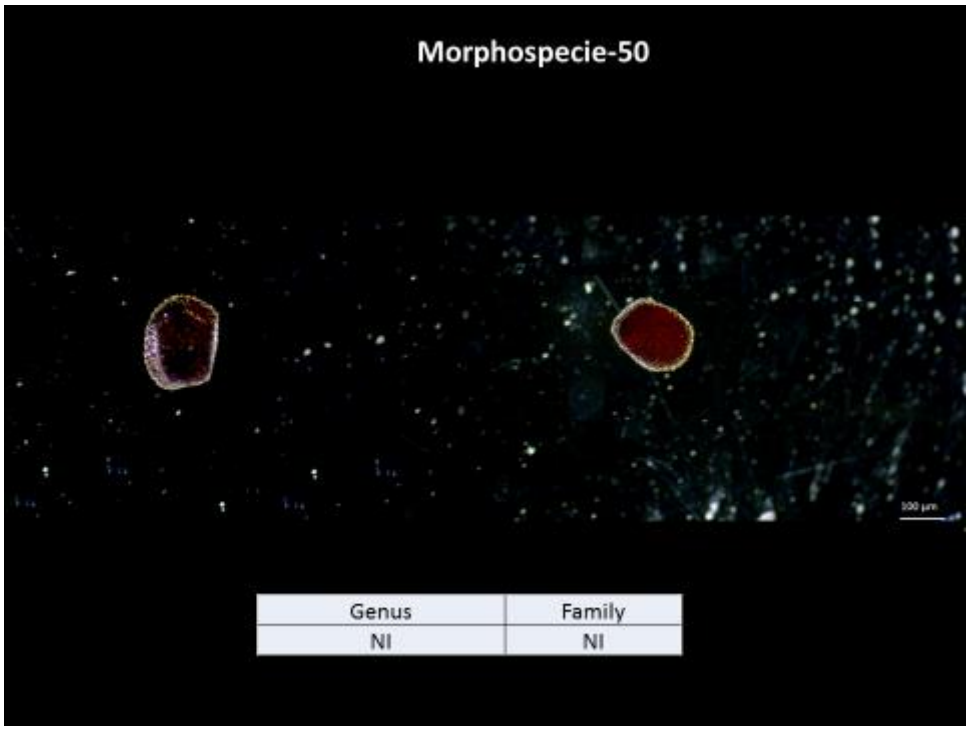


Genus	Family
Lychnophora	Asteraceae

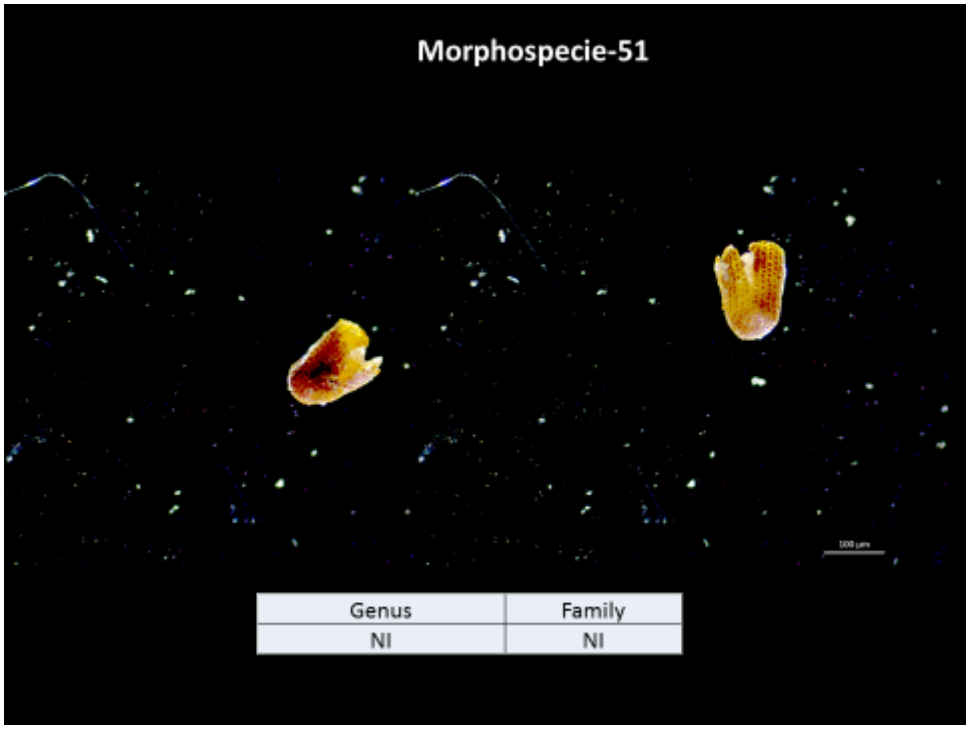
Morphospecie-49



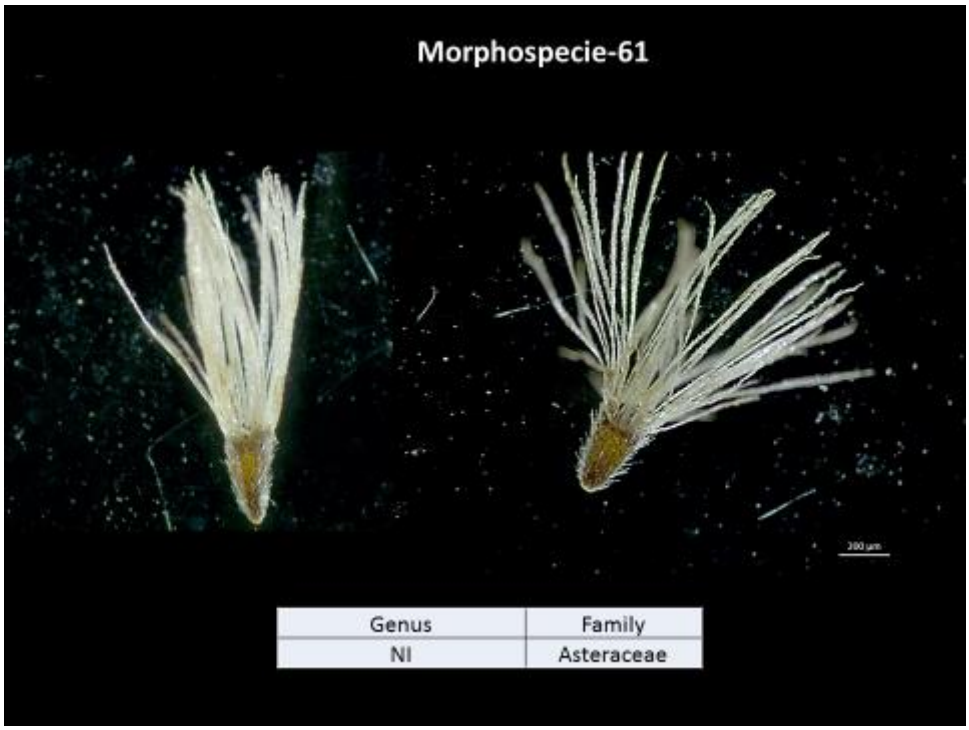
Genus	Family
Rhynchospora	Cyperaceae

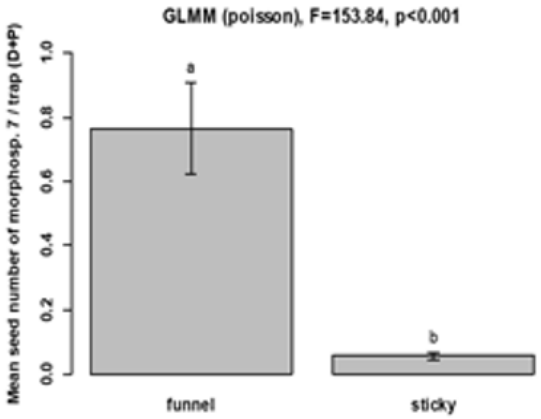


Genus	Family
NI	NI

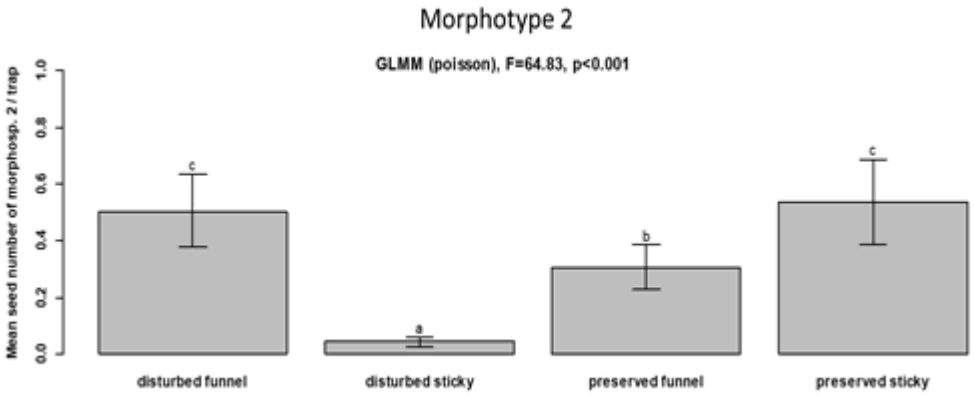


Genus	Family
NI	NI

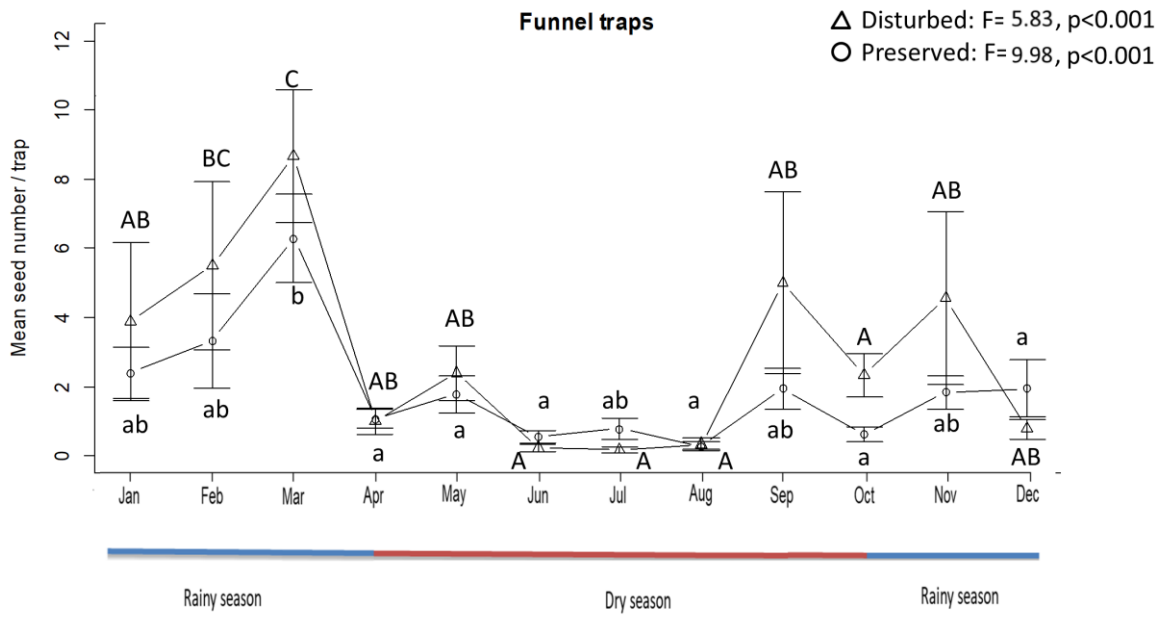




Appendix S6: Mean seed number collected for Morphospecies-7 according to trap types.



Appendix S7: Mean seed number collected for Morphospecies-2 according to trap and plot types.



Appendix S8: Monthly mean seed number per trap for funnel traps in disturbed and preserved sites.

Appendix S9: Plant species occurrence according to plot type considering all sites (PR= registered exclusively in preserved plots; DI= registered exclusively in disturbed plots; BOTH= registered in both plot types).

Family	Species	PR	DI	BOTH
Amaranthaceae	*Gomphrena sp*	x		
Apocynaceae	*Hemipogon hatschbachii*	x		
Asteraceae	*Eremanthus erythropappus*		x	
	Lessingianthus linearis	x		
	Lychnophora ericoides	x		
	Lychnophora passerina		x	
	Porophyllum obscurum		x	
	Prestelia eriopus	x		
	Richterago polyphylla	x		
Calophyllaceae	*Kielmeyera petiolaris*		x	
	kielmeyera sp	x		
Convolvulaceae	*Evolvulus lithospermoides*	x		
Cyperaceae	*Bulbostylis capillaris*	x		
	Bulbostylis junciformis	x		

	Bulbostylis paradoxa	x		
	Lagenocarpus rigidus			x
	Lagenocarpus tenuifolius			x
	Rhynchospora brasiliensis			x
	Rhynchospora consaguinea			x
	Rhynchospora pilosa			x
	Rhynchospora recurvata			x
	Rhynchospora riedeliana			x
	Rhynchospora terminalis			x
	Scleria sp	x		
Ericaceae	*Gaylussacia cinerea**	x		
Eriocaulaceae	*Paepalanthus nigrescens*		x	
Euphorbiaceae	*Croton sp*		x	
	Euphorbia sp	x		
	Sebastiana sp	x		
Fabaceae	*Calliandra linearis*			x
	Chamaecrista ochnacea	x		

	Chamaecrista papillata	x		
	Chamaecrista sp1		x	
	Chamaecrista sp2		x	
Iridaceae	*Trimezia juncifolia*	x		
Lamiaceae	*Hyptis proteoides*	x		
	Hyptis sp	x		
Lythraceae	*Diplusodon orbicularis*			x
Malpighiaceae	*Byrsonima sp**	x		
	Tetrapteris sp	x		
Malvaceae	*Sida aurantiaca*	x		
Melastomataceae	*Lavoisiera sp*	x		
	Marcetia taxifolia	x		
	*Miconia ferruginata**		x	
	Microlicia sp		x	
Myrtaceae	*Myrcia sp**			x
Nyctaginaceae	*Neea theifera**	x		
Phyllanthaceae	*Phyllanthus klotzschianus*		x	

Poaceae	*Andropogon leucostachyus*	x		
	Andropogon sp		x	
	Apochloa euprepes	x		
	Aristida setifolia		x	
	Aristida sp			x
	Ctenium brevispicatum	x		
	Echinolaena inflexa			x
	Homolepis longispicula	x		
	Mesosetum exaratum	x		
	Mesosetum loliforme			x
	Panicum nigrescens		x	
	Panicum sp		x	
	Paspalum erianthum	x		
	Paspalum pectinatum	x		
	Poaceae sp2	x		
	Tatianyx arnacites			x
	Trachypogon spicatus			x

Polygalaceae	*Polygala paniculata*		x	
	Polygala sp		x	
Rubiaceae	*Diodella sp*			x
Solanaceae	*Schwenckia americana*		x	
Velloziaceae	*Vellozia albiflora*	x		
	Vellozia nivea	x		
	Vellozia variabilis	x		
Verbenaceae	*Lippia lupulina*	x		
Vochysiaceae	*Vochysia pygmaea*	x		
Total	74	39	18	17

* Indicates species that produce vertebrate-dispersed berries.

CHAPTER 4: Topsoil disturbance reshapes diaspore interactions with ground foraging animals in an edaphic megadiverse grassland

1. Abstract

Questions

Anthropogenic disturbances, such as topsoil removal, are known to be followed by extremely poor recovery in edaphic grasslands. However, the role of interactions with seed predators and secondary dispersers in modulating plant recovery remains overlooked. We performed field experiments to investigate how soil disturbances affect diaspore removal and interactions with the ground foraging fauna to better examine establishment limitation.

Location

Campo rupestre vegetation, megadiverse edaphic grasslands, southeastern Brazil.

Methods

We used natural diaspores from five native species to compare removal rates between disturbed and preserved adjacent sites. We controlled invertebrate and vertebrate access to determine diaspore removal and dispersal distance. Moreover, we assessed distinct types of ant-diaspore interactions in preserved and disturbed sites and analyzed their structure through network based-approach.

Results

The removal rates varied significantly among species, being negligible for two species and relatively high for three species (between 50 and 100%). Soil disturbance reduced the interactions with invertebrates, and overall removal rates by 20%. Ants were the most important removal agents in both disturbed and preserved sites. Seed predator ants (*Pheidole* and *Dorymyrmex*) were more common in disturbed sites. Nearly 40% of the diaspores in disturbed sites were transported to preserved sites, but no diaspore was transported from preserved to disturbed sites. Additionally, ant-diaspore networks in preserved sites were more diverse and more robust compared to disturbed ones.

Conclusions

Although topsoil disturbance did not collapse diaspore removal, it resulted in contrasting outcomes of ant-diaspore interactions in disturbed and preserved sites. The lack of diaspore dispersal by ants towards disturbed sites indicates that disturbance modifications on secondary removal dynamics is a critical step for understand plant recovery capacity. Our findings help to explain the biological filters involved in natural recovery limitation after topsoil removal. Disturbance negatively affects diaspore fate by changing interactions with potential ground

foraging secondary seed dispersers and predators, constraining formation of seed bank and thus vegetation dynamics and resilience.

Keywords: ants, *campo rupestre*, degradation, diaspore removal, mirmecocory, natural recovery, seed limitation, seed predation

2. Introduction

Understanding the relative importance of diaspore-animal interactions in vegetation dynamics in natural and disturbed ecosystems is a critical aspect for better practices in restoration and conservation (MacMahon & Jordan 1994; Török 2018), especially in seed-limited system (Dayrell et al. 2016). Seeds within diaspores constitute a highly nutritive food resource for animals (Thorsen at al. 2011; Schowalter 2016) and ground foraging animals may affect seed fate by interacting with diaspores handled and discarded or dispersed by primary dispersers (Roberts & Heithaus 1986; Chambers & MacMahon 1994; Vander Wall et al. 2005). Examining interactions between ground foraging animals and diaspores is thus crucial to better understand plant recruitment (Herrera et al. 2011; Martinson & Fagan 2014).

Empirical studies examining post-dispersal seed fate have shown important variability among species and sites (Schupp & Fuentes 1995; Chapman & Chapman 1996, Lambert 2002). In disturbed ecosystems, seed predation can act as a biotic filter that affects natural recovery and restoration outcomes (Calviño-Cancela 2007; Denham 2008). Seed predators may also target specific species (Roselli 2014), influencing in different ways the role of seed limitation in natural recovery. However, seed predators can accidentally work as seed dispersers and even facilitate seed germination (Rico-Gray & Oliveira 2007; Gómez et al. 2019), thus promoting regeneration. Seed dispersal is characterized by the horizontal displacement of seeds in space and is an important mechanism for plant colonization in disturbed sites, which may also increase seed and seedling survival (Grubb 1977; Schupp 1988; Dennis 2007).

The outcomes of diaspore interactions with vertebrates and invertebrates have been crucial to the development of theoretical models regarding seed fate (Hammond & Brown 1998). By moving seeds, ground foraging animals may expand seed shadows generated by primary dispersers (Christianini & Oliveira 2009) and provide additional opportunity for seeds to escape predation (Giladi 2006; Rico-Gray & Oliveira 2007), fire (Rico-Gray & Oliveira 2007) and seed deposition in nutrient-rich soils (Sternberg et al. 2007, Arnan et al. 2012). Post-dispersal diaspore interactions with vertebrates have been mainly attributed to rodents, and are known to influence seedling recruitment (Feer & Forget 2002). While rodents are known as seed predators of large-seeded species (Feer & Forget 2002), it has been also shown that small mammals could also provide important benefits to plants since many of the small seeds can pass unharmed through their guts (Lessa et al. 2013; Genrich et al. 2017). Ants, although known as potential seed predators (Retana et al. 2004; Costa et al. 2017), can play a prominent role as seed dispersers in Neotropical savannas (Christianini & Oliveira 2009, 2010) often providing seed survival and germination by foraging on fruit and cleaning seeds, which become less vulnerable to decay (Passos & Oliveira 2003; Christianini et al. 2007).

Diaspore-animal interactions in tropical ecosystems often involves different agents of dispersal in subsequent steps (Vander Wall and Longland, 2004; Camargo et al. 2019). While most diaspores in Neotropical savannas do not present any apparent characteristics that promote ant dispersal (Christianini & Oliveira 2010), some studies have shown ants as important seed dispersers of several non-myrmecochorous plant species (Leal & Oliveira 1998; Christianini et al. 2007; Christianini & Oliveira 2009, 2010; Lima et al. 2013; Guerra et al. 2018). However, the role of ants as secondary diaspore dispersers and the consequences for plant recruitment across different habitats are still poorly understood (Magalhães et al. 2018; Camargo et al. 2019). Most seed dispersal studies in Neotropical savannas have focused on primary seed dispersal in few plant species typically vertebrate-dispersed (Faustino & Machado 2006; Silveira et al. 2012; Guerra & Pizo 2014, Guerra et al. 2017), and diaspore interactions with

invertebrates still need to be better explored, to better characterize their potential in modulating natural regeneration in disturbed sites.

The Brazilian *campo rupestre*, an Old Climatically-Buffered Infertile Landscape (OCBIL *sensu* Hopper et al. 2016), encompasses old-growth fire-prone tropical grasslands, associated to extremely poor soils on ancient mountaintops that harbors a highly diversified flora with remarkable levels of plant endemism (Echternacht et. al. 2011; Silveira et al. 2016; Colli-Silva et al. 2019). Nevertheless, along the past decades, the vegetation dynamics in these unique landscapes has been hampered by anthropogenic disturbances, especially topsoil removal associated to quarrying and mining activities (Silveira et al. 2016; Fernandes et al. 2018). Plant communities from *campo rupestre*, although highly resilient to endogenous disturbances, such as fires (Le Stradic et al. 2018a), are extremely vulnerable to human-caused exogenous soil disturbances (Le Stradic et al. 2018b; Buisson et al. 2019), which can be partially explained by dispersal limitation (Morellato & Silveira 2018).

In old-grow grasslands, plant species disperse poorly and at low rates, making community re-assembly a lengthy process (Bond & Parr 2010; Parr et al. 2014; Veldman et al. 2015; Buisson et al. 2019). Seed limitation is thus an important driver of vegetation dynamics and resilience in old-grow grasslands (Buisson et al. 2019). In seed-limited ecosystems, any reduction in seed quantity (e.g., seed predation) may compromises plant recruitment, while processes that increase seed dispersal success may prompt plant recruitment (Calviño-Cancela 2007). However, only a handful studies have addressed the influence of diaspore-animal interactions on vegetation dynamics and recovery in *campo rupestre* (Lima et al. 2013; Fernandes 2016; Guerra et al. 2018).

A better understanding of animal diaspore interactions can be crucial to unveil complex mechanisms involved in seed dispersal and plant recovery (Chamberlain et al 2014; Camargo et al. 2019). In this study, to ascertain how topsoil disturbances affect diaspore removal and interactions with ground foraging fauna in *campo rupestre*, we compared diaspore removal

rates and qualitatively evaluated animal-diaspore interactions between disturbed and preserved sites. We recorded different interaction outcomes to build disperser-diaspore multilayer networks aiming to explore distinct patterns of diaspore-animal interactions according to disturbance and diaspore type. We also compared seed dispersal effectiveness among ant species in disturbed and preserved sites to unveil how disturbance affects diaspore-animal interactions outcomes for plant community assembly and recovery. More specifically, we expected that diaspore removal rates should be lower on disturbed sites due to lower presence of ground foraging animals. We also expected diaspore removal rates to be higher when accessible to both vertebrates and invertebrates than when accessible exclusively to invertebrates.

3. Material and methods

3.1.*Study area*

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 (43º 35'W, 19º 17'S). The annual precipitation averages around 1,400 mm and the climate is markedly seasonal with most rainfall occurring in the hot summers (from October to March; Silveira et al. 2016). Altitude at the study site ranges between 1,150 and 1,300 m a.s.l. The main vegetation comprises *campo rupestre* vegetation, an old-growth, fire-prone grassland established on quartzite-derived rocks, with shallow and severely nutrient-impoverished sandy soils in mountaintops (Veldman et al. 2015; Silveira et al. 2016, Mucina 2018). The landscape encompasses a mosaic formed by patches of rocky outcrops and boulders where sclerophyllous treelets and shrubs grow amongst an herbaceous stratum, dominated by monocots and sparsely distributed shrubs (Le Stradic et al. 2015; Guerra et al. 2017).

During the paving of the MG010 highway in 2002 small quarries were exploited for soil extraction, destroying vegetation and virtually removing all topsoil horizons. These disturbed sites had their topsoil and vegetation removed and, consequently, the community composition between preserved and disturbed sites is drastically different (Chapter 3; Le Stradic et al. 2018b). For the present study, we chose four paired disturbed and preserved sites of at least 100 m^2 each. In all sites, the paired disturbed and preserved sites were adjacent at approximately 30 meters away from each other (i.e. thus away from site margins) and paired sites spaced by at least 2 km from each other. All permissions 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.

3.2.*Diaspores from native species*

We used diaspores from five native species commonly found in the study area to compare removal rates in disturbed and adjacent preserved sites (Appendix S1). We chose these species because of the potential attractiveness of their diaspores for frugivores (both vertebrates and invertebrates), but also due to diaspores availability (between 600 to 1000 diaspores per species approximately), and phylogenetic and morphological diversity. We used seeds of *Stryphnodendron gracile* (Fabaceae) and *Davilla elliptica* (Dilleniaceae) in our experiment because their dry fruits are not consumed by the ground-foraging fauna. We used berries of three species: *Miconia irwinii* (Melastomataceae), *Byrsonima vacciniifolia* (Malpighiaceae) and *Coccoloba cereifera* (Polygonaceae), all of which a primarily dispersed by vertebrates.

Stryphnodendron gracile is a Brazilian endemic small shrub, found exclusively in the cerrado biome (Occhioni 1990). It typically produces seeds characterized by a very rigid seed coat (De Lima 1985). *Davilla elliptica* is widespread species in the cerrado biome (Fraga 2012*)* and is and produces fruits that contain up to two seeds surrounded by an aril (Pott & Pott 1994). *Miconia irwinii* is a Brazilian endemic treelet found exclusively in rocky outcrops at *campo*

rupestre, with single plants producing up to 4,000 small, water- and sugar-rich purplish-blackberries that are primarily dispersed by birds, and secondarily dispersed by ants (Guerra et al. 2017, 2018). *Byrsonima vacciniifolia* is a Brazilian endemic treelet found exclusively in the biomes cerrado and caatinga (Mamede & Francener 2015). *Byrsonima vacciniifolia* typically produce water- and sugar-rich yellow fruits with a striking aroma (Leal et al. 2007). *Coccoloba cereifera* is a narrowly distributed endemic species from sandy soils at *campo rupestre* in southeastern Brazil (Ribeiro & Fernandes 1999). It typically produces small fleshy violet fruits (Barroso et al. 1978). We collected the native diaspores during the fruiting period of each species and set them in the refrigerator (-4°C) until the moment of the experiment.

3.3.*Sampling design*

We performed randomized block factorial experiments to compare diaspore removal between disturbed and preserved sites, and between vertebrate-exclosure and control treatments. We used wire frames to create a treatment accessible to invertebrates and vertebrates (control treatment) and wired cages to create treatments to exclude vertebrates but accessible to invertebrates (vertebrate-exclosure treatment) (Appendix S2). We performed five experiments separately, one for each species, but simultaneously in all sites for each species. We paired our samples in four disturbed sites and four preserved neighboring sites. In each disturbed site, we set 12 sampling stations forming six blocks with the 12 sampling stations placed in the neighboring preserved site, totaling six samples for each treatment level in each site. Blocks consisted of four sampling stations, 1) controls in disturbed sites, 2) vertebrate-exclosure in disturbed sites, 3) controls in preserved site, and 4) vertebrate-exclosure in preserved sites. Sampling stations were distant nearly 1 m from each other within each site. To exclude vertebrates, we constructed wire exclosure cages (17 × 17 × 8 cm) fenced with wire mesh (1.2 cm) and wire frames constructed without mesh was used for controlling (i.e., access

to both vertebrates and invertebrates) possible effects of wire presence on diaspore removal (Guerra et al. 2018).

We conducted the experiment in the summer from December 2016 to March 2017, the period of higher foraging activity of ground-dwelling animals (Costa et al. 2018; Pol et al. 2011). Each sampling station consisted of diaspore piles placed in the ground over a filter paper, with the number of diaspores varying according to diaspore availability, but always controlling for the number of diaspores for each species. We placed 10 diaspores per sampling unit totaling 960 diaspores per species, with the exception of *Byrsonima* and *Davilla*, in which we placed eight and six diaspores per cage treatment, totaling 768 and 576 diaspores respectively. We evaluated diaspore removal rates in both cage treatments exhaustively searching for diaspores in the stations after 48h of exposure in the field (Guerra et al. 2018). The proportion of diaspores removed from each cage treatment (PDR) was calculated as: *PDR = (Nrc or Nre)/No*; where *No* is the number of offered diaspores, and *Nrc* and *Nre* are the number of recovered diaspores in the control treatment and in the cage treatment (vertebrate exclosure), respectively. No rain or strong winds occurred during the observation periods, reducing the possibility that seeds were lost due to abiotic factors.

To determine the identity of species interacting with diaspores, the frequency of interactions, and their behavior towards the diaspores, we performed direct observation on diaspore piles in disturbed and preserved sites. To do that, we used diaspore of the three species that presented the most significant removal rates in the first experiment: *Byrsonima vacciniifolia*, *Davilla elliptica* and *Miconia irwinii*.

For these species, we performed direct observations bouts totaling 20 hours for each species, equally distributed between the four disturbed and preserved sites. We obtained and handled the diaspores as described in the first experiment, but placed five diaspores of each species directly on the ground and without any structures around. We performed the observation bouts during the daytime, always between 10:00 AM and 05:00 PM. Each bout comprised 50

minutes of continuous observation performed by a single observer. We followed the animals that effectively removed diaspores from piles and recorded dispersal distances and the final destination (ant nest and preserved or degraded site) whenever possible. Ants were the only group of animals observed, thus were the focus of our subsequent experiments. Specimens were fixed in alcohol 70% and prepared for identification using the key provided by Baccaro et al. (2015) and compared the specimens to a reference collection from Insect Ecology Lab UFMG (Costa et al. 2016). Ant behavior was classified as follows: (1) removal, when displacing the diaspore further than 5 cm; (2) depulping, when consuming or removing diaspore pulp, with no removal; (3) interaction, when inspecting or manipulating diaspore, without removal (<5 cm).

3.4.*Statistical analyses*

We employed generalized linear mixed effects models (GLMMs, *glmer* function for non-normal datasets, with *lme4* package in R) with fixed and random effects to analyze the datasets of diaspore removal experiments (Bolker 2015). In each model, site types (disturbed *vs.* preserved), cage treatments (vertebrate-exclosure *vs.* control) and possible interactions among these factors were considered as predictive variables of fixed effects. Sampling blocks were nested within sites and grouped as random effects to account for the spatial heterogeneity of samples (Bates et al. 2014). The response variables were the proportions of diaspore removed after 48h of exposure in the field, separately for each species. We performed analyses assuming a binomial distribution error of response variable. Regarding the interactions between variables, when significant, we run post-hoc Tukey tests (Crawley 2013).

To test if network structure differs between site types (disturbed *vs.* preserved), we used two network metrics: interactions' Shannon diversity per site (H2 – see Bersier et al. 2002; Blüthgen et al. 2008) and robustness with regard to cumulative random extinctions of ant species (Memmot et al. 2004; Burgos et al. 2007). In our local networks (i.e., site level), consistent with other studies on ant-plant interactions (e.g., Costa et al. 2018), each interaction

frequency was computed based on the interaction between a disperser species with an individual diaspore, not the number of workers recruited per diaspore type. Hence, in each site we included all records from all interaction types that occurred between dispersers and the three diaspores types, to build weighted matrices with diaspore types as rows and dispersers species as columns and filled cells with the number of events observed between one diaspore species *i* and one disperser species *j*. Each matrix was used to compute the diversity of interactions and robustness. In total, we had eight matrices/networks that correspond to each site (n=4 per site type). Network metrics were obtained with the package bipartite for R-software (Dormann et al. 2008). Thus, each metric was fitted as response variable and site type as predictive variable in generalized linear models (GLM) (Crawley 2013). Furthermore, we computed multilayer networks comprising all types of events recorded between dispersers and diaspores to assess how disturbance might prompt distinct patterns of interactions outcomes. Hence, each layer corresponded to a distinct type of disperser-diaspora association, i.e., removal, depulping and interaction (see Costa et al., 2016 for a similar approach).

To test for differences in the seed dispersal effectiveness (SDE) between ant species present in disturbed and preserved sites, we constructed the SDE landscape (Schupp et al. 2010). We combined two variables involved in diaspore dispersal protection into an index of 'seed dispersal effectiveness' (SDE). This index ultimately defines the position of each ant on the overall SDE landscape characteristic of site type. We estimated the SDE for each ant species in each site using the formula: SDE = QTC x QLC. The quantitative component (QTC) corresponded to the frequency of interactions between each ant species and all available diaspores in each site type. The qualitative component (QLC) corresponded to the frequency of diaspore removed by each ant species. Thus, each ant species was classified according to the values of SDE. For each ant species, we computed a measure of total dispersal service that integrates the quality and quantity components of service offered. We considered highly-effective dispersers those species that had high values of SDE (high quantitative and qualitative

values); inefficient dispersers as those species that had low SDE values (low quantitative and qualitative values); and lowly-effective dispersers as those species that had intermediate SDE values (low quantitative values and high qualitative values or high quantitative values and low qualitative values).

We performed all statistical analyses in R (R Core Team 2017) and networks' drawings were prepared in Pajek 4.09 (Batagelj & Mrvar 1998).

4. Results

4.1. *48-hour removal trial experiment*

We found a great variation on removal rates between diaspore types, according to cage treatments and site types (Table 1). The removal for *Coccoloba* and *Stryphnodendron* were negligible, smaller than 0.1% in all sites and treatments (Table 1). Conversely, the percentage of diaspore removal was significantly higher for the other three plant species. For *Byrsonima* we found a total percentage of 32% of diaspore removal, ranging from 27% to 43% across site types and cage treatments (Table 1). For *Davilla*, we found a total percentage of 67% of diaspore removed, ranging from 48% to 83% (Table 1). For *Miconia*, we found a total of 52% of diaspore removal, which ranged from 47% to 57% along site types and treatments (Table 1). For *Byrsonima*, *Davilla* and *Miconia*, when considering the data of both cage treatments together, we have observed higher rates of diaspore removal in preserved sites (Table 1). For all diaspores types, the removal rates in the control treatments were 6% to 16% higher than in exclusion treatments, being the higher difference of 16% noted for *Byrsonima* in preserved sites (Table 1).

Considering the generalized linear mixed model (GLMM) tested, we found significant interactions between cage treatments and site types for *Byrsonima* (GLMM, $P<0.05$), with significantly high removal rates in control treatments in preserved sites (Fig. 1). *Davilla*

presented significant differences only between site types (GLMM, P < 0.01) and cage treatments (GLMM, P < 0.001), with significantly high removal rates in preserved sites and in control treatments (Fig. 1). *Miconia* presented significant difference only between cage treatments (GLMM, P < 0.001), with high removal rates in control treatments (Fig. 1).

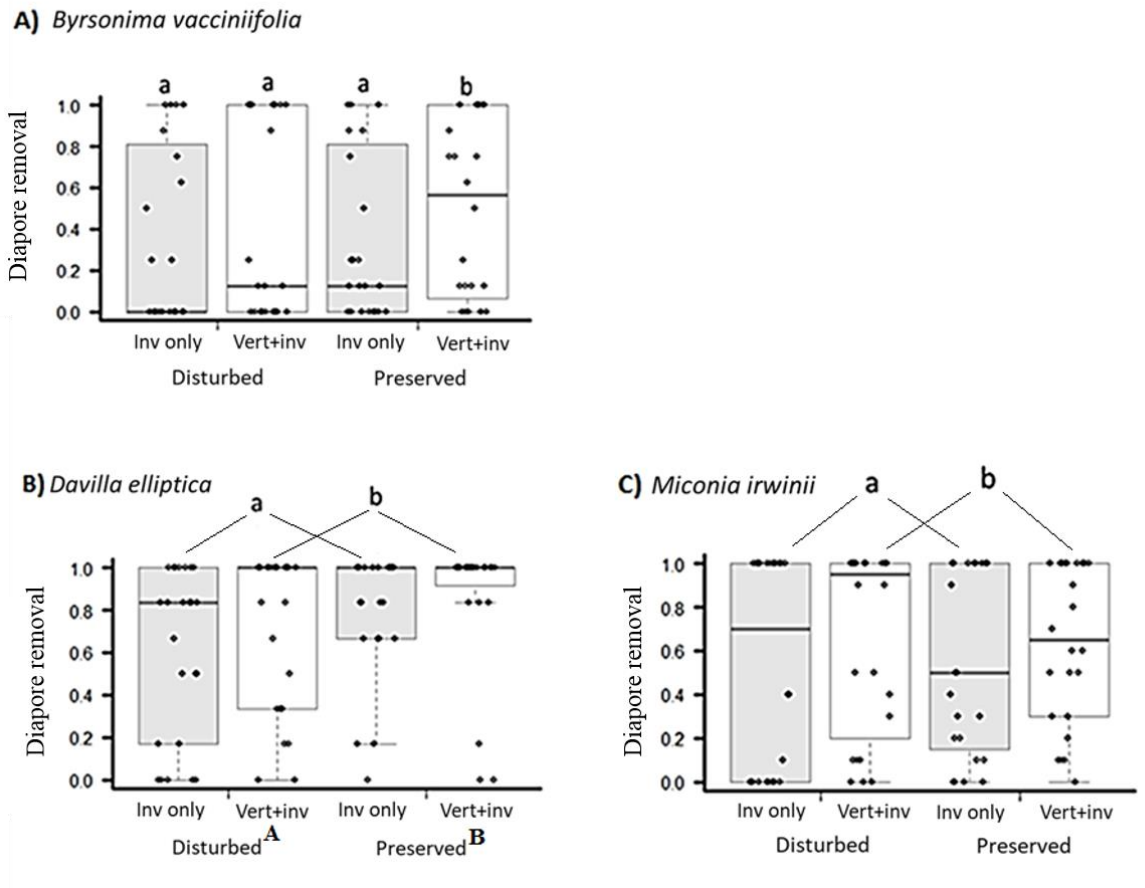


Figure 1. Diaspore removal rates in each cage treatment along disturbed and preserved sites of *campo rupestre* vegetation, eastern Brazil. (Dark gray squares: median; Rectangles: 25-75% percentile; Black Circles: outliers; Bars: non-outlier range). Different letters indicate statistical differences among treatments for each species (Upper case letters placed above the name of the site type indicate differences between site types; lowercase letters preceded by a line indicate differences between treatments; lowercase letters directly above bloxplots indicate significant interactions between treatments and sites). Inv only = access to invertebrates only = vertebrate

exclusion (or wired cage treatment); Vert+Inv = access to both vertebrates and invertebrates (wired frame control treatment).

4.2. *Diaspore observation experiment*

Ants were the only group of animals recorded interacting with the diaspores during the direct diurnal observations. For all diaspore types, we observed a higher number of diaspore-ant interactions in preserved sites (Table 2), with a significant difference between site types for all diaspores (GLMM, $P < 0.001$; Fig. 2).

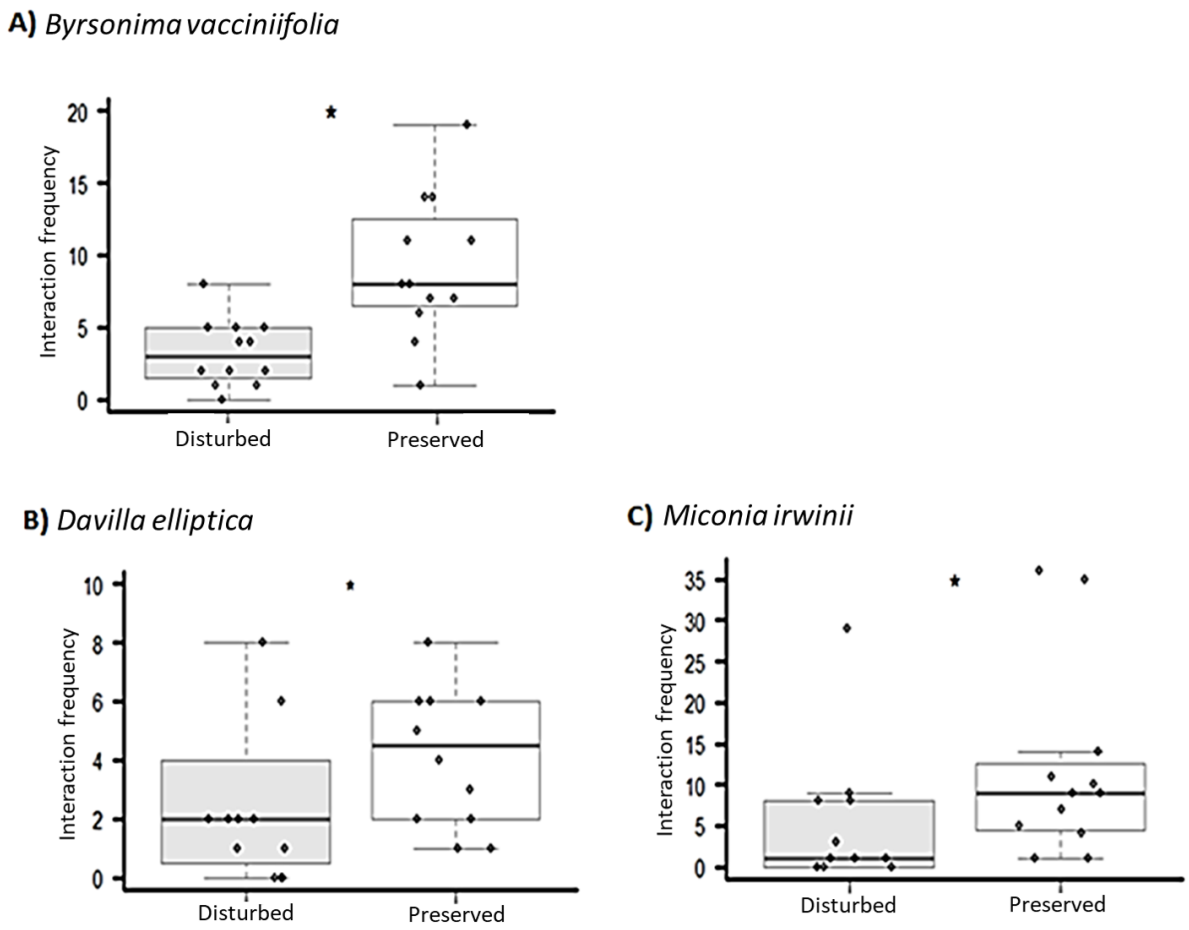


Figure 2: Interactions frequency at network level according to site type for three species from of *campo rupestre* vegetation, eastern Brazil (Black squares: median; Rectangles: 25-

75% percentile; Black Circles: outliers; Bars: non-outlier range; * represents significant differences between sites).

Byrsonima presented the higher number of depulping events (three in each site type), as well the higher number of diaspores taken into ant nests (two in disturbed sites and one in preserved sites) (Table 2). All diaspore types had at least one event of diaspore displacement from disturbed to preserved site, but no diaspore was transported from preserved to disturbed sites (Table 2). The maximum dispersal distance (42 meters) was observed for *Byrsonima* in a preserved site. We found that the mean dispersal distances were generally longer for *Byrsonima*, in preserved sites (Fig. 3), but no significant differences were found between site types for *Davilla* and *Miconia*.

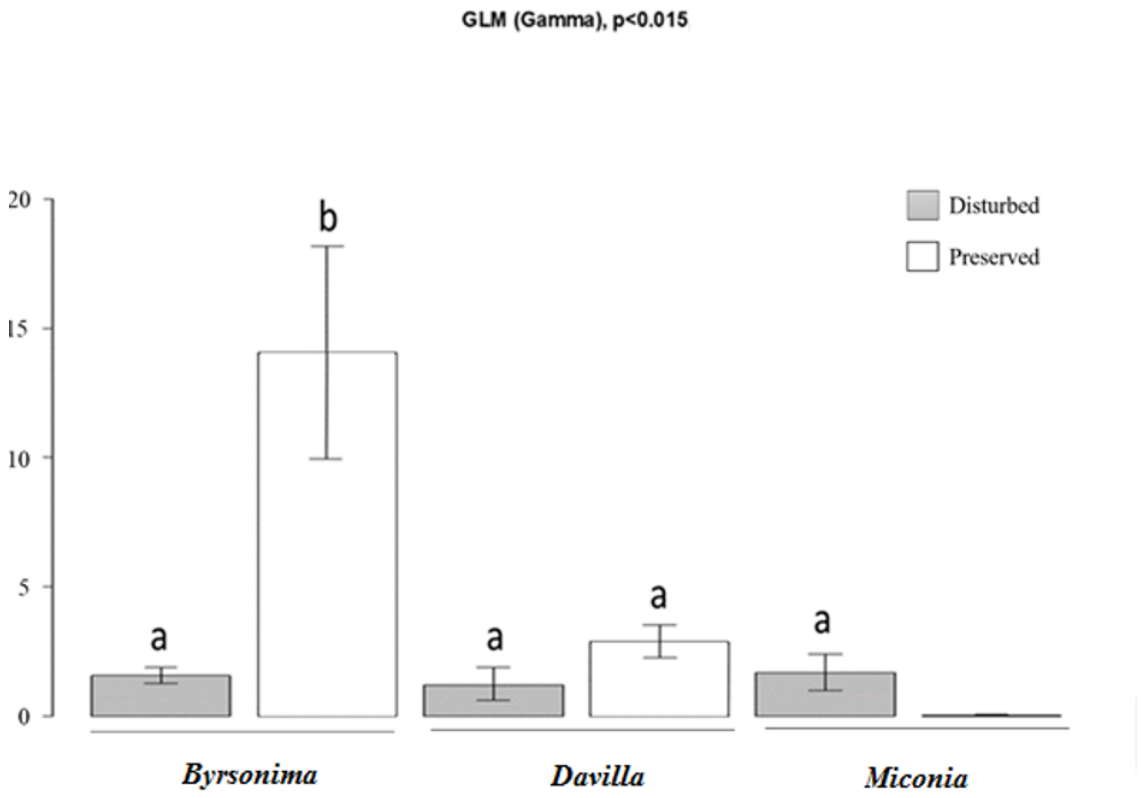


Figure 3: Mean dispersal distances for diaspores of *Byrsonima*, *Davilla* and *Miconia* in disturbed and preserved *campo rupestre* sites, southeastern Brazil.

4.3. *Ant-diaspore interactions*

We registered 20 ant species performing 477 interaction events with the diaspores studied, of which 65% were recorded in preserved sites (Appendix S3). From all records, 7% represent diaspore removal events and nearly 2% correspond to diaspore depulping (Table 2). The species *Crematogaster* sp1, *Pheidole oxyops*, *Pheidole triconstricta* had the highest total number of removals across all diaspores and sites (Appendix S3). Three ant species were only registered in disturbed sites: *Brachymyrmex cordemoyi*, *Dolichoderinae sp.* and *Ectatomma tuberculatum* (Appendix 3). *Pheidole triostricta* was the species responsible for 46% of diaspore interactions in disturbed sites (Appendix S3). We found that seed predator ants (*Pheidole* and *Dorymyrmex*) had their activities not reduced by the disturbance, with *Pheidole triostricta* even presenting higher number of interactions with diaspores in disturbed sites. Seven ant species were registered exclusively in preserved sites: *Brachymyrmex pictus*, *Camponotus rufipes*, *Camponotus sp1*, *Crematogaster sp1*, *Pheidole sp2*, *Pheidole sp3*, and *Pheidole sp4* (Appendix 3). *Crematogaster sp1* and *Pheidole triconstricta* were the species with the highest number of interactions with diaspores in preserved sites, representing 22% and 21% of all interactions records, respectively (Appendix S3). There was remarkable reduction by 100% and 88% of the activity of the seed predators *Crematogaster sp1* and *Dorymyrmex pyramicus* in disturbed sites, respectively (Appendix S3).

The seed removal effectiveness landscapes indicated that according to site type, different ant species are more effective in respect of the dispersal service provided to plants (Fig. 4).

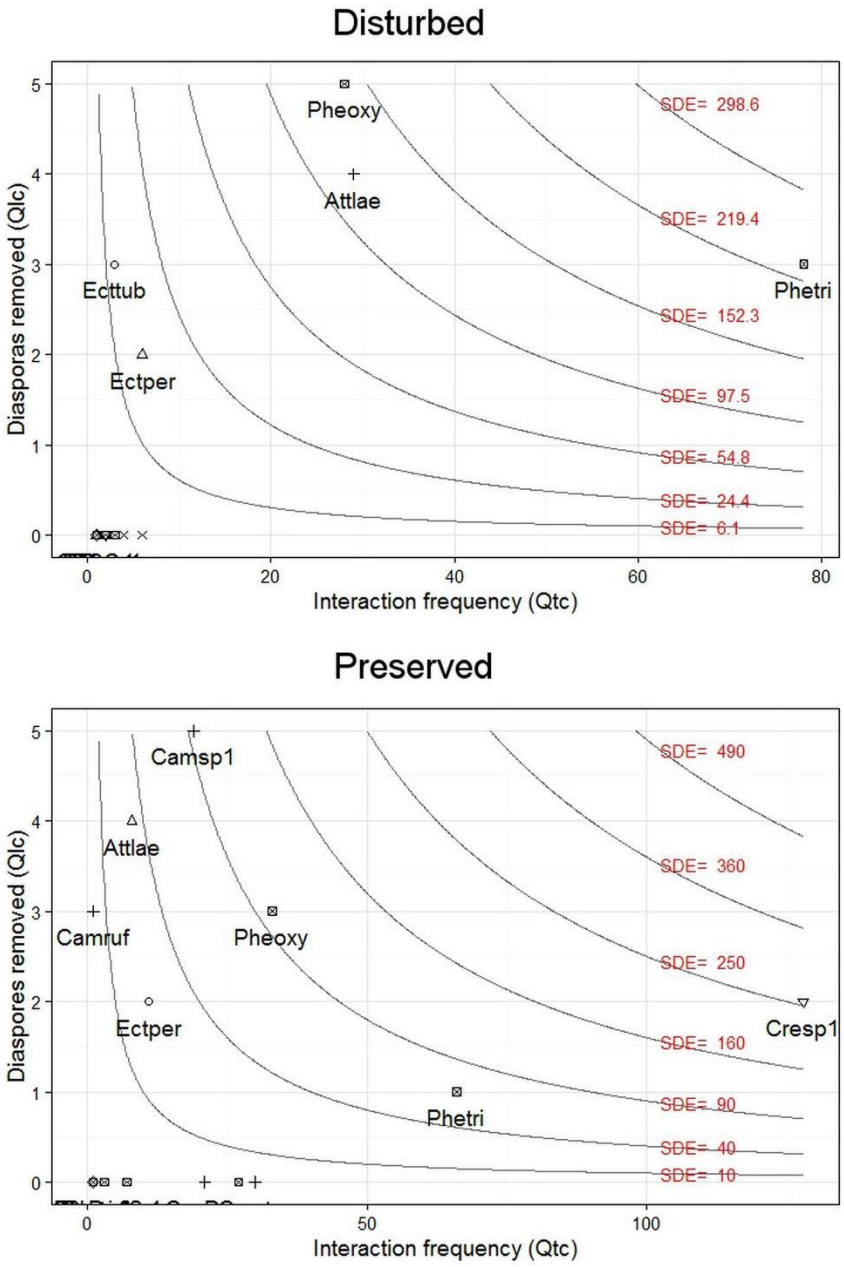


Figure 4: Diasporas dispersal effectiveness landscape (SDE=Quantity x Quality) of all ant species interacting with diaspores of *Byrsonima*, *Davilla* and *Miconia* in preserved and disturbed *campo rupestre* sites in Eastern Brazil. Isoclines represent all combinations of quantity and quality components with the same value of SDE. Symbols represent distinct functional groups of ant species Ants' codes and symbols definition can be found in Appendix S3.

In preserved sites *Pheidole oxyops*, *Camponotus sp1* and *Pheidole triconstricta* were highly-effective dispersers with high values of SDE (high quantitative and qualitative components values). On the opposite, in disturbed sites, the species *Pheidole oxyops*, *Atta laevigata* and *Ectatomma permagnum* were highly-effective dispersers with high values of SDE (high quantitative and qualitative values). The species *Pheidole oxyops* and *Atta laevigata* presented high values of qualitative component in both site types. *Ectatomma tuberculatum* presented high values of qualitative component only in disturbed sites, while *Camponotus sp1* reached high values of in preserved sites. For the quantitative component, we have found that *Crematogaster* sp1 and *Pheidole triconstricta* were the species that more contributed in preserved sites, while *Pheidole triconstricta, Atta laevigata* and *P. oxyops* presented the higher values in disturbed sites. *Atta laevigata* achieved distinct SDE values when comparing site types, with an average value of 3 times higher in disturbed than on preserved sites. The remaining species that do not appear in the landscape analysis presented very low SDE values.

We found that ant-diaspore networks are formed by three distinct types of interactions, which represent distinct ant behaviors upon the food source (Fig. 5).

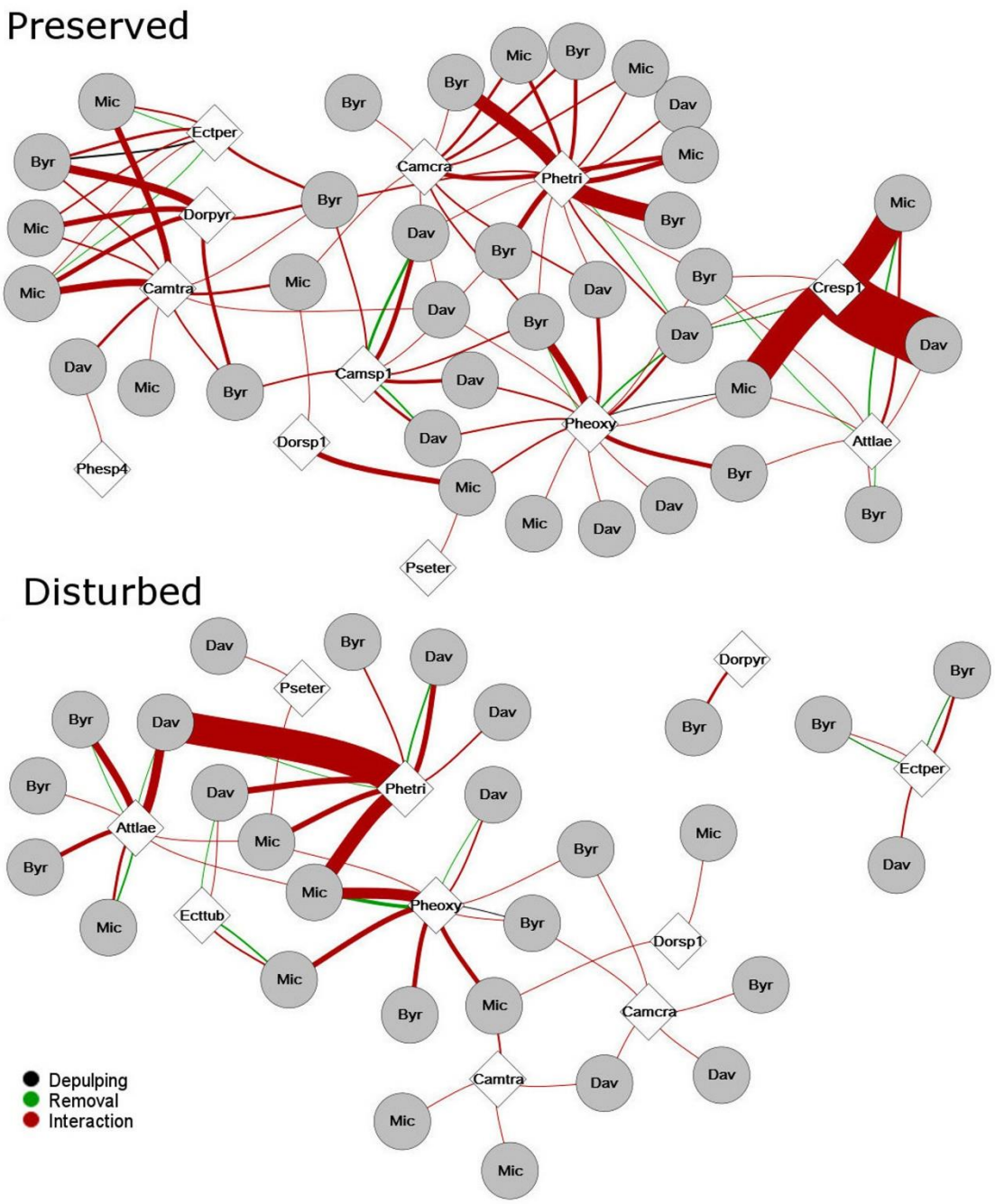


Figure 5: Ant-diaspore networks in preserved and disturbed *campo rupestre* sites, considering distinct types of interactions according to ant behavior (represented by distinct colors). Line width represents the frequency of interactions. Diamonds depict ant species, with ant codes provided in Appendix S3. Circles represent distinct stations inside each site. (*Interaction* = diaspore inspection or manipulation without pulp removal or diaspore displacement, *Depulping* = diaspore pulp removal; *Removal* = diaspore displacement further than 5 cm; Byr = *Byrsonima*; Dav = *Davilla*; Mic = *Miconia*).

Networks in preserved sites presented higher diversity of interactions and more robustness (i.e., a more stable structure under random and cumulative extinctions of ant species) when compared to networks from disturbed sites (Fig. 6).

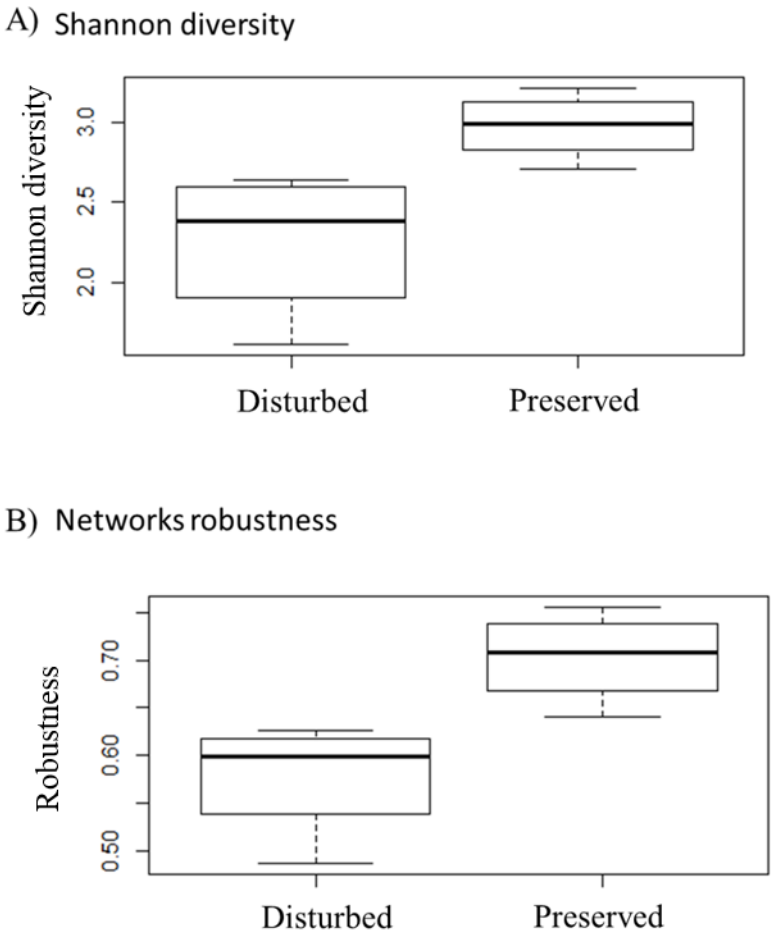


Figure 6: A) Interactions' Shannon diversity index and B) networks robustness under ant species cumulative extinctions for ant-diaspores networks in preserved and disturbed *campo rupestre* sites, eastern Brazil.

5. Discussion

We found that interactions between ground foraging animals and diaspores in *campo rupestre* can strongly influence plant dynamics and contribute to the typical low natural vegetation recovery observed after soil disturbance (Le Stradic et al. 2018b). Our data reveals

that the quantity and quality of secondary seed dispersal can greatly shift between plant species and site conditions. Our data supports that foraging animals can target specific diaspore species (Roselli 2014) and that ground foraging animals can respond in different ways to particular disturbances (Schowalter et al. 1999; Wikars & Schimmel 2001). Therefore, it seems that soil disturbance reshape the role of ground-foraging ants, which become a negative biological filter affecting the resilience of disturbed edaphic grasslands.

Soil disturbance seems to reshape diaspore interactions with ground foraging animals in *campo rupestre*, intensifying seed limitation and promoting dispersal limitation by: 1) decreasing the proportion of mutualistic agonistic interactions (seed depulping and dispersal); 2) increasing the activity of seed predator ants; 3) moving removed seeds from disturbed to preserved sites; 4) decreasing overall seed dispersal distance. The low diversity and robustness of animal-diaspore networks in disturbed sites reveals less stable and conserved animal-diaspore networks (Mello et al. 2011). The remarkable difference in the species composition between sites (35% of the ant species were exclusively found in preserved sites) and the significant intra- and interspecific differences in the seed dispersal effectiveness (SDE) between sites, reinforces that disturbance can strongly influence foraging animal population composition and behavior (Schoereder et al. 2004).

The remarkable removal rates for *Byrsonima* (almost a third of all diaspores removed), *Davilla* and *Miconia* (both with more than half of all diaspores removed), indicate that animal-diaspore interactions comprise an important ecological filter driving post-dispersal seed fate for these species and probably for many other plant species in *campo rupestre*. The marked reduction in the removal rates for *Davilla* in disturbed sites, indicates the higher vulnerability of the foraging animals' species that interact with *Davilla* seeds after soil disturbance. *Byrsonima* presented the higher quantity of depulping events and diaspores-taken-to-ant-nest observed. Likewise, *Byrsonima* diasporas accounted with the higher dispersal distances observed, signalizing a strong role of ground foraging animals on the diaspore fate for this

species, placing its diaspores as a good target for future studies exploring the role of seed-animal interaction outcomes on plant recruitment. The negligible removal rates for *Coccoloba* and *Stryphnodendron* do not mean that their diasporas do not represent a potential resource for ground foraging animals, but that in a period of 48 hours these diaspores are unlike to be removed in *campo rupestre*.

Diaspore animal interactions are commonly attributed to vertebrates in the literature (Schowalter 2016; Benítez-Malvido et al. 2016). However, our results reveal that secondary diaspores removal and interactions in *campo rupestre* seems to be mostly attributed to ants. Our findings are in accordance with studies that show ants as effective seed dispersers in Neotropical savannas (Christianini and Oliveira, 2009, 2010), as well in other OCBILs, such as fynbos and *kwongan* (Milewski & Bond 1982; Traveset & Rodríguez-Pérez 2008). Nevertheless, while our diaspore observation experiment was diurnal only, post-dispersal diaspore interactions with vertebrates in *campo rupestre* cannot be neglected. In fact, it is sustained by the significant differences found between cage treatments in both sites, with high removal rates for *Byrsonima*, *Davilla* and *Miconia* in the treatment where diasporas were accessible to both vertebrates and invertebrates. Post-dispersal diaspore interactions with vertebrates in *campo rupestre* are expected to be related manly to birds, small mammals and lizards (Lessa et al. 2013; Guerra et al. 2018). Such interactions likely occurred during the night and were not assessed by our methodology. Nevertheless, understanding the role played by vertebrates as secondary seed dispersers in natural regeneration should be examined in the future.

The ant species observed interacting with diaspores encompass well-represented ant genera in *campo rupestre*, such as *Pheidole*, *Camponotus*, *Crematogaster* and *Dorymyrmex* (Costa et al. 2015). The significant differences in the SDE value between ant species suggest specificity in the provision of important ecological services during this critical stage of plant recruitment (e.g. depulping, directed dispersal). The genera *Pheidole* displayed the highest interaction frequency and the highest number of removals and relevant values in SDE in both

site types, which place this ant genus as a key secondary disperser in campo *rupestre*. The high values of QLC (quality component) for the genus *Pheidole* and *Atta* in both sites, place these genera as well as key biotic agents (both as potential negative biotic filter) on seed fate in *campo rupestre*. The genera *Crematogaster*, *Pheidole* and *Componotus* presented the highest values for SDE in preserved sites, being crucial in structuring the network between diaspores and ground foraging animals in preserved areas of *campo rupestre*.

Pheidole triconstricta and *Pheidole oxyops* were the species with the highest number of interactions and SDE values in disturbed sites. As a common seed predator (Traniello 2010), *Pheidole* seems to be highly tolerant to soil removal disturbance in *campo rupestre* and may hamper natural recovery by seed predation (Denham 2008). Likewise, *Atta laevigata* presented a strong dispersal effectiveness in disturbed sites (with an average of SDE values 3 times higher in disturbed than in preserved sites), suggesting that this species benefits from disturbance (Vieira-Neto et al 2016). This species was largely responsible for moving diaspores from degraded to preserved sites, suggesting that it may deplete soil seed banks in degraded sites, and thus hamper natural regeneration.

The predominance of short dispersal distances in our experiments are in accordance with previous studies that show ants as short distances seed dispersers (Christianini & Oliveira 2010; Gómez & Spadaler 2013; Camargo et al. 2016). The majority of species observed are small ant species, endorsing that dispersal body size is a key trait to the outcomes of ant-plant interactions (Warren and Giladi 2014; Magalhães et al. 2018). Small ants usually only consume fruit pulp on the spot and do not remove the diaspores far away (Ness et al. 2004), which is confirmed by the low SDE values found for the majority of the small ant species. Our single observation of a long dispersal distance for *Byrsonima* (42 meters) was carried by *Atta laevigata*, the largest ant species observed, reinforcing that large ants are able to provide greater distances of dispersal than smaller ants (Ness et al. 2004). *Ectatomma* carried dispersal at relevant distances as well (approximately 10 meters), but different from *Atta* only appears in

the SDE in preserved sites. Still, depulping, that were mainly carried out by *Ectatomma*, *Crematogaster* and *Pheidole*, is an important service provided by ants because it decreases the chances of fungal attack (Ohkaware & Akino 2005), and creates conditions for germination of light-demanding seeds, as the case of the *Miconia* (Lima et al. 2013).

6. Conclusion

Our study is the first to look for the interspecific differences in dispersal removal by foraging animals in *campo rupestre*. Moreover, it comprises the first attempt to access the possible effects of topsoil disturbance on such key interactions for ecosystem recovery. Our results suggest that soil disturbance modify ant-diaspore interactions by decreasing positive and increasing negative interactions' outcomes, and therefore, precluding regeneration from seeds in soil seed banks. Our results also reinforces how habitat and species diversity are important for plant community assumptions on seed dispersal (Tilman, 1997). Consistent with our first expectation, disturbance resulted in structurally different networks involving diaspores and ground foraging animals. Our findings are in accordance with empirical studies that found great variability on post-dispersal seed fate among plant species and sites, depending on preservation level (Schupp & Fuentes 1995; Chapman & Chapman 1996; Lambert 2002).

Our results become even more relevant considering that *campo rupestre* is a seed-limited ecosystem (Dayrell et al. 2016), where any reduction in seed quantity may strongly compromises plant recruitment and natural regeneration (Calviño-Cancela 2007; Silveira et al. 2016; Chapter 3). Although topsoil disturbance apparently did not collapse diaspore animal interactions, it created contrasting outcomes in disturbed and preserved sites and affected robustness in diaspore animals network, which may be an indicative of collapse under disturbance intensity. These findings help to explain, at least partially, why natural recovery can be compromised after topsoil removal. The lack of ants nests in disturbed sites and diaspore

dispersal by ants towards disturbed sites may strong influence dispersal limitation and hamper natural recovery, providing a mechanistic explanation for high vulnerability to soil removal in edaphic grasslands (Hopper et al. 2016, Buisson et al. 2019). In summary, our study comes to integrate the development of better restoration and conservation practices, for example in maximizing establishment and persistence of desired species using seed sowing techniques (Turnbull et al. 2000; Chambers & MacMahon 1994) or by reintroducing ants dispersing seeds on disturbed areas (Bulot et al. 2014).

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Table 1: Percentage of diaspore removal for five plant species from *campo rupestre* vegetation in cage treatments (vertebrate-exclosure and control, i.e. open to both vertebrate and invertebrate) in preserved and disturbed sites, at Serra do Cipó, southeastern Brazil. See Figure 1 for differences between site types and cage treatments for species in bold.

	Cage treatments	Preserved	Disturbed
Byrsonima	Exclosure	27%	27%
	Control	43%	32%

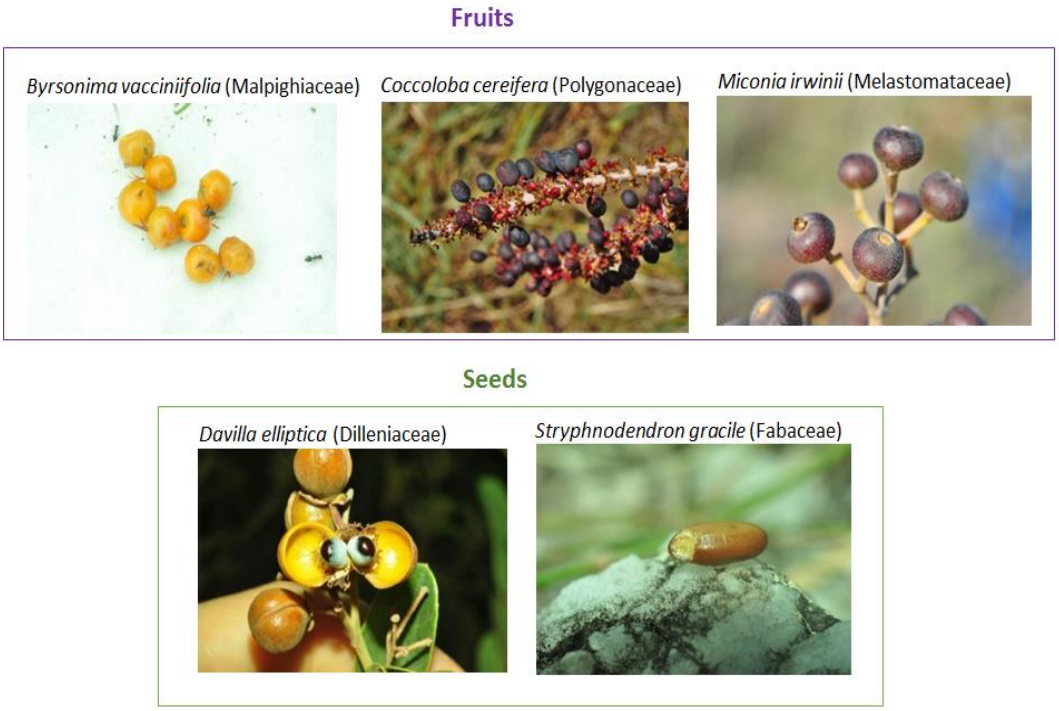
Davilla	Exclosure	78%	48%
	Control	83%	58%
Coccoloba	Exclosure	<0.1%	<0.1%
	Exclosure	<0.1%	<0.1%
Miconia	Exclusion	51%	47%
	Exclosure	57%	57%
Striphnodendron	Exclusion	<0.1%	<0.1%
	Control	<0.1%	<0.1%

Table 2: Number of ant-diaspore interaction types observed for three plant species from disturbed and preserved *campo rupestre* sites, eastern Brazil. Interaction types were classified as: *Interaction* = total number of animal diaspore associations without pulp or diaspore removal; *Depulping*: total number of events were ants removed diaspores pulp; *Site change*= total number of observations when the final diaspore destination was different from the site of origin; *Ants nest*= total number of diaspores taken into an ant nest.

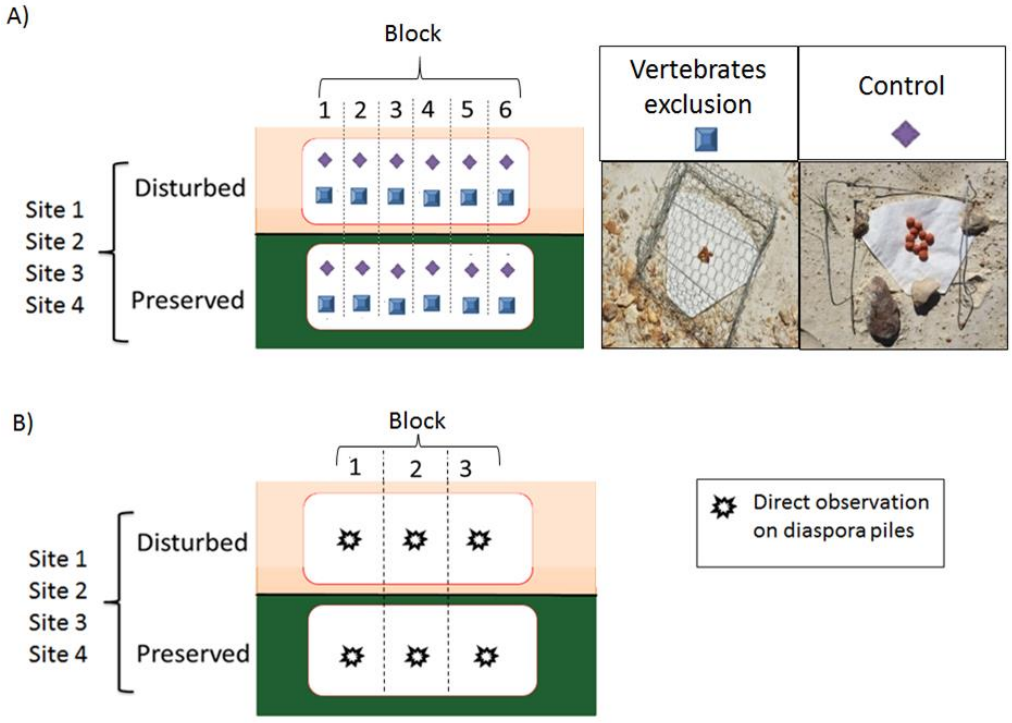
		Interaction	Removal	Depulping	Site change	Ants nest
Byrsonima	Disturbed	37	3	3	2	2
	Preserved	110	3	2	0	1
Davilla	Disturbed	68	6	0	1	1
	Preserved	108	13	1	0	1
Miconia	Disturbed	60	8	0	1	0
	Preserved	142	4	1	0	0

Supporting Information

Data S1. Photos of the diaspore species



Data S2. Experiment sampling design for the 48 hours' diaspore removal experiment and the focal experiment in disturbed and preserved *campo rupestre* sites.



Data S3. List of ant species interacting with diaspore of three plant species from disturbed and preserved *campo rupestre* sites, presenting: ant code used in the figures, functional group, number of interactions and/or removal events per ant species for each diaspore type between sites, and total number of interactions and/or removal events per ant species considering all diaspore types between sites (PR= preserved, DI=disturbed).

Ant species	**Ant code**	**Functional group**	***Byrsonima***		***Davilla***		***Miconia***		**TOTAL**	
			PR	**DI**	**PR**	**DI**	**PR**	**DI**	**PR**	**DI**
Atta laevigata	Attlae	Fungivorous leaf cutters	5	15	1	11	6	7	12	33
Brachymyrmex cordemoyi	Brapic	Ground and arboreal opportunistic	0	1	0	0	0	0	0	1
Brachymyrmex pictus	Brapic	Ground and arboreal opportunistic	1	0	0	0	0	0	1	0
Camponotus crassus	Camcra	Generalist patrol camponotines	7	3	3	2	11	0	21	5
Camponotus rufipes	Camruf	Generalist patrol camponotines	0	0	4	0	0	0	4	0
Camponotus sp1	Camsp1	Generalist patrol camponotines	7	0	17	0	0	0	24	0
Camponotus trapeziceps	Camtra	Generalist patrol camponotines	5	0	4	1	21	4	30	5
Crematogaster sp1	Cresp1	Omnivorous arboreal	0	0	0	0	66	0	66	0

Dolichoderinae sp1	Dorsp1	-	0	1	0	0	0	0	0	1
Dorymyrmex pyramicus	Dorpyr	Omnivorous ground dominants	16	3	0	0	11	0	27	3
Dorymyrmex sp1	Dorsp1	Omnivorous ground dominants	0	0	0	0	7	2	7	2
Ectatomma permagnum	Ectper	Epigaeic generalist predators	6	4	0	4	5	0	11	8
Ectatomma tuberculatum	Ecttub	Epigaeic generalist predators	0	0	0	1	0	2	0	3
Gnaptogenys sp1	Gnasp1	Epigaeic generalist predators	1	1	0	0	0	0	1	1
Pheidole oxyops	Pheoxy	Omnivorous ground dominants	15	2	14	2	4	24	33	28
Pheidole sp2	Phesp2	Omnivorous ground dominants	0	0	1	0	0	0	1	0
Pheidole sp3	Phesp3	Omnivorous ground dominants	0	0	2	0	0	0	2	0
Pheidole sp4	Phesp4	Omnivorous ground dominants	0	0	1	0	0	0	1	0
Pheidole triconstricta	Phetri	Omnivorous ground dominants	48	7	5	49	12	22	65	78
Pseudomyrmex termitarius	Pseter	Ground specialist	0	0	0	1	1	1	1	2

GENERAL DISCUSSION

1. Studying seed dispersal in grasslands

Our data supports that seed dispersal is a complex process influenced by a vast diversity of intrinsic (e.g. poor dispersal ability) and extrinsic variables (e.g. human disturbance), making direct measurements a challenging and timing consuming process (Robledo-Arnuncio et al. 2014). Our study not only simply quantify dispersal rates and kernels of single species, but assess how seed dispersal metrics can vary across plant species, environmental conditions and time, which is crucial for seed dispersal inferences for the plant community (Ronce 2007).

Our results show that biological (e.g. diaspore species), temporal (e.g. seasons) and spatial aspects (e.g. disturbance) can greatly influence the qualitative and quantitative aspects of seed dispersal, reinforcing them as critical aspects to understand relevant biotic and abiotic factors driving plant dispersal (Kraft & Ackerly 2014; Thomson et al. 2011; Tamme et al. 2014; Robledo-Arnuncio et al. 2014).

The guidelines and protocols for methodology and data reporting proposed in this thesis come to foster the quality, transparency, reproducibility, and value of seed dispersal studies. We clearly demonstrated that the lack of justification for the employment of different methods and incomplete data reporting can strongly hampers our ability to compare results among studies, hence preventing a better appreciation of the role played by seed dispersal in plant community assembly.

2. **Seed dispersal in *campo rupestre***

2.1 *Seed rain*

Our study about seed rain in *campo rupestre* represents an important contribution for seed rain studies on tropical grasslands. 1) The seed image bank presented in this work is the first effort to build a database for *campo rupestre* plant community and future efforts are needed

to build a more robust image bank. 2) The overall low averages of seed rain density per day registered for *campo rupestre* and the remarkable low number of seeds found for 90% of morphospecies, corroborates with the predictions of OCBIL theory, which expect an accentuated dispersal limitation on these ancient ecosystems (Hopper et al. 2016; Silveira et al. 2016). These findings support the idea that strong dispersal limitation is linked with low resilience in *campo rupestre* and is reinforced by significant low averages of seed rain in disturbed areas. On the other hand, some relatively abundant plant genera and families in the seed rain, placing them as good targets for future research on seed ecology and restoration projects.

The seed limitation in the seed rain in *campo rupestre* may be closely related to life-history traits associated with the low dispersal ability of plant communities considering that most plant species in *campo rupestre* are resprouters after fire (Le Stradic et al. 2018a). Resprouters normally present low seed production in comparison to nonsprouting species (Lamont & Wiens 2003; Lamont et al. 2011). Additionally, *campo rupestre*, as an OCBIlL (Hopper et al. 2009), may present a predominance of short distance dispersal events, hampering seed migration to disturbed areas (Thomson et al. 2011; Török et al 2018). Thus, on short timescales, community assembly in *campo rupestre* seems to be dispersal limited and new seeds that would arrive from external seed sources to disturbed sites occurs only in a low frequency and in small numbers.

The lack of effective dispersal mechanisms in most *campo rupestre* species may explain the low rates of anemochorus seeds retrieved in disturbed areas, from which long distance dispersal is expected (Thomson et al. 2011). Additionally, the fact that only few diaspores of five species producing berries were retrieved in seed traps, suggests that even species dispersing seeds for long distances seem not to arrive frequently in disturbed sites (Guerra et al. 2017; Chen et al. 2019). On the other hand, water-dispersed species may be a key element plant

community assembly in disturbed sites in *campo rupestre*, which is in accordance with studies that shows the great participation of water as a seed dispersal vector (Ozinga et al. 2009; Merritt et al. 2010; Fraaije et al. 2015). We should thus expect that adaptations promoting seed flotation (Van der Pijl 1972) may be crucial for many seeds in *campo rupestre* to travel large distances and may play a key role on plant community assembly, although at this point, this remains speculative.

2.2 *Secondary seed dispersal*

Our study is the first to look for the differences of disperser species role in dispersal effectiveness under the effect of soil disturbance in *campo rupestre*. Our findings are in accordance with empirical studies that found great variability on post-dispersal seed fate among plant species and sites depending on conservation status (Schupp & Fuentes 1995; Chapman & Chapman 1996; Lambert 2002). Our results reinforce that in seed dispersal systems involving seed-harvesting ants, the patterns of seed predation and seed dispersal are highly dependent on seed attributes (Schupp et al. 2010; Arnan et al. 2012). Consistent with our first expectation, disturbance prompted structurally different networks of interactions between diaspores and ground foraging animals, reinforcing that disturbance can strongly influence foraging animal population composition and behavior (Schoereder et al. 2004).

The significant rates of ground foraging animal interactions found for some diaspores in *campo rupestre,* indicates that secondary seed dispersal can strongly influence plant dynamics and contribute to the typical low natural vegetation recovery observed after soil disturbance (Le Stradic et al. 2018b). Although topsoil disturbance apparently did not collapse diaspore animal interactions, it created contrasting outcomes in disturbed and preserved sites and affected robustness which may be indicative of collapse under disturbance intensity (Mello et al. 2011). Our data reveals that the quantity and quality of secondary seed dispersal can greatly shift between plant species and site conditions, which is in accordance with studies that

shows that foraging animals can target specific diaspore species (Roselli 2014) and that ground foraging animals can respond in different ways to particular disturbances (Schowalter et al. 1999; Wikars & Schimmel 2001). Therefore, it seems that soil disturbance reshapes the role of ground-foraging ants, which can become a negative biological filter affecting the resilience of disturbed edaphic grasslands. Our results are in accordance with studies that show rapid change of ant communities along different habitats conditions (Retana & Cerda 2000; Manzaneda et al 2007).

However, it still unclear to what extent plant community assembly is influenced by secondary seed removal in *campo rupestre* and future efforts are need to better understand of how animal-diaspore interactions in *campo rupestre can* favor or hamper plant recovery in disturbed areas. Further experiments should study seed dispersal 1) at night, when animals other than ants may be active, and 2) during the day using camera traps, in order to avoid animals being scared by the presence of observers watching seed removal. Finally seed removal should be studied on more plant species, such as Cyperaceae and Poaceae, which are dominant in such grasslands (Le Stradic et al. 2012).

3. *Implications for conservation and restoration*

Despite considerable progress in recent decades, we still face great challenges to improve our knowledge and increase the use of seed dispersal studies to better support grassland restoration (Arruda et al. 2018). More research on restoration of tropical and subtropical grasslands is required due to the fact that techniques used for temperate grassland restoration are not successful in restoring tropical ones (Le Stradic et al. 2018b). Species spontaneous colonization is a fundamental process in community dynamics and of high relevance for a better understanding of ecosystem resilience (Bakker et al. 1996; Török et al. 2018). Unveiling

ecological process linked to grasslands resilience, such as where, when and why seed limitation can occur, is extremely important for developing successful restoration strategies.

In some disturbed areas of *campo rupestre*, due to intense disturbances linked to soil removal by gravel exploitation, the natural recovery success strongly relies on the arrival of new diaspores and in the seed fate after primary removal. Patterns and outcomes of seed rain and seed bank dynamics are thus crucial in plant community assembly and are highly relevant processes that have to be carefully incorporated in restoration planning (Török et al. 2018). Our study about seed rain in *campo rupestre* brings the first information about the reliability of spontaneous seed dispersal to preserved and disturbed areas of *campo rupestre*, and represents an important step to understand how disturbance can hamper plant recovery in these disturbed areas. Additionally, we provide important information about the fate of seeds that have managed to arrive in the disturbed sites, which may be picked up by ants and brought back to native grassland areas. This is another important component of dispersal limitation towards community reassembly (Török et al. 2018).

Our findings indicate that the intrinsic seed dispersal limitation of *campo rupestre* can be even stronger in disturbed areas, reinforcing that prior to restoration, seed rain and pre- and post-dispersal seed predation need to be evaluated in order i) to assess the potential for regeneration or passive restoration and ii) to plan restoration actions; which should also be included in long-term post-restoration monitoring programs whenever possible (Jacquemyn et al. 2011; Pardini et al. 2017). Our data also sustain that the distinction of seed dispersal patterns between preserved and disturbed areas may be crucial to directing subsequent management and restoration efforts, evaluating: the loss of propagule sources, dispersal vectors, connectivity and animal interactions with diaspores; which all may influence seed dispersal success (Brederveld et al. 2011; Fraaije et al. 2015).

Advances in plant dispersal research will be determined by our ability to surmount challenges of not only temporal and spatial scale (e.g. habitat heterogeneity) but system complexity (e.g. evolutionary background) (Robledo-Arnuncio et al. 2014). Future collaborative efforts, increasingly pool data and expertise from multiple disciplines, are paramount for a global assessment of the role played by seed dispersal in restoration ecology. Future perspectives for restoration and conservation practices in *campo rupestre* may include seed sowing techniques, and maximizing establishment and persistence of desired species (Turnbull et al. 2000; Chambers & MacMahon 1994).

A better understanding of seed rain and secondary seed removal interactions outcomes, analyzing the effects of such interactions on plant community assembly, represents an exciting challenge for future experimental studies of seed dispersal in *campo rupestre*. Reintroducing seed-dispersing ants into disturbed areas has been carried out previouly (Bulot et al. 2014; Török et al. 2018). It could also be tested in *campo rupestre*, but seed dispersal directions should then be studied. Considering the viability of seed sowing practices, it has been demonstrated that the seeds of several species in *campo rupestre* are dormant, unviable, or empty (Le Stradic et al. 2018b; Dayrell et al. 2017). Further studies about seed quality are necessary to shed a light on this matter, which may allow a better understanding of the reliability of seed sowing to recover *campo rupestre* vegetation after human-caused disturbance.

4. **Final remarks**

I consider that in this thesis I could critically evaluate our current knowledge about seed rain in grasslands, proposing better practices in seed dispersal studies and contributing to our knowledge about mechanisms and factors that influence the dispersal filter in a threatened and old-growth grasslands (Fig. 1).

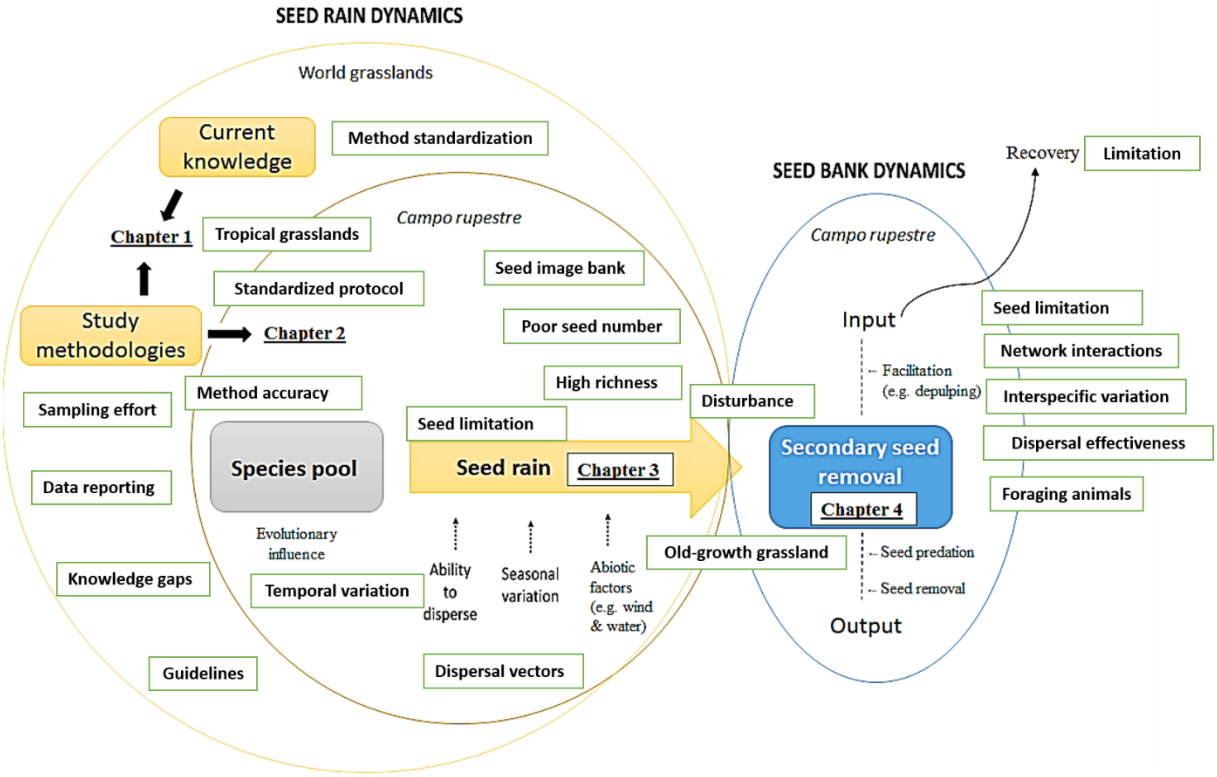


Figure 1: Spatial representation of the main thesis findings according to thesis chapters and knowledge areas, indicated by key words in green boxes.

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