



Rodent suppression of seedling establishment in tropical pasture

Crystal A. Guzmán¹ · Henry F. Howe² · David H. Wise³ · Rosamond I. Coates⁴ · Jenny Zambrano⁵

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Abstract

Grasses are recognized as a critical regeneration barrier in tropical pastures, yet the effects of rodents and rodent–grass interactions are not well understood. As selective foragers, rodents could shape tree communities, moderating biodiversity in regenerating tropical landscapes. We utilized a fully crossed two-way factorial design to examine the effect that grasses, rodents, and their interaction had on tree seedling establishment in pasture habitat. We followed two separate tree cohorts for 1 year each within the experimental framework. Multiple cohorts were used to better represent successional tree species variation and responses. Trees species were characterized by a gradient of seed masses and as pioneer or persistent successional type. Both cohort seedlings were altered when rodents were present compared to control treatments. In Cohort 1, rodents adversely affected seedlings of persistent tree species only in the absence of grass. In Cohort 2, seedlings of persistent tree species were decimated by rodents in the absence or presence of grass. In both cohorts, seedlings of persistent species established better in grass treatments, while seedlings of pioneer tree species were strongly suppressed. Tree species seed mass positively correlated with seedling establishment across all treatments except no grass–rodent treatments. Strong suppression of tree seedlings by rodents (*Sigmodon toltecus*) is a novel result in tropical land recently released from agriculture. One implication is that selective foraging by rodents on large-seeded persistent tree species may be facilitated by the removal of grass. Another implication is that temporary rodent control in pastures may permit higher establishment of deep-forest persistent species.

Keywords Regeneration barriers · Rodent exclosure · Seed mass · *Sigmodon toltecus* · Tropical forest

Introduction

Worldwide, escalating tropical deforestation results in a significant loss of biodiversity. Subsequent secondary-growth forest regeneration does not consistently recover that biodiversity (Chazdon 2014; Song et al. 2018). Diminished forest recovery can be characterized by reduced tree diversity and attributed to the limited movement of larger

seeded, mid to late-succession trees from forests into regenerating habitat (Saavedra et al. 2015). Since seed dispersal manifests as a leading regeneration bottleneck (Cole et al. 2011), the introduction of non-pioneer or persistent tree species into regenerating habitat can offer a means to tree diversity recovery. Seed arrival alone, however, does not secure tree establishment (Reid and Holl 2013). Post-dispersal regeneration barriers regularly impede tree establishment (Hooper et al. 2002). The challenge of

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✉ Crystal A. Guzmán
crystal.a.guzman@gmail.com

Henry F. Howe
hfhowe@uic.edu

David H. Wise
dhwise@uic.edu

Rosamond I. Coates
rcoates@ib.unam.mx

Jenny Zambrano
jenny.zambrano@wsu.edu

¹ 71 Potomac Ave SE Apt 815, Washington, DC 20003, USA

² Liberal Arts and Sciences, University of Illinois At Chicago, 11219 HiddenView Farm Road, Marysville, IN 47141, USA

³ University of Illinois At Chicago, 901 W Taylor St, Chicago, IL 60607, USA

⁴ Jefa de La Estacion de Biologia Tropical “Los Tuxtlas”, Instituto de Biologia, UNAM, San Andres Tuxtla, Apdo. Pos. 94, Veracruz, Mexico

⁵ School of Biological Sciences, Washington State University, PO Box 644236, Pullman, WA 99164-4236, USA

assisted tree diversity recovery then becomes to identify post-dispersal barriers in regenerating habitats.

Tree establishment can be defined as the transition from seed to seedling, in which biotic and abiotic habitat filtering reduces overall survival. Among regenerating habitat, poor tree establishment can be especially pronounced in pasture habitat, where animal-mediated seed rain of many non-pioneer species is limited (Martínez-Garza et al. 2009). Post-dispersal, increased exposure to seasonal drought, high temperatures, and high irradiance contribute to a low probability of establishment in pastures (Martínez-Garza et al. 2013). Above- and below-ground competition for resources with grasses also plays a critical role in diminishing establishment. The removal of grass in pasture habitat, for instance, often increases tree seedling survival (Román-Dañobeytia et al. 2012). A much-less studied potential barrier to tree colonization of abandoned pastures is the abundance of rodent populations (Howe and Davlantes 2017). Whether rodents strongly affect tree seedling establishment in tropical pastures is an open question.

Rodents shape succession in a variety of grassland habitats. Voles (*Microtus pennsylvanicus* Ord) influence prairie development by seed predation (Howe and Brown 2001), herbivory (Sullivan and Howe 2009), or both (Howe et al. 2006). In temperate old fields, voles selectively kill seedlings of some hardwood tree species but not others, shaping the course of forest succession (Manson et al. 2001). Similarly, in the absence of ungulate grazers, small rodent herbivores have substantial effects on savanna vegetation in Kenya (Keesing 2000). A comparable role by neotropical rodents on the establishment of tree species could pose a significant regeneration barrier in tropical pastures released from livestock grazing.

Potential effects that grass and rodents have on tree establishment may occur simultaneously or independently in early pasture habitat succession. Given the role second-growth trees have in shaping tropical biodiversity, it is pertinent to determine if rodent foraging (Randolph and Cameron 2001), in combination with grass suppression, favors some tree species establishment over others in pasture habitat. In early succession, tree seedlings diverge along major adaptive axes, including light requirement, resource use, and defense capability. Pioneer species require high irradiance to recruit, grow rapidly, and produce leaves with minimal defenses, making them vulnerable to herbivory (Goodale et al. 2014). Persistent species, are typically shade-tolerant, grow more slowly, and deter herbivory with physical and chemical defenses (Kitajima et al. 2012). Both successional types are also distinguished by contrasting seed masses, with larger seed masses, greater in maternal energy reserves, being typical of persistent tree species (Moles and Westoby 2004).

Existing evidence that grasses impede succession and that rodents shape other ecological communities introduce two questions with widespread implications. First, to what degree do rodents reduce tree establishment in tropical pastures released from grazing? Second, does suppression of trees by grasses and rodents strongly favor colonization of some forest tree species over others? Here, we address these questions using experimental exclosures and seed additions.

Methods

Study site

The Los Tuxtlas region, located in the southern state of Veracruz, Mexico (95°00' W, 18°25' N), represents the northernmost extent of New World tropical rainforest (Dirzo and Miranda 1991). This region is of volcanic origin, with topography ranging from 0 to 1780 m above sea level. The mean annual precipitation is 4,560 mm, and annual temperature varies between 24 and 26° C (Soto and Gama 1997). Deforestation between 1972 and 1993 eliminated ~84% of the forest, converting the landscape into an agricultural mosaic of crops, orchards, pastures, and forest fragments (Castillo-Campos and Laborde 2004). Regional census has recorded *Sigmodon* as the most abundant rodent in pastures (Estrada et al. 1994). In 1998, the Los Tuxtlas Biosphere Reserve was enacted as a protected forest of ~155,122 ha (Gutierrez-Garcia and Ricker 2011). Our study site (18°35'27" N, 95°6'49.02" W) was located adjacent to the forest of the Los Tuxtlas Tropical Biology Field Station on land heavily grazed by cattle from 1995 to 2010. Characteristic vegetation included exotic African Star Grass [*Cynodon plectostachyus* (K. Schum.)], which dominated most of the site, and native Grama [*Paspalum conjugatum* (Bergius)]. Released from grazing, these grasses provided a groundcover 50–100 cm high.

Experimental design

The effect that pasture grass, rodents, and any interaction between them had on tree establishment was evaluated using a fully crossed two-way factorial experiment. Seedling establishment was defined as survival after 1 year and evaluated for different treatments. Rodent exclosures were established in June 2010 (Fig. 1). Wire mesh (6.4 mm²) extended 140 cm above and 50 cm below the soil surface to prevent rodent access. Smooth metal flashing 20 cm wide on the outside prevented rodents from scaling the wire mesh. Four 1.5-m wooden poles supported each 2 × 4 m exclosure frame from the inside. In no grass treatments (NG), above-ground vegetation was removed monthly by hand. Treatments with grass cover (G) were not manipulated and simulated

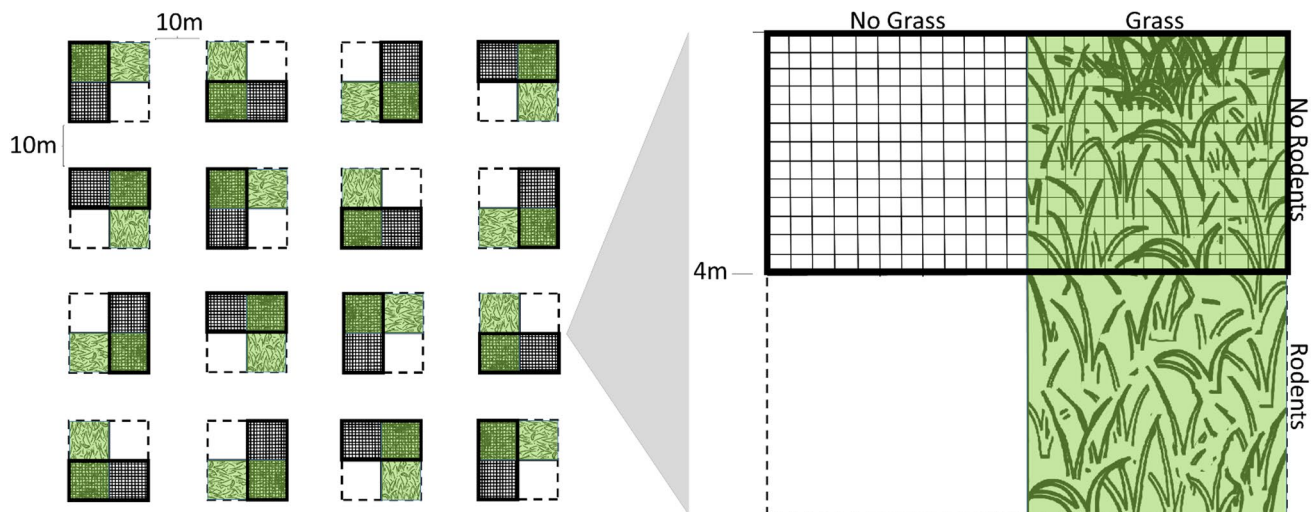


Fig. 1 Fully crossed factorial experiment treatment plot arrangement. Amplified block shows rodent mesh enclosure (bold), open access (dashed), and grass in absence–present combinations

grass succession. Sixteen 4 m² blocks set 10 m apart were arranged in a 4 × 4 matrix in the pasture. Each block subdivided into four 2 m² plots (Fig. 1). Each plot had four treatments: no grass–no rodents (NG–NR), no grass–rodent access (NG–R), grass–no rodents (G–NR), and grass–rodent access (G–R).

Grass and rodent effects on tree establishment were investigated in two consecutive experiments of 1 year each. Two species cohorts were used to best represent species variation and responses. Experiment 1 operated from June 2010 to June 2011, Experiment 2, from August 2011 to August 2012. Seeds for 10 tree species in Experiment 1 were collected from May to June 2010; seeds of 16 tree species in Experiment 2 were collected from June to August 2011 (Table 1). Tree species represented a gradient of seed masses and were identified as a pioneer or persistent successional type. Successional types were represented in approximately equal quantities in both cohorts. Differences between tree cohorts reflect the limited availability of fruiting tree species. Seeds were collected from three or more individual trees per species. Fruits were cleaned, pulp decanted off, and seeds were air-dried. Seeds were stored in paper bags for 1–6 weeks in cool storage until the start of each experiment. Plant nomenclature and seed mass data follows the Kew Royal Botanic Gardens Database (WCSP 2017). Successional type and dispersal syndrome follow Ibarra-Manríquez and Oyama (1992) and Ibarra-Manríquez et al. (2001).

In June of 2010, 50 seeds from each of the 10 tree species were sown into each 2 m² treatment plot and monitored until June 2011. After this period, tree seedlings were removed from plots. Pasture vegetation was then permitted to recolonize the experiment plots for one month. We conducted an additional experiment in August of 2011, using the same

experimental design and treatments from June 2010; 40 seeds for each 16 tree species were sown in each 2 m² treatment plots and monitored until August 2012. August 2011 per species seed counts were decreased, relative to cohort 1, to offset the increase of total tree species. To avoid trampling grass, monthly seedling survivorship was monitored only within non-grass treatments throughout each 12-month period. Each month, newly emerged seedlings were tagged and identified to species.

Rodents were trapped over 4 days and nights in monthly intervals, using 57 Sherman™ live-traps (10–12 h/trap) to estimate activity (Fig. 2) in the experimental area vicinity. Four live-traps, at a 5 m distance, were placed in a grid around each experimental block. Live-traps were also placed in each rodent enclosure to assure rodent absence. Bait weighed ~8 g and comprised of a mix of peanut butter and sunflower seeds. Captured rodents were identified, sexed, weighed, and released. Released rodents were marked by clipping 1–2 cm² of fur on the nape to record recaptures. All applicable institutional and/or national guidelines for the care and use of animals were followed (permit ACC 10-013).

Statistical analysis

To determine the effect that grass and rodent presence had on tree seedling establishment, a multivariate permutational analysis of variance (PerMANOVA, Anderson et al. 2008) with 9,999 random permutations was utilized in each experiment. PerMANOVA is a nonparametric multivariate statistical test used to compare groups or treatments and test a null hypothesis (Anderson 2001). Using rodent and grass variables as fixed factors, Bray–Curtis dissimilarity measures were applied to each experiment log-transformed [$\log(x + 1)$]

Table 1 Tree species characteristics organized by increasing seed mass

Scientific name ^a	Family ^a	Seed mass ^a (g)	Dispersal syndrome ^b	Successional type ^b
Experiment 1: 2010–2011				
<i>Piper umbellatum</i>	Piperaceae	0.000073	Animal	Pioneer
<i>Cecropia obtusifolia</i>	Urticaceae	0.000700	Animal	Pioneer
<i>Eupatorium galeottii</i>	Asteraceae	0.000900	Wind	Pioneer
<i>Heliocarpus appendiculatus</i>	Malvaceae	0.001351	Wind	Pioneer
<i>Ficus yoponensis</i>	Moraceae	0.001900	Animal	Pioneer
<i>Trema micrantha</i>	Cannabaceae	0.007000	Animal	Pioneer
<i>Tetrorchidium rotundatum</i>	Euphorbiaceae	0.048000	Animal	Persistent
<i>Bursera simaruba</i>	Burseraceae	0.117000	Animal	Persistent
<i>Cupania glabra</i>	Sapindaceae	0.149211	Animal	Persistent
<i>Sapindus saponaria</i>	Sapindaceae	0.767000	Animal	Persistent
Experiment 2: 2011–2012				
<i>Cecropia obtusifolia</i>	Urticaceae	0.000700	Animal	Pioneer
<i>Eupatorium galeottii</i>	Asteraceae	0.000900	Wind	Pioneer
<i>Ficus yoponensis</i>	Moraceae	0.001900	Animal	Pioneer
<i>Trema micrantha</i>	Cannabaceae	0.007000	Animal	Pioneer
<i>Ochroma pyramidale</i>	Malvaceae	0.008000	Wind	Pioneer
<i>Cordia stellifera</i>	Boraginaceae	0.010000	Animal	Persistent
<i>Albizia tomentosa</i>	Fabaceae	0.029546	Wind	Pioneer
<i>Stemmadenia donnell-smithii</i>	Apocynaceae	0.070000	Animal	Persistent
<i>Hampea nutricia</i>	Malvaceae	0.110000	Animal	Pioneer
<i>Bursera simaruba</i>	Burseraceae	0.117000	Animal	Persistent
<i>Cordia megalantha</i>	Boraginaceae	0.149200	Wind	Persistent
<i>Guarea grandifolia</i>	Meliaceae	0.800000	Animal	Persistent
<i>Brosimum alicastrum</i>	Moraceae	1.375000	Animal	Persistent
<i>Couepia polyandra</i>	Chrysobalanaceae	Animal	Persistent	
	2.100000			
<i>Cynometra retusa</i>	Fabaceae	6.500000	Animal	Persistent
<i>Unknown</i>	Celastraceae	n/a	n/a	n/a

^aNomenclatures and seed mass follows Kew Royal Botanic Gardens Database

^bDispersal syndrome and successional type follows (Ibarra-Manriquez and Oyama 1992; Ibarra-Manriquez et al. 2001)

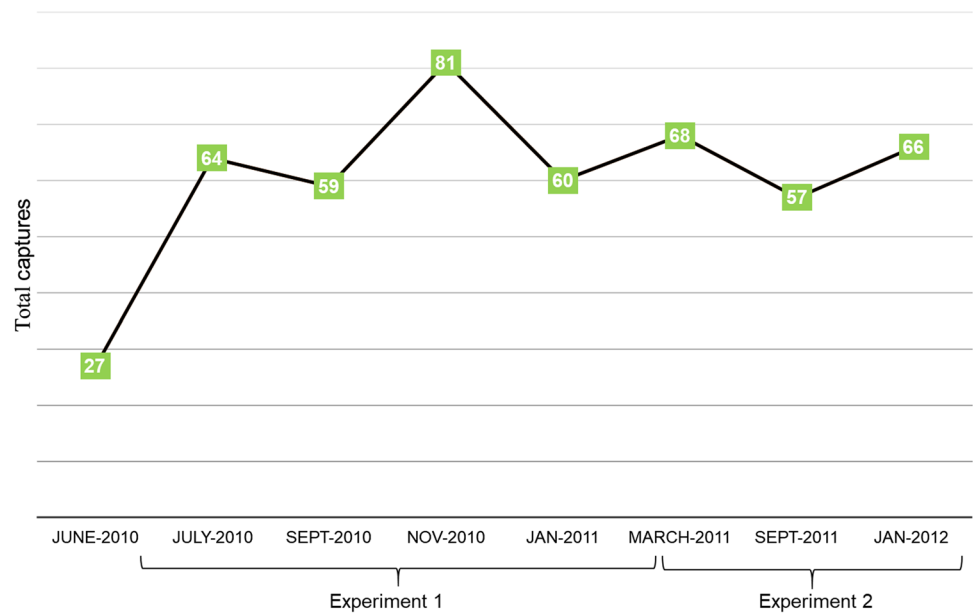
seedling abundance data (Anderson et al. 2008). Multivariate data was log-transformed to reduce the influence of highly abundant species. MANOVA and related analyses (e.g., GLMM) were not utilized as the multivariate datasets were not normal and did not meet model assumptions.

The multivariate dissimilarity matrixes were then used to visualize the association of successional types to each respective rodent–grass treatment group. Dissimilarity matrixes calculated the pairwise dissimilarities between the observations. To achieve this, canonical analysis of principal coordinate (CAP) ordinations were employed with a vector overlay (explanatory variable) of Spearman rank correlations ($r > 0.5$) (Anderson and Willis 2003). Ordinations were constrained by rodent and grass factors and visualized clustering or separation among seedlings. Clustering represents similarity among treatment plots. Ordinations are a multivariate technique used to interpret site or population

data point patterns in a multidimensional space. The first two components, with the two primary axes CAP 1 and CAP 2, which accounted for a large sum of the total variance, were used to plot the ordination. Supplemental univariate PerANOVA tests, with log-transformed $[\log(x + 1)]$ seedling abundance data, were conducted to explore the successional types and rodent–grass interaction further.

To evaluate the effect seed mass had on seedling establishment, data from both experiments were pooled. Established seedling abundance and seed mass were log-transformed $[\log(x + 1)]$. Tree species seed mass was examined for each treatment using four PerANOVAs with over 9,999 permutations. Univariate and multivariate tests used the *adonis* function (vegan package) in R version 3.6.2 (R Core Team 2019). Canonical analysis of principal coordinate ordinations were performed in Primer-E/PerMANOVA + software (Anderson et al. 2008).

Fig. 2 Experiments 1 and 2 cotton rat (*Sigmodon toltecus*) total captures in the Los Tuxtlas, Mexico pasture site (56 m²). Fifty-seven Sherman™ live-traps were placed in the experiment area for 4 days and nights (10–12 h/trap) in monthly intervals. An average of 60 *S. toltecus* individuals were captured throughout both experiments



Results

Overview of grass and rodent effects

All but one rodent captured was the cotton rat (*Sigmodon toltecus*) Saussure (Cricetidae), a genetically-defined cryptic species of the *Sigmodon hispidus* complex (Bradley et al. 2008). *Peromyscus mexicanus* was the other species recorded in the census. Overall, there were 482 *S. toltecus* captured in the experiment vicinity (Fig. 2). During trapping periods, no rodents were captured or observed in exclosures.

Tree species with the largest seed mass experienced higher establishment across all treatments. In Experiment 1, 7 of 10 study species established with a total of 174 individual seedlings, representing 0.02% of 8,000 seeds sowed. In Experiment 2, 8 of 16 study species established in treatments, with a total of 659 individual seedlings representing 0.06% of 10,240 seeds sowed. The establishment of *Sapindus saponaria* was disproportionately high, representing 57% of 174 seedlings in Experiment 1. Comparable dominance by individual species was not observed in Experiment 2. Species with the highest seedling densities included *C. polyandra* and *B. alicastrum*. For more information on per species abundance, refer to Electronic Supplemental Material (ESM) Online Resource 1 and 2.

Rodent and grass effects on the seedling community establishment by successional type

Seedling composition varied across Experiment 1 treatments (Table 2a). Pairwise tests indicated that differences in seedling abundances occurred between NG–NR vs. NG–R

treatments ($p = 0.043$) and NG–NR vs. G–NR ($p = 0.006$). These results were consistent with the clustering patterns observed in the Canonical Analysis of Principal Coordinate (CAP), which explained 38% of the total observed variance. Separation of experimental treatments in the ordination space occurred primarily between NG–NR and NG–R centroids (explanatory variables) (Fig. 3).

Table 2 Experiment permutation multivariate analysis of variance (PerMANOVA) results based on Bray–Curtis dissimilarity measure for standardized abundance data with grass, rodents as fixed factors and experiment block (16) as a random factor

Source	df	SS	MS	Pseudo-F	P(perm)
a. Experiment 1: 2010–2011					
Grass	1	3850.9	3850.9	6.8152	0.003
Rodent	1	1159.3	1159.3	2.6332	0.078
Block	15	23,797	1586.5	2.8117	0.003
Grass × rodent	1	2510.4	2510.4	4.4493	0.014
Grass × block	15	8475.6	565.04	1.0014	0.477
Rodent × block	15	6603.9	440.26	0.78028	0.774
Res	15	8463.5	564.23		
Total	63	54,860			
b. Experiment 2: 2011–2012					
Grass	1	12,638	12,638	13.211	0.001
Rodent	1	23,298	23,298	28.234	0.001
Block	15	16,147	1076.5	1.7121	0.028
Grass × rodent	1	2181.7	2181.7	3.4699	0.037
Grass × block	15	14,349	956.63	1.5215	0.098
Rodent × block	15	12,377	825.16	1.3124	0.215
Res	15	9431.2	628.74		
Total	63	90,422			

In CAP, successional seedling type vector association to treatments indicated increased abundance. In Experiment 1, both successional seedling type vectors positively correlated with NG–NR treatments but separated along both CAP axes displaying increased seedlings densities among persistent and pioneer species in Grass and NG–R treatments, respectively (Fig. 3). Univariate PerANOVA analyses supported CAP findings, and documented successional seedling type [$\text{Pseudo}F_{(1,127)} = 11.22$, $p = 0.001$; ESM Resource 3] and seedling type–grass interactions [$\text{Pseudo}F_{(1,127)} = 3.82$, $p = 0.023$; ESM Resource 3].

When analyzed by successional type (Fig. 4a), seedlings of persistent tree species were 4 times the abundance in grass vs. non-grass plots when rodents were present (GR vs. NG–R), uncovering a significant interaction [$\text{Pseudo}F_{(1,63)} = 4.26$, $p = 0.009$; ESM Resource 3]. Pairwise treatment comparisons also found seedlings of persistent to be 3 times more numerous in NG–NR compared to NG–R plots ($p = 0.05$). In contrast, pioneer species (Fig. 4b) experienced a strong grass effect and had 22 times more seedlings in NG–NR than G–NR plots [$\text{Pseudo}F_{(1,63)} = 12.14$, $p = 0.001$; ESM Resource 3].

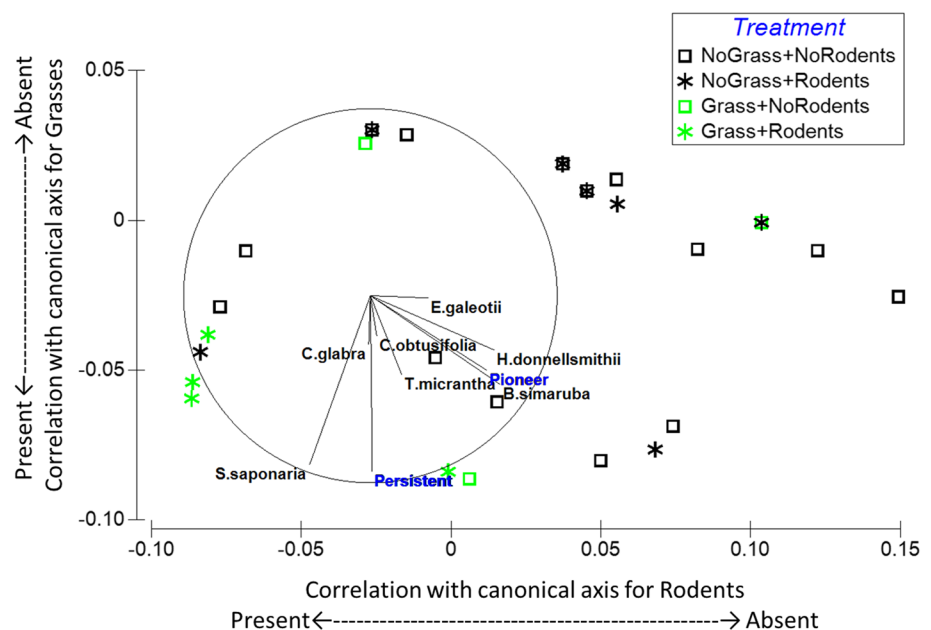
In Experiment 2, seedling configuration also differed among grass and rodent treatments (Table 2b). As determined by pairwise tests, seedling abundance differed in NG–NR vs. NG–R ($p = 0.001$), NG–NR vs. G–NR ($p = 0.001$), and G–NR vs. GR ($p = 0.001$) treatments. A CAP plot further expanded on the influence of rodent–grass factors by illustrating different treatment clustering patterns within the seedling treatments and explaining 72% of the total observed variance (Fig. 5). Overall, NG–NR displayed the greatest extent of clustering

within the ordination space and clearly separated from NG–R centroids (Fig. 5).

In the CAP multivariate space, Experiment 2 successional seedling type vectors and treatment associations elaborated on seedling assemblage differences (Fig. 5). Seedling type vectors, for instance, positively associated with NG–NR treatments but diverged with persistent species being most associated with G–NR and pioneers with NG–R plots. Follow-up PerANOVA analyses supported these results, and recorded successional seedling type [$\text{Pseudo}F_{(1,127)} = 148.44$, $p = 0.001$; ESM Resource 4] and a grass–seedling type interaction [$\text{Pseudo}F_{(1,127)} = 24.148$, $p = 0.001$; ESM Resource 4] among seedlings.

Between successional types (Fig. 6a), persistent seedlings were 11 times more abundant in NG–NR plots than in NG–R [$\text{Pseudo}F_{(1,63)} = 50.54$, $p = 0.001$; ESM Resource 4]. This rodent effect was also observed in grass (G–NR vs. GR), where by a factor of 2.6 there were fewer persistent seedlings [$\text{Pseudo}F_{(1,63)} = 5.827$, $p = 0.023$; ESM Resource 4]. Similar to persistent seedlings, by a factor of 3, more pioneer seedlings (Fig. 6b) were present when rodents were absent in NG–NR vs. NG–R plots [$\text{Pseudo}F_{(1,63)} = 5.736$, $p = 0.025$; ESM Resource 4]. However, unlike persistent seedlings, pioneers were strongly inhibited by grasses and were 3 times more abundant in NG–NR than G–NR plots [$\text{Pseudo}F_{(1,63)} = 49.07$, $p = 0.001$; ESM Resource 4].

Fig. 3 Experiment 1 Canonical Analysis of Principal Coordinate (CAP) ordination for rodents (x axis, black squares vs. asterisks) and grasses (y axis, green squares vs. asterisks) with tree species and seedling types correlation (red) vectors. Interactive effects for rodents and grasses are illustrated within the circle



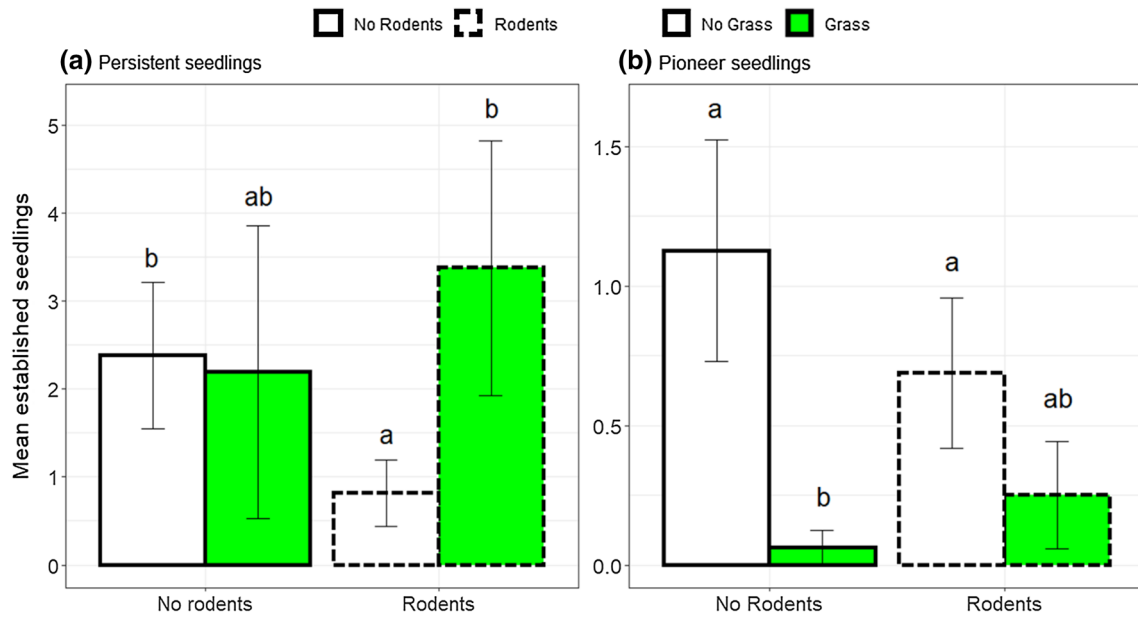
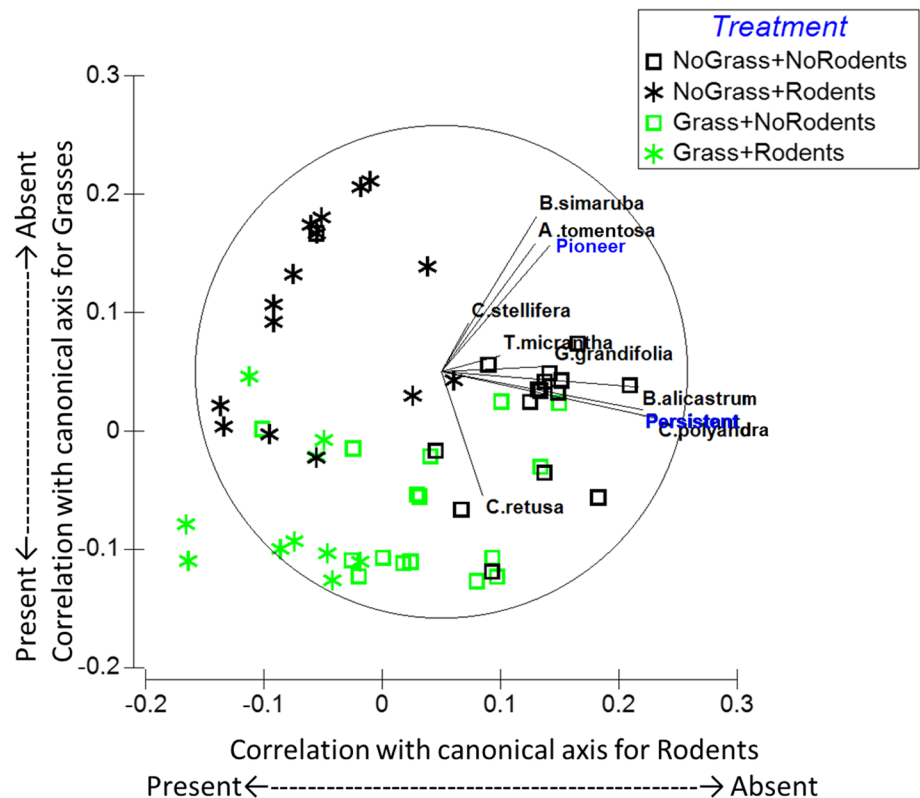


Fig. 4 Experiment 1 by treatment **a** persistent and **b** pioneer successional type establishment. Dashed bars indicate rodent presence, and bold bars indicate rodent absence. Green bars represent grass pres-

ence and no color grass absence. Error bars are one standard error ($n=16$ for each category). Bars that do not share a letter are significantly different ($p \leq 0.05$)

Fig. 5 Experiment 2 Canonical Analysis of Principal Coordinate (CAP) ordination for rodents (x axis, black squares vs. asterisks) and grasses (y axis, green squares vs. asterisks) with tree species and seedling types correlation (red) vectors. Interactive effects for rodents and grasses are illustrated within the circle



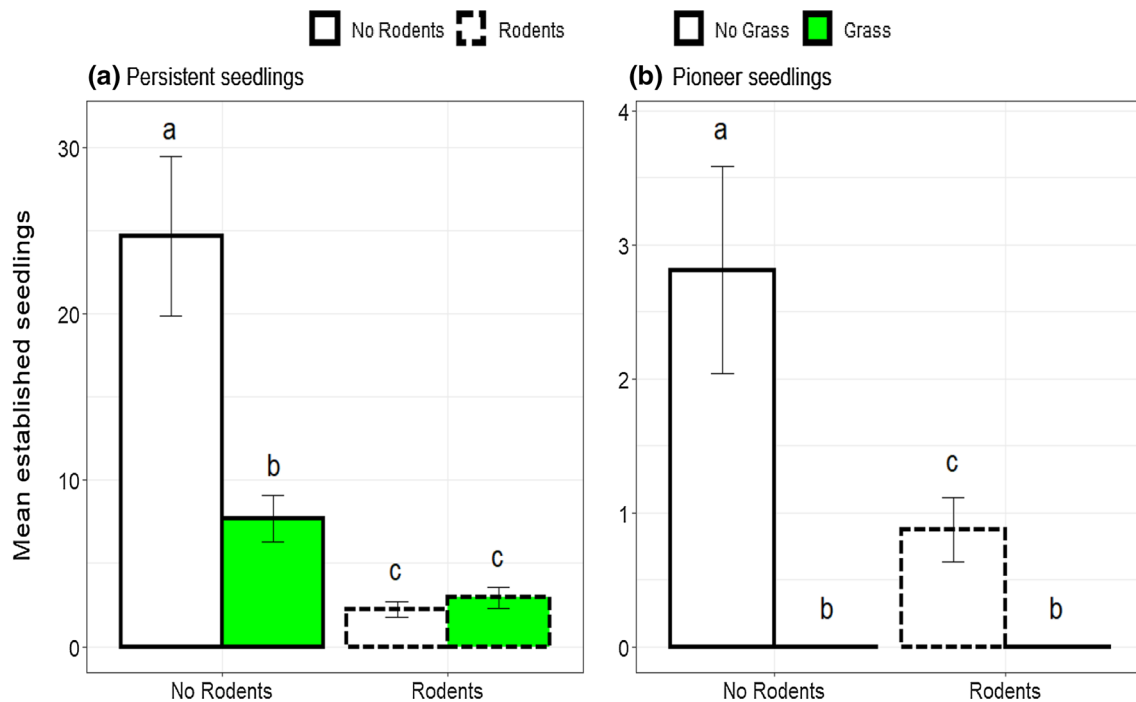


Fig. 6 Experiment 2 by treatment **a** persistent and **b** pioneer successional type establishment. Dashed bars indicate rodent presence, and bold bars indicate rodent absence. Green bars represent grass pres-

ence and no color grass absence. Error bars are one standard error ($n = 16$ for each category). Bars that do not share a letter are significantly different ($p \leq 0.05$)

Seed mass effects on seedling community establishment

To evaluate the role that seed mass had on establishment, seedlings from Experiment 1 and 2 were pooled. Univariate analysis on the 15 established tree species, found that treatment and seed mass significantly interacted [PerMANOVA, $\text{Pseudo}F_{(3,59)} = 2.50$, $p = 0.044$]. The effect of seed mass on seedling establishment was then evaluated by treatment (Fig. 7; ESM Resource 5). Across 15 species, seed mass had a positive relationship with seedling establishment in NG–NR [$\text{Pseudo}F_{(1,14)} = 7.93$, $r^2 = 0.38$, $p = 0.015$], G–NR [$\text{Pseudo}F_{(1,14)} = 64.97$, $r^2 = 0.83$, $p = 0.001$] and G–R treatments [$\text{Pseudo}F_{(1,14)} = 8.67$, $r^2 = 0.40$, $p = 0.013$]. The NG–R treatment was the exception; there was no relationship between seed mass and seedling establishment [$\text{Pseudo}F_{(1,14)} = 1.14$, $r^2 = 0.08$, $p = 0.313$].

Discussion

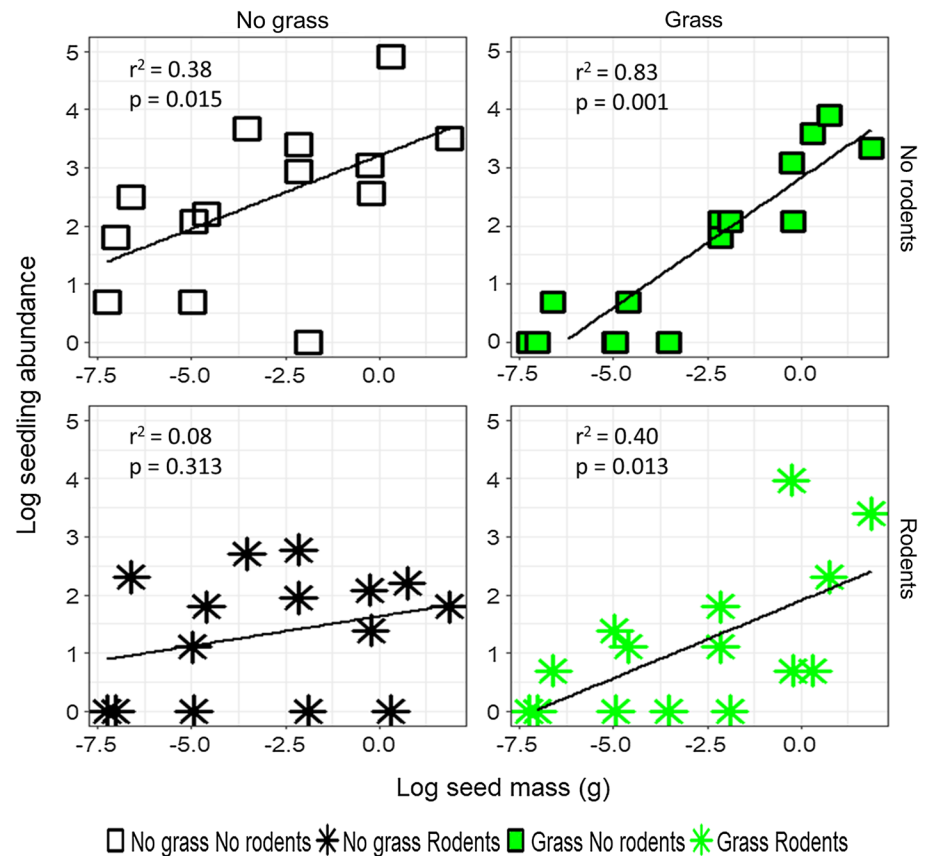
Rodent effects on seedling establishment

Experimental restriction of rodent activity from tree species cohorts uncovered greater seedling establishment. Except for Experiment 1 pioneers, the inclusion of rodents in non-grass plots (NG–NR vs. NG–R) revealed a significant decrease in

seedling counts in both experiments. Rodent inclusion into grass plots (G–NR vs. G–R) also saw less persistent seedling establishment in Experiment 2 grass treatments. The experimental framework demonstrated that a likely monocot diet (e.g., grass) by *Sigmodon toltecus* is supplemented by dicots (i.e., trees) in tropical pastures and that lack of grass cover does not deter seedling foraging. A monocot-dicot diet by the genus has been previously documented in temperate habitats, including coastal prairies just north of Mexico (Cameron and Eshelman 1996) by the hispid cotton rat (*S. hispidus*), a close relative of *S. toltecus*.

Sparse vegetation cover imposes a significant predation risk on most rodents (Jacob 2008). Nevertheless, hispid cotton rats (*S. hispidus*) in North America travel up to 400 m across open matrices in fragmented landscapes, moving across open matrices (e.g., burned areas, short grass) (Bowne et al. 1999). In a Los Tuxtlas landscape, juvenile and adult *S. toltecus* travel across open pastures to ungrazed patches of grasses and taller vegetation (Howe and Davlan-tes 2017). Foraging in the open may still impose at least a partial microhabitat barrier to *S. toltecus*. However, evidence of rodent seedling suppression with and without grass cover observed here indicates that predation risk can be circumvented. Furthermore, while it is evident that grasses generally suppress seedling abundance, removal of grasses without rodent mitigation may impede intended seedling establishment and, ultimately, local forest recovery.

Fig. 7 Influence of seed mass on seedling survival for pooled experiment treatments. Treatments included no grass–no rodents, no grass–rodents, grass–no rodents and grass–rodents. Each plot symbol represents 1 of 14 log-transformed species seed mass



The role of grass and rodent presence on successional seedling establishment

Grass and rodent mitigation altered establishment of both successional seedling types. Pioneer dicot establishment, for instance, was substantially lower in the presence of grass. The highest pioneer seedling abundance, in both experiments, was achieved in non-grass treatments. Pioneer seedlings are characterized as having foliaceous cotyledons or highly photosynthetic leaves (Strauss-Debenedetti and Bazzaz 1991). Consistent with Foster and Janson (1985), it is likely that pioneer seedlings experienced lower mortality under the direct sun as compared to shady conditions. In a Los Tuxtlas study, pioneer species acclimatized better by growing faster compared to other seedlings types, when exposed to increasing light (Popma and Bongers 1988). This successional strategy can explain why pioneer seedlings established in higher numbers under non-grass treatments and succumbed under grasses, which at 5 cm from the soil surface can have light levels similar to the forest interior (Holl 1999). Furthermore, release from above and below-ground competition for resources (e.g., nutrients, space) may also contribute to greater establishment in non-grass treatments (Meli et al. 2015).

Seedling abundance of persistent species was also altered, but differed from the general pioneer response to grass and rodent presence. In both experiments, persistent seedlings, compared to pioneers, established in higher numbers in grass treatments. *S. saponaria* drove this trend in Experiment 1, recruiting heavily in grass treatments. The distinction in establishment between successional types is likely due to persistent seedlings' ability to recruit under shaded conditions similar to those present under grass cover in our treatments (Paz et al. 2005). Persistent seedlings may also be fundamentally better above- and below-ground competitors, persisting while gathering essential resources otherwise exploited by the dominant pasture competitor, grass. Persistent species, for instance, commonly have hypogeal reserves that dedicate more resources for energy storage to compensate for carbon deficits or damage (Kitajima 2003). In particular, persistent seedlings frequently have higher concentrations of nitrogen, relying on this energy reserve for more extended periods during the initial stage of seedling growth (Kitajima 2002). Consequently, persistent seedlings inherently have more metabolic resources available (Kidson and Westoby 2000) to successfully compete with grasses for soil nutrients or maintain seedling growth when resources are in deficit within degraded soil characteristic of pasture habitat.

With rodents present, persistent successional types experienced a marked reduction in seedling establishment. This decline was most pronounced when seedlings were uncovered in non-grass plots (NG–NR to NG–R). In Experiments 1 and 2, for instance, persistent seedlings declined by a factor of 11 and 3, respectively. Once germinated, it is likely that sizeable and high energy hypogeal reserves, common in persistent species (Kitajima 2003), attracted rodent predation. Furthermore, for many persistent species, germination is positively correlated with high nitrogen and lipid concentrations in seeds (Soriano et al. 2011). The comparably higher concentrations of vital nutrients and a more significant maternal energy source among the persistent seedlings may have made this successional type the preferred food choice for foraging rodents. This manner of selective foraging for plants prominent in protein and lipids has been recorded in the closely related *S. hispidus* in North America (Randolph et al. 1995).

Overall, cumulative establishment saw persistent seedlings establish in higher numbers than pioneer seedlings in both experiments. In the presence of rodents, pioneers showed some susceptibility, but persistent seedlings were generally targeted, suggesting that they were the preferred food choice. The common practice of grass removal with no rodent enclosure may minimize establishment of persistent tree species. If the performance of successional types applies widely, the prevalence of pioneers in pasture habitat, as is commonly interpreted a consequence of their higher seed rain rather than establishment potential. Once introduced in pasture habitat, we expect that persistent species, with typically greater maternal reserves and better defense traits such as high tissue density and fracture toughness (Alvarez-Clare and Kitajima 2007), will experience higher overall seedling establishment.

The role of seed mass on seedling establishment

At the seed life-stage, interspecific variation in seed mass can determine the probability of seedling establishment (Moles and Westoby 2004). In our experiment, in the absence of grass and rodents, interspecific seed mass were positively correlated with establishment, explaining approximately 38% of the total observed variation. Although small-seeded tree species germinate rapidly under high irradiance (Norden et al. 2009), they are less likely to germinate under low water potential than large-seeded species (Daws et al. 2008). Exposed to high irradiance in NG–NR treatments, smaller-seeded species may be more vulnerable to desiccation. In contrast, large-seeded species, with more substantial maternal reserves, can better manage dry season and drought abiotic extremes (Moles and Westoby 2004). Furthermore, seedlings of large-seeded species are more likely to re-sprout after defoliation (Green and Juniper 2004), a

condition sometimes induced by drought. Extreme abiotic filtering in the pasture habitat appears to favor establishment of large-seeded over small-seeded tree species.

Seed removal was anticipated to have also shaped seedling establishment. Relative to forest habitat, seed removal can be extensive in pastures (Cole 2009). Small-seeded species, in particular, can experience high removal rates by ants (Holl and Lulow 1997). We observed that among non-grass treatments (NG–NR vs. NG–R), large-seeded species establishment decreased substantially when rodents were present. Effectively, the foraging of large-seeded species by rodents in NG–R plots countered their maternal-resource advantage and put them on par with smaller seeded tree species (Fig. 7). This seed mass-mediated outcome suggests that rodent foraging may have included seeds as well as seedlings. Further investigation will be needed to address rodent seed to seedling foraging preferences directly.

In pastures recently released from cattle grazing, dispersed seeds are often embedded underneath grass vegetation. Our experimental framework approximated the role that seed mass had under this common outcome and found that seedling establishment in grass cover, with or without rodent foraging, favored large-seeded tree species. Tree species seed mass explained forty percent of the total variation observed in seedling establishment when grass and rodents were present and eighty-three percent of the total variation when only grass was present. Notably, within grass treatments, small-seeded species saw little or no establishment, which left large-seeded species to dominate in G–NR plots. These findings are consistent with research that demonstrated a positive relationship between tree establishment and seed mass under shady conditions (Paz et al. 2005). The increased establishment potential of large-seeded seedlings can be attributed to their superior ability to resist physical and biotic damage, including predation (Janzen 1969) in shady conditions (Daws et al. 2008). All in all, the ability for large-seeded species to establish at higher rates than small-seeded species in the pasture habitat supports research that indicates that seed dispersal limitation of large-seeded species is largely responsible for the lack of tree diversity observed in regenerating tropical landscapes (Costa et al. 2012).

Early regeneration and restoration implications

In our experimental design, rodents shaped tree establishment in early tropical succession to degrees not previously fully explored. Here, we introduced Toltec cotton rats as potential ecosystem engineers in regenerating tropical systems, with broad implications for succession and restoration in Mexico and northern Central America. One implication posits that rodents may diminish tree diversity in regenerating landscapes by preferably consuming

large-seeded persistent or deep-forest species. Since deep-forest tree species are underrepresented in regenerating habitat and abundant in primary forest, it is critical to prioritize their restoration. Our results suggest that the sowing of large-seeded persistent species in early regenerating habitat can be a successful tree establishment strategy with initial protection from rodents. The sowing of tree species can also represent an inexpensive and logistically feasible option for reforestation in a degraded landscape. Other economic and logistically practicable approaches aimed at forest recovery such as nucleation (Zahawi et al. 2013), artificial bird benches (Reid and Holl 2013), or bat roosts (Kelm et al. 2008) can accelerate seed input into regenerating habitat but fail to increase late-successional species abundance. With fragmented tropical landscapes increasingly characterized by the hyperabundance of pioneer species (Tabarelli et al. 2010), it is critical that forest restoration efforts make tree diversity recovery a top conservation priority.

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Author contribution statement CAG and HFH designed experiments. CAG conducted fieldwork, analyzed data, and wrote the manuscript. HFH, DHW, RIC, and JZ helped edit and finalize the manuscript.

Compliance with ethical standards

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

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