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

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1 **Running title: Reliability of seed rain estimates**

2

3

4 **A simple standardized protocol to evaluate the reliability of seed rain**

5 **estimates**

6

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15

16

17 **Abstract**

18 Seed dispersal has key implications for community dynamics and restoration ecology.
19 However, estimating seed rain (the number and diversity of seeds arriving in a given area)
20 is challenging, and the lack of standardization in measurement prevents cross-site
21 comparisons. Seed trap effectiveness and accuracy of seed sorting methods are key
22 components of seed rain estimates in need of standardization. We propose and describe
23 a standardized protocol for evaluating the effectiveness of two seed trap types (sticky and
24 funnel traps), and the accuracy of a seed sorting method. We used widely available seeds
25 (arugula, quinoa, sesame and sunflower) to produce a gradient of seed size, weight and
26 color. Proof-of-concept was tested in a tropical grassland, where traps were set for 30
27 days. Our results suggest that we underestimate dispersal of small seeds (less than 2mm
28 width) that can be easily mistaken for debris and soil particles or that fail to adhere to
29 sticky traps. Seeds on sticky traps may be more vulnerable to removal by wind and rain,
30 whereas seeds in funnel traps are more susceptible to decay. We found no evidence of
31 observer bias on seed sorting for funnel trap samples. However, accuracy on seed sorting
32 for funnel trap samples tended to decline for seeds with less than 2mm width, suggesting
33 a size-dependence in seed retrieval success. Our standardized protocol addressing trap
34 effectiveness and seed sorting methods will increase reliability of data obtained in seed
35 rain studies in grasslands and allow more reliable comparisons between datasets.

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

37

38 **Introduction**

39 Seed dispersal studies are vital to understanding plant distribution and community
40 resilience, and they guide conservation and restoration activities (Török et al., 2018). A
41 useful way of studying seed dispersal is to estimate seed rain —*i.e.* the number and
42 diversity of new seeds reaching a given area (Baskin and Baskin 2014) — using seed
43 traps to collect propagules at particular locations, then identifying and counting them.
44 However, measuring seed rain is challenging, and a lack of methodological
45 standardization persists, compromising the accuracy of seed rain estimates and impairing
46 comparison of data between studies (Arruda et al., 2018; Wolfe et al., 2019). Seed rain
47 has long been used by ecologists to address ecological succession and factors limiting
48 regeneration in tropical forests (Holl 1999; Saulei and Swaine, 1988). In open
49 environments, however, seed dispersal by wind and water run-off poses additional
50 challenges to estimate seed rain. . For example, despite seed rain in temperate grasslands
51 has been relatively well studied, we not only need more research in other grasslands types
52 (e.g. tropical grasslands), but to improve many crucial aspects related to methods
53 standardization and data reporting (Arruda et al. 2018). Determining the effectiveness of
54 seed traps, and of seed sorting methods for samples, is crucial for improving
55 reproducibility, but it is rarely tested in seed rain studies (Thompson and Mcginnes, 1963;
56 Jackel and Poschlod, 1994; Kollmann and Goetze, 1998; Stevenson and Vargas, 2008).

57 Evaluation of seed trap effectiveness involves the evaluation of two processes:
58 trap capacity to capture seeds (seed catch), and trap capacity to retain seeds (seed
59 retention) and avoid seed loss (Box 1; Fig. 1). Additionally, the accuracy of seed sorting
60 methods for trap samples depends not only on seed size—small seeds are harder to find—
61 but also on the ability to separate seed material from debris, which can strongly affect
62 seed retrieval rates (Cottrell, 2004). Knowing the seed retrieval rate of seed sorting is

63 important for determining the influence of seed size, of observer effect, and of sample
64 composition, or more precisely, the color and size of soil particles, debris and litter often
65 present in samples (Debussche and Isenmann, 1994).

66 Among other invertebrates, ants are known for their ability to collect large
67 amounts of seeds and can have a major impact on seed trap effectiveness (Predavec,
68 1997). Seed decay can vary greatly between seed types and is also modulated by other
69 biotic and abiotic conditions such as pathogens, humidity and the amount of litter/soil
70 accumulated within traps (Roberts, 1972; Box 1). Additionally, seed loss by wind or
71 water run-off can vary greatly between trap types, seasons and plant communities, thereby
72 influencing seed retrieval rates. Therefore, to maximize seed catch and minimize seed
73 loss in open ecosystems, the use of complementary seed trap types is recommended
74 (Chabrierie and Alard, 2005).

75 Funnel traps can be used to study local seed rain and the transportation of seeds
76 by water run-off (Jackel and Poschlod, 1994). Funnel traps are effective in seed catch, but
77 seed loss to predation and to decay caused by excessive moisture arise as potential
78 problems (Schott, 1995; Kollmann and Goetze, 1998; Jensen, 1998). Sticky traps, in turn,
79 are more suitable for studying wind-dispersed species (Jefferson and Usher 1989). While
80 sticky traps carry a lower risk of seed predation, checking them is often hindered by
81 trapped insects and debris (Kollmann and Goetze, 1998). Both sticky and funnel traps
82 may also bias the seed catch towards larger seeds that are more easily detected by visual
83 assessment, while soft seeds may easily rot, and smooth seeds may be lost through rain
84 or wind action (Kollmann and Goetze, 1998; Cottrell, 2004). Despite the current state of
85 knowledge, the influence of seed size and weight on the retention rate of seed traps is still
86 overlooked.

87 Among the possible methods for sorting seeds in funnel-trap samples, the most
88 effective is direct seed inspection after sieving to separate seeds from debris (Kollmann
89 and Goetze, 1998; Cottrel, 2004). Seed identification requires training; however, one’s
90 capacity to find and sort seeds also depends on seed traits, such as size and color (Martin
91 and Barkley, 1961; Cottrell, 2004). Considering that results can be biased by differences
92 in one’s capacity to find and sort seeds from the samples, it is important to obtain, prior
93 to data interpretation in seed rain studies, an estimate of how many seeds are missed in
94 the sorting procedure. Our goal is to propose and describe a simple standardized protocol
95 to evaluate the effectiveness in seed retention of two types of seed trap (Box 1, Fig. 1),
96 and the accuracy of a seed sorting method in assessing seed rain. The standardized
97 protocol should be run along with every seed rain study using these traps to allow more
98 reliable comparisons between studies. We tested these standardized protocols in a tropical
99 grassland as a proof-of-concept.

100

101 **Description and implementation**

102

103 **SEED TRAPS**

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

109

110 **STANDARDIZED PROTOCOL**

111 We used four species: arugula (*Eruca sativa*), quinoa (*Chenopodium quinoa*),
112 sesame (*Sesamum indicum*) and sunflower (*Helianthus annuus*). Here, the sunflower
113 achenes are called seeds to facilitate the terminology throughout the manuscript. These
114 seeds are available in most market or garden center worldwide and provide variation in
115 size, weight and colors that can be tested across ecosystems (Table 1). To assess seed
116 retention, the number of traps and the length of time that traps should be left in the field
117 can be adapted to each study and grassland type. Killing the seeds before using them in
118 the field is a mandatory step to 1) prevent the seeds from germinating on the seed traps,
119 2) avoid contamination by pathogens that may be associated with these seeds (Godefroid
120 et al., 2017), 3) prevent invasion of exotic species (Estévez et al., 2015). We placed all
121 seeds in a drying oven at 120°C for an hour, as treatments heating seeds above 85°C for
122 more than 8 min have shown to kill most seeds (Hess et al. 2018). We conducted all seed
123 measurements (e.g. weight and size) after the heat treatment.

124 On each trap, we gently dropped 10 seeds of each species, totaling 40 seeds per
125 trap (Table 1). On funnel traps, we put all seeds straight into the bag collectors. It is best
126 to run the standardized protocol either during the same season(s) as the study or, if run
127 over one year, during the season in which conditions are most challenging for
128 preservation of the seeds on the traps. For the sticky traps, all samples collected from each
129 trap are examined under magnification. For the funnel traps, bags are collected separately
130 from each trap, and their content washed in a 250- μ m sieve to reduce the amount of fine
131 soil particles, then examined under a magnifying glass to count and identify seeds. All
132 seeds are counted and any signs of damage recorded.

133 To evaluate seed loss during the seed sorting of funnel traps samples, a second
134 experiment examines the sorting accuracy with funnel traps samples. We chose to test the
135 seed sorting accuracy with only funnel trap samples because the traps can accumulate

136 much litter and soil in the field, making it difficult to retrieve seeds. For this test, a given
137 number of seeds of the four species are mixed in soil, in a proportion equal to that
138 commonly found inside the funnel trap bags. The soil used must be taken from the study
139 area to control for color, debris and litter composition. The number of seeds in each
140 sample (with a minimum of 3 and maximum of 30 seeds per species) should be
141 randomized and noted; this number is not known to the observers. A minimum of three
142 previously trained observers then sort the samples, searching for, identifying and counting
143 the seeds. For ethical aspects, observers' anonymity must be respected when collecting
144 and reporting the data.

145 The proportion of seeds retrieved at the end of the experiments (retrieval success)
146 is obtained by calculating the percentage of seeds retrieved by each observer. Both
147 experiments are analyzed using generalized linear models that assume a quasibinomial
148 distribution and use retrieval success as the response variable. For the seed trap
149 effectiveness experiment, trap type and species are the categorical variables (interaction
150 was tested). For the seed sorting experiment, species and experimenters are the
151 categorical variables (interaction was tested). In both cases, pairwise contrast
152 comparisons with a Tukey adjustment can be run. We here performed these analyses with
153 R (R Core Team, 2018), packages *base* and *emmeans*.

154

155 PROOF OF CONCEPT

156 In order to proof-of-concept our protocol, we conducted fieldwork in the southern
157 part of the Espinhaço mountain range, southeastern Brazil (43° 35' W, 19° 17' S). The
158 annual precipitation averages around 1,400 mm, and climate is markedly seasonal, with
159 most rainfall occurring in the hot summers (Silveira et al., 2016). We conducted the
160 experiment in March 2017, at the end of the raining season, when high temperatures,

161 strong winds and rainy days prevail, creating the most challenging conditions in the study
162 area. The main vegetation comprises the mountaintop *campo rupestre*, fire-prone open
163 grasslands that establish on quartzite-derived rocks, with shallow and severely nutrient-
164 poor sandy soils (Silveira et al., 2016).

165 We randomly placed six of each type of seed trap on a pristine site (50 m²), and
166 retrieved seeds after a period of one month, as this the most common timeframe used to
167 sample seed rain in grasslands (Arruda et al., 2018). For the second experiment, testing
168 seed sorting accuracy with funnel trap samples, we ran the protocol with three previously
169 trained observers in the laboratory.

170

171 **Results of the proof-of-concept**

172 In the proof-of-concept experiment, we found no difference between the two trap types
173 in sunflower seed retention, with both performing well (98.6% for funnel trap and 100%
174 for sticky trap) (Fig. 3). Funnel traps were ineffective in retaining quinoa seeds under the
175 field conditions, while the sticky trap had a good retention rate for quinoa seeds (88.6%;
176 LM quasibinomial, $p < 0.001$; Fig. 3). We found no significant difference in the
177 performance between seed traps for sesame seeds (Fig. 3). Funnel traps performed better
178 than sticky traps in the retention of arugula seeds (41.4% more efficient than sticky traps)
179 (Fig. 3). Quinoa and arugula seeds had the lowest retention rates, with only 1.4% and
180 42.9% of the arugula seeds on sticky and funnel traps, respectively, and no quinoa seeds
181 retrieved from funnel traps, after one month in the field. Most sesame seeds and sunflower
182 seeds were retrieved from both sticky and funnel traps (> 88%) (Fig. 3).

183 We found no evidence to support an observer effect (GLM quasibinomial,
184 $p > 0.05$), but, while finding most seeds during seed sorting, there was a slight difference
185 in retrieval success between species, arugula being significantly lower: 88.6% of arugula

186 seeds, 97.9% of quinoa seeds, 96.9% of sesame seeds and 100% of sunflower seeds (Fig.
187 4; GLM quasibinomial, $p < 0.001$).

188

189 **Discussion**

190 Improving the accuracy and precision of seed rain estimates is necessary to further
191 our understanding of both seed dispersal and seed limitation, and to support ecological
192 restoration (Török et al., 2018). However, few studies test seed trap effectiveness in open
193 ecosystems (Arruda et al., 2018), thus precluding attempts to understand these processes
194 on a global scale. Our results show that, under the tested field conditions, both trap types
195 underperformed for species having seeds smaller than 2mm width, and so that we are
196 underestimating seed dispersal of small seeds. Despite finding no observer bias on seed
197 sorting for funnel trap samples, we found that accuracy tended to decline for seeds under
198 2mm width size, suggesting that size-dependence in seed retrieval success is more
199 common than previously thought (Kollmann and Goetze, 1998).

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

210 The high loss rates found for quinoa and arugula seeds in funnel traps indicate
211 alarming losses for small seeds in general. The weak structure of the quinoa seed coat can
212 make these seeds more vulnerable to mechanical stress, fluctuations in humidity and
213 temperature, and growth of microorganisms (Mohamed-Yasseen et al., 1994). The loss
214 of quinoa seeds are thus probably linked with humidity within the funnel traps and seed
215 decay is likely the main cause of seed loss in funnel traps (seed removal by animals is
216 unlikely due to the shape of the funnel trap). It shows that when reporting results, one
217 should be aware that seed rain may presents diaspores vulnerable to decay resulting in
218 underestimation on seed rain surveys, especially during data collection on rainy seasons.
219 Seeds with hard seed coats, such as sesame and arugula, are generally long-lived and have
220 high retention rates by funnel traps (Priestley, 1986). Hard seed coats may negative
221 influence the retention capacity of small seeds on sticky traps, as most arugula seeds were
222 probably washed off by rain or removed by strong winds from the glue.

223 Despite finding no observer bias on seed sorting for funnel trap samples, we found
224 that accuracy tended to decline with decreasing seed size and for seeds of darker color.
225 Soils with high content of organic particles, like where we tested the proof-of-concept,
226 may directly impact the accuracy of seed sorting methods because soil particles are
227 similar in color to some seeds; sorting methods should account for this similarity.

228

229 **Conclusion**

230 Our study clearly demonstrates that the traits of seeds influence their retrieval from seed
231 traps used in seed rain studies. We provide a detailed standardized protocol that can be
232 easily implemented in any seed rain study in grasslands using sticky and funnel traps that
233 can be tested and used in globally distributed experiments (Borer et al. 2014). When
234 discussing the results of a seed rain study using the proposed protocol, one must offer the

235 caveat that the methodology applied likely underestimates seed rain considering seed
236 traits, traps types and environmental conditions. Under our field conditions, our seed rain
237 study would not allow us to conclude that small, soft seeds cannot be dispersed by water
238 run-off, as they may decay in funnel traps; nor could we infer anything regarding small,
239 dark, smooth seeds, as they can be lost from sticky traps or not found by observers.
240 Finally, we argue that our standardized protocol addressing trap effectiveness and seed
241 sorting methods will increase reliability of data obtained in seed rain studies in grasslands
242 and allow more reliable comparisons between datasets.

243

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249 no conflict of interest.

250

251 **Authors' contributions**

252 All authors conceived the ideas and designed methodology; A.J.A. and E.B. collected the
253 data; A.J.A. and E.B. analysed the data; A.J.A. led the writing of the manuscript. All
254 authors contributed critically to the drafts and gave final approval for publication.

255

256 **Supporting Information**

257 Additional Supporting Information may be found in the online version of this article.

258 **Data S1.** Sticky traps construction guidelines (Sticky_trap.csv).

259 **Data S2.** Funnel traps construction guidelines (Funnel_trap.csv).

260

261 **References**

- 262 Arruda AJ, Buisson, E, Poschlod P and Silveira FAO (2018) How have we studied seed
263 rain in grasslands and what do we need to improve for better restoration? *Restoration*
264 *Ecology* **26**, S84-S91. doi:10.1111/rec.12686
- 265 Borer ET, Harpole WS, Adler PB, Lind EM, Orrock JL, Seabloom ER and Smith MD
266 (2014) Finding generality in Ecology: a model for globally distributed experiments.
267 *Methods in Ecology and Evolution* **5**, 65-73.
- 268 Chabrierie O and Alard D (2005) Comparison of three seed trap types in a chalk
269 grassland: toward a standardised protocol. *Plant Ecology* **176**, 101–112.
270 doi:10.1007/s11258-004-0024-2
- 271 Cook RE (1980) The biology of seeds in the soil. In *Demography and evolution in plant*
272 *populations*, pp. 107–129 in O.T. Solbrig (Eds), *Botanical Monographs* **15**, University
273 of California Press, Berkeley, California.
- 274 Cottrell TR (2004) Seed rain traps for forest lands: Considerations for trap construction
275 and study design. *BC Journal of Ecosystems and Management*, **5** (1). Retrieved from
276 <http://jem-online.org/index.php/jem/article/view/283>
- 277 Debussche M and Isenmann M (1994) Bird-dispersed seed rain and seedling
278 establishment in patchy Mediterranean vegetation. *Oikos* **69**, 414–426.
279 doi:10.2307/3545854
- 280 Estévez RA, Anderson CB, Pizarro JC and Burgman MA (2015) Clarifying values, risk
281 perceptions, and attitudes to resolve or avoid social conflicts in invasive species
282 management. *Conservation Biology* **29**, 19-30. doi:10.1111/cobi.12359
- 283 Godefroid S, Vyver A Van De, Stoffelen P and Vanderborght T (2017) Effectiveness of
284 dry heat as a seed sterilisation technique: Implications for ex situ conservation, *Plant*

285 Biosystems - An International Journal Dealing with all Aspects of Plant Biology
286 **151(6)**, 1054-1061. doi:10.1080/11263504.2016.1231140

287 Hess MCM, De Wilde M, Yavercovski N, Willm L, Mesléard F and Buisson E (2018)
288 Microwave soil heating reduces seedling emergence of a wide range of species
289 including invasives. *Restoration Ecology* **26**: S160-S169. doi:10.1111/rec.12668

290 Holl KD (1999) Factors Limiting Tropical Rain Forest Regeneration in Abandoned
291 Pasture: Seed Rain, Seed Germination, Microclimate, and Soil. *Biotropica* **31**: 229-
292 242. doi:10.1111/j.1744-7429.1999.tb00135.x

293 Howe HF and Smallwood J (1982) “Ecology of Seed Dispersal.” *Annual Review of*
294 *Ecology and Systematics*, **13**, 201–228.

295 Jackel AK and Poschlod P (1994) Diaspore production and the influence of the size of
296 diaspore traps on the quantitative result of seasonal diaspore rain in two calcareous
297 grassland sites, pp. 123–132. *Berichte des Institutes für Landschafts-und*
298 *Pflanzenökologie der Universität Hohenheim* **3**.

299 Jefferson RG and Usher M.B (1989) Seed rain dynamics in disused chalk quarries in the
300 Yorkshire Wolds, England, with special reference to nature conservation. *Biological*
301 *Conservation* **47**, 123-136. doi:10.1016/0006-3207(89)90095-5

302 Jensen K (1998) Species composition of soil seed bank and seed rain of abandoned wet
303 meadows and their relation to above ground vegetation. *Flora*, **193**, 345-359.
304 doi:10.1016/S0367-2530(17)30860-5

305 Kollmann J and Goetze D (1998) Notes on seed traps in terrestrial plant communities.
306 *Flora*, **193**, 31–40. doi:10.1016/S0367-2530(17)30813-7

307 Martin AC and Barkley WD (1961) *Seed identification manual*. University of California
308 Press, Berkeley, California.

309 Mohamed-Yasseen Y (1991) Onion seed aging and plant regeneration in vitro. Ph.D.
310 diss., University of Illinois, Urbana.

311 Mohamed-Yasseen Y, Barringer SA, Splittstoesser WE and Costanza S (1994) The Role
312 of Seed Coats in Seed Viability. *The Botanical Review* **60** (4), 426-439. Retrieved
313 from <http://www.jstor.org/stable/4354239>

314 Predavec M (1997) Seed removal by rodents, ants and birds in the Simpson Desert, central
315 Australia. *Journal of Arid Environments* **36**, 327–332. doi:10.1006/jare.1996.0156

316 Priestley DA (1986) Seed aging. Cornell University Press, Ithaca, New York.

317 Roberts EH (1972) Loss of viability and crop yields, pp. 307-359 in E. H. Roberts (ed.),
318 Viability of seeds. Chapman and Hall, London.

319 Saulei SM and Swaine MD (1988) Rain forest seed dynamics during succession at Gogol,
320 Papua New Guinea. *Journal of Ecology* **76** (4): 1133-152. doi:10.2307/2260639.

321 Schott GW (1995) A seed trap for monitoring the seed rain in terrestrial communities.
322 *Canadian Journal of Botany* **73**, 794–796. doi:10.1139/b95-087

323 Silveira FAO, Negreiros D, Barbosa NPU, Buisson E., Carmo FF, Carstensen DW,
324 Conceição AA, Cornelissen TG, Echternacht L, Fernandes GW, Garcia QS, Guerra T
325 J, Jacobi CM, Lemos Filho JP, LeStradic S, Morellato LPC, Neves FS, Oliveira RS,
326 Schaefer CE, Viana PL, Lambers H (2016) Ecology and evolution of plant diversity
327 in the endangered campo rupestre: a neglected conservation priority. *Plant Soil* **403**,
328 129–152. doi:10.1007/s11104-015-2637-8

329 Stevenson PR and Vargas IN (2008) Sample size and appropriate design of fruit and seed
330 traps in tropical forests. *Journal of Tropical Ecology* **24**, 95-105.
331 doi.org/10.1017/s0266467407004646

332 Thompson RL and Mcginnes BS (1963) A comparison of eight types of mast traps.
333 *Journal of Forestry* **61**, 679-680.

- 334 Török P, Helm A, Kiehl K, Buisson E and Valkó O (2018) Beyond the species pool:
335 modification of species dispersal, establishment, and assembly by habitat restoration.
336 *Restoration Ecology* **26**, S65-S72. doi:10.1111/rec.12825
- 337 Wolfe BT, Macchiavelli R, Van Bloem SJ (2019) Seed rain along a gradient of
338 degradation in Caribbean dry forest: Effects of dispersal limitation on the trajectory of
339 forest recovery. *Applied Vegetation Science* **22**, 423-434. doi: 10.1111/avsc.12444

340 **Table 1:** Seed average width, length and weight for the four species that should be used in the protocol from measurements made from 15 seeds of
341 each species after heat treatment at 120°C for an hour.

342

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

343

Box 1 – Definitions of terms related to seed rain estimates

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

Seed rain – the number and diversity of seeds reaching a given area

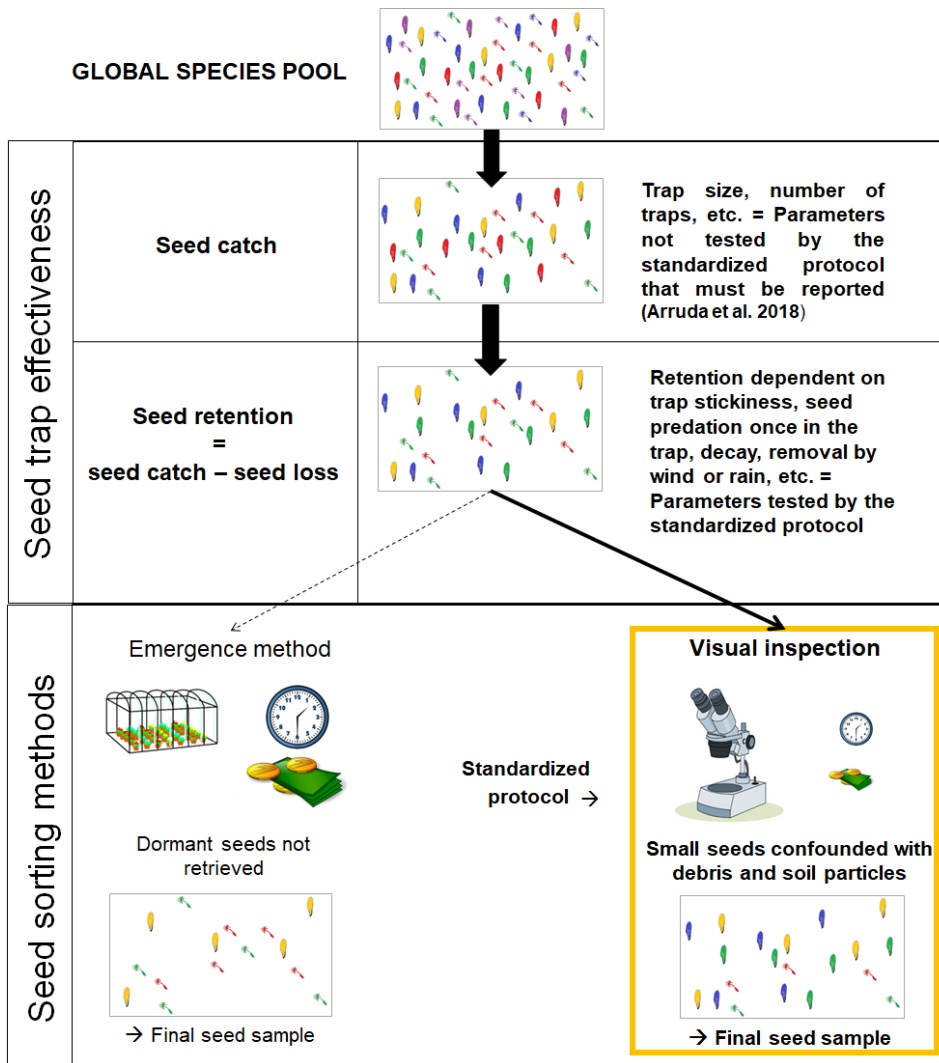
Seed trap effectiveness – the ability of seed traps to accurately and precisely estimate seed rain. Seed trap effectiveness is determined by seed catch and seed retention.

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

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

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

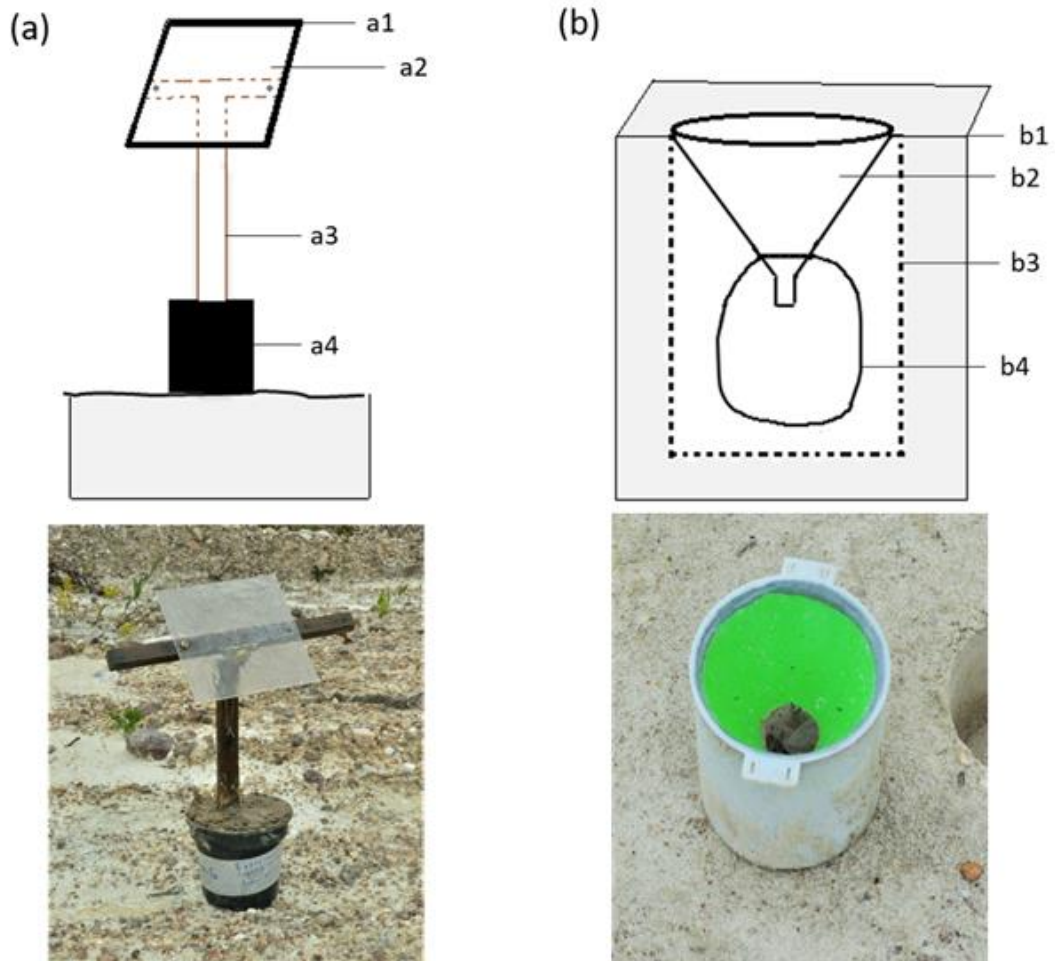
Seed decay - the progressive deterioration of the structures and functions of the seed over time, and which will ultimately leads to seed death



347

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

357

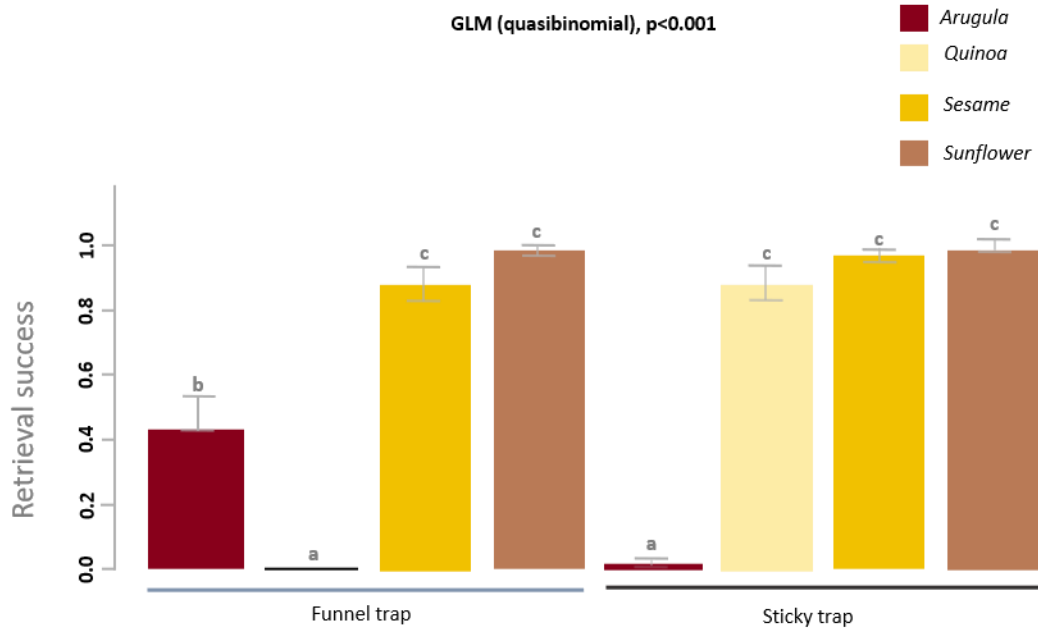


358

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

363

364



365

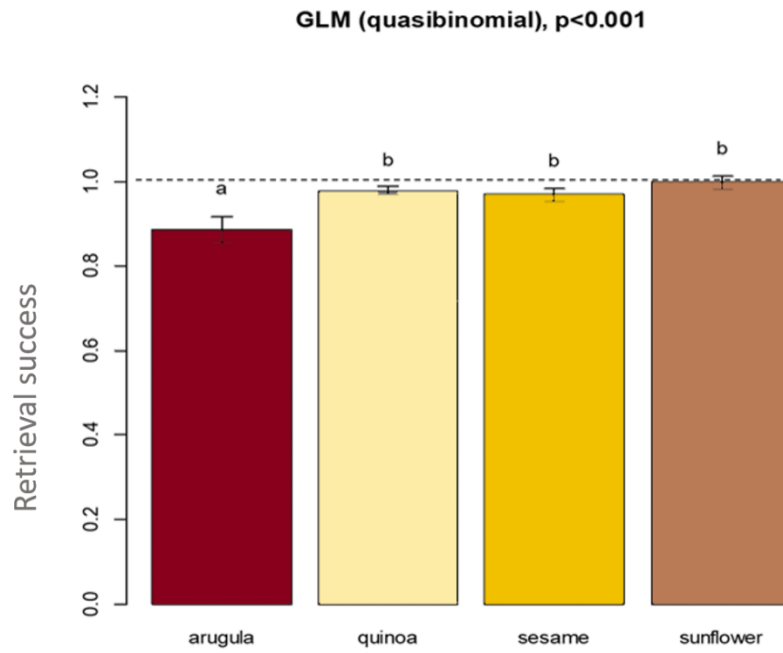
366 **Fig. 3.** Retrieval success for four species in two seed trap types over a 30-day period

367 (***)= $p < 0.001$). For each species, 10 seeds were placed in each trap type (indicated by

368 the dashed line).

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371

372 **Fig. 4.** Retrieval success on the sorting accuracy test of the funnel trap samples for the

373 four species.

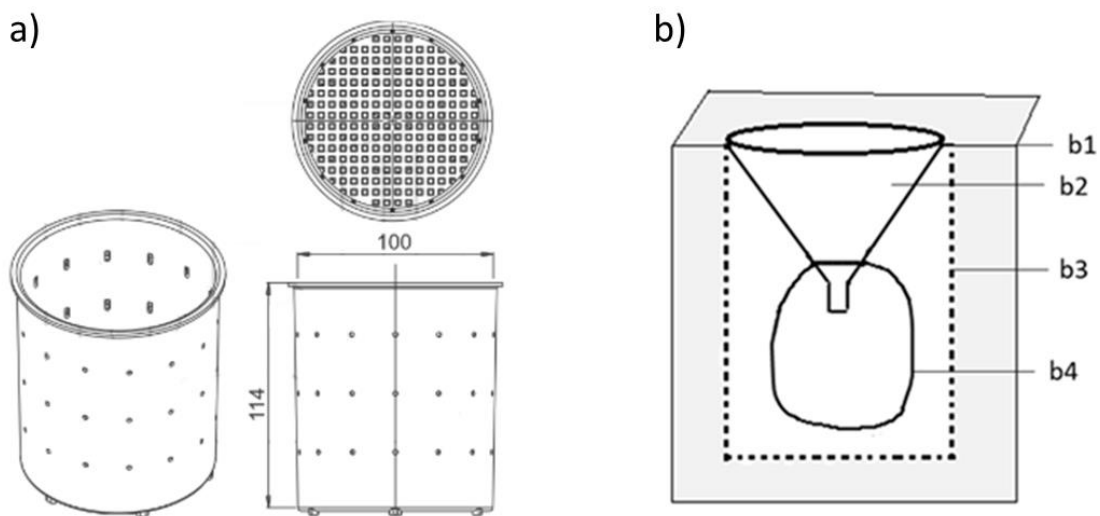
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376 **Data S2. Funnel traps construction guidelines (Funnel_trap.csv).**

377 Use a PVC pipe measuring 11.4 cm high \times 15.0 cm wide (top) \times 10 cm long, originally
378 designed for cheese production. This cylindrical plastic structure should have holes, 1 cm
379 in diameter, on the sides and bottom to allow drainage. Attach a conical PVC plastic
380 funnel, 15 cm in diameter, to the top end of the cylindrical plastic structure
381 (approximately 0.0177 m² in area). At the bottom of the funnel, the seeds are caught in a
382 disposable polypropylene bag, with pores smaller than 0.1 mm, fixed with an elastic band
383 to the base of the funnel. This polypropylene bag is originally produced as a disposable
384 hygienic hair cap, and it retains its integrity over the period of exposure in the field,
385 independent of weather conditions (TALGE, Santa Catarina, Brazil).

386



387

388 **Appendix S2:** a) External structure of the funnel trap before PVC funnel installation b)
389 Internal structure of the funnel trap installed in the field; b1: ground level; b2: PVC plastic
390 funnel (15 cm² upper end area); b3: PVC pipe with bore holes 1 cm diameter; b4: bag of
391 $<$ 0.1mm mesh size.