Tree species richness effects on pre-dispersal seed predation are mediated by tree fruit type

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Abstract

Forest BEF experiments are only now reaching a stage at which natural tree regeneration can be studied, offering new opportunities to understand how biodiversity shapes trophic interactions during early demographic filtering. Here, we quantified seed productivity and insect-mediated pre-dispersal seed predation on 12 tree species across a tree species richness gradient from 1 to 16 in the BEF-China experiment, using 589 seed traps across two sites and 2,356 seasonal samples. Across 12 tree species, tree diversity did not exert a uniform effect on pre-dispersal seed predation. Instead, tree richness effects varied strongly among species and were significantly influenced by fruit type. Pre-dispersal seed predation rate on nuts declined with increasing tree species richness (95% CI: 10.96-30.89%), whereas fleshy fruits (95% CI: 10.34-25.37%) and capsules (95% CI: 7.78-17.12%) showed the opposite tendency. Seed productivity followed similar fruit-type-specific patterns and seed predation was generally negatively related to seed productivity. Seed predation was also negatively related to canopy cover, whereas seed productivity declined with increasing soil pH. Here, we are one of the first regeneration-feedback studies in mature forest BEF systems that shows that pre-dispersal seed predation, as one of the earliest demographic filters in forest regeneration, is interactively affected by tree species richness and fruit type. Together with future insights into seed predator identity and their resource specialisation, our data open new avenues for data-based forest restoration.

Keywords: biodiversity-ecosystem functioning, BEF-China, demographic filtering, forest regeneration, fruit type, insect seed predators, seed productivity, seed mass loss, community assemblage

Introduction

Biodiversity-Ecosystem-Functioning (BEF) experiments in forests have now been running for nearly three decades (Liu et al., 2026). While numerous studies from these platforms showed tree diversity to shape forest productivity and functioning via multitrophic interactions such as herbivory, fungal infection, or litter decomposition (Grossman et al., 2018; Li et al., 2023), there is little evidence on how tree diversity influences the regeneration processes of trees in forest stands and, consequently, regeneration and rejuvenation of forests. Coming of age, the world's largest forest BEF experiment, BEF-China, now includes reproducing and fruit-bearing trees and thus allows to study how tree species richness affects regeneration in trees varying in fruit and seed traits.

There is a wealth of evidence from observational studies showing that tree species richness can reorganize consumer pressure and multitrophic interactions from the beginning of forest development: for example, studies in comparative forest stands demonstrated that insect herbivory on seedling recruits increases with tree diversity (Schuldt et al., 2010, 2015), and subsequent studies reported similar diversity effects on canopy and leaf damage during early stand development (Brezzi et al., 2017). This leaf-centred perspective was later extended to linked pathogen damage and tree growth responses, as well as to the diversity of herbivores, predators and parasitoids across trophic levels (Staab et al., 2014; Schuldt et al., 2017; Guo et al., 2021; Li et al., 2025). Whether similar diversity effects also occur at the tree reproductive stage, when insects attack seeds, remains largely unresolved.

Seed predation may be classified into two modes: pre- and post-dispersal seed predation (Lewis & Gripenberg 2008). While the later stages of seed fate in subtropical forests, especially rodent-mediated post-dispersal seed predation have received a lot of attention (Xiao et al., 2006; Yang et al., 2020; Feng et al., 2021), pre-dispersal seed predation is a critical but often overlooked component of plant-consumer interactions. As this trophic interaction removes propagules before the seed rain, it represents one of the earliest demographic filters in forest regeneration. It occurs while seeds are still attached to the mother plant and is typically caused by specialized insects, especially beetles, flies, or moths (Kolb et al., 2007; Stemmelen et al., 2022). In forests, this means that insect attack on seeds may represent a direct top-down pathway linking biodiversity at consumer level to regeneration failure or success at the producer level.

Existing research on pre-dispersal seed predation is still dominated by single-species or small sets of species of trees (Espelta et al., 2009; Chen et al., 2025) and restricted to short-term demographic studies that are able to go to the trouble of painstakingly tracking the fate of individual seeds all the way to the growing seedling (Elwood et al., 2018). Broad comparative analyses of coexisting forest trees are rare (Kolb et al., 2007). Yet, one of the few community-level forest studies showed that pre-dispersal seed predation occurred in 17 of 44 woody species, that roughly three-quarters of cases were caused by insects, and that attack rates were related to fruit type and linked to seed mass and seed production dynamics (Xu et al., 2015).

Seeds and fruits are highly heterogeneous resources (Gripenberg et al., 2019). Their traits such as seed exposure, protective tissue, nutritional quality, fruiting phenology, or olfactory or visual cues determine how easily consumers can locate, access and exploit developing propagules (Beckman & Muller-Landau, 2011; Espelta et al., 2009; Xu et al., 2015; Palmer et al., 2022). Given this variety, tree species richness may influence pre-dispersal seed predation through several, even potentially opposing mechanisms. On the one hand, increasing tree diversity can reduce the density and apparency of conspecific host trees, thereby disrupting host-finding cues and producing associational resistance against specialized seed predators (Jactel et al., 2021; Castagneyrol et al., 2013). On the other hand, diverse tree species can provide more food resources for more generalist seed predators potentially increasing their abundance and activity (Staab & Schuldt, 2020; Stemmelen et al., 2022). In addition to resources, tree diversity can influence local environmental conditions such as light availability, temperature or humidity via canopy cover, which in turn may affect host visibility or insect movement, thus seed detectability and predator activity (Nakamura et al., 2017). Microclimatic conditions may further be modified by topography, whereas soil properties such as pH and C:N ratio can affect tree reproductive allocation, fruit maturation and seed productivity (Fernández-Martínez et al., 2017).

Here, we quantified insect-mediated pre-dispersal seed predation among 12 subtropical tree species along a tree species richness gradient within the BEF-China experiment. Selected tree species differing in fruit type, allowing us to test whether reproductive traits mediate tree diversity effects on seed fate. We also considered seed productivity and local environmental conditions in their effects on pre-dispersal seed predation. Specifically, we hypothesised that (i) rather than uniformly affecting pre-dispersal seed predation, tree species richness effects would be tree-species specific, (ii) variability in effects of tree species richness on pre-dispersal seed predation would depend on fruit type, and (iii) seed productivity and local environmental conditions would further modulate pre-dispersal seed fate.

Methods

Study area

This study utilized the BEF-China platform (Bruelheide et al., 2014), located in Xingangshan, Jiangxi Province, southeastern China (29◦08′ - 29◦11′N, 117◦90′ - 117◦93′E). The region experiences an average annual temperature of 16.7 °C and an average annual precipitation of 1,821 mm (Yang et al., 2013). The platform consists of two study sites, planted in 2009 (Site A) and 2010 (Site B), covering a total area of over 50 ha. A total of 566 plots of 25.8 × 25.8 m^2 was randomly distributed across these two sites, with 271 plots in site A and 295 plots in site B. Each plot contains 400 trees, arranged in a 20-row × 20-column grid. Using a ‘broken-stick’ design, 40 native deciduous and evergreen broad leaf tree species were planted to create gradients of tree species richness ranging from 1 to 24 tree species on each site. Sites A and B differed in their species pools including an overlap of 8 species. Each site encompasses 40 monocultures, 44 2-species mixtures, 28 4-species mixtures, 20 8-species mixtures, 16 16-species mixtures and 2 24-species mixtures totalling 150 each which are available (Klein et al., 2026).

Experiment design

We installed seed traps in early September 2024 before the forest seed rain to quantify pre-dispersal seed predation in selected tree species covering a wide range of seed size, fruit type and phylogenetic background. The tree species were selected from the BEF-China species pool of 40 native deciduous and evergreen broad-leaved tree species and encompassed *Choerospondias axillaris, Koelreuteria bipinnata, Liquidambar formosana, Quercus fabri, Quercus serrata, Alniphyllum fortunei,* *Castanopsis fargesii, Elaeocarpus chinensis, Manglietia fordiana, Cyclobalanopsis glauca, Lithocarpus glaber* and *Schima superba* (Table 1). Among these 12 target tree species, five occurred only at Site A, four occurred only at Site B, and three were present at both sites. Out of the 150 plots per site, we selected three plot replicates for each level of planted tree species richness per target tree species (due to constraints of the overall design monocultures were only represented twice). In plots with high tree species richness (with a limited number of replicates in the overall design of the BEF-China platform), we had to use the same plot for multiple target species, resulting in 72 plots at site A and 57 plots at site B. The final number of plots sampled was 27, 37, 32, 17 and 16 for the tree species richness levels of 1, 2, 4, 8 and 16, respectively. Three seed traps were set up for each tree species in each plot, resulting in a total of 675 traps. Due to constraints such as terrain and tree mortality, a total of 589 seed traps was successfully installed and subsequently monitored. Following Huang et al. (2018), the seed traps were made of nylon net, with a square trapping area of 85×85 cm, that was placed over a frame (built of PVC tubes). One seed trap each was installed under a selected tree individual of a target tree species and set 1 m above the ground to avoid post-dispersal seed predation by vertebrates. Seed sampling started in September with repeated seed collections in October, November, January and April to account for varying fruiting phenology and seed dispersal times, obtaining a total of 2,356 samples.

TABLE 1 Characteristics of the 12 target tree species used in this study

Species	Family	Site	Fruit type	No. plots	No. seed traps	Seed size (mm)	Seed mass (g)
Choerospondias axillaris	Anacardiaceae	A	Fleshy	14	42	23 * 14	3.65
Koelreuteria bipinnata	Sapindaceae	A	Capsules	11	29	6 * 5	0.22
Liquidambar formosana	Altingiaceae	A	Capsules	14	42	9 * 2	1.31
Quercus fabri	Fagaceae	A	Nut	13	31	17 * 10	0.37
Quercus serrata	Fagaceae	A	Nut	15	45	19 * 10	0.37
Alniphyllum fortunei	Styraceae	B	Nut	14	40	7 * 5	0.08
Castanopsis fargesii	Fagaceae	B	Nut	14	42	13 * 10	0.27
Elaeocarpus chinensis	Elaeocarpaceae	B	Fleshy	14	42	12 * 6	0.09
Manglietia fordiana	Magnoliaceae	B	Fleshy	14	42	8 * 6	0.05
Cyclobalanopsis glauca	Fagaceae	A/B	Nut	13/12	39/36	13 * 12	0.53/0.60
Lithocarpus glaber	Fagaceae	A/B	Nut	14/13	42/39	19 * 12	0.89/0.69
Schima superba	Theaceae	A/B	Capsules	14/12	42/36	9 * 5	0.07/0.02

Note: seed size was expressed as length * width, with data derived from the Flora of China. For species without data, including: *Elaeocarpus chinensis* and *Schima superba*, we measured the size of 100 mature seeds. Seed mass was calculated as the average value of all the seeds collected in the seed trap.

Seed identity, seed mass and seed predation

Seeds for each target tree species per sample were checked for the existence of pre-dispersal predation marks under a magnifier. Seeds were classified into two categories: (1) healthy seeds (attaining mature seed size with intact outer shell); (2) attacked seeds (having a hole or gnawing mark caused by insect predation, or excrement left by insect larvae) and their respective numbers were counted. Seeds were then oven dried at 60° C for 48 h before measuring the dry mass individually for each seed. In addition, seeds of non-target species were identified and counted to quantify total seed number per trap. For analyses, we considered the sum of all time samples as the seasonal cumulative seed count per seed trap and target tree.

Environmental factors

Canopy cover was calculated for each plot using elevation-normalized drone-based LiDAR data (Deng et al., 2025). The drone-based LiDAR data was acquired during the leaf-on season of September 2024 using a Hesai Pandar 40P laser scanner (LiAir 220N system, GreenValley International). Canopy cover was approximately the inverse of the gap fraction and estimated as the proportion of the ground covered by forest canopy (above 2 m). Other environmental data, such as the slope, northness, soil pH and soil C:N, were retrieved from the BEF-China platform (Scholten et al., 2017).

Data analysis

We calculated the sum of all time samples as the seasonal cumulative seed count per seed trap and target tree. Response variables used for analyses were:

I) **Seed number** = seed number$_{\text{Oct}}$ + seed number$_{\text{Nov}}$ + seed number$_{\text{Jan}}$ + seed number$_{\text{Apr}}$;
II) **Seed mass** = (seed mass$_{\text{Oct}}$ + seed mass$_{\text{Nov}}$ + seed mass$_{\text{Jan}}$ + seed mass$_{\text{Apr}}$) / 4
III) **Seed mass loss** = (healthy seed mass - attacked seed mass) / healthy seed mass
IV) **Seed productivity =** (healthy seed number + attacked seed number) * healthy seed mass
V) **Seed predation rate** = attacked seed number / (healthy seed number + attacked seed number).

All analyses were performed in R program version 4.2.2 (R Core Team, 2022). The general effect of tree species richness on pre-dispersal seed fate was tested fitting generalized linear mixed effects models (GLMMs) using 'glmmTMB' (Brooks et al., 2017), with seed productivity, pre-dispersal seed predation rate and seed mass loss as response variables, respectively. Before model fitting, tree species richness was log2-transformed to linearize its relationships with response variables. For pre-dispersal seed predation rate, we modelled it as a binomial response, with attacked seeds as successes and healthy seeds as failures. We fitted the models with a beta-binomial error distribution and a logit link to account for overdispersion. For seed productivity and seed mass loss, we used a tweedie distribution with a log link. We standardized all continuous predictors before the analysis. For visualization, predictor values on the x-axis were back-transformed and displayed on their original scales to improve interpretability. Residuals were assessed using 'DHARMa' package (Hartig, 2020), Wald χ^2 and p-values were obtained by using the Anova (III) function from package 'car' (Fox & Weisberg, 2019).

First, we considered only those species for which complete quantified responses were available across all diversity levels, i.e., to better fit the model, we excluded five species (*Liquidambar formosana*, *Koelreuteria bipinnata*, *Manglietia fordiana*, *Castanopsis fargesii*, *Alniphyllum fortunei*). We fitted three GLMMs with seed productivity, pre-dispersal seed predation rate and seed mass loss as response variables, respectively. For each model, tree species richness, tree species, and the tree species richness × tree species interaction included as fixed effects. Plot was nested within site and included as a random effect to account for the hierarchical sampling design.

Second, to further identify the environmental and biotic factors associated with variation in seed predation and seed productivity, we fitted another set of GLMMs. Compared to the first set of models, tree species was modelled here as a random effect with random slopes for tree species richness, allowing the effect of tree species richness to vary among tree species while including all 12 target tree species. In all three models, tree species richness was included as a fixed effect. In the seed predation rate model, canopy cover, seed productivity, slope, and northness were included as covariates; in the seed productivity model, we included northness, slope, and additionally soil pH, and soil C:N as covariates; and in the seed mass loss model, seed productivity, northness, and slope were included to covary with tree species richness. In all models, plot nested within site was included as random intercept to account for the hierarchical sampling design.

Third, to test whether fruit type mediated the effect of tree species richness on pre-dispersal seed fate, we fitted the third set of GLMMs. For each model, tree species richness, fruit type, and their interaction were included as fixed effects. In the seed predation rate model, seed productivity, canopy cover, slope, and northness were included as covariates; in the seed productivity model, we included northness, slope, soil pH, and soil C:N as covariates; and in the seed mass loss model, seed productivity was a covariate. To account for the hierarchical sampling design, plot nested within site was included as a random effect, and tree species nested within fruit type was included as an additional random effect to account for variation among tree species within fruit types.

Results

Interactive effects of tree species richness and tree species on pre-dispersal seed fate

Based on the reduced set of 7 species, tree species richness did not exert a consistent effect on pre-dispersal seed fate. Instead, the effects of tree species richness depended strongly on tree species identity (Appendix 1). While the overall tree richness effect on pre-dispersal seed predation rate was not significant, seed mass loss and seed productivity increased with tree species richness (Fig. 1). However, these overall richness effects did not capture the inter-specific variation, as indicated by the significant interaction effects between tree species richness and tree species for all three response variables.

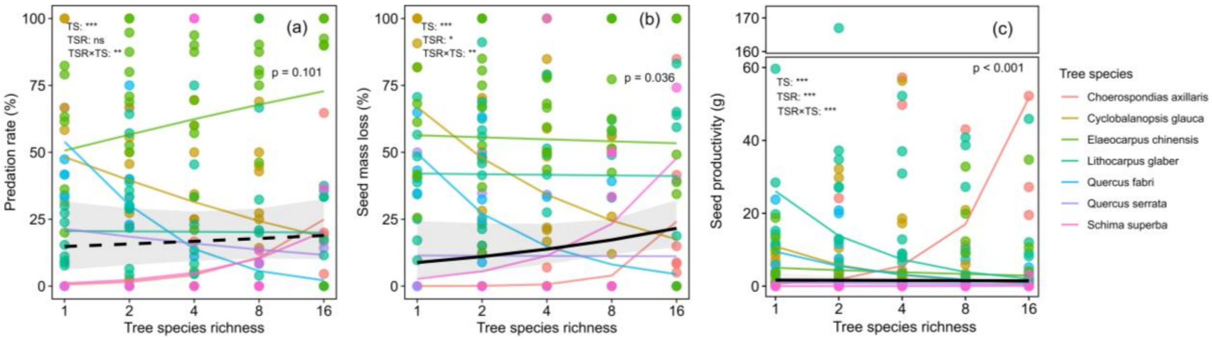

Figure 1. Effects of tree species richness, tree species, and their interaction on pre-dispersal seed predation rate

(a), seed mass loss (b), and seed productivity (c). Coloured points indicate observed values for different tree species, and coloured lines show species-specific fitted relationships from the GLMMs. The black line shows the overall fitted relationship, with the grey band indicating the 95% confidence interval. P-values indicate the significance of the effect of tree species richness in each model. TS: tree species; TSR: tree species richness. *, * * and *** indicate statistically significant effects at *p* < 0.05, *p* < 0.01 and *p* < 0.001, respectively.

Tree species-specific responses of pre-dispersal seed fate to tree species richness

In the second set of GLMMs, fitted slopes for the 12 target tree species exhibited significant differences in pre-dispersal seed predation rate, seed mass loss and seed productivity, again showing that tree diversity did not exert a common response across species (Fig. 2a-c, Appendix 2). The extended GLMMs showed that differences in the fate of tree seeds can additionally be attributed to environmental factors. Seed predation rate declined with increasing canopy cover (p = 0.020, Fig. 2d), seed mass loss was negatively related to seed productivity (p = 0.013, Fig. 2e) and seed productivity was negatively related to soil pH (p = 0.008, Fig 2f).

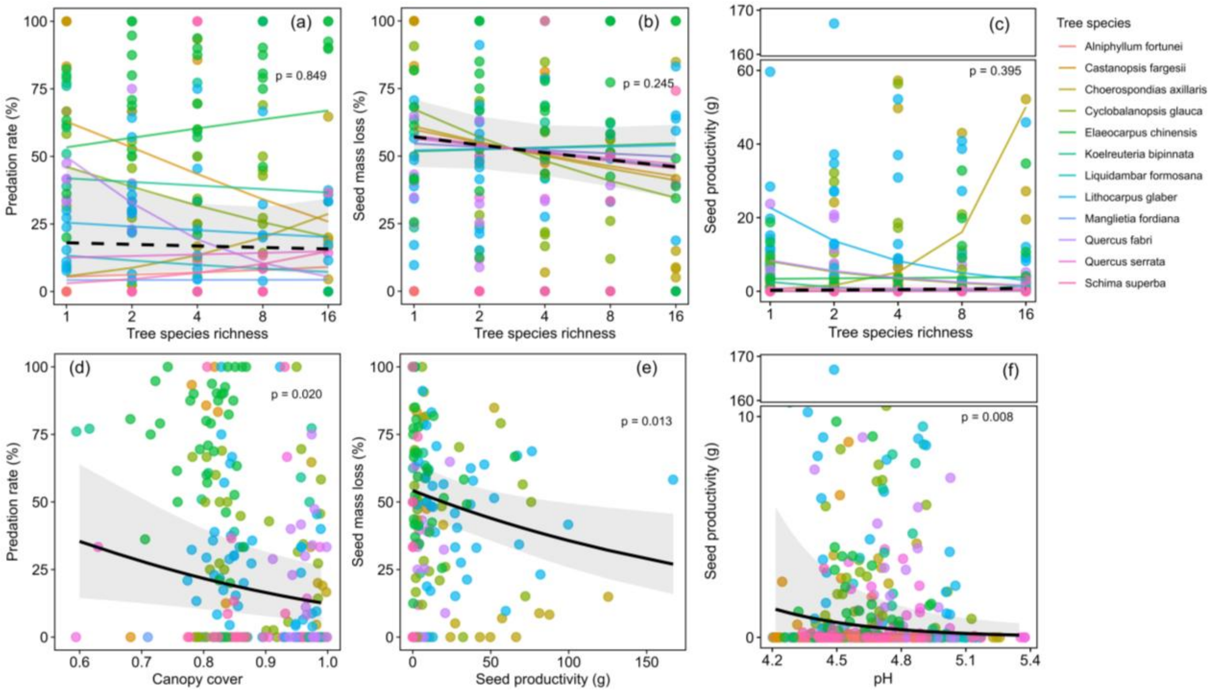

Figure 2. Tree species-mediated relationships between tree species richness and pre-dispersal seed predation rate (a), seed mass loss (b), and seed productivity (c) and additional environmental variables found significant in the GLMMs (d, e, f). Coloured points indicate observations for different tree species, and coloured lines show species-specific fitted relationships from the GLMMs. In panel (a-c), the black line shows the overall fitted relationship from GLMMs; in panel (d-f), the black line shows fitted relationships between significant predictors and their response variables, and grey bands indicate 95% confidence intervals. P-values indicate the significance of the predictor shown in each model.

Fruit type-specific responses of pre-dispersal seed fate to tree species richness

The third set of GLMMs showed that fruit type explained part of the variation in how pre-dispersal seed fate responded to tree species richness (Fig. 3, Appendix 3). Fleshy-, nut- and capsule-bearing species showed contrasting richness-dependent responses, particularly for seed predation rate and seed productivity. For pre-dispersal seed predation rate, fitted relationships suggested that capsule- and fleshy-fruited species tended to increase with increasing tree species richness, whereas nut-bearing species showed a declining trend along the richness gradient (Fig. 3a). By contrast, fruit type differences in seed mass loss were weaker: fitted lines for all three fruit types were generally neutral to slightly negative, indicating only limited divergence in richness effects for this variable (Fig. 3b). Seed productivity showed a positive response to tree species richness in fleshy-bearing species, while it showed a relatively weak response in capsule-bearing species, and tended to decline with increasing tree species richness in nut-bearing species (Fig. 3c). In addition, pre-dispersal seed predation rate decreased with increasing canopy cover (p = 0.003, Fig. 3d) and seed productivity (p = 0.047, Fig. 3e), but increased with northness (p = 0.026, Fig. 3f). Seed mass loss declined with seed productivity (p = 0.010, Fig. 3g), while seed productivity decreased with soil pH (p = 0.047, Fig. 3h).

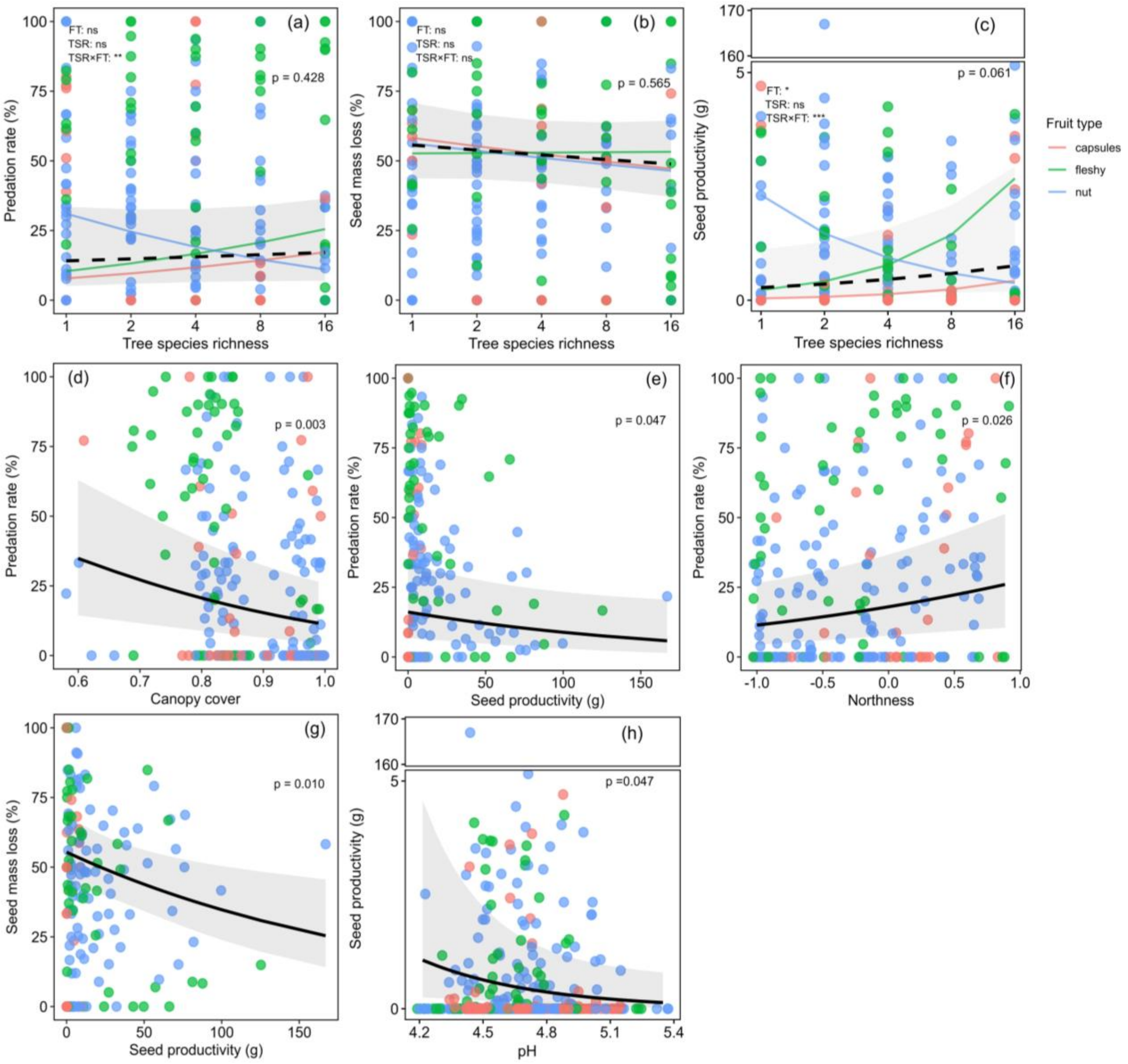

Figure 3. Fruit type-mediated relationships between tree species richness and pre-dispersal seed predation rate (a), seed mass loss (b), and seed productivity (c) and the variables found significant in the GLMMs (d, e, f, g, h). Coloured points indicate observations for different fruit types, and coloured lines show fruit types-specific fitted relationships from the GLMMs. In panel (a-c), the black line shows the overall fitted relationship from GLMMs; in panel (d-h), the black line shows fitted relationships between significant predictors and their response variables, and grey bands indicate 95% confidence intervals. P-values indicate the significance of the predictor shown in each model.

Discussion

For the first time, a BEF forest platform was used to study pre-dispersal seed predation at a community level. Our results from BEF-China show that tree species richness did not impose a consistent effect on pre-dispersal seed fate across tree species. Instead, richness effects were strongly dependent on species identity, and part of this heterogeneity was explained by fruit type. This implies that examining the impact of tree species richness on pre-dispersal seed predation solely as a simple community-average effect would fail to capture the ecological variability in highly diverse forests of subtropical China. These findings extend forest BEF research into the reproductive stage and show that biodiversity effects can propagate into species-specific demographic processes during forest generation through trait-dependent interactions.

While tree species vary in many traits, fruit type emerged as an important mediator of tree diversity effects on pre-dispersal seed predation. Fruit type integrates multiple functional traits that determine enemy access and performance, including seed exposure, protective tissues, fruit structure, crop presentation and reproductive phenology (Terborgh et al., 2008; Ramírez & Traveset, 2010; Beckman & Muller-Landau, 2011). Previous work showed that community-level pre-dispersal seed predation differs among fruit types and can be linked to seed mass and seed production dynamics (Xu et al., 2015). Oak systems inter-specific differences in pre-dispersal seed predation are further shaped by the joint effects of seed phenology, seed size and predator traits (Espelta et al., 2009), highlighting the importance of functional seed traits in structuring predator responses. Trait-mediated bottom-up effects on seed consumers have furthermore been shown to influence seedling recruitment in fleshy-fruited species (Muñoz et al., 2017), suggesting that functional seed traits may affect regeneration processes beyond the seed stage. Our results extend these findings by indicating, that diversity-mediated neighbourhood interact with fruit traits in shaping pre-dispersal seed predation.

In our study, nut-bearing species tended to show lower pre-dispersal seed predation with increasing tree species richness, whereas fleshy- and capsule-bearing species showed neutral to positive trends. These contrasting patterns suggest that seed predators responded differently to tree diversity depending on fruit types and associated predator guilds. One possible explanation is that hard-coated nuts, which are often attacked by specialized weevils, benefit more strongly from reduced host apparency or disrupted host location in diverse stands, consistent with the associational resistance and plant apparency hypothesis (Jactel et al., 2021; Farinha et al., 2024). By contrast, fleshy fruits and capsules may remain more accessible or detectable in diverse neighbourhoods, or may be attacked by broader enemy guilds that are less sensitive to neighbour heterogeneity (Basset et al., 2021; Beckman & Muller-Landau, 2011). Because seed predators were not identified directly here, these mechanisms should be regarded as hypotheses rather than demonstrated processes and corroborated by multi-seasonal studies. Nevertheless, our results indicate that tree diversity effects on tree pre-dispersal seed predation were structured by reproductive trait syndromes, that require further investigation, rather than by tree species identity alone.

The negative relationships of seed predation rate and seed mass loss with seed productivity further suggest that trees producing more seeds may escape insect attack more effectively, although species traits still influence predation intensity (Xiao et al., 2017). This pattern is consistent with the predator satiation theory, which predicts that producing larger seeds or increasing seed crops can reduce per-seed attack proportion and seed attack rates by exceeding consumer capacity (Kelly, 1994; Zwolak et al., 2022). It is also consistent with a study from species-rich forests showing that pre-dispersal seed predation can decline with seed density and that dominant species can partially escape attack through large reproductive output (Xu et al., 2015). However, predator satiation theory alone cannot fully explain the pattern observed in our study. If seed production was the dominant mechanism, richness effects should have been more similar among species. Instead, species-specific responses remained highly divergent, implying that seed production may buffer predator pressure in certain cases, but does not override the trait-mediated differences among tree species. From this view, pre-dispersal seed fate in subtropical forests appears to be shaped by an interaction between seed production and species identity, rather than by a universal density effect.

Local environmental conditions added another layer of filtering mechanisms, particularly through canopy cover. Pre-dispersal seed predation declined with increasing canopy cover. One possible explanation is that denser canopies may reduce the apparency of fruits and developing seeds by lowering light conditions and weakening visual or chemical host-location cues. In more open plots, by contrast, fruits may be more exposed and easier for insects to detect, especially for seed predators that rely on host visibility or short-distance orientation cues. Denser canopies may also buffer temperature and humidity, creating cooler microclimatic conditions that could alter insect flight activity, oviposition behaviour or larval performance (Nakamura et al., 2017; Chen et al., 2025). This interpretation is consistent with fruit-removal trials from BEF-China showing that canopy cover can have stronger effects on fruit-related processes than tree diversity itself (Rehling et al., 2026). In the fruit-type models, seed predation rate increased with northness, suggesting that aspect-related differences in solar radiation and moisture may also influence seed-feeding insect activity or the phenology of developing fruits. This effect should be interpreted cautiously because northness may integrate several microclimatic conditions rather than representing a direct mechanism. More broadly, studies on forest diversity and insect enemies suggests that tree diversity may alter both bottom-up and top-down regulation, but that increases in enemy diversity do not necessarily transfer into stronger enemy activity and seed predation in forest ecosystems (Staab & Schuldt, 2020; Stemmelen et al., 2022). Our results therefore most likely reflect a combination of microclimatic buffering and modified enemy search capabilities rather than a single mechanism. The negative association between soil pH and seed productivity also points in the same direction, indicating that pre-dispersal seed fate not only responds to tree diversity and fruit traits, but also depends on local abiotic context that may influence reproductive allocation and fruit maturation (Fernández-Martínez et al., 2017, 2019).

These findings have broader implications for forest regeneration theory. Since the Janzen-Connell hypothesis was proposed, species coexistence theory has emphasized that host-specific natural enemies can stabilize diversity by imposing negative density dependence on common species, a framework later expanded and reinterpreted through modern coexistence theory (Janzen, 1970; Comita & Stump, 2020). Most tests of this theory have focused on post-dispersal seeds, seedlings or foliar damage (Germany et al., 2019). Our study suggests that part of this filtering may begin even earlier, before seed rain reaches the forest floor. At the same time, our results indicate that this early enemy filter is asymmetric among coexisting species. Rather than all tree species experiencing a similar diversity-dependent reduction in reproductive success, some species may escape attack whereas others remain vulnerable or even become more exposed. Such asymmetry is ecologically important because it can alter not only total recruitment but also the composition of the effective seed rain. In other words, pre-dispersal seed predation may help determine which species enter the next regeneration stage at all, thereby linking tree diversity effects directly to community assembly. Importantly, our study does not test species coexistence directly, but it identifies a plausible mechanism by which biodiversity effects on multitrophic interactions could extend to the seed pre-dispersal stages. Finally, linking pre-dispersal seed loss to post-dispersal survival and seedling establishment would help reveal how strongly these early demographic filters propagate through subsequent demographic stages.

Appendix 1 ANOVA (type III Wald chi-square tests) of fixed factors of the first set GLMMs.

		TSR	TS	TSR * TS
Predation rate	χ^2	2.693	24.396	20.872
	p	0.101	**<0.001**	**0.002**
Seed mass loss	χ^2	4.420	25.472	21.210
	p	**0.036**	**<0.001**	**0.002**
Seed productivity	χ^2	15.571	50.923	38.845
	p	**<0.001**	**<0.001**	**<0.001**

Statistically significant values are in boldface.

Appendix 2 ANOVA (type III Wald chi-square tests) of fixed factors of the second set GLMMs.

		TSR	Canopy cover	Seed productivity	Northness	Slope	pH	Soil C:N
Predation rate	χ^2	0.036	5.378	3.303	3.271	0.804	/	/
	p	0.849	**0.020**	0.069	0.071	0.370	/	/
Seed mass loss	χ^2	1.354	/	6.202	1.282	0.048	/	/
	p	0.245	/	**0.013**	0.258	0.827	/	/
Seed productivity	χ^2	0.724	/	/	0.191	0.118	7.125	0.680
	p	0.395	/	/	0.662	0.731	**0.008**	0.409

Statistically significant values are in boldface.

Appendix 3 ANOVA (type III Wald chi-square tests) of fixed factors of the third set GLMMs.

		TSR	FT	TSR * FT	Canopy cover	Seed productivity	Northness	Slope	pH	Soil C:N
Predation rate	χ^2	0.629	2.393	11.190	8.786	3.936	4.931	0.301	/	/

	p	0.428	0.302	**0.004**	**0.003**	**0.047**	**0.026**	0.583	/	/
Seed mass loss	χ^2	0.332	0.180	0.538	/	6.646	/	/	/	/
	p	0.565	0.914	0.764	/	**0.010**	/	/	/	/
Seed productivity	χ^2	3.512	6.748	24.119	/	/	0.722	0.054	3.943	0.089
	p	0.061	**0.034**	<**0.001**	/	/	0.395	0.817	**0.047**	0.766

Statistically significant values are in boldface.

Acknowledgements

We acknowledge the BEF-China platform and the support of Shan Li and Bo Yang in maintaining the research station. We specially thank Helge Bruelheide, Keping Ma. and Bernhard Schmid for initiating the BEF-China platform, and Alexandra-M. Klein and Chao-Dong Zhu for their combined efforts in coordinating MultiTroph (Klein et al., 2026). We acknowledge Shan Li and Bo Yang for their efforts in maintaining the research station. We are grateful to student helpers Julius Kühn, Leif Pantléon, Lauritz Runge, and Alexandra Williams, and local helpers who greatly supported the implementation and data collection within this project.

Financial support

This work is part of the MultiTroph research unit (FOR 5281, grant no. 452861007), funded by the Deutsche Forschungsgemeinschaft (DFG).

Author contributions

Feilong Ji: investigation, data curation, methodology (equal), formal analysis, visualization, writing - original draft preparation. **Noga Abecassis Monteyne**: methodology (equal). **Xiao-Juan Liu**: investigation (support), methodology (equal), writing - review & editing (equal). **Finn Rehling**: formal analysis (support), writing – review & editing (equal). **Alexandra Erfmeier**: conceptualization (equal), funding acquisition (equal), project administration (equal), resources (equal), supervision (equal), validation (equal), writing – original draft preparation (support), writing - review & editing (equal). **Tim Diekötter**: conceptualization (equal), funding acquisition (equal), project administration (equal), supervision (equal), resources (equal), validation (equal), writing – original draft preparation (support), writing – review & editing (equal).

Competing interests

The authors declare no competing interests

Data availability

The data will be made publicly available in an open data repository upon acceptance of the manuscript in a peer-reviewed journal.

References

Basset, Y., Jorge, L. R., Butterill, P. T., Lamarre, G. P. A., Dahl, C., Ctvrtecka, R., Gripenberg, S., Lewis, O. T., Barrios, H., Brown, J. W., Bunyavejchewin, S., Butcher, B. A., Cognato, A. I., Davies, S. J., Kaman, O., Klimes, P., Knížek, M., Miller, S. E., Morse, G. E., ... Segar, S. T. (2021). Host specificity and interaction networks of insects feeding on seeds and fruits in tropical rainforests. Oikos, 130, 1462–1476. https://doi.org/10.1111/oik.08152

Beckman, N. G., & Muller-Landau, H. C. (2011). Linking fruit traits to variation in predispersal vertebrate seed predation, insect seed predation, and pathogen attack. Ecology, 92, 2131-2140. https://doi.org/10.1890/10-2378.1

Brezzi, M., Schmid, B., Niklaus, P. A., & Schuldt, A. (2017). Tree diversity increases levels of herbivore damage in a subtropical forest canopy: Evidence for dietary mixing by arthropods? Journal of Plant Ecology, 10, 13-27. https://doi.org/10.1093/jpe/rtw038

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler, M., & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal, 9(2), 378–400. https://doi.org/10.32614/RJ-2017-066

Bruelheide, H., Nadrowski, K., Assmann, T., Bauhus, J., Both, S., Buscot, F., Chen, X.-Y., Ding, B., Durka, W., Erfmeier, A., Gutknecht, J. L. M., Guo, D., Guo, L.-D., Härdtle, W., He, J.-S., Klein, A.-M., Kühn, P., Liang, Y., Liu, X.-J., ... Schmid, B. (2014). Designing forest biodiversity experiments: General considerations illustrated by a new large experiment in subtropical China. Methods in Ecology and Evolution, 5, 74-89. https://doi.org/10.1111/2041-210X.12126

Castagneyrol, B., Giffard, B., Péré, C., & Jactel, H. (2013). Plant apparency, an overlooked driver of associational resistance to insect herbivory. Journal of Ecology, 101, 418–429. https://doi.org/10.1111/1365-2745.12055

Chen, J.-T., Wang, M.-Q., Luo, A., Zhang, F., Chesters, D., Liu, S., Li, Y., von Oheimb, G., Kunz, M., Zhou, Q.-S., Bruelheide, H., Liu, X.-J., Ma, K.-P., Schuldt, A., & Zhu, C.-D. (2025). Bottom-up and top-down effects combine to drive predator-prey interactions in a forest biodiversity experiment. Journal of Animal Ecology, 94, 2035-2046. https://doi.org/10.1111/1365-2656.70103

Chen, X., Li, X., & Li, Q. (2025). Interspecific variation in seed traits facilitates divergent pre-dispersal seed predation among five stone oak species. Ecological Processes, 14, Article 42. https://doi.org/10.1186/s13717-025-00607-y

Comita, L. S., & Stump, S. M. (2020). Natural enemies and the maintenance of tropical tree diversity: Recent insights and implications for the future of biodiversity in a changing world. Annals of the Missouri Botanical Garden, 105, 377-392. https://doi.org/10.3417/2020591

Deng, X., Schmid, B., Bruelheide, H., Chen, C., Li, Y., Li, S., Morsdorf, F., Ray, T., Schuman, M. C., Tang, T., von Oheimb, G., Ma, K., & Liu, X. (2025). Forest biodiversity increases productivity via complementarity from greater canopy structural complexity. Proceedings of the National Academy of Sciences of the United States of America, 122, Article e2506750122. https://doi.org/10.1073/pnas.2506750122

Elwood, E. C., Lichti, N. I., Fitzsimmons, S. F., & Dalgleish, H. J. (2018). Scatterhoarders drive long- and short-term population dynamics of a nut-producing tree, while pre-dispersal seed predators and herbivores have little effect. Journal of Ecology, 106, 1191–1203. https://doi.org/10.1111/1365-2745.12902

Espelta, J. M., Bonal, R., & Sánchez-Humanes, B. (2009). Pre-dispersal acorn predation in mixed oak forests: Interspecific differences are driven by the interplay among seed phenology, seed size and predator size. Journal of Ecology, 97, 1416-1423. https://doi.org/10.1111/j.1365-2745.2009.01564.x

Farinha, A., Castagneyrol, B., van Halder, I., Branco, M., Meredieu, C., & Jactel, H. (2024). Reduction of damage by the exotic invasive conifer seed bug Leptoglossus occidentalis in mixed pine-birch plantations: A case of biotic resistance. Forest Ecology and Management, 569, Article 122193. https://doi.org/10.1016/j.foreco.2024.122193

Feng, L., Chen, S., & Wang, B. (2021). Fine-scale spatiotemporal variation in seed-rodent interactions: A potential contribution to species coexistence. Forest Ecology and Management, 498, Article 119566. https://doi.org/10.1016/j.foreco.2021.119566

Fernández-Martínez, M., Pearse, I., Sardans, J., Sayol, F., Koenig, W. D., LaMontagne, J. M., Bogdziewicz, M., Collalti, A., Hacket-Pain, A., Vacchiano, G., Espelta, J. M., Peñuelas, J., & Janssens, I. A. (2019). Nutrient scarcity as a selective pressure for mast seeding. Nature Plants, 5, 1222-1228. https://doi.org/10.1038/s41477-019-0549-y

Fernández-Martínez, M., Vicca, S., Janssens, I. A., Espelta, J. M., & Peñuelas, J. (2017). The role of nutrients, productivity and climate in determining tree fruit production in European forests. New Phytologist, 213, 669-679. https://doi.org/10.1111/nph.14193

Fox, J., & Weisberg, S. (2019). An R companion to applied regression (3rd ed.). Sage.

Germany, M. S., Bruelheide, H., & Erfmeier, A. (2019). Janzen-Connell effects in a forest BEF experiment: Strong distance-dependent seedling establishment of multiple species. Ecology, 100, Article e02736. https://doi.org/10.1002/ecy.2736

Grossman, J. J., Vanhellemont, M., Barsoum, N., Bauhus, J., Bruelheide, H., Castagneyrol, B., Cavender-Bares, J., Eisenhauer, N., Ferlian, O., Gravel, D., Hector, A., Jactel, H., Kreft, H., Mereu, S., Messier, C., Muys, B., Nock, C., Paquette, A., Parker, J., Verheyen, K. (2018). Synthesis and future research directions linking tree diversity to growth, survival, and damage in a global network of tree diversity experiments. Environmental and Experimental Botany, 152, 68-89. https://doi.org/10.1016/j.envexpbot.2017.12.015

Gripenberg, S., Basset, Y., Lewis, O. T., Terry, J. C. D., Wright, S. J., Simón, I., Fernández, D. C., Cedeño-Sanchez, M., Rivera, M., Barrios, H., Brown, J. W., Calderón, O., Cognato, A. I., Kim, J., Miller, S. E., Morse, G. E., Pinzón-Navarro, S., Quicke, D. L. J., Robbins, R.

K., Salminen, J.-P., & Vesterinen, E. (2019). A highly resolved food web for insect seed predators in a species-rich tropical forest. Ecology Letters, 22, 1638–1649. https://doi.org/10.1111/ele.13359

Guo, P.-F., Wang, M.-Q., Orr, M., Li, Y., Chen, J.-T., Zhou, Q.-S., Staab, M., Fornoff, F., Chen, G.-H., Zhang, N.-L., Klein, A.-M., & Zhu, C.-D. (2021). Tree diversity promotes predatory wasps and parasitoids but not pollinator bees in a subtropical experimental forest. Basic and Applied Ecology, 53, 134-142. https://doi.org/10.1016/j.baae.2021.03.007

Hartig, F. (2020). DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models (R package version 0.3.3.0).

Huang, Y., Ma, K., Niklaus, P. A., & Schmid, B. (2018). Leaf-litter overyielding in a forest biodiversity experiment in subtropical China. Forest Ecosystems, 5, Article 38. https://doi.org/10.1186/s40663-018-0157-8

Jactel, H., Moreira, X., & Castagneyrol, B. (2021). Tree diversity and forest resistance to insect pests: Patterns, mechanisms, and prospects. Annual Review of Entomology, 66, 277-296. https://doi.org/10.1146/annurev-ento-041720-075234

Janzen, D. H. (1970). Herbivores and the number of tree species in tropical forests. The American Naturalist, 104, 501-528. https://doi.org/10.1086/282687

Kelly, D. (1994). The evolutionary ecology of mast seeding. Trends in Ecology & Evolution, 9, 465-470. https://doi.org/10.1016/0169-5347(94)90310-7

Klein, A.-M., Bruelheide, H., Chesters, D., Diekötter, T., Erfmeier, A., Feldhaar, H., Fornoff, F., Grozinger, C., Kranke, N., Liang, Y., Liu, X., Luo, A., Oelmann, Y., Orr, M., Petermann, J. S., Qiao, H., Rehling, F., Sann, M., Scholten, T., Schuldt, A., Seitz, S., Staab, M., Thorn, S., Wang, M.-Q., Xiao, Z.-S., Zhang, N., & Zhu, C.-D. (2026). MultiTroph: Multi-trophic interactions in a forest biodiversity experiment in China. Research Ideas and Outcomes, 12, e181743. https://doi.org/10.3897/rio.12.e181743

Kolb, A., Ehrlén, J., & Eriksson, O. (2007). Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. Perspectives in Plant Ecology, Evolution and Systematics, 9, 79-100. https://doi.org/10.1016/j.ppees.2007.09.001

Lewis, O. T., & Gripenberg, S. (2008). Insect seed predators and environmental change. Journal of Applied Ecology, 45, 1593-1599. https://doi.org/10.1111/j.1365-2664.2008.01575.x

Li, Y., Schmid, B., Schuldt, A., Li, S., Wang, M.-Q., Fornoff, F., Staab, M., Guo, P.-F., Anttonen, P., Chesters, D., Bruelheide, H., Zhu, C.-D., Ma, K., & Liu, X. (2023). Multitrophic arthropod diversity mediates tree diversity effects on primary productivity. Nature Ecology & Evolution, 7, 832-840.

https://doi.org/10.1038/s41559-023-02049-1

Li, Y., Schuldt, A., Bauhus, J., Belluau, M., Berthelot, S., Burghardt, K. T., Bruelheide, H., Castagneyrol, B., Chu, C., Eisenhauer, N., Ferlian, O., Fründ, J., Gebauer, T., Gravel, D., Jactel, H., Li, S., Liang, Y., Parker, J. D., Parker, W. C., ... Liu, X. (2025). The tree growth-herbivory relationship depends on functional traits across forest biodiversity experiments. Nature Ecology & Evolution, 9, 2014-2024. https://doi.org/10.1038/s41559-025-02835-z

Liu, X., Schuldt, A., Cavender-Bares, J., Paquette, A., Schmid, B., & Ma, K. (2026). Ecological insights from three decades of forest biodiversity experiments. Nature Reviews Biodiversity, 2, 9-23. https://doi.org/10.1038/s44358-025-00112-2

Muñoz, M. C., Schaefer, H. M., Böhning-Gaese, K., Neuschulz, E. L., & Schleuning, M. (2017). Phylogenetic and functional diversity of fleshy-fruited plants are positively associated with seedling diversity in a tropical montane forest. Frontiers in Ecology and Evolution, 5, Article 93. https://doi.org/10.3389/fevo.2017.00093

Nakamura, A., Kitching, R. L., Cao, M., Creedy, T. J., Fayle, T. M., Freiberg, M., Hewitt, C. N., Itioka, T., Koh, L. P., Ma, K., Malhi, Y., Mitchell, A., Novotny, V., Ozanne, C. M. P., Song, L., Wang, H., & Ashton, L. A. (2017). Forests and their canopies: Achievements and horizons in canopy science. Trends in Ecology & Evolution, 32, 438-451. https://doi.org/10.1016/j.tree.2017.02.012

Palmer, J. B., Hahn, P. G., Metcalf, E. C., & Maron, J. L. (2022). Seed size of co-occurring forb species predicts rates of pre-dispersal seed loss from insects. Ecosphere, 13, Article e4032. https://doi.org/10.1002/ecs2.4032

R Core Team. (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Ramírez, N., & Traveset, A. (2010). Predispersal seed-predation by insects in the Venezuelan Central Plain: Overall patterns and traits that influence its biology and taxonomic groups. Perspectives in Plant Ecology, Evolution and Systematics, 12, 193-209. https://doi.org/10.1016/j.ppees.2010.04.001

Rehling, F., Senger, L. M., Niedernhoefer, F. T., Anderson, N., Diekötter, T., Erfmeier, A., Li, Y & Klein, A.-M. (2026). Removal and decomposition of fruit respond in opposite ways to canopy cover in a biodiversity experiment [Preprint]. EcoEvoRxiv. https://doi.org/10.32942/X2G381

Scholten, T., Goebes, P., Kühn, P., Seitz, S., Assmann, T., Bauhus, J., Bruelheide, H., Buscot, F., Erfmeier, A., Fischer, M., Härdtle, W., He, J.-S., Ma, K., Niklaus, P. A., Scherer-Lorenzen, M., Schmid, B., Shi, X., Song, Z., von Oheimb, G., ... Schmidt, K. (2017). On the combined effect of soil fertility and topography on tree growth in subtropical forest ecosystems: A study from SE China. Journal of Plant Ecology, 10, 111-127. https://doi.org/10.1093/jpe/rtw065

Schuldt, A., Baruffol, M., Böhnke, M., Bruelheide, H., Härdtle, W., Lang, A. C., Nadrowski, K., von Oheimb, G., Voigt, W., Zhou, H., & Assmann, T. (2010). Tree diversity promotes insect herbivory in subtropical forests of south-east China. Journal of Ecology, 98, 917-926. https://doi.org/10.1111/j.1365-2745.2010.01659.x

Schuldt, A., Bruelheide, H., Härdtle, W., Assmann, T., Li, Y., Ma, K., von Oheimb, G., & Zhang, J. (2015). Early positive effects of tree species richness on herbivory in a large-scale forest biodiversity experiment influence tree growth. Journal of Ecology, 103, 563-571. https://doi.org/10.1111/1365-2745.12396

Schuldt, A., Hönig, L., Li, Y., Fichtner, A., Härdtle, W., von Oheimb, G., Welk, E., & Bruelheide, H. (2017). Herbivore and pathogen effects on tree growth are additive, but mediated by tree diversity and plant traits. Ecology and Evolution, 7, 7462-7474. https://doi.org/10.1002/ece3.3292

Staab, M., & Schuldt, A. (2020). The influence of tree diversity on natural enemies: A review of the “enemies” hypothesis in forests. Current Forestry Reports, 6, 243-259. https://doi.org/10.1007/s40725-020-00123-6

Staab, M., Schuldt, A., Assmann, T., & Klein, A.-M. (2014). Tree diversity promotes predator but not omnivore ants in a subtropical Chinese forest. Ecological Entomology, 39, 637-647. https://doi.org/10.1111/een.12143

Stemmelen, A., Jactel, H., Brockerhoff, E., & Castagneyrol, B. (2022). Meta-analysis of tree diversity effects on the abundance, diversity and activity of herbivores’ enemies. Basic and Applied Ecology, 58, 130-138. https://doi.org/10.1016/j.baae.2021.12.003

Terborgh, J., Nuñez-Iturri, G., Pitman, N. C. A., Cornejo Valverde, F. H., Alvarez, P., Swamy, V., Pringle, E. G., & Paine, C. E. T. (2008). Tree recruitment in an empty forest. Ecology, 89, 1757-1768. https://doi.org/10.1890/07-0479.1

Xiao, Z., Jansen, P. A., & Zhang, Z. (2006). Using seed-tagging methods for assessing post-dispersal seed fate in rodent-dispersed trees. Forest Ecology and Management, 223, 18-23. https://doi.org/10.1016/j.foreco.2005.10.054

Xiao, Z., Mi, X., Holyoak, M., Xie, W., Cao, K., Yang, X., Huang, X., & Krebs, C. J. (2017). Seed-predator satiation and Janzen-Connell effects vary with spatial scales for seed-feeding insects. Annals of Botany, 119, 109-116. https://doi.org/10.1093/aob/mcw224

Xu, Y., Shen, Z., Li, D., & Guo, Q. (2015). Pre-dispersal seed predation in a species-rich forest community: Patterns and the interplay with determinants. PLOS ONE, 10, Article e0143040. https://doi.org/10.1371/journal.pone.0143040

Yang, X., Bauhus, J., Both, S., Fang, T., Härdtle, W., Kröber, W., Ma, K., Nadrowski, K., Pei, K., Scherer-Lorenzen, M., Scholten, T., Seidler, G., Schmid, B., von Oheimb, G., &

Bruelheide, H. (2013). Establishment success in a forest biodiversity and ecosystem functioning experiment in subtropical China (BEF-China). European Journal of Forest Research, 132, 593-606. https://doi.org/10.1007/s10342-013-0696-z

Yang, X., Yan, C., Gu, H., & Zhang, Z. (2020). Interspecific synchrony of seed rain shapes rodent-mediated indirect seed-seed interactions of sympatric tree species in a subtropical forest. Ecology Letters, 23, 45-54. https://doi.org/10.1111/ele.13405

Zwolak, R., Celebias, P., & Bogdziewicz, M. (2022). Global patterns in the predator satiation effect of masting: A meta-analysis. Proceedings of the National Academy of Sciences of the United States of America, 119, Article e2105655119. https://doi.org/10.1073/pnas.2105655119