

Frugivore gut passage increases seed germination: an updated meta-analysis

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Short title: Frugivore gut passage impacts on germination

Keywords: mutualism, seed dispersal, scarification, gut passage, ecosystem functioning, plant-animal interactions, seed dispersal effectiveness

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Authorship: HSR conceived of the project, organized data collection and designed the database with AK, and wrote the first draft of the manuscript. ECF conducted analyses and produced figures. All authors contributed to data collection and revision of the manuscript.

1 **ABSTRACT**

2 Many plants rely on animal mutualists for reproduction. Quantifying how animal mutualists
3 impact plant performance provides a foundation for modelling how change in animal
4 communities affects the composition and functioning of plant communities. We performed a
5 meta-analysis of 2539 experiments, 6 times more than the last comprehensive meta-analysis,
6 examining how gut passage by frugivores influences seed germination. We simultaneously
7 analyzed multiple predictor variables related to study methodology, location, and frugivore
8 identity to disentangle methodological from ecological impacts on effect sizes. We found that gut
9 passage by birds, fish, reptiles, bats, primates, and other mammals on average increased seed
10 germination, but that the magnitude varied across vertebrate groups. The positive effects of gut
11 passage were largely explained by the de-inhibitory effects of pulp removal rather than by the
12 scarification of seed tissues. Some previous studies and meta-analyses that found no effect of gut
13 passage only tested scarification or did not distinguish between these tests of scarification and
14 pulp removal. We found that, for a typical fleshy-fruited plant species, the lack of gut passage
15 reduces germination by 60%. From an evolutionary perspective, this indicates a large risk
16 associated with reliance on animal mutualists that is balanced against the benefits of animal-
17 mediated seed dispersal. From a conservation perspective, this highlights the potential for large
18 demographic consequences of frugivore declines on plant populations. Our database and findings
19 advance quantitative predictions for the role of fruit-frugivore interactions in shaping plant
20 communities in the Anthropocene.

21 INTRODUCTION

22 Interactions among species support the functioning of ecosystems (Loreau *et al.* 2001).
23 Mutualisms like pollination and seed dispersal affect plant communities by impacting both plant
24 reproduction and the movement of genetic material (Kremen *et al.* 2007). Mutualists thus shape
25 the ecosystem services that plants provide to people (Garibaldi *et al.* 2013; Egerer *et al.* 2017).
26 Mutualist declines under anthropogenic change threaten plant diversity, ecosystem functioning,
27 and ecosystem services (Potts *et al.* 2010; Rogers *et al.* 2021). Generalizing knowledge of the
28 functional impacts of such species interactions informs how mutualistic interactions affect
29 community dynamics and the consequences of ongoing declines of animal mutualists (Brodie *et*
30 *al.* 2018). The outcome of a species interaction for ecosystem function depends on the frequency
31 of interaction (quantity component) as well as the net impact of that interaction on individual
32 plant performance (quality component), which could be positive, neutral, or negative even for
33 putatively ‘mutualistic’ interactions. Formalized in the effectiveness framework (Schupp *et al.*
34 2017), the product of the quantity and quality components gives an estimate of the total impact
35 of the interactions among a species pair on ecosystem function. Whereas the quantity component
36 may be more easily measured (i.e., via direct observation), measuring the quality component
37 requires intensive experiments that track impacts on reproduction or survival over months or
38 years.

39 Seed dispersal by animals is widespread across the phylogeny of seed plants (Jordano
40 2000; Rogers *et al.* 2021). Roughly half of the ~350,000 angiosperm species producing fleshy
41 fruit (Aslan *et al.* 2013) are adapted for consumption and dispersal by mutualistic partners
42 including birds, non-avian reptiles, bats, primates, and invertebrates. Global change factors
43 including overhunting, species invasion, and fragmentation are causing declines in many seed-

44 dispersing mutualists (McConkey *et al.* 2012), with particularly striking declines in large-bodied
45 mammals and birds (McConkey *et al.* 2012; Galetti & Dirzo 2013; Dirzo *et al.* 2014). Changes
46 in frugivore abundance and diversity may affect plant populations through mechanisms such as
47 reduced seedling recruitment due to insufficient seed consumption and gut passage, reduced
48 movement away from areas of high mortality near conspecific parents or to areas suitable for
49 germination, and reduced colonization ability (Farwig & Berens 2012; Aslan *et al.* 2019; Rogers
50 *et al.* 2021).

51 The impact of frugivore gut passage on seed germination has been studied for more than
52 a century (Barrows & Schwarz 1895 p. 85-87; Troup 1921; Ridley 1930; Krefting & Roe 1949).
53 Experiments measuring these impacts compare the probability of germination of gut-passed
54 seeds to that of seeds that are not gut-passed (Samuels & Levey 2005). These experiments
55 address several basic ecological and evolutionary questions. First, they address some of the
56 fundamental costs and benefits to plants that result from engaging in mutualistic seed dispersal
57 interactions, specifically the cost of seed destruction and benefit of increased seed germination
58 by frugivores. Second, they examine aspects of seed and reproductive biology including the
59 impact on the probability or timing of germination due to removal of inhibitory cues through
60 pulp removal (de-inhibition effect) versus that of mechanical and chemical changes to seed
61 tissues (scarification effect). Third, they can reveal how phylogenetically or morphologically
62 distinct animal partners vary in their impacts on germination, offering insights into
63 coevolutionary processes among mutualists. Further, these experiments also have direct
64 applications in conservation contexts. Studies on individual plant or animal species elucidate the
65 demographic consequences of frugivore declines; plant species more heavily dependent on
66 frugivore gut passage are more vulnerable to mutualism disruption (Rogers *et al.* 2021) and

67 frugivores that provide the largest functional benefits are of particular importance in
68 conservation or restoration settings (Samuels & Levey 2005).

69 Previous reviews and meta-analyses have addressed the impact of gut passage on
70 germination, covering studies across all frugivores (Traveset 1998 [315 experiments from 80
71 studies]; Traveset & Verdu 2002 [351 experiments from 83 studies]; Verdu & Traveset 2004
72 [216 experiments]; Soltani *et al.* 2018 [581 experiments from 76 studies]) and particular
73 taxonomic groups of frugivores (primates - Fuzessy *et al.* 2016 [460 experiments from 19
74 studies]; bats - Saldaña-Vázquez *et al.* 2019 [106 experiments from 33 studies]). In general,
75 these studies support a positive effect of gut passage for most plant species, with variation
76 between frugivore groups. However, bats and reptiles were poorly represented in the last
77 comprehensive meta-analysis (Traveset & Verdu 2002 [bats - 19 studies, reptiles - 39 studies])
78 and fish and insects were not included due to a lack of studies. A recent meta-analysis on bats
79 (Saldaña-Vázquez *et al.* 2019) covered 5 times more experiments than Traveset and Verdu
80 (2002) and concluded that gut passage by bats had a neutral effect on germination, which differs
81 from the positive effect found by Traveset and Verdu (2002). In addition to the large number of
82 studies published in the years since the last meta-analysis, updated analytic approaches
83 (Viechtbauer 2010) allow for more robust insights. In particular, previous meta-analyses assess a
84 single predictor variable at a time, whereas the metafor package (Viechtbauer 2010) facilitates
85 the incorporation of multiple predictor variables in a single model.

86 A fundamental methodological limitation of prior meta-analyses, and of the vast majority
87 of experiments included in the meta-analyses, is the use of manually de-pulped seeds for the
88 control treatment, or even more problematically, the lack of distinction between manually de-
89 pulped seeds and seeds within whole fruit. An experimental design that only uses manually de-

90 pulped seeds for comparison with gut-passed poorly represents the ecosystem functioning
91 provided by frugivores—or consequences of mutualist loss—because animals are responsible for
92 both pulp removal and scarification in nature (Samuels & Levey 2005; Costa-Pereira 2017).
93 Comparisons between gut-passed and manually de-pulped seeds, without comparison to whole
94 fruit, fail to quantify the de-inhibitory effects of frugivores and may lead to incomplete
95 conclusions on the impacts of gut passage. This is likely to lead to inaccurate conclusions if
96 studies on specific animal groups disproportionately employ methods focused only on
97 scarification or de-inhibition, because the confounded effects of study design and animal group
98 would obscure differences between groups. The recent meta-analysis of studies involving bats
99 was only able to include comparisons between gut-passed and manually de-pulped seeds, due to
100 limited studies involving comparisons to whole fruits, and showed no overall effect of gut
101 passage on germination (Saldaña-Vázquez *et al.* 2019). However, this only tested the
102 scarification component of gut passage so it is premature to conclude that gut passage by bats
103 does not affect germination.

104 Here, we compiled a database of all available studies testing the effect of gut passage on
105 germination of fleshy-fruited plant species. The database includes 2539 experimental
106 comparisons from 339 publications, an increase of 2188 experiments and 256 studies since the
107 last comprehensive meta-analysis. By conducting the first meta-analysis to simultaneously
108 analyze multiple predictor variables related to study methodology, location, and frugivore
109 identity, we disentangle methodological from ecological impacts on effect sizes. This allows us
110 to 1) compare the magnitude of the de-inhibition and scarification effects, 2) understand the
111 effects of different frugivore taxa on mutualistic ecosystem functioning, and 3) examine coarse
112 macroecological variation in effect sizes.

113

114 **METHODS**

115 Database compilation

116 We aimed to compile all primary experiments published through the end of 2017 on the
117 impacts of animal gut passage on germination. To identify potential papers for inclusion in the
118 meta-analysis, we performed a SCOPUS search using the search terms: “TITLE-ABS-KEY
119 (germinat* AND ("seed dispers*" OR frugivor* OR "gut pass*" OR "ingest*" OR
120 "endozoochor*))”. We supplemented this with studies cited in, or that cited, Traveset (1998).
121 Among the 2,410 potential papers, we selected studies that compared germination of ingested
122 seeds against a control, either whole fruit or manually de-pulped seeds for inclusion in this paper.
123 We included studies where seeds were regurgitated following ingestion in addition to the great
124 majority of cases where seeds were defecated. We analyzed data for studies where the proportion
125 of seeds germinating could be discerned, such as the number of seeds sown and germinated in
126 the gut-passed and control treatments or percent of seeds germinated. When data were only
127 presented in figures, we used WebPlotDigitizer (Rohatgi 2017) to obtain quantitative values. We
128 include a list of the 339 studies used in our meta-analysis in the Supplemental Materials (Table
129 S1).

130 Along with data to characterize effect sizes from each study, we recorded several other
131 variables related to the study or focal species. For methodological variables, we recorded the
132 control that gut-passed seeds were compared to (whole fruit or mechanically cleaned seeds).
133 Other less common treatments or experimental setups (e.g., comparisons to chemically scarified
134 seeds) were excluded from analysis. We noted whether gut-passed seeds were collected by
135 searching for scat in the field (field-collected) or were collected during feeding trials with captive

136 animals (captive trial samples). We recorded the medium in which seeds were sown, either petri
137 dishes, greenhouse soil, field soil, or other planting mediums (such as tree branches for mistletoe
138 seeds). We recorded the plant and animal names to the finest taxonomic resolution available
139 down to the species level, resolving taxonomy using the Taxonstand package in R (Cayuela *et al.*
140 2012). We assigned animal species to several animal groups: bird, reptile, bat, primate, other
141 mammal, fish, and invertebrates. Using the Global Invasive Species Database, we determined
142 whether each plant or animal is known to occur as an invasive species in any part of its current
143 range. We noted the latitudinal region in which the study occurred (tropical, subtropical,
144 temperate) and whether it occurred on an island or mainland ecosystem. We sought to
145 understand how the number of animal species studied relates to the total number of frugivorous
146 species. Focusing on birds and mammals, we recorded the IUCN Red List status of each studied
147 animal species and for all bird and mammal species that have fruit in their diet (>5%) based on
148 the EltonTraits 1.0 database (Wilman *et al.* 2014).

149

150 Meta-analysis methods

151 We fit meta-analytic multivariate mixed effects models using the ‘*rma.mv*’ function in
152 the *metafor* package in R (Viechtbauer 2010). The effect sizes were calculated as an odds ratio
153 based on the number of seeds and germinants in the gut-passed and control treatments. When
154 only the proportion germinating—not the absolute number—was reported, we assumed that the
155 number of seeds in the experiment equaled the median number of seeds across experiments
156 where these data were reported. Although this decision could influence sampling variances
157 estimated for each experiment, this decision likely did not affect our conclusions because models
158 run after excluding these cases gave qualitatively equivalent results. In a full model where we

159 allowed random intercepts by plant and animal species, we included fixed effects describing the
160 control type, feeding trial type, sowing medium, frugivore taxon, invasive status, latitude region,
161 and mainland vs. island study location. To develop a best-fit model, we compared all nested
162 models with fewer fixed effects and removed variables that did not improve AIC by 2 units. To
163 evaluate the potential for phylogenetic non-independence to bias our conclusion, we ran
164 equivalent models with a variance-covariance matrix based on the plant phylogeny. We
165 constructed the dated plant phylogenetic tree using Phylomatic (Webb & Donoghue 2005) and
166 the *bladJ* algorithm (Webb *et al.* 2008).

167 Using the best-fit model, we made specific comparisons outlined in the introduction
168 using linear hypothesis testing in the ‘multcomp’ package in R (Hothorn *et al.* 2008). We
169 assessed differences between the de-inhibition effect (difference between whole fruit and
170 manually de-pulped germination) and the scarification effect (difference between de-pulped and
171 gut-passed germination). We likewise tested for differences across the methodological factors
172 (e.g., whether effect sizes differed among each combination of planting mediums), species-level
173 factors (e.g., whether effect sizes differed among each pair of animal groups), and variables
174 related to study location. For visualization of linear hypothesis test results, we obtained model
175 estimates for a combination of levels of the categorical variables that characterize the total gut
176 passage effect (de-inhibition and scarification) for a typical experiment. Specifically, this
177 combination of levels represents a trial involving birds in the tropics, comparing to a whole fruit
178 control, using captive feeding trials, and with seeds sown in petri dishes. Other combinations of
179 levels would give identical statistical results for the linear comparisons because we did not allow
180 interaction terms in the meta-analytic model.

181 We used two approaches to assess potential publication bias. We present histograms of
182 the log odds effect size across all combinations and this effect size weighted by the inverse of the
183 variance. These can indicate publication bias against studies with small effect sizes if depressed
184 near zero. We also present a funnel plot, which can indicate publication bias if asymmetric. As a
185 statistical test of funnel plot asymmetry, we present a rank correlation test (Begg and Mazumdar
186 1994).

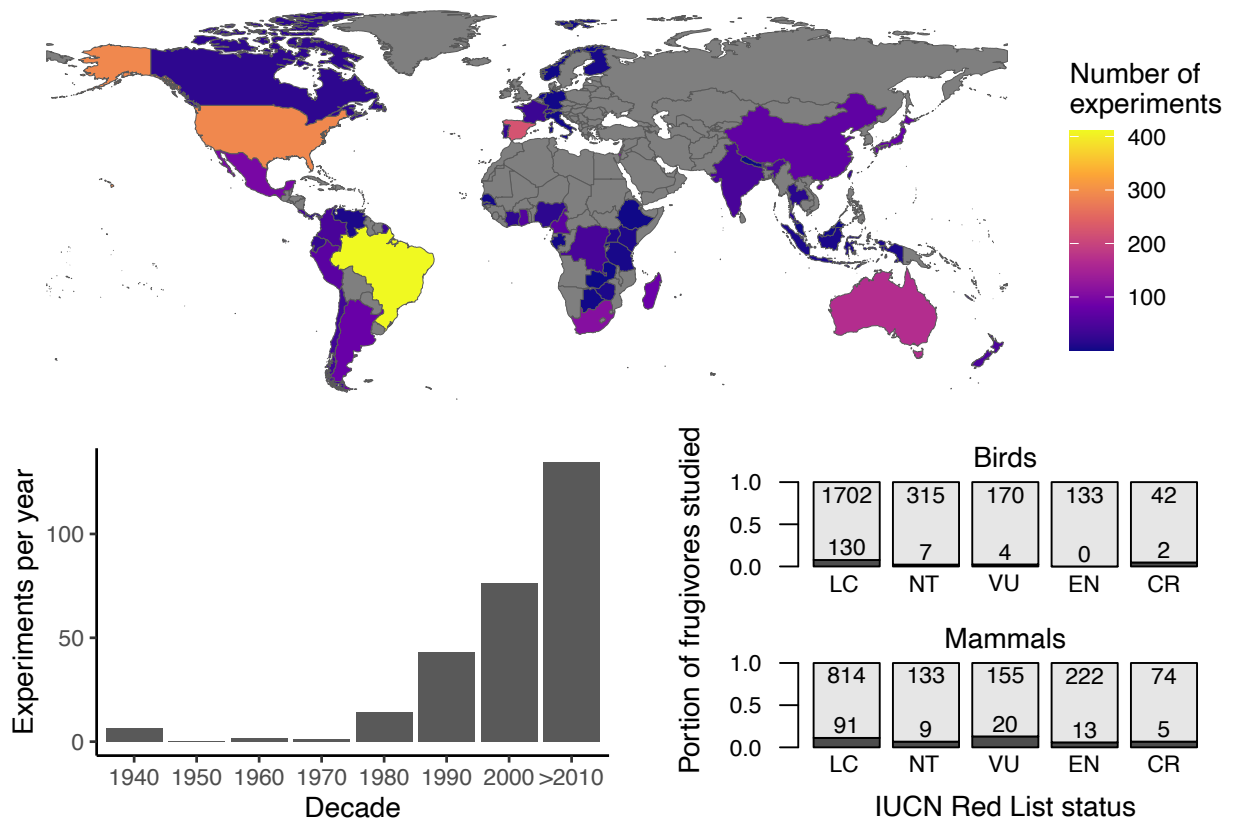
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188 **RESULTS**

189 We analyzed data from 339 publications reporting the results of 2539 experimental
190 comparisons between gut-passed and control seeds involving 1622 unique plant-frugivore
191 interactions from 446 plant genera and 226 animal genera. The countries in which experiments
192 were performed exhibit spatial heterogeneity, with the most well-studied countries including
193 Brazil, the United States of America, Spain, Australia, and South Africa (Fig. 1a). The number of
194 experiments per year has increased over time (Fig. 1b). Out of the total number of frugivorous
195 bird and mammal species, the portion that has been the focus of a gut passage experiment is
196 small (Fig. 1c). Whereas mammals have been studied roughly in proportion to IUCN Red List
197 status, birds that are more threatened are disproportionately poorly studied (Fig. 1c).

198 We fit a meta-analytic mixed effects model using all predictor variables related to study
199 methods, frugivore taxon, plant and animal invasive species status, and study location (Fig. S1,
200 Table S1). An equivalent model with a covariance matrix based on plant phylogeny showed
201 qualitatively and quantitatively similar results (Fig. S2, Table S2), suggesting that plant
202 phylogenetic non-independence is unlikely to bias our conclusions. The one difference was a
203 relative inflation of confidence intervals around the model intercept in the phylogenetic model.

204 This may be due to closely related species, or the same species, exhibiting variable effect sizes in
205 different experiments. The best fit model included all predictor variables except the variables
206 describing whether the plant or animal species was an invasive species; neither variable
207 predicted the effect of gut passage on germination (Fig. S1). We used this best fit model for
208 linear hypothesis tests. The funnel plot did not suggest bias against publications with small effect
209 sizes (Fig. S1d), but we did find evidence for funnel plot asymmetry (Kendall's tau = 0.1025, $p <$
210 0.001).

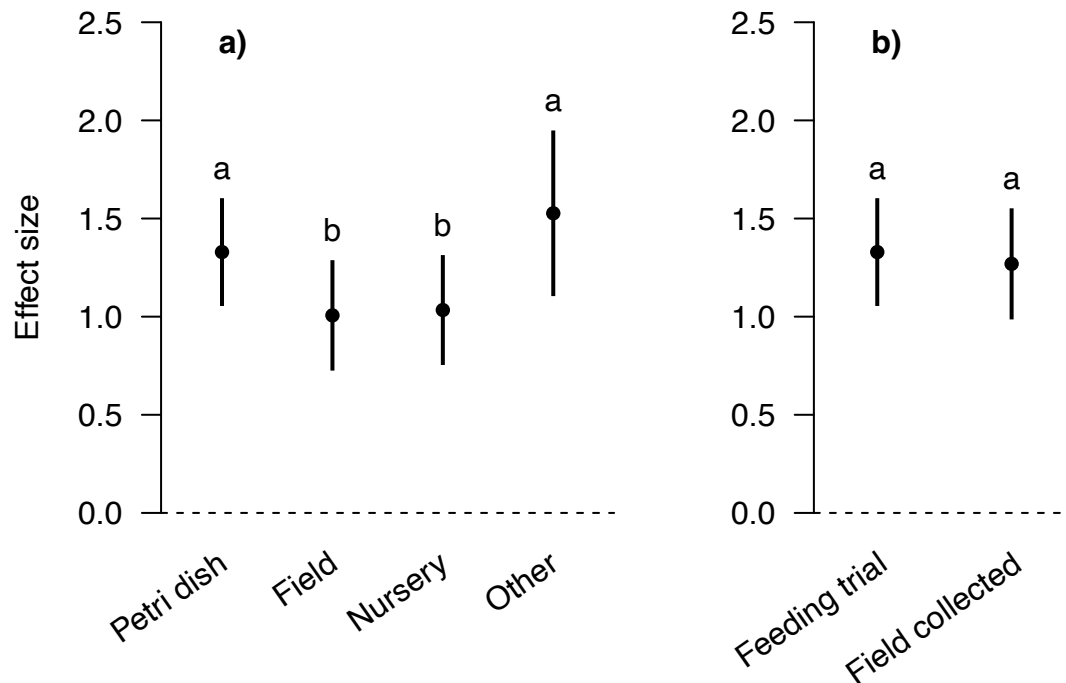


211

212 Figure 1. Study intensity in relation to space, time, and IUCN Red List status of frugivores. a)
213 The number of experiments per country is shown on a color gradient; countries with no studies in
214 the meta-analysis are shown in grey. b) The number of experiments per year has increased since
215 the 1980s. c) The portion of bird and mammal species with fruit in their diet that have been
216 assessed in a gut passage germination experiment (shown in dark grey; species count shown by
217 numbers at bottom of each bar) is small relative to the total number of frugivorous species
218 (shown in light grey; species counts at top of bar) and varies across IUCN Red List status.

219

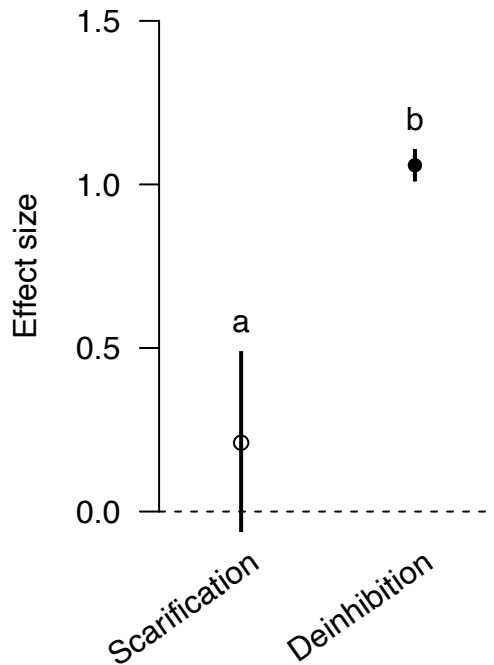
220 Aspects of study design altered the measured impact of gut passage on germination (Fig.
221 2, Table S3). Effect sizes varied with the planting medium in which experimental seeds were
222 sown, with seeds sown in petri dishes and other locations (e.g., tree branches for mistletoe seeds)
223 showing more positive effect sizes than seeds sown in nursery or field soil (Fig. 2a). There was
224 no significant difference in gut passage effects between studies where gut-passed seeds were
225 collected during feeding trials with captive animals and where gut-passed seeds were collected
226 from scat in the field (Fig. 2b).



227

228 Figure 2. Impacts of study methodology on estimated effect sizes. Points represent model-
229 estimated impacts of gut passage on germination (log-odds scale), bars indicate confidence
230 intervals, and letters show statistically significant differences. Estimates were developed using
231 values for the reference predictor combination and varying either (a) the planting medium or (b)
232 whether the test used seeds from a feeding trial or field-collected seeds.
233

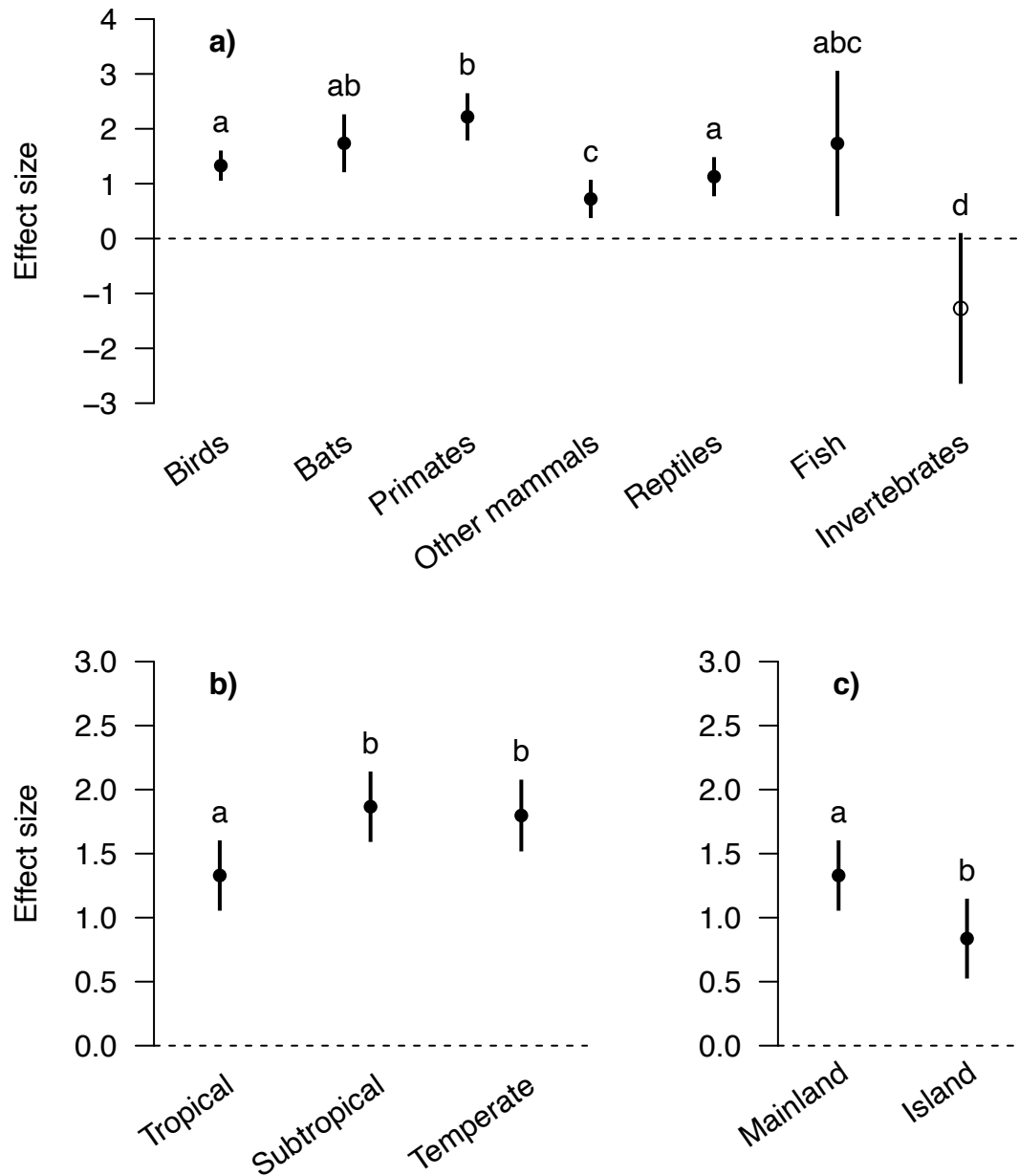
234 We modeled the scarification effect as the difference between the germination of gut-
235 passed seeds versus seeds that were manually de-pulped and the de-inhibition effect as the
236 difference between the germination of seeds in whole fruit versus manually de-pulped seeds. The
237 scarification effect was significantly smaller than the de-inhibition effect (Fig. 3, Table S3).



238
239 Figure 3. Disentangling the scarification and de-inhibition effects. Effects of scarification
240 (caused by gut passage) were smaller than de-inhibition effects caused by pulp removal
241 (independent of scarification via gut passage).
242

243 Other differences in effect sizes were explained by the frugivore group and study location
244 (Fig. 4, Table S3). When considering the comprehensive effect of gut passage (both de-inhibition
245 and scarification effects) on germination, birds exhibited positive effect sizes (Fig. 4a). Primates
246 showed more positive effect sizes than birds, and bat effect sizes were similar to those of both
247 birds and primates. Other mammals exhibited smaller but positive effect sizes. Reptiles had
248 effect sizes similar to those of birds and bats. The positive effect sizes of fish could not be

249 distinguished from the effect sizes of other vertebrate taxa. Invertebrates had negative mean
250 effects exhibiting a marginally significant difference from zero. Latitudinal zone impacted effect
251 sizes, with subtropical and temperate effect sizes similar to each other but both larger than
252 tropical effect sizes (Fig. 4b). Mainland effect sizes were more positive than island effect sizes
253 (Fig. 4c).



254

255 Figure 4. Differences in effect sizes explained by (a) frugivore taxon and (b, c) location.
256

257 **DISCUSSION**

258 Fruit-frugivore interactions are widespread and increasingly affected by global change
259 factors such as overhunting, invasive species, and habitat fragmentation (McConkey *et al.* 2012;
260 Fricke & Svenning 2020; Rogers *et al.* 2021); however, the quantitative effect of these changes
261 in animal communities on plant ecosystem functioning is poorly known. To quantify how
262 frugivores influence plant performance through impacts of gut passage on germination, we
263 performed a meta-analysis of more than 2500 experiments comparing germination of gut-passed
264 seeds to seeds that were not passed by frugivores. Using the first meta-analytic approach to take
265 multiple explanatory variables into account simultaneously, we found that frugivorous
266 vertebrates exhibited on average strong positive effects of gut passage on germination whereas
267 the few experiments with invertebrates showed a negative effect. Since about half of all plant
268 species have fleshy fruit adapted for animal dispersal largely by vertebrates, defaunation of
269 vertebrate frugivores will cascade to affect plant germination and recruitment.

270 Our meta-analysis is the first to separate the effects of de-inhibition through pulp removal
271 from those of scarification from gut passage. Studies typically compare germination of gut-
272 passed seeds to two types of ‘control’ treatments: either seeds that were mechanically cleaned of
273 pulp by researchers or seeds that were left within whole fruits. The former quantifies the
274 scarification effect—caused by physical or chemical changes to seed tissues—whereas the latter
275 quantifies the sum of the scarification effect and de-inhibition effect—caused by removal of pulp
276 and chemical cues within it (Samuels & Levey 2005; Robertson *et al.* 2006). Ultimately,
277 frugivores both remove pulp and scarify the seed, so the most ecologically relevant comparison
278 for testing the impact of frugivores on germination is between gut-passed seeds and seeds

279 remaining within intact fruit. We found that experiments involving comparisons between gut-
280 passed and manually de-pulped seeds had substantially smaller effect sizes than those involving
281 comparisons to whole/intact fruit. Although our meta-analysis showed large and statistically
282 significant total effect sizes across all vertebrate groups, other recent taxon-specific meta-
283 analyses have shown no statistically significant effect for bats (Saldaña-Vázquez *et al.* 2019) or
284 small positive effects for neotropical primates (Fuzessy *et al.* 2016). However, these meta-
285 analyses focused on studies that made comparisons either exclusively to manually de-pulped
286 seeds or primarily to manually de-pulped seeds without accounting for study methods (Saldaña-
287 Vázquez *et al.* 2019)(Fuzessy *et al.* 2016). By separately testing the effect of de-inhibition and
288 scarification, we demonstrate that the total influence of bats and primates on germination is
289 likely positive. These findings amplify previous calls encouraging researchers to include
290 comparisons to seeds within whole fruit in order to characterize the total performance impacts of
291 gut passage and to estimate the impacts of frugivore loss on plant populations (Samuels & Levey
292 2005; Costa-Pereira 2017; Fricke *et al.* 2019).

293 The experimental design of gut passage effect studies may offer an incomplete
294 understanding of the role of fruit-frugivore interactions on germination for multiple reasons, in
295 addition to the failure to test de-inhibition effects discussed above. First, most gut passage
296 studies are conducted on fleshy-fruited plants with frugivores that are considered to be good seed
297 dispersers. Seeds of species lacking fleshy fruit can germinate after ingestion by herbivores
298 (Jaroszewicz *et al.* 2009; Lovas-Kiss *et al.* 2020). Such plant-animal interactions could be
299 considered mutualistic with foliage serving as the reward to the animal partner (Janzen 1984).
300 Likewise, animal species that are commonly understood to be herbivores or granivores often pass
301 seeds of fleshy- and non-fleshy-fruited species intact and contribute to seed dispersal

302 effectiveness (van Leeuwen *et al.* 2020). However, their total role in seed dispersal is poorly
303 known because they are seldom the focus of study. Second, researchers typically report the
304 proportion of gut-passed seeds that germinate, but use the number of intact seeds recovered from
305 feces, rather than the number of seeds ingested, as the denominator. This causes a positive bias
306 on estimated effect sizes when seeds are destroyed during gut passage. We recommend that
307 future studies quantify the number of seeds ingested relative to number of seeds that pass intact
308 and germinate to thoroughly characterize the effect of gut passage on germination. Future
309 research that characterizes the total impacts from ingestion to germination can help generalize
310 knowledge of plant species' dependence on animals, and the importance of diverse animal
311 vectors, for seed dispersal.

312 Our data synthesis spurs recommendations for taxa that should be prioritized for future
313 study. We found that—despite the decades of relatively intensive experimental research to
314 quantify gut passage effects—only a small portion of frugivorous birds and mammals have been
315 tested. The ecological impacts of many vulnerable and endangered frugivore species are poorly
316 known, especially among birds. The same knowledge gap exists for plants: Aslan *et al.* (2013)
317 estimated that 156,900 angiosperm species are vertebrate dispersed yet only 446 plant genera
318 have been tested to determine how frugivore gut passage affects germination. These insights
319 suggest that plant and animal species of conservation concern should be prioritized for future
320 research. On the other hand, we note that measured effect sizes for a given frugivore species can
321 be highly variable across experiments, even when the same plant-animal combination is tested.
322 This suggests that individual studies on plant-animal pairs may only provide an approximate
323 understanding of the functional importance of a given frugivore species. Thus, this meta-analysis
324 and a future examination of the relationship between plant traits and the effects of gut passage

325 may provide sufficient information to predict effects without conducting extensive labor-
326 intensive experiments.

327 Trait-based approaches have potential for predicting gut passage effects on unstudied
328 plant-animal combinations, and developing a quantitative understanding of gut passage effects
329 across frugivores globally. Traits related to animal diet, body mass, and morphology of mouth
330 and gut could predict gut passage effects by different frugivores. Fuzessy et al. (2016) showed
331 substantial variation in effect sizes among neotropical primates explained by their primary diet
332 and gut complexity. Functional traits of plants such as seed size, flesh-to-seed ratio, and shade
333 tolerance may predict the benefits that plants receive from animal gut passage, and plant
334 functional groups may be useful for predicting gut passage effects (Aslan *et al.* 2019; Rogers *et*
335 *al.* 2021). A trait-based analysis of fruit consumed by fish found that fish are more likely to
336 disperse fleshy-fruited species than dry-fruited species, but did not find a relationship between
337 fruit traits related to color, shape, or size and the probability of dispersal (Correa *et al.* 2015).
338 However, gut passage by fish is severely understudied, and the methods have been inconsistent
339 (Costa-Pereira 2017), therefore limited conclusions can be drawn without additional data.

340 We found that plant or animal species included in the Global Invasive Species Database,
341 indicating they are considered invasive in at least a portion of their range, do not differ in their
342 gut passage effects from plants or animals that lack invasive populations. A priori, one could
343 imagine multiple possible relationships between invasiveness and gut passage effects for plants.
344 On one hand, plants with invasive populations may offer more flesh rewards to encourage seed
345 dispersal, establishment, and expansion, and also be more dependent upon these dispersers for
346 germination (Richardson et al. 2000). On the other hand, reduced dependence on mutualistic
347 interactions may contribute to a plant's ability to invade. Our analysis did not support either

348 alternative, suggesting dependence on gut passage for germination is uncoupled from their
349 propensity to be invasive. Invasive frugivore species exhibited similar impacts of gut passage on
350 germination as non-invasive frugivores, suggesting that invasive frugivores also provide a
351 similar quality of dispersal on average as their native counterparts (Vizentin-Bugoni *et al.* 2019).
352 Overall, this suggests that traits may be more important than species origin in predicting gut
353 passage effects.

354 The effects of frugivores on gut passage were more positive in temperate and subtropical
355 locations than in the tropics, and on mainland systems than on islands. We caution that these
356 effect sizes could be confounded by the study species targeted by researchers in these areas, yet
357 find the patterns intriguing nonetheless. The increased benefit of gut passage in temperate and
358 subtropical areas is surprising given that vertebrate seed dispersal is more common in tropical
359 areas (Rogers *et al.* 2021). It is possible that the smaller number of temperate studies are more
360 biased towards species most likely to benefit from dispersal. Alternatively, there may be limited
361 successful life history strategies for fleshy-fruited species in temperate and subtropical areas
362 compared to tropical areas. For example, tropical species may include many large-seeded plants
363 with reduced dependence on frugivores for gut passage. Species on oceanic islands may be
364 expected to exhibit smaller effect sizes because species with fewer dependencies on species
365 interactions for reproduction and survival may be more likely to establish or persist in species-
366 poor systems.

367 Because our meta-analysis shows that the total benefit of gut passage was large, the loss
368 of these benefits may pose substantial demographic constraints for plant reproduction in
369 ecosystems facing frugivore declines. For the average fleshy-fruited plant species studied, the
370 mean effect size corresponds to more than a 60% reduction in germination probability for seeds

371 not ingested by frugivores. We suggest that the loss of benefits of gut passage are
372 underappreciated relative to other mechanisms that could negatively affect plant populations
373 experiencing disperser loss. The loss of benefits associated with escape from conspecific
374 negative distance- or density-dependent mortality (CNDD) is often highlighted as the primary
375 negative consequence of seed disperser loss on plant populations. Yet a meta-analysis of
376 experiments measuring the strength of CNDD (Comita et al. 2010) showed mean effect sizes,
377 which were unrelated to study duration, corresponding to a roughly 25% reduction in survival for
378 undispersed individuals. Although the effects of CNDD accrue over life stages, CNDD primarily
379 impacts plant survival at the earliest life stages (Green & Harms 2017). The loss of gut passage
380 benefits appears to be sizable relative to the loss of benefits associated with escape from CNDD.

381 Our meta-analysis brings renewed attention to a widespread yet under-appreciated
382 ecological interaction. The simple act of removing flesh from a seed likely provides significant
383 benefits for over half of the world's plants (Aslan *et al.* 2013; Rogers *et al.* 2021). Many
384 populations of avian, mammalian, reptilian, and fish seed dispersers are in decline in systems
385 around the world; fewer individual frugivores, even in common species, means more fruits are
386 left unconsumed and thus have a reduced chance of germination. When these condition-related
387 benefits are combined with movement-related benefits of dispersal, the impacts on plant
388 populations and communities are likely to be significant (Rogers *et al.* 2021). However, our
389 finding that the primary benefits of frugivory come from de-inhibition rather than scarification,
390 provides some optimism. First, most species do not require special treatment in the gut by a
391 particular frugivore to germinate. Rather, any frugivore that consumes a given species and passes
392 the seeds intact may confer some level of benefit, which increases the potential for compensation
393 by remaining frugivores, even non-native species. Second, in the short-term and on an extremely

394 limited spatial scale, humans may be able to maintain some plant species of conservation
395 concern through fruit collection, manual de-pulping, and seed sowing. However, restoring fruit-
396 frugivore mutualisms through rewilding will be necessary to restore this ecological function at
397 larger taxonomic and geographic scales.
398

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