

## ORIGINAL ARTICLE OPEN ACCESS

# Defaunation Increases Clustering and Fine-Scale Spatial Genetic Structure in a Small-Seeded Palm Despite Remaining Small-Bodied Frugivores

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## ABSTRACT

Anthropogenic pressures such as hunting are increasingly driving the localised functional extinctions of large- and medium-sized wildlife in tropical forests, a phenomenon broadly termed 'defaunation'. Concurrently in these areas, smaller-bodied species benefit from factors such as competitive release and increase in numbers. This transformation of the wildlife community can impact species interactions and ecosystem services such as seed dispersal and seed-mediated gene flow with far-reaching consequences. Evidence for negative genetic effects following defaunation is well-documented in large-seeded plants that require large frugivores for long-distance seed dispersal. However, how defaunation affects plants with small or medium-small seeds (< 1.5 cm), which tend to be consumed and dispersed by frugivorous mutualists of a range of body sizes and responses to anthropogenic threats, is not well understood. To better understand defaunation's impacts on tropical plant communities, we investigated spatial and genetic patterns in a hyperabundant medium-to-small-seeded palm, *Euterpe precatoria* in three sites with different defaunation levels. Results indicate that defaunation is associated with higher fine-scale spatial genetic structure among seedlings and increased spatial clustering within seedling cohorts and between seedlings and conspecific adults, as well as a reduction in nearest-neighbour distances between seedlings and conspecific adults. There were no clear effects on inbreeding or genetic diversity. However, we caution these trends may indicate that defaunation reduces seed dispersal services for species previously presumed to be robust to deleterious effects of losing large frugivores by virtue of having smaller seeds and broad suites of dispersal agents, and negative downstream effects on genetic diversity could occur.

## 1 | Introduction

Understanding the consequences that biodiversity loss will have on ecosystem functioning is a longstanding focus of ecological

and conservation research, particularly in light of ongoing global species declines (Ceballos, Ehrlich, and Raven 2020; Gonzalez et al. 2020; van der Plas 2019). In tropical forests, biodiversity loss in the form of *defaunation*, or the decline and local

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extirpation of wildlife species, presents a multi-faceted threat to ecosystem functioning by affecting species interactions (e.g., seed dispersal), changing the relative abundances of large- versus small-bodied animals and decreasing wildlife diversity. In particular, defaunation is associated with decreases in the abundance of large-bodied animals and increases in smaller-bodied vertebrates that benefit from release from predators and competition as well as supplementary resources from nearby human settlements (Dirzo et al. 2014; Peres and Dolman 2000; Pires and Galetti 2023). While defaunation is often intertwined with disturbances such as fragmentation and logging, it can be independently pervasive when heavy hunting and poaching have impacted animal communities while the plant community remains structurally intact. Some estimates posit that hunting-driven defaunation affects a greater area of tropical forests than logging and fragmentation combined (Benítez-López et al. 2019; Harrison et al. 2016; Pires and Galetti 2023). Despite the ubiquity of defaunation, it remains unclear (1) the extent to which animals remaining in defaunated forests compensate for interactions and services previously performed by the larger extirpated species and (2) the functional outcomes of these altered or lost interactions (Bueno et al. 2013; Culot et al. 2017; Fricke, Tewksbury, and Rogers 2018; Sekar and Sukumar 2013). Developing nuanced insight into how defaunation per se impacts ecological processes is an important step toward understanding the consequences of anthropogenic change in tropical forests. Further, this offers insight into the functional roles that frugivores differentially impacted by defaunation (e.g., small vs. large-bodied species) play in shaping tropical forest plant communities.

Seed dispersal is a co-evolutionary, mutualistic relationship between plants and animals that is often disrupted by defaunation. In exchange for important food resources, the consumption of fruit and/or seeds by animal species can result in the deposition of seeds away from maternal plants. Many specialised pathogenic and invertebrate seed predators are associated with soil communities beneath maternal plants, making the dispersal of seeds to new microhabitats an important aspect of increasing the survival of seeds and forest regeneration as a whole (Chesson 2000; Connell 1971; Hazelwood et al. 2020; Janzen 1970; Swamy 2017). From a micro-evolutionary perspective, seed dispersal across a large spatial scale can promote migration between populations to minimise the effects of drift and inbreeding and increase genetic diversity in populations (Aguilar et al. 2008; Dick et al. 2008; Oddou-Muratorio et al. 2001; Pérez-Méndez et al. 2016). At a more local scale, the movement of seeds by diverse frugivore assemblages to a variety of deposition sites serves to decrease the relatedness of neighbouring plants and thereby limiting fine-scale spatial genetic structure as well as biparental inbreeding (Browne, Ottewell, and Karubian 2015; Carvalho et al. 2021; Choo, Juenger, and Simpson 2012; Karubian et al. 2010). As a result, seed dispersal is a critical ecosystem function carried out by fruit-eating animals and is especially important in tropical forests where the majority of plant species rely on animals to disperse their seeds (Howe and Smallwood 1982).

Large-bodied frugivores are capable of moving seeds across large distances and are most likely to promote long-distance dispersal between plant populations (Fragoso, Silviu, and Correa 2003;

Goebel et al. 2023; Link and Fiore 2006; Naniwadekar et al. 2019; Ong, McConkey, and Campos-Arceiz 2021). In some instances, there are close mutualistic relationships between plants with large seeds and large-bodied frugivore species with gape sizes large enough to swallow the seeds whole (Brockelman et al. 2022; Giombini, Bravo, and Tosto 2016; Giombini et al. 2017; Landim, Fernandez, and Pires 2022). Most research investigating anthropogenic effects on seed dispersal services in tropical forests has focused on large-seeded plants (but see Carvalho et al. 2021; Galetti et al. 2013) because they are seen as the most vulnerable to losing dispersal services from the large-bodied mutualistic partners they specialise on, which tend to disappear first after human disturbances (Dirzo et al. 2014; Kurten 2013). Such studies have linked the loss of large-bodied frugivores to decreased dispersal and recruitment success of larger seeded plants (Bagchi et al. 2018; Holbrook and Loiselle 2009), changes in allele frequencies and lower allelic richness (Giombini et al. 2017), as well as higher spatial genetic structure and lower genetic diversity (Browne, Ottewell, and Karubian 2015; Diaz-Martin and Karubian 2021; Giombini et al. 2017). However, animal-dispersed plants that are generalists, that is, those that produce fruits that appeal to multiple taxa and have seeds small enough to be handled and swallowed whole by frugivores across a range of body sizes, are extremely common in tropical forests but have not been broadly evaluated for deleterious effects following defaunation. Smaller-sized seeds (e.g., < 1.5 cm diameter) are likely able to be passed intact by many frugivores not typically targeted by hunters, such as toucanets and tamarins in neotropical forests, in addition to being consumed by a spectrum of medium- and large-bodied animals alike (Howe and Smallwood 1982; Malhado et al. 2015; McConkey and Brockelman 2011). As such, it stands to reason that generalist species may be relatively unaffected by defaunation, but little research has used a population genetics lens to evaluate this (Kurten 2013).

Accurately predicting the net effect of defaunation on seed dispersal and population genetic patterns in non-large seeded plants is difficult. Plants dispersed by a range of frugivores with varying levels of sensitivity and vulnerability to human activities and disturbances may be robust to vertebrate losses associated with defaunation thanks to redundant seed-disperser roles played by multiple small-, medium- and large-bodied frugivore mutualists. Animals that persist in high densities in defaunated forests such as some small primates, small or medium frugivorous birds and agoutis or other rodents can facilitate effective seed dispersal in defaunated tropical forests (Carvalho et al. 2021; Culot et al. 2010; Godó et al. 2022; Heymann, Fuzessy, and Culot 2022; Hirsch et al. 2012; Mittelman et al. 2020). Indeed, rats can be important seed dispersers in tropical forests because they have the ability to handle small and large seeds and occupy central positions within seed-dispersal networks (Ong, McConkey, and Campos-Arceiz 2021). In addition, small and medium-sized frugivores, particularly birds, may remain and sometimes thrive in defaunated forests where they can serve as important seed dispersers capable of maintaining local plant genetic diversity and the movement and mixing of maternal genotypes (Carvalho et al. 2021; Heymann et al. 2019; Mittelman et al. 2020). However, smaller frugivores remove seeds in smaller numbers and typically disperse across shorter distances than their larger counterparts (Bagchi et al. 2018;

Heymann, Fuzessy, and Culot 2022). Larger-bodied frugivores are often predicted to be disproportionately important within seed-dispersal networks even for smaller-seeded plants because they facilitate long-distance seed dispersal and consume large numbers of seeds in a single foraging bout (Donoso et al. 2020; Goebel et al. 2023; Naniwadekar et al. 2019; Vidal, Pires, and Guimarães 2013). Because defaunation causes downsizing in the size and diversity of the frugivore community (Koerner et al. 2017; Poulsen, Clark, and Palmer 2013), it may effectively eliminate long-distance dispersal events for animal-dispersed plants in defaunated forests (Donoso et al. 2020). Distinguishing empirical genetic and spatial patterns resulting from seed dispersal in defaunated relative to faunally intact forests from the myriad of theoretical outcomes will advance our understanding of how defaunation impacts seed-mediated gene flow and genetic structure of generalist plant populations.

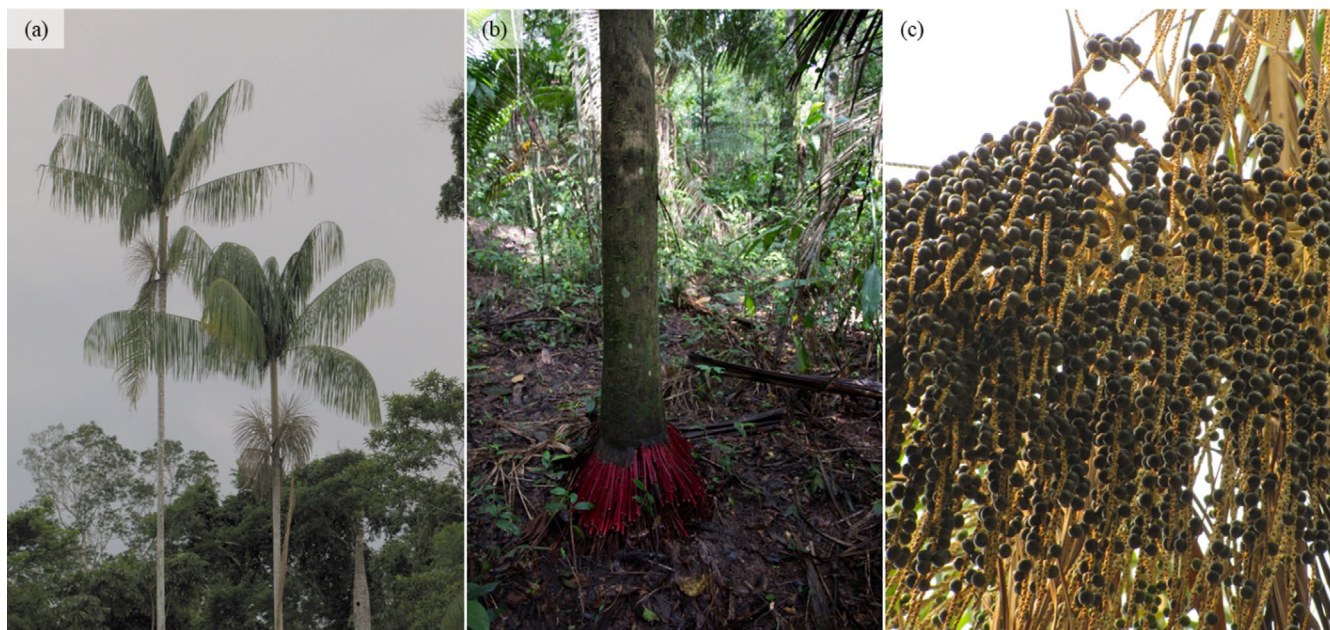
Here we investigate potential genetic and spatial consequences for a medium-to-small-seeded (0.8–1.2 cm diameter) generalist palm, *Euterpe precatoria*, following defaunation. Across three comparable continuous mature forest sites impacted by varying levels of hunting pressures, we compare inter- and intra-cohort: (1) spatial clustering, (2) fine-scale spatial genetic structure and (3) genetic diversity and inbreeding. We predicted that if defaunation does remove a suite of frugivores that play significant roles in fruit removal and seed dispersal for our study species in faunally intact areas, then defaunation will be associated with increased spatial clustering of seedlings around conspecific adults and, relatedly, decreased pairwise spatial distances between seedlings and their nearest conspecific neighbours resulting from seeds being moved shorter distances or failing to be dispersed at all from parent plants; further, we predict this may also result in higher genetic similarities between adults and seedlings and an increase in fine-scale spatial genetic structure due to increased spatial aggregations of related individuals. However, differences in genetic diversity and inbreeding are not expected to be detectable. Genetic diversity and inbreeding

measures are typically robust to anthropogenic change over shorter timescales (e.g., Browne, Ottewell, and Karubian 2015). Disruptions to gene flow would have to be extremely severe for these effects to occur in our study species, for which time-since-defaunation is estimated to be less than five generations in our sites affected by defaunation. Examining the effects of defaunation on a generalist, non-large-seeded plant will further our understanding of how changes in animal communities impact micro-evolutionary processes in the plants that rely on their ecosystem services.

## 2 | Materials and Methods

### 2.1 | Study Species

*Euterpe precatoria* Mart. (Arecaceae) (Figure 1) is a widespread palm spanning from the edge of the southwestern Amazon Basin in Bolivia through Peru, Ecuador, Colombia and Brazil (Ramos et al. 2021). The species is adapted to both nutrient-poor, dry soils as well as seasonally flooded alluvial soils and has been classified as the most abundant palm in the Amazon Basin (ter Steege et al. 2013). Because of this, *E. precatoria* represents an important resource for frugivorous animals. Although extraction by humans for the sale and consumption of palmhearts can affect *E. precatoria* population demography and size (Avalos, Fernández Otárola, and Engeln 2013; Lopes et al. 2019), it has not been harvested by humans across our study sites. *E. precatoria* is monoecious and while self-pollination may occur, existing studies document temporally distinct male and female flower phases (Ramos et al. 2019). Pollination can occur with wind, but primarily a mixed-species guild of beetles (primarily in the Curculionidae, Chysomelidae and Staphylinidae families) and bees (primarily in the Halictidae family) pollinate *E. precatoria* (Küchmeister, Silberbauer-Gottsberger, and Gottsberger 1997). Individuals in our study areas reached heights of approximately 20–30 m. While we expect genetic effects of defaunation in the



**FIGURE 1** | Images of the study species showing (a) adult *Euterpe precatoria*, (b) red surface roots characteristic of *E. precatoria* and (c) *E. precatoria* inflorescence with mature fruits. Photo credit: Rainer W. Bussmann & Narel Y. Paniagua Zambrana.



seedling cohort, we do not necessarily anticipate this to be the case in the adult cohort because hunting only began affecting our defaunated and recovering sites in the early- to mid-1980s and *E. precatória* is a long lived, relatively slow growing palm and can take over a decade to reach reproductive maturity (which occurs at about 10m) (Avalos 2016; Avalos, Fernández Otárola, and Engeln 2013).

We collected 6–13 seeds from 6 maternal *E. precatória* individuals to estimate seed diameter ranges, which were 0.8–1.2cm with a mean diameter of 1.01 cm (0.01 cm standard deviation) (Supporting Information Figure S1). When ripe, seeds are surrounded by a thin dark purple or black mesocarp (Figure 1c). Fruits are dispersed by a range of small and large frugivores birds, primates and rodents (Avalos, Fernández Otárola, and Engeln 2013; Bagchi et al. 2018; Ramos et al. 2021).

Seed size is a functional trait that determines the range of frugivore species that can swallow and thereby disperse a species (Galetti et al. 2013; Holbrook and Loiselle 2009). Because of this, seed size is a trait used to categorically group plant species when considering what frugivores are likely to comprise their primary seed-dispersal partners. In general, large-seeded plants are considered specialists requiring large-bodied animals to swallow intact seeds while species with small and medium-sized seeds

(e.g., as in the case of our study species, species with seeds  $\leq 1.2$  cm diameter) are considered generalists because a wide range of frugivores can swallow and disperse their seeds (Galetti et al. 2013; Kurten 2013; Malhado et al. 2015).

## 2.2 | Study Sites and Defaunation History

Our study was conducted in three sites hosting contiguous mature forests along a defaunation gradient in the Madre de Dios River Basin in southeastern Peru (Figure 2). Euclidean distances between sites range from 82 km between closest sites to 113 km between farthest sites. Rainfall in the Madre de Dios River Basin is typically 2500–3500 mm annually and punctuated by a dry season from June through September (Gentry 1993; Tobler, Carrillo-Percegue, and Powell 2009). Data on our focal palm species was collected from within long-term forest dynamics plots measuring 4 ha within each site that was established in 2008. All sites are unlogged primary floodplain forests with similar soil types, and which historically hosted similar communities of key seed dispersers (i.e., large- and medium-sized frugivores such as tapirs, spider monkeys, capuchins, toucans, guans and trumpeters). Uniquely, our study sites are not fragmented or otherwise disturbed (e.g., logged) and as such allow insight into the effects of defaunation per se without additional



**FIGURE 2** | (a) Study region in Peru and (b) enlargement of the Madre de Dios region with study sites labelled (Google Earth Pro 2022).

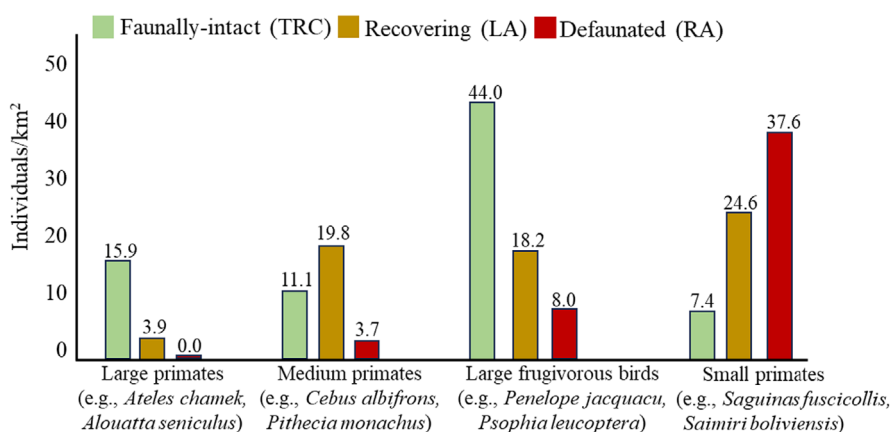
confounding sources of disturbance; this is important given the widespread and ubiquitous nature of defaunation even in intact forests across the tropics (Benítez-López et al. 2019; Harrison 2011; Harrison et al. 2013; Terborgh et al. 2008). Data on the frugivore communities in our study sites documents the transition from a diverse seed disperser community that includes several large-bodied frugivores in our faunally intact site into a downsized and depauperate community in our defaunated site, which is currently and has been historically subjected to frequent hunting (Figure 3, Supporting Information Table S1). While we cannot rule out natural (i.e., not caused by hunting) species turnover in frugivore communities between our three study sites, the composition of the frugivore communities, especially at the functional level, are thought to have been comparable prior to hunting given that all sites share a common ecological region (the Madre de Dios River Basin), age and a lack of logging and other non-hunting disturbances, and they exhibit similar forest composition and structure with three main habitat types in all: flooded palm swamp, terra firma and seasonally flooded forests (Bagchi et al. 2018; Rosin and Swamy 2013; Swamy 2017; Tobler et al. 2008). Importantly, the dominant large- and medium-sized frugivore groups (e.g., large primates such as spider monkeys and large birds such as toucans), which, when present, have strong impacts on seed dispersal and plant regeneration patterns (Choo, Juenger, and Simpson 2012; Giombini, Bravo, and Tosto 2016; Holbrook and Loiselle 2009; Naniwadekar et al. 2019; Nuñez-Iturri and Howe 2007) have common species ranges that encompass all three sites and anecdotally are said to at one time have been present at all sites (V. Swamy, personal communication). There are two exceptions in primate species that are present at the intermediately defaunated, recovering site but were likely not historically (and are not currently) present at the other two sites: the bald-faced saki monkey (*Pithecia irrorate*), which an arboreal seed predator and disperser (Palminteri, Powell, and Peres 2012) and the emperor tamarin (*Saguinus imperator*).

We note that our results must be interpreted with caution due to a lack of replication. The processes and effects of defaunation occur at landscape scales, creating logistical constraints that limit our ability to intensively sample multiple sites across remote and rugged regions, resulting in a lack of multiple replicated sites within each defaunation level. While we believe

that our study design and the similarly replication-limited study designs of related studies on defaunation offer valuable insights (Aliaga-Rossel, Fragoso, and Moraes 2022; Bagchi et al. 2018; Boissier et al. 2020; Boiten et al. 2023; Hazelwood et al. 2020; Holbrook and Loiselle 2009; Nuñez-Iturri and Howe 2007; Rosin and Swamy 2013), this limitation reduces our ability to draw strong conclusions about defaunation causing difference between sites. However, we minimise confounding factors where possible in our study design by, for example, working in forests within the same region and habitat types with known shared frugivore and plant communities, and such commonalities across these same sites have been evaluated and leveraged in related studies (e.g., Bagchi et al. 2018; Rosin et al. 2017) and by exhaustively sampling within relatively large (4 ha) plots.

### 2.2.1 | Defaunated Site: Reserva Amazonica

Reserva Amazonica (RA) [12°32'4" S, 69°3'13" W] has been impacted by significant hunting pressure since the mid-1980s (Bagchi et al. 2018) and likely exposed to moderate hunting pressure since the early 1900's. RA is a small private reserve located adjacent to the Tambopata National Reserve. It was founded in 1977 by the ecotourism company Asociación Inkaterria. Despite noncontinuous tenure as a protected reserve due to lapses in its reserve status in the 1980s, RA encompasses about 1500 ha of structurally intact mature forest (Bagchi et al. 2018). RA is ~1 km from the nearest human settlement and only 16 km from the regional capital Puerto Maldonado (Figure 2), which was founded in 1902 to facilitate resource extraction (e.g., rubber) in the area (Markham 1903). Although RA adjoins some contiguous mature forest, considerable amounts of the surrounding area have been converted for human uses such as small-scale agriculture (Bagchi et al. 2018; Rosin and Swamy 2013). RA's historically low protection from hunting and proximity to human settlements, along with recent increases in hunting pressure associated with human population growth in the area have resulted in a defaunated animal community. Specifically, RA lacks large-bodied mammal species that act as important seed dispersers (e.g., tapirs and spider monkeys, as well as the seed-predating/occasional seed-dispersing white lipped peccaries) and has a low abundance of large frugivorous birds and medium-bodied frugivores (e.g., guans, toucans, capuchins) (Bagchi et al. 2018;



**FIGURE 3** | Densities (individuals/km<sup>2</sup>) in each study site of individuals belonging to four key frugivore groups (data procured from Rosin and Swamy 2013; Bagchi et al. 2018). Frugivore and granivore species lists can be found in Supporting Information, Table S1.

Rosin et al. 2017) (Figure 3). In contrast, RA hosts high densities of small vertebrates including secondary seed dispersers and seed predators such as agouties, bird species not heavily affected by hunting, and a hyperabundance of saddleback tamarins (*Saguinus fuscicollis*) which have benefitted notably from competitive release in the area (Rosin and Swamy 2013).

### 2.2.2 | Intermediately Defaunated, Recovering Site: Los Amigos

Los Amigos Biological Field Station/Centrode Investigación y Capacitación Rio Los Amigos (LA) [12°34'10" S, 70°4'52" W] is categorised as having an intermediately defaunated, recovering faunal community. LA is situated in a 453-ha reserve adjoined by a 46,000-ha conservation concession. Los Amigos is just 2 km from the nearest human settlement and until the early 1990s hosted a large gold mining camp during which time it was subject to heavy hunting (Bagchi et al. 2018; Rosin and Swamy 2013). The area has been officially protected since 2001 and monitoring efforts have nearly eliminated hunting in the area since 2003 (Tobler, Carrillo-Percestequi, and Powell 2009). Los Amigos now hosts low abundances of some large frugivores (e.g., spider monkeys, saki monkeys, toucans and guans) as well as white-lipped peccaries and relatively high numbers of medium- and small-sized frugivores (e.g., capuchins and tamarins) relative to the faunally intact site, which may be a remaining effect of predation/competition release due to virtually all large predators and large frugivores being previously hunted out of the area (Figure 3). Small-scale mining activities continue in the area which is likely accompanied by some hunting (Rosin and Swamy 2013).

### 2.2.3 | Faunally Intact Site: Tambopata Research Station

Tambopata Research Center (TRC) [13°7'9" S, 69°36'59" W] is located within ~17,000 km<sup>2</sup> of protected forest at a junction between the Tambopata National Reserve and Bahuaja-Sonene National Park. The area has been formerly protected from hunting since the early 1990s; however, before that time, hunting was limited due to the station's remote location (> 50 km from human settlements and difficult to access) (Rosin and Swamy 2013). Because of this, TRC hosts a faunally intact vertebrate community (Bagchi et al. 2018; Rosin et al. 2017). For example, TRC hosts about 4× more large primates (e.g., spider monkeys) than LA (while RA has no large primates) and more than 2× as many large frugivorous birds (e.g., toucans) along with high densities of white-lipped peccaries.

## 2.3 | Data Collection

We exhaustively mapped and sampled all *E. precatoria* seedlings and adult individuals in each 4 ha plot in each site (Supporting Information Figure S2d). Adult densities in each plot ranged from 23 to 49 individuals per 4 ha plot and seedling densities ranged from 172 to 427 individuals (Table 1). We collected genetic tissue primarily from attached, healthy (e.g., not covered in visible fungus) leaves when accessible, otherwise fresh root tissue was taken. Samples were collected from all individuals in

**TABLE 1** | The number of individuals found in reach cohort in each 4-ha plots and the total number of individuals successfully genotyped.

Cohort	Site	Total individuals	N genotyped
Adults	Defaunated (RA)	33	25
	Recovering (LA)	49	38
	Faunally intact (TRC)	23	22
Seedlings	Defaunated (RA)	267	115
	Recovering (LA)	172	119
	Faunally intact (TRC)	427	122

two cohorts: adults  $\geq 10$  cm dbh and seedlings < 1 m stem height (measured along the stem from the ground to the base of the first leaf). Individuals < 1 m tall in this species are typically considered seedlings because at ~1 m tall, *E. precatoria* begins to shift ontogenetic stages, becomes more woody, and may change growth speed (Avalos, Fernández Otárola, and Engeln 2013; Avalos and Fernández Otárola 2010). We consider individuals < 1 m to be recent recruits likely dispersed by the frugivore community currently persisting in these sites.

## 2.4 | Genotyping

We only included adults and seedlings in genetic analyses because we expect signatures of defaunation to be most detectable in seedlings (relative to sapling or juvenile cohorts), and we use adults to help understand if there were pre-existing genetic differences in individuals between sites. While all seedlings were initially mapped and sampled in the field, we randomly selected a subset of 125 seedlings from each site to genotype. Because adults existed at low densities, all adults were mapped, sampled and genotyped (Table 1). We extracted genomic DNA from leaf or root tissue using Qiagen DNeasy 96 Plant Kit following the manufacturer's instructions (Qiagen, Germany). We used polymerase chain reaction (PCR) to test 11 microsatellite loci developed for *E. precatoria* (Arias et al. 2016). After evaluating the fragment size for each locus, we create two PCR multiplexes, one with forward and reverse primers for Ep03, Ep05, Ep08, Ep13, Ep25 and Ep30; and the other with forward and reverse primers for Ep02, Ep06, Ep32, Ep35 and Ep36. To make Multiplex 1, for each forward and reverse primer of Ep05, Ep25 and Ep08, 5  $\mu$ L (1  $\mu$ Molar concentration) were used; for Ep13, Ep30 and Ep03, 8  $\mu$ L (1.6  $\mu$ M) were used. To make Multiplex 2, 10  $\mu$ L (2  $\mu$ M concentration) were used for all primers for each forward and reverse. Each PCR multiplex master mix was prepared in an Eppendorf tube with 315  $\mu$ L deionised water, 525  $\mu$ L Multiplex PCR Qiagen Master Mix and 105  $\mu$ L of the multiplexed primer mixture. We then aliquoted 9  $\mu$ L of the PCR multiplex master mix across a 96-well plate and added 10 ng DNA (volume required to



obtain this varied slightly depending on the individual but was typically 0.5–1  $\mu$ L). PCR was run on an Eppendorf ProS thermal cycler following programming outlined in the Qiagen Multiplex PCR Kit, with annealing temperatures of 60C for the first multiplex and 61.1C for the second multiplex. Post PCR, multiplexes were pooled and run on an ABI 3100 capillary sequencer. We then analysed amplified fragments and genotyped samples using the program GENEMARKER v. 1.85 (SoftGenetics).

Of the 11 microsatellites, two were not polymorphic in our sampled sites and were excluded from subsequent analyses, leaving a set of nine loci. We evaluated marker quality for the remaining nine loci using GenePop (Rousset et al. 2023) in R v. 4.2.2 (R Core Team 2022) (all subsequent analyses were conducted in R unless otherwise noted). First, we excluded all individuals with missing data in  $\geq 30\%$  of loci. Then in each site, we tested each locus for departure from the Hardy–Weinberg Equilibrium (HWE) using exact tests with a Markov chain method and Bonferroni correction (Benjamini and Hochberg 1995; Guo and Thompson 1992); this was evaluated across all samples (adults and seedlings). No markers significantly deviated from HWE across all sites and 4 of the 21 marker x site combinations showed significant HWE departure within a site (Ep06 in RA, Ep35 and Ep32 in LA and Ep32 in TRC) (Supporting Information, Table S2). We estimated null allele frequencies through maximum likelihood (Dempster, Laird, and Rubin 1977). Null allele frequency estimates ranged from 0 to 0.24 (average = 0.058) (Supporting Information, Table S3). One marker, Ep25, had high null allele frequencies ( $> 0.9$ ) and was excluded from all analyses. We then tested for linkage disequilibrium between pairs of loci at each site to avoid pseudoreplication (Hill and Weir 1994). No marker pairs showed significant linkage disequilibrium in all three sites. Potential linkage was detected between Ep32 and Ep02 in LA ( $p = 0.01$ ) and in TRC ( $p = 0.04$ ), and between Ep03 and Ep06 in RA ( $p = 0.04$ ). We re-amplified and re-genotyped approximately 7% of our samples ( $n = 28$ ) to assess allele identification error rates. Mean allele typing error per locus was low (average = 0.44%; Supporting Information, Table S4), a rate lower than or consistent with data used in similar studies (Browne, Ottewell, and Karubian 2015; Giombini et al. 2017). In addition, we found marker Ep36 to have a high percentage of missing data ( $> 25\%$ ) in the adult cohort due to amplification failure. We excluded Epr36 leaving a final panel of seven microsatellites markers that were used in our subsequent analyses of seedlings. In adult cohorts, however, TRC adults were all homozygous for the same allele for Epr02 and LA adults were all homozygous for the same allele for Epr35. We ran analyses with and without these markers included for all analyses that involved adults, and results were qualitatively unchanged; however, we conservatively excluded these markers from all analyses involving adults (including the inter-cohort fine-scale spatial genetic structure analyses, for which these markers were removed from the seedling cohorts as well).

## 2.5 | Genetic Diversity and Inbreeding

We used the function `basic.stats` in the package `hierfstat` to compute allelic richness ( $Ar$ , the raw number of alleles present

per loci rarified to  $n = 115$  seedlings and  $n = 22$  adults), observed heterozygosity ( $Ho$ ) and gene diversity ( $Hs$ ) in each cohort at each site for each locus.  $Ho$  measures the observed genetic variability as  $Ho = 1 - \sum \sum \frac{Pkii}{np}$  where  $np$  is the number of individuals in the sample,  $k$  and  $ii$  are the proportion of homozygote  $i$  in sample  $k$ . Gene diversity ( $Hs$ ) is also often referred to as expected heterozygosity and was calculated based on what would be predicted for a population in Hardy–Weinberg equilibrium (HWE) (Goudet 2005; Nei 1987; Nei and Chesser 1983). These heterozygosity measures are used to evaluate study site subpopulations for evidence of events such as a significantly reduced breeding population size (i.e., a population bottleneck) which would result in very low  $Ho$ . Evaluating  $Ho$  relative to  $Hs$ , or, the heterozygosity expected under HWE, helps gain information regarding factors at play in a subpopulation such as inbreeding, which can contribute to lower-than-expected heterozygosity (Nei 1987; Ritland 1996). We also report the number of effective alleles ( $NAe$ ) computed through the program SPAGeDi (Spatial Pattern Analysis of Genetic Diversity v1.5) (Vekemans and Hardy 2004), which estimates the number of alleles with equal frequencies needed for the specific gene diversity ( $Hs$ ) observed; with  $NAe$ , infrequent alleles contribute little and  $NAe$  helps facilitate comparisons between subpopulations even if allelic richness and frequencies vary widely (Nielsen, Tarpay, and Reeve 2003). Multilocus individual inbreeding coefficients were also computed in SPAGeDi and significance was tested based on two-tailed  $t$ -tests of observed values versus null model expectations after permutations of 10,000 randomisations of gene copies among individuals. Per locus values of  $Ar$ ,  $Ho$ ,  $Hs$ ,  $NAe$  and  $F_i$  allowed us to test for differences in mean values between the three defaunation levels (sites RA, LA and TRC) using nonparametric Friedman tests which accommodate  $> 2$  groups in unreplicated complete block designs (Hollander, Wolfe, and Chicken 2013). In this case, locus was the blocking factor (Browne, Ottewell, and Karubian 2015; Giombini et al. 2017) and defaunation level (site) was the treatment; a separate Friedman test was run for each dependent variable ( $Ar$ ,  $Ho$ ,  $Hs$ ,  $NAe$  and  $F_i$ ).

To estimate if reducing seed dispersal distances and fruit removal rates defaunation resulted in lower genetic differentiation between seedlings and adults, we compared intercohort (seedling–adult) multilocus average pairwise kinship coefficients  $F_{ij}$  between sites (Loiselle et al. 1995). Average  $F_{ij}$  estimates and standard errors were computed in SPAGeDi by jackknifing across loci.

## 2.6 | Spatial Clustering and Intercohort Nearest Neighbour Distances

If defaunation is reducing seed dispersal services, seedlings should be found in greater frequency nearer to conspecific adults (presumably the parent individuals) in defaunated sites compared to faunally intact sites (Bagchi et al. 2018; Giombini et al. 2017; Ismail et al. 2017), and, relatedly, spatial clustering of individuals is expected to increase with decreased dispersal services. To investigate if defaunation could be reducing seed dispersal enough to affect the spatial distribution of *E. precatioria* in our sites, we first examine the distribution of pairwise

distances between seedlings and nearest conspecific adults with the expectation that if defaunation is negatively affecting seed dispersal, inter-cohort pairwise distances between seedlings and their nearest conspecific adult neighbour will be reduced. We compare differences in mean pairwise distances between the three sites using a Kruskal–Wallis test, which allows for nonparametric dependent data and >2 treatment groups (in this case, the three sites of varying defaunation levels). Because Kruskal–Wallis only indicates the presence of a significant treatment effect (for our purposes, defaunation level) and does not identify which pairs of treatments differ significantly from one another, we also ran a post hoc Dunn test to evaluate specifically which pairs of sites (e.g., RA vs. TRC or RA vs. LA) hosted significant differences in nearest neighbour inter-cohort spatial distances (Dunn 1961).

We used Ripley's K-function to summarise the intensity of within- and between-cohort clustering of conspecific individuals across a range of spatial scales,  $r$ . Ripley's K-function corrects for edge effects and variation in overall abundances of individuals across sites, making comparisons of spatial clustering across our sites possible despite different densities of individuals. Using the package 'spatstat' (Baddeley, Rubak, and Turner 2015), we computed within-cohort univariate K-functions to assess clumping of seedlings around conspecific seedlings and adults around other conspecific adults and bivariate K-functions to assess clustering of seedlings around conspecific adults. Permutations of individuals among locations ( $n = 999$  permutations) were performed to gain estimates and 95% confidence intervals for the expected inhomogeneous Poisson processes (also called complete spatial randomness, CSR) in each site. K-functions above the CSR envelope indicate significant clustering while values below the envelope indicate dispersion. We focus our spatial clustering analysis on pairwise distance scales of <25 m because spatial aggregation as a result of reduced seed dispersal services would be expected at such relatively small scales (Bagchi et al. 2018; Hubbell et al. 2001).

## 2.7 | Fine-Scale Spatial Genetic Structure

We evaluated if defaunation was associated with increased fine-scale spatial genetic structure (FSGS) of *Euterpe precatoria* by analysing spatial autocorrelation of the pairwise kinship coefficient  $F_{ij}$  (Loiselle et al. 1995). The kinship coefficient  $F_{ij}$  is computed between pairs of individuals and gives the correlation in the frequencies of like alleles,  $F_i$  and  $F_j$  at a given locus in pairs of individuals  $i$  and  $j$  (Loiselle et al. 1995). If FSGS exists in the sampled plots, we would expect pairs of near-neighbour individuals to be associated with higher kinship coefficients relative to pairs of individuals that are farther apart. If defaunation is negatively affecting the dispersal of our focal species, the consequences will likely be a decrease in the removal and dispersal of seeds away from parent plants, a lower frequency of medium- and long-distance seed dispersal events due to a lack of large frugivores (Giombini et al. 2024; Link and Fiore 2006), and, ultimately, this would lead to less mixing of genotypes across the landscape and a reduction in the deposition of novel genotypes and less-related propagules to the area. From a spatial genetic structure perspective, we

would expect these changes to strengthen the pattern of FSGS in the defaunated site and lead to greater kinship coefficients among near-neighbour individuals relative to individuals that recruited in a faunally intact context. FSGS was analysed in each site for seedling and adult cohorts separately as well as between seedling and adult cohorts. While we expect seedlings to reflect dispersal and recruitment under the current frugivore communities at each site, the adult populations are likely a mixture of individuals that recruited during pre- and post-defaunation in RA and LA.

We used SPAGeDi to compute kinship coefficients,  $F_{ij}$ , between pairs of individuals across increasing pairwise distance intervals. We chose distance intervals to achieve the finest-scale analysis possible while meeting the SPAGeDi program guidelines and recommendations by Vekemans and Hardy (2004). For example, all distance intervals had >50% participation of individuals and, with some exceptions specified below, all intervals included >100 pairwise comparisons of individuals when computing kinship coefficient estimates. Reference allele frequencies were computed based on the whole sample (all individuals) from each site and pairwise  $F_{ij}$  coefficients were calculated between all possible pairs of individuals that were within the specified distance intervals. Mean  $F_{ij}$  and standard errors were estimated by jackknifing across loci. To test the null hypothesis of no spatial genetic structure, we ran 10,000 random permutations within each specified distance interval for which genotypes were randomly assigned to the locations of mapped individuals to construct 95% confidence intervals. One- and two-tailed tests and associated  $p$ -values were also computed in SPAGeDi reflecting greater or lower kinship coefficients than expected by chance for each distance interval. Distance intervals used in the seedling FSGS analysis in RA and TRC were: 7, 14, 18, 22, 30, 35, 40, 50, 70, 90, 110, 130, 150 and 195 m. We aimed to assign the same pairwise distance intervals for each site for like cohorts and for the between cohort analysis to facilitate comparisons between sites; however, the LA seedling cohort analysis differed slightly to achieve the recommended sample sizes and participation of individuals in each of the distance intervals (with the exception of the first distance interval in LA which held 99 comparisons instead of the recommended 100), which were: 8, 16, 20, 25, 30, 35, 40, 50, 70, 90, 110, 130, 150 and 195 m. Distance intervals for the adult cohorts analyses were 70, 100, 130, 160 and 205 m. Because adult sample sizes were low, RA and TRC both have <100 pairwise comparisons per distance interval ( $n$  pairs ranged from 26 to 76). We therefore cannot be certain of the accuracy of results for adults in RA and TRC and recommend interpreting comparisons of adult SGS across sites with caution. Distance intervals for the intercohort analysis between seedlings and adults were 25, 35, 45, 55, 75, 95, 115, 130, 150 and 215 m.

In each site, we also used SPAGeDi to compute the mean slope of observed  $F_{ij}$  over the natural logarithm of pairwise distances within each distance interval. This slope, denoted as  $b_{\text{Flog}}$ , is expected to be negative if spatial genetic structure is present, meaning, mean  $F_{ij}$  should decrease as pairwise distance intervals increase. Significance of  $b_{\text{Flog}}$  was determined through one-tailed and two-tailed tests of the observed values compared to the null model's 95% confidence intervals. To test for differences in the strength of FSGS between sites for adults and seedlings as well as the strength of intercohort FSGS, we used the statistic  $Sp$ . This metric was proposed



by Vekemans and Hardy (2004) and has been used to summarise the strength of fine-scale spatial genetic structure in a subpopulation and can be used for comparisons across groups (Vekemans and Hardy 2004).  $Sp$  is calculated as  $-b_{F_{log}}/(1-F_{ij})$  where  $F_{ij}$  is the mean observed kinship coefficient of the first pairwise distance interval (i.e., between nearest conspecific neighbours). Importantly,  $Sp$  statistics are robust to variation in the distance and number of pairwise intervals used in spatial genetic structure analysis. In addition to multilocus estimates, SPAGeDi computes  $F_{ij}$  and  $b_{F_{log}}$  estimates per locus. Variation in  $Sp$  across sites can be tested using a randomised block analysis of variance using  $Sp$  estimates per locus, with loci as the blocking factor and site as the treatment factor (Browne, Ottewell, and Karubian 2015). We also perform *post hoc* Tukey Honest Significant Differences tests to check pairwise differences between sites. We evaluated models to check that assumptions were met and used Shapiro–Wilk Goodness-of-Fit-Test to verify normality.

### 3 | Results

Adult and seedling densities (number of individuals/4-ha) varied across sites (Table 1). Notable differences in densities include  $>2\times$  as many adults in LA relative to TRC (49 vs. 23, respectively), but LA had substantially fewer seedlings (172 seedlings in LA vs. 267 in RA, for example). TRC had by far the highest density of seedlings with 427 in 4 ha but the lowest density of adults. There was also some variation in the number of individuals successfully genotyped (Table 1). We found the majority of seedlings in all sites were under 0.25 m tall and the frequency distributions of seedling heights in each site were mostly comparable, though the intermediately defaunated site, LA, showed a relatively more uniform height distribution of individuals throughout the  $<1$  m height range and may therefore represent a greater number of seedling cohorts and consequently a broader age range than the seedling populations sampled in RA and TRC. Mean seedling height in RA was 0.23 m and TRC was 0.26 m, while LA seedlings tended to be slightly taller with a mean height of 0.39 m (Supporting Information Figure S3 for detailed information summarising each sites' seedling heights and seedling height frequency distributions).

**TABLE 2** | Means  $\pm$  standard error of genetic diversity metrics for each cohort and site across loci. Allelic richness ( $A_r$ ) (rarified to  $n=115$  seedlings and  $n=22$  adults), number of effective alleles ( $NA_e$ ), observed heterozygosity ( $H_o$ ), and gene diversity ( $H_s$ ) did not differ significantly between sites for either cohort. Per-locus values can be found in Supporting Information, Table S5; Friedman test outputs comparing mean values between groups can be found in Supporting Information, Table S6.

Cohort	Defaunation status	Rarefied allelic richness ( $A_r$ )	Effective number of alleles ( $NA_e$ )	Observed heterozygosity ( $H_o$ )	Expected heterozygosity ( $H_s$ )
Adult	Defaunated (RA)	3.93 ( $\pm 0.31$ )	1.91 ( $\pm 0.24$ )	0.37 ( $\pm 0.02$ )	0.45 ( $\pm 0.06$ )
	Recovering (LA)	3.46 ( $\pm 0.45$ )	2.34 ( $\pm 0.34$ )	0.46 ( $\pm 0.07$ )	0.52 ( $\pm 0.09$ )
	Faunally intact (TRC)	3.00 ( $\pm 0.63$ )	1.92 ( $\pm 0.48$ )	0.35 ( $\pm 0.14$ )	0.35 ( $\pm 0.13$ )
Seedling	Defaunated (RA)	4.86 ( $\pm 0.63$ )	1.92 ( $\pm 0.28$ )	0.40 ( $\pm 0.05$ )	0.43 ( $\pm 0.05$ )
	Recovering (LA)	4.85 ( $\pm 0.51$ )	1.99 ( $\pm 0.32$ )	0.40 ( $\pm 0.1$ )	0.41 ( $\pm 0.10$ )
	Faunally intact (TRC)	4.85 ( $\pm 0.63$ )	1.78 ( $\pm 0.37$ )	0.30 ( $\pm 0.09$ )	0.33 ( $\pm 0.10$ )

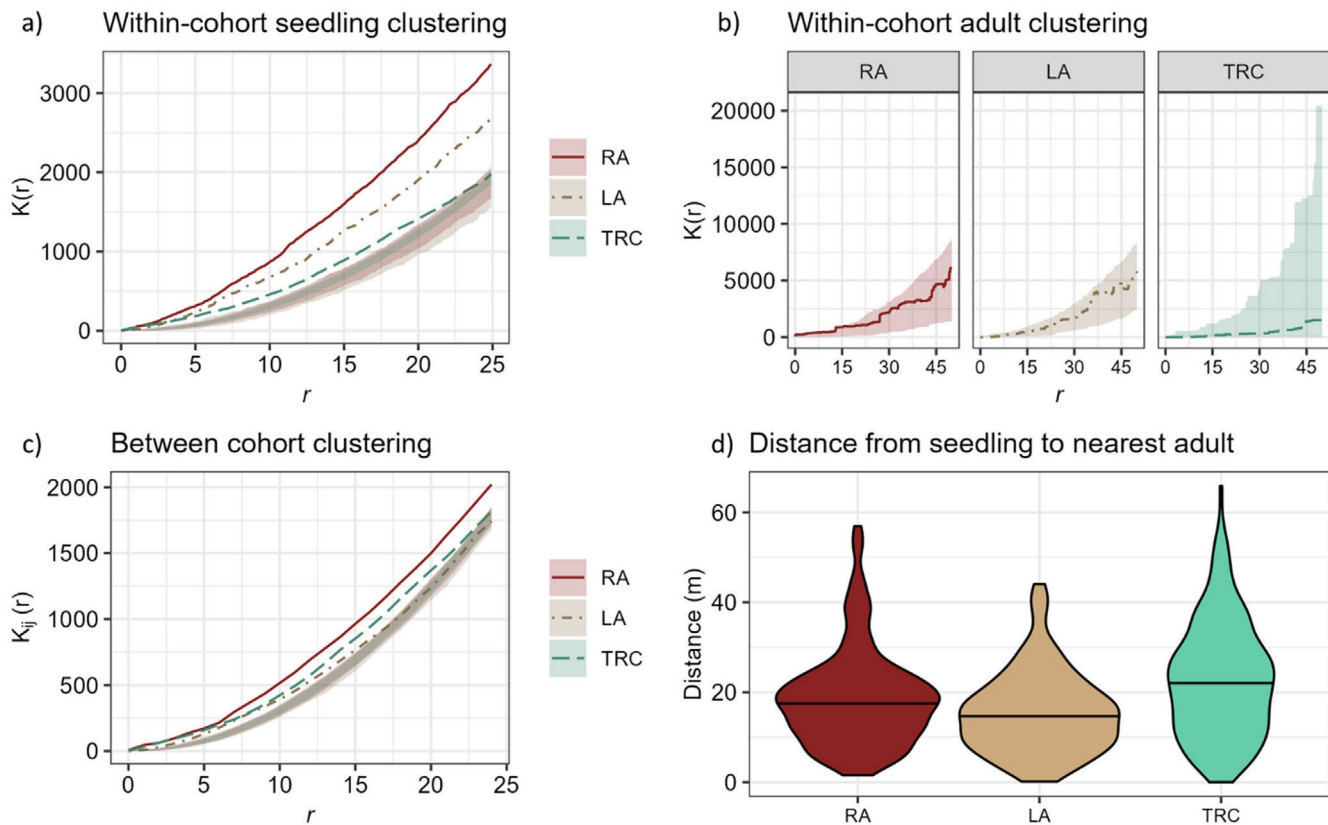
### 3.1 | Genetic Diversity, Inbreeding and Intercohort Relatedness

All genetic diversity metrics were lowest in the faunally intact site (Table 2); however, Friedman tests indicate no significant differences in mean estimates of genetic diversity or inbreeding metrics across sites (full test outputs can be found in Supporting Information Table S6). For seedlings, allelic richness ( $A_r$ ) was rarefied to 115 individuals (the lowest number seedling abundance of any of the sites) and mean  $A_r$  was just under five for all sites. Adult  $A_r$  ranged from 3.0 to 3.9 and was lowest in the faunally intact site (Table 2). Number of effective alleles ( $NA_e$ ) was low (1.9–2.3) for all sites and cohorts (Table 2). Mean observed heterozygosity ( $H_o$ ), also showed little variation across sites and was highest for seedlings and adults in LA (Table 2). Mean gene diversity ( $H_s$ ) was also highest in the hunting-impacted sites (0.43 for RA seedlings and 0.52 for LA adults). Multilocus individual inbreeding coefficients ( $F_i$ ) were significant (based on two-tailed tests comparing observed values to null models of 10,000 permutations of randomised gene copies among individuals) in adult cohorts in RA ( $F_i=0.14$ ,  $p=0.04$ ) and LA ( $F_i=0.12$ ,  $p=0.03$ ), but not in TRC ( $F_i=-0.04$ ,  $p=0.69$ ). Seedling cohort  $F_i$  was significant in RA ( $F_i=0.07$ ,  $p=0.01$ ) and TRC ( $F_i=0.07$ ,  $p=0.02$ ), but not in LA ( $F_i=-0.002$ ,  $p=0.94$ ).

Genetic differentiation between adults and seedlings, as estimated by mean pairwise kinship coefficients for all seedling-adult pairs per site, did not differ significantly between sites. Jackknifed estimates of average kinship coefficients  $F_{ij}$  between adults and seedlings was lowest in RA  $-0.0011 \pm 0.0009$  SE and LA  $F_{ij}=-0.0013 \pm 0.0019$  and TRC  $F_{ij}=-0.0001 \pm 0.001$ .

### 3.2 | Spatial Clustering and Intercohort Nearest Neighbour Distances

Distance between *E. precatorea* seedlings and nearest conspecific adults varied significantly with defaunation status (Kruskal–Wallis rank sum test  $\chi^2=48.73$ ,  $p<0.001$ ) (Figure 4d, Supporting Information, Table S7). On average the distance between a seedling and its nearest conspecific adult neighbour



**FIGURE 4** | *Euterpe precatoria* seedling and adult spatial data. (a) Within-cohort univariate K-functions for seedlings (i.e., estimates of clustering of seedlings with conspecific seedlings) in each site (RA: defaunated, LA: recovering, TRC: faunally intact). Shaded regions show null model confidence envelopes (Poisson process spatial randomness based on 999 simulations). K-functions above shaded envelopes indicate significant clustering, (b) within-cohort univariate K-functions for adults in each site; note that each site is displayed in a separate panel to improve clarity due to overlap in values across sites, (c) between-cohort (seedling-adult) bivariate K-functions for each site (again, shaded regions indicate null model envelopes and values above this are significantly clustered) and (d) pairwise distances between seedlings and the nearest conspecific adult for all seedlings in each plot.

was greatest in the faunally intact site, TRC, at approximately 23m, compared to about 19m in the defaunated site, RA, and approximately 16m in the recovering site, LA. Post hoc Dunn tests indicate these differences were significant between all sites (TRC vs. RA:  $Z = -4.16$ ,  $p < 0.001$ ; TRC vs. LA:  $Z = -6.67$ ,  $p < 0.001$  and LA vs. RA:  $Z = -2.84$ ,  $p < 0.01$ ).

Within-cohort estimates of K-functions show significantly greater clustering among seedlings than expected by chance at all distances in all sites, and clustering was significantly greater among seedlings in the defaunated site (RA) than in the other two sites at spatial scales  $> 3$  m, while the recovering site (LA) showed clustering values midway between the faunally intact site (TRC, which had the lowest degree of within-cohort seedling-seedling clustering) and the defaunated site (Figure 4a). Within-cohort adult univariate K-functions indicated adults were not significantly clustered in any sites, although adults in RA showed near-significant clustering with other conspecific adults at spatial scales  $< 10$  m (Figure 4b). Intercohort (adult-seedling) bivariate K-functions summarising clustering of seedlings around conspecific adults indicate that seedlings in all sites at all spatial scales are significantly clustered and that at distances  $> 7$  m, this pattern is strongest in the defaunated site relative to the other two sites (Figure 4c).

### 3.3 | Defaunation and Fine-Scale Spatial Genetic Structure

#### 3.3.1 | Within-Cohort Seedling Spatial Genetic Structure

Seedling kinship coefficients  $F_{ij}$  were evaluated across 14 intervals of pairwise distances up to 195m. In the defaunated site (RA), there was evidence of fine-scale spatial genetic structure (FSGS). Seedling-seedling kinship values showed some decay with increasing pairwise distances across the first several distance classes (up to 70m). Among these, only the third distance class (20m) showed significantly higher kinship than expected (one-tailed test: observed values (obs)  $>$  expected values under null permutation outcome (exp),  $p = 0.002$ ; two-sided test: obs  $<$  exp.,  $p = 0.004$ ).  $b_{Flog}$  (the regression of  $F_{ij}$  coefficients between seedlings on the natural logarithm of distance) in RA was significant ( $b_{Flog} = -0.005 \pm 0.003$  SE) (one-sided test: obs  $<$  exp.,  $p = 0.03$ ; two-sided test: obs  $<$  exp.  $p = 0.06$ ) (Figure 5a).

In LA, the previously defaunated but currently recovering site, there was also evidence of FSGS and kinship coefficients decreased across distances, although the only significant departure from the null model was in the 11th distance interval

(130 m) in which seedling kinship was significantly lower than expected (one-sided test:  $\text{obs} < \text{exp.}$ ,  $p = 0.005$ ; two-sided test:  $\text{obs} < > \text{exp.}$ ,  $p = 0.009$ ).  $b_{F_{\log}}$  was also significantly lower than null expectations ( $b_{F_{\log}} = -0.006 \pm -0.003$ ; one-sided test:  $\text{obs} < \text{exp.}$ ,  $p = 0.03$ ; two-sided test,  $p = 0.06$ ) (Figure 5a).

The faunally intact site, TRC, there was marginally significant kinship between seedlings in the first distance class (7 m; one-sided test:  $\text{obs} > \text{exp.}$ ,  $p = 0.048$ ; two-sided test:  $\text{obs} < > \text{exp.}$ ,  $p = 0.09$ ) but no other intervals differed significantly from null expectations. After the first distance interval, intracohort seedling kinship values decreased sharply and remained lower than the first two distance classes at all scales  $< 90$  m; however, slope  $b_{F_{\log}}$  was not significant (one-sided test:  $\text{obs} < \text{exp.}$ ,  $p = 0.54$ ; two-sided test:  $\text{obs} < > \text{exp.}$ ,  $p = 0.92$ ;  $b_{F_{\log}} = 0.0003 \pm 0.0009$ ) (Figure 5a).

Strength of seedling intracohort FSGS as summarised by  $Sp$  statistics differed significantly according to defaunation status ( $F = 4.52$ ,  $p = 0.03$ ) such that  $Sp$  was significantly higher in the defaunated site relative to the faunally intact site (RA multilocus mean  $Sp = 0.0049 \pm 0.003$  versus TRC multilocus mean  $Sp = -0.0047 \pm 0.003$ , while LA  $Sp$  metrics fell between the two sites) (Figure 6a).

### 3.3.2 | Within-Cohort Adult Spatial Genetic Structure

Within-cohort kinship coefficients between pairs of adult *E. precatatoria* were evaluated across five distance intervals (up to 205 m). Note that due to low densities of adults within study plots, adult sample sizes are low and consequently fewer than recommended pairwise comparisons exist per distance interval in RA and TRC.

In RA,  $b_{F_{\log}} = -0.03 \pm 0.04$ , which was significantly lower than expected under the null model (one-sided test:  $\text{obs} < \text{exp.}$ ,  $p = 0.03$ ; two-sided test:  $\text{obs} < > \text{exp.}$ ,  $p = 0.06$ ); however, kinship coefficient estimates did not deviate significantly from the null model expectations in any distance interval (Figure 5b).

In LA, adult kinship coefficients did show some decay across increasing distance intervals (Figure 5b).  $b_{F_{\log}} = -0.02 \pm 0.01$  and was marginally significantly lower than null expectations (one-sided test:  $\text{obs} < \text{exp.}$ ,  $p = 0.049$ ; two-sided test:  $\text{obs} < > \text{exp.}$ ,  $p = 0.09$ ). However, the final distance class (205 m) was the only significant deviation of  $F_{ij}$  from the null model (one-sided test:  $\text{obs} < \text{exp.}$ ,  $p = 0.01$ , two-sided test:  $\text{obs} < > \text{exp.}$ ,  $p = 0.03$ ).

In TRC, adults showed a pattern of decay in kinship with increasing pairwise distances across the first three distance classes (up to 130 m) and kinship was significantly lower than expected in that third interval (one-sided test:  $\text{obs} < \text{exp.}$ ,  $p = 0.009$ ; two-sided test:  $\text{obs} < > \text{exp.}$ ,  $p = 0.02$ ) (Figure 5b). The slope  $b_{F_{\log}} = -0.02 \pm 0.03$  and did not differ from null expectations (one-sided:  $\text{obs} < \text{exp.}$ ,  $p = 0.23$ ; two-sided:  $\text{obs} < > \text{exp.}$ ,  $p = 0.46$ ).

The strength of fine-scale spatial genetic structure (FSGS) as summarised by  $Sp$  statistics was qualitatively lowest in the faunally intact site and highest in the defaunated site, but this difference was not significant ( $F = 0.076$ ,  $p = 0.58$ ; Figure 6c).

### 3.3.3 | Intercohort Seedling-Adult Spatial Genetic Structure

Kinship coefficients were evaluated for seedling-adult pairs across 10 pairwise distance intervals up to 215 m. In RA, there was no clear trend of intercohort spatial genetic structure between seedlings and adults across increasing pairwise distances. Kinship values only departed from null expectations in one interval (one-sided test:  $\text{obs} < \text{exp.}$ ,  $p = 0.04$ ; two-sided test:  $\text{obs} < > \text{exp.}$ ,  $p = 0.08$ ).  $b_{F_{\log}} = 0.002 \pm 0.004$  and did not differ from null expectations (one-sided test:  $\text{obs} < \text{exp.}$ ,  $p = 0.06$ ; two-sided test:  $\text{obs} < > \text{exp.}$ ,  $p = 0.71$ ).

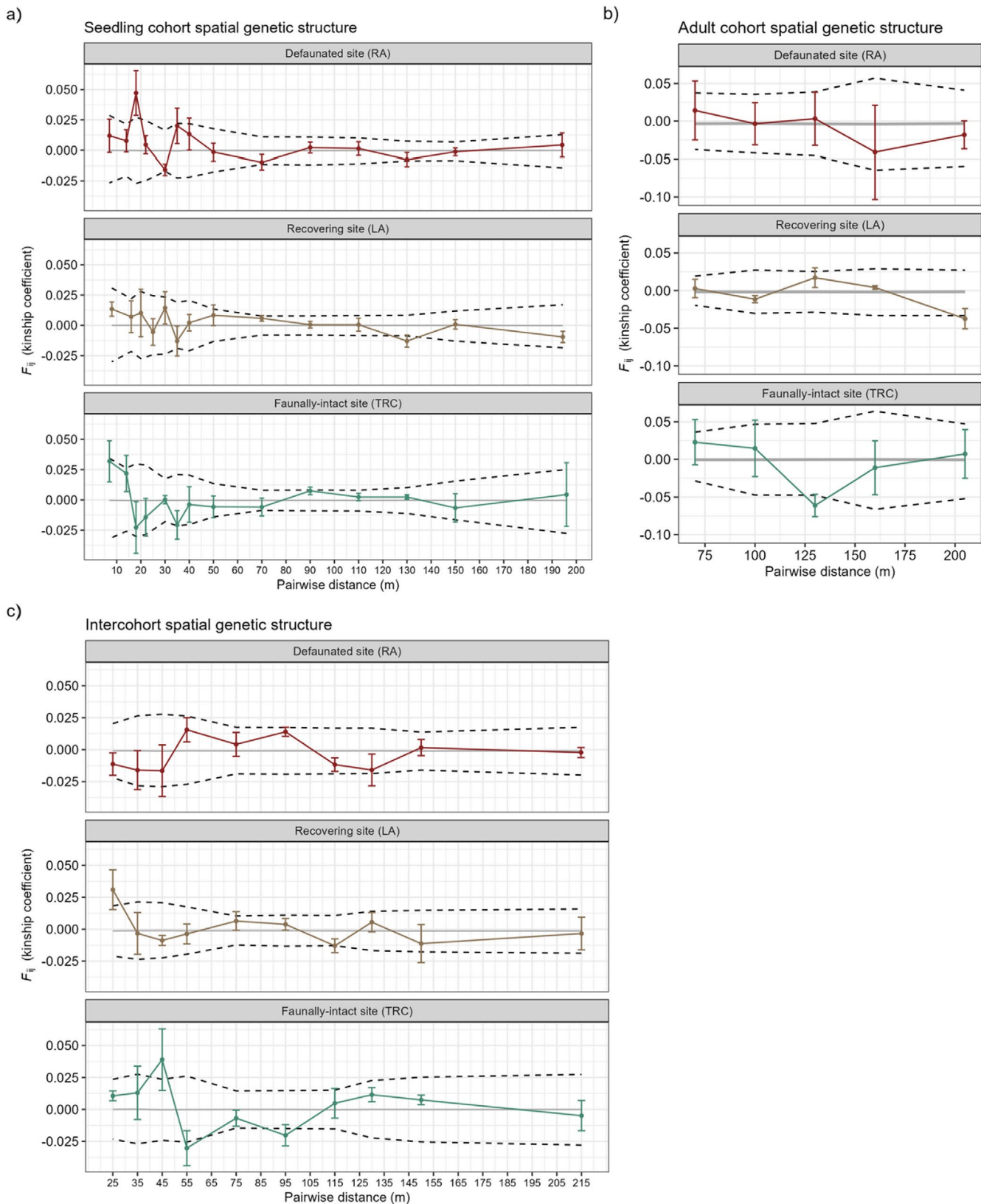
In LA, kinship decayed quickly across the smallest pairwise distance intervals (those  $< 55$  m). Kinship coefficients were significantly higher than expected in the first distance class (8 m) (one-sided test:  $\text{obs} > \text{exp.}$ ,  $p = 0.002$ ; two-sided test:  $\text{obs} < > \text{exp.}$ ,  $p = 0.003$ ), and was significant  $b_{F_{\log}} = -0.013 \pm 0.01$  (one-sided test:  $\text{obs} < \text{exp.}$ ,  $p = 0.002$ ; two-sided test:  $\text{obs} < > \text{exp.}$ ,  $p = 0.003$ ).

In TRC, kinship was significant in the third distance interval (45 m) where it was higher than the null expectations (one-sided test:  $\text{obs} > \text{exp.}$ ,  $p = 0.0008$ ; two-sided test:  $\text{obs} < > \text{exp.}$ ,  $p = 0.002$ ). Mean kinship dropped below expected values in the next distance interval (55 m) (one-sided test:  $\text{obs} < \text{exp.}$ ,  $p = 0.01$ ; two-sided test:  $\text{obs} < > \text{exp.}$ ,  $p = 0.02$ ) and again in the 6th distance interval (95 m) (one-sided test:  $\text{obs} < \text{exp.}$ ,  $p = 0.01$ ; two-sided test:  $\text{obs} < > \text{exp.}$ ,  $p = 0.02$ ). Slope  $b_{F_{\log}}$  was not significant, however, based on comparisons with null model expectations ( $b_{F_{\log}} = -0.007 \pm 0.005$ ; one-sided test:  $\text{obs} < \text{exp.}$ ,  $p = 0.12$ ; two-sided test:  $\text{obs} < > \text{exp.}$ ,  $p = 0.24$ ).

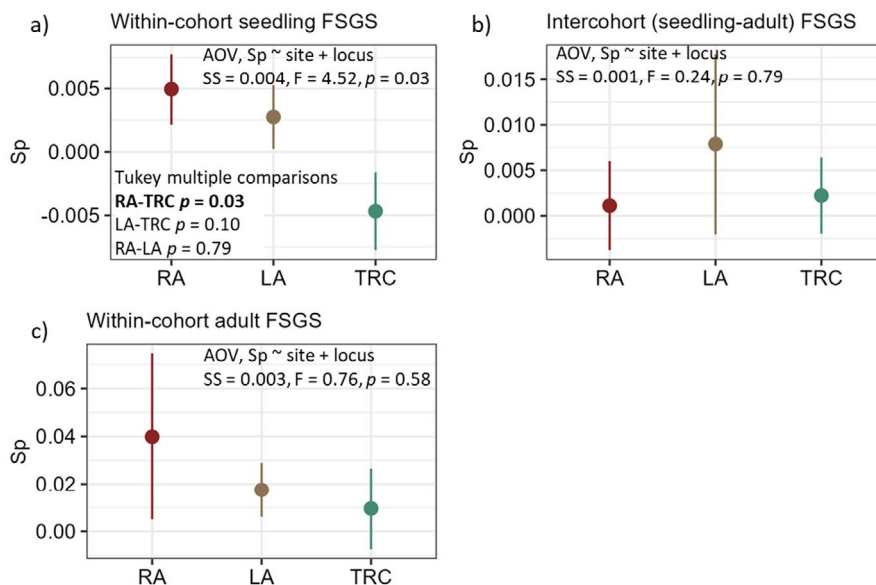
## 4 | Discussion

We investigated the effects of defaunation on seed dispersal services by evaluating fine-scale spatial genetic structure (FSGS), genetic diversity and spatial patterns in a hyperabundant, generalist palm species that produces seeds small enough to be consumed and dispersed by a variety of frugivore species not negatively impacted by hunting and defaunation. Despite the presence of multiple viable dispersers, however, we found that for our study species defaunation was associated with increased fine-scale spatial genetic structure between seedlings, increased within- and between-cohort clustering of seedlings, and decreased distances between seedlings and nearest conspecific neighbouring adults. While our results should be interpreted with caution due to the lack of replication, our findings generally suggest that defaunation is reducing seed dispersal services and fruit removal for our focal species despite it being a generalist plant with a range of frugivore mutualists present in both intact and defaunated forests. We caution that while large-seeded plants are the most obviously vulnerable species to defaunation and seed disperser loss, smaller-seeded species may be more impacted than previously thought and we urge further research across a broader suite of plant groups, especially given that the patterns detected here of increased fine-scale spatial genetic structure and clustering in seedling cohorts in the short term may affect population genetics and consequently demographic dynamics in the long term.





**FIGURE 5** | Spatial autocorrelation of pairwise kinship coefficients for each site between (a) seedlings, (b) adults and (c) seedling and adult pairs. Points are mean observed kinship coefficients  $F_{ij}$  over pairwise distance intervals (maximum pairwise distance allowed within each interval is given on the x-axis in meters) with jackknifed standard errors shown by vertical bars. Mean permuted  $F_{ij}$  across pairwise distances for the null model is shown as solid horizontal grey line and upper and lower 95% confidence intervals are shown in dashed lines. Full details of plotted values and per-distance-interval sample sizes can be found in [Supporting Information, Table S8](#).



**FIGURE 6** | Mean (points) and standard error (bars) of  $S_p$  values computed per loci in SPAGeDi for each site: RA (defaunated), LA (recovering), TRC (faunally intact). Summarised output is given for the randomised block analysis of variance with loci as the blocking factor and site (defaunation level) as the treatment factor in upper right hand corner of plots. SS: sum of squares. A Tukey multiple comparisons test was performed for the within-seedling cohort  $S_p$  data and adjusted  $p$ -values are given in the plot (bold values indicate significant pairwise differences). See [Supporting Information](#), Table S9 for full test output.

#### 4.1 | Caveats and Further Research

While restricted fruit removal and subsequent seed movement is among the most cited explanations for the presence of a high spatial genetic structure, we recommend a conservative interpretation of our results (Grimm and Railsback 2005; Levin 1992) and a number of caveats follow. First, it should be noted that our study uses a limited number of microsatellite markers and alleles, which may reduce the robustness of our genetic results. Another consideration is that our seedling size category included individuals up to 1 m tall, which is a large enough range to encompass multiple seedling cohorts. This could bias inferences about differences in spatial genetic structure between sites if variation existed in the number of cohorts and in the age structures of seedlings at each site; however, seedling height frequency distributions between plots were similar and indicated that we primarily sampled from one, recent cohort in both of these sites ([Supporting Information](#) Figure S3a–c). Although the intermediately defaunated, recovering site did exhibit a more uniform distribution of seedling heights than the other two sites, the majority of sampled seedlings within LA were still <0.25 m as in the other two sites. While age class and seedling mortality (which would be expected to have had more time to occur in an older seedling cohort) do not necessarily affect spatial genetic structure (Hampe, El Masri, and Petit 2010; Sandurska et al. 2024), we cannot rule out that the intermediately defaunated site could have included more cohorts and consequently a different age structure than seedlings from the defaunated and faunally intact sites, and this may have diluted or otherwise affected differences detected in fine-scale spatial genetic structure between this site and the others. Seedling densities likewise differed between sites, which could affect spatial genetic structure patterns. However, we do not believe this to have influenced our qualitative conclusions because if density were to bias results, the most likely outcome would have been opposite of the

observed trend because our faunally intact site had substantially higher densities of seedlings (~420 seedlings/ha) compared to the defaunated site (~260 seedlings/ha) and higher density is typically positively associated with the strength of spatial genetic structure (Goncalves et al. 2022). Next, we acknowledge it could be argued that our focal species, *E. precatorea*, is not the best subject to address how defaunation may affect species with small-seeds, as many more, smaller-seeded species exist. Because our work indicates that defaunation affects not only large-seeded species but also generalists, we urge further related research to consider truly small-seeded species (i.e., those <0.5 cm in diameter), such as the many keystone species belonging to the Melastomataceae and Moraceae families (e.g., *Ficus* species). Such small-seeded groups have been presumed to be unimpacted by defaunation, but this remains to be tested. Incorporating plant species representative of a wider range of functional groups will be important to gain a more comprehensive and community-level understanding of defaunation's downstream effects. Again, our results are based on a limited number of study sites with only one site per defaunation level (defaunated, intermediately defaunated/recovering and faunally intact), and should be interpreted with this in mind. We recommend that our results be viewed as impetus for further research on the effects of defaunation on dispersal services for plants with varying fruit and seed morphologies that leverage higher replication.

#### 4.2 | Consequences of Defaunation on Seedling Spatial Distributions and Spatial Genetic Structure

The downsizing of the frugivore community through defaunation is a well-documented phenomenon and may explain the observed changes in spatial aggregation and increased clustering of seedlings around each other and around conspecific adults, as

well as the increase in seedling FSGS in the defaunated site and in intercohort FSGS in the recovering, previously defaunated site. The defaunated site (and to a lesser degree the recovering site) lacked or had low abundances of species such as toucans, guans and trumpeters, large- and medium-sized primates such as spider monkeys and capuchins, and large terrestrial secondary dispersers such as tapirs and brocket deer. Relative to other frugivores, these species remove and disperse the greatest diversities and quantities of seeds across the longest distances and their foraging behaviours are known to shape spatial and genetic patterns in animal-dispersed plant populations (Browne and Karubian 2018a; Choo, Juenger, and Simpson 2012; Giombini, Bravo, and Tosto 2016; Holbrook and Loiselle 2009; Link and Fiore 2006; Wehncke and Domínguez 2007). For example, spider monkeys remove large quantities of seeds from fruiting trees and disperse them over 1250m, with an average distance estimated to be ~450m, and tapirs disperse seeds >2km (Fragoso 1997; Karubian et al. 2015; Link and Fiore 2006) and both species shape genetic patterns for plants whose fruits they consume (Giombini, Bravo, and Tosto 2016; Karubian et al. 2015). In the defaunated site in our study, these extirpated frugivore groups have been replaced by high abundances of tamarins and rodents (such as agoutis). Although tamarins are frugivorous and can disperse seeds upwards of 500m, as smaller-bodied animals, they remove fewer fruits and most of their seed dispersal is over much shorter distances, generally under 200m (Culot et al. 2010; Heymann, Fuzessy, and Culot 2022). Agoutis can also be effective short-distance secondary dispersers and will cache seeds in clusters, although they are also seed predators (Cao et al. 2011; Mittelman et al. 2021). The loss of large-bodied frugivores has likely not only reduced the portion of fruits that are removed from fruiting trees in the defaunated site but also dampened long-distance seed dispersal and reshaped the spatial distribution and aggregation of seed deposition on the forest floor, resulting in recruiting seedlings being nearer to conspecific adults.

Less well-studied are the effects of defaunation, which decreases the diversity of the frugivore community by decreasing the abundance and evenness of species. We posit that this may be an additional facet behind the increased seedling FSGS we found in the defaunated site and the increased intercohort FSGS we found in the recovering, previously defaunated site. Decreased frugivore diversity should lead to a decrease in the diversity of seed deposition sites given that different frugivore species vary in their movement patterns, habitat use and foraging behaviours, all of which can create differential seed dispersal services and variation in dispersal to different microhabitats (Carvalho et al. 2021; Razafindratsima and Dunham 2015; Rumeu et al. 2020). For example, small terrestrial vertebrates tend to re-use paths and particular sections of the forest as they forage and move throughout the day while larger vertebrates are more wide ranging (Cao et al. 2011; Lichti, Steele, and Swihart 2017; Russo, Portnoy, and Augspurger 2006). We therefore posit that by reducing frugivore diversity, defaunation not only reduces long distance dispersal but also dampens dispersal at small scales such that near neighbours become more related. If the current seedlings establish and become reproductively active adults, bi-parental inbreeding could increase in subsequent generations and increase the expression of deleterious alleles (Jones and Hubbell 2006; Quesada et al. 2011; Sebbenn et al. 2011). Partitioning the effects of the

downsizing of the frugivore community versus the decrease in diversity would significantly advance our understanding of how defaunation impacts animal-mediated seed dispersal.

### 4.3 | No Short-Term Consequences of Defaunation for Genetic Diversity

Genetic diversity metrics were moderate to low in all sites and cohorts, and one reason for this could be potentially because of low polymorphism in our markers and as such we draw no strong conclusions regarding qualitative trends across sites. As predicted, defaunation was not associated with a decrease in genetic diversity (including allelic richness, effective number of alleles, observed heterozygosity or gene diversity). This is likely because a severe genetic bottleneck would be required in order for these metrics to be impacted during the timescale of our study (an estimated <5 generations in our study species have passed since defaunation in RA). The impact of defaunation alone does not cause an immediate reduction in effective population size, which would be a prerequisite to decreases in metrics such as genetic diversity in the short term (Aldrich et al. 1998; Carvalho et al. 2021; Kettle et al. 2008; Kramer et al. 2008). Indeed, studies on the negative effects of forest loss and fragmentation (which represent more severe and rapid reductions in the gene pool than defaunation) on genetic diversity have found effects can take 50–100years to become detectable (Aguilar et al. 2008; Vranckx et al. 2012). However, increases in fine-scale spatial genetic structure serve as early warning signs that seed-mediated geneflow is being interrupted (Carvalho et al. 2021). One study examining another canopy palm species, *Oenocarpus bataua*, found that forest loss has a direct, negative effect on genetic diversity, most likely by decreasing effective population size  $N_e$  and that this effect was much greater than minor disruptions to seed dispersal services (Diaz-Martin and Karubian 2021). Additionally, continued pollen flow in our system will likely continue to help maintain genetic diversity and related metrics irrespective of diminished seed dispersal. The extensive nature of pollen dispersal makes it a key ecological process that maintains gene flow and therefore genetic diversity in many tropical and temperate plant species (Browne and Karubian 2018a; Ennos 1994; Hamrick 2004; Parejo Farnés et al. 2017; Petit et al. 2005; Sork et al. 2015). As our study sites were in contiguous forests, we do not anticipate that pollinator communities have been impacted. Even in severely deforested landscapes, beetle and bee pollinators of another South American palm have been found to maintain gene flow (Diaz-Martin and Karubian 2021). Although we do not find that defaunation has a direct effect on genetic diversity, we similarly caution that over much longer timescales than the study at hand, dampened seed dispersal could increase inbreeding to eventually impact genetic diversity. Indeed, the defaunated site was the only site that showed significant inbreeding in both adult and seedling cohorts, while the recovering site only showed significant inbreeding in the adult cohorts. While this could align with the expected effects of defaunation and increased reproduction between related individuals following increased spatial genetic structure, we draw no strong conclusions from this pattern, particularly given that the faunally intact site had significant inbreeding in seedlings as well (though not among adults).



## 5 | Conclusion

Our results corroborate recent works that suggest defaunation may increase the importance of small- and medium-bodied frugivores, but that they will likely not be able to compensate fully for the loss of ecological functions performed by extirpated species (Carvalho et al. 2021; Fricke, Tewksbury, and Rogers 2018; Goebel et al. 2023; McConkey and Brockelman 2011). The effect of defaunation may be particularly damaging for rare plants which represent a large portion of tropical plant species, as they may rely on farther-ranging dispersers to regenerate in isolated habitat niches. In a broader context, the results reported here are an indication of how defaunation may interact with other ongoing global change patterns by damaging plant's abilities to persist with multiple threats. For example, increased spatial genetic structure resulting from decreased fruit removal and the absence of long-distance dispersal events can eventually affect genetic diversity and increase inbreeding in a population, which are tied to a species' population-level ability to maintain robust immune systems and mount effective defences against plant pathogens and adapt to threats such as climate change (Aguilar et al. 2008; Browne and Karubian 2018b; Carvalho et al. 2016; Fricke et al. 2022). Over multiple generations spanning several decades into the future, these consequences could become more severe and have long-term and irreversible effects on fitness and demography of affected tree species, with cascading ecosystem-level effects on higher and lower trophic levels.

### Author Contributions

T.L. conceived the project idea and initial study design, carried out field data collection, lab work, genetic and statistical analyses and manuscript writing. Z.D.-M. contributed to lab work, genetic analysis and manuscript writing and revisions. V.S. contributed to the study design, field data collection and manuscript revision. J.K. contributed reagents and materials to lab work and contributed to study design and manuscript revision. J.C. contributed reagents and materials to lab work and contributed to genetic analysis, results interpretation and troubleshooting and manuscript revision. A.E.D. contributed to project framing, study design and manuscript revision.

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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

Data (including genotype data) and code are available on GitHub: <https://github.com/Jlamperty/Defaunation-SeedDisp-MEC/tree/main>. The curated and published dataset additionally available on Dryad: <https://doi.org/10.5061/dryad.wwpzgm5v6>.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section.