

Universidade Federal de São Carlos
Centro de Ciências Biológicas e da Saúde
Programa de Pós-graduação em Ecologia e Recursos Naturais

Vanessa Mariano da Silva

Efeitos de distúrbios antrópicos na regeneração de plantas

São Carlos -SP

2020

Universidade Federal de São Carlos
Centro de Ciências Biológicas e da Saúde
Programa de Pós-graduação em Ecologia e Recursos Naturais

Vanessa Mariano da Silva

Efeitos de distúrbios antrópicos na regeneração de plantas

Tese de Doutorado

Tese apresentada ao Programa de Pós-graduação
em Ecologia e Recursos Naturais da Universidade
Federal de São Carlos, para obtenção do título de
Doutora em Ciências

Orientador: Alexander V. Christianini

São Carlos - SP

2020



UNIVERSIDADE FEDERAL DE SÃO CARLOS

Centro de Ciências Biológicas e da Saúde
Programa de Pós-Graduação em Ecologia e Recursos Naturais

Folha de Aprovação

Assinaturas dos membros da comissão examinadora que avaliou e aprovou a Defesa de Tese de Doutorado da candidata Vanessa Mariano da Silva, realizada em 19/03/2020:

Prof. Dr. Alexander Vicente Christianini
UFSCar

Profa. Dra. Sonia Cristina Juliano Gualtieri
UFSCar

Profa. Dra. Andrea Lucia Teixeira de Souza
UFSCar

Profa. Dra. Camila de Toledo Castanho
UNIFESP

Profa. Dra. Alessandra Tomaselli Fidelis
UNESP

Certifico que a defesa realizou-se com a participação à distância do(s) membro(s) Sonia Cristina Juliano Gualtieri, Camila de Toledo Castanho, Alessandra Tomaselli Fidelis e, depois das arguições e deliberações realizadas, o(s) participante(s) à distância está(ão) de acordo com o conteúdo do parecer da banca examinadora redigido neste relatório de defesa.

Prof. Dr. Alexander Vicente Christianini

Índice

Agradecimentos	6
Resumo	7
Abstract.....	9
Introdução geral.....	11
Objetivos.....	14
Métodos	14
Referências	17
Capítulo 1: <i>Meta-analysis of human disturbance effects on seed germination under field conditions</i>	21
Abstract	22
Introduction	24
Methods	27
Results	30
Discussion	31
Supporting Information	36
Acknowledgments	37
Literature Cited.....	38
Figures	47
Appendix A	48
Appendix B.....	63
Capítulo 2: <i>Meta-analysis of human disturbance effects on seedling survival under field conditions</i>	66
Abstract	67
Introduction	69
Methods	72
Results	76
Discussion	78
Acknowledgements	83
References	84
Figures	89
Appendix A	92
Appendix B.....	96

Capítulo 3: <i>Microhabitat characteristics, seed germination and seed removal in small Atlantic Forest fragments and restoration areas</i>	108
Abstract	109
Implications for practice.....	110
Introduction	111
Methods	113
Results	118
Discussion	119
References	124
Figures	132
Appendix A	134
Conclusões gerais	138

Agradecimentos

Agradeço à toda a minha família pelo grande apoio desde sempre, em especial aos meus pais Lucia e Ademir, ao meu marido Matheus, ao meu irmão Lucas, à minha querida avó Amélia (*in memoriam*) e à minha tia-avó Iá. Aos meus grandes amigos da vida e da pós-graduação pelo incentivo constante e pela ajuda em discussões científicas e filosóficas.

Ao meu orientador Alexander Christianini pela oportunidade de desenvolver mais um projeto de pesquisa e por todas as conversas, sugestões e discussões produtivas. Agradeço por compartilhar conhecimentos e por incentivar e apoiar novas experiências e conquistas acadêmicas.

À minha supervisora Jessica Gurevitch pela recepção em seu laboratório de pesquisa durante todo o período do doutorado-sanduíche e por compartilhar seu grande conhecimento e experiência. Agradeço também à Stony Brook University e aos membros do Department of Ecology and Evolution, em especial à Donna, Martha e Bob pela oportunidade e disposição em ajudar sempre que foi necessário. Sou grata aos membros do laboratório que frequentei, assim como aos colegas Kristjan, Nicolle, Liza e Mihir pela companhia e auxílio durante todo esse período.

Agradeço ao Programa de Pós-Graduação em Ecologia e Recursos Naturais e à Universidade Federal de São Carlos pela oportunidade do doutorado e pelo apoio institucional. À CAPES pela bolsa de pesquisa concedida e por possibilitar a realização do doutorado-sanduíche no exterior (PDSE). À todos os docentes e discentes que ajudaram na minha formação e na definição e desenvolvimento desse projeto de pesquisa. Aos docentes que compuseram as bancas de qualificação e defesa, Camila Castanho, Sônia Gualtieri, Andreia de Souza, Tatiana Cornelissen, Marco Batalha e Luciano Lopes, pelas grandes contribuições para essa tese.

Resumo

Distúrbios antrópicos, como a fragmentação de habitats, os efeitos de borda, invasão biológica, corte seletivo e pastoreio, frequentemente alteram condições bióticas e abióticas, potencialmente afetando a regeneração de plantas. Porém, não há sínteses da literatura avaliando como os distúrbios antrópicos afetam a germinação de sementes e a sobrevivência de plântulas. Também há pouca informação sobre os efeitos dos distúrbios no recrutamento inicial de plantas da Mata Atlântica. Nós conduzimos duas meta-análises com dados globais para avaliar os efeitos dos distúrbios antrópicos na germinação e sobrevivência de plântulas e a influência de fatores como o tipo de distúrbio, vegetação, características das espécies e filogenia na resposta das espécies aos distúrbios. Além disso, realizamos experimentos em campo para comparar características de micro-habitats, germinação e remoção de sementes de plantas nativas no interior e bordas de pequenos fragmentos de Mata Atlântica e em áreas sujeitas à restauração florestal. Nossos resultados indicam um efeito geral negativo de atividades humanas na germinação de sementes, sendo que invasão biológica apresentou o efeito mais negativo. A germinação em savanas e fisionomias campestres foi afetada negativamente em locais perturbados, assim como a emergência de plântulas de espécies não-arbóreas. Por outro lado, distúrbios antrópicos não apresentaram um efeito consistente na germinação de espécies florestais ou em árvores. Síndrome de dispersão e peso da semente não influenciaram a resposta das espécies. De acordo com nossa meta-análise sobre sobrevivência de plântulas, as respostas das espécies vegetais foram altamente heterogêneas e os distúrbios antrópicos não apresentaram um efeito geral na sobrevivência de plântulas. A variação dos efeitos não foi relacionada com nenhuma das características das espécies, da vegetação ou com a interação entre elas. Relações filogenéticas entre espécies não afetaram os resultados das nossas meta-análises. Nossos

experimentos de campo em pequenos fragmentos de Mata Atlântica do sudeste do Brasil indicaram que a cobertura do dossel e a umidade de solo diferiram entre o interior de pequenos fragmentos, bordas e áreas de restauração, sendo que as áreas de restauração apresentaram os menores valores. A germinação de sementes foi extremamente baixa em todos os tipos de habitat para todas as 13 espécies de plantas estudadas. Os índices de remoção de sementes foram maiores do que 45% de todas as sementes adicionadas e variaram de acordo com a espécie de planta e com o tratamento de exclusão de vertebrados. No geral, nossos resultados sugerem que distúrbios antrópicos geralmente diminuem a germinação de sementes e espécies não-arbóreas de fisionomias não-florestais provavelmente são o grupo mais susceptível. Os efeitos dos distúrbios na sobrevivência de plântulas foram difíceis de prever usando abordagens baseadas em nichos ecológicos, indicando que a estocasticidade e a variabilidade em uma escala de micro-habitat podem ser importantes fontes de heterogeneidade na resposta das plântulas. Nossos experimentos de campo corroboraram a variação de condições ambientais em micro-habitats e as restrições para a emergência de plântulas e recrutamento em pequenos fragmentos e áreas de restauração florestal. Portanto, práticas de conservação e restauração que focam no recrutamento inicial de plantas, como a adição direta de sementes, devem considerar a remediação de distúrbios antrópicos para com isso aumentar o sucesso destas iniciativas.

Palavras-chave: germinação de sementes, sobrevivência de plântulas, Mata Atlântica, remoção de sementes, micro-habitats, fragmentação, invasão biológica, efeitos de borda, restauração ecológica.

Abstract

Human disturbances like habitat fragmentation, edge effects, biological invasions, selective logging and grazing often alter biotic and abiotic conditions, potentially affecting plant regeneration. However, there was no research synthesis evaluating how human disturbances affect germination and seedling survival and there is also scarce information about disturbance effects on plant regeneration in biomes such as the Atlantic Forest. We performed two global meta-analyses to evaluate human disturbance effects on seed germination and seedling survival and the influence of disturbance type, vegetation, species traits and phylogeny on species responses to disturbance. In addition, we conducted field experiments to compare microhabitat conditions, seed germination and seed removal of native plant species in the interior and edges of small Atlantic Forest fragments and areas under ecological restoration. Our results indicate an overall negative effect of human disturbance on seed germination, and biological invasion had the largest negative impact. Germination in savannas, shrublands and grasslands was negatively affected by disturbed conditions, as well as seedling emergence of non-tree species. On the other hand, human disturbance did not have a consistent effect on germination of forest species and trees. Dispersal syndrome and seed mass had no effect on species responses. According to our meta-analysis about seedling survival, species responses were highly heterogeneous and human disturbances had no overall effect on seedling survival. The variation in effect sizes was not related to any of the species traits, habitat characteristics or their interactions. Phylogenetic relationships between species did not bias the results in any of our meta-analysis. Our field experiments in an Atlantic Forest landscape in southeastern Brazil indicated that canopy cover and soil moisture differed among the interior of small forest fragments, edges, and restoration areas, with the latter presenting the lowest mean values. Seed

germination rates were extremely low in all habitat types for all 13 species studied. Seed removal rates were over 45% of all added seeds and varied according to species and vertebrate exclusion treatment. Our main results suggest that human disturbances generally decrease seed germination, and non-trees from open physiognomies are probably the most susceptible group. Seedling survival responses to disturbance were hard to predict using niche-based approaches, indicating that stochasticity and variability at a microhabitat scale might be important sources of heterogeneity on seedling responses. Our field experiments corroborated the variation of abiotic conditions at a microhabitat scale and the constraints to seedling emergence and recruitment at small fragments and restoration areas. Therefore, conservation practices or restoration efforts focusing on early recruitment, such as direct sowing of seeds, should consider the amelioration of disturbed conditions to enhance the success of those initiatives.

Keywords: seed germination, seed addition, seedling survival, Atlantic Forest, seed removal, microhabitat conditions, fragmentation, biological invasion, edge effects, ecological restoration.

Introdução geral

A germinação de sementes e o recrutamento de plântulas são considerados gargalos na regeneração de diversas espécies vegetais (NATHAN; MULLER-LANDAU, 2000; SALAZAR et al., 2012; WANG; SMITH, 2002). Diversos fatores bióticos e abióticos como luz, umidade, serapilheira e herbivoria podem afetar o recrutamento, variando de acordo com as tolerâncias e requerimentos de cada espécie (DONOHUE et al., 2010). Interações interespecíficas como predação e dispersão de sementes também podem apresentar um profundo efeito no estabelecimento de plantas, influenciando taxas de germinação e a distribuição espacial dos propágulos (WANG; SMITH, 2002; WRIGHT et al., 2007). Os padrões de dispersão e predação de sementes e de emergência e sobrevivência de plântulas influenciam a densidade e a distribuição espacial de juvenis e adultos, afetando as dinâmicas das populações e comunidades (NATHAN; MULLER-LANDAU, 2000; WANG; SMITH, 2002).

Atividades humanas têm causado alterações globais nos ecossistemas, levando à extinção de espécies e mudanças em condições bióticas e abióticas (CHAPIN III et al., 2000). A perda e a fragmentação de habitats são importantes causas de mudanças ecossistêmicas e de perda de biodiversidade (LAURANCE, 2008; LAURANCE; COCHRANE, 2001). A criação de fragmentos também aumenta os efeitos de borda, os quais resultam da interação entre a borda dos fragmentos e a matriz adjacente, modificando condições bióticas e abióticas nessas áreas (HADDAD et al., 2015; MURCIA, 1995). A composição de espécies vegetais pode variar de acordo com o tamanho dos fragmentos de habitat, assim como entre a borda e interior (HADDAD et al., 2015). Dessa forma, pequenos fragmentos e bordas são frequentemente dominados por algumas espécies de plantas mais resistentes ou que se beneficiam de distúrbios (LIEBSCH; MARQUES; GOLDENBERG, 2008; TABARELLI; PERES; MELO, 2012).

A fragmentação de habitat também tem o potencial de alterar interações entre as espécies, modificando a composição e funcionamento das comunidades (FONTÚRBEL et al., 2015; HADDAD et al., 2015; IBÁÑEZ et al., 2014; LAURANCE, 2008; MURCIA, 1995).

Além disso, outros distúrbios antrópicos, como invasão biológica, corte seletivo e pastoreio são importantes causas de degradação ambiental, provocando mudanças na estrutura e composição das comunidades (CHAPIN III et al., 2000; COLTMAN et al., 2003; MCINTYRE; HOBBS, 1999). Por exemplo, espécies de plantas invasoras podem alterar propriedades do solo e a incidência de luz, assim como a riqueza de plantas nativas (RUWANZA et al., 2013; TRUSCOTT et al., 2008). O corte seletivo pode provocar mudanças na cobertura do dossel, influenciando a disponibilidade de luz, a umidade e o recrutamento de plantas (ASNER et al., 2005; FORGET; MERONA; JULLIOT, 2001). Os distúrbios no solo e pisoteio causados pelo pastoreio e criação de gado também podem alterar a regeneração de plantas nativas (e.g. STEPHENS; CASTRO-MORALES; QUINTANA-ASCENCIO, 2012).

De modo geral, a fragmentação e a degradação de habitat podem afetar a abundância e diversidade de comunidades de animais e plantas, modificar o funcionamento dos ecossistemas, a estrutura da vegetação e as interações entre espécies (FONTÚRBEL et al., 2015; HADDAD et al., 2015; IBÁÑEZ et al., 2014; WRIGHT, 2005). Portanto, locais afetados por distúrbios antrópicos geralmente apresentam diferentes características bióticas e abióticas quando comparados à locais sem distúrbios (HADDAD et al., 2015; TABARELLI; PERES; MELO, 2012), potencialmente influenciando taxas de remoção e germinação de sementes e a sobrevivência de plântulas (e.g. CHRISTIANINI; GALETTI, 2007; GUERRERO; BUSTAMANTE, 2009). Porém, os efeitos desses distúrbios na regeneração de espécies vegetais podem variar amplamente

de acordo com características das espécies, as quais podem ser favorecidas ou prejudicadas em áreas perturbadas (IBÁÑEZ et al., 2014; LIEBSCH; MARQUES; GOLDENBERG, 2008; NEUSCHULZ et al., 2016). Por exemplo, as condições ambientais de áreas sujeitas à distúrbios antrópicos são frequentemente relacionadas com uma menor abundância de espécies zoocóricas, não-pioneiras e típicas de sub-bosque, como pode ser visto em locais fragmentados (e.g. LIEBSCH; MARQUES; GOLDENBERG, 2008; TABARELLI; PERES; MELO, 2012).

Estudos em paisagens fragmentadas e degradadas trazem importantes percepções de seus efeitos sobre espécies e processos ecológicos (LAURANCE; COCHRANE, 2001; SANTOS; KINOSHITA; SANTOS, 2007). Apesar da presença de diversos estudos de caso, ainda não é possível identificar padrões globais sobre os efeitos das atividades humanas na emergência e sobrevivência de plântulas devido à falta de revisões sistemáticas e meta-análises sobre o tema. Além disso, há lacunas na literatura sobre os efeitos de distúrbios em diversos biomas do mundo. Na Mata Atlântica, por exemplo, apesar dos grandes efeitos da fragmentação de habitat (HADDAD et al., 2015; RIBEIRO et al., 2009; TABARELLI et al., 2010), possíveis diferenças na regeneração de plantas e condições abióticas entre borda e interior de pequenos fragmentos, assim como áreas de restauração ecológica, ainda são pouco exploradas na literatura. Portanto, a presente tese apresenta informações inéditas sobre os efeitos de distúrbios antrópicos na regeneração de plantas, as quais podem ser utilizadas na definição de prioridades de conservação e restauração.

Objetivos

O objetivo geral dessa tese foi avaliar os efeitos de distúrbios antrópicos na regeneração de plantas. Os objetivos específicos foram: (a) avaliar os efeitos de distúrbios antrópicos na germinação de sementes e na sobrevivência de plântulas e a influência de características das espécies e do ambiente na resposta das espécies aos distúrbios (Capítulos 1 e 2); e (b) comparar a germinação e remoção de sementes, além de condições ambientais, em áreas de restauração florestal e de interior e borda de pequenos fragmentos de Mata Atlântica (Capítulo 3).

Métodos

O uso de meta-análises em ecologia tem possibilitado a descrição de processos e padrões, identificação de lacunas na literatura e comparações em diversas escalas espaciais e temporais, entre espécies e ecossistemas (KORICHEVA; GUREVITCH, 2014). Nos capítulos 1 e 2 conduzimos meta-análises para avaliar os efeitos de distúrbios antrópicos na germinação de sementes e na sobrevivência de plântulas, respectivamente. Dessa forma, fizemos revisões da literatura e criamos bancos de dados com informações globais, os quais foram incorporados nas análises. Selecionamos previamente palavras-chave, assim como critérios de busca e triagem dos artigos científicos. As palavras-chave, refinamento da busca, critérios de inclusão de artigos e outras informações relacionadas estão especificados nos métodos e Apêndices de cada capítulo. Todas as fases da revisão e das análises, desde a busca de artigos até a seleção e interpretação de resultados dos modelos, seguiram as diretrizes e sugestões de literatura especializada, como o PRISMA Statement (MOHER et al., 2009), Koricheva & Gurevitch (2014) e Koricheva et al. (2013).

No Capítulo 1 utilizamos o estimador *Hedges' d*, empregando valores de proporção média de germinação, desvios padrões e tamanhos amostrais em áreas com e sem distúrbio, para avaliar o efeito dos distúrbios antrópicos na germinação de sementes. Para estimar o valor de *Hedges' d* de cada estudo de caso utilizamos as fórmulas abaixo (HEDGES; OLKIN, 1985; ROSENBERG; ROTHSTEIN; GUREVITCH, 2013), nas quais os valores de \bar{Y} indicam as proporções médias de germinação e os valores de n e s representam o tamanho amostral e o desvio padrão das médias, respectivamente. Os subscritos 1 e 2 representam as informações de locais com e sem distúrbios, respectivamente. J representa um fator de correção para tamanhos amostrais pequenos, o qual utiliza valores de n_1 e n_2 .

$$d = \frac{\bar{Y}_1 - \bar{Y}_2}{\sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}} J$$

$$J = 1 - \frac{3}{4(n_1 + n_2 - 2) - 1}$$

No Capítulo 2 utilizamos o estimador *Odds Ratio*, o qual incorpora o número de plântulas que sobreviveram e que morreram em áreas com e sem distúrbio, para avaliar o efeito de distúrbios antrópicos na sobrevivência de plântulas. O valor de *Odds Ratio* (OR) de cada estudo de caso foi estimado utilizando a fórmula abaixo (ROSENBERG; ROTHSTEIN; GUREVITCH, 2013), na qual os valores de S e D indicam os números de plântulas que sobreviveram e morreram, respectivamente. Os subscritos *dist* e *und* representam as informações de locais com e sem distúrbios, respectivamente.

$$OR = \frac{S_{dist} \cdot D_{und}}{S_{und} \cdot D_{dist}}$$

Ambos estimadores são extensamente utilizados em meta-análises e propiciam estimativas do tamanho do efeito (*effect size*) para cada estudo de caso. Valores positivos

e negativos indicam que os distúrbios antrópicos afetaram a germinação ou a sobrevivência de plântulas de forma positiva ou negativa, respectivamente. Todos modelos meta-analíticos incorporaram os valores desses *effect sizes* para determinar o efeito geral dos distúrbios antrópicos ou de cada moderador (ex: peso de semente, tipo de vegetação) na germinação ou sobrevivência de plântulas. Mais detalhes sobre os modelos e a análise de dados estão presentes nos métodos e Apêndices de cada capítulo.

No capítulo 3 realizamos experimentos em campo em três pequenos fragmentos de Mata Atlântica e duas áreas contíguas sujeitas à restauração ecológica. Foram utilizadas sementes de 13 e 5 espécies de plantas nativas em experimentos de germinação e remoção de sementes, respectivamente. As sementes foram adicionadas em parcelas no interior e borda dos fragmentos, assim como nas áreas de restauração. Cobertura do dossel, profundidade da serapilheira e umidade do solo também foram avaliados nesses locais. Mais detalhes estão especificados nos métodos do Capítulo 3.

Referências

- ASNER, G. P. et al. Selective Logging in the Brazilian Amazon. *Science*, v. 310, n. 2005, p. 480–482, 2005.
- CHAPIN III, F. S. et al. Consequences of changing biodiversity. *Nature*, v. 405, n. June, p. 234–242, 2000.
- CHRISTIANINI, A. V.; GALETTI, M. Spatial variation in post-dispersal seed removal in an Atlantic forest: Effects of habitat, location and guilds of seed predators. *Acta Oecologica*, v. 32, p. 328–336, 2007.
- COLTMAN, D. et al. Undesirable evolutionary consequences of trophy hunting. *Nature*, v. 426, n. December, p. 655–658, 2003.
- DONOHUE, K. et al. Germination, postgermination adaptation, and species ecological ranges. *Annual Review of Ecology, Evolution, and Systematics*, v. 41, p. 293–319, 2010.
- FONTÚRBEL, F. E. et al. Meta-analysis of anthropogenic habitat disturbance effects on animal-mediated seed dispersal. *Global Change Biology*, v. 21, n. 11, p. 3951–3960, 2015.
- FORGET, P.; MERONA, J. M. R.; JULLIOT, C. The effects of forest type, harvesting and stand refinement on early seedling recruitment in a tropical rain forest. *Journal of Tropical Ecology*, v. 17, p. 593–609, 2001.
- GUERRERO, P. C.; BUSTAMANTE, R. O. Abiotic alterations caused by forest fragmentation affect tree regeneration: A shade and drought tolerance gradient in the remnants of Coastal Maulino forest. *Revista Chilena de Historia Natural*, v. 82, n. 3, p. 413–424, 2009.
- HADDAD, N. M. et al. Habitat fragmentation and its lasting impact on Earth's ecosystems. *Science Advances*, v. 1, p. 1–10, 2015.

IBÁÑEZ, I. et al. Assessing the integrated effects of landscape fragmentation on plants and plant communities: The challenge of multiprocess-multiresponse dynamics. *Journal of Ecology*, v. 102, n. 4, p. 882–895, 2014.

KORICHEVA, J.; GUREVITCH, J. Uses and misuses of meta-analysis in plant ecology. *Journal of Ecology*, v. 102, n. 4, p. 828–844, 2014.

KORICHEVA, J.; GUREVITCH, J.; MENGERSEN, K. *Handbook of Meta-analysis in Ecology and Evolution*. Princeton, USA, Princeton University Press, 2013.

LAURANCE, W. F. Theory meets reality: How habitat fragmentation research has transcended island biogeographic theory. *Biological Conservation*, v. 141, n. 7, p. 1731–1744, 2008.

LAURANCE, W. F.; COCHRANE, M. A. Synergistic effects in fragmented landscapes. *Conservation Biology*, v. 15, p. 1488–1489, 2001.

LIEBSCH, D.; MARQUES, M. C. M.; GOLDENBERG, R. How long does the Atlantic Rain Forest take to recover after a disturbance? Changes in species composition and ecological features during secondary succession. *Biological Conservation*, v. 141, p. 1717–1725, 2008.

MCINTYRE, S.; HOBBS, R. A framework for conceptualizing human effects on landscapes and its relevance of management and research models. *Conservation Biology*, v. 13, n. 6, p. 1282–1292, 1999.

MOHER, D. et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Medicine*, v. 6, p. 889–896, 2009.

MURCIA, C. Edge effects in fragmented forests: implications for conservation. *Trends in Ecology & Evolution*, v. 10, n. 2, p. 58–62, 1995.

NATHAN, R.; MULLER-LANDAU, H. C. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology & Evolution*, v. 15, n. 7, p. 278–285, 2000.

NEUSCHULZ, E. L. et al. Pollination and seed dispersal are the most threatened processes of plant regeneration. *Scientific Reports*, v. 6, p. 6–11, 2016.

RIBEIRO, M. C. et al. The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biological Conservation*, v. 142, n. 6, p. 1141–1153, 2009.

RUWANZA, S. et al. The effectiveness of active and passive restoration on recovery of indigenous vegetation in riparian zones in the Western Cape, South Africa: A preliminary assessment. *South African Journal of Botany*, v. 88, p. 132–141, 2013.

SALAZAR, A. et al. Differential seedling establishment of woody plants along a tree density gradient in Neotropical savannas. *Journal of Ecology*, v. 100, p. 1411–1421, 2012.

SANTOS, K. DOS; KINOSHITAB, L. S.; SANTOS, F. A. M. DOS. Tree species composition and similarity in semideciduous forest fragments of southeastern Brazil. *Biological Conservation*, v. 135, p. 268–277, 2007.

STEPHENS E.L.; CASTRO-MORALES L.; QUINTANA-ASCENCIO, P.F. Post-dispersal seed predation, germination, and seedling survival of five rare Florida scrub species in intact and degraded habitats. *The American Midland Naturalist*, v. 167, p. 223–239, 2012.

TABARELLI, M.; PERES, C. A.; MELO, F. P. L. The “few winners and many losers” paradigm revisited: Emerging prospects for tropical forest biodiversity. *Biological Conservation*, v. 155, p. 136–140, 2012.

- TRUSCOTT, A. M. et al. Consequences of invasion by the alien plant *Mimulus guttatus* on the species composition and soil properties of riparian plant communities in Scotland. *Perspectives in Plant Ecology, Evolution and Systematics*, v. 10, n. 4, p. 231–240, 2008.
- WANG, B. C.; SMITH, T. B. Closing the seed dispersal loop. *Trends in Ecology & Evolution*, v. 17, n. 8, p. 379–385, 2002.
- WRIGHT, S. J. Tropical forests in a changing environment. *Trends in Ecology and Evolution*, v. 20, n. 10, p. 553–560, 2005.
- WRIGHT, S. J. et al. The plight of large animals in tropical forests and the consequences for plant regeneration. *Biotropica*, v. 39, n. 3, p. 289–291, 2007.

1 **Capítulo 1**

2 Formatado de acordo com as normas do periódico *Perspectives in Plant Ecology,*

3 *Evolution and Systematics*

4

5

6 **Meta-analysis of human disturbance effects on seed germination under**

7 **field conditions**

8 **Abstract**

9 Human disturbances, such as habitat fragmentation, edge effects, biological invasions,
10 and selective logging can affect abiotic conditions that influence the likelihood of seed
11 germination, an important bottleneck for plant regeneration. However, there is no
12 research synthesis evaluating how human disturbances affect germination. We
13 performed a global meta-analysis comparing seed germination on disturbed and
14 undisturbed sites. We evaluated the influence of disturbance type, vegetation, growth
15 form, dispersal syndrome, seed mass and phylogeny on germination responses to
16 disturbance. We obtained information about 63 plant species from 19 studies. We found
17 an overall negative effect of human disturbance on seed germination. Biological
18 invasion had the largest negative impact, while habitat fragmentation, edge effects,
19 selective logging and grazing had no consistent effect on seed germination. Germination
20 of forest species was not generally affected by disturbances, but plants from savannas,
21 shrublands and grasslands exhibited a negative response. Likewise, human disturbances
22 did not affect seedling emergence of trees, but non-tree species (shrubs, herbs and
23 grasses) were negatively affected. Dispersal syndrome and seed mass had no effect on
24 species responses. Phylogenetic relationships between species did not bias the results.
25 Human disturbances generally decrease seed germination, and non-trees from open
26 physiognomies are probably the most susceptible group. Therefore, conservation
27 practices or restoration efforts focusing on early recruitment of those plants, such as
28 direct sowing of seeds, should pay special attention to decreases in seed germination
29 due to habitat alterations following disturbances. Amelioration of conditions, such as
30 control of plant invaders, should be considered to enhance the germination success of
31 sown species.

32

- 33 **Keywords:** habitat fragmentation; anthropic degradation; seedling emergence;
- 34 biological invasion; edge effects; selective logging.

35 **Introduction**

36 Seed germination is an important bottleneck in the plant life cycle, influencing
37 seedling, sapling and adult numbers and distributions (Nathan & Muller-Landau 2000;
38 Wang & Smith 2002). Abiotic factors like light availability, moisture, temperature
39 fluctuation, litter layer, and biotic factors such as seed predators, pathogens and seed
40 ingestion by vertebrate frugivores are often determinant to germination success under
41 natural conditions (Nathan & Muller-Landau 2000; Donoso et al. 2004; Donohue et al.
42 2010). Since all of these abiotic and biotic factors are important triggers of seed
43 germination, human disturbances that degrade the original conditions of the
44 environment and biodiversity may affect germination rates of several plant species in a
45 positive or in a negative way (e.g. Ibáñez et al. 2014; Myster 2015; Culot et al. 2017).
46 Anthropogenic disturbances can thus alter environmental conditions, plant community
47 composition and intra- and inter-specific interactions, affecting species differently in
48 accordance to their tolerance to degraded conditions (Murcia 1995; Laurance 2008;
49 Liebsch et al. 2008; Tabarelli et al. 2010; Ibáñez et al. 2014).

50 For instance, edges of remnant tropical forest fragments are drier and experience
51 higher temperatures and vapor pressure deficits than sites that are distant from forest
52 edges (Camargo & Kapos 1995). Biological invasions and selective logging often alter
53 microhabitat conditions such as light availability and vegetation structure, influencing
54 recruitment of native plants (Ruwanza et al. 2013; Osazuwa-Peters et al. 2015). Human
55 disturbance can increase the biotic homogenization of plants communities in habitat
56 remnants since only plants able to deal with these changes can survive and recruit in the
57 long term (Tabarelli et al. 2012). In some neotropical forests, short-lived and small-
58 seeded pioneer plants and abiotic dispersed species often increase in importance after
59 disturbances (Tabarelli & Peres 2002). In addition, reductions in abundance and

60 diversity of frugivores may cascade to plants that rely on animals for seed dispersal,
61 decreasing not only the abundance of recruits, but also modifying seed size and
62 susceptibility to seed desiccation (Galetti et al. 2013).

63 Surprisingly, the overall effect of human disturbance on seed germination is
64 unclear (Camargo et al. 2002; Guerrero & Bustamante 2009) and there is no global
65 synthesis evaluating how species traits influence seed germination responses to
66 disturbances in the field. The results of seed germination experiments using germination
67 chambers or common gardens are abundant in the literature, but such experiments are
68 performed under controlled conditions (e.g. regularly watered, under controlled
69 temperature and light incidence, etc), which sometimes produces conflicting results
70 with those performed under natural conditions (Rodríguez-Pérez et al. 2005). Therefore,
71 results of germination experiments performed under field conditions would be
72 preferable to measure the real impact of disturbance on seed germination.

73 Understanding how anthropogenic activities affect seed germination can help us
74 to assess the consequences of disturbance to early plant recruitment and which traits
75 would make a species more susceptible. Therefore, we carried out a meta-analysis to
76 evaluate the effect of human disturbances on seed germination under field conditions.
77 We also examined if disturbance affects germination differently according to
78 disturbance type, vegetation type, growth form, dispersal syndrome and seed mass. In
79 addition, since phylogenetic relationships between plant species might influence their
80 germination responses to habitat conditions (e.g. Zhang et al. 2014), we also considered
81 the potential impact of phylogeny in our analysis.

82 Human disturbances such as habitat fragmentation, edge effects, biological
83 invasions, selective logging and grazing often decreases local species richness and
84 diversity. Moreover, a few resilient species might dominate disturbed areas, replacing

85 native species that cannot tolerate those conditions (e.g. Oliveira et al. 2004; Liebsch et
86 al. 2008; Tabarelli et al. 2012). Therefore, we hypothesized that human disturbances
87 decrease seed germination generally and factors such as vegetation type, growth form,
88 dispersal syndrome and seed mass may influence species responses. For example, it is
89 likely that seed germination of light demanding lianas would respond differently to
90 degraded conditions compared to large-seeded trees adapted to deep shade inside
91 mature forest. Due to the high heterogeneity of species responses to altered conditions
92 in forests (e.g. Neuschulz et al. 2016), we expected a nonsignificant effect of human
93 disturbances on germination in those habitats. Human disturbances can also alter
94 species composition and resource availability in open physiognomies such as grasslands
95 and savannas (MacDougall & Turkinson 2005; Ruwanza et al. 2013). However, positive
96 germination responses to disturbed conditions are not commonly found in open
97 physiognomies (but see Stephens et al. 2012) and we expected that germination is
98 negatively affected by human disturbances in open physiognomies, following the
99 overall effect. In addition, plants with different growth forms might have different
100 germination and survival requirements, such as light incidence, which can influence
101 their response to disturbed conditions (e.g. MacDougall & Turkington 2005; Luna &
102 Moreno 2009). Since seed manipulation by animals and frugivore gut passage can also
103 affect germination probabilities (Camargo et al. 2016; Fricke et al. 2019), we also
104 expected that biotic dispersed plants would be more susceptible to disturbance in terms
105 of germination, compared to abiotic dispersed ones. Finally, seed mass often influences
106 early plant recruitment (e.g. Moles & Westoby 2006; Zhang et al. 2014). Large-seeded
107 species usually have more seed reserves, which might decrease their dependence on
108 abiotic factors for germination, such as light incidence, and increase seedling resistance
109 to abiotic stress (Milberg et al. 2000; Kitajima 2002; Moles & Westoby 2006).

110 Therefore, we expected that small-seeded species would be more susceptible to
111 disturbed conditions, compared to large-seeded ones.

112

113 **Methods**

114 *Literature Survey*

115 To obtain a broad picture about the effects of human disturbances on seed
116 germination, we searched the literature by topic using Web of Science (from 1945 to
117 December 2018) using the following combination of search terms: germination* AND
118 degrad* OR fragment* OR edge_effect* OR selective_logging OR
119 biological_invasion*. We filtered the results of the research areas ‘Plant Sciences’,
120 ‘Environmental Sciences’, ‘Ecology’ and ‘Biodiversity Conservation’. The search
121 resulted in 6,532 articles. We selected studies based on the following criteria: (a) studies
122 that described the effects of habitat degradation (defined as selective logging, hunting,
123 grazing and biological invasion) and/or habitat fragmentation (studies in habitat
124 remnants, forest edges and/or secondary forests) on seed germination percentage and/or
125 probability; (b) studies reporting data from field experiments (we did not consider
126 laboratory or greenhouse trials); (c) comparing at least one natural or near-natural area
127 (control site) with another site under human disturbance conditions, or the same site
128 before and after disturbance; (d) presented mean germination percentage or number of
129 germinated seeds for at least two sample unities (to access variation measures on seed
130 germination). Based on abstract content we selected 103 manuscripts according to those
131 criteria (see Appendix A for more details). After full-text reading, 31 manuscripts
132 remained. Authors were contacted when some required information was not provided in
133 the paper. We excluded studies comparing natural areas to crops, bare soil or isolated
134 trees. When the manuscript presented data from a gradient of disturbance, such as

135 replicates in different habitat fragment sizes, we selected the most disturbed plot or
136 category (e.g. smaller fragment) and the least disturbed (e.g. larger fragment) for the
137 meta-analysis. If an article presented data for more than one species, we consider each
138 species as an independent case study. When two or more years or periods were sampled
139 in the same study, we randomly selected one of them to include in our database. We
140 retained 19 studies from which we could extract mean germination values, standard
141 error and sample sizes for disturbed and undisturbed sites. Disturbance type, vegetation
142 type (forest and non-forest ecosystems), growth form (trees and non-trees), dispersal
143 syndrome (biotic and abiotic) and seed mass were obtained for each species or case
144 study based on information provided by the original articles or the literature. We also
145 searched the databases TRY and Seed Information Database to access information about
146 species traits (Kattge et al. 2012; Royal Botanic Gardens Kew, 2019).

147

148 *Data analysis*

149 We calculated the effect size of case studies using Hedges' unbiased
150 standardized mean difference (Hedges' d , Hedges & Olkin 1985), which compared
151 mean values of disturbed and undisturbed sites. Negative and positive values indicated
152 that seed germination was reduced or increased, respectively, by human disturbance. A
153 random effects model was used to analyze the overall effect of human disturbance
154 because we assume variation of effects between and within studies. To evaluate the
155 amount of heterogeneity the model can explain, we analyzed the model heterogeneity
156 statistics, Q and I^2 , and their p values (Rosenberg 2013). Mixed-effects models tested
157 the influence of different moderators on germination and disturbance type, vegetation
158 type, growth form, dispersal syndrome and seed mass were used as moderators. For
159 each mixed-effects model we evaluated the Wald-type test of coefficients results, Q_M

160 and its p-value, which indicates if there is a relationship between effect sizes and the
161 moderator. For categorical moderators (disturbance type, vegetation type, growth form,
162 dispersal syndrome) we obtained the mean effect sizes and CI for each moderator
163 category excluding the intercept from the models (see Viechtbauer 2010). To evaluate
164 the relationship between effect sizes and the continuous moderator (seed mass), in
165 addition to the results of the Wald-type test, we also analyzed the R^2 value. To evaluate
166 if the interaction between species traits and/or habitat conditions can influence
167 germination responses to disturbance, we specified multiple moderators and their
168 interaction in the same mixed-effect model (see Viechbauer 2010). We analyzed the
169 interaction between (i) vegetation type and growth form; (ii) seed mass and growth
170 form; and (iii) seed mass and dispersal syndrome. We used the estimator restricted
171 maximum-likelihood (REML) in all models. Meta-analyses were performed in R (R
172 Core Team 2017) using the metafor package (Viechtbauer 2010) and OpenMEE
173 (Wallace et al. 2017).

174 To evaluate publication bias we used the Rosenthal approach to calculate the
175 fail-safe number (Rosenthal 1979) and a funnel plot to verify potential asymmetry
176 between positive and negative values distributions (Hedges & Vevea 1996). We also
177 performed a trim-and-fill procedure to evaluate the estimated number of missing studies
178 and recalculate indexes according to that number (Duval & Tweedie 2000; Jennions &
179 Møller 2002). Phylogenetic independence was analyzed using the software phyloMeta
180 (Lajeunesse 2011; version 1.3), which generates models with and without phylogenetic
181 information. The best model was selected based on the lowest Akaike's Information
182 Criterion (AIC; see Lajeunesse 2009). The phylogenetic tree at species level was
183 obtained from Zanne et al. (2014) and was extracted from Phylomatic (Webb &
184 Donoghue 2005; Version 3, 2012).

185

186 **Results**

187 The selected papers provided 63 case studies comparing seed germination rates of 63
188 plant species from 32 botanical families in disturbed and undisturbed sites (see
189 Appendix A for details). Studies addressing habitat degradation (biological invasion,
190 selective logging and grazing) and fragmentation (habitat fragments, secondary forests
191 and edge effects) provided data for 33 and 30 plant species, respectively. Data was
192 concentrated on tree species and forests and several geographic regions were under
193 investigated, especially in temperate areas (Appendix A).

194 There was a negative overall effect of human disturbance on seed germination
195 ($d = -0.63$; $Q = 290.92$, $p < 0.01$, $I^2 = 86.22\%$; Fig. 1). When human disturbance types were
196 analyzed separately ($Q_M = 19.09$, $p < 0.01$, $I^2 = 83.40\%$; Fig. 1), only biological invasion
197 had a significant negative impact on germination. There was great variation on species
198 responses to disturbance, and fragmentation, edge effects, selective logging and grazing
199 presented no significant effect (Fig. 1). Most species subjected to fragmentation or
200 selective logging presented negative or neutral germination responses to those disturbed
201 conditions (see Appendix A). Since the available information for edge effects and
202 grazing impacts on germination corresponded to only five species each, those results
203 should be interpreted with caution. No consistent effect was found for forest species, but
204 plants from open physiognomies (such as savannas, scrub, shrublands and grasslands)
205 presented a strong negative influence of disturbance on germination ($Q_M = 6.35$, $p = 0.01$,
206 $I^2 = 86.37\%$, Fig. 2). When examining different growth forms, we found a negative effect
207 of disturbance on germination of non-tree species (shrubs, herbs and grasses combined)
208 but no effect for trees ($Q_M = 6.17$, $p = 0.01$, $I^2 = 86.91\%$; Fig. 1). Dispersal syndromes did
209 not modulate germination responses ($Q_M = 0.08$, $p = 0.77$, $I^2 = 86.83\%$; Fig. 1) and both

210 biotic- and abiotic-dispersed plants were negatively affected by human disturbances.
211 Seed mass also did not influence germination responses to disturbances ($Q_M=0.24$,
212 $p=0.62$, $I^2=85.35\%$, $R^2=0\%$). There was no significant influence of the interaction
213 between vegetation type and growth form ($Q=1.08$; $p=0.30$), seed mass and growth
214 form ($Q=1.38$; $p=0.78$), and seed mass and dispersal syndrome ($Q=0.08$; $p=0.78$) on
215 germination responses to disturbed conditions (see Appendix A for more details).

216 We detected no publication bias since trim-and-fill procedures estimated no
217 missing studies and the funnel plot analysis followed the same trend (Appendix B). In
218 addition, Rosenthal's fail-safe number indicated that 1,387 studies detecting no human
219 disturbance effect on germination would be necessary to change the overall effect. The
220 model that did not include phylogeny had the lowest AIC value (AIC= 221.65),
221 compared to the model that incorporated phylogenetic information (AIC= 260.68,
222 Appendix B). Therefore, phylogenetic relatedness among species is not driving
223 germination responses to disturbance.

224

225 **Discussion**

226 Our results demonstrated that human disturbance has an overall negative impact on seed
227 germination, and the effect of disturbance varies especially according to species,
228 disturbance type, vegetation physiognomy and growth form. Therefore, seed
229 germination might be an important constraint to plant recruitment in disturbed sites,
230 with consequences potentially cascading to further recruitment stages (Bruna 2002)
231 affecting population dynamics, community composition and ecosystem processes
232 (Donohue et al. 2010; Tabarelli et al. 2012; Jiménez-Alfaro et al. 2016). Our findings
233 highlight the importance of disturbance mitigation to enhance early recruitment of
234 native species. In addition, seedling emergence responses to disturbance differed

235 according to growth form and vegetation type, which could guide the definition of
236 conservation priorities and seed sowing practices to restore disturbed areas.

237 Only biological invasion had a clear and consistent negative effect on seed
238 germination. It also presented the most negative mean effect size, indicating that
239 invasive species are an important threat to germination of native plants. Studies are
240 often concerned about the direct impacts of invasive species, such as replacement of
241 native species, changes in species interactions and in habitat structure (MacDougall &
242 Turkington 2005; Rogers et al. 2017; Zimmermann et al. 2017). However, there are
243 often subtle but important effects of invasions that can be easily overlooked (Rogers et
244 al 2017, and references therein). For example, invasive plants may increase the
245 accumulation of litter above soil, change soil properties and produce allelopathic
246 compounds that inhibit seed germination of native species (Zimmermann et al. 2017).
247 The intensity and frequency of further disturbances (e.g. fire) may also play a role in the
248 replacement of native species (see D'Antônio & Vitousek 1992).

249 Contrary to our expectations, habitat fragmentation, edge effects, selective
250 logging and grazing do not present a significant overall effect on seed germination.
251 Species responses to those disturbed conditions were highly heterogeneous in terms of
252 germination rates, especially when considering fragmentation and edge effects. Habitat
253 fragmentation and edge effects can lead to alterations on abiotic factors that are triggers
254 for seed germination, such as light, temperature, and vapor pressure deficits, in addition
255 to frequent changes in biotic interactions and vegetation structure (Asquith & Mejía-
256 Chang 2005; Asner et al. 2006; Fontúrbel et al. 2015; Stephens & Quintana-Ascencio
257 2015). These disturbed conditions enhance seedling emergence of some species (e.g.
258 *Caryocar villosum* and *Simarouba amara*) whilst being extremely negative to
259 germination of others (e.g. *Aristotelia chilensis* and *Nothofagus glauca*; references are

260 available in Appendix A). Therefore, seedling emergence can still be affected at
261 community level, which could help to explain why areas subjected to fragmentation
262 often present different successional trajectories and species composition from
263 undisturbed sites (Liebsch et al. 2008; Tabarelli et al. 2012). In this context, studies at a
264 community level, especially those considering several functional traits, are extremely
265 important.

266 As expected, seed germination in forests is generally not affected by human
267 disturbance. There is a suggestion that anthropogenic disturbance has a neutral general
268 effect on early plant recruitment in forests (i.e. seed germination, seedling and sapling
269 survival combined), despite the detrimental influence on pollination and seed dispersal
270 (Neuschulz et al. 2016). Early recruitment can be increased or decreased after
271 disturbance according to species light incidence requirements, as can be seen in forest
272 species adapted to vegetation gaps and highly disturbed sites versus species that require
273 deep shade, respectively (Hubbel et al. 1999; Liebsch et al. 2008; Tabarelli et al. 2010,
274 2012). Therefore, the neutral effect of anthropogenic disturbance on seed germination of
275 forest plants is probably related to the heterogeneity of species responses to disturbed
276 conditions. Since the data available for forest species included mostly trees, our
277 conclusion should be confined to this growth form. Future studies should include more
278 tests with plants from non-dominant growth forms, such as herbs, shrubs, lianas and
279 epiphytes in forests and trees in savannas and grasslands (see below) to allow a broader
280 generalization about the effects of disturbance on seed germination in different
281 vegetation types.

282 Seed germination responses to disturbance can also vary largely among species
283 from open physiognomies (e.g. Stephens et al. 2012), but the overall effect is negative
284 as hypothesized. Grasslands and savannas are usually adapted to frequent natural

285 disturbances, especially by fire (Parr et al. 2014). However, anthropogenic disturbances
286 probably alter the environment in different ways, decreasing seedling emergence. For
287 example, invasive annual species of grasses can decrease soil moisture faster than native
288 perennial grasses, negatively affecting seed germination (Gordon & Rice 2000). It is not
289 clear which biotic and abiotic factors are the most important constraint to seedling
290 emergence in open physiognomies, but light incidence at the soil level and litter cover
291 are suggested as important factors (MacDougall & Turkington 2005; Salazar et al.
292 2012). These effects agree with the negative impact of invasive species on germination,
293 as invasive plants often change soil cover.

294 Germination of plant species from non-forest physiognomies, especially shrubs,
295 herbs and grasses, are the most negatively affected by disturbance activities, and
296 respond differently to disturbances compared to trees. Germination time and percentage
297 can vary according to growth form in some habitats, which might be associated to their
298 different growth strategies (Bu et al. 2008). However, since most studies including trees
299 were performed on forests, and the remaining plant growth forms were mostly studied
300 on other physiognomies, we cannot disentangle the effect of growth form and
301 vegetation type.

302 Seed mass represents the maternal investment in an individual offspring, and it
303 generally has a positive association to the nutrient content available in the seed (see
304 Leishman et al. 2000). Some large-seeded species might be less dependent on abiotic
305 factors to germinate, such as light incidence, when compared to small-seeded ones at
306 laboratory conditions (Milberg et al. 2000). Nevertheless, contrary to expectations the
307 effect of human disturbance on germination is not related to seed mass. Previous studies
308 performed in undisturbed sites also found no relationship between seed mass and seed
309 germination, despite the positive influence of this trait on further recruitment stages

310 such as seedling survival (Moles & Westoby 2004, 2006). Seed mass may be more
311 influential to some indirect effects of disturbances. For instance, defaunation often leads
312 to the loss of seed dispersal by large-bodied frugivorous birds, which can compromise
313 regeneration of large-seeded plants through dispersal limitation (Galetti et al. 2013).
314 This highlights the importance to consider bottlenecks to different recruitment stages
315 and the interspecific interactions that may be changed due to direct and indirect effects
316 of disturbances.

317 As human activities can also affect seed dispersers and lead to drastic decreases
318 in seed removal and plant recruitment of at least some animal-dispersed plants (Markl
319 et al. 2012; Fontúrbel et al. 2015; Culot et al. 2017), we expected a higher impact on
320 zoochoric species. Contrary to our expectations, the negative effect of disturbance on
321 germination is consistent among different dispersal syndromes, and abiotic and biotic
322 dispersed species have lower seed germination under disturbed conditions. However, it
323 is important to highlight that germination experiments in the field often employ seeds
324 without previous interactions with animals (e.g. gut-passed seeds are not used). Gut
325 passage can enhance percentage and speed of seed germination (Traveset 1998; Fuzessy
326 et al. 2016; Soltani et al. 2018; Fricke et al. 2019). Then, seeds that previously
327 interacted with animals may present different germination responses to disturbance. We
328 recommend future studies to include seeds manipulated by animals in germination trials
329 in the field to enhance our understanding of the role of seed disperser extinction in plant
330 regeneration under disturbed conditions.

331 We also identified some knowledge gaps in the literature. Most field data
332 available so far are focused on tropical areas, forest ecosystems and tree species, and
333 Europe, Asia and Australia are under investigated. There is also a scarcity of
334 information about the effect of disturbances such as edge effects and grazing on seed

335 germination in the field. For instance, the available data about the effects of grazing on
336 germination included only 5 case studies of 5 herb and shrub species from the same
337 study site (Stephens et al. 2012; Stephens & Quintana-Ascencio 2015). Although these
338 species belong to different families, additional information would be worthwhile.
339 Studies investigating the influence of fragmentation and edge effects on seed
340 germination are focused on trees and/or zoochoric dispersed plants. Thus, future studies
341 should also evaluate species with different traits. Information about non-tree species in
342 forests and tree species in open physiognomies would be important to disassociate the
343 influence of growth form and vegetation type in future analysis.

344 Decreased seed germination in disturbed sites may help to explain some changes
345 in plant species composition observed with time in habitat remnants (e.g. Tabarelli et al.
346 2010) and the difficulties to restore plant composition of small fragments and sites
347 invaded by aggressive exotic species with the use of seeding methods. The large
348 heterogeneity on species responses to disturbance suggests that information about the
349 germination requirements of target species in the field might be especially important for
350 the success of species conservation or population restoration programs. Amelioration of
351 conditions, such as control of plant invaders, should be considered to enhance the
352 germination success of target species (e.g. Sampaio et al. 2019). Despite their global
353 extension and high biodiversity, savannas and grasslands have long been neglected in
354 research and conservation initiatives (Parr et al. 2014). Our analysis highlights that seed
355 germination of native species from those open vegetations may be especially susceptible
356 to human disturbance, which should be observed in the evaluation of conservation and
357 restoration priorities.

358

359 **Supporting Information**

360 PRISMA flow diagram, information about study species, categories classification,
361 species traits, effect size estimators and heterogeneity indexes for each meta-analytic
362 model, full references for all original articles (Appendix A); and funnel plot for
363 publication bias analysis and information about phylogenetic models and analysis
364 (Appendix B) are available online. The authors are solely responsible for the content
365 and functionality of these materials. Queries (other than absence of the material) should
366 be directed to the corresponding author.

367

368 **Acknowledgments**

369 We thank the authors of all case studies for providing us information. We thank L.
370 Lopes, T. Cornelissen and M. Batalha for their valuable suggestions on the previous
371 versions of this manuscript. This study was financed by Coordenação de
372 Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001.
373 The study has been supported by the TRY initiative on plant traits (<http://www.try->
374 [db.org](http://www.try-db.org)). The TRY initiative and database is hosted, developed and maintained by J.
375 Kattge and G. Bönisch (Max Planck Institute for Biogeochemistry, Jena, Germany).
376 TRY is currently supported by DIVERSITAS/Future Earth and the German Centre for
377 Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig.

378 **Literature Cited**

- 379 Asner GP, Asner GP, Knapp DE, Broadbent EN, Oliveira PJC, Keller M, Silva JN.
380 2005. Selective logging in the Brazilian Amazon. *Science* 310:480–482.
- 381 Asquith NM, Mejía-Chang M. 2005. Mammals, edge effects, and the loss of Tropical
382 Forest diversity. *Ecology* 86:379–390.
- 383 Bu H, Du G, Chen X. 2008. Community-wide germination strategies in an alpine
384 meadow on the eastern Qinghai-Tibet plateau: phylogenetic and life-history
385 correlates. *Plant Ecology* 195:87–98.
- 386 Bruna EM. 2002. Effects of forest fragmentation *Heliconia acuminata* on seedling
387 recruitment in central Amazonia. *Oecologia* 132:235–243.
- 388 Camargo JLC, Ferraz IDK, Imakawa AM. 2002. Rehabilitation of degraded areas of
389 Central Amazonia using direct sowing of forest tree seeds. *Restoration Ecology*
390 10:636–644.
- 391 Camargo JLC, Kapos V. 1995. Complex edge effects on soil moisture and microclimate
392 in central Amazonian forest. *Journal of Tropical Ecology* 11: 205-221.
- 393 Camargo PH, Martins MM, Feitosa RM, Christianini A V. 2016. Bird and ant synergy
394 increases the seed dispersal effectiveness of an ornithochoric shrub. *Oecologia*
395 181:507–518.
- 396 Chapin III FS et al. 2000. Consequences of changing biodiversity. *Nature* 405:234–242.
- 397 Culot L, Bello C, Batista JLF, Do Couto HTZ, Galetti M. 2017. Synergistic effects of
398 seed disperser and predator loss on recruitment success and long-term
399 consequences for carbon stocks in tropical rainforests. *Scientific Reports* 7:1–8.
- 400 D'Antonio CM, Vitousek PM 1992. Biological invasions by exotic grasses, the grass
401 fire cycle, and global change. *Annual Review of Ecology and Systematics* 23:
402 63–87.

- 403 Dirzo R, Young HS, Galetti M, Ceballos G, Issac NJB, Collen B. 2014. Defaunation in
404 the Anthropocene. *Science* 345:401–406.
- 405 Donohue K, Casas RR De, Burghardt L, Kovach K, Willis CG. 2010. Germination,
406 postgermination adaptation, and species ecological ranges. *Annual Review of*
407 *Ecology, Evolution, and Systematics* 41:293–319.
- 408 Donoso DS, Grez AA, Simonetti JA. 2004. Effects of forest fragmentation on the
409 granivory of differently sized seeds. *Biological Conservation* 115:63–70.
- 410 Duval S, Tweedie R. 2000. A nonparametric “Trim and Fill” method of accounting for
411 publication bias in meta-analysis. *Journal of the American Statistical Association*
412 95:89–98.
- 413 Fontúrbel FE, Candia AB, Malebrán J, Salazar DA, González-Browne C, Medel R.
414 2015. Meta-analysis of anthropogenic habitat disturbance effects on animal-
415 mediated seed dispersal. *Global Change Biology* 21:3951–3960.
- 416 Fricke EC, Bender J, Rehm EM, Rogers HS. 2019. Functional outcomes of mutualistic
417 network interactions: A community-scale study of frugivore gut passage on
418 germination. *Journal of Ecology* 107:757–767.
- 419 Fuzessy LF, Cornelissen TG, Janson C, Silveira FAO. 2016. How do primates affect
420 seed germination? A meta-analysis of gut passage effects on neotropical plants.
421 *Oikos* 125:1069–1080.
- 422 Galetti M, Guevara R, Côrtes MC, Fadini R, Von Matter S, Leite AB, Labecca F,
423 Ribeiro T, Carvalho CS, Collevatti RG, Pires MM, Guimarães Jr. PR, Brancalion
424 PH, Ribeiro MC, Jordano P. 2013. Functional extinction of birds drives rapid
425 evolutionary change in seed size. *Science* 340:1086-1090.
- 426 Gordon DR, Rice KJ. 2000. Competitive suppression of *Quercus douglasii* (Fagaceae)
427 seedling emergence and growth. *American Journal of Botany* 87:986–994.

- 428 Guerrero PC, Bustamante RO. 2009. Abiotic alterations caused by forest fragmentation
429 affect tree regeneration: A shade and drought tolerance gradient in the remnants of
430 Coastal Maulino forest. *Revista Chilena de Historia Natural* 82:413–424.
- 431 Hedges L V, Vevea JL. 1996. Estimating effect size under publication bias: small
432 sample properties and robustness of a random effects selection model. *Journal of*
433 *Educational and Behavioral Statistics* 21:299–332.
- 434 Hubbell SP, Foster RB, O’Brien ST, Harms KE, Condit R, Wechsler B, Wright SJ, Loo
435 De Lao S. 1999. Light-gap disturbances, recruitment limitation, and tree diversity
436 in a neotropical forest. *Science* 283:554–557.
- 437 Ibáñez I, Katz DSW, Peltier D, Wolf SM, Connor Barrie BT. 2014. Assessing the
438 integrated effects of landscape fragmentation on plants and plant communities:
439 The challenge of multiprocess-multiresponse dynamics. *Journal of Ecology*
440 102:882–895.
- 441 Jennions MD, Møller AP. 2002. Publication bias in ecology and evolution: an empirical
442 assessment using the ‘trim and fill’ method. *Biological Reviews* 77:211–222.
- 443 Jiménez-Alfaro B, Silveira FAO, Fidelis A, Poschod P, Commander LE. 2016. Seed
444 germination traits can contribute better to plant community ecology. *Journal of*
445 *Vegetation Science* 27:637–645.
- 446 Kattge, J., Bönsch, G., Günther, A., Wright, I., Zanne, A., Wirth, C., Reich, P.B. and
447 the TRY Consortium. 2012. TRY - Categorical Traits Dataset. Data from: TRY -
448 a global database of plant traits. Available from [www.try-](http://www.try-db.org/TryWeb/Data.php#3)
449 [db.org/TryWeb/Data.php#3](http://www.try-db.org/TryWeb/Data.php#3) (accessed March 2019).
- 450 Kitajima K. 2002. Do shade-tolerant tropical tree seedlings depend longer on seed
451 reserves? Functional growth analysis of three Bignoniaceae species. *Functional*
452 *Ecology* 16:433–444.

- 453 Lajeunesse MJ. 2009. Meta-Analysis and the comparative phylogenetic method. The
454 American Midland Naturalist 174:369–381.
- 455 Lajeunesse MJ. 2011. phyloMeta: A program for phylogenetic comparative analyses
456 with meta-analysis. *Bioinformatics* 27:2603–2604.
- 457 Laurance WF. 2008. Theory meets reality: How habitat fragmentation research has
458 transcended island biogeographic theory. *Biological Conservation* 141:1731–
459 1744.
- 460 Laurance WF, Cochrane MA. 2001. Synergistic effects in fragmented landscapes.
461 *Conservation Biology* 15:1488–1489.
- 462 Liebsch D, Marques MCM, Goldenberg R. 2008. How long does the Atlantic Rain
463 Forest take to recover after a disturbance? Changes in species composition and
464 ecological features during secondary succession. *Biological Conservation*
465 141:1717–1725.
- 466 Luna B, Moreno J. 2009. Light and nitrate effects on seed germination of Mediterranean
467 plant species of several functional groups. *Plant Ecology* 203:123–135.
- 468 MacDougall AS, Turkington R. 2005. Are invasive species the drivers or passengers of
469 change in degraded ecosystems? *Ecology* 86:42–55.
- 470 Markl JS, Schleuning M, Forget PM, Jordano P, Lambert JE, Traveset A, Wright SJ,
471 Böhning-Gaese K. 2012. Meta-Analysis of the Effects of Human Disturbance on
472 Seed Dispersal by Animals. *Conservation Biology* 26:1072–1081.
- 473 McIntyre S, Hobbs R. 1999. A framework for conceptualizing human effects on
474 landscapes and its relevance of management and research models. *Conservation*
475 *Biology* 13:1282–1292.

- 476 Milberg P, Andersson L, Thompson K. 2000. Large-seeded species are less dependent
477 on light for germination than small-seeded ones. *Seed Science Research* 10:99–
478 104.
- 479 Moles AT, Westoby M. 2004. What do seedlings die from and what are the implications
480 for evolution of seed size? *Oikos* 106:193–199.
- 481 Moles AT, Westoby M. 2006. Seed size and plant strategy across the whole life cycle.
482 *Oikos* 113:91–105.
- 483 Murcia C. 1995. Edge effects in fragmented forests: implications for conservation.
484 *Trends in Ecology & Evolution* 10:58–62.
- 485 Myster RW. 2015. Seed predation, pathogens and germination in primary vs. secondary
486 cloud forest at Maquipucuna Reserve, Ecuador. *Journal of Tropical Ecology*
487 31:375–378.
- 488 Nathan R, Muller-Landau HC. 2000. Spatial patterns of seed dispersal, their
489 determinants and consequences for recruitment. *Trends in Ecology & Evolution*
490 15:278–285.
- 491 Neuschulz EL, Mueller T, Schleuning M, Böhning-Gaese K. 2016. Pollination and seed
492 dispersal are the most threatened processes of plant regeneration. *Scientific*
493 *Reports* 6:6–11.
- 494 Nogales M, Castañeda I, López-Darias M, Medina FM, Bonnaud E. 2015. The
495 unnoticed effect of a top predator on complex mutualistic ecological interactions.
496 *Biological Invasions* 17:1655–1665.
- 497 Oliveira MA, Grillo AS, Tabarelli M. 2004. Forest edge in the Brazilian Atlantic forest:
498 drastic changes in tree species assemblages. *Oryx* 38:389–394.

- 499 Parr CL, Lehmann CER, Bond WJ, Hoffmann WA, Andersen AN. 2014. Tropical
500 grassy biomes: Misunderstood, neglected, and under threat. *Trends in Ecology
501 and Evolution* 29:205–213.
- 502 Pereira HM et al. 2010. Scenarios for global biodiversity in the 21st Century. *Science*
503 330:1496–1501.
- 504 Osazuwa-Peters O, Jiménez I, Oberle B, Chapman CA, Zanne AE. 2015. Selective
505 logging: Do rates of forest turnover in stems, species composition and functional
506 traits decrease with time since disturbance? A 45-year perspective. *Forest Ecology
507 and Management* 337: 10-21.
- 508 Rodríguez-Pérez J, Riera N, Traveset A. 2005. Effect of seed passage through birds and
509 lizards on emergence rate of mediterranean species: differences between natural
510 and controlled conditions. *Functional Ecology* 19:699–706.
- 511 Rogers H, Buhle ER, HilleRisLambers J, Fricke EC, Miller RH, Tewksbury JJ. 2017.
512 Effects of an invasive predator cascade to plants via mutualism disruption. *Nature
513 Communications* 8:14557.
- 514 Rosenberg MS, Rothstein HR, Gurevitch J. 2013. Effect sizes: conventional choices and
515 calculations. Pages 61-71 in Koricheva J, Gurevitch J, Mengersen K, editors.
516 *Handbook of Meta-Analysis in Ecology and Evolution*. Princeton University
517 Press.
- 518 Rosenthal R. 1979. The “fail-drawer problem” and tolerance for null results.
519 *Psychological Bulletin* 86:638–641.
- 520 Royal Botanic Gardens Kew. 2019. Seed Information Database (SID). Version 7.1.
521 Available from data.kew.org/sid/ (accessed March 2019).
- 522 Ruwanza S, Gaertner M, Esler KJ, Richardson DM. 2013. The effectiveness of active
523 and passive restoration on recovery of indigenous vegetation in riparian zones in

- 524 the Western Cape, South Africa: A preliminary assessment. *South African Journal*
525 *of Botany* 88:132–141.
- 526 Salazar A, Goldstein G, Franco AC, Miralles-wilhelm F. 2012. Differential seedling
527 establishment of woody plants along a tree density gradient in Neotropical
528 savannas. *Journal of Ecology* 100:1411–1421.
- 529 Sampaio AB, Vieira DLM, Holl KD, Pellizzaro KF, Alves M, Coutinho AG, Cordeiro
530 A, Felipe J, Schmidt IB. 2019. Lessons on direct seeding to restore Neotropical
531 savanna. *Ecological Engineering* 138:148–154.
- 532 Soltani E, Baskin CC, Baskin JM, Heshmati S, Mirfazeli MS. 2018. A meta-analysis of
533 the effects of frugivory (endozoochory) on seed germination: role of seed size and
534 kind of dormancy. *Plant Ecology* 219:1283–1294.
- 535 Stephens EL, Castro-Morales L, Quintana-Ascencio PF. 2012. Post-dispersal seed
536 predation, germination, and seedling survival of five rare Florida scrub species in
537 intact and degraded habitats. *The American Midland Naturalist* 167:223–239.
- 538 Stephens EL, Quintana-Ascencio PF. 2015. Effects of habitat degradation, microsite,
539 and seed density on the persistence of two native herbs in a subtropical shrubland.
540 *American Journal of Botany* 102:1978–1995.
- 541 Tabarelli M, Peres CA. 2002. Abiotic and vertebrate seed dispersal in the Brazilian
542 Atlantic forest: implications for forest regeneration. *Biological Conservation* 106:
543 165-176.
- 544 Tabarelli M, Aguiar A V., Girão LC, Peres CA, Lopes A V. 2010. Effects of pioneer
545 tree species hyperabundance on forest fragments in Northeastern Brazil.
546 *Conservation Biology* 24:1654–1663.

- 547 Tabarelli M, Peres CA, Melo FPL. 2012. The “few winners and many losers” paradigm
548 revisited: Emerging prospects for tropical forest biodiversity. *Biological*
549 *Conservation* 155:136–140.
- 550 Traveset A. 1998. Effect of seed passage through vertebrate frugivores’ guts on
551 germination: a review. *Perspectives in Plant Ecology, Evolution and Systematics*
552 1:151–190.
- 553 Truscott AM, Palmer SC, Soulsby C, Westaway S, Hulme PE. 2008. Consequences of
554 invasion by the alien plant *Mimulus guttatus* on the species composition and soil
555 properties of riparian plant communities in Scotland. *Perspectives in Plant*
556 *Ecology, Evolution and Systematics* 10:231–240.
- 557 Viechtbauer W. 2010. Conducting meta-analyses in R with the metafor package.
558 *Journal of Statistical Software* 36.
- 559 Wallace BC, Lajeunesse MJ, Dietz G, Dahabreh IJ, Trikalinos TA, Schmid CH,
560 Gurevitch J. 2017. OpenMEE : Intuitive, open-source software for meta-analysis
561 in ecology and evolutionary biology. *Methods in Ecology and Evolution* 8:941–
562 947.
- 563 Wang BC, Smith TB. 2002. Closing the seed dispersal loop. *Trends in Ecology &*
564 *Evolution* 17:379–385.
- 565 Webb CO, Donoghue MJ. 2005. Phylomatic: Tree assembly for applied phylogenetics.
566 *Molecular Ecology Notes* 5:181–183.
- 567 Zhang C, Willis CG, Burghardt LT, Qi W, Liu K, Souza-Filho PRM, Ma Z, Du G.
568 2014. The community-level effect of light on germination timing in relation to
569 seed mass: a source of regeneration niche differentiation. *New Phytologist*
570 204:496–506.

571 Zimmermann TG, Andrade ACS, Richardson DM. 2017. Abiotic barriers limit tree
572 invasion but do not hamper native shrub recruitment in invaded stands. *Biological*
573 *Invasions* 19:109–129.
574

Figures

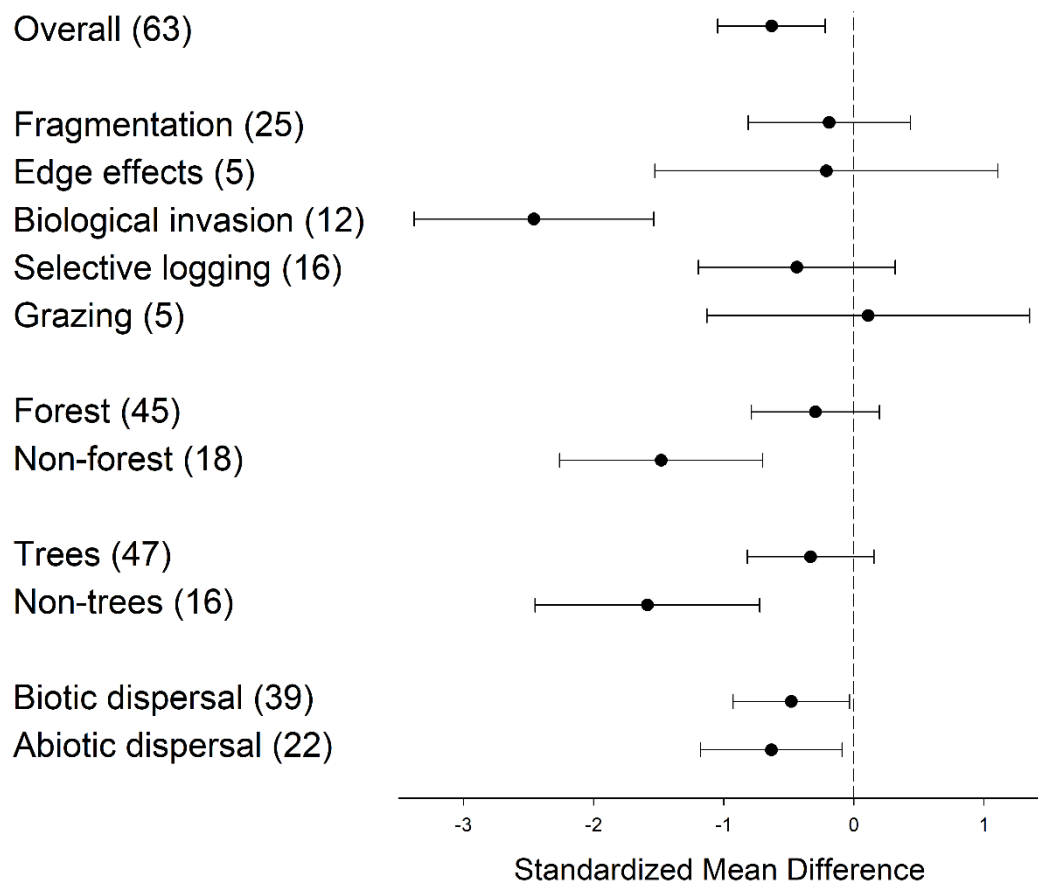


Fig. 1. Human disturbances effects on seed germination. Mean effect sizes (Standardized Mean Difference Hedges' d), 95% confidence intervals (CI) and sample sizes are given for the overall effect, different disturbance types, vegetation types (forests and non-forest physiognomies like savannas, shrublands and grasslands), growth forms (trees and non-tree species like grasses, herbs and shrubs) and dispersal syndromes. Positive and negative values of effect sizes indicate a positive or negative effect of human disturbance on seed germination, respectively. CI (horizontal lines) overlapping zero (vertical dashed line) indicates that germination is not significantly affected by disturbance.

Capítulo 1 - Apêndices

Meta-analysis of human disturbance effects on seed germination under field conditions

Appendix A

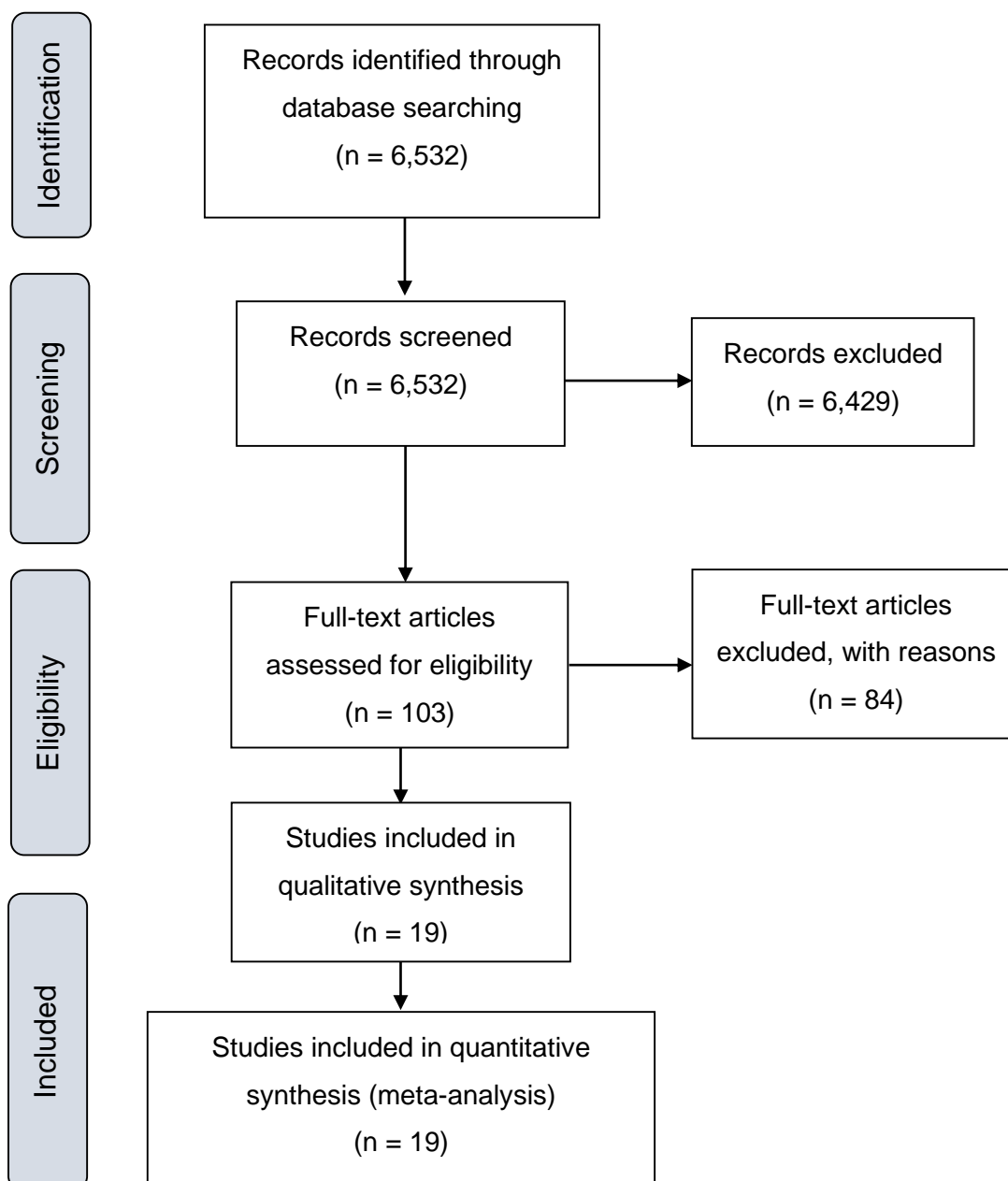


Fig. 1. PRISMA flow diagram describing the number of articles included in each phase of our review and meta-analysis about human disturbance effects on seed germination. We searched the Web of Science platform to obtain information about seed germination and human disturbance (see more details in the main text).



Fig. 2. Locations of all case studies included in the meta-analysis of human disturbance effects on seed germination ($n=19$) are represented by black circles (see Table 1 for more details about the original papers).

Table 1. Original papers included in the meta-analysis about human disturbance effects on germination. Plant species and disturbance type are specified for each case study. Full references of all original papers are provided below.

Original paper	Species	Disturbance type
Asquith & Meija-Cheng 2005	<i>Astrocaryum standleyanum</i>	Habitat fragmentation
Asquith & Meija-Cheng 2005	<i>Calophyllum longifolium</i>	Habitat fragmentation
Asquith & Meija-Cheng 2005	<i>Dipteryx panamensis</i>	Habitat fragmentation
Asquith & Meija-Cheng 2005	<i>Gustavia superba</i>	Habitat fragmentation
Asquith & Meija-Cheng 2005	<i>Oenocarpus mapora</i>	Habitat fragmentation
Asquith & Meija-Cheng 2005	<i>Prioria copaifera</i>	Habitat fragmentation
Asquith & Meija-Cheng 2005	<i>Protium panamense</i>	Habitat fragmentation
Asquith & Meija-Cheng 2005	<i>Swartzia simplex</i>	Habitat fragmentation
Barik et al 1996	<i>Lithocarpus dealbatus</i>	Selective logging
Barik et al 1996	<i>Quercus graffithii</i>	Selective logging
Barik et al 1996	<i>Schima khasiana</i>	Selective logging
Bruna 2002	<i>Heliconia acuminata</i>	Habitat fragmentation
Camargo et al 2002	<i>Buchenavia grandis</i>	Habitat fragmentation
Camargo et al 2002	<i>Cariniana micrantha</i>	Habitat fragmentation
Camargo et al 2002	<i>Caryocar villosum</i>	Habitat fragmentation
Camargo et al 2002	<i>Cochlospermum orinocense</i>	Habitat fragmentation
Camargo et al 2002	<i>Dinizia excelsa</i>	Habitat fragmentation
Camargo et al 2002	<i>Jacaranda copaia</i>	Habitat fragmentation
Camargo et al 2002	<i>Ochroma pyramidale</i>	Habitat fragmentation
Camargo et al 2002	<i>Parkia multijuga</i>	Habitat fragmentation
Camargo et al 2002	<i>Parkia pendula</i>	Habitat fragmentation
Camargo et al 2002	<i>Simarouba amara</i>	Habitat fragmentation
Camargo et al 2002	<i>Triplaris surinamensis</i>	Habitat fragmentation
Christianini & Oliveira 2013	<i>Erythroxylum pelleterianum</i>	Edge effect
Granados et al. 2017	<i>Dryobalanops lanceolata</i>	Selective logging
Granados et al. 2017	<i>Hopea nervosa</i>	Selective logging
Granados et al. 2017	<i>Parashorea tomentella</i>	Selective logging

Granados et al. 2017	<i>Shorea leprosula</i>	Selective logging
Granados et al. 2017	<i>Shorea macrophylla</i>	Selective logging
Guariguata & Sàenz 2002	<i>Quercus costaricensis</i>	Selective logging
Guerreiro & Bustamante 2009	<i>Aristotelia chilensis</i>	Habitat fragmentation
Guerreiro & Bustamante 2009	<i>Cryptocarya alba</i>	Habitat fragmentation
Guerreiro & Bustamante 2009	<i>Nothofagus glauca</i>	Habitat fragmentation
Guerreiro & Bustamante 2009	<i>Nothofagus obliqua</i>	Habitat fragmentation
Herget et al. 2015	<i>Poa secunda</i>	Biological invasion
Lehouck et al 2009	<i>Xymalos monospora</i>	Habitat fragmentation
López-Barrera & Newton 2005	<i>Quercus crassifolia</i>	Edge effect
López-Barrera & Newton 2005	<i>Quercus laurina</i>	Edge effect
López-Barrera & Newton 2005	<i>Quercus rugosa</i>	Edge effect
Lopez-Gallego 2013	<i>Zamia fairchildiana</i>	Selective logging
Mariano & Christianini 2019	<i>Dalbergia miscolobium</i>	Edge effect
Ruwanza et al. 2013	<i>Diospyros glabra</i>	Biological invasion
Ruwanza et al. 2013	<i>Euclea tomentosa</i>	Biological invasion
Ruwanza et al. 2013	<i>Kiggelaria africana</i>	Biological invasion
Ruwanza et al. 2013	<i>Leonotis leonurus</i>	Biological invasion
Ruwanza et al. 2013	<i>Melianthus major</i>	Biological invasion
Ruwanza et al. 2013	<i>Metalasia muricata</i>	Biological invasion
Ruwanza et al. 2013	<i>Olea europaea sub africana</i>	Biological invasion
Ruwanza et al. 2013	<i>Searsia angustifolia</i>	Biological invasion
Stephens & Quintana-Ascencio 2015	<i>Balduina angustifolia</i>	Grazing
Stephens & Quintana-Ascencio 2015	<i>Chamaecrista fasciculata</i>	Grazing
Stephens et al 2012	<i>Hypericum cumulicola</i>	Grazing
Stephens et al 2012	<i>Liatris ohlingerae</i>	Grazing
Stephens et al 2012	<i>Polygonella basiramia</i>	Grazing
Vieira & Scariot 2006	<i>Astronium fraxinifolium</i>	Selective logging
Vieira & Scariot 2006	<i>Cavanillesia arborea</i>	Selective logging
Vieira & Scariot 2006	<i>Erythrina</i> sp.	Selective logging

Vieira & Scariot 2006	<i>Eugenia dysenterica</i>	Selective logging
Vieira & Scariot 2006	<i>Swartzia multijuga</i>	Selective logging
Vieira & Scariot 2006	<i>Tabebuia impetiginosa</i>	Selective logging
Walker & Vitousek 1991	<i>Metrosideros polymorpha</i>	Biological invasion
Zimmermann et al 2017	<i>Clusia hilariana</i>	Biological invasion
Zimmermann et al 2017	<i>Maytenus obtusifolia</i>	Biological invasion

Table 2. Case studies included in our meta-analysis about human disturbance effects on germination. We specified species, dispersal agent, seed mass (g), vegetation type, growth form, effect size Hedges' d and effect sizes variance for each case study included on our database. Dashes represent information that was not provided by the authors of the original papers and that were not available in the literature. Positive and negative values of Hedges' d indicate an increase or decrease in seed germination in disturbed conditions, respectively.

Species	Dispersal	Seed mass (g)	Vegetation	Growth form	Hedges' d	Variance
<i>Aristotelia chilensis</i>	biotic	0.029	forest	tree	-2.4945	0.13097
<i>Astrocaryum standleyanum</i>	biotic	9.6	forest	tree	-0.1167	0.66959
<i>Astronium fraxinifolium</i>	abiotic	0.02	forest	tree	0.46043	0.10292
<i>Balduina angustifolia</i>	biotic	-	non-forest	herb	-0.2511	0.25257
<i>Buchenavia grandis</i>	biotic	0.10	forest	tree	3.73844	2.12718
<i>Calophyllum longifolium</i>	biotic	13.70	forest	tree	0.56419	0.73498
<i>Cariniana micrantha</i>	abiotic	0.17	forest	tree	-3.3233	1.78584
<i>Caryocar villosum</i>	biotic	6.20	forest	tree	5.5068	4.03064
<i>Cavanillesia arborea</i>	abiotic	7.99	forest	tree	-1.3311	0.12444
<i>Chamaecrista fasciculata</i>	biotic	0.0084	non-forest	herb	0.5701	0.23362
<i>Clusia hilariana</i>	biotic	0.05	non-forest	shrub	-0.5593	0.35224
<i>Cochlospermum orinocense</i>	abiotic	0.03	forest	tree	0.9632	0.60802
<i>Cryptocarya alba</i>	biotic	1	forest	tree	-1.3875	0.08985

<i>Dalbergia miscolobium</i>	abiotic	0.32	non-forest	tree	-0.5273	0.36354
<i>Dinizia excelsa</i>	abiotic	0.20	forest	tree	-1.8163	0.88409
<i>Diospyros glabra</i>	biotic	0.10	non-forest	shrub	-3.4956	0.74811
<i>Dipteryx panamensis</i>	biotic	7.54	forest	tree	0.6773	0.76511
<i>Dryobalanops lanceolata</i>	biotic	3.27	forest	tree	-0.1836	0.6739
<i>Erythrina</i> sp.	abiotic	0.17	forest	tree	-0.1153	0.10018
<i>Erytroxylum pelleterianum</i>	biotic	0.04	non-forest	shrub	-0.568	0.7359
<i>Euclea tomentosa</i>	biotic	0.52	non-forest	shrub	-0.2547	0.25264
<i>Eugenia dysenterica</i>	biotic	0.62	forest	tree	0.29033	0.20259
<i>Gustavia superba</i>	biotic	12.50	forest	tree	-0.3061	0.68677
<i>Heliconia acuminata</i>	biotic	0.0775	forest	herb	-3.1619	2.09469
<i>Hopea nervosa</i>	biotic	0.65	forest	tree	-2.3859	1.88827
<i>Hypericum cumulicola</i>	abiotic	-	non-forest	herb	-0.3899	0.38419
<i>Jacaranda copaia</i>	abiotic	0.01	forest	tree	1.04342	0.62676
<i>Kiggelaria africana</i>	-	0.04	non-forest	tree	-1.8607	0.39113
<i>Leonotis leonurus</i>	biotic	0.0028	non-forest	shrub	-7.3188	2.43357
<i>Liatris ohlingerae</i>	abiotic	-	non-forest	herb	0	0.25
<i>Lithocarpus dealbatus</i>	biotic	-	forest	tree	3.31876	3.03033

<i>Maytenus obtusifolia</i>	biotic	0.06	non-forest	shrub	-0.3065	0.33901
<i>Melianthus major</i>	abiotic	0.03	non-forest	shrub	-13.882	8.10598
<i>Metalasia muricata</i>	abiotic	0.0006	non-forest	shrub	-3.4972	0.74857
<i>Metrosideros polymorpha</i>	abiotic	0.00006	forest	tree	-1.7326	0.1414
<i>Nothofagus glauca</i>	abiotic	0.5316	forest	tree	-1.8709	0.10492
<i>Nothofagus obliqua</i>	abiotic	0.0115	forest	tree	-0.493	0.07375
<i>Ochroma pyramidale</i>	abiotic	0.008	forest	tree	0.31894	0.51184
<i>Oenocarpus mapora</i>	biotic	6.40	forest	tree	0.30455	0.68657
<i>Olea europaea sub africana</i>	biotic	0.17	non-forest	tree	-1.9169	0.39979
<i>Parashorea tomentella</i>	biotic	2.95	forest	tree	-2.5982	2.1154
<i>Parkia multijuga</i>	biotic	7.40	forest	tree	-0.8611	0.58632
<i>Parkia pendula</i>	biotic	0.12	forest	tree	-2.3031	1.11754
<i>Poa secunda</i>	abiotic	0.0004	non-forest	grass	-0.9575	1.91673
<i>Polygonella basiramia</i>	abiotic	-	non-forest	herb	0.58607	0.264
<i>Prioria copaifera</i>	biotic	58.82	forest	tree	-0.4879	0.71776
<i>Protium panamense</i>	biotic	0.61	forest	tree	0.84104	0.81847
<i>Quercus costaricensis</i>	biotic	24.39	forest	tree	-0.6894	0.07089
<i>Quercus crassifolia</i>	biotic	1.99	forest	tree	-2.7911	0.80415

<i>Quercus graffithii</i>	biotic	0.69	forest	tree	3.32452	3.03854
<i>Quercus laurina</i>	biotic	1.80	forest	tree	0.93657	0.38635
<i>Quercus rugosa</i>	biotic	2.94	forest	tree	1.4873	0.46702
<i>Schima khasiana</i>	abiotic	0.095	forest	tree	0	0.66667
<i>Searsia angustifolia</i>	biotic	-	non-forest	shrub	-10.457	4.70756
<i>Shorea leprosula</i>	biotic	0.74	forest	tree	-2.9986	2.59622
<i>Shorea macrophylla</i>	biotic	13.80	forest	tree	-1.1821	0.96656
<i>Simarouba amara</i>	biotic	0.26	forest	tree	4.37144	2.72486
<i>Swartzia multijuga</i>	biotic	8.97	forest	tree	-0.6772	0.2141
<i>Swartzia simplex</i>	biotic	1.09	forest	tree	-0.2363	0.67865
<i>Tabebuia impetiginosa</i>	abiotic	0.11	forest	tree	-0.2958	0.10121
<i>Triplaris surinamensis</i>	abiotic	0.11	forest	tree	1.99148	0.96175
<i>Xymalos monospora</i>	biotic	0.16	forest	tree	0.15818	1.02502
<i>Zamia fairchildiana</i>	abiotic	-	forest	tree	-0.9485	0.14092

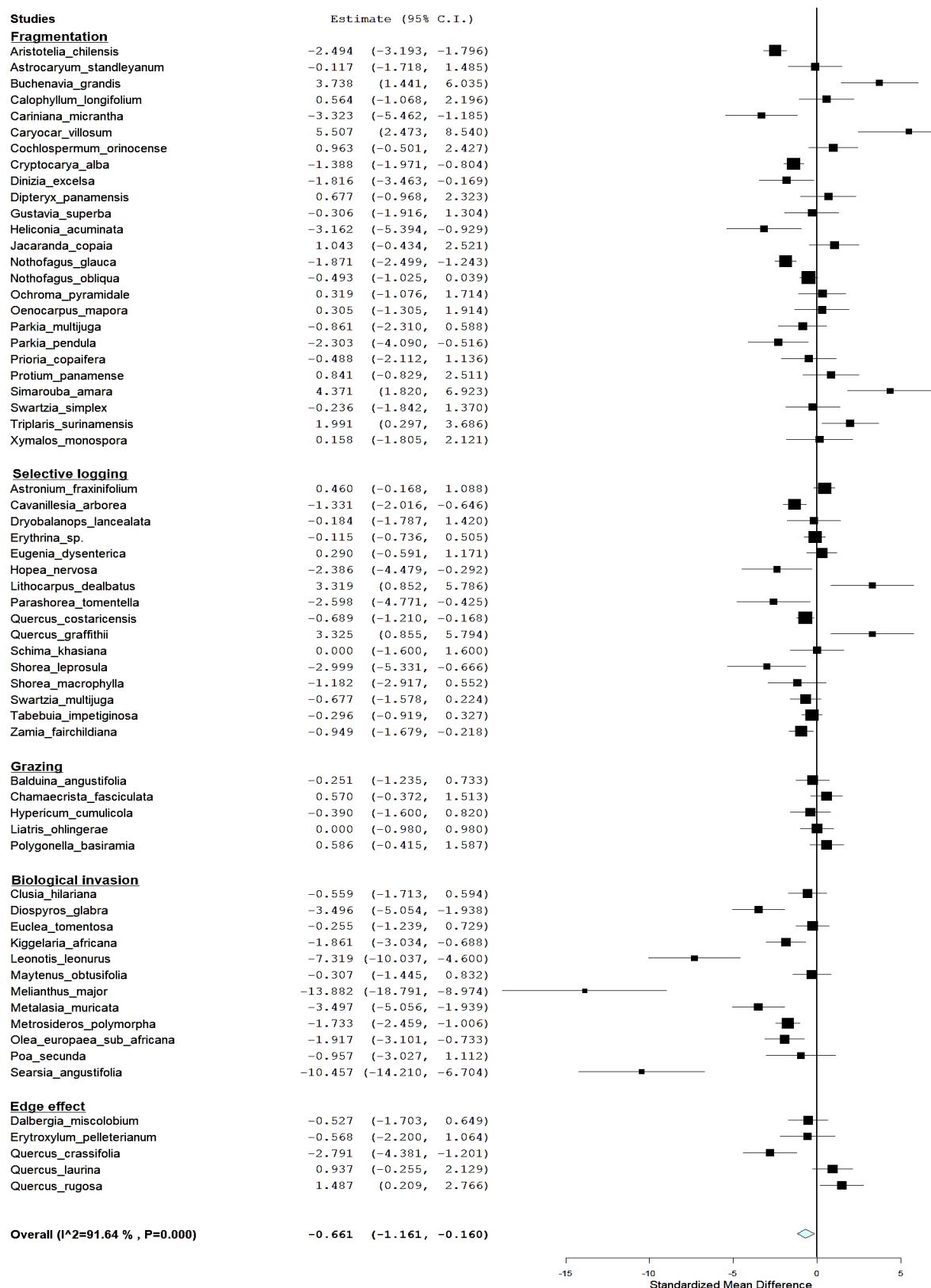


Figure 3. Forest plot containing individual effect sizes (Hedges' d) for each species included in our study. Species are ordered by disturbance type. Values of Hedges' d (black squares) and corresponded confidence intervals are specified. Confidence intervals overlapping zero (vertical line) indicate that human disturbance does not affect germination rates of the referred species.

Table 3. Estimated effect sizes (Hedges' d) and its respective standard error (SE) and 95% confidence interval (CI), according to disturbance activities, habitat and species traits. Bold values represent effect sizes that are significantly different from zero.

	Hedges' d	SE	CI	Lower CI	Upper CI
Overall	-0.6326	0.2108	0.41	-1.0458	-0.2195
Fragmentation	-0.1891	0.3182	0.62	-0.8127	0.4345
Edge effects	-0.2102	0.6725	1.32	-1.5282	1.1077
Biological invasion	-2.459	0.4697	0.92	-3.3795	-1.5385
Selective logging	-0.4376	0.3861	0.76	-1.1943	0.3191
Grazing	0.1096	0.6324	1.24	-1.1298	1.349
Forest	-0.2953	0.2512	0.49	-0.7876	0.197
Non-forest	-1.4809	0.3976	0.78	-2.2603	-0.7016
Trees	-0.3324	0.2483	0.49	-0.8191	0.1542
Non-trees	-1.587	0.4398	0.86	-2.4491	-0.7249
Biotic dispersal	-0.4809	0.2283	0.45	-0.9284	-0.0334
Abiotic dispersal	-0.6339	0.2775	0.54	-1.1777	-0.09

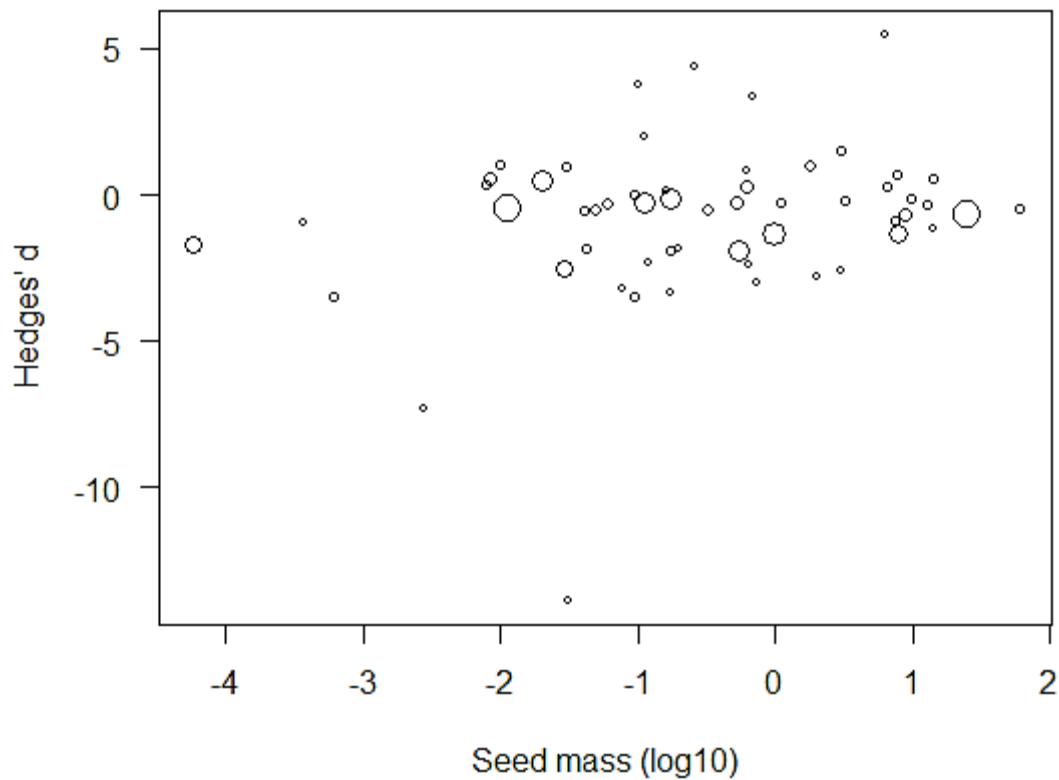


Figure 4. Seed mass and effect sizes of human disturbance effects on seed germination (Hedges' d). Each open circle represents a different species. The size of each circle is related to the variation on effect sizes. There is no relationship between seed mass and germination responses to disturbance ($Q_M=0.25$, $p=0.62$; $I^2=85.35\%$; $R^2=0\%$).

Table 4. Mixed-effect models considering the effects of species traits and/or habitat characteristics and their interaction on seed germination responses to disturbance (Hedges' d). Bold p-values represent the factor significantly affects Hedges' d.

	Q	p
Growth form	7.81	<0.01
Vegetation type	0.48	0.49
Growth form:Vegetation type	1.08	0.30
Seed mass	0.27	0.60
Growth form	8.02	<0.01
Seed mass:Growth form	1.38	0.24
Seed mass	0.22	0.64
Dispersal syndrome	0.49	0.48
Seed mass: Dispersal syndrome	0.08	0.78

Original publications included in the meta-analysis

- Asquith NM, Mejia-chang M. 2005. Mammals, edge effects, and the loss of Tropical Forest diversity. *Ecology* **86**:379–390.
- Barik SK, Tripathi RS, Pandey HN, Rao P. 1996. Tree Regeneration in a Subtropical Humid Forest: Effect of Cultural Disturbance on Seed Production, Dispersal and Germination. *Journal of Applied Ecology* **33**:1551–1560.
- Bruna EM. 2002. Effects of forest fragmentation on *Heliconia acuminata* seedling recruitment in central Amazonia. *Oecologia* **132**:235–243.
- Camargo JLC, Ferraz IDK, Imakawa AM. 2002. Rehabilitation of degraded areas of Central Amazonia using direct sowing of forest tree seeds. *Restoration Ecology* **10**:636–644.
- Christianini A V., Oliveira PS. 2013. Edge effects decrease ant-derived benefits to seedlings in a neotropical savanna. *Arthropod-Plant Interactions* **7**:191–199.
- Granados A, Brodie JF, Bernard H, O'Brien MJ. 2017. Defaunation and habitat disturbance interact synergistically to alter seedling recruitment: Ecological Applications **27**:2092–2101.
- Guariguata MR, Sáenz GP. 2002. Post-logging acorn production and oak regeneration in a tropical montane forest, Costa Rica. *Forest Ecology and Management* **167**:285–293.
- Guerrero PC, Bustamante RO. 2009. Abiotic alterations caused by forest fragmentation affect tree regeneration: A shade and drought tolerance gradient in the remnants of Coastal Maulino forest. *Revista Chilena de Historia Natural* **82**:413–424.
- Herget ME, Hufford KM, Mummey DL, Shreading LN. 2015. Consequences of Seed Origin and Biological Invasion for Early Establishment in Restoration of a North American Grass Species. *PLoS ONE* **10**:1–16.
- Lehouck V, Spanhove T, Gonsamo A, Cordeiro N, Lens L. 2009. Spatial and temporal effects on recruitment of an Afromontane forest tree in a threatened fragmented ecosystem. *Biological Conservation* **142**:518–528.
- López-Barrera F, Newton A. 2005. Edge type effect on germination of oak tree species in the Highlands of Chiapas, Mexico. *Forest Ecology and Management* **217**:67–79.
- Lopez-Gallego C. 2013. Genotype-by-Environment Interactions for Seedling Establishment Across Native and Degraded-Forest Habitats in a Long-Lived Cycad. *Botanical Review* **79**:542–558.
- Mariano V, Rebolo IF, Christianini A V. 2019. Fire-sensitive species dominate seed rain after fire suppression: Implications for plant community diversity and woody encroachment in the Cerrado. *Biotropica* **51**:5–9.
- Ruwanza S, Gaertner M, Esler KJ, Richardson DM. 2013. The effectiveness of active and passive restoration on recovery of indigenous vegetation in riparian zones in the

Western Cape, South Africa: A preliminary assessment. *South African Journal of Botany* **88**:132–141.

Stephens EL, Castro-Morales L, Quintana-Ascencio PF. 2012. Post-dispersal seed predation, germination, and seedling survival of five rare Florida scrub species in intact and degraded habitats. *The American Midland Naturalist* **167**:223–239.

Stephens EL, Quintana-Ascencio PF. 2015. Effects of habitat degradation, microsite, and seed density on the persistence of two native herbs in a subtropical shrubland. *American Journal of Botany* **102**:1978–1995.

Vieira DLM, Scariot A. 2006. Effects of logging, liana tangles and pasture on seed fate of dry forest tree species in Central Brazil. *Forest Ecology and Management* **230**:197–205.

Walker LR, Vitousek PM. 1991. An Invader Alters Germination and Growth of Native Dominant Tree in Hawai'i. *Ecology* **72**:1449–1455.

Zimmermann TG, Andrade ACS, Richardson DM. 2017. Abiotic barriers limit tree invasion but do not hamper native shrub recruitment in invaded stands. *Biological Invasions* **19**:109–129. Springer International Publishing.

Meta-analysis of human disturbance effects on seed germination under field conditions

Appendix B

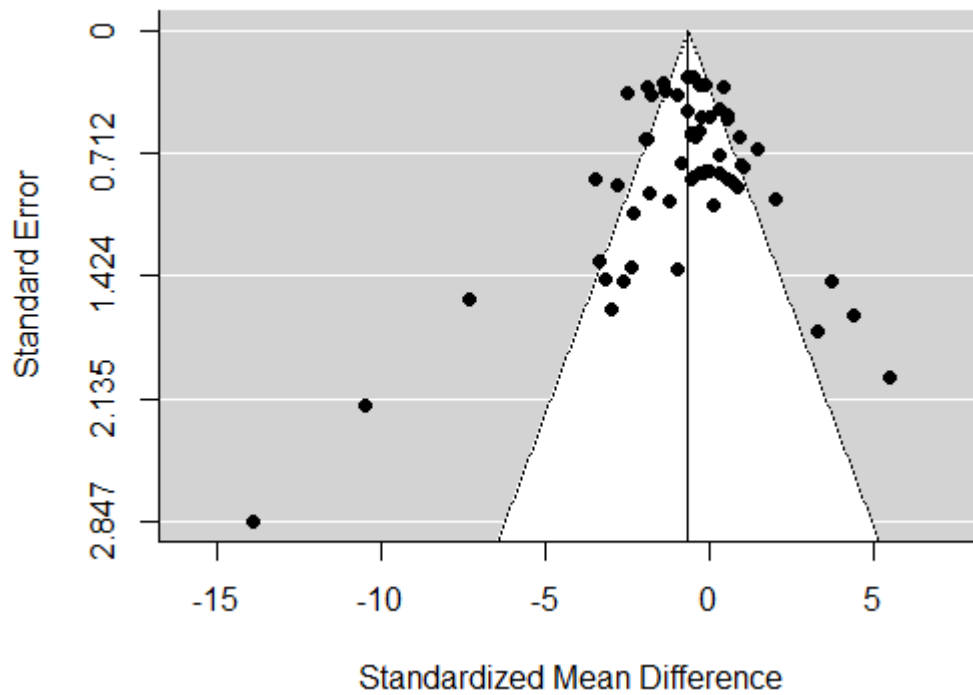


Fig. 1. Funnel plot demonstrating the absence of publication bias in the meta-analysis database. The individual effect sizes Hedges' d are represented on the x-axis and their corresponding standard errors on the y-axis. The vertical line indicates the mean effect size and the dashed line around it indicates the confidence interval (± 1.96 SE). Each black dot represents a plant species.

Table 1. Phylogenetically independent meta-analysis about human disturbance effects on seed germination. Number of species, effect size Hedges' d, effect size variance, 95% confidence interval (CI), z and p-values are provided for analysis considering all study cases and according to different disturbance types. Bold p-value indicates an effect size significantly different from zero.

Group	N	Hedges' d	Variance	95%CI	Z	p
All studies	63	-0.232	0.0736	(-0.764,0.299)	0.73	0.3915
Degradation	44	0.772	0.0993	(0.155,1.390)	6.01	0.0142
Fragmentation	19	-0.453	0.1878	(-1.303,0.396)	1.09	0.2955

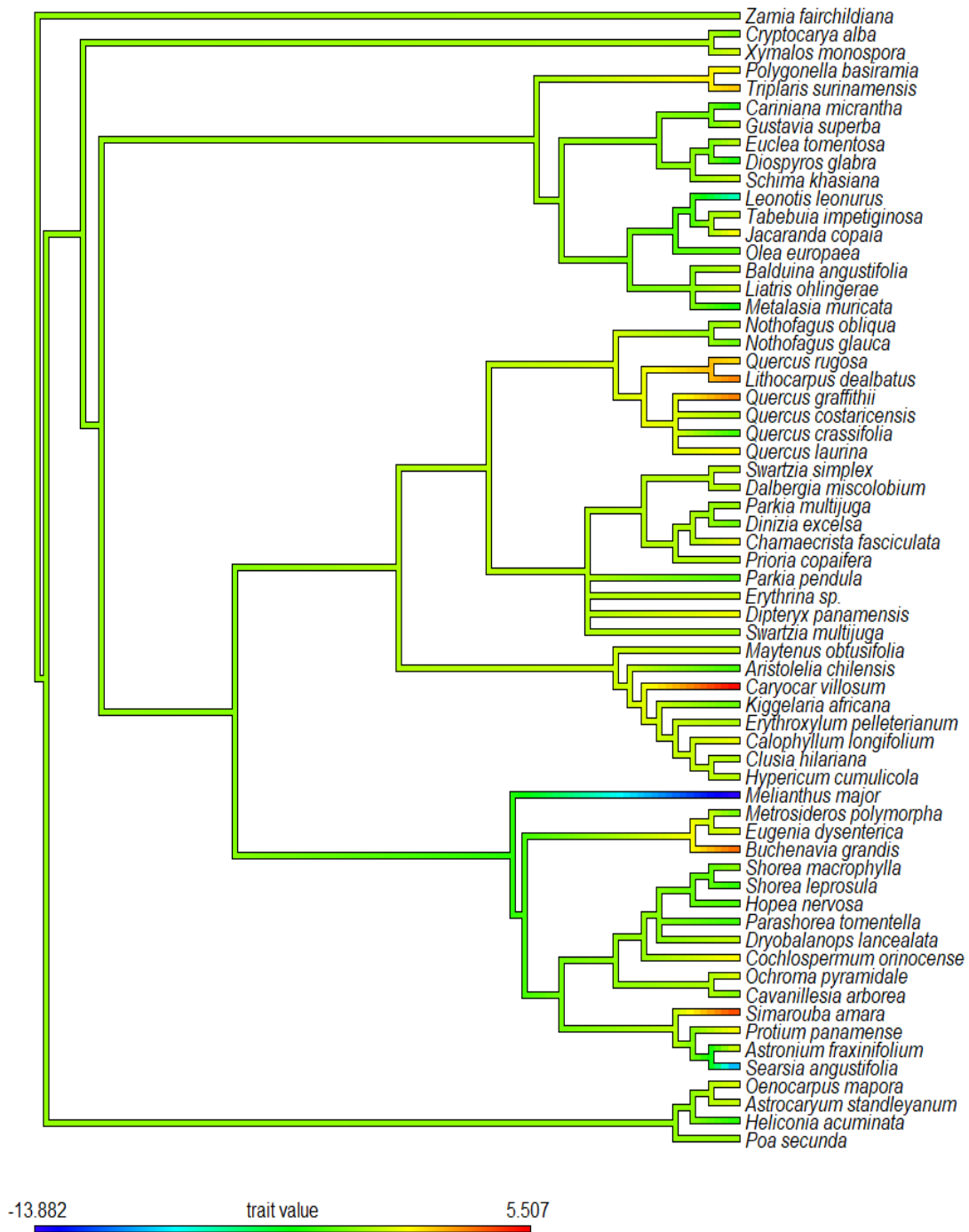


Fig. 2. Phylogenetic tree of 63 species included in the meta-analysis about human disturbance effects on seed germination. Colors in the end of each branch demonstrate the effect size (Hedges' d) of each species. Positive and negative values indicate a positive or negative effect of disturbance on seed germination, respectively. Effect size varies according to the legend (trait value). More information about the Hedges' d value for each species and their variance are provided in Appendix A.

1 **Capítulo 2**

2 Formatado de acordo com as normas do periódico *Journal of Ecology*

3

4

5 **Meta-analysis of human disturbance effects on seedling survival under field**

6 **conditions**

7 **Abstract**

8 1. Seedling survival is one of the most important bottlenecks on plant recruitment.

9 Human disturbances can alter abiotic and biotic factors, potentially changing plant
10 regeneration and species composition. Seedling survival responses to disturbance can be
11 extremely heterogeneous, and it is still not clear if species traits and environmental
12 characteristics are able to predict species responses or if they are essentially random.

13 2. We carried out a global meta-analysis to evaluate the impact of human disturbance in
14 seedling survival in the field. We analyzed whether disturbance type, plant growth form,
15 dispersal syndrome, ecological successional category, seed mass, SLA, leaf thickness,
16 vegetation type, latitude and interactions between those characteristics are related to
17 seedling responses to disturbance. We also accounted for phylogenetic relatedness
18 among species.

19 3. We identified 51 case studies provided by 22 papers. Available information was
20 concentrated on forest tree species from tropical and subtropical areas. Species
21 responses were highly heterogeneous and human disturbances had no overall effect on
22 seedling survival. The variation in effect sizes was not related to any of the species
23 traits, habitat characteristics, their interactions or phylogeny.

24 4. *Synthesis*. Our global meta-analysis demonstrated that seedling survival responses to
25 disturbance are hard to predict. Species traits and habitat characteristics are not able to
26 explain seedling responses to disturbed conditions, suggesting a limitation of niche-
27 based approaches. Alternatively, stochasticity and variability of biotic and abiotic
28 factors at a microsite scale might be important sources of heterogeneity on seedling
29 responses.

30

- 31 **Keywords:** plant recruitment, habitat fragmentation, habitat degradation, biological
- 32 invasion, edge effects, selective logging, stochasticity, species traits.

33 **Introduction**

34 Seedling survival is an important bottleneck to plant recruitment. Even in a plant
35 population in which seeds disperse and colonize new patches, only a few seedlings
36 survive and become saplings (e.g. Gomez-Aparicio, 2008). Many hypotheses have been
37 suggested to explain variation in seedling recruitment and survival. Characteristics of
38 the species or individual plants (such as seed mass; Moles & Westoby, 2006; Neuschulz
39 et al., 2016), and those of the biotic and abiotic environment (such as the surrounding
40 vegetation; Lett & Dorrepaal, 2018) in which seedlings find themselves, may constrain
41 opportunities for successful recruitment. Alternatively, the chance of a seedling
42 surviving to maturity might be so low that when looked at in a general context, it might
43 be an essentially random event, in which no characteristics of the plants or their
44 environment can offer a general explanation for successful recruitment (e.g. Hubbell et
45 al., 1999).

46 Seedling survival might be one of the main limiting factors for plant
47 regeneration, with the potential to affect community composition and diversity (Clark,
48 Poulsen, & Levey, 2013; Gómez-Aparicio, 2008; Herrera, Jordano, Lopez-Soria, &
49 Amat, 1994). The concepts of “safe sites” for seedling survival and the “regeneration
50 niche” were recognized as critical factors in plant population dynamics over 40 years
51 ago and are still foundational concepts in plant ecology (Grubb, 1977). Environmental
52 heterogeneity can influence recruitment at a range of spatial scales and variation in
53 microsite conditions can affect seeds, seedlings and saplings differently (Gómez-
54 Aparicio, 2008; Turnbull, Crawley, & Rees, 2000). Microsite characteristics and species
55 niche differentiation have been reported as determinant to seedling emergence and
56 survival (Clark, Poulsen, & Levey, 2012). In addition to abiotic conditions, biotic
57 factors like herbivory, pathogens, competition between seedlings and interactions with

58 the surrounding vegetation can also play an important role on plant recruitment (Janzen,
59 1970; Connell, 1971; Broker et al., 2008; Cadotte & Tucker, 2017; Clark et al., 2012;
60 Lett & Dorrepaal, 2018). Moreover, some species traits, like seed mass and growth
61 form, are often used to predict regeneration likelihood (e.g. Moles & Westoby 2002;
62 Ibáñez et al., 2014), and plant strategies or syndromes of traits may help to explain
63 which seedlings survive in which environments and under which conditions. It is often
64 assumed that disturbed conditions such as those found in fragmented habitats and on
65 forest edges favor ruderal and other ‘disturbance-adapted’ species, while ‘interior’
66 species are harmed by disturbance.

67 Nevertheless, microsite characteristics and species traits sometimes do not
68 explain interspecific variation in plant recruitment (Clark, Poulsen, Levey, & Osenberg,
69 2007; Hubbell et al., 1999; Moles & Westoby, 2006). Other approaches used to explain
70 variation in plant regeneration include stochastic and null models. According to the
71 lottery model, for example, temporal variation in environmental conditions can favor
72 different species at different times, depending on species competitive and dispersal
73 abilities and propagule production (Sale, 1977; Chesson & Warner, 1981; Turnbull et
74 al., 2000). On the other hand, the neutral theory assumes that trophically similar species
75 are functionally equivalent, thus stochasticity would be an important source of variation
76 in plant regeneration rather than niche differences between species (Hubbell, 2005).
77 Other authors propose that neutral and niche-based models can both be predictive and
78 plant communities can be affected by synergistic interactions among stochastic
79 processes, species tolerances to abiotic factors and interspecific interactions (see Lortie
80 et al., 2004).

81 Human disturbance can be a critical factor for plant regeneration. Disturbance
82 can alter environmental conditions, changing community structure and causing species

83 loss and alterations in ecosystem functions (Chapin III et al., 2000; Hooper et al., 2005;
84 Haddad et al., 2015). Habitat fragmentation, edge effects and degradation activities,
85 such as biological invasion, selective logging and grazing are some of the main
86 disturbance causes (McIntyre & Hobbs, 1999; Murcia, 1995; Fisher et al., 2007; Morris,
87 2008). Anthropogenic disturbance can alter species richness and composition and
88 several abiotic factors, such as light availability, soil moisture, air temperature and litter
89 accumulation (Laurance & Cochrane, 2001; Lima, Lima, Santos, Tabarelli, & Zickel,
90 2015; Uriarte, Bruna, Rubim, Anciaes, & Jonckheere, 2010). In addition, some plant
91 species can increase their densities in disturbed areas, dominating edges and small
92 fragments and changing conditions for seed germination and seedling establishment of
93 their own or for other species (Aguiar & Tabarelli, 2010). Human disturbance impacts
94 on ecosystems are extensive and pervasive worldwide, then understanding their effects
95 on ecological processes is crucial to the definition of disturbance mitigation and
96 conservation strategies (Haddad et al., 2015; Neuschulz et al., 2012).

97 Species responses to disturbance can be highly variable and attributing
98 differences among plants to different functional groups and vegetation types is difficult
99 (e.g. Ibáñez et al., 2014). Degradation and fragmentation can have negative effects on
100 different plant regeneration processes, like pollination and seed dispersal, and there is
101 variation in species responses according to disturbance type, seed size and geographical
102 region (Fontúrbel, Jordano, & Medel, 2015; Markl et al., 2012; Neuschulz, Mueller,
103 Schleuning, & Böhning-Gaese, 2016). According to recent meta-analysis from
104 Neuschulz et al. (2016), human disturbance in forests has no overall effect on plant
105 regeneration processes like recruitment, seed predation and herbivory. Despite its
106 importance to plant regeneration and community assembly, it is still not clear how

107 disturbance generally affects seedling survival and if there is variation on species
108 responses according to species traits and environmental characteristics.

109 Detecting the reasons behind changes in species composition has profound
110 consequences for our understanding of the reassembly of ecological communities after
111 disturbances. As seedlings produce the template of future plant communities, we
112 conducted a global meta-analysis of field experiments on seedling survival responses to
113 disturbance to evaluate whether species and environmental characteristics can explain
114 the variations observed. We evaluated whether a series of intrinsic species traits related
115 to resource acquisition and life-history strategies (growth form, dispersal syndrome,
116 seed mass, ecological succession category, specific leaf area (SLA) and leaf thickness)
117 can predict positive or negative responses to disturbance, and if extrinsic factors such as
118 disturbance type or environmental characteristics (vegetation type and latitude) create
119 positive or negative conditions for seedling survival in response to anthropic
120 disturbance. Since some species can be favored by disturbed conditions and others can
121 be severely harmed, it is important to evaluate which characteristics and traits of the
122 species and the environment may favor or constrain seedling survival under different
123 disturbance conditions or if species survival responses are indistinguishable from
124 random. No previous studies have synthesized the results of such experiments in the
125 field to address those questions.

126

127 **Methods**

128 *Literature Review*

129 We searched the literature on Web of Science (1945 to August 2018) using a
130 combination of search terms: seedling* AND degrad* OR fragment* OR disturb*OR
131 edge_effect* OR selective_logging OR biological_invasion*. These terms were selected

132 to include publications addressing human disturbance effects on seedling survival.
133 Results were filtered according to the research areas ‘Environmental Sciences’,
134 ‘Ecology’, ‘Biodiversity Conservation’ and ‘Plant Sciences’. Studies were selected
135 according to the following criteria: (a) studies describing effects of habitat degradation
136 (e.g. selective logging, biological invasion, hunting and grazing) and/or habitat
137 fragmentation (studies involving habitat fragments, secondary forests and/or edge
138 effects) on seedling survival; (b) studies using field experiments or field observations
139 (we did not include laboratory or greenhouse trials); (c) comparing at least one
140 undisturbed or relatively undisturbed natural area (control site) to one or more sites
141 affected by human disturbance conditions; and (d) studies providing mean seedling
142 survival or the number of surviving seedlings after some defined period of time for
143 disturbed and undisturbed sites. To increase our dataset, we also screened the papers
144 included in the meta-analysis of Neuschulz et al. (2016) about disturbance effects on
145 forest plant regeneration. We accessed their database about plant recruitment (including
146 germination, seedling survival, seedling density and sapling establishment) to select
147 studies that fulfilled the criteria described above. Full-text reading was conducted in all
148 selected papers, and studies that did not fit in our criteria were excluded. We contacted
149 authors when there was some missing information in the manuscript. We followed the
150 PRISMA Statement and guidelines of quality criteria for meta-analysis (Moher et al.
151 2009; Koricheva & Gurevitch, 2014; Appendix A). If the manuscript presented data
152 from a gradient of degradation or fragmentation, we selected seedling survival data for
153 the most disturbed plot or category and the least disturbed one for calculating the
154 response (effect size) to disturbance. If a paper presented data for more than one
155 species, each species was considered as an independent case (see below for how these
156 were analyzed). When an experiment was replicated in more than one year, we used

157 data from only one of those periods, chosen at random, to include in our database. We
158 did not include studies that started the experiment with one-year-old saplings or older
159 individuals since they do not contain information about seedling survival at early stages.
160 From each selected paper we extracted seedling survival proportions, the number of
161 seedlings that survived and died and the sample size under disturbed and undisturbed
162 conditions. We also recorded information about disturbance type (fragmentation, edge
163 effects, biological invasion, selective logging, etc), species traits (growth form, dispersal
164 syndrome, ecological succession category, seed mass, SLA and leaf thickness),
165 vegetation type, geographic coordinates, experimental method (seed addition, seedling
166 addition and naturally emerged seedlings) and duration of the seedling monitoring from
167 the original papers. Since some information about species traits was not available in all
168 manuscripts, we included data from the TRY Plant Trait Database and Seed Information
169 Database (Kattge et al., 2011; Royal Botanic Gardens Kew, 2019; see Appendix B for
170 more details). The classification of species into an ecological successional category
171 followed information presented by the original papers. We broadly classified species as
172 pioneers (also including species described as early successional) and non-pioneers (also
173 including old-growth or shade tolerant species).

174

175 *Data analysis*

176 Effect sizes of case studies were calculated using ln odds ratios to compare the
177 number of seedlings that survived and died on disturbed and undisturbed sites. Odds
178 ratios are a commonly used metric of effect size in medical meta-analyses concerned
179 with morbidity and mortality (Rosenberg et al., 2013) but have been less frequently
180 used in ecological meta-analyses (Koricheva & Gurevitch, 2014). Odds ratios
181 calculations incorporate a 2x2 contingency table, which includes data about seedling

182 survival and mortality rates in disturbed and undisturbed sites. Significant negative and
183 positive values indicate if seedling survival is reduced or increased, respectively, by
184 human disturbance. To estimate the overall effect of human disturbance on seedling
185 survival we used a random-effects model since we assume true effect size variation
186 between species. The estimator restricted maximum-likelihood (REML) was used to
187 estimate the true heterogeneity (τ^2), and the significance and proportion of heterogeneity
188 explained by the model was analyzed using the model heterogeneity statistics Q , p -
189 value, and I^2 (Rosenberg 2013, Higgins and Thompson 2002). Mixed-effects meta-
190 regression models were applied to individually evaluate the influence of disturbance
191 type, species traits and habitat characteristics on species response to human disturbance.
192 For each meta-regression we included one of the following factors as a moderator (see
193 Appendix B for details): disturbance type, growth form, dispersal syndrome, ecological
194 succession category, seed mass, SLA, leaf thickness, vegetation type, latitude,
195 experimental method and duration of the experiment. To evaluate if interactions
196 between two moderators influenced seedling survival responses to disturbance, we
197 applied additional mixed-effects meta-regressions using two moderators in the same
198 model. We tested the following pairs of moderators: (a) seed mass and ecological
199 succession, (b) seed mass and growth form, and (c) vegetation type and growth form.
200 Sample sizes of all previously described models are presented below (see Appendix B
201 for more details). For each mixed-effects model (containing one or two moderators) we
202 also evaluated the results of the Wald-type test of coefficients, Q_M and its p -value,
203 which indicates if the moderator(s) significantly affects effect sizes variability. We
204 excluded the intercept from models that involved categorical moderators to obtain mean
205 effect sizes and Confidence Intervals (CI) for each category within the moderators (see
206 Viechtbauer, 2010). Effect sizes (ln odds ratios) were considered significant if 95% CI

207 did not overlap zero. Meta-analyses were performed using OpenMEE (Wallace et al.
208 2016) and the metafor R package (R Core Team 2017, Viechtbauer 2010).

209 To evaluate the estimated number of studies that might be missing due to
210 publication bias, we used the trim-and-fill procedure. We also analyzed a funnel plot to
211 assess potential asymmetry between positive and negative values distributions. We
212 evaluated the influence of evolutionary relatedness on species responses to disturbance
213 performing a phylogenetic autocorrelation analysis using the software phyloMeta
214 (Lajeunesse 2011; version 1.3), which compared models with and without phylogenetic
215 information. The best fitted model was selected based on the lowest Akaike's
216 Information Criterion (AIC; Lajeunesse, 2009). We obtained the phylogenetic tree at
217 species level from Zanne et al. (2014) using Phylomatic (Webb & Donoghue, 2005;
218 Version 3, 2012).

219

220 **Results**

221 Our search resulted in 1906 papers and 22 were selected according to our criteria,
222 corresponding to 51 case studies and 50 species (Appendix A). Our database included
223 species from temperate and tropical forests, shrublands and grasslands, but study sites
224 were unequally distributed across the globe, with far better representation in the New
225 World tropics and subtropics than some previous global ecological meta-analyses (e.g.
226 Lowry et al., 2013; Fig. 1). Studies addressing fragmentation and habitat degradation
227 represented 24 and 27 species, respectively (Appendix B). Disturbance had neutral,
228 positive and negative effects on seedling survival for 28, 12 and 11 case studies,
229 respectively.

230 The mean overall effect of human disturbance on seedling survival was slightly
231 negative, but it was not significantly different than zero (Fig. 2; $\ln OR = -0.20$, $CI = 0.50$,

232 $p=0.43$) and was highly heterogeneous ($Q=521.73$, $p<0.01$, $I^2=92.24\%$, $n=51$); that is,
233 human disturbance had no overall generalizable effect on seedling survival, and results
234 exhibited high heterogeneity in species responses to disturbance. Furthermore, none of
235 the analyzed species traits and habitat characteristics explained the variation in
236 responses to human disturbance. In addition, effect sizes variation was not related to the
237 experimental method ($Q_M=0.98$, $p=0.61$, $I^2=91.40\%$, $n=51$) or the duration of seedling
238 survival monitoring ($Q_M=0.03$, $p=0.86$, $I^2=92.42\%$, $n=47$).

239 Neither biological invasion, selective logging, habitat fragmentation or edge
240 effects significantly affected seedling survival on average, compared to undisturbed
241 conditions, with high unexplained heterogeneity among studies (Fig. 2, $Q_M=1.51$,
242 $p=0.68$, $I^2=89.91\%$, $n=42$). Growth form did not explain the variation in species
243 response to disturbance ($Q_M=4.99$, $p=0.29$, $I^2=89.78\%$, $n=51$) and mean effect sizes for
244 trees, herbs, shrubs and grasses were not different from each other (Fig. 2). Dispersal
245 syndrome did not influence seedling survival responses to disturbed conditions
246 ($Q_M=1.03$, $p=0.31$, $I^2=90.01\%$, $n=45$). Both pioneer and non-pioneer species had neutral
247 responses (Fig. 2), and ecological successional category did not affect disturbance
248 effects on seedling survival ($Q_M=0.02$, $p=0.90$, $I^2=87.42\%$, $n=30$). Seed mass did not
249 explain variation in effect sizes ($R^2=0$, $Q_M=0.01$, $p=0.93$, $I^2=87.77\%$, $n=39$, Fig. 3) and
250 seedling survival responses to disturbance were also not related to SLA ($R^2=0$, $Q_M=0.1$,
251 $p=0.75$, $I^2=87.73\%$, $n=10$) and leaf thickness ($R^2=0$, $Q_M=0.03$, $p=0.86$, $I^2=83.99\%$,
252 $n=14$).

253 There was no influence of vegetation type on seedling responses to disturbed
254 conditions, when comparing forests and non-forest physiognomies (Fig. 2, $Q_M=0.09$,
255 $p=0.77$, $I^2=92.19\%$, $n=51$). Moreover, human disturbance effects on seedling survival
256 were not related to absolute latitude (Fig. 2, $Q_M=0.03$, $p=0.86$, $I^2=92.23\%$, $n=51$).

257 When testing the interactions between moderators, seed mass and ecological
258 succession category combined were not related to effect sizes variation ($Q_M=0.14$,
259 $p=0.93$, $I^2=84.11\%$, $R^2=0$, $n=23$). Seedling survival responses to disturbance were also
260 not related to the interaction between seed mass and growth form ($Q_M=8.44$, $p=0.08$,
261 $I^2=84.1\%$, $R^2=17.37\%$, $n=39$) and vegetation type and growth form ($Q_M=3.31$, $p<0.51$,
262 $I^2=92.02\%$, $R^2=0$, $n=51$).

263 Our database included a wide range of negative, neutral and positive effect sizes.
264 The funnel plot indicated there was no publication bias and no asymmetry between
265 negative and positive values (see Appendix A) and the trim-and-fill analysis also
266 demonstrated that there are no missing studies.

267 When analyzing phylogenetic autocorrelation, the model that did not consider
268 phylogenetic information presented the lowest AIC value (155.13), compared to the
269 model that incorporated phylogeny (162.85; see Appendix A for more results); that is,
270 accounting for phylogeny did not improve the fit of the model.

271

272 **Discussion**

273 Human disturbance had no consistent overall effect on seedling survival, and we found
274 great heterogeneity in species responses. None of the intrinsic (seed mass, SLA, leaf
275 thickness, successional category), extrinsic (latitude, vegetation type) and evolutionary
276 (phylogenetic relatedness) factors generally assumed to affect seedling survival had a
277 general effect on differential survival in disturbed compared to less disturbed
278 environments in experimental field studies.

279 There are several possible explanations for this. First, a global meta-analysis of
280 field experimental results may be at the wrong scale to detect differential responses, and
281 microsite differences within studies may be where such differences exist. Second, while

282 none of the presumed explanatory factors actually explain variation in survival
283 responses to disturbance, there may be other untested factors responsible for the high
284 heterogeneity that we found in responses (see below). Third, it may be that seedling
285 survival is such a rare event that causal factors cannot be distinguished from random, at
286 least as a general explanation for how seedling survival responds to anthropogenic
287 disturbance. Fourth, seedling survival is indeed so limited that it is not what determines
288 species distributions or dominance regarding disturbance. Instead, it may be that the
289 other factors such as seed production and dispersal determine where species are found;
290 that is, the sensitivity of population growth (λ) to other factors overshadows that
291 to seedling survival. If that is true, the existence of species- or syndrome-specific
292 seedling safe sites or regeneration niches is uncertain, at least with respect to seedling
293 survival.

294 While we cannot distinguish between these explanations, our results do cast into
295 serious question the common explanations for the regeneration niche and seedling
296 strategies for survival in response to disturbance. It is also unlikely that our inability to
297 detect the effects of explanatory factors is due to low statistical power. An examination
298 of the figures and results shows broad overlap between the responses due to
299 hypothesized explanatory factors, and all p values are far from statistical significance.
300 The only exceptions would be the models that included SLA and leaf thickness because
301 of the relatively low sample size. While phylogenetic resemblance does not explain
302 species' survival responses to disturbance, we note that there is essentially no
303 replication of species among these studies, and further experiments that replicated
304 species responses in different sites and conditions would be valuable.

305 The microhabitat immediately surrounding a seedling can be determinant to its
306 survival (Gomez-Aparicio 2008, Rodríguez-García, Bravo & Spies 2011). Factors such

307 as canopy openness, light irradiance, litter cover and surrounding vegetation can have a
308 profound impact in the suitability of a microsite to seedling establishment and survival
309 (Fowler 1988, Dalling & Hubbel 2002, Comita et al 2009, Gomez-Aparicio et al 2005).
310 In addition, all those factors can also alter biotic interactions like herbivory and
311 pathogens that also influence survival (Dalling & Hubbell 2002, Krishnadas & Comita
312 2018). Therefore, variation at a microsite level could explain the high heterogeneity on
313 species responses to disturbance and the lack of influence of species traits and habitat
314 characteristics on survival responses. Further information about microsite characteristics
315 of each experimental plot, like litter cover, light incidence, soil characteristics and
316 herbivory rates, might help us understand the importance of the microsite-scale factors
317 to seedling survival responses to disturbance. In that context, studies addressing
318 seedling establishment at different scales, from regional scale to microhabitat, are also
319 important (e.g. Gómez-Aparicio 2008).

320 Growth form, dispersal syndrome, ecological successional category and seed
321 mass are traits often used to predict plant recruitment variation among different species,
322 and some of those traits might affect the susceptibility of certain plant recruitment
323 stages to disturbance (Ibáñez et al., 2014; Neuschulz et al., 2016). For example, seed
324 mass influences human disturbance effects on seed dispersal, and large-seeded species
325 are the most affected ones (Markl et al., 2012). In addition, traits like SLA and leaf
326 thickness can be associated to resource acquisition of seedlings and adult plants and
327 their responses to environmental conditions (Grotkopp et al. 2002; Cingolani et al.
328 2007). However, all tested species traits and habitat characteristics are not able to
329 explain how human disturbance affects seedling survival. Other untested characteristics
330 may affect survival responses, such as seedling-related traits like growth rates (see
331 Dalling & Hubbel 2002), but such data is unavailable for most species. Since the

332 surrounding vegetation can also affect seedling survival (e.g. Lett & Dorrepaal, 2018),
333 local species richness and diversity could also influence effect sizes. Unfortunately,
334 there is not enough information to analyze the effect of any of those factors on seedling
335 responses. In addition, additional studies on vegetation types such as grasslands,
336 shrublands and savannas would elucidate if plants from different open physiognomies
337 respond differently to disturbance.

338 Seedling mortality can be extremely high for some species. Most seedlings die
339 from desiccation, herbivory and pathogens attack and just a few of them become
340 saplings and juveniles (Moles & Westoby 2004, Herrera et al. 1994). In addition, abiotic
341 and biotic factors that affect seedling survival are often variable across space and time,
342 such as temperature, humidity, litter cover and herbivory, which could add more
343 heterogeneity in survival responses to disturbance. Therefore, the combination of low
344 seedling survival rates and spatial and temporal variability of related factors could
345 generate the unpredictable and essentially random responses of seedling survival to
346 disturbance. Functional differences among species and niche differentiation are often
347 used to explain species responses to a heterogeneous environment (e.g. Clark et al.,
348 2012), but our analysis demonstrates that species traits and habitat characteristics are
349 not good predictors of seedling survival responses to disturbed conditions in a global
350 scale. Although we cannot elucidate the relative importance of niche-based and
351 stochastic processes to seedling responses to disturbance, stochasticity should also be
352 considered as a possible explanation to variation in those plant recruitment processes.

353 Since seedling responses to disturbance are hard to predict, generalizations
354 probably are not efficient in this case. Despite the neutral overall effect of human
355 disturbance on seedling survival that we found in our meta-analysis, some case studies
356 indicate that species are individually affected in positive or negative ways (see

357 Appendix B). For example, seedling survival rates of *Parkia multijuga*, *Pectocarya*
358 *linearis* and other 21 species were lower higher in disturbed sites, compared to
359 undisturbed ones (see Appendix B for references and details). However, we caution that
360 these are unreplicated results from individual studies; further information on these
361 species would be very valuable for confirming those results. For endangered and
362 ecologically important species, it might be especially important to analyze effect sizes at
363 multiple sites (e.g. Yates & Broadhurst 2002), highlighting the importance of each case
364 study.

365 Our review also revealed important knowledge gaps involving disturbance and
366 seedling survival. Information about non-tree forest species and trees on non-forest
367 physiognomies would be extremely helpful to dissociate the effects of growth form and
368 vegetation type on species responses. Grasses, herbs and shrubs are underrepresented on
369 literature, as desert and semi-arid plant species. Some geographical regions are
370 underrepresented, especially Europe and Asia, and most study cases are concentrated on
371 tropical and subtropical areas. Moreover, because there was no data replication for the
372 same species (except for the two case studies for *Quercus acutifolia*), it is not possible
373 to distinguish whether seedling survival responses to disturbance vary according to
374 species, study site or both combined. We also identified some issues with the data
375 report. For example, some papers describe the methods vaguely and do not specify
376 important information, such as the number of experimental units and how many seeds
377 or seedlings were added in the field. Variation metrics (SD, SE or CI) and species traits
378 like growth form, dispersal syndrome and seed mass could be easily provided by most
379 of the studies, but they are not reported in some of them.

380 Predictive factors, such as species traits, could be extremely useful to understand
381 ecological processes and to define restoration and conservation priorities. However, in

382 the case of seedling survival responses to disturbance we were not able to identify any
383 generalizations involving the species traits and habitat characteristics, when analyzing at
384 a global scale. We suggest that further studies evaluate seedling responses to
385 disturbance of several plant species, as well as microhabitat characteristics (e.g. litter
386 layer, soil moisture, light incidence, etc). Moreover, studies evaluating seedlings of
387 plant species already described in the literature, i.e. providing species replication, would
388 help us understand how different sites and disturbance types can affect species
389 responses (Nakagawa & Parker, 2015). Our results indicate that there is high
390 heterogeneity on seedling survival responses to anthropogenic disturbance, and the
391 limitation of niche-based approaches to properly explain seedling responses at a global
392 scale. Our findings highlight the importance to test approaches that consider
393 stochasticity and heterogeneity on microhabitat conditions as potential sources of
394 variation on seedling survival.

395

396

397 **Acknowledgements**

398 We thank R. Tacker for his valuable comments about phylogenetic analysis and D.
399 Digiovanni, M. Nolan and Stony Brook University for their assistance. This study was
400 financed by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil
401 (CAPES) – Finance Codes 001 and PDSE 88881.188844/2018-01, and Conselho
402 Nacional de Desenvolvimento Científico e Tecnológico (CNPq; proc. 486113/2013-2).
403 The study has been supported by the TRY initiative on plant traits ([http://www.try-
404 db.org](http://www.try-
404 db.org)). The TRY initiative and database is hosted, developed and maintained by J.
405 Kattge and G. Bönisch (Max Planck Institute for Biogeochemistry, Jena, Germany).

406 TRY is currently supported by DIVERSITAS/Future Earth and the German Centre for
407 Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig.

408

409 **References**

410 Aguiar, A. V., & Tabarelli, M. (2010). Edge Effects and Seedling Bank Depletion: The
411 Role Played by the Early Successional Palm *Attalea oleifera* (Arecaceae) in the
412 Atlantic Forest. *Biotropica*, *42*(2), 158–166.

413 Brooker, R. W., Maestre, F. T., Callaway, R. M., Lortie, C. L., Cavieres, L. A.,
414 Kunstler, G., ... Michalet, R. (2008). Facilitation in plant communities: the past,
415 the present, and the future. *Journal of Ecology*, *96*, 18–34.

416 Cadotte, M. W., & Tucker, C. M. (2017). Should Environmental Filtering be
417 Abandoned? *Trends in Ecology and Evolution*, *32*(6), 429–437. doi:
418 10.1016/j.tree.2017.03.004

419 Chapin III, F. S., Zavaleta, E. S., Eviner, V. T., Naylor, R. L., Vitousek, P. M.,
420 Reynolds, H. L., ... Díaz, S. (2000). Consequences of changing biodiversity.
421 *Nature*, *405*(June), 234–242. doi: 10.1038/35012241

422 Clark, C. J., Poulsen, J., Levey, D., & Osenberg, W. (2007). Are Plant Populations Seed
423 Limited? A Critique and Meta-Analysis of Seed Addition Experiments. *The*
424 *American Naturalist*, *170*(1), 128–142. doi: 10.2307/4541065

425 Clark, C. J., Poulsen, J. R., & Levey. (2012). Vertebrate herbivory impacts seedling
426 recruitment more than niche partitioning or density-dependent mortality.
427 *Ecological Monographs*, *93*(3), 554–564. Retrieved from
428 <http://www.esajournals.org/doi/abs/10.1890/11-0894.1>

429 Clark, C. J., Poulsen, J. R., & Levey, D. J. (2013). Roles of Seed and Establishment
430 Limitation in Determining Patterns of Afrotropical Tree Recruitment. *PLoS ONE*,

- 431 8(5), 1–12. doi: 10.1371/journal.pone.0063330
- 432 Fontúrbel, F. E., Jordano, P., & Medel, R. (2015). Scale-dependent responses of
433 pollination and seed dispersal mutualisms in a habitat transformation scenario.
434 *Journal of Ecology*, 103(5), 1334–1343. doi: 10.1111/1365-2745.12443
- 435 Gómez-Aparicio, L., Gómez, J. M., & Zamora, R. (2005). Microhabitats shift rank in
436 suitability for seedling establishment depending on habitat type and climate.
437 *Journal of Ecology*, 93(6), 1194–1202. doi: 10.1111/j.1365-2745.2005.01047.x
- 438 Gómez-Aparicio, L. (2008). Spatial patterns of recruitment in Mediterranean plant
439 species: Linking the fate of seeds, seedlings and saplings in heterogeneous
440 landscapes at different scales. *Journal of Ecology*, 96(6), 1128–1140. doi:
441 10.1111/j.1365-2745.2008.01431.x
- 442 Haddad, N. M., Brudvig, L. A., Clobert, J., Davies, K. F., Gonzalez, A., Holt, R. D., ...
443 Townshend, J. R. (2015). Habitat fragmentation and its lasting impact on Earth's
444 ecosystems. *Science Advances*, 1(2), 1–10.
- 445 Herrera, C. M., Jordano, P., Lopez-Soria, L., & Amat, J. A. (1994). Recruitment of a
446 Mast-Fruiting, Bird-Dispersed Tree: Bridging Frugivore Activity and Seedling
447 Establishment. *Ecological Monographs*, 64(3), 315–344.
- 448 Hooper, D. U., Chapin III, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., ...
449 Wardle, D. A. (2005). Effects of biodiversity on ecosystem functioning: a
450 consensus of current knowledge. *Ecological Monographs*, 75(1), 3–35. doi:
451 10.1890/04-0922
- 452 Hubbell, S. P. (2005). Neutral theory in community ecology and the hypothesis of
453 functional equivalence. *Functional Ecology*, 19(1), 166–172. doi: 10.1111/j.0269-
454 8463.2005.00965.x
- 455 Hubbell, S. P., Foster, R. B., O'Brien, S. T., Harms, K. E., Condit, R., Wechsler, B., ...

- 456 Loo De Lao, S. (1999). Light-gap disturbances, recruitment limitation, and tree
457 diversity in a neotropical forest. *Science*, 283(5401), 554–557. doi:
458 10.1126/science.283.5401.554
- 459 Ibáñez, I., Katz, D. S. W., Peltier, D., Wolf, S. M., & Connor Barrie, B. T. (2014).
460 Assessing the integrated effects of landscape fragmentation on plants and plant
461 communities: The challenge of multiprocess-multiresponse dynamics. *Journal of*
462 *Ecology*, 102(4), 882–895. doi: 10.1111/1365-2745.12223
- 463 Koricheva, J., & Gurevitch, J. (2014). Uses and misuses of meta-analysis in plant
464 ecology. *Journal of Ecology*, 102(4), 828–844. doi: 10.1111/1365-2745.12224
- 465 Laurance, W. F., & Cochrane, M. A. (2001). Synergistic effects in fragmented
466 landscapes. *Conservation Biology*, 15, 1488–1489.
- 467 Lett, S., & Dorrepaal, E. (2018). Global drivers of tree seedling establishment at alpine
468 treelines in a changing climate. *Functional Ecology*, 32(7), 1666–1680. doi:
469 10.1111/1365-2435.13137
- 470 Lima, P. B., Lima, L. F., Santos, B. A., Tabarelli, M., & Zickel, C. S. (2015). Altered
471 herb assemblages in fragments of the Brazilian Atlantic forest. *Biological*
472 *Conservation*, 191, 588–595. doi: 10.1016/j.biocon.2015.08.014
- 473 Lortie, C. J., Brooker, R. W., Choler, P., Kikvidze, Z., Michalet, R., Pugnaire, F. I., &
474 Callaway, R. M. (2004). Rethinking plant community theory. *Oikos*, 107, 433–438.
475 Retrieved from
476 [http://www.ingentaconnect.com/content/mksg/oki/2004/00000107/00000002/art00](http://www.ingentaconnect.com/content/mksg/oki/2004/00000107/00000002/art00022%5Cnhttp://dx.doi.org/10.1111/j.0030-1299.2004.13250.x)
477 [022%5Cnhttp://dx.doi.org/10.1111/j.0030-1299.2004.13250.x](http://dx.doi.org/10.1111/j.0030-1299.2004.13250.x)
- 478 Lowry, E., Rollinson, E. J., Laybourn, A. J., Scott, T. E., Aiello-Lammens, M. E., Gray,
479 S. M., ... Gurevitch, J. (2013). Biological invasions: A field synopsis, systematic
480 review, and database of the literature. *Ecology and Evolution*, 3(1), 182–196. doi:

- 481 10.1002/ece3.431
- 482 Markl, J. S., Schleuning, M., Forget, P. M., Jordano, P., Lambert, J. E., Traveset, A., ...
483 Böhning-Gaese, K. (2012a). Meta-Analysis of the Effects of Human Disturbance
484 on Seed Dispersal by Animals. *Conservation Biology*, 26(6), 1072–1081. doi:
485 10.1111/j.1523-1739.2012.01927.x
- 486 McIntyre, S., & Hobbs, R. (1999). A framework for conceptualizing human effects on
487 landscapes and its relevance for management and research models. *Conservation*
488 *Biology*, 13(6), 1282–1292. doi: 10.1046/j.1523-1739.1999.97509.x
- 489 Moles, A. T., & Westoby, M. (2002). Seed addition experiments are more likely to
490 increase recruitment in larger-seeded species. *Oikos*, 99, 241–248.
- 491 Moles, A. T., & Westoby, M. (2006). Seed size and plant strategy across the whole life
492 cycle. *Oikos*, 113, 91–105.
- 493 Murcia, C. (1995). Edge effects in fragmented forests: implications for conservation.
494 *Trends in Ecology & Evolution*, 10(2), 58–62. doi: 10.1016/S0169-5347(00)88977-
495 6
- 496 Nakagawa, S., & Parker, T. H. (2015). Replicating research in ecology and evolution:
497 feasibility, incentives, and the cost-benefit conundrum. *BMC Biology*, 13, 88. doi:
498 10.1186/s12915-015-0196-3
- 499 Neuschulz, E. L., Mueller, T., Schleuning, M., & Böhning-Gaese, K. (2016). Pollination
500 and seed dispersal are the most threatened processes of plant regeneration.
501 *Scientific Reports*, 6, 6–11. doi: 10.1038/srep29839
- 502 Rodríguez-García, E., Bravo, F., & Spies, T. A. (2011). Effects of overstorey canopy,
503 plant-plant interactions and soil properties on Mediterranean maritime pine
504 seedling dynamics. *Forest Ecology and Management*, 262, 244–251. doi:
505 10.1016/j.foreco.2011.03.029

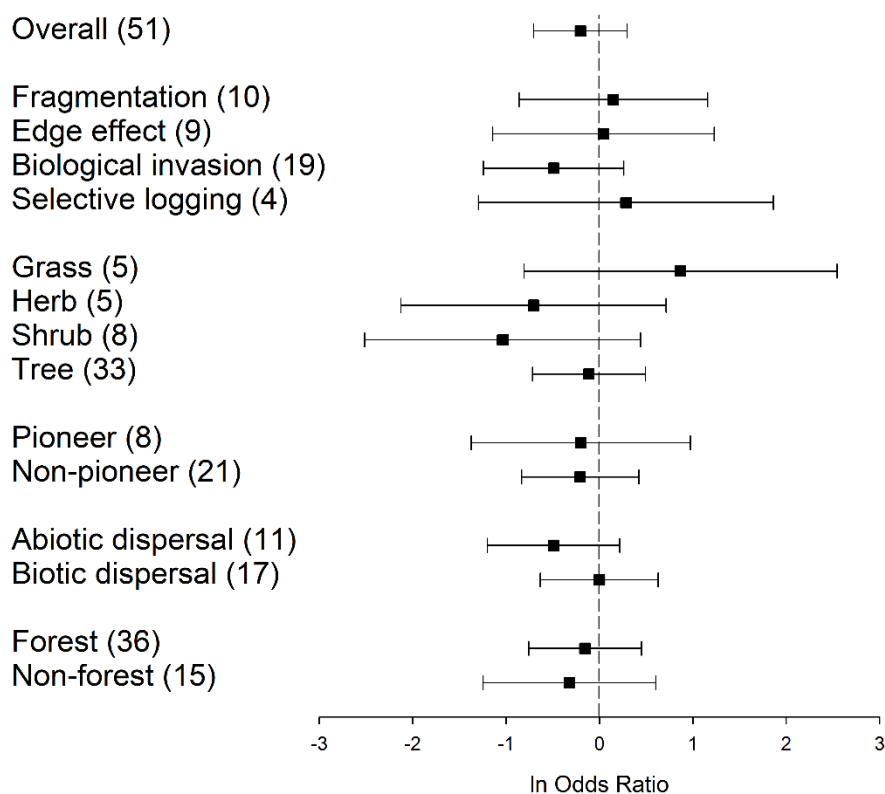
- 506 Turnbull, L. A., Crawley, M. J., & Rees, M. (2000). Are plant populations seed-limited?
507 A review of seed sowing experiments. *Oikos*, 88(2), 225–238. doi: 10.1034/j.1600-
508 0706.2000.880201.x
- 509 Uriarte, M., Bruna, E. M., Rubim, P., Anciaes, M., & Jonckheere, I. (2010). Effects of
510 forest fragmentation on the seedling recruitment of a tropical herb: assessing seed
511 vs. safe-site limitation. *Ecology*, 91(5), 1317–1328.
- 512 Viechtbauer, W. (2010). Conducting meta-analyses in R with the metafor package.
513 *Journal of Statistical Software*, 36(3).
- 514
- 515

516 **Figures**

517

518 Fig. 1. Map of all study sites included in our global meta-analysis of human disturbance
519 effects on seedling survival (n=22). Each black circle represents a study site.

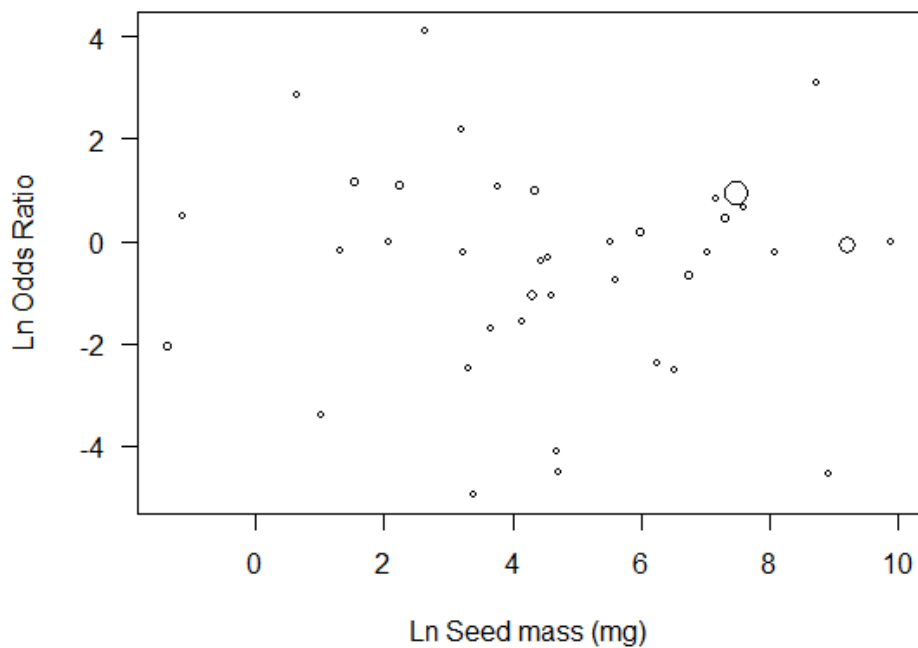
520



521

522 Fig. 2. Human disturbances effects on seedling survival. Mean effect sizes (ln Odds
 523 Ratio; black squares), 95% confidence intervals (CI) and sample sizes are given for the
 524 overall effect, different disturbance types, growth form, ecological succession
 525 categories, dispersal syndromes and vegetation types. CIs overlapping zero (vertical
 526 line) indicate that human disturbance does not affect seedling survival significantly.

527



528

529 Fig. 3. Effect sizes variation (Ln Odds Ratio) according to seed mass (mg),
530 demonstrating the lack of a significant relationship between those variables. Effect sizes
531 represent seedling survival responses to human disturbance and each circle represents
532 case study included in our meta-analysis (n=39). Circle sizes vary according to the
533 effect size variance.

534

Capítulo 2 - Apêndices

Appendix A

Meta-analysis of human disturbance effects on seedling survival under field conditions

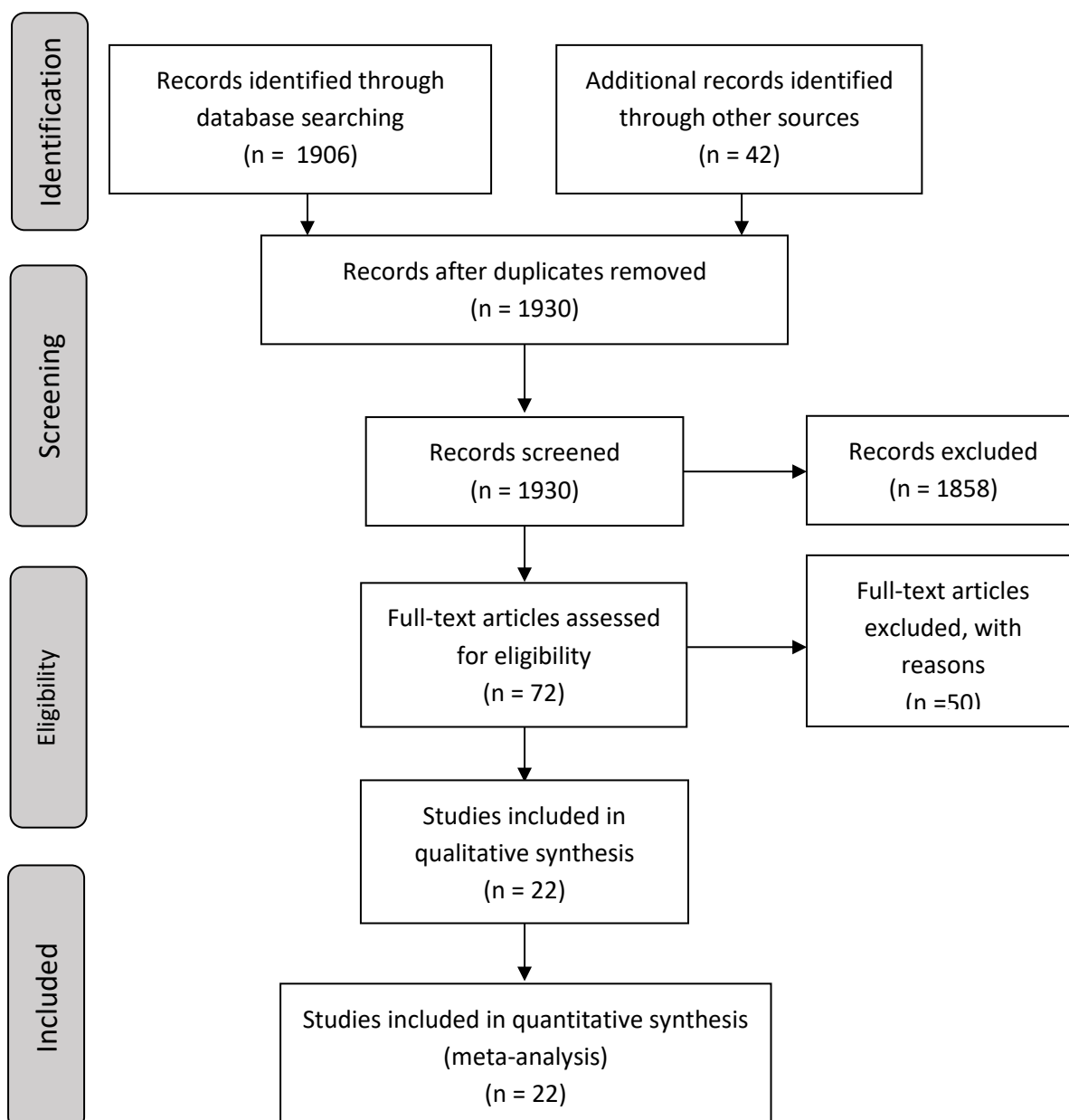


Fig. 1. PRISMA flow diagram describing the information and number of papers included in each phase of our literature review and meta-analysis about human disturbance effects on seedling survival.

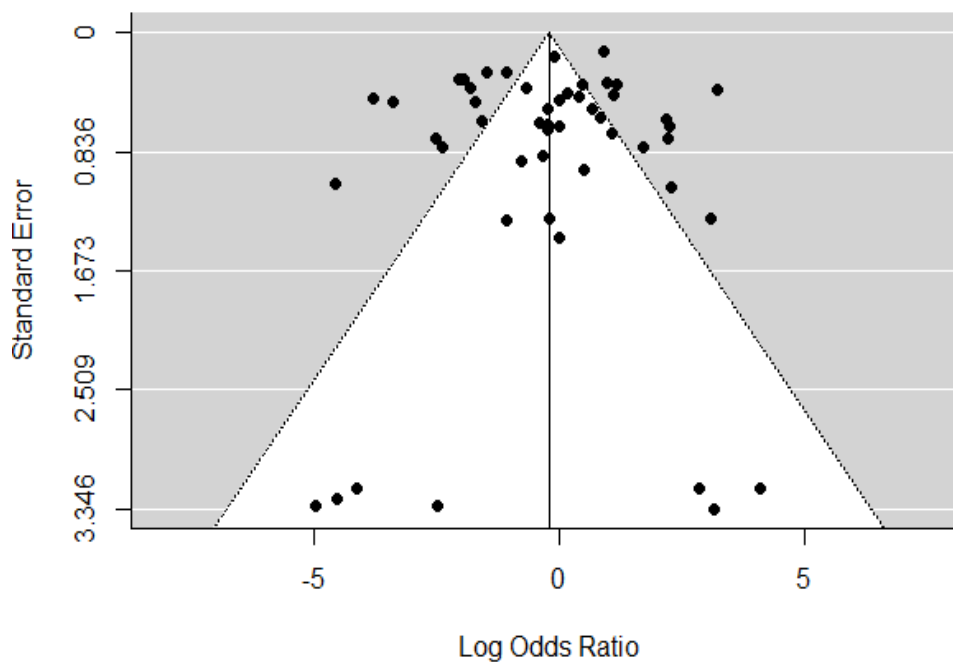


Fig. 2. Funnel plot including effect sizes (log Odds Ratio) and standard errors for all case studies ($n=51$) included in our meta-analysis of human disturbance effects on seedling survival. This graph illustrates the presence of positive, neutral and negative effect sizes, indicating there is no publication bias. Each black circle represents a case study. Vertical line represents the overall effect size of human disturbance on seedling survival and the white triangle delimitates the confidence interval (± 1.96 SE).

Table 1. AIC values, ln odds ratio (ln OR) and its respective confidence interval (CI and p-value) of the traditional and the phylogenetic meta-analyses. The traditional meta-analysis did not include any phylogenetic information and the phylogenetic meta-analysis incorporated the phylogenetic tree at species level. We selected the traditional meta-analysis because it presented the lowest AIC value.

	Traditional meta-analysis	Phylogenetic meta-analysis
AIC	155.13	162.85
ln OR	-0.2	-0.15
CI	0.5	0.66
p-value	0.43	0.64

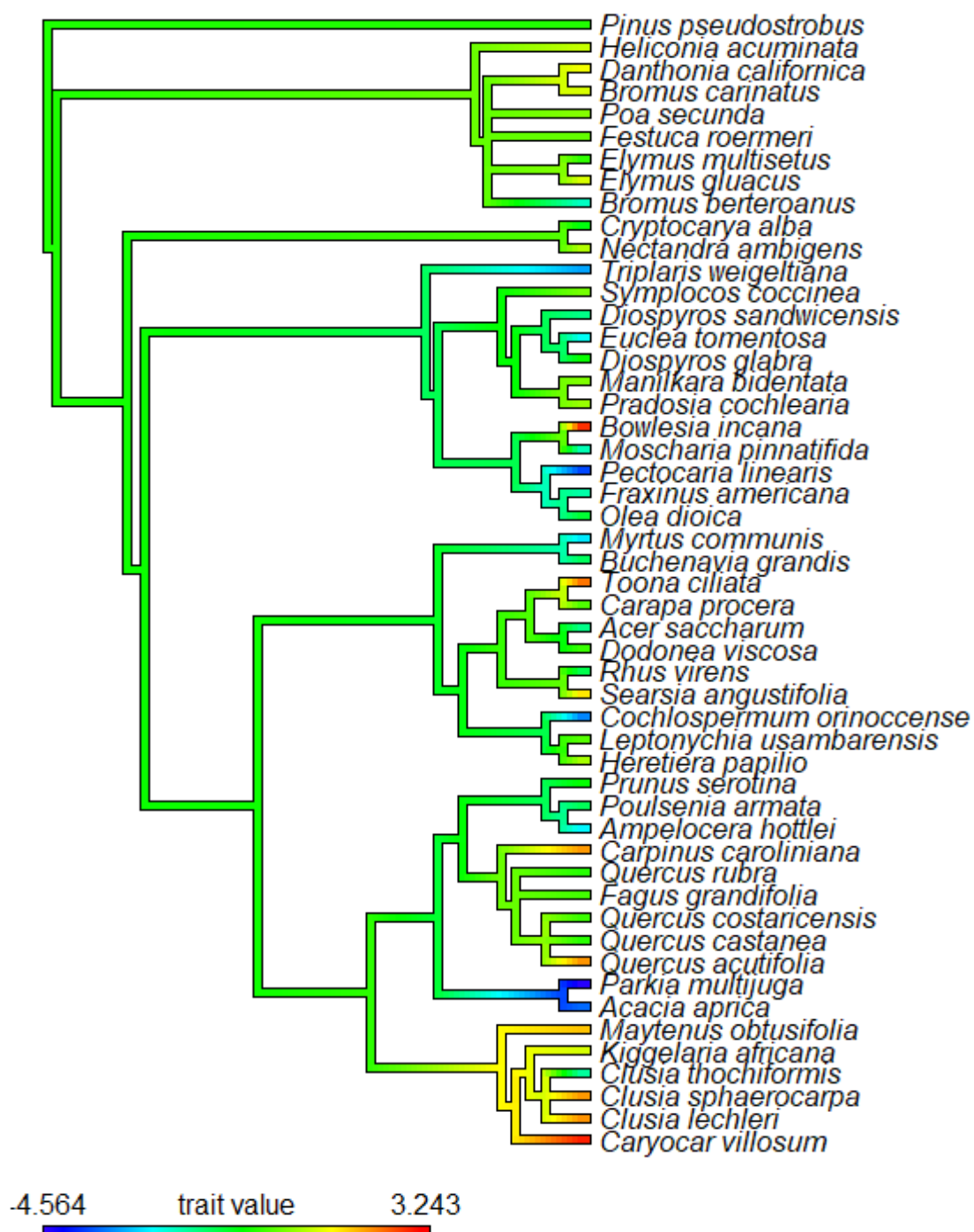


Fig. 3. Phylogenetic tree containing the 50 plant species included in our meta-analysis of human disturbance effects on seedling survival. Colors in the end of each branch represent the effect size (Odds Ratio) of each species. Positive and negative values indicate that human disturbance affects seedling survival in a positive or negative way, respectively. Effect sizes are represented according to the legend (trait value). Additional information about effect sizes for each species and their variance are provided in Appendix B. This figure was formulated using the R package phytools (Revel, 2012).

Appendix B

Meta-analysis of human disturbance effects on seedling survival under field

conditions

Mariano et al. 2019

Table 1. Original publication, species and botanical family of each case study included in our meta-analysis about human disturbance effects on seedling survival. Full references of the original publications are provided below.

Original publication	Species	Family
Álvarez-Aquino et al. 2004	<i>Carpinus carolinia</i>	Betulaceae
Álvarez-Aquino et al. 2004	<i>Quercus acutifolia</i>	Fagaceae
Álvarez-Aquino et al. 2004	<i>Fagus grandifolia</i>	Fagaceae
Álvarez-Aquino et al. 2004	<i>Symplocos coccinea</i>	Symplocaceae
Asbjornsen et al. 2004	<i>Dodonaea viscosa</i>	Sapindaceae
Asbjornsen et al. 2004	<i>Pinus pseudostrobus</i>	Pinaceae
Asbjornsen et al. 2004	<i>Quercus acutifolia</i>	Fagaceae
Asbjornsen et al. 2004	<i>Quercus castanea</i>	Fagaceae
Asbjornsen et al. 2004	<i>Rhus virens</i>	Anacardiaceae
Bruna 2002	<i>Heliconia acumita</i>	Heliconiaceae
Cabin et al. 2000	<i>Diospyros sandwicensis</i>	Ebenaceae
Camargo et al. 2002	<i>Buchevia grandis</i>	Combretaceae
Camargo et al. 2002	<i>Caryocar villosum</i>	Caryocaraceae
Camargo et al. 2002	<i>Cochlospermum orinocense</i>	Bixaceae
Camargo et al. 2002	<i>Parkia multijuga</i>	Fabaceae
Camargo et al. 2002	<i>Triplaris weigeltia</i>	Polygonaceae
Castro et al. 2010	<i>Bowlesia inca</i>	Apiaceae
Castro et al. 2010	<i>Bromus berterioanus</i>	Poaceae
Castro et al. 2010	<i>Moscharia pintifida</i>	Asteraceae
Castro et al. 2010	<i>Pectocarya linearis</i>	Boraginaceae
Chávez-Pesqueira & Núñez-Farfan 2016	<i>Nectandra ambigens</i>	Lauraceae
Cordeiro et al. 2009	<i>Leptonychia usambarensis</i>	Malvaceae
Forget et al. 2001	<i>Carapa procera</i>	Meliaceae
Forget et al. 2001	<i>Manilkara bidentata</i>	Sapotaceae
Forget et al. 2001	<i>Pradosia cochlearia</i>	Sapotaceae
Gallegos et al. 2015	<i>Clusia lechleri</i>	Clusiaceae
Gallegos et al. 2015	<i>Clusia sphaerocarpa</i>	Clusiaceae
Gallegos et al. 2015	<i>Clusia trochiformis</i>	Clusiaceae
González-Di Pierro et al. 2011	<i>Ampelocera hottlei</i>	Ulmaceae

González-Varo et al. 2012	<i>Myrtus communis</i>	Myrtaceae
Gorchov & Trisel 2003	<i>Acer saccharum</i>	Sapindaceae
Gorchov & Trisel 2003	<i>Fraxinus americana</i>	Oleaceae
Gorchov & Trisel 2003	<i>Prunus seroti</i>	Rosaceae
Gorchov & Trisel 2003	<i>Quercus rubra</i>	Fagaceae
Guariguata & Sáenz 2002	<i>Quercus costaricensis</i>	Fagaceae
Guerrero & Bustamante 2009	<i>Cryptocarya alba</i>	Lauraceae
Krishnadas & Comita 2018	<i>Heritiera papilio</i>	Malvaceae
Krishnadas & Comita 2018	<i>Olea dioica</i>	Oleaceae
Krishnadas & Comita 2018	<i>Toona ciliata</i>	Meliaceae
Leger & Goergen 2017	<i>Elymus multisetus</i>	Poaceae
Leger & Goergen 2017	<i>Poa secunda</i>	Poaceae
MacDougall & Turkington 2005	<i>Bromus caritus</i>	Poaceae
MacDougall & Turkington 2005	<i>Danthonia californica</i>	Poaceae
MacDougall & Turkington 2005	<i>Elymus glaucus</i>	Poaceae
Ruwanza et al. 2013	<i>Diospyros glabra</i>	Ebenaceae
Ruwanza et al. 2013	<i>Euclea tomentosa</i>	Ebenaceae
Ruwanza et al. 2013	<i>Kiggelaria africana</i>	Achariaceae
Ruwanza et al. 2013	<i>Searsia angustifolia</i>	Anacardiaceae
Yates & Broadhurst 2002	<i>Acacia aprica</i>	Fabaceae
Zambrano et al. 2014	<i>Poulsenia armata</i>	Moraceae
Zimmermann et al. 2017	<i>Maytenus obtusifolia</i>	Celastraceae

Table 2. Case studies included in our meta-analysis about human disturbance effects on seedling survival. We specified species, disturbance type, growth form, dispersal syndrome, succession category, seed mass (mg), SLA, leaf thickness, vegetation type, effect size ln odds ratio (ln OR) and effect sizes variance (vi) for each case study included on our database. Superscripts indicate trait databases that provided information when it was not available in the original paper (full references are specified at the bottom of the table). Dashes represent information that was not provided by the original papers and that were not available in the searched trait databases.

Species	Disturbance type	Growth form	Dispersal syndrome	Succession category	Seed mass	SLA ¹	Leaf thickness ¹	Vegetation type	ln OR	vi
<i>Acacia aprica</i>	Biological invasion	shrub	abiotic	-	2.76 ²	-	-	non-forest	2.20	0.38
<i>Acer saccharum</i>	Biological invasion	Tree	abiotic ¹	non-pioneer	62.52	318.17	117.395	forest	2.28	1.18
<i>Ampelocera hottlei</i>	Fragmentation	Tree	biotic	-	670 ¹	-	-	forest	0.00	0.23
<i>Bowlesia inca</i>	Biological invasion	Herb	-	-	-	-	-	non-forest	0.40	0.20
<i>Bromus berterioanus</i>	Biological invasion	Herb	abiotic	-	-	-	-	non-forest	0.00	2.08
<i>Bromus caritus</i>	Biological invasion	grass	abiotic	-	9.2	66.33	202.65	non-forest	-0.23	0.46
<i>Buchevia grandis</i>	Habitat degradation	Tree	biotic ¹	non-pioneer	100	-	173.18	forest	0.00	0.43
<i>Carapa procera</i>	Selective logging	Tree	biotic	non-pioneer	20000	-	242.29	forest	-0.22	0.29
<i>Carpinus carolinia</i>	Fragmentation	Tree	abiotic ¹	-	24.5 ¹	429.64	94	forest	-2.48	11.08
<i>Caryocar villosum</i>	Habitat degradation	Tree	biotic	non-pioneer	6200	-	-	forest	0.99	0.12
<i>Clusia lechleri</i>	Habitat degradation	Tree	biotic	non-pioneer	-	-	-	forest	-1.48	0.08
<i>Clusia sphaerocarpa</i>	Habitat degradation	Tree	biotic	non-pioneer	-	-	-	forest	-1.06	1.73
<i>Clusia trochiformis</i>	Habitat degradation	Tree	biotic	non-pioneer	-	-	-	forest	3.09	1.72
<i>Cochlospermum orinocense</i>	Habitat degradation	Tree	abiotic ¹	pioneer	30	-	-	forest	-4.95	11.02
<i>Cryptocarya alba</i>	Fragmentation	Tree	biotic	non-pioneer	833 ¹	-	300	forest	-4.56	1.14
<i>Danthonia californica</i>	Biological invasion	grass	abiotic	-	1.9	89.37	220.78	non-forest	-4.53	10.78
<i>Diospyros glabra</i>	Biological invasion	shrub	biotic	pioneer	94.36 ¹	-	-	non-forest	3.24	0.16
<i>Diospyros sandwicensis</i>	Habitat degradation	Tree	biotic	-	-	-	-	forest	-1.94	0.11

<i>Dodonaea viscosa</i>	Edge effect	shrub	biotic ²	pioneer	8.0 ²	-	-	forest	-2.05	0.11
<i>Elymus glaucus</i>	Biological invasion	grass	abiotic	-	4.6	101.93	190.85	non-forest	-3.80	0.21
<i>Elymus multisetus</i>	Biological invasion	grass	abiotic	-	3.7 ¹	-	-	non-forest	0.92	0.02
<i>Euclea tomentosa</i>	Biological invasion	shrub	-	pioneer	524.7 ¹	-	-	non-forest	0.18	0.18
<i>Fagus grandifolia</i>	Fragmentation	Tree	biotic	non-pioneer	251.5 ¹	410.29	146	forest	0.00	2.06
<i>Fraxinus americana</i>	Biological invasion	Tree	abiotic ¹	non-pioneer	38.5 ¹	398.23	131.98	forest	0.47	0.14
<i>Heliconia acumita</i>	Fragmentation	Herb	biotic	-	77	-	-	forest	0.67	0.28
<i>Heritiera papilio</i>	Edge effect	Tree	abiotic	-	1290	-	-	forest	2.26	0.43
<i>Kiggelaria africana</i>	Biological invasion	Tree	-	pioneer	43.13 ²	-	-	non-forest	2.21	0.56
<i>Leptonychia usambarensis</i>	Edge effect	Tree	biotic	-	400	-	-	forest	-1.79	0.15
<i>Manilkara bidentata</i>	Selective logging	Tree	biotic	non-pioneer	1500	-	-	forest	-2.52	0.56
<i>Maytenus obtusifolia</i>	Biological invasion	shrub	biotic	-	-	-	-	non-forest	-4.11	10.25
<i>Moscharia pintifida</i>	Biological invasion	Herb	abiotic	-	0.25 ¹	-	-	non-forest	-1.58	0.39
<i>Myrtus communis</i>	Fragmentation	shrub	biotic	-	108	122.9	-	forest	-1.72	0.24
<i>Nectandra ambigens</i>	Fragmentation	Tree	biotic	non-pioneer	1800 ¹	-	-	forest	-0.40	0.40
<i>Olea dioica</i>	Edge effect	Tree	biotic	-	270	-	-	forest	-0.21	0.42
<i>Parkia multijuga</i>	Habitat degradation	Tree	abiotic	non-pioneer	7400	-	-	forest	-0.09	0.03
<i>Pectocarya linearis</i>	Biological invasion	Herb	-	-	-	-	-	non-forest	-0.65	0.16
<i>Pinus pseudostrobus</i>	Edge effect	Tree	abiotic	-	24.9 ¹	-	-	forest	0.84	0.35
<i>Poa secunda</i>	Biological invasion	grass	abiotic	-	0.32 ¹	-	-	non-forest	-0.77	0.82
<i>Poulsenia armata</i>	Fragmentation	Tree	biotic	non-pioneer	75 ¹	112.1	249.81	forest	4.11	10.27
<i>Pradosia cochlearia</i>	Selective logging	Tree	biotic	non-pioneer	2000 ¹	-	268.18	forest	-0.19	1.70
<i>Prunus seroti</i>	Biological invasion	Tree	biotic ²	non-pioneer	83.9 ²	-	-	forest	0.50	0.93
<i>Quercus acutifolia</i>	Fragmentation	Tree	abiotic	non-pioneer	-	-	-	forest	1.10	0.19
<i>Quercus acutifolia</i>	Edge effect	Tree	abiotic	non-pioneer	-	-	-	forest	2.86	10.24
<i>Quercus castanea</i>	Edge effect	Tree	abiotic ²	non-pioneer	1128.3 ¹	-	-	forest	1.18	0.13
<i>Quercus costaricensis</i>	Selective logging	Tree	biotic	pioneer	10100	-	347.035	forest	-0.32	0.75
<i>Quercus rubra</i>	Biological invasion	Tree	abiotic ¹	non-pioneer	3216 ¹	183.43	160.26	forest	-2.38	0.65
<i>Rhus virens</i>	Edge effect	shrub	-	pioneer	27.59 ¹	-	-	forest	1.07	0.49
<i>Searsia angustifolia</i>	Biological invasion	shrub	-	pioneer	-	-	-	non-forest	1.73	0.65

<i>Symplocos coccinea</i>	Fragmentation	Tree	biotic	non-pioneer	-	-	-	forest	-3.38	0.24
<i>Toona ciliata</i>	Edge effect	Tree	abiotic ²	-	14	-	-	forest	-1.06	0.08
<i>Triplaris weigeltia</i>	Habitat degradation	Tree	abiotic ¹	non-pioneer	110	-	-	forest	3.18	11.20

¹ Kattge, J., Bönsch, G., Günther, A., Wright, I., Zanne, A., Wirth, C., Reich, P.B. and the TRY Consortium. (2012). TRY - Categorical Traits Dataset. Data from: TRY - a global database of plant traits. Available from www.try-db.org/

² Royal Botanic Gardens Kew. (2019) Seed Information Database (SID). Version 7.1. Available from: <http://data.kew.org/sid/>

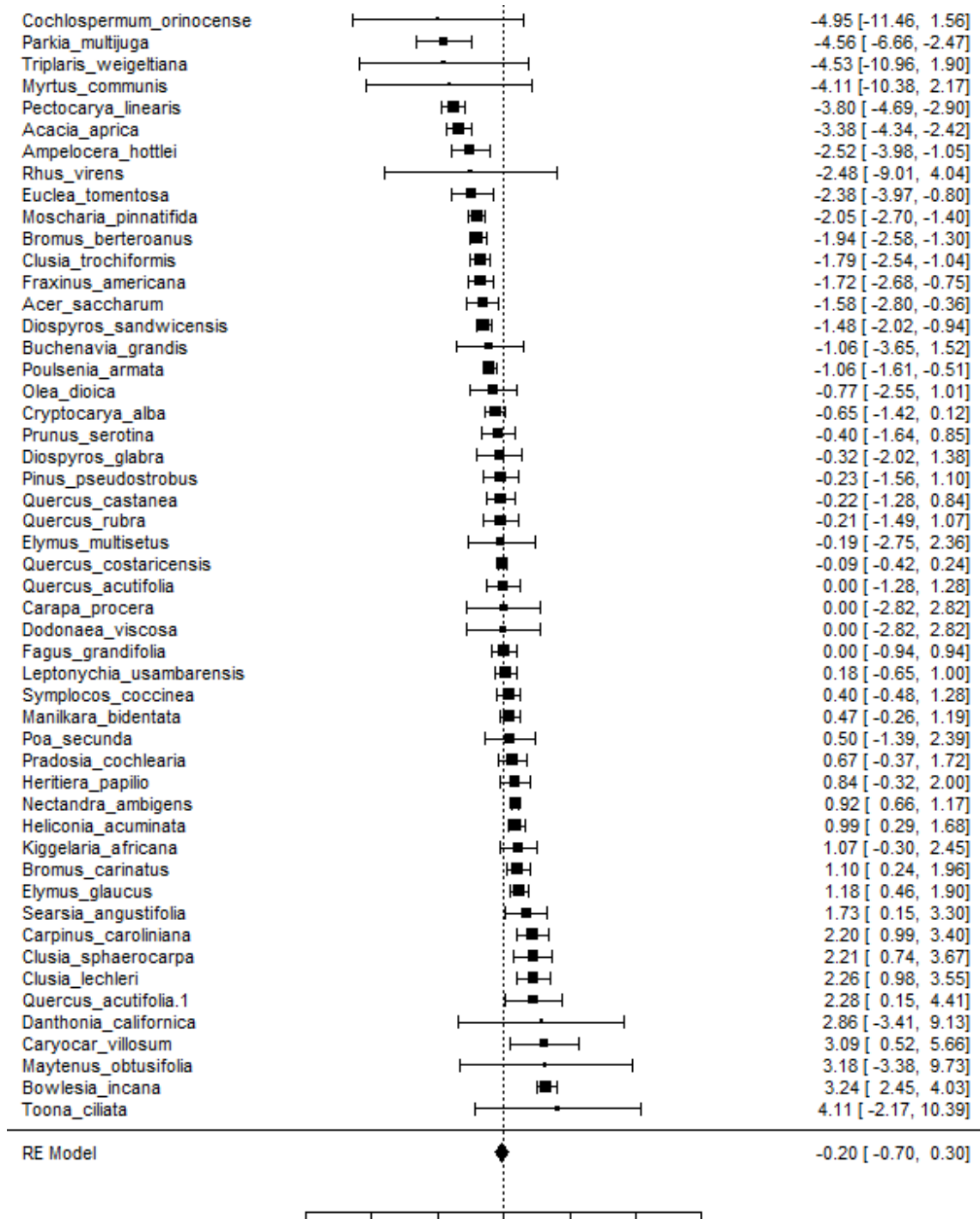


Fig. 1. Forest plot containing all 51 case studies included in our meta-analysis about human disturbance effects on seedling survival. Effect sizes (ln Odds Ratio; squares) and confidence intervals (horizontal lines and values inside the brackets) are specified for each case study. Vertical line indicates zero value of ln Odds Ratio. Case studies are ordered from the largest to the smallest effect size.

Table 3. Heterogeneity statistics of each meta-analytic model included in our analysis of human disturbance effects on seedling survival.

We specified the number of case studies (n), true heterogeneity (τ^2), its respective standard error (SE) and I^2 for the overall effect (random-effects model). For each mixed-effects model we also specified the included categories and Q_M , Q_E and their p-values. Each mixed-effects model is identified by the moderator(s) used in the analysis (disturbance type, growth form, ecological succession, dispersal syndrome, seed mass, specific leaf area, leaf thickness, vegetation type, absolute latitude, and the interactions between moderators). See Table 4 for information about sample sizes and the effect sizes for each category.

Model	Categories	n	τ^2	SE	I^2 (%)	Q_M	p	Q_E	p
Overall effect	-	51	2.51	0.63	92.24	-	-	-	-
Disturbance type	Fragmentation, Edge effects, Biological invasion, Selective logging	42	2.16	0.62	89.91	1.51	0.68	366.56	< 0.001
Growth form	Grass, Herb, Shrub, Tree	51	2.48	0.65	91.9	3.36	0.34	459.76	< 0.001
Ecological succession	Pioneer, Non-pioneer	30	1.72	0.62	87.42	0.02	0.90	176.78	< 0.001
Dispersal syndrome	Abiotic dispersal, Biotic dispersal	45	1.87	0.53	90.01	1.03	0.31	340.96	< 0.001
Seed mass	-	39	1.76	0.55	87.77	0.01	0.93	271.54	< 0.001

Specific leaf area (SLA)	-	10	1.97	1.27	87.73	0.1	0.75	63.06	< 0.001
Leaf thickness	-	14	1.19	0.65	83.99	0.03	0.86	66.71	< 0.001
Vegetation type	Forest, Non-forest	51	2.56	0.65	92.19	0.09	0.77	502.07	< 0.001
Latitude	-	51	2.57	0.65	92.23	0.03	0.86	512.67	< 0.001
Seed mass + Ecological succession	-	23	1.29	0.59	84.11	0.14	0.93	118.74	< 0.001
Seed mass + Growth form	-	39	1.41	0.49	84.1	8.47	0.08	198.11	< 0.001
Vegetation type + Growth form	-	51	2.54	0.67	92.02	3.31	0.51	443.25	< 0.001

Table 4. Estimated effect sizes of human disturbance effect on seedling survival (Odds Ratio, OR), number of case studies (n), standard errors (SE), z and p-values, and 95% confidence intervals (CI) for the overall effect and different disturbance types (fragmentation, edge effect, biological invasion and selective logging), growth form (grass, herb, shrub and tree), ecological succession categories (pioneer and non-pioneer), dispersal syndrome (biotic and abiotic dispersal) and vegetation types (forest and non-forest).

	n	OR	SE	z	p	CI lb	CI up
Overall	51	-0.2033	0.2553	-0.7962	0.4259	-0.7037	0.2971
Fragmentation	10	0.149	0.5154	0.2891	0.7725	-0.8611	1.1591
Edge effect	9	0.0433	0.605	0.0715	0.943	-1.1425	1.2291
Biological invasion	19	-0.4903	0.3841	-1.2764	0.2018	-1.2432	0.2626
Selective logging	4	0.2841	0.8057	0.3526	0.7244	-1.2951	1.8633
Grass	5	0.8689	0.8548	1.0165	0.3094	-0.8065	2.5443
Herb	5	-0.7058	0.724	-0.9748	0.3297	-2.1249	0.7133
Shrub	8	-1.0363	0.7534	-1.3754	0.169	-2.513	0.4404
Tree	33	-0.1118	0.3089	-0.3619	0.7174	-0.7173	0.4937
Pioneer	8	-0.199	0.5987	-0.3324	0.7396	-1.3724	0.9744
Non-pioneer	21	-0.2061	0.3207	-0.6427	0.5204	-0.8347	0.4225
Abiotic dispersal	11	-0.4908	0.3612	-1.3588	0.1742	-1.1987	0.2172
Biotic dispersal	17	-0.0007	0.3217	-0.0022	0.9982	-0.6312	0.6298
Forest	36	-0.1538	0.3072	-0.5006	0.6167	-0.7559	0.4484
Non-forest	15	-0.3202	0.472	-0.6785	0.4974	-1.2452	0.6048

Full references of the original publications included in our meta-analysis about human disturbance effects on seedling survival

- Alvarez-Aquino, C., Williams-linera, G., & Newton, A. C. (2004). Experimental Native Tree Seedling Establishment for the Restoration of a Mexican Cloud Forest. *Restoration Ecology*, *12*(3), 412–419.
- Asbjornsen, H., Vogt, K. A., & Ashton, M. S. (2004). Synergistic responses of oak, pine and shrub seedlings to edge environments and drought in a fragmented tropical highland oak forest, Oaxaca, Mexico. *Forest Ecology and Management*, *192*.
- Bruna, E. M. (2002). Effects of forest fragmentation *Heliconia acuminata* on seedling recruitment in central Amazonia. *Oecologia*, *132*(2), 235–243.
- Cabin, R. J., Weller, S. G., Lorence, D. H., Flynn, T. I. M. W., Sakai, A. N. N. K., Sandquist, D., & Hadway, L. J. (2000). Effects of Long-Term Ungulate Exclusion and Recent Alien Species Control on the Preservation and Restoration of a Hawaiian Tropical Dry Forest. *Conservation Biology*, *14*(2), 439–453.
- Camargo, J. L. C., Ferraz, I. D. K., & Imakawa, A. M. (2002). Rehabilitation of degraded areas of Central Amazonia using direct sowing of forest tree seeds. *Restoration Ecology*, *10*(4), 636–644.
- Castro, S. A., & Badano, E. (2010). Biological invasion of a refuge habitat: *Anthriscus caucalis* (Apiaceae) decreases diversity, evenness, and survival of native herbs in the Chilean matorral. *Biological Invasions*, *12*, 1295–1303.
- Chávez-Pesqueira, M., & Núñez-Farfán, J. (2016). Habitat fragmentation changes the adaptive value of seed mass for the establishment of a tropical canopy tree. *Biotropica*, *48*(5), 628–637.
- Cordeiro, N. O. J., Ndangalasi, H. E. J., McEntee, J. A. Y. P., & Howe, H. (2009).

- Disperser limitation and recruitment of an endemic African tree in a fragmented landscape. *Ecology*, *90*(4), 1030–1041.
- Forget, P., Merona, J. M. R., & Julliot, C. (2001). The effects of forest type, harvesting and stand refinement on early seedling recruitment in a tropical rain forest. *Journal of Tropical Ecology*, *17*, 593–609.
- Gallegos, S. C., Hensen, I., Saavedra, F., & Schleuning, M. (2015). Forest Ecology and Management Bracken fern facilitates tree seedling recruitment in tropical fire-degraded habitats. *Forest Ecology and Management*, *337*, 135–143.
- González-Di Pierro, A. M., Benítez-Malvido, J., Méndez-Toribio, M., Zermeño, I., Arroyo-Rodríguez, V., & Stoner, K. E. (2011). Effects of the Physical Environment and Primate Gut Passage on the Early Establishment of *Ampelocera hottlei* Standley in Rain Forest Fragments. *Biotropica*, *43*(4), 459–466.
- González-Varo, J. P., Nora, S., & Aparicio, A. (2012). Bottlenecks for plant recruitment in woodland remnants: An ornithochorous shrub in a Mediterranean “relictual” landscape. *Perspectives in Plant Ecology, Evolution and Systematics*, *14*, 111–122.
- Gorchov, D. L., & Trisel, D. E. (2003). Competitive effects of the invasive shrub, *Lonicera maackii* (Rupr.) Herder (Caprifoliaceae), on the growth and survival of native tree seedlings. *Plant Ecology*, *166*, 13–24.
- Guariguata, M. R., & Sáenz, G. P. (2002). Post-logging acorn production and oak regeneration in a tropical montane forest, Costa Rica. *Forest Ecology and Management*, *167*(1–3), 285–293.
- Guerrero, P. C., & Bustamante, R. O. (2009). Abiotic alterations caused by forest fragmentation affect tree regeneration: A shade and drought tolerance gradient in the remnants of Coastal Maulino forest. *Revista Chilena de Historia Natural*, *82*(3), 413–424.

- Krishnadas, M., & Comita, L. S. (2018). Influence of soil pathogens on early regeneration success of tropical trees varies between forest edge and interior. *Oecologia*, *186*(1), 259–268.
- Leger, E. A., & Goergen, E. M. (2017). Invasive *Bromus tectorum* alters natural selection in arid systems. *Journal of Ecology*, *105*, 1509–1520.
- MacDougall, A. S., & Turkington, R. (2005). Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology*, *86*(1), 42–55.
- Ruwanza, S., Gaertner, M., Esler, K. J., & Richardson, D. M. (2013). The effectiveness of active and passive restoration on recovery of indigenous vegetation in riparian zones in the Western Cape, South Africa: A preliminary assessment. *South African Journal of Botany*, *88*, 132–141.
- Yates, C. J., & Broadhurst, L. M. (2002). Assessing limitations on population growth in two critically endangered *Acacia* taxa. *Biological Conservation*, *108*, 13–26.
- Zambrano, J., Coates, R., & Howe, H. F. (2014). Effects of forest fragmentation on the recruitment success of the tropical tree *Poulsenia armata* at Los Tuxtlas, Veracruz, Mexico. *Journal of Tropical Ecology*, *30*, 209–218.
- Zimmermann, T. G., Andrade, A. C. S., & Richardson, D. M. (2017). Abiotic barriers limit tree invasion but do not hamper native shrub recruitment in invaded stands. *Biological Invasions*, *19*(1), 109–129.

Capítulo 3

Formatado de acordo com as normas do periódico *Restoration Ecology*

**Microhabitat characteristics, seed germination and seed removal in small
Atlantic Forest fragments and restoration areas**

1 **Abstract**

2 Small forest fragments, forest edges and areas under ecological restoration often experience
3 different biotic and abiotic conditions that may lead to changes in trajectories of succession
4 and species composition. We compared seed germination, seed removal and microhabitat
5 conditions among interior and edges of three small forest fragments and two adjacent sites
6 undergoing active restoration in Atlantic Forest from southeastern Brazil. Propagules of 13
7 and 5 native species were added in our seed germination and removal experiments,
8 respectively, and canopy cover, litter layer depth and soil moisture were evaluated in all
9 plots. Canopy cover and soil moisture differed among habitat types and restoration areas
10 presented the lowest mean values. Seed germination was extremely low (from 0 to 0.7%)
11 for all 13 species irrespective of habitat type. Over 45% and 63% of all added propagules
12 were removed from treatments with and without selective exclosures, respectively. Soil
13 moisture negatively influenced removal rates, but canopy cover, litter layer and habitat
14 were not related to seed removal. Our results indicate that interior and edges of small
15 Atlantic Forest fragments and 8-10 years old restoration areas present different
16 microhabitat conditions, potentially affecting early plant regeneration and further species
17 composition. Low rates of seed germination indicate that establishment limitation is an
18 important constraint to plant recruitment at all sites. Seed removal by invertebrates and
19 vertebrates potentially enhance regeneration constraints to all species. Complementary
20 management strategies, such as enrichment plantings, might be necessary to maintain
21 successional trajectories in restorations in a long term.

22 **Keywords:** Edge effect, establishment limitation, seedling emergence, seed dispersal, seed
23 limitation, seed predation, tropical rainforest, canopy cover, soil moisture

25 **Implications for practice**

- 26 • Low seed germination rates indicate the limitations of relying on natural seed rain or
27 seed addition practices to restore biodiversity in small Atlantic Forest fragments and
28 restoration areas.
- 29 • Areas under ecological restoration (8-10 y.o.) still presented lower canopy cover and
30 soil moisture than the interior of forest fragments, potentially affecting early stages of
31 plant regeneration. Complementary restoration efforts, such as enrichment plantings of
32 additional species/or and saplings, might be necessary to increase plant recruitment and
33 keep going the successional trajectories in those areas.

34 **Introduction**

35 Habitat loss and fragmentation are among the most important threats to global
36 biodiversity (Laurance et al. 2002; Laurance & Cochrane 2001; Haddad et al. 2015). The
37 remaining forest fragments and their edges are subjected to increasing alterations in abiotic
38 conditions following a decrease in fragment size, such as light incidence, wind, humidity,
39 and soil moisture, which can lead to changes in species richness and composition (Laurance
40 et al. 2002; Guerrero & Bustamante 2009; Tabarelli et al. 2012; Harper et al. 2005). Those
41 alterations in biotic and biotic factors can have a profound impact on plant regeneration.
42 Small forest fragments and forest edges often suffer from a replacement of old-growth tree
43 species by a small number of disturbance-tolerant pioneer plant species (Oliveira et al.
44 2004; Tabarelli et al. 2012). In this context, ecological restoration initiatives are important
45 ways to increase habitat availability, biodiversity and vegetation structure and to maintain
46 ecosystem services in degraded areas worldwide (Crouzeilles et al. 2016; Benayas et al.
47 2009).

48 The Atlantic Forest is a highly diverse tropical forest and it is considered a
49 biodiversity hotspot (Myers et al. 2000). It was one of the largest rainforests of the
50 Americas, but extensive anthropogenic pressure and high levels of habitat loss and
51 fragmentation have reduced this biome to only 16% of its original cover (Joly et al. 2014;
52 Ribeiro et al. 2009). Unfortunately, at least half of Atlantic Forest remnants is up to a 100
53 m from the nearest forest edge and 80% of the fragments are smaller than 100 ha (Ribeiro
54 et al. 2009; Haddad et al. 2015). Edge effects might be one of the main causes of decreased
55 species richness of animals and plants in Atlantic Forest landscapes (Püttker et al. 2019). In
56 addition, areas once covered by Atlantic forests subjected to ecological restoration might
57 take a long time to recover species composition and the ecosystem characteristics of their

58 nearby forest fragments (Rodrigues et al. 2009; Souza & Batista 2004; Liebsch et al. 2008).
59 Many Atlantic Forest restoration sites present low natural plant regeneration, which might
60 be associated to the low potential of seed deposition (or seed dispersal from the surrounding
61 fragments), and/or the low rates of early recruitment of the deposited seeds (Rodrigues et
62 al. 2009). Low natural regeneration may compromise the long-term maintenance and
63 successional trajectories of restorations.

64 Seedling emergence and survival can constraint plant recruitment and might be
65 affected positively or negatively by habitat fragmentation, according to species
66 requirements and tolerances to disturbed conditions (Peña-Domene et al. 2017). For
67 example, small forest fragments can present higher temperature, lower humidity and
68 increased litter accumulation, which can alter triggers of seed germination and seedling
69 survival rates (Bruna 2002; Guerrero & Bustamante 2009; Benitez-Malvido 1998). Early
70 recruitment of pioneer species is often increased in small fragments and edges, while non-
71 pioneers and understory plants can present higher recruitment in mature forests (Peña-
72 Domene et al. 2017; Bruna 2002). In addition, animal composition and ecological
73 interactions, like seed dispersal and predation, can also be modified in small fragments
74 compared to continuous forests, potentially influencing plant recruitment, spatial
75 distribution and establishment of plant populations (Bihn et al. 2008; Fontúrbel et al. 2015).
76 Vertebrate and invertebrate granivores often avoid areas with low vegetation cover such as
77 forest gaps and edges due to the increased exposure to predators and hazardous
78 microclimate conditions, which often lead to higher levels of seed predation in shaded
79 portions of forest interiors (Christianini & Galetti 2007; Restrepo & Vargas 1999).

80 Despite the local importance of small Atlantic Forest fragments and ecological
81 restorations to the maintenance of biodiversity, especially in highly disturbed landscapes

82 (Tabarelli et al. 2010; Joly et al. 2014; Santos et al. 2007), there is a scarcity of information
83 about the drivers of plant regeneration and species composition in those areas. Microhabitat
84 conditions, seed removal and germination are often important predictors of plant
85 regeneration (Oda et al. 2019). Therefore, the aim of this study is to analyze microhabitat
86 conditions (canopy cover, litter layer and soil moisture), seed germination and seed removal
87 of native Atlantic Forest plants in ecological restoration areas compared to interior and
88 edges of small fragments of secondary forests. Since Atlantic Forest edges and early
89 restorations often present harsh conditions for natural regeneration, we expected they would
90 present different microhabitat conditions and lower seed germination compared to the
91 interior of forest fragments. That is, canopy cover, litter layer and soil moisture would be
92 higher in forest interiors, as well as seed germination. Since granivores often avoid areas
93 with lower vegetation cover, we also expected that seed removal would be higher in the
94 interior of forest fragments than in edges and restoration areas.

95

96 **Methods**

97 *Study site*

98 The study was carried out in three small Atlantic Forest fragments (~ 2 ha) and two
99 adjacent ecological restoration areas (1-2 ha). All sites are located in the Universidade
100 Federal de São Carlos, Sorocaba Campus, Sorocaba, Brazil (23°58'42"S, 47°51'90"W).
101 Mean annual temperature in the region is 21.2°C and mean annual precipitation is 1339 mm
102 (Abreu & Tonello 2017). The vegetation is classified as Seasonal Semideciduous Forest
103 (SSF), and contains at least 166 woody species from 47 families, including ten endangered
104 ones (Kortz et al. 2014). Myrtaceae, Fabaceae and Lauraceae are the most representative
105 families (Kortz et al. 2014). The region is in a transition zone between SSF and the

106 Cerrado, a neotropical savanna (Albuquerque & Rodrigues 2000, Kortz et al. 2014). The
107 three secondary forest fragments are surrounded by a matrix of pasturelands mainly
108 composed by African grasses *Urochloa decumbens* (Syn. *Brachiaria decumbens*). The
109 ecological restorations are 8-10 years old and are composed mainly by native tree species,
110 which were planted as saplings in a 2 x 3m design. The canopy height is around 4.5 m and
111 is not completely closed and the original planting design is still distinguishable in these
112 areas due to the small number of naturally regenerating individuals.

113

114 *Experimental design*

115 We delimited 28 experimental quadrats (2 x 1 m) in three habitat types: (a) the
116 interior of three small forest fragments (N = 10, all at least 30 m distant from closest edge);
117 (b) edges of the same forest fragments, which were in direct contact with pasturelands (N =
118 10); and (c) ecological restoration areas adjacent to the forest fragments (N = 8). These
119 experimental quadrats were used in the seed addition and seed removal experiments as well
120 as in the assessment of the microhabitat conditions (described below). Each forest fragment
121 contained the same number of interior and edge quadrats, with quadrats within the same
122 habitat category (interior, edge or restoration) been located at least 10 m apart from each
123 other. The number and location of the quadrats were determined based on the availability of
124 interior and restoration areas. The location of each quadrat in the forest fragments and
125 restoration areas is specified in Appendix A.

126

127 *Microhabitat characteristics*

128 To evaluate if there are differences in microhabitat conditions among forest interior,
129 forest edges and restoration areas, we measured canopy cover, litter layer depth and soil

130 moisture in all quadrats. To evaluate canopy cover we took pictures of the canopy
131 immediately above our experimental quadrats using a digital camera (12 MP, DFOV 77°).
132 Photos were taken 10 cm above the ground using a tripod and a ground level. Canopy cover
133 percentage was used as an estimation of light incidence and it was determined using the
134 software ImageJ (Rueden et al. 2007). Litter layer depth was estimated using a pachymeter,
135 which measured the amount of litter above the soil surface around our plots. We measure
136 the litter depth in two different locations of each plot, in two randomly determined
137 directions; the corresponding mean value was calculated for all plots. Soil moisture content
138 was estimated comparing the fresh and dry weights of core soil samples. Each sample
139 corresponded to ~100 cm³ of soil, which was collected and stored on sealed plastic
140 containers until their fresh weight was recorded (up to a maximum of one and a half hour
141 later). Then all samples were dried at 70°C for 24h to obtain their dry weight. Weight loss
142 was considered the amount of water in soil. All samples were collected in the same day.

143

144 *Seed germination and seedling survival*

145 To compare seed germination and seedling survival among the restorations, edges
146 and interior of forest fragments we performed seed addition experiments. We used
147 diaspores from 13 Atlantic Forest plants: *Aristolochia labiata* Willd. (Aristolochiaceae),
148 *Baccharis dracunculifolia* DC. (Asteraceae), *Cecropia hololeuca* Miq. (Urticaceae), *Cissus*
149 *sp. L.* (Vitaceae), *Euterpe edulis* Mart. (Arecaceae), *Gochnatia polymorpha* (Less.) Cabrera
150 (Asteraceae), *Guazuma ulmifolia* Lam. (Malvaceae), *Pleroma granulosum* (Desr.) D. Don
151 (Melastomataceae), *Schinus terebinthifolia* Raddi (Anacardiaceae), *Styrax camporum* Pohl
152 (Styracaceae), *Syagrus romanzoffiana* (Cham.) Glassman (Arecaceae), *Tabernaemontana*
153 *catharinensis* A.DC. (Apocynaceae) and *Zanthoxylum riedelianum* Engl. (Rutaceae).

154 Species were selected according to seed availability in our study sites. We included species
155 that differed in seed mass, dispersal syndrome and growth form. Species traits and the
156 number of added seeds are presented in Table 1. Seeds were added in the 28 previously
157 described experimental quadrats in forest interiors, forest edges and restoration areas. We
158 only included seeds without any signs of predation marks or pathogens. We established 14
159 seed addition plots (20 x 20 cm) in each one of the 28 experimental quadrats (located at the
160 interior and edges of forest fragments and restoration areas), one plot for each one of the 13
161 plant species and one control plot. We did not add any seeds in the control plot to assess
162 germination rates from naturally fallen seeds. All propagules were placed directly above the
163 soil, simulating naturally fallen fruits and seeds. We avoided the removal of the litter layer
164 while placing the propagules in the soil. We recorded seed germination biweekly for five
165 months (May to October 2019) and all recently emerged seedlings were tagged, and their
166 survival was followed until the end of the experiment. Seeds were collected in the study
167 site, where all plant species naturally occur. The exception were the seeds from the palm
168 *Euterpe edulis*, which were collected from a protected site in the region (Parque Estadual
169 Carlos Botelho, 38,705 ha, 24°06'10"S, 47°98'92"W) because this species no longer occurs
170 in the study site due to intensive harvesting. Seed addition experiments started during the
171 fruiting period of those species and the number of added seeds varied according to their
172 availability.

173

174 *Seed removal*

175 To assess seed removal, we conducted field experiments using propagules of 5
176 species: *A. labiata*, *G. ulmifolia*, *S. terenithifolius*, *S. romanzoffiana* and *Z. riedelianum*. All
177 species were also used in the experiment involving seed germination (see Table 1 for

178 details) and are well distributed in our forest fragments. Species were selected based on
179 seed availability. We added propagules of those species in 8 of the previously described
180 quadrats located at forest edges, 8 in forest interiors and 8 in ecological restoration areas.
181 The exception was the addition of *G. ulmifolia* seeds, which were placed in 3 quadrats in
182 each habitat (9 in total) due to the low seed availability. Each quadrat included two
183 0.5x0.3m plots. One plot received an exclusion of medium and large vertebrates, while the
184 other plot was a control without exclusion (allowing the access of vertebrates and
185 invertebrates). Vertebrates were excluded using plastic frames covered by 1.5 cm plastic
186 mesh, which were secured to the ground by wooden stakes. All plastic meshes had four
187 2x5cm openings to allow removal of the large seeded species such as *S. romanzoffiana*.
188 Those openings probably allow the access of small rodents. Propagules were placed on the
189 ground and their number varied according to their availability in the field (Table 1). Seed
190 removal was verified biweekly for 2 months and seeds that have been moved at least 6 cm
191 were considered as removed.

192

193 *Data analysis*

194 We used one-way ANOVAs to analyze potential differences on microhabitat
195 conditions (canopy cover, litter layer depth and soil moisture) between forest interior, forest
196 edges and restoration areas. To evaluate the general effect of vertebrate exclusion and
197 habitat (forest interior, edge and restoration) on seed removal of all five species, we used a
198 binomial Generalized Linear Mixed-Effects Model (GLMM), which included vertebrate
199 exclusion and habitat as fixed factors and species and quadrat as random factors. To
200 analyze the effect of vertebrate exclusion and habitat on seed removal proportion of each
201 species, we performed GLMMs for each one of the studied species including vertebrate

202 exclusion and habitat as fixed factors and quadrat as random factor. The relationship
203 between seed removal and microhabitat conditions was evaluated using a binomial GLMM.
204 Seed removal proportion was included as the dependent variable, canopy cover, litter layer
205 depth and soil moisture as fixed factors, and species and quadrat as random factors.
206 GLMMs families were determined based on quantile-comparison plots, which were
207 obtained using the R package car (Fox & Weisberg 2011). The best fitted distributions for
208 the response variables were determined by comparing gaussian, lognormal, binomial,
209 Poisson and gamma distributions. When gaussian distribution was the best fit, we used
210 Linear Mixed-Effects Models (LMMs; see more details in Bates et al. 2015). GLMM and
211 LMMs were performed using the R package lme4 (Bates et al. 2015). Variation on seed
212 germination and seedling survival was not analyzed due to the low number of emerged
213 seedlings (see Results section).

214

215 **Results**

216 Restoration areas had lower canopy cover compared to forest interior and edges ($F_{(2, 25)} = 16.07$, $p < 0.01$; Figure 1). Litter layer depth did not differ between restoration, forest
217 interior and forest edges ($F_{(2, 25)} = 0.33$, $p = 0.72$), but soil moisture was higher in forest
218 interior compared to edges and restoration areas ($F_{(2, 25)} = 47.92$, $p < 0.01$; Figure 1).

220 Only 6 out of 26,488 seeds germinated during our experiment, 3 of *S. terebinthifolia*
221 and 3 of *G. ulmifolia*, corresponding to only 0.5% and 0.7% of the total number of seeds
222 added for each of those species, respectively. Emerged seedlings were found in only two
223 plots, one in forest interior and the other in the forest edge. None of the seedlings survived
224 more than 15 days after emergence, and all of *S. terebinthifolia* seedlings presented signs of

225 herbivory. There was no seed germination in any of the control plots, indicating that added
226 seeds were the sources of emerged seedlings found on experimental plots.

227 Seed removal varied according to vertebrate exclusion treatment when we
228 considered all five species, but there was no significant effect of habitat type (forest
229 interior, edge and restoration areas) or the interactions between these factors (see Table 2).
230 Vertebrate exclusion decreased seed removal in general (Figure 2), indicating that
231 vertebrates are important seed removal agents. Removal percentages with and without
232 vertebrate exclusion corresponded to $45.4\% \pm 39.6\%$ (mean \pm SD) and $63.4\% \pm 34.5\%$ of
233 all propagules, respectively. Removal rates were significantly influenced by soil moisture
234 but were not related to canopy cover and litter layer depth (Table 3).

235 When species were analyzed individually, vertebrate exclusion decreased seed
236 removal of *S. terebinthifolia* and *G. ulmifolia*, indicating that vertebrates are important
237 removal agents for these species. Exclusion did not affect removal of the other three species
238 (see Appendix 1 for more details). Habitat type only influenced seed removal of *S.*
239 *romanzoffiana*, with restoration areas presenting higher removal compared to forest interior
240 sites (see Fig. 2 and Appendix 1). The interaction of exclusion and habitat type did not
241 affect any of the analyzed species. Invertebrates and small vertebrates removed a relevant
242 proportion of propagules of all studied species, from the wind-dispersed liana *A. labiata* to
243 the large-seeded palm *S. romanzoffiana* (Fig. 2).

244

245 **Discussion**

246 The interior and edges of small forest fragments have mild microhabitat conditions
247 compared to restorations, potentially affecting early plant regeneration. However, seed
248 germination rates are extremely low at all sites, at least when considering our 13 studied

249 species, potentially constraining plant recruitment at all sites. Furthermore, the lack of
250 seedling emergence in all control plots suggests that natural recruitment of plants is also
251 reduced, which can be related to low seed germination rates/seedling establishment from
252 naturally fallen seeds (establishment limitation) and/or a small number of seeds deposited
253 on the ground (seed limitation). Finally, considering that seeds removed are more likely
254 preyed on (Fleury & Galetti 2004), the high seed removal potentially affects plant
255 recruitment at all sites.

256 Restoration sites present lower canopy cover than forest interior and edges, and soil
257 moisture is lower when compared to forest interior. Those results highlight the harsh
258 environments for seed germination and the relatively long time required to properly restore
259 microhabitat conditions in Atlantic forest restoration areas in our region (e.g. Rodrigues et
260 al. 2009), even when taking small secondary forest fragments as a reference, as is the case
261 here. Increased light availability and decreased soil moisture might affect early plant
262 recruitment in restorations, influencing seed germination, seedling and sapling densities
263 according to species tolerances to these conditions (e.g. Guerrero & Bustamante 2009). In
264 addition, forest interior presents higher soil moisture than edges, indicating microhabitat
265 heterogeneity within small fragments and the importance of the maintenance of those core
266 areas in a long term. We expected that forest interior would present higher levels of canopy
267 cover, litter layer depth and soil moisture, when compared to edges and restoration areas.
268 However, each abiotic condition varied in a different way between habitats, suggesting
269 complex microhabitat spatial heterogeneity (see also Oliveira et al. 2019; Guerrero &
270 Bustamante 2009).

271 Our results suggest a strong constraint to germination in small forest fragments and
272 restoration sites. For example, *E. edulis* attain germination rates up to 60% in larger

273 Atlantic Forest fragments and continuous areas (Soares et al. 2015; Pizo et al. 2006), but
274 there was no seed germination in our plots. In addition, even if a seed germinates, several
275 factors (such as seedling herbivory in the case of *S. terebinthifolia*) can decrease the
276 survival of seedlings and saplings. Some herbivores, like leaf-cutting ants, might present a
277 higher impact in disturbed and early successional habitats, which can be an important
278 constraint to plant recruitment in those areas, including sites under restoration (Leal et al.
279 2014; Wirth et al. 2007; Klippel et al. 2015; Brancalion et al. 2015). The low percentage of
280 seedling emergence and survival indicate high levels of establishment limitation in our
281 small Atlantic Forest fragments and restoration areas. Although the seeds of some of the
282 species might remain viable after a few months in the soil, such as *C. hololeuca* (IPEF
283 2019) and *S. romanzoffina* (Soares-Oliveira et al. 2015), seed viability is often highly
284 reduced after a few months. Direct seed sowing has been considered a restoration
285 alternative to nursery-raised seedling plantings due to its lower costs (Raupp et al. 2020).
286 However, pre-germinative treatments (such as seed scarification), physical protection of
287 sown seeds, and selection of species might be necessary to increase seed sowing efficacy
288 (Engel & Parrotta 2001; Cole et al. 2011; Ceccon et al. 2015). Unfortunately, only a few
289 studies explore seedling emergence and survival of Atlantic Forest species in the field (e.g.
290 Souza & Valio 2001; Pizo et al. 2006; Rother et al. 2013), and there is scarce information
291 about early plant recruitment in small forest fragments and areas under restoration to use in
292 comparisons. Therefore, more information about germination of Atlantic Forest species
293 under field conditions and comparisons of recruitment in natural, restored, and unrestored
294 sites would be worthwhile.

295 Seed dispersal and predation are important factors for plant regeneration, potentially
296 changing recruitment probability, spatial distribution, and diversity of plant communities

297 (Wang & Smith 2002; Camargo et al. 2016; Soares et al. 2015). Animal composition and
298 the surrounding vegetation may influence seed removal (Garrote et al. 2019; Pinto et al.
299 2009). Habitat loss and fragmentation often affect animal communities and plant-animal
300 interactions (Markl et al. 2012; Fontúrbel et al. 2015). In addition, seed dispersal and
301 predation of Atlantic Forest species can vary according to microhabitat conditions and the
302 level of human disturbance (Rother et al. 2013; Soares et al. 2015; Christianini & Galetti
303 2007; Fleury & Galetti 2004). For example, seed removal of large-seeded trees can be
304 higher in forest edges and under fruiting trees (Pinto et al. 2009). Fragment size and animal
305 abundance also seem to affect seed removal and predation of *S. romanzoffiana* (Fleury &
306 Galetti 2004). Contrary to expectations, habitat type does not seem to influence seed
307 removal levels of most of our studied species. On the other hand, our results highlight the
308 influence of microhabitat conditions, especially soil moisture, on seed removal. Soil
309 moisture and water evaporation are often associated with vegetation structure (e.g. Wang et
310 al. 2018), which can influence granivores and seed removal and predation (Fleury & Galetti
311 2006; García et al. 2011). In addition, invertebrates and vertebrates seem to be important
312 seed removal agents for all studied species. Studies of relatively large-seeded plants, like
313 the palm *S. romanzoffiana*, often highlight seed removal by vertebrates, especially rodents
314 and mammals, and invertebrates like beetles often prey on seeds on site (e.g. Soares et al.
315 2015; Meiga & Christianini 2015). Our results indicate that invertebrates and small rodents
316 play a role on seed removal of those species, at least in small forest fragments and
317 restoration areas. In addition, the anemochoric propagules of the liana *A. labiata* and the
318 seeds of the zoochoric tree *G. ulmifolia* also present high removal rates by invertebrates and
319 small vertebrates, suggesting that seed predation by those animals might be an important
320 constraint to recruitment of these small-seeded species.

321 This study highlights several biotic and abiotic differences among the core areas of
322 small Atlantic forest fragments, fragment edges and restorations, from microhabitat
323 conditions to seed removal. Between species variation reinforces the importance of studies
324 at a community level. Potential differences in abiotic factors and plant regeneration can
325 affect plant species persistence in small fragments and restorations in the long term,
326 changing community composition at local and regional scale (Püttker et al. 2019;
327 Rodrigues et al. 2009; Metzger et al. 2009), and influencing successional trajectories
328 (Tabarelli et al. 2012). The low levels of seed germination in small fragments and
329 restoration sites, high seed removal rates and harsh conditions specially in restorations
330 suggest strong constraints to seed and seedling survival. Then, strategies of direct seed
331 augmentation might not be effective to restore biodiversity in those areas (Ceccon et al.
332 2016). Alternatively, enrichment plantings are probably necessary in several Atlantic Forest
333 areas under restoration since natural regeneration seems to be limited even when
334 restorations are surrounded by forest fragments, as in our landscape (see also Rodrigues et
335 al. 2009).

336 **References**

- 337 Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models
338 Using lme4. *Journal of Statistical Software* 67:1–48
- 339 Benitez-Malvido J (1998) Impact of Forest Fragmentation on Seedling Abundance in a
340 Tropical Rain Forest. *Conservation Biology* 12:380–389
- 341 Bihn JH et al. (2008) Do secondary forests act as refuges for old growth forest animals?
342 Recovery of ant diversity in the Atlantic forest of Brazil. *Biological Conservation*
343 114:733–743
- 344 Brancalion PHS, Gandolfi S, Rodrigues RR (2015) Restauração Florestal. Oficina de
345 Textos 190-202
- 346 Bruna EM (2002) Effects of forest fragmentation *Heliconia acuminata* on seedling
347 recruitment in central Amazonia. *Oecologia* 132:235–243
- 348 Camargo PH et al. (2016) Bird and ant synergy increases the seed dispersal effectiveness of
349 an ornithochoric shrub. *Oecologia* 181:507–518
- 350 Ceccon E, González EJ, Martorell C (2016) Is direct seeding a biologically viable strategy
351 for restoring forest ecosystems? Evidences from a meta-analysis. *Land Degradation &*
352 *Development* 27:511-520
- 353 Christianini A V, Galetti M (2007) Spatial variation in post-dispersal seed removal in an
354 Atlantic forest: Effects of habitat, location and guilds of seed predators. *Acta*
355 *Oecologica* 32:328–336
- 356 Cole RJ et al. (2011) Direct seeding of late-successional trees to restore tropical montane
357 forest. *Forest Ecology and Management* 261:1590–1597
- 358 Engel VL, Parrotta JA. An evaluation of direct seeding for reforestation of degraded lands
359 in central São Paulo state, Brazil. *Forest Ecology & Management* 152:169-181

- 360 Fox J, Weisberg S (2019) *An R Companion to Applied Regression*, Third edition. Sage,
361 Thousand Oaks CA
- 362 Fleury M, Galetti M (2004) Effects of microhabitat on palm seed predation in two forest
363 fragments in southeast Brazil. *Acta Oecologica* 26:179–184
- 364 Fontúrbel FE et al. (2015) Meta-analysis of anthropogenic habitat disturbance effects on
365 animal-mediated seed dispersal. *Global Change Biology* 21:3951–3960
- 366 Garrote PJ et al. (2019) Extrinsic factors rather than seed traits mediate strong spatial
367 variation in seed predation. *Perspectives in Plant Ecology, Evolution and Systematics*
368 38:39–47
- 369 Guerrero PC, Bustamante RO (2009) Abiotic alterations caused by forest fragmentation
370 affect tree regeneration: A shade and drought tolerance gradient in the remnants of
371 Coastal Maulino forest. *Revista Chilena de Historia Natural* 82:413–424
- 372 Haddad NM et al. (2015) Habitat fragmentation and its lasting impact on Earth's
373 ecosystems. *Science Advances* 1:1–10
- 374 Harper KA et al. (2005) Edge influence on forest structure and composition in fragmented
375 landscapes. *Conservation Biology* 19:768–782
- 376 Joly CA, Metzger JP, Tabarelli M (2014) Experiences from the Brazilian Atlantic Forest:
377 ecological findings and conservation initiatives. *New Phytologist* 204:459–473
- 378 Klippel VH et al. (2015) Evaluation of forest restoration methods of Tableland Forest, ES.
379 *Revista Árvores* 39:69–79
- 380 Kortz AR et al. (2014) Wood vegetation in Atlantic rain forest remnants in Sorocaba (São
381 Paulo, Brazil). *Check List* 10:344–354
- 382 Laurance WF et al. (2002) Ecosystem decay of Amazonian forest fragments: a 22-years
383 investigation. *Conservation Biology* 16:605–618

- 384 Laurance WF, Cochrane MA (2001) Synergistic effects in fragmented landscapes.
385 Conservation Biology 15:1488–1489
- 386 Leal IR, Wirth R, Tabarelli M (2014) The Multiple Impacts of Leaf-Cutting Ants and Their
387 Novel Ecological Role in Human-Modified Neotropical Forests. *Biotropica* 46:516–
388 528
- 389 Liebsch D, Marques MCM, Goldenberg R (2008) How long does the Atlantic Rain Forest
390 take to recover after a disturbance? Changes in species composition and ecological
391 features during secondary succession. *Biological Conservation* 141:1717–1725
- 392 Markl JS et al. (2012) Meta-Analysis of the Effects of Human Disturbance on Seed
393 Dispersal by Animals. *Conservation Biology* 26:1072–1081
- 394 Meiga AY, Christianini A V. (2015) Changes in seed predation of *Attalea dubia* in a
395 gradient of atlantic forest disturbance. *Palms* 59:135–144
- 396 Metzger JP et al. (2009) Time-lag in biological responses to landscape changes in a highly
397 dynamic Atlantic forest region. *Biological Conservation* 142:1166–1177
- 398 Myers N et al. (2000) Biodiversity hotspots for conservation priority. *Nature* 403:853–858
- 399 Oda GA et al. (2019) Micro- or macroscale ? Which one best predicts the establishment of
400 an endemic Atlantic Forest palm? *Ecology and Evolution* 9:7284–7290
- 401 Oliveira MA, Grillo AS, Tabarelli M (2004) Forest edge in the Brazilian Atlantic forest:
402 drastic changes in tree species assemblages. *Oryx* 38:389–394
- 403 Oliveira RAC, Marques R, Marques MCM (2019) Plant diversity and local environmental
404 conditions indirectly affect litter decomposition in a tropical forest. *Applied Soil
405 Ecology* 134:45–53
- 406 Peña-Domene M De et al. (2017) Seed to seedling transitions in successional habitats
407 across a tropical landscape. 410–419

- 408 Pinto S, Santos A, Tabarelli M (2009) Seed predation by rodents and safe sites for large-
409 seeded trees in a fragment of the Brazilian Atlantic forest. *Brazilian Journal of Biology*
410 69:763–771
- 411 Pizo MA, Von Allmen C, Morellato LPC (2006) Seed size variation in the palm
412 *Euterpe edulis* and the effects of seed predators on germination and seedling survival.
413 *Acta Oecologica* 29:311–315
- 414 Püttker T et al. (2019) Indirect effects of habitat loss via habitat fragmentation: A cross-
415 taxa analysis of forest-dependent species. *Biological Conservation* 241
- 416 Raupp PP et al. (2020) Direct seeding reduces the costs of tree planting for forest and
417 savanna restoration. *Ecological Engineering* 148:105788
- 418 Restrepo C, Vargas A (1999) Seeds and seedlings of two neotropical montane understory
419 shrubs respond differently to anthropogenic edges and treefall gaps. *Oecologia*
420 119:419–426
- 421 Ribeiro MC et al. (2009) The Brazilian Atlantic Forest: How much is left, and how is the
422 remaining forest distributed? Implications for conservation. *Biological Conservation*
423 142:1141–1153
- 424 Rodrigues RR et al. (2009) On the restoration of high diversity forests : 30 years of
425 experience in the Brazilian Atlantic Forest. *Biological Conservation* 142:1242–1251
- 426 Rother DC et al. (2013) Demographic bottlenecks in tropical plant regeneration: A
427 comparative analysis of causal influences. *Perspectives in Plant Ecology, Evolution*
428 *and Systematics* 15:86–96
- 429 Santos K, Kinoshita LS, Santos FAM (2007) Tree species composition and similarity in
430 semideciduous forest fragments of southeastern Brazil. *Biological Conservation*
431 135:268–277

- 432 Soares LASS et al. (2015) Implications of habitat loss on seed predation and early
433 recruitment of a keystone palm in anthropogenic landscapes in the Brazilian Atlantic
434 Rainforest. PLoS ONE 10:1–14
- 435 Soares-Oliveira TG et al. (2015). Longevity and germination of *Syagrus romanzoffiana*
436 (Arecaceae) seeds and its ecological implications. Revista de Biología Tropical
437 63:333-340
- 438 Souza FM, Batista JLF (2004) Restoration of seasonal semideciduous forests in Brazil:
439 Influence of age and restoration design on forest structure. Forest Ecology and
440 Management 191:185–200
- 441 Souza RP De, Valio FM (2001) Seed size, seed germination, and seedling survival of
442 Brazilian tropical tree species differing in successional status. Biotropica 33:447–457
- 443 Tabarelli M et al. (2010) Prospects for biodiversity conservation in the Atlantic Forest:
444 Lessons from aging human-modified landscapes. Biological Conservation 143:2328–
445 2340
- 446 Tabarelli M, Peres CA, Melo FPL (2012) The ‘few winners and many losers’ paradigm
447 revisited: Emerging prospects for tropical forest biodiversity. Biological Conservation
448 155:136–140
- 449 Wang BC, Smith TB (2002) Closing the seed dispersal loop. Trends in Ecology &
450 Evolution 17:379–385
- 451 Wirth R et al. (2007) Increasing densities of leaf-cutting ants (*Atta* spp.) with proximity to
452 the edge in a Brazilian Atlantic forest. Journal of Tropical Ecology 23:501–505
453

Table 1. Atlantic Forest plant species used in our experiments comparing seed germination and seed removal in interior and edges of small forest fragments and adjacent ecological restoration areas. Propagule type (F= fruit; S=seed), seed mass (mg), dispersal syndrome, growth form, ecological succession category (suc.= successional), and total number of seeds included in all experimental blocks of seed germination (n=28) and removal experiments (n=24; n=9 for *G. ulmifolia*) were provided for each species. Numbers inside parenthesis indicate mean values of seed germination and seed removal percentages for each species.

Species	Propagule type	Seed mass (mg)	Dispersal syndrome	Growth form	Succession	Seeds added (% germinat.)	Seeds added (% removal)
<i>Aristolochia labiata</i>	F	2.9	anemochoric	liana	-	840 (0)	720 (57.1)
<i>Baccharis dracunculifolia</i>	F	0.07	anemochoric	shrub	early suc.	560 (0)	-
<i>Cecropia hololeuca</i>	S	0.52	zoochoric	tree	early suc.	3640 (0)	-
<i>Cissus</i> sp.	F	38.1	zoochoric	liana	-	140 (0)	-
<i>Euterpe edulis</i>	S	750.5	zoochoric	palm	-	280 (0)	-
<i>Gochnatia polymorpha</i>	F	3.7	anemochoric	tree	early suc.	560 (0)	-
<i>Guazuma ulmifolia</i>	S	6.1	zoochoric	tree	early suc.	420 (0.7)	180 (61.9)
<i>Pleroma granulosum</i>	S	0.04	anemochoric	tree	early suc.	17920 (0)	-
<i>Schinus terebinthifolia</i>	F	14.2	zoochoric	tree	early suc.	560 (0.5)	480 (41.1)
<i>Styrax camporum</i>	F	147.7	zoochoric	treelet	early suc.	168 (0)	-
<i>Syagrus romanzoffiana</i>	F	3100.5	zoochoric	palm	-	280 (0)	480 (48.9)
<i>Tabernaemontana catharinensis</i>	S	65.7	zoochoric	tree	early suc.	560 (0)	480 (67.8)
<i>Zanthoxylum riedelianum</i>	S	37.9	zoochoric	tree	early suc.	560 (0)	-

Table 2. Main results of the binomial Generalized Linear Mixed-Effects Model (GLMM) relating seed removal proportions to habitat type (forest interior, forest edge and restoration areas) and medium- and large-sized vertebrate exclusion treatment (with and without exclusion). Bold text represents significant p-values.

Fixed factors	Estimate	SE	z	p
(Intercept)	-0.36	0.32	-1.15	0.25
Without exclusion	0.87	0.29	2.97	<0.01
Interior	-0.44	0.36	-1.20	0.23
Restoration	-0.07	0.36	-0.20	0.84

Table 3. Main results of the Generalized Linear Mixed-Effects Model (GLMM) relating seed removal rates and microhabitat conditions (canopy cover, litter layer depth and soil moisture) in our experimental quadrats in small Atlantic Forest fragments and areas under ecological restoration. Bold text indicates significant p-values.

	Estimate	SE	z	p-value
(Intercept)	-0.51	1.57	-0.33	0.75
Canopy cover	0.03	0.02	1.33	0.18
Litter depth	0.12	0.19	0.64	0.52
Soil moisture	-0.15	0.08	-2.02	0.04

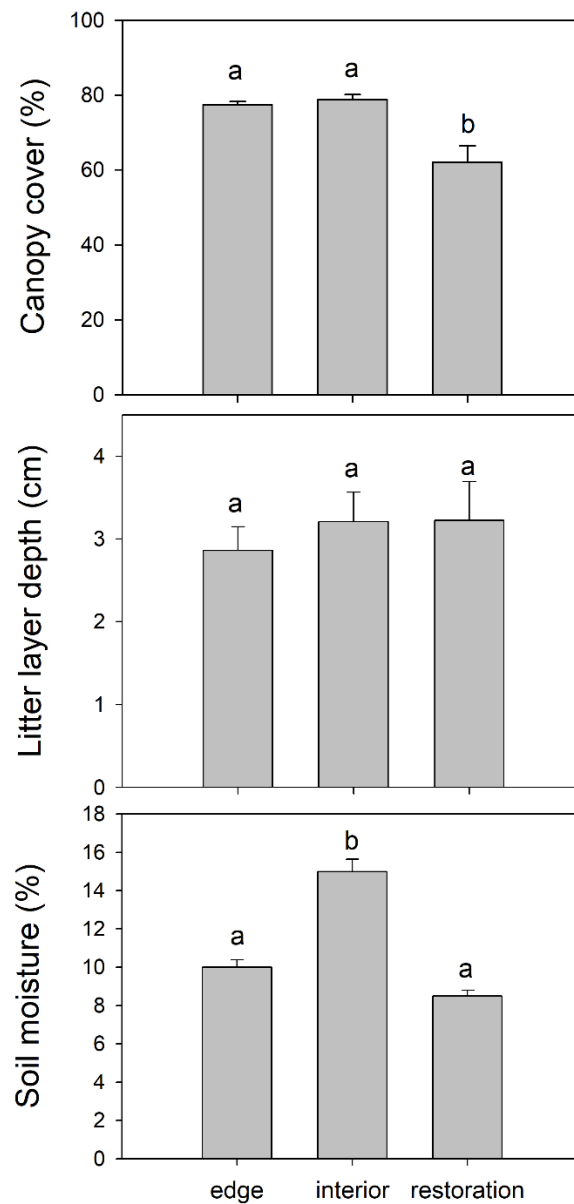
Figures

Fig 1. Microhabitat conditions in small Atlantic Forest interiors (n=10) and edges (n=10) and adjacent ecological restoration areas (n=8). Mean values of canopy cover percentage, litter layer depth and soil moisture percentage for different habitat types are represented by gray bars. Error bars represent standard errors. Different letters above each bar represent a significant difference.

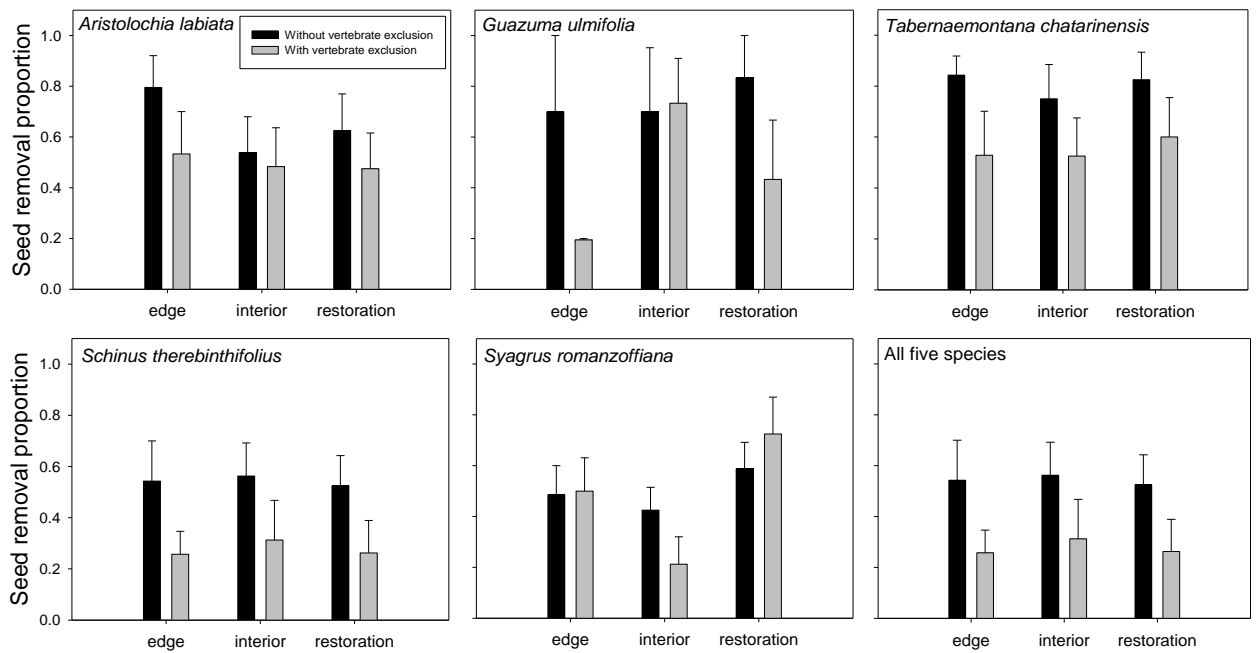


Fig. 2. Seed removal proportion in plots with and without exclusion of medium- and large-sized vertebrates for (a) *Aristolochia labiata*, (b) *Guazuma ulmifolia*, (c) *Tabernaemontana catharinensis*, (d) *Schinus terebinthifolius*, (e) *Syagrus romanzoffiana* and (f) the mean of all five studied species in the interior and edges of small Atlantic Forest fragments and restoration areas.

Microhabitat characteristics, seed germination and seed removal in small Atlantic Forest fragments and restoration areas

Mariano, V. & Christianini, A.V.

Appendix A

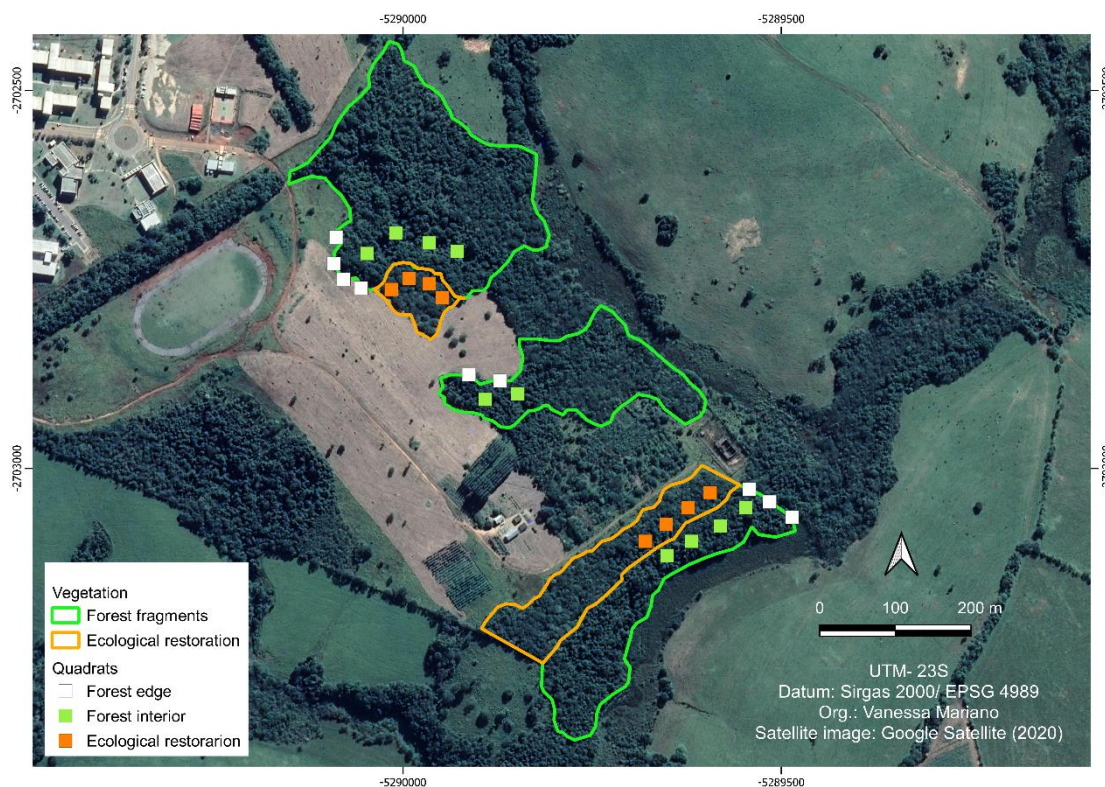


Figure S1. Distribution of the Atlantic Forest fragments and ecological restorations in our study site at Sorocaba, São Paulo, southeastern Brazil. The approximate location of each one of the 28 experimental quadrats (2 x 1 m) used in our study are also indicated in the map. Forest fragments and restoration areas are surrounded by a pastureland matrix dominated by the African grass *Urochloa decumbens*. More information about the study site and the experimental design are provided in the main text.

Table S1. Main results of the Linear Mixed-Effects Models (LMMs) and Generalized Linear Mixed-Effects Models (GLMMs) relating seed removal proportions of each species to habitat type (forest interior, forest edge and restoration areas), medium- and large-sized vertebrate exclusion treatment (with and without exclusion) and the interaction between factors. These results are from independent analysis for each plant species included in our experiment and quadrat was used as random effect in all models. We specified the family used in each model after species names. LMMs were used for Gaussian distributions of seed removal proportions of each species and GLMMs were used for non-Gaussian distributions. Values of t and z (and the respective p-values) are provided for each LMM and GLMM models, respectively. Chi-square tests results, including p-values, are provided for gaussian models in Table S2. More details and results are provided in the main text.

	Estimate	SE	t	z	p-value
<i>Aristolochia labiata</i> (binomial)					
(Intercept)	-0.63	0.50	-	-1.25	0.21
Without exclusion	0.40	0.65	-	0.61	0.538
Interior	-0.10	0.71	-	-0.14	0.89
Restoration	-0.12	0.71	-	-0.16	0.871
Without exclusion:Interior	-0.29	0.94	-	-0.31	0.756
Without exclusion:Restoration	-0.12	0.94	-	-0.13	0.895
<i>Schinus terebinthifolia</i> (gaussian)					
(Intercept)	0.26	0.13	2.00	-	-
Without exclusion	0.29	0.13	2.22	-	-
Interior	0.06	0.18	0.31	-	-
Restoration	0.01	0.18	0.03	-	-
Without exclusion:Interior	-0.04	0.18	-0.20	-	-
Without exclusion:Restoration	-0.02	0.18	-0.13	-	-
<i>Guazuma ulmifolia</i> (gaussian)					
(Intercept)	0.20	0.20	1.02	-	-
Without exclusion	0.50	0.26	1.96	-	-
Interior	0.53	0.25	2.10	-	-
Restoration	0.23	0.25	0.92	-	-
Without exclusion:Interior	-0.53	0.33	-1.62	-	-
Without exclusion:Restoration	-0.10	0.33	-0.30	-	-
<i>Syagrus romanzoffiana</i> (gaussian)					
(Intercept)	0.50	0.11	4.40	-	-
Without exclusion	-0.01	0.15	-0.10	-	-
Interior	-0.29	0.16	-1.85	-	-
Restoration	0.23	0.16	1.45	-	-

Without exclusion:Interior	0.23	0.20	1.11	-	-
Without exclusion:Restoration	-0.12	0.20	-0.60	-	-
<i>Tabernaemontana catharinensis</i> (binomial)					
(Intercept)	-0.64	0.47	-	-1.35	0.18
Without exclusion	0.47	0.63	-	0.74	0.46
Interior	-0.01	0.64	-	-0.01	0.99
Restoration	0.13	0.69	-	0.19	0.85
Without exclusion:Interior	-0.11	0.84	-	-0.13	0.90
Without exclusion:Restoration	-0.15	0.93	-	-0.16	0.87

Table S2. Type II Wald chi-square tests for Linear Mixed Effects Models (LMMs) results relating seed removal proportions of *Schinus terebinthifolia*, *Guazuma ulmifolia* and *Syagrus romanzoffiana* to habitat type (forest interior, forest edge and restoration areas), medium- and large-sized vertebrate exclusion treatment (with and without exclusion) and the interaction between factors. More results and details are provided in Table S1 and in the main text. χ^2 , degrees of freedom (df) and p-values are provided for each model.

	χ^2	df	p
<i>Schinus terebinthifolia</i>			
exclusion	12.13	1	<0.01
habitat	0.09	2	0.96
exclusion:habitat	0.04	2	0.98
<i>Guazuma ulmifolia</i>			
exclusion	4.21	1	0.04
habitat	1.93	2	0.38
exclusion:habitat	3.30	2	0.19
<i>Syagrus romanzoffiana</i>			
exclusion	0.07	1	0.79
habitat	8.89	2	0.01
exclusion:habitat	3.22	2	0.20

Conclusões gerais

O presente trabalho trouxe informações importantes sobre os efeitos distúrbios antrópicos nos estágios iniciais de regeneração de plantas nativas, os quais variaram de acordo com cada fase do processo de regeneração. Após analisarmos dados globais, constatamos que, em geral, a germinação de sementes é afetada negativamente pelas atividades humanas. Ou seja, locais sujeitos a esses distúrbios apresentam menores taxas de germinação quando comparados à locais sem distúrbios. Porém, quando analisamos esses efeitos na sobrevivência de plântulas vimos que há mais variação na resposta das espécies aos distúrbios, dificultando a identificação de padrões. Além disso, nossos experimentos em pequenos fragmentos de Mata Atlântica e de áreas adjacentes sujeitas à restauração ecológica sugerem que a germinação de sementes pode ser extremamente baixa nesses locais, indicando uma limitação potencialmente importante no estabelecimento. A remoção de sementes por invertebrados e vertebrados foi importante para as todas as cinco espécies analisadas, com o potencial de alterar os padrões de deposição e a sobrevivência de sementes e recrutamento de plantas.

Além disso, algumas características das espécies e do ambiente provavelmente tornam algumas espécies mais suscetíveis aos distúrbios antrópicos. Por exemplo, o efeito dos distúrbios na germinação variou de acordo com a forma de vida e o tipo de vegetação, sendo que gramíneas, ervas e arbustos e espécies de fisionomias não-florestais foram as mais susceptíveis. Por outro lado, nenhuma das características das espécies e da vegetação avaliadas foi boa preditora dos efeitos dos distúrbios na sobrevivência de plântulas, sugerindo uma limitação nas abordagens baseadas em nichos ecológicos nesse caso. Assim, sugerimos a importância de se incluir a estocasticidade e a heterogeneidade de fatores bióticos e abióticos em nível de micro-habitat como fontes importantes de variação na resposta das plântulas à distúrbios.

Os resultados dos nossos experimentos em campo reforçam a importância de se avaliar características abióticas dos micro-habitats. Os baixos níveis de germinação e sobrevivência de plântulas, inclusive de espécies pioneiras, indicam que pequenos fragmentos e áreas de restauração apresentam condições bióticas e abióticas que dificultam o recrutamento de plântulas. Os altos níveis de remoção de sementes sugerem que a dispersão e predação têm o potencial de alterar a regeneração das espécies avaliadas. Nossos resultados reforçam estudos anteriores em áreas sujeitas à restauração ecológica da Mata Atlântica, ressaltando as diferenças nas condições abióticas, como menores índices de cobertura de dossel e umidade do solo, as quais podem levar aos baixos níveis de regeneração natural nessas áreas.

Todos os tipos de distúrbios antrópicos incluídos em nossas meta-análises tiveram efeitos variados na germinação de sementes e na sobrevivência de plantas, apresentando influência positiva, negativa ou neutra nas espécies avaliadas. Invasão biológica se destacou pelo seu forte efeito negativo na germinação de sementes, mas teve efeito mais heterogêneo e inconsistente na sobrevivência de plântulas. Fragmentação de habitats, efeitos de borda, corte seletivo e pastoreio afetaram as espécies de forma variável em ambas as fases do recrutamento, fazendo com que o efeito geral não fosse significativo. Esses resultados reforçam a importância de estudos sobre diferentes distúrbios antrópicos no mesmo bioma e/ou que avaliem estágios de vida distintos e em diferentes espécies.

Os resultados apresentados nessa tese sugerem que a regeneração de plantas pode ser afetada por distúrbios antrópicos, de forma positiva ou negativa, reforçando a importância da mitigação desses impactos para a manutenção da composição e diversidade de espécies a longo prazo. Os efeitos predominantemente negativos desses distúrbios na germinação podem indicar uma influência mais forte e consistente de atividades antrópicas nessa fase do recrutamento, quando comparada à sobrevivência de

plântulas. Além disso, nossas análises sugerem que práticas de adição de sementes são menos efetivas em locais perturbados. Portanto, outras abordagens, como a adição de juvenis (por meio de mudas), provavelmente serão mais eficientes nesses casos, em especial para espécies não arbóreas e de fisionomias não-florestais. Plantios complementares de enriquecimento de áreas em processo de restauração também poderiam acelerar a recomposição de fatores bióticos e abióticos que afetam a regeneração natural nessas áreas.

Recomendamos que estudos futuros sobre os estágios iniciais da regeneração de plantas considerem avaliar condições abióticas nos micro-habitats e interações interespecíficas associadas ao recrutamento. Além disso, a análise de espécies já exploradas anteriormente na literatura traria novas perspectivas sobre a variação intraespecífica nas respostas aos distúrbios. Estudos sobre espécies não-arbóreas em florestas e espécies arbóreas em fisionomias campestres, quando possível, também trariam informações complementares à literatura existente. Dessa forma, poderemos avaliar com mais precisão quais fatores bióticos e abióticos influenciam a resposta das espécies aos distúrbios antrópicos.