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Herbivory Rather than Root Competition and Environmental Factors Determines Plant Establishment in Fragmented Forests

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Abstract: In fragmented forests, many factors can affect plant community establishment, including abiotic factors, below-ground root competition, aboveground seed predation, and seedling herbivory. Little is known about the relative effects of biotic and abiotic factors affecting the initial stage of seedling establishment: seed germination and early seedling survival. Here, we carried out a root competition exclusion experiment and a herbivory (including seed predation) exclusion experiment on 11 islands in Thousand Island Lake, China, using four native woody plant species that differed in functional traits (e.g., seed mass and dominance). The dominant species on islands showed the highest seedling survival, and there was no significant linear relationship between the proportion of surviving seedlings and island area under either treatment for any species. Compared to the control and excluding root competition treatments, excluding seed predation and herbivory significantly increased seedling survival after controlling for the environmental factors. However, abiotic factors had no effect on early seedling establishment. Our results suggest that seedling regeneration of rare species in fragmented ecosystems may be limited and that seedlings may be more susceptible to predators and herbivores in fragmented ecosystems. These results have significant implications for the conservation of plant diversity in fragmented forests.

Keywords: seedling recruitments; below-ground competition; herbivory; plant establishment; habitat fragmentation; islands; fragmented forest; biodiversity



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1. Introduction

Habitat loss and degradation caused by human land use have become the major threats to biodiversity [1,2]. In particular, about 70% of the planet's forests are fragmented and within 1 km of the forest's edge, subject to the degrading effects of habitat fragmentation [3]. Habitat fragmentation has become one of the main factors affecting the dynamics of forest communities [2,4,5]. Seed germination and early seedling recruitment are the initial stages of plant establishment [6,7], therefore, represent a crucial bottleneck in the dynamics of fragmented forest communities [8–11]. Habitat fragmentation, in particular, can alter environmental conditions (e.g., the edge effect) [12], which can have cascading effects on biotic interactions (e.g., seed predation, herbivory, etc.) [13–15], potentially altering seed germination and seedling survival. To restore and conserve forest communities in fragmented habitats, dispersal limitations can be overcome by supplementing forest fragments with seeds, but far less is known about how to overcome limitations of plant establishment (i.e., seed germination and seedling growth) [16]. It is important to understand the main ecological processes affecting plant establishment in fragmented ecosystems [8,17–19].

Forest fragmentation mainly affects the abiotic conditions and biotic interactions close to the edge of the patches (i.e., edge effects) [20–22]. Seedling establishment may be directly limited by changes in soil conditions (e.g., soil depth and litter depth) [23], light intensity

(e.g., canopy openness), and topographical factors (e.g., altitude and aspect) [24,25], or indirectly by altering the strength of herbivory and competitive interactions [20,26]. For example, insect herbivory, which alters recruitment, can mediate the edge effect on seedling diversity [12]. Meanwhile, species with different functional traits (e.g., shade tolerance) may be affected differently by changes in the environment of fragments. For example, seedlings of shade-tolerant species may be more likely to survive in large patches than would seedlings of shade-intolerant species [27,28].

Competition for soil nutrients and water resources is also thought to be one of the most important biotic filters restricting seedling establishment and growth, especially at lower nutrient levels [6,29,30]. As soil resources become more limiting with decreasing patch size and increasing edge effects, root competition could become more intense. However, compared with aboveground ecological processes (e.g., herbivory), belowground ecological processes, such as root competition, have been largely ignored. It remains unclear whether belowground root competition is important for determining the survival and growth of establishing species in fragmented forests.

Furthermore, plant establishment may be limited at different ontogenetic stages, especially during the first growing season when they are most vulnerable (i.e., seed germination and seedling recruitment) [6]. Seeds could be eaten by seed predators during the seed-to-seedling transition, which is one of the main factors affecting the final rates of seed germination and seedling recruitment [31–33]. More isolated and smaller patches can lead to the extinction of top predators due to resource constraints, thus resulting in the ecological release of small mammal populations (such as the rodents) [34–36], which would exacerbate seed predation in smaller patches [15], especially for large seeds [37]. When seeds germinate, seedlings could also be eaten by small mammals or herbivores, limiting successful establishment [20]. Therefore, understanding the relative roles of abiotic (e.g., soil conditions, light intensity, and the distance to patch edge) and biotic factors (e.g., seed predation, herbivory, and belowground competition) during initial plant establishment is crucial for distilling the mechanisms driving community assembly and restoration success in fragmented forests.

Here, we investigated the relative importance of belowground competition, herbivory (including seed predation), and environmental factors on early plant establishment across early life stages (i.e., seed-to-seedling) for four species on the islands in the Thousand Island Lake, Zhejiang Province, China. Specially, we aimed to answer the following questions: (1) are there differences in successful early establishment for species with different functional traits (e.g., shade tolerance) on islands when island size is considered; (2) what are the key factors that affect the early plant establishment on islands; and (3) are the key factors affecting plant establishment consistent among species?

2. Materials and Methods

2.1. Study Site

The study was conducted at the Thousand Island Lake (TIL) in Zhejiang Province, eastern China (Figure 1). The TIL is a hydroelectric reservoir, which was formed by the construction of the Xin'an River Dam in 1959 [38]. The lake has a total area of 581 km² and has 1078 land-bridge islands (>0.25 ha), and a designed highest water level of 108 m above the sea level. All islands were isolated at the same time, beginning with the damming of the Xin'an River and subsequent flooding of the landscape [23]. Forests were completely clear-cut during the construction of the reservoir dam, and contemporary forests on the islands are of similar succession age (i.e., about 60 years) [39]. The major vegetation type is a secondary successional forest dominated by Masson pine (*Pinus massoniana*) with broad-leaved plants (e.g., *Loropetalum chinense*) in the sub-canopy and understory [40]. The study area is characterized by a subtropical monsoon climate. The mean annual temperature in the TIL region is 17.0 °C, with a frost-free season of 241–296 days, and daily temperatures ranging from −7.6 °C in January to 41.8 °C in July. The annual precipitation varies from 1148 to 2015 mm, with 155 days of precipitation per year, mostly between April and June [40].

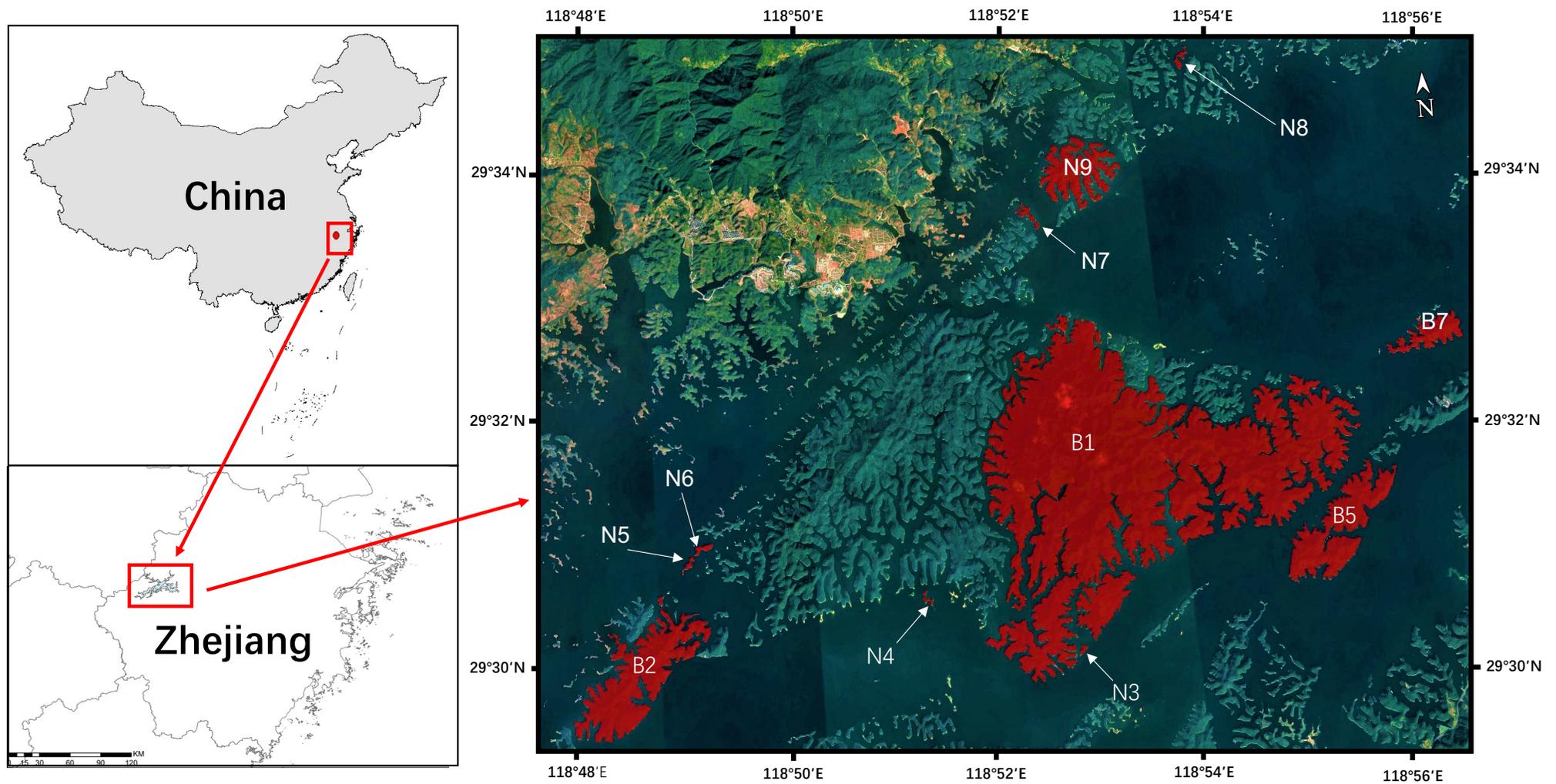


Figure 1. Map of the Thousand Island Lake (TIL) region and the distribution of studied islands (red color).

2.2. Experimental Design

To determine how belowground competition and herbivory (including seed predation) affect plant establishment in fragmented forests, we used a large-scale experiment consisting of 11 islands ranging in area from 1.06–1153.88 ha to ensure that sampling plots were representative of the range of forest fragment attributes (Figure 1). The island area was measured using ArcGis 10.8.

For seed germination and seedling growth (i.e., early plant establishment) experiments, we set up two experimental sites on each small island and four experimental sites on each large island, for a total of 32 sites on all islands. At each experimental site, we set up a 20 m × 20 m experiment plot. According to previous research in TIL, each plot was classified as an “edge plot” if it was within 40 m of the edge of the island; otherwise, it was classified as an “interior plot”, because plant species composition showed differences between the edge and interior plots [27]. Since all the plots were close to the island edge, two edge plots were established on islands smaller than 5 ha (here, we call these islands small islands). Two replicated plots were established on the interior and edge of each island on islands larger than 20 ha (i.e., large islands) (Figure 2). Among these islands, six small islands are less than 5 ha, and five large islands are greater than 20 ha. In total, 32 plots were established on all islands.

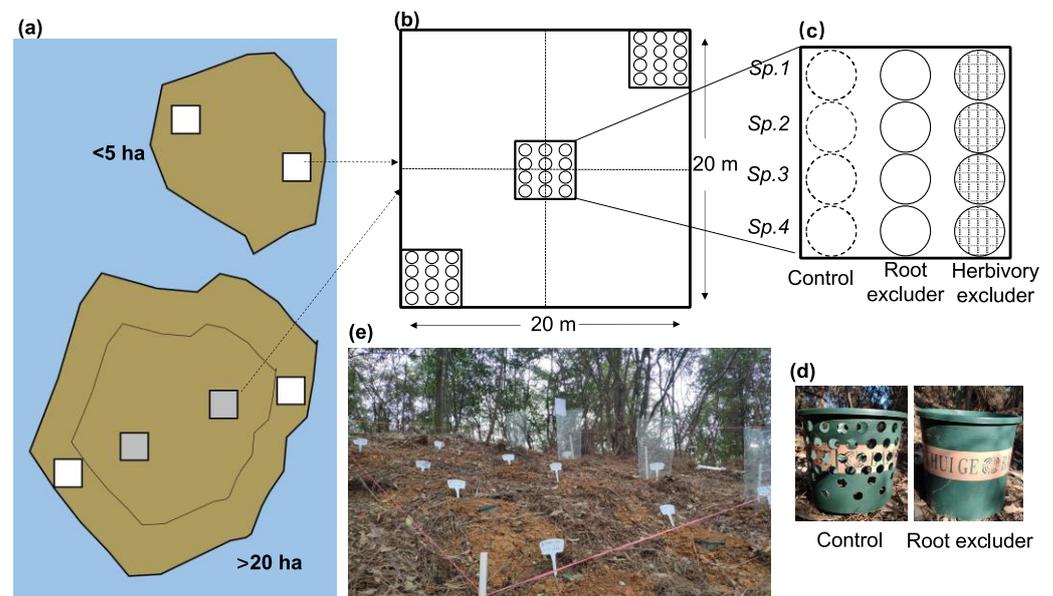


Figure 2. The experiment was conducted on small islands (<5 ha) and large islands (>20 ha) in the TIL (a). The experiment contained two edge plots (<40 m to island edge) on all islands and an additional two interior plots (>40 m) on large islands (a). Each plot contained three subplots (b) with 12 flowerpots that manipulated belowground competition (dotted line signifies perforated flowerpots to permit other plant roots to grow in; solid line signifies root-excluding flowerpots to prevent root competition) and aboveground herbivory (including seed predation) (circle mesh signifies a fence over the flowerpots to exclude seed predation and seedling herbivory) for four species (c). Root-excluding flowerpots were 21 cm high × 23 cm wide, and all flowerpots were open on the bottom (d). A photograph of a subplot in the experiment appears in (e).

Each plot contained three subplots that were evenly distributed along the diagonal of the plot. In each of the subplots, three seed germination and seedling growth treatment devices were set up c. 0.5 m apart in a loose spatial block for each of the four plant species (Figure 2): (1) a “control” treatment: the side of a flowerpot was drilled with 2 cm holes to allow other plant roots to grow inward and simulate the growth environment of seeds germinating in natural conditions; (2) a “root exclusion” treatment: the sides of the flowerpots were remained intact to prevent roots of other plants from entering

the flowerpot, thus preventing root competition; (3) a “full-cage or herbivory exclusion” treatment: the sides of the flowerpots were remained intact, and mask cages and fences were placed above the flowerpots to prevent predation of seeds and seedlings by animals (e.g., rodents [15]). This setup gave a total of 1152 flowerpots across all sites: (6 small islands \times 2 plots \times 3 subplots + 5 large islands \times 4 plots \times 3 subplots) \times 4 species \times 3 treatments. We measured the effect of belowground competition on establishing seedlings by comparing the number of surviving seedlings between the “Excluding root competition” and the “Control” treatments and by measuring herbivory from animals by comparing the number of surviving seedlings between the “Control”, “Excluding root competition”, and the “Excluding herbivory” treatments.

Flowerpots were installed from December 2020 to January 2021, prior to adding seeds of target species, in order to allow root growth from the surrounding community into the perforated flowerpots.

2.3. Species Selection and Sampling

We selected four species (i.e., *Cinnamomum camphora*, *Cyclobalanopsis glauca*, *Loropetalum chinense*, and *Schima superba*) (Table 1). Two species (*L. chinense* and *S. superba*) are generalist species on islands in the TIL, and the other two species are remnant specialists on islands. All seeds were collected in autumn 2020 before the sowing experiment in the TIL region. We added 20 individuals of all four species to each flowerpot in early March 2021 by removing the leaf litter and existing rooted plants, scarifying the soil surface, and gently tamping the seeds into the soil to increase seed-soil contact. All seeds were seen to be vital, based on exposure to 5% TTC dye (2,3,5-triphenyltetrazolium chloride) solution prior to planting.

Table 1. The attributes of experimented species.

Species	Seed Mass (g) ¹	Shade Tolerance	Dominance ²
<i>Cinnamomum camphora</i>	12.75	Shade intolerant	Rare
<i>Cyclobalanopsis glauca</i>	180.34	Shade tolerant	Rare
<i>Loropetalum chinense</i>	2.43	Shade intolerant	Dominant
<i>Schima superba</i>	0.66	Shade tolerant	Common

¹ Seed mass were calculated by the average weight of 100 seeds. ² The abundance of species on islands according to the field observations.

In April 2022, all surviving seedlings were identified and counted in each flowerpot. The seed germination and early seedling survival monitoring experiment lasted for 4 months. After the last measurement of the germination rate in July, no new seedlings appeared in the flowerpots, which was marked the end of the experiment. At the end of the experiment, the number of seedlings that were alive in the pots was used as a measure of the success of the early establishment of the species (Table S1).

2.4. Measurement of Environmental Factors

To test how environmental factors affected plant establishment, we used six environmental variables in each experimental subplot that focused on key limiting environmental attributes thought to be the most important for plants in this system [23,27] or other studies [24]: slope, altitude, soil depth, litter depth, irradiance, and edge distance in each experimental subplot (Table S2). Irradiance (or canopy openness) was quantified for each subplot with hemispherical photographs taken at 1.3 m above the soil surface. Photographs were taken with a Canon 6D MARK II digital camera connected to a Sigma 4.5-mm fisheye lens mounted on a tripod. Hemispherical photographs were processed using Hemiview v. 2.1 software. Photos were taken in the early morning from 9 July through to 15 July when skies were uniformly overcast. Environmental variables were averaged for three subplots to estimate the values for the entire plot.

Google Earth Pro (V7.3) was used to calculate the distance from the middle of the plot to the nearest edge of the island (i.e., edge distance), which was assumed to be an indicator of the edge effect [27] (Table S2).

2.5. Data Analysis

To test for differences in plant establishment (i.e., the number of surviving seedlings) among the four species under each treatment, we used a one-way ANOVA and Tukey's post-hoc comparisons to test for differences among species in the proportion of surviving seedlings in each plot. We also used linear regression analysis to test whether the island area influenced plant establishment success among different treatments for each species.

Analysis of covariance (ANCOVA) was used to test whether the difference in the proportion of surviving seedlings in each plot was related to the effect of the treatments (independent variable) while controlling for the influence of the environmental factors (covariate). Before analysis, a principal component analysis (PCA) was performed on all standardized environmental variables (i.e., altitude, soil depth, litter depth, slope, irradiance, and edge distance) to identify the major differences in environmental variables between plots. The first two principal components (PC1 and PC2), which accounted for 62.94% of the total variance, were used separately in the ANCOVA analysis as environmental factors. In addition, ANCOVA was conducted for each environmental variable alone as a covariate. Because the results were similar when using either the PCs or the single environmental variables, we reported only the results using PCs. To better visualize the differences in plant establishment between the three treatments, we also plotted the relationship between the number of surviving seedlings and PCs for each species.

Finally, we tested the combined effects of abiotic and biotic (excluding herbivory and predation treatments) factors on plant establishment using linear mixed-effects models (LME) [41]. Multicollinearity between variables (i.e., soil depth, litter depth, slope, irradiance, edge distance, island area, and treatment) was tested using variance inflation factors (<10), and the response variable (the proportion of surviving seedlings) for each species was found to be normally distributed using a Shapiro–Wilk test. In the LME models, environmental factors and treatments were taken as fixed effects, and the nested relationship between plots and islands was taken as a random effect to account for the lack of independence among plots on each island. All numeric variables were log-transformed to induce normality in models.

All analyses were conducted using R software (version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria) with the packages *lmerTest*, *multcompView*, and *Agricolae*.

3. Results

3.1. Differences in Seed Germination and Early Seedling Survival among Species

The proportion of surviving seedlings differed significantly among the four species, with *L. chinense*, a dominant species on the islands, having the highest proportion of surviving seedlings under all three treatments (Figure 3). *C. camphora* and *S. superba* had higher proportions of surviving seedlings than *C. glauca* under the control and excluding root competition treatments (Figure 3a,b), while *C. camphora* had a higher proportion of surviving seedlings than *C. glauca* and *S. superba* (Figure 3c). Meanwhile, there was no linear relationship between germination rates of the four species and island area in the control (Figure 3d), excluding root competition (Figure 3e), or excluding herbivory (Figure 3f) treatments, indicating that island size did not affect differences between species.

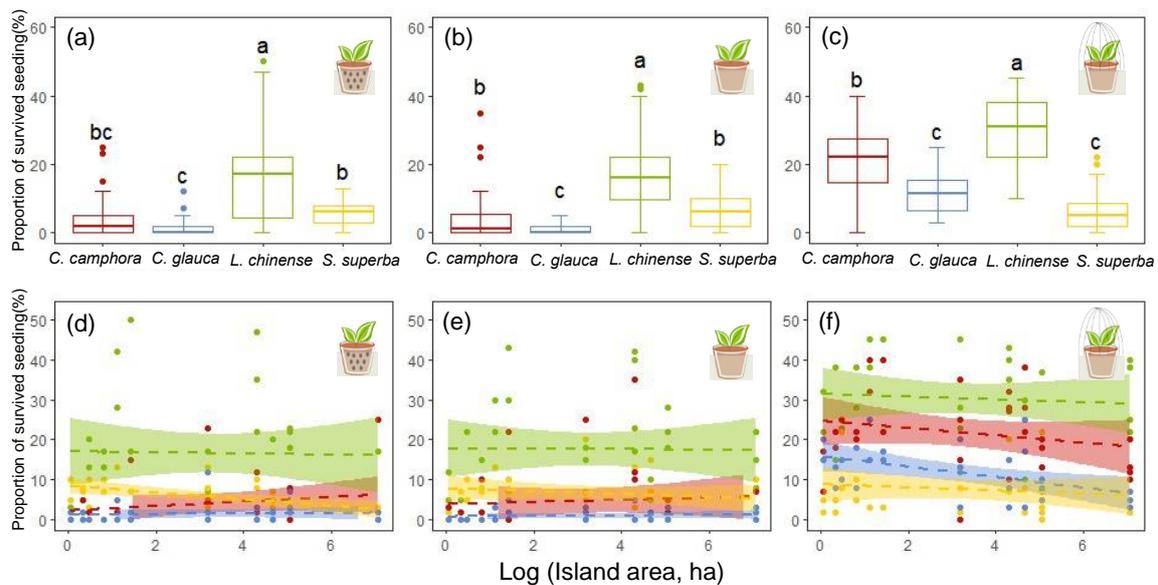


Figure 3. Differences in the proportion of surviving seedlings among the four species, and the relationship between the proportion of surviving seedlings and island area for species in the control (a,d), excluding root competition (b,e), and excluding herbivory (c,f) treatments. In (a–c), letters denote the significance ($\alpha = 0.05$) of pairwise comparisons assessed using Tukey’s post-hoc comparisons. The dotted lines in (d–f) are the best fit linear regression lines and the shading indicates the 95% confidence intervals around the regression, the red dotted lines refer to the relationships for *Cinnamomum camphora*, blue dotted lines refer to the relationships for *Cyclobalanopsis glauca*, green dotted lines refer to the relationships for *Loropetalum chinense*, and yellow dotted lines refer to the relationships for *Schima superba*.

3.2. Differences among Treatments in Seed Gemination and Early Seedling Survival

The first two axes of the PCA explained c. 62% of the variation in environmental variables (Figure 4): the first PC axis (PC1) explained 33.99% of the variance and was positively correlated with irradiance and negatively correlated with altitude, edge distance, and slope. Because PC1 was associated with environmental changes along the edge gradient, we refer to PC1 as the “edge effect” axis. The second PC axis (PC2) explained 28.95% of the variance and was positively related to soil depth and litter depth. Because these variables indicate soil resources, we refer to PC2 as the “soil resource” axis.

There was a significant positive effect of excluding herbivory on the proportion of surviving seedlings after controlling for PC1 for *C. camphora* ($F_{3,92} = 35.19, p < 0.001$), *C. glauca* ($F_{3,92} = 46.46, p < 0.001$), and *L. chinense* ($F_{3,92} = 9.218, p < 0.001$) (Table 2), and after controlling for PC2 (Table A1). Compared to the control and excluding root competition treatments, the excluding herbivory treatment led to significantly more seedling survival (Table 2, Figure 5), and there was no significant relationship between the proportion of surviving seedlings and PC1 or PC2 (Figure 5).

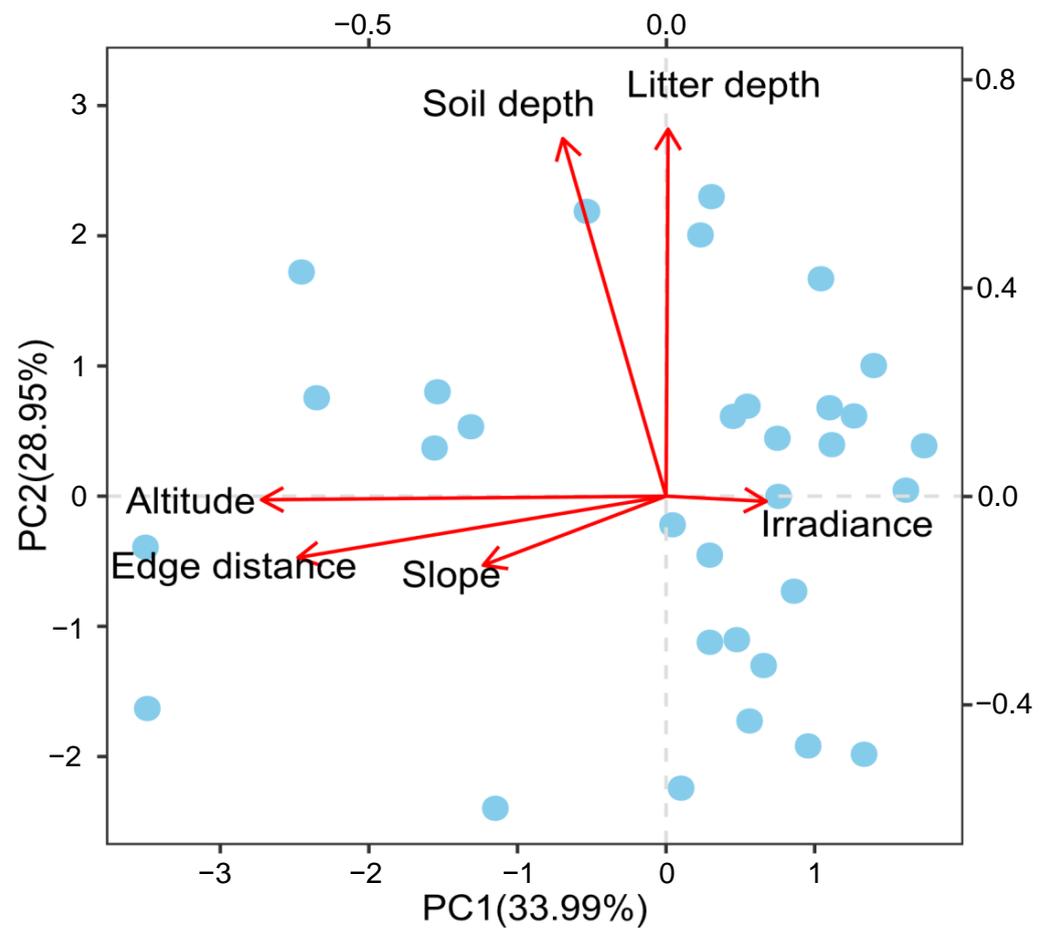


Figure 4. Principal component analysis (PCA) for six environmental factors.

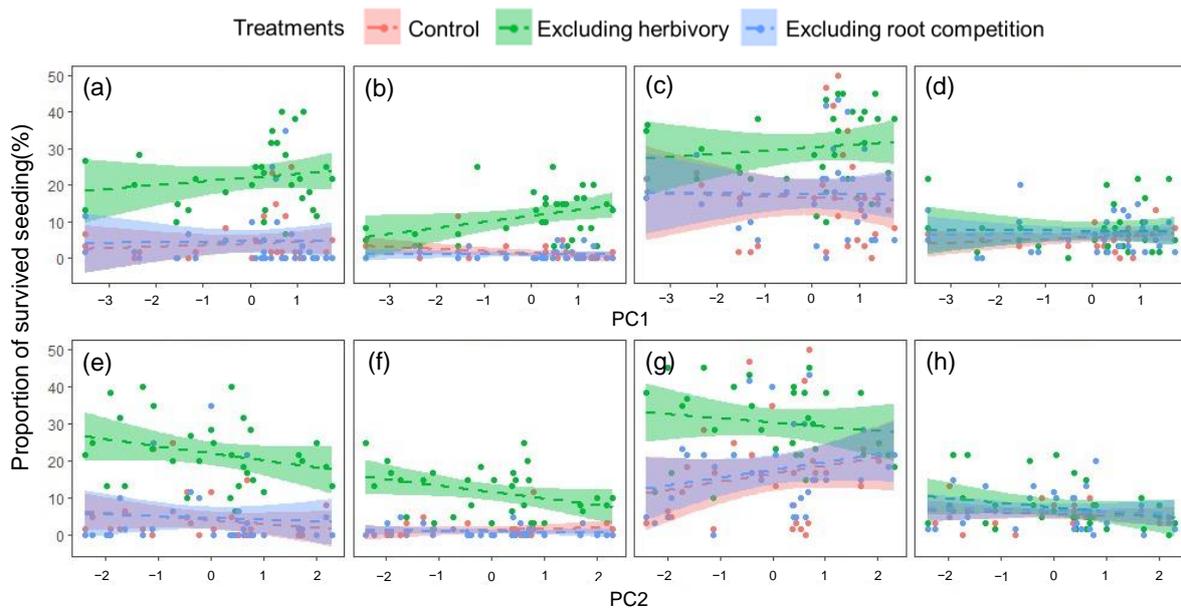


Figure 5. Relationships between the proportion of surviving seedlings and PC1 and PC2 for *Cinnamomum camphora* (a,e), *Cyclobalanopsis glauca* (b,f), *Loropetalum chinense* (c,g), and *Schima superba* (d,h). The dotted lines and shading are the best fit linear regressions and the 95% confidence intervals around the regressions.

Table 2. Analysis of covariance (ANCOVA) for the four species. The dependent variables were the proportion of surviving seedlings in each plot for each species, the independent variable was a categorical variable indicating treatment (control, excluding herbivory, and excluding root competition), and PC1 was included as a covariate. The significance of the regression coefficients and the adjusted R-squared (R^2_{adj}) values are also presented. Significance level: *** $p < 0.001$.

	Excluding Herbivory	Excluding Root Competition	PC1	R^2_{adj}	$F_{3, 92}$	p
<i>C. camphora</i>	18.012 ***	0.625	0.528	0.519	35.19	<0.001
<i>C. glauca</i>	10.194 ***	−0.422	0.363	0.589	46.46	<0.001
<i>L. chinense</i>	13.744 ***	1.041	0.143	0.206	9.218	<0.001
<i>S. superba</i>	1.755	0.841	0.161	−0.010	0.681	0.566

3.3. The Effect of Abiotic and Biotic Factors on Early Seedling Survival

The biotic variable (i.e., excluding herbivory) rather than abiotic variables significantly affected the proportion of surviving seedlings for *C. camphora* ($t = 10.670, p < 0.001$), *C. glauca* ($t = 10.175, p < 0.001$), and *L. chinense* ($t = 8.031, p < 0.001$), but none of the biotic and abiotic factors showed any significant effect on the proportion of surviving seedling for *S. superba* (Table 3).

Table 3. Linear mixed-effects model (LME) results. The response variable was the proportion of surviving seedlings for each species. Six abiotic variables (soil depth, litter depth, slope, irradiance, edge distance, and island area) and one categorical variable indicating the treatment were used as explanatory variables.

	Estimate	Std. Error	t Value	Pr ($> t $)
<i>Cinnamomum camphora</i>				
(Intercept)	4.806	15.795	0.304	0.764
Soil depth	−0.627	5.867	−0.107	0.916
Litter depth	−4.784	6.913	−0.692	0.495
Slope	4.010	2.754	1.456	0.157
Irradiance	−9.724	7.423	−1.310	0.201
Edge distance	−3.510	2.020	−1.738	0.094
Island area	0.259	0.528	0.491	0.628
Excluding herbivory	17.939	1.681	10.670	<0.001
Excluding root competition	0.5518	1.681	0.328	0.744
<i>Cyclobalanopsis glauca</i>				
(Intercept)	6.063	6.548	0.926	0.357
Soil depth	−0.006	2.505	−0.002	0.998
Litter depth	−4.450	2.893	−1.555	0.124
Slope	0.416	1.158	0.360	0.720
Irradiance	−3.013	3.109	−0.969	0.335
Edge distance	−0.478	0.840	−0.569	0.571
Island area	−0.432	0.219	−1.976	0.051
Excluding herbivory	10.194	1.002	10.175	<0.001
Excluding root competition	−0.422	1.002	−0.421	0.675
<i>Loropetalum chinense</i>				
(Intercept)	−4.915	26.364	−0.186	0.854
Soil depth	9.131	9.465	0.965	0.342
Litter depth	−1.790	11.499	−0.156	0.878
Slope	2.772	4.302	0.644	0.525
Irradiance	−17.321	11.995	−1.444	0.161
Edge distance	−4.158	3.103	−1.340	0.196
Island area	0.251	1.118	0.225	0.827
Excluding herbivory	13.544	1.687	8.031	<0.001
Excluding root competition	0.841	1.687	0.499	0.620

Table 3. Cont.

	Estimate	Std. Error	t Value	Pr (> t)
<i>Schima superba</i>				
(Intercept)	19.113	11.460	1.668	0.108
Soil depth	−5.494	4.229	−1.299	0.203
Litter depth	3.684	5.010	0.735	0.469
Slope	−1.144	1.939	−0.590	0.560
Irradiance	3.856	5.338	0.722	0.476
Edge distance	1.172	1.340	0.838	0.413
Island area	−0.559	0.425	−1.317	0.231
Excluding herbivory	1.687	1.015	1.662	0.101
Excluding root competition	0.752	1.015	0.741	0.461

4. Discussion

Exploring factors limiting plant establishment is important for understanding forest community assembly and the maintenance of species diversity in fragmented forests [20,26]. By experimentally manipulating seed and seedling herbivory and belowground root competition on subtropical islands, we showed that species with different functional traits (i.e., shade tolerance and dominance) have different regeneration rates and that herbivory, rather than root competition and environmental factors, determines establishment in fragmented forests during the seed-to-seedling stage.

On these islands, *L. chinense* had the highest relative abundance in the understory [23]. We further found that *L. chinense* had the highest proportion of seedling survival compared to the other three species studied. The higher seedling survival rate of dominant species on islands may be related to the fact that current environmental conditions on islands are suitable for seed germination and seedling survival of dominant species. In addition, maintaining a high abundance or having a high seed production rate may reduce the probability of being eaten by predators or herbivores (i.e., the predator satiation hypothesis) [39,42,43].

Our results further showed that herbivory rather than belowground root competition and environmental factors determined early seedling recruitment for the other three species. Many studies have indicated that habitat fragmentation can have cascading effects on the seed dispersal and predation; for example, habitat loss and fragmentation may drive large vertebrates to extinction, and the loss of predators may have indirect effects on seed predation by releasing rodents from top-down limitation and allowing them to more effectively consume seeds [34,35]. Similarly, in our study system, defaunation by large mammals has been shown to alter competition for food among rodents and, thus, change seed dispersal [15]. We also found that preventing seed predation and seedling herbivory could significantly increase seedling recruitment (Table 2): Recruitment increased by 18.02% in *C. camphora*, by 10.20% in *C. glauca*, and by 13.75% in *L. chinense*, but there was no increase for *S. superba* (Figures 4 and 5). Our results, along with those from previous studies [8], suggest that post-dispersal seed predation and seedling herbivory may be the main factor limiting seedling recruitment on islands, but that the effect may depend on seed traits, such as seed mass. Vertebrates tend to feed on larger seeds due to higher visibility and nutrient content [44], such as the large seeds of Fagaceae (e.g., *C. glauca*). For species like *S. superba*, which have small seeds, the seed predation may not be the main factor limiting seedling recruitment [45].

Contrary to our expectations and other studies (e.g., [6]), we did not find that belowground root competition influenced seedling recruitment. Belowground competition could be more important to survival and growth in resource-poor environments, such as those with limited soil water availability, than in resource-rich environments [46]. Unlike other research sites, our study site was subtropical, with frequent rainfall from April to July. Adequate water probably mitigated the effect of root competition on seedling survival [47].

The island area may also affect the functional trait composition. For example, at our research site, larger islands have more late-successional or shade-tolerant woody

plant species than smaller islands [27]. However, in the current study, we found that there was no simple linear relationship between seedling recruitment and the island area (Figure 2, Table 3), although species varied in shade tolerance. Similarly, no significant linear relationships between fragment size and seed germination or seedling growth have been found for a bird-pollinated shrub [9]. The lack of a clear relationship may be due to the fact that our study focused on the early germination stage, while the results of Liu et al. [27] were focused mainly on individuals with DBH above 1 cm. Thus, although the island area had no effect on seedling establishment in the early stage, the island area combined with the edge effect might influence seedling survival over the longer term, such as for shade-tolerant species [28]. Meanwhile, local environmental factors (e.g., edge effects and soil resources) did not have an effect on the early seedling establishment (Table 3). Many studies have found similar results; for example, Burgos et al. [8] also found that increased post-dispersal seed predation determined seed germination but not canopy openness or soil humidity in small remnants of the Maulino forest, and Krishnadas and Comita [12] found that edge effects on seedling diversity are mediated by impacts of fungi and insects on seedling recruitment but not on survival.

Our findings have significant implications for the restoration and conservation of plant diversity in forest fragments. For species with larger seeds that are easier to be preyed upon by mammals such as rodents, seed germination and regeneration can be significantly improved by excluding seed predation and seedling herbivory. However, for species with small seeds that have low germination rates, direct seedling transplantation may improve seedling recruitment. In addition, more attention should be paid to rare species in fragmented ecosystems due to their limited regeneration and the possibility that rare species may be more susceptible to predation and herbivory [39].

5. Conclusions

Our study excluded roots and herbivory to alter competition and predation (including seed predation and seedling herbivory) to test the main factors determining early seedling establishment. Our results showed that rare species on islands have a relatively low establishment and that biotic factors (i.e., seed predation and seedling herbivory) are the main drivers of seed germination and seedling survival during the initial stages of seedling establishment, especially for species with large seeds. These results indicate that vegetation restoration in fragmented habitats may be more successful by excluding seed predators and seedling herbivores. In addition, the limited establishment of rare species and herbivory affect the dynamics of fragmented forest communities over the long term.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f13050767/s1>, Table S1: The number of survived seedlings in 32 plots on 11 islands; Table S2: The environmental variables in 32 plots on 11 islands.

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Appendix A

Table A1. The analysis of covariance (ANCOVA) results for four species. For the ANCOVA analysis, the dependent variables were the proportion of survived seedlings in each plot for each species, the independent variable was a categorical variable (treatment: control, excluding herbivory, and excluding root competition) and the PC2 was included as a covariate. The significance of the regression coefficients and the adjusted R-squared (R^2_{adj}) values are also presented. Significance level: *** $p < 0.001$.

	Excluding Herbivory	Excluding Root Competition	PC2	R^2_{adj}	$F_{92, 3}$	p
<i>Cinnamomum camphora</i>	18.012 ***	0.625	−1.106	0.531	36.85	<0.001
<i>Cyclobalanopsis glauca</i>	10.194 ***	−0.422	−0.529	0.595	47.48	<0.001
<i>Loropetalum chinense</i>	13.744 ***	1.041	1.044	0.217	9.776	<0.001
<i>Schima superba</i>	1.775	0.841	−0.634	0.014	1.453	0.233

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