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ORIGINAL ARTICLE

Exploring rodent-seed interaction experimental designs: a comparative analysis of mixed versus single release of multiple seed species

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Abstract

Rodents play a crucial role in seed dispersal through scatter-hoarding behavior. In natural ecosystems, rodents demonstrate selective foraging behavior among co-occurring heterospecific and conspecific seeds. Current studies on rodent-seed interactions primarily employ two contrasting experimental approaches: releasing seeds of each plant species separately and releasing mixed seeds of multiple species, representing fundamentally distinct paradigms that engage different dimensions of optimal foraging theory. Specifically, single-species releases primarily isolate optimal diet selection processes based on energy-to-handling-time ratios, while mixed-species releases incorporate more complex ecological dynamics including patch-use strategies and trait-mediated neighbor effects. This fundamental divergence in experimental design has profound implications for research outcomes, as each approach captures different aspects of rodent foraging decision-making under varying ecological contexts. Surprisingly, most current studies overlook the potential impact of these two experimental methods on research outcomes. Here, we selected three tree species in a subtropical forest in southwest of China and compared the differences in the seed predation and dispersal by rodents of each species between two seed releasing methods. Our study revealed that methodological differences in seed release significantly affect seed predation and dispersal by rodents. These methodological effects are not only manifested in the seed fates of specific species and the effects of seed size, but also in the preferential selection of different species of seeds by rodents. Therefore, we recommend that the choice between mixed releasing of multiple species and individual releasing of each species in actual research should be comprehensively considered based on the research objectives and experimental environmental context.

Significance statement

Our study highlights the critical impact of experimental design on understanding rodent-seed interactions in natural ecosystems. By comparing two common seed releasing methods, separately releasing seeds of each plant species versus mixed releasing of multiple species, we found significant differences in seed predation and dispersal patterns. These differences are not only manifested in the fates of specific seed species and the effects of seed size, but also in the preferential selection of different seed species by rodents. Our results underscore the importance of considering the neighbor effect when studying rodent-seed interactions and emphasize the need for researchers to carefully choose experimental methods based on their specific research objectives and environmental contexts. This has broad implications for other plant-animal interaction studies, including herbivory and pollination systems.

Keywords Foraging preference · Interspecific comparison · Plant-animal interaction · Seed predation · Seed dispersal

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Kun He and Jinyu Zhang contributed equally to this work and should be considered co-first authors.

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Introduction

Rodents play a crucial role in seed dispersal for a variety of plants through their scatter-hoarding behavior (Vander Wall 2010; Lichti et al. 2017). From the perspective of optimal foraging theory (OFT), this behavior reflects trade-offs between immediate energy gain (seed consumption) and future benefits (caching for later use), shaped by factors such as seed handling time, energy content, and predation risk (Kotler et al. 1999; van der Merwe et al. 2014). In various forest ecosystems, multiple plant species coexist, and their fruiting periods sometimes partially overlap (Garzon-Lopez et al. 2015; Yang et al. 2020). Consequently, during seed maturation, rodents encounter seeds either in single-species aggregations or multi-species mixtures, engaging fundamentally different foraging decisions. Here, rodents face a classic diet selection problem (Stephens and Krebs 1986): they must prioritize seeds based on energy-to-handling-time ratios (e/h), while also evaluating patch depletion dynamics and nutrient complementarity when seeds co-occur (Brown and Mitchell 1989; Vincent et al. 1996).

Current studies have conducted numerous in-depth explorations regarding the selection preferences of rodents for different seeds during their scatter-hoarding behavior (Vander Wall 2010; Lichti et al. 2017). These explorations cover various aspects, such as the differences in proportion of seeds being removed and cached, and dispersal distances among different species of seeds, as well as how seed traits like size influence seed predation and dispersal, with comparisons made across different plant species (Lichti et al. 2014; Chen et al. 2022; Xiao et al. 2022). However, fewer studies have explicitly linked these behaviors to OFT predictions. For example, optimal diet theory predicts that larger seeds, typically with higher e/h values, should be preferentially harvested and cached (Vickery 1984), while patch use theory suggests that mixed-seed patches may alter foraging efficiency due to diminishing returns or short-term apparent competition (Holt and Kotler 1987). When designing experiments to investigate the interactions between rodents and seeds, the current mainstream experimental designs primarily focus on two distinct seed releasing methods to compare rodents' selection preferences and the differences in seed predation and dispersal. The first method involves releasing seeds of each plant species separately at a single seed release point (Vander Wall 2003; Moore et al. 2007; Yang et al. 2012, 2019); the second method involves mixing seeds of multiple species at the same release point (Xiao et al. 2005; Chang et al. 2012; Zhang et al. 2016; Feng et al. 2021). From a theoretical standpoint, these methods represent different foraging scenarios: single-species releases primarily reflect the optimal diet theory when seeds are easily accessible (e.g., cafeteria-style presentation), where seeds are ranked by their energy-to-handling-time ratio (e/h), but can incorporate patch use dynamics if seeds require search effort (e.g., mixed within substrate). Similarly, mixed-species releases engage both diet selection and patch use processes, though cafeteria-style arrangements may emphasize pure diet selection by eliminating search costs, while clustered presentations enhance patch depletion effects. Furthermore, mixed-species releases may also trigger potential neighbor interference (Holt and Kotler 1987), and alter foraging decisions (Brown and Mitchell 1989). Additionally, incidental predation during inter-patch movements (Schmidt et al. 2001) may differentially affect seed fates between the two methods. These behavioral differences suggest that methodological choices could fundamentally influence research outcomes.

Here, we would further emphasize that trait-mediated neighbor effects (e.g., seed size or toxicity contrasts within multi-species assemblages) represent a critical but understudied dimension of optimal foraging theory in seed dispersal systems. When seeds of multiple species co-occur, rodents may adjust their foraging strategies due to trait contrasts between seeds (e.g., size, toxicity) or nutrient complementarity (Garb et al. 2000; Schmidt 2000; Liu et al. 2023). For instance, a high-value seed (high e/h) surrounded by low-value neighbors may be harvested more intensively due to reduced search costs, whereas its caching fate could depend on trade-offs between present and future value (Kotler et al. 1999). Additionally, the presence of high-value seeds in a patch may increase the harvest of neighboring low-value seeds through associational susceptibility (Barbosa et al. 2009). Surprisingly, no study has systematically evaluated how these methodological choices, specifically single species versus mixed species releases, affect inferences about rodent-seed interactions. This gap is problematic because the two methods may yield divergent results even for the same research question, potentially biasing our understanding of seed fate mechanisms.

In this study, we selected three tree species within a subtropical evergreen broad-leaved forest in Ailao Mountains of southwest of China, all of which primarily depend on rodents for seed dispersal. By comparing the differences in seed fate (in terms of predation and dispersal by scatter-hoarding rodents) of each species between two seed-releasing methods (i.e., releasing each species' seeds alone versus releasing all the species' seeds mixed together), we aim to explore the following three questions: (1) Compared to the mixed seed-releasing method, do differences exist in the seed fate (including harvest rate, proportion of seeds harvested, eaten in situ, removed and cached, and dispersal distance) of each species when released alone? (2) Compared to the mixed seed-releasing method, do differences exist in the intensity and direction of the influence of seed size on the fate of each species' seeds when they are released alone? (3) Is there a difference in rodents' preference for the seeds of the three plant species when using the two seed-releasing methods?

Materials and methods

Study site

In this study, we selected a subtropical montane wet evergreen broad-leaved forest located within the Ailao Mountains National Nature Reserve in Yunnan Province (24°32′N, 101°01′E) as our experimental site. The elevation is approximately 2500 m above sea level, with an annual average temperature of 11.3 °C and an annual average rainfall of 1930 mm, which is concentrated mainly from May to October. The forest is predominantly composed of tree species belonging to families such as Fagaceae, Lauraceae, Theaceae, and Magnoliaceae. Dominant species in the tree layer, such as *Lithocarpus hancei* and *Lithocarpus xylocarpus*, primarily rely on the scatter-hoarding behavior of rodents for their seed dispersal (Xiao and Zhang 2012; Lang and Wang 2016). The main rodent species within the forest include *Niviventer confucianus*, *Dremomys rufigenis*, *Niviventer andersoni*, and *Apodemus draco* (Xiao and Zhang 2012; Lang and Wang 2016; Liu et al. 2023).

Study species and experimental design

Three tree species within the forest were selected, namely *L. hancei* (mean seed mass ± SD = 1.33 ± 0.89 g), *L. xylocarpus* (2.65 ± 1.23 g), and *Lithocarpus pachyphyllus* (2.27 ± 0.69 g), all of which primarily rely on the scatter-hoarding behavior of rodents for their seed dispersal. The seed maturation periods of the selected tree species are concentrated between August and November. Seed collection was conducted during this period, and the collected mature and intact seeds were stored in a refrigerator (at 4 °C) for subsequent seed release experiments.

In late November 2023 (during the final stage of seed maturation), a total of 16 seed release points (spaced 30 m apart) were randomly set up within the forest for the seed release experiments. Previous experiments have shown that the rodents within the selected forest typically transport seeds within a distance of 20 m (Lang and Wang 2016; Chen et al. 2022; Liu et al. 2023), hence, the 30-meter spacing between release points was used in this study. For the single species release method, 60 seeds of the same species were released at each release point (total seed mass per point: *L. hancei* = 82.91 ± 10.75 g, *L. xylocarpus* = 153.08 ± 134.45 g, *L. pachyphyllus* = 134.45 ± 5.75 g), while for the mixed-species release method, each point contained 20 seeds of each of species (60 seeds total; mean total mass = 130.75 ± 16.27 g). Field seed rain surveys confirmed that the three focal species co-occurred with overlapping canopies, creating local seed assemblages ranging from single-species patches to mixed-species clusters. Seed rain data showed high spatiotemporal variability: densities fluctuated from nearly 0 seeds/m² (non-mast years) to > 100 seeds/m² (mast years), with mixed-species patches frequently observed under overlapping crowns. While natural seed densities are highly heterogeneous, our experimental design captures realistic density scenarios without exceeding natural extremes. There were four replicates for each release method (i.e., 4 single-species release points × 3 species + 4 mixed-species release points = 16 release points). Prior to seed release, each seed was weighed individually and marked using the tagging method. The tagging method involved drilling a hole (0.5 mm in diameter) in each seed and attaching a plastic tag (2.5 cm × 0.7 cm) using a thin iron wire (0.2 mm in diameter) approximately 15 cm long. Each seed was specifically numbered on the tag for easy identification during the subsequent fate tracking process. The drilling was done at the base of the seed to minimize its impact; preliminary tests showed that drilled seeds could germinate normally. During the experiment, rodents would disperse and cache some seeds in the soil or litter, usually leaving the plastic tags on the surface, which facilitated our tracking of their fate. The fate of all the seeds was checked after releasing them on days 1, 2, 3, 4, 6, 8, 12, 16, and 24. A complete systematic search was conducted within a 30-m radius of each release point, supplemented by haphazard searches beyond this area. Typically, a three-researcher team performed these searches, with duration (30–60 min per point) adjusted according to the number of seeds removed and cached by rodents. Our unpublished infrared camera data confirmed that rodents typically transported and cached seeds individually. The seed fates included remaining intact at the original release points, eaten in situ, removed and scatter-hoarded by rodents, as well as the removal distance. Detailed experimental methods can be referred to in (Wang and Chen 2009; Wang and Ives 2017; Liu et al. 2023). It was not possible to record data blind because our study involved focal animals in the field.

Data analyses

All statistical analyses were performed using R software, version 4.4.1 (R Core Team 2024). Firstly, we tested the effect of seed releasing methods on seed fate for each species. A Cox proportional hazards model (function "coxme" in package "coxme") was used to test the differences of seed survival time between the two seed-releasing methods (i.e., mixed-releasing versus single-releasing) (Therneau 2022). A generalized linear mixed model (GLMM) with binomial error distribution and logit link function was used to analyze the effects of seed-releasing method on the probability of seeds being harvested versus ignored, the probability of seeds removed versus eaten in situ, and the probability of removed seeds being eaten versus cached by rodents. A linear mixed model (LMM) with Gaussian error distribution was conducted to analyze the differences in dispersal distance (log-transformed) between seed-releasing methods. In all the models, seed releasing methods were considered as fixed effects, with seed releasing point as a random effect.

Secondly, we used GLMM to analyze the effect of seed mass on the probability of seeds being harvested versus ignored, the probability of seeds removed versus eaten in situ, and the probability of removed seeds being eaten versus cached by rodents for each species using mixed-releasing method and single-releasing method, respectively. A LMM was used to analyze the effect of seed mass on dispersal distance (log-transformed) for each species. In all the models, seed size was considered as fixed effects, with seed releasing point as a random effect.

Furthermore, we tested the foraging preference among the three plant species of seeds in each seed-releasing method separately. The Cox proportional hazards model was used to test the difference of harvest time by rodents among species. The GLMM was used to compare the probability of seeds being harvested, removed, and cached by rodents. Additionally, the LMM was performed to test the difference in dispersal distance among species. The statistical significance for both the GLMMs and LMMs was estimated using the Wald χ^2 type II and F test (function “Anova” in package “car”), respectively. Post-hoc pairwise comparisons were conducted using the Bonferroni method.

Results

Seed fate of each species

Seeds of *L. xylocarpus* were harvested more quickly when released together with other species of seeds (mean survival time was 10.12 ± 0.15 days, mean ± SD), compared with when released alone (14.73 ± 0.09 days, $z = -2.610$, $p = 0.009$; Fig. 1b), while *L. hancei* and *L. pachyphyllus* showed no significant differences in survival time between the two seed releasing methods. Seeds were more likely to be harvested in the mixed-species release treatment than in the single-species release treatment for *L. xylocarpus* (63.75% vs. 42.92%, $z = -2.613$, $p = 0.009$; Fig. 1e), but not for the other two species. No statistically significant differences were observed in the proportion of seeds being removed or cached between the two seed releasing methods for all species (all P values > 0.09, Fig. 1g-l). For the dispersal distance, seeds of *L. xylocarpus* were dispersed farther in the mixed-species release treatment than in the single-species release treatment (3.85 vs. 1.54 m, $t = -2.793$, $p = 0.034$; Fig. 1n) and so were seeds of *L. pachyphyllus* (5.97 vs. 3.35 m, $t = -2.382$, $p = 0.043$; Fig. 1o), but not for *L. hancei* (3.26 vs. 3.09 m, $t = 0.768$, $p = 0.479$; Fig. 1m).

Seed size effects on seed fate

For *L. hancei*, a significant positive correlation between seed size and the probability of seeds being harvested was observed in the single-species release treatment, but this correlation was not significant in the mixed-species release treatment (Fig. 2a). In contrast, for *L. xylocarpus*, a significant positive effect of seed size on the probability of seeds being harvested was detected in both seed release treatments, and the trend line was steeper in the mixed-species release treatment compared to the single-species release treatment (Fig. 2b). For *L. hancei*, a significant positive effect of seed size on the probability of seeds being removed rather than eaten in situ was detected in both seed release treatments (Fig. 2d); while for the other two species, no correlations between seed size and removal probability were detected in either seed release treatment (Fig. 2e, f). For all the three species, seed size showed no effects on the probability of seeds being cached in either seed release treatment (Fig. 2d-i). The effects of seed size on dispersal distance differed between seed release treatments for all the species. For *L. hancei* and *L. pachyphyllus*, a positive correlation was detected between seed size and dispersal distance in the single species release treatment but not in the mixed-species release treatment; while for *L. xylocarpus*, the positive seed size effect on dispersal distance was detected in the mixed-species release treatment but not in the single species release treatment.

Interspecific comparisons

Significant interspecific differences were detected in most of the scatter-hoarding processes in both seed releasing treatments (Fig. 3). *L. pachyphyllus* seeds were harvested the most quickly in both seed releasing treatments; however, in the single-releasing treatment, *L. hancei* seeds were harvested significantly more quickly than *L. xylocarpus* seeds, whereas this difference was not observed in the mixed-releasing treatment (Fig. 3a, b). Specifically, when considering seeds at release points, *L. xylocarpus* seeds were less likely to be harvested than *L. hancei*, and *L. pachyphyllus* (with percentages of 63.75% vs. 85% and 100%, and 42.92% vs. 88.75% and 100% in both treatments, respectively)

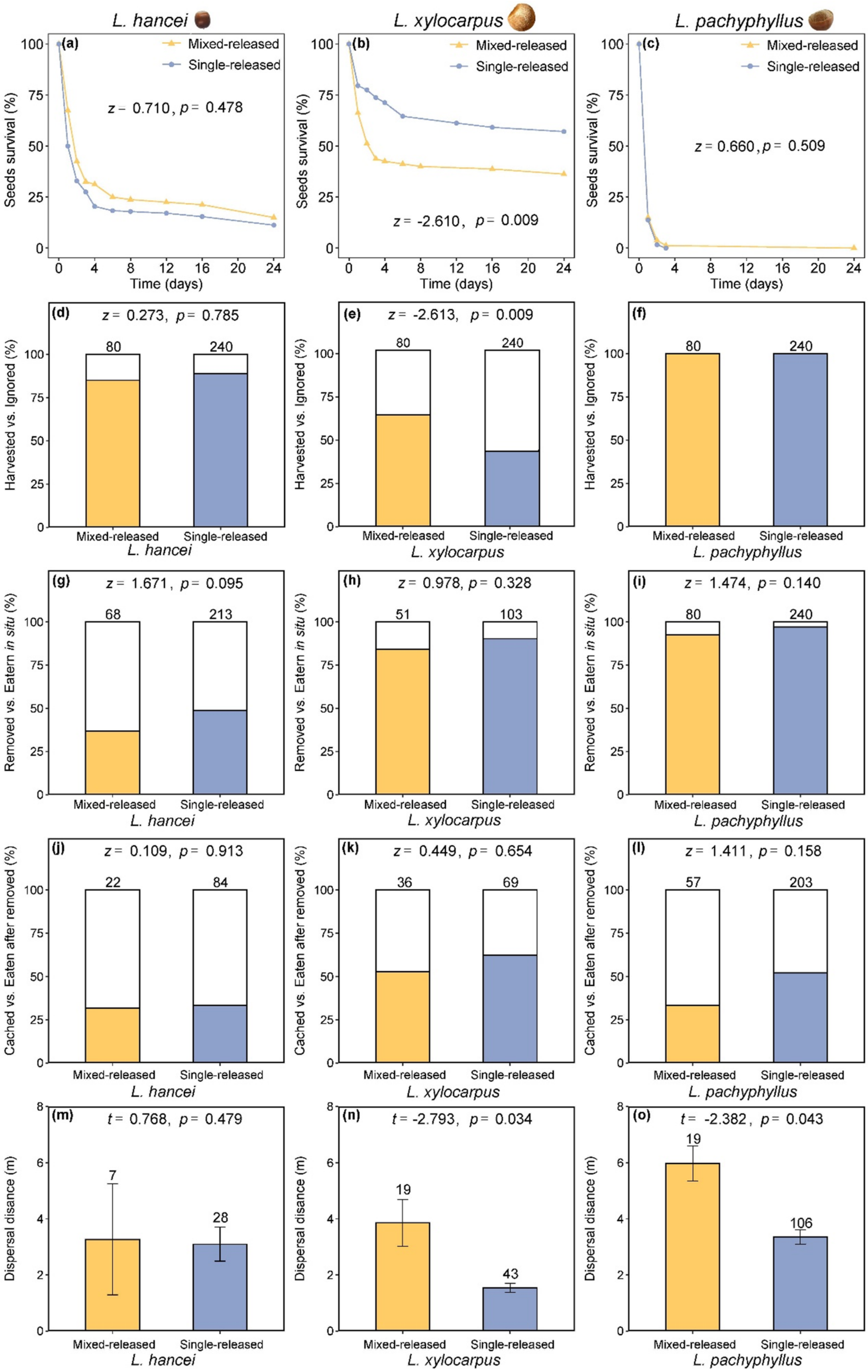
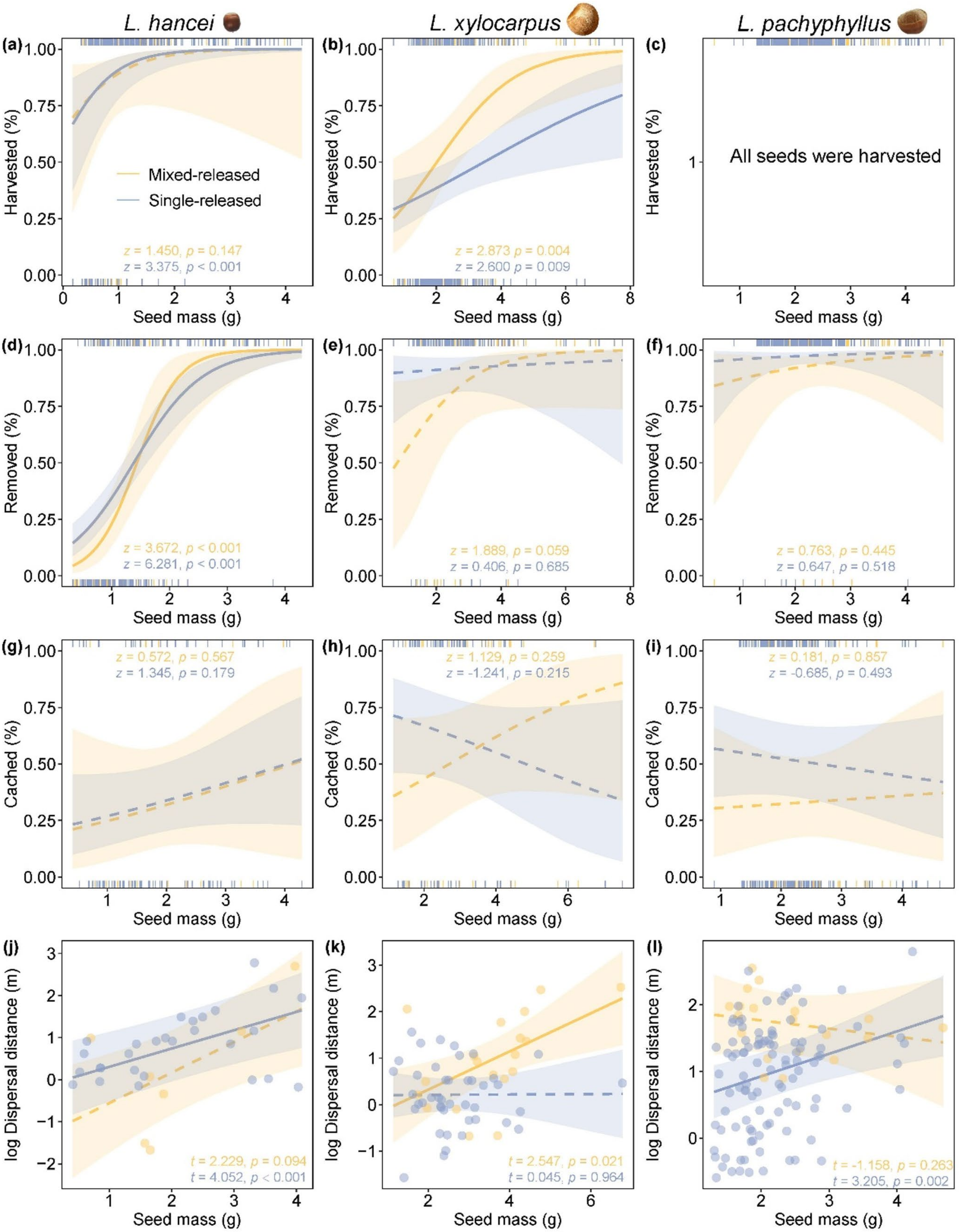


Fig. 1 Comparison of seed fate for *L. hancei*, *L. xylocarpus*, and *L. pachyphyllus* between the two seed-releasing treatments (mixed-releasing versus single-releasing): (**a**-**c**) seed survival time, (**d**-**f**) the probability of seeds being harvested versus ignored, (**g**-**i**) the probability of seeds removed versus eaten in situ, (**j**-**l**) the probability of seeds being eaten versus cached after being removed by rodents, and (**m**-**o**) dispersal distance (mean ± SE). Numbers in the panels are the sample sizes, i.e., the total number of seeds analysed. Detailed model statistics please see Table S1 in the Supporting materials

(Fig. 3c, d). Among harvested seeds, in both treatments, *L. hancei* seeds were less likely to be removed compared to *L. xylocarpus* and *L. pachyphyllus* seeds (36.76% vs. 84.31% and 92.50%, and 48.83% vs. 90.29% and 97.08%, respectively); however, a significant difference in the seed removal probability was observed between *L. xylocarpus* and *L. pachyphyllus* seeds only in the single-releasing treatment, with no significant difference detected in the mixed-releasing treatment (Fig. 3e, f). While for the probability of seeds being cached after removal, no differences were detected


L. hancei
L. xylocarpus
L. pachyphyllus
(a)
(b)
(c)
Harvested (%)
Mixed-released
Single-released
z = 1.450, p = 0.147
z = 3.375, p < 0.001
z = 2.873 p = 0.004
z = 2.600 p = 0.009
All seeds were harvested
Seed mass (g)
(d)
(e)
(f)
Removed (%)
z = 3.672, p < 0.001
z = 6.281, p < 0.001
z = 1.889, p = 0.059
z = 0.406, p = 0.685
z = 0.763, p = 0.445
z = 0.647, p = 0.518
(g)
(h)
(i)
Cached (%)
z = 0.572, p = 0.567
z = 1.345, p = 0.179
z = 1.129, p = 0.259
z = -1.241, p = 0.215
z = 0.181, p = 0.857
z = -0.685, p = 0.493
(j)
(k)
(l)
log Dispersal distance (m)
t = 2.229, p = 0.094
t = 4.052, p < 0.001
t = 2.547, p = 0.021
t = 0.045, p = 0.964
t = -1.158, p = 0.263
t = 3.205, p = 0.002

◀**Fig. 2** Comparisons of the effects of seed mass on seed fate for each species between the two seed-releasing treatments (mixed-releasing in yellow versus single-releasing in blue): (**a**-**c**) the probability of seeds being harvested, (**d**-**f**) the probability of seeds removed, (**g**-**i**) the probability of seeds being cached after removal, and (**j**-**l**) dispersal distance (log-transformed). Shaded areas indicate 95% confidence intervals. Detailed model statistics please see Table S2

among species in either treatment (Fig. 3g, h). Regarding dispersal distance, seeds of *L. pachyphyllus* (5.97 ± 2.74 m, *n* = 19) were dispersed significantly farther than both *L. hancei* (3.26 ± 5.23 m, *n* = 7) and *L. xylocarpus* (3.85 ± 3.63 m, *n* = 19) in the mixed-releasing treatment (Fig. 3i). In contrast, in the single-releasing treatment, there was no significant difference in dispersal distance among the three species (Fig. 3j).

Discussion

Our study showed that selecting different seed releasing methods would result in significant differences in seed predation and dispersal by rodents. These differences are not only evident in the seed fates of specific species and the effects of seed size on seed fates, but also in the preferential selection of different species of seeds by rodents. These contrasting outcomes likely stem from fundamentally different foraging decision-making processes engaged by each experimental approach, as predicted by optimal foraging theory.

Central to these differing foraging decisions is the neighbor effect, a phenomenon that alters rodent behavior based on interspecific seed associations. The neighbor effect exhibits species specificity, meaning that some species are significantly influenced by the neighbor effect, while others may be less affected (Wang 2020; Liu et al. 2023). This, to a certain extent, explains our research findings: why the difference in seed fate between the two seed releasing methods is relatively small for *L. hancei* but larger for *L. xylocarpus*. A crucial factor contributing to this interspecific variation in the neighbor effect is the trait contrast between neighboring seeds and target seeds (Wang 2020; Xiao et al. 2022). For example, Liu et al. (2023) showed that the larger the size of the target seeds compared to the neighboring seeds, the higher the probability of the target seeds being preferred by rodents. In our study, *L. xylocarpus* seeds were much larger than those of the other two species, thus, when neighboring smaller seeds, *L. xylocarpus* seeds were more prominent and therefore were more likely to be harvested and dispersed farther, compared to when they were released alone. This aligns with optimal diet theory (Holt and Kotler 1987), which predicts that larger seeds (like those of *L. xylocarpus*) with higher e/h ratios will be preferentially selected by rodents. However, the probability of *L. xylocarpus* seeds being removed and cached was similar in both seed release treatments, indicating that neighbor effects did not alter rodents' assessment of trade-offs between immediate energy gain (consumption) and future benefits (caching) for this species.

Our results demonstrated that neighbor effects varied among scatter-hoarding processes (e.g., harvested vs. ignored, and removed vs. eaten in situ, etc.) and plant species, and that the size difference between neighboring seeds and target seeds alone could not fully account for our findings. This complexity likely arises because other seed traits - particularly nutrient and tannin content - also significantly influence neighbor effect, with their impacts varying across different scatter-hoarding processes (Yi and Wang 2015; Bogdziewicz et al. 2019; Yang et al. 2020; Xiao et al. 2022; Liu et al. 2023). Notably, while within-species selection followed optimal diet theory (larger seeds preferred due to higher e/h ratios), between-species patterns revealed no consistent preference for the largest-seeded species (*L. xylocarpus*) over smaller congeners (Figs. 2a-b and 3c-d). This suggests that interspecific selection incorporates additional trade-offs, such as toxin avoidance or handling constraints, which may override pure size-based energy optimization. Furthermore, seed availability in the forest may exhibit heterogeneity, as seed production typically demonstrates considerable variation among individual trees (Wang and Ives 2017). This heterogeneous seed availability could potentially impact our findings. Nevertheless, our results consistently demonstrated significant methodological differences in rodent-mediated seed predation and dispersal patterns. These context-dependent outcomes can be understood through multiple optimal foraging theory frameworks: (1) the trade-off between immediate consumption and future caching benefits (Kotler et al. 1999); (2) the balance between energy acquisition (seed size/nutrients) and handling costs (toxin) (Stephens and Krebs 1986); (3) patch use dynamics featuring diminishing returns and short-term apparent competition (Holt and Kotler 1987); and (4) incidental predation during inter-patch movements (Samuni-Blank et al. 2013). The interplay of these mechanisms explains the divergent seed fates between single-species and mixed-species experimental designs. Future studies could further explore how these theoretical frameworks interact under different ecological contexts to better understand their relative contributions.

These two seed releasing methods represent fundamentally distinct experimental approaches that engage different aspects of optimal foraging theory, where single-species releases primarily reflect optimal diet theory (enabling clear evaluation of seed traits through energy-to-handling-time ratios) whereas mixed-species releases incorporate more complex patch-use dynamics and neighbor effects (Holt and Kotler 1987). Critically, the choice between methods should

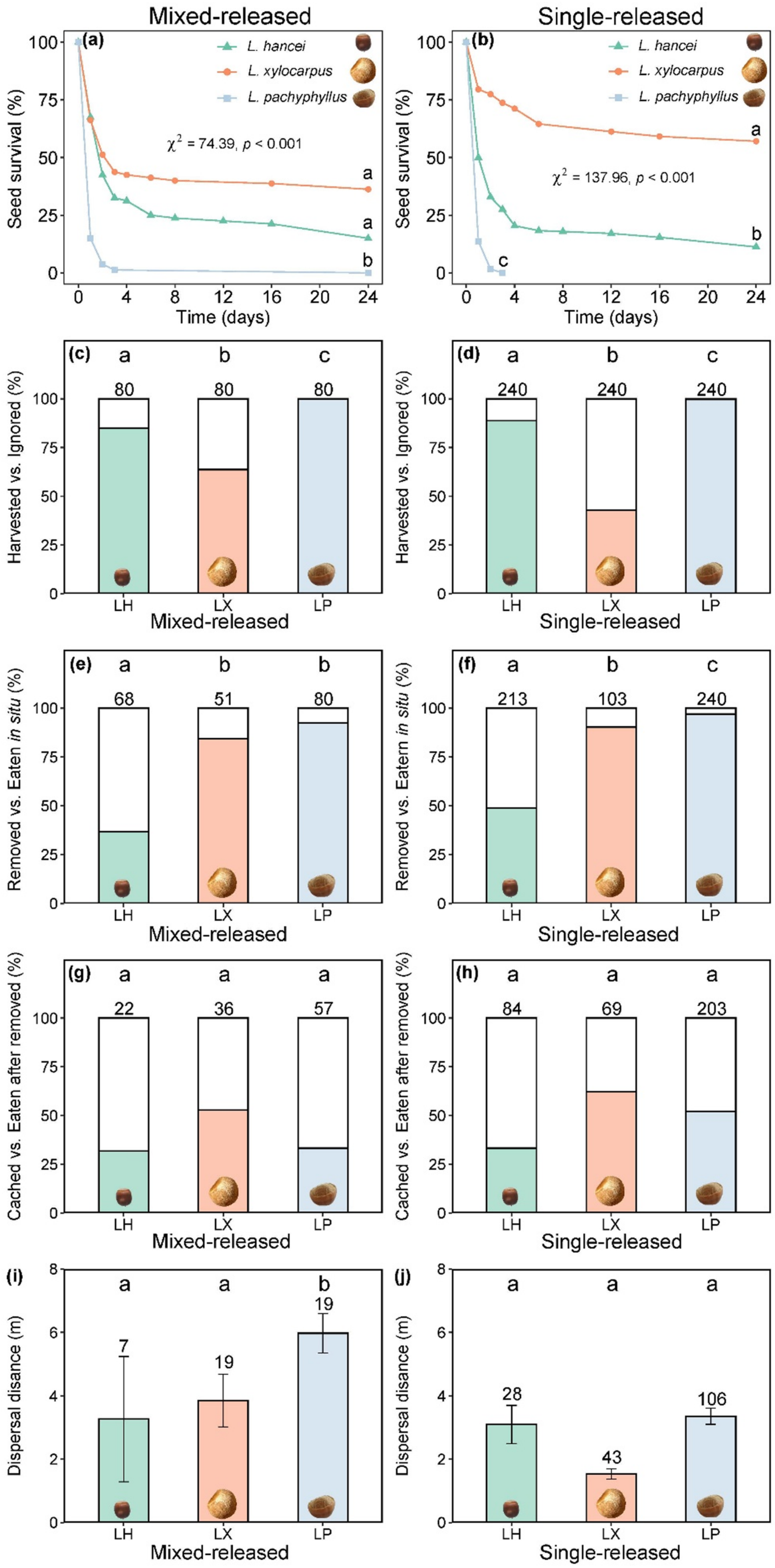


Fig. 3 Comparison of the preferential selection of different species of seeds by rodents between mixed-releasing treatment and single-releasing treatment: (**a**-**b**) seed survival time, (**c**-**d**) the probability of seeds being harvested versus ignored, (**e**-**f**) the probability of seeds removed versus eaten in situ, (**g**-**h**) the probability of seeds being eaten versus cached after being removed by rodents, and (**i**-**j**) dispersal distance (mean ± SE). LH represents *L. hancei*, LX represents *L. xylocarpus*, and LP represents *L. pachyphyllus*. Numbers in the panels are the sample sizes, i.e., the total number of seeds analysed. Different letters indicate significant differences among variables ($P < 0.05$). Detailed model statistics please see Table S3, S4

be guided by specific research objectives. For instance, if the aim is to explore the impact of seed traits on the feeding and dispersal preferences of rodents and how this impact differs among different plant species, it may be better to release each species of seed individually because mixed releasing may introduce neighbor effects. On the other hand, if the goal is to compare the preferences of rodents for seeds of several sympatric species, especially when these species have overlapping fruiting periods and intertwining canopies, then mixed releasing is more aligned with natural conditions. Notably, our results demonstrate that these methodological differences can profoundly influence conclusions about rodent-seed interactions. The complementary use of both approaches provides a powerful framework to deconstruct foraging behavior: single-species releases isolate diet selection based on e/h ratios, while mixed-species releases additionally capture patch use dynamics and neighbor effects on depletable resources. For a comprehensive understanding, we strongly recommend adopting this dual-method approach when feasible, implementing both single species releases (to focus on trait-specific effects on seed fate) alongside mixed-species releases (to examine combined influences of intrinsic traits and neighbor effects). This comparative design enables researchers to partition and separately analyze these distinct components of foraging decisions, a strategy particularly valuable for understanding complex scatter-hoarding systems. A comparative analysis of results from these two methods will provide a more comprehensive understanding of the complex trade-offs governing rodent scatter-hoarding behavior and its consequences for plant recruitment and species coexistence.

The neighbor effect not only exists in the interactions between rodents and plant seeds but also widely prevails in other animal-plant interactions, including herbivory systems and pollination systems (Feldman et al. 2004; Alm Bergvall et al. 2006; dos Santos et al. 2018; Underwood et al. 2020). Therefore, similar discrepancies in results due to differences in experimental methods are likely to exist in such studies as well. Hence, we recommend that attention be paid to the selection of experimental methods in these studies. The various interactions established between animals and plants constitute one of the critical components of ecosystems, thereby attracting substantial research attention (Li et al. 2020; Proesmans et al. 2021; Wetzel et al. 2023). However, when investigating the same scientific question, different studies often yield inconsistent results; consequently, many studies have attempted to integrate these disparate findings to identify a general pattern and analyze the underlying causes of these varying results (Morales and Traveset 2009; Fuzessy et al. 2016; Dylewski et al. 2020). Yet, current studies have rarely focused on the issue of experimental methods, such as the difference mentioned in this study between mixed releasing of multiple species of seed and single-species seed releasing. Therefore, we propose that future meta-analyses should systematically account for methodological differences through the lens of optimal foraging theory, as this approach will provide both a conceptual framework for understanding variation across studies and practical guidance for designing more comparable experiments in animal-plant interaction research.

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Author contributions BW conceived and designed the experiments. KH performed the experiments. KH, JZ, and BW analyzed the data and wrote first draft of the manuscript.

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Data availability All the datasets generated during the current study are included in this article as supplementary material.

Declarations

Ethical approval Not applicable.

Conflict of interest The authors declare that they have no conflicts of interest.

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