Received: 16 November 2023 | Accepted: 26 January 2024

DOI: 10.1111/1365-2745.14280

Journal of Ecology | BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Animal-mediated seed dispersal and the demo-genetic configuration across plant colonization gradients

Jorge Isla[1] | Miguel Jácome-Flores[2,1] | Cristina Rigueiro[1] | Juan Miguel Arroyo[1] | Pedro Jordano[1,3] | Cristina García[4]

[1]Department Integrative Ecology, Estación Biológica de Doñana, EBD-CSIC, Sevilla, Spain

[2]CONAHCYT-Centro del Cambio Global y la Sustentabilidad, Calle Centenario del Instituto Juárez S/N, Villahermosa, Tabasco, Mexico

[3]Department of Biología Vegetal y Ecología. Facultad de Biología., Universidad de Sevilla, Sevilla, Spain

[4]Department of Biological Science, Centre for Ecology, Evolution, and Behaviour, Royal Holloway University of London, Egham, UK

Correspondence
Jorge Isla
Email: jorgeislaescudero1992@gmail.com

Funding information
European Regional Development Fund, Grant/Award Number: SUMHAL, LIFEWATCH-2019-09-CSIC-13 and POPE 2014-2020; Spanish Ministry of Science and Innovation, Grant/Award Number: CGL2017-82847-P, PID2022-136812NB-I00 and PRE2018-085916; Investigadores por México-CONAHCYT, Grant/Award Number: 73215; VI Plan Propio de Investigación of the Universidad de Sevilla, Grant/Award Number: VI PPIT-US; Research Application Support Programme (RHUL); Royal Society (Research Application Support Programme) RaSP

Handling Editor: Michał Bogdziewicz

Abstract

1. Ecologists have long recognized that seed dispersal mutualisms trigger natural regeneration and range expansion of animal-dispersed trees. Yet we lack empirical studies addressing whether frugivore activity influences founder effects, which reduce genetic diversity at the colonization front of expanding populations.
2. Here, we evaluate the contribution, from both demographic and genetic perspectives, of animal frugivores dispersing seeds across an expansion gradient. We used DNA barcoding for frugivores identification and highly polymorphic genetic markers (SSRs) for maternal analysis of juniper seeds to investigate how (1) stand maturity, (2) microhabitat types and (3) foraging patterns shape the distribution of the maternal progenies along this gradient.
3. We expect both reduced seed rain density and low numbers of source trees contributing to the seed rain at the colonization front, with limited availability of local fruiting trees. We also anticipated that large-sized frugivore species would promote maternally rich seed rain due to their ability to mix seed progenies during digestive processing and move further distances across the landscape.
4. Contrary to our expectations, we found that all identified frugivores produced dense and genetically diverse seed rains across the expansion gradient, even at the colonization front, characterized by scarce fruiting trees.
5. *Synthesis*: Our findings shed light on the fundamental and applied implications of plant–frugivore interactions in shaping highly diverse second-growth forests. These results emphasize the necessity of preserving plant–animal mutualistic interactions to ensure the persistence and expansion of natural tree populations, particularly in formerly fragmented landscapes

KEYWORDS
frugivore's forging patterns, maternal progenies, Mediterranean forests, microhabitat, natural regeneration, plant–animal mutualisms, second-growth expanding forest, seed dispersal, seed rain, stand maturity

1 | INTRODUCTION

Deforestation has led to a global reduction in forest cover over decades, but recent environmental policies and widespread rural land abandonment now foster forest regeneration and expansion worldwide (Chazdon et al., 2020). As second-growth forests expand, our dependence on them to support our well-being increases, but regrowth forests have long been considered less capable of providing ecosystem services comparable to those obtained from old-growth forests (Chazdon, 2014). Yet, recent studies reveal that naturally regenerating forests on formerly tree-depleted rural lands exhibit comparable carbon uptake and host similar levels of plant and insect diversity (Espelta et al., 2020; Poorter et al., 2016). These results encourage further empirical studies to understand the regeneration dynamics underlying fast-paced forest natural expansion and, more importantly, to identify the factors that shape diverse, functional and resilient future forests.

Natural regeneration and the expansion of forest fragments commence with the formation of the seed rain, that is, the spatial distribution throughout the landscape of all seeds dispersed in each reproductive event (Harper, 1977; Figure 1). Seed rains, thus, serve as the starting template for plant regeneration in mature stands and for plant expansion in colonization front stands (Jordano & Godoy, 2002). Frugivores provide dispersal services to animal-dispersed plants—including 70%–94% of woody species in tropical forests and 50%–75% in local Mediterranean habitats (Jordano, 2014)—in exchange for food resources. Frugivore foraging patterns form strongly aggregated seed rain patterns, where seeds are disproportionately dispersed to few highly preferred deposition sites (see Figure 1 for further discussion on seed rain patterns, foraging behaviour and microhabitat preferences). The same foraging patterns also determine the distribution of maternal progenies within and across deposition sites, that is, whether deposition sites receive seeds from one/few fruiting trees or, instead, they accumulate seeds from many different mother trees (Figure 1) (Carvalho et al., 2020; García & Grivet, 2011; Karubian et al., 2010; Scofield et al., 2012). In this way, the demographic and genetic consequences of animal-mediated seed dispersal are tightly intertwined. For example, Karubian et al. (2010) found that the foraging behaviour of a long-wattled umbrellabird clumped maternal progenies of a palm tree at leks where males spend up to 80% of their time during their breeding season (see also Wenny & Levey, 1998). Likewise, García et al. (2009) demonstrated that vegetation cover determined frugivores' site preferences, with more maternal progenies found in covered microhabitats compared with open ones, a pattern corroborated in other studies (e.g. Lavabre et al., 2016). The spatial distribution of the maternal progenies across deposition sites is expected to influence the establishment of dispersed seeds and early stages of seedling recruitment (García et al., 2009; Grivet et al., 2005), but this aspect remains unexplored across population expansion gradients. For example, colonization fronts often exhibit sparse fruiting tree populations, potentially diminishing maternal source tree diversity in the seed rain while aggregating maternal progenies at a limited number of suitable deposition sites.

The reduced diversity found at the colonization front of expanding populations has been attributed to founder effects coupled with rapid expansion, allowing a few colonizing individuals (or genes) to establish and reproduce over vacant lands while hampering the arrival of later individuals (Waters et al., 2013). However, recent advanced models reveal that landscape heterogeneity, density-dependent processes (such as Allee effects) and biotic interactions (namely competition) may facilitate the sequential arrival of individuals promoting high diversity at the colonization front (Gandhi et al., 2019; Paulose & Hallatschek, 2020; Roques et al., 2012). Moreover, when long-distance dispersal events (LDD) occur frequently, genetic diversity persists across the landscape because the homogenizing effect of surfing is prevented due to a highly stratified dispersal process (Le Corre & Kremer, 1998). The increasing data on forest regeneration and expansion dynamics offer us a timely opportunity to examine whether natural plant expansion promotes high diversity levels as suggested previously (Espelta et al., 2020; García et al., 2020) and, if so, to identify the underlying factors driving these patterns.

In this study, we explore whether a frugivore assemblage influences seed rain patterns along an expansion gradient, spanning from mature forest to the colonization front edge. Our objective is to investigate whether plant–frugivore seed dispersal interactions promote high levels of genetic diversity in expanding plant populations. We focus on a juniper expanding population within the Doñana Biological Reserve (SW Spain), a protected area where juniper woodland patches have flourished over the past five decades (García et al., 2014), attributed mainly to frugivore activity (Isla, Jácome-Flores, Arroyo, et al., 2023) coupled with changes in land use management. Resident and migrant birds, as well as generalist mammals, forage on the fleshy cones (galbules) when other food resources are scarce in the area. Here, we used DNA barcoding for frugivores identification along with highly polymorphic genetic markers (SSRs) for maternal analysis of dispersed seeds to: (1) investigate how stand maturity influences the density and distribution of maternal progenies in the seed rain across a colonization gradient; (2) evaluate the impact of microhabitat type on the density and distribution of maternal progenies in the seed rain across this gradient; (3) identify the main frugivores contributing seeds to the seed rain across the colonization gradient and compare their relative contribution across stands and microhabitats; and (4) examine whether known foraging patterns of identified frugivores explain the distribution of maternal progenies in the seed rain. We expect that stand maturity will strongly determine seed density and maternal progeny distribution, because high-density seed rains are associated with mature forests that produce abundant crops (e.g., Armesto et al., 2001; Butler & Chazdon, 1998; Carlo & Morales, 2008; Carvalho et al., 2020). We anticipate decreased levels of seed density and a low number of fruiting trees contributing to the seed rain at the colonization front, with limited availability of local fruiting trees. It is known that different frugivores species may generate distinctive seed rain and maternal progeny patterns due to differences in their body sizes, foraging patterns and movement patterns across the landscape (González-Varo et al., 2023; Jordano et al., 2007; Schupp et al., 2010). Larger frugivores exhibit longer seed retention times and move larger distances through space while feeding on fruits and dispersing seeds compared with smaller frugivores. Thus, we expect that medium-sized

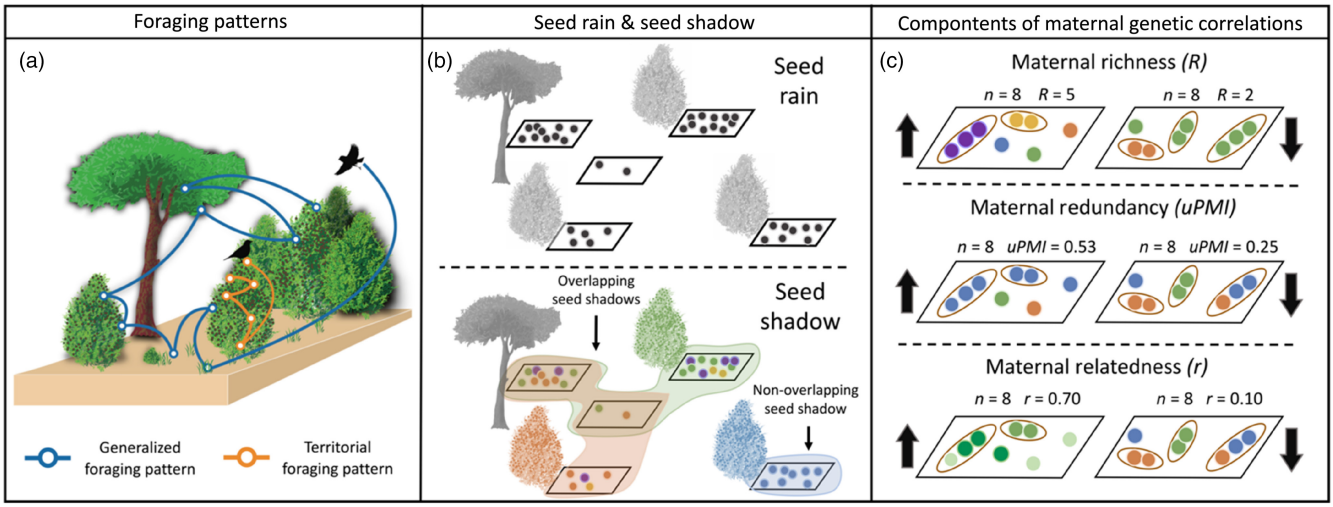

FIGURE 1 Distribution of maternal progenies in frugivore-generated seed rains. Frugivores transition from fruiting trees, where they forage on mature fruits, to deposition sites, where they rest, perch or nest. In this process, they disperse seeds into deposition sites at preferred microhabitats (e.g. Jordano & Schupp, 2000). Frugivores exhibit different foraging patterns defined by the combination of microhabitat preferences and foraging behaviour that encompasses the sequence of movements among fruiting trees and deposition sites. (a) Territorial species typically display limited mobility, favouring specific preferred sites where dispersed seeds are clumped (a, orange line). In contrast, other species or individuals exhibit broader foraging behaviour, traversing various fruiting trees and widely distributed microhabitats throughout the landscape (a, blue line). (b) Frugivore foraging patterns imprint the seed rain, that is, the spatial distribution of all seeds dispersed throughout the landscape from all fruiting trees that contribute seeds. The seed rain functions as the spatial template that shapes subsequent recruitment patterns of maternal progenies across the landscape, quantified as the number of seeds in a deposition site (expressed as seed density, no. seeds/m^2). When the fruiting tree that produced dispersed seeds is known, we can depict the distribution of the maternal progenies throughout the landscape. This will show the extent of the seed shadows, which are the spatial distribution of all dispersed seeds from one specific mother tree (coloured shadows in b; Janzen, 1970). Overlapping seed shadows arise when different fruiting trees share deposition sites where they disperse progeny (b, green and orange seed shadows), while non-overlapping seed shadows occur when fruiting trees do not share deposition sites (b, blue seed shadow). (c) The maternal composition of the seed rain (distribution of progenies) is described using maternal genetic correlations at a given study unit that might include scats (c, brown ellipses), deposition sites (c, black polygons), microhabitats or a nested combination of these study units (García et al., 2009). Indices describing the fine-scale genetic structure of these contributions to the seed rain include: Maternal Richness (*R*) refers to the number of distinct contributing source trees at the study unit (e.g. deposition site). Maternal Redundancy (*uPMI*) represents the maternal redundancy at the study unit and it is measured as the probability that two random seeds from the same deposition site come from the same tree (sensu Grivet et al., 2005). Maternal Relatedness (*r*) is the mean genetic relatedness (allele sharing probability) among the trees contributing seeds to a deposition site. Maternal relatedness increases when seeds come from nearby trees (seeds coloured in green tones) and decreases when seeds come from distant trees because adult trees show strong spatial genetic correlations (Vekemans & Hardy, 2004).

frugivores will have a greater ability to mix maternal progenies during digestive processing in the gut and disperse them widely across the landscape, which will favour maternally richer seed rains, than small-sized species. We expect our findings to shed light on the fundamental implications of plant–frugivore interactions during forest regeneration processes. Furthermore, we discuss broader applied implications of our research to advance our understanding of the role of biotic interactions in shaping the diversity of expanding second-growth forests.

2 | MATERIALS AND METHODS

2.1 | Study system

Our study focuses on an expanding population of *Juniperus phoenicea* subsp. *turbinata* and its frugivore seed dispersers in Doñana National Park (37°0′ N, 6°30′ W, SW Spain). *Juniperus phoenicea* is considered a foundation species (sensu Whitham et al., 2006) and is one of the clearest examples of a successful colonization process in the area, generating a maturity gradient from the remnant old-growth patches towards recently established stands (García et al., 2014). This is an anemophilous small tree characterized by productive mast seasons occurring over years with low cone production (Jordano, 1993). The species has an extended fruiting period (September–May), during which it produces red-brown fleshy cones when ripe, with an average of five seeds per cone (S.D. = 1.2, range 1–10, *n* = 5014).

2.2 | Sampling design

We conducted our study in three 1-ha plots (stands, hereafter) representing different maturity stages of plant population colonization (Table S1). The mature stand is a dense, old-growth juniper forest

(882 junipers/ha) with a high abundance of other fleshy fruited species, and scarce pine trees and open areas (Figure S1). The intermediate-maturity stand has an intermediate density (700 junipers/ha) and a low abundance of other fleshy fruited species but a significant presence of pine trees, open areas and low, dry-fruited, shrubby vegetation. The colonization front stand presents low densities of recently established juniper individuals (126 junipers/ha). Here, sandy open areas and dry-fruited species are abundant, with intermediate densities of pines and other sparse fleshy fruited species (Figure S1). Within each stand, we differentiated five microhabitats where we sampled the frugivore-generated seed rain of *J. phoenicea* during two fruiting seasons (2018–2019 and 2019–2020). The five microhabitats were undercover with (*Pinus pinea*, *Juniperus phoenicea*), other fleshy fruited shrubs, mainly *Phillyrea angustifolia* and *Pistacia lentiscus*, non-fleshy fruited vegetation, namely *Halimium halimifolium* and *Salvia rosmarinus*, and sandy open areas without vegetation cover. We characterized the coverage of each microhabitat by linear transects within the stands. See Table S1 in Supporting Information. for a detailed description of the main characteristics and composition of the three stands. We placed seed traps to collect frugivore scats and regurgitated seeds in 45 deposition sites per microhabitat, 15 per stand, except in open area, where we sampled by transects ($n = 539$ deposition sites; *P. pinea* = 45, *J. phoenicea* = 45, fleshy fruited shrubs = 45, non-fleshy fruited shrubs = 45, open area = 359). See *Seed rain survey protocol* section in Supporting Information. for sampling details. We collected samples every 10 days following the protocol described by González-Varo et al. (2014). We estimated the seed rain density (no. seeds/m^2) by dividing the number of seeds by the area sampled in each deposition site. We randomly selected a subset of deposition sites ($n = 105$, black-ringed circles in Figure 2a) in which we both analysed the contribution of frugivore species and identified the maternal progeny composition of the seed rain.

2.3 | Molecular procedures: Frugivore contributions and maternal genetic composition of the seed rain

We identified the frugivore species dispersing the seeds sampled in the seed traps by DNA barcoding of animal scats and regurgitated seeds following the extraction and PCR protocols described in González-Varo et al. (2014), see *DNA-Barcoding* section in Supporting Information. We obtained a reliable identification for 518 samples (regurgitated seeds and scats containing one or more seeds) after discarding those without a reliable identification from the analyses (8%). We extracted the seeds and identified their maternal progeny by genotyping the seed endocarp (maternally inherited) using a set of 12 polymorphic microsatellite markers following the protocols described in García and Escribano-Ávila (2016), see *Seed progenies identification* section in Supporting Information for details. After reamplification, we obtained a reliable genotype for 1000 seeds (99.6%), discarding those without DNA amplification or more than two null alleles. We identified maternal progenies (seeds from the same mother tree) by matching endocarp genotypes using the R package *ALLELEMATCH* v 2.5.1 (Galpern et al., 2012). We

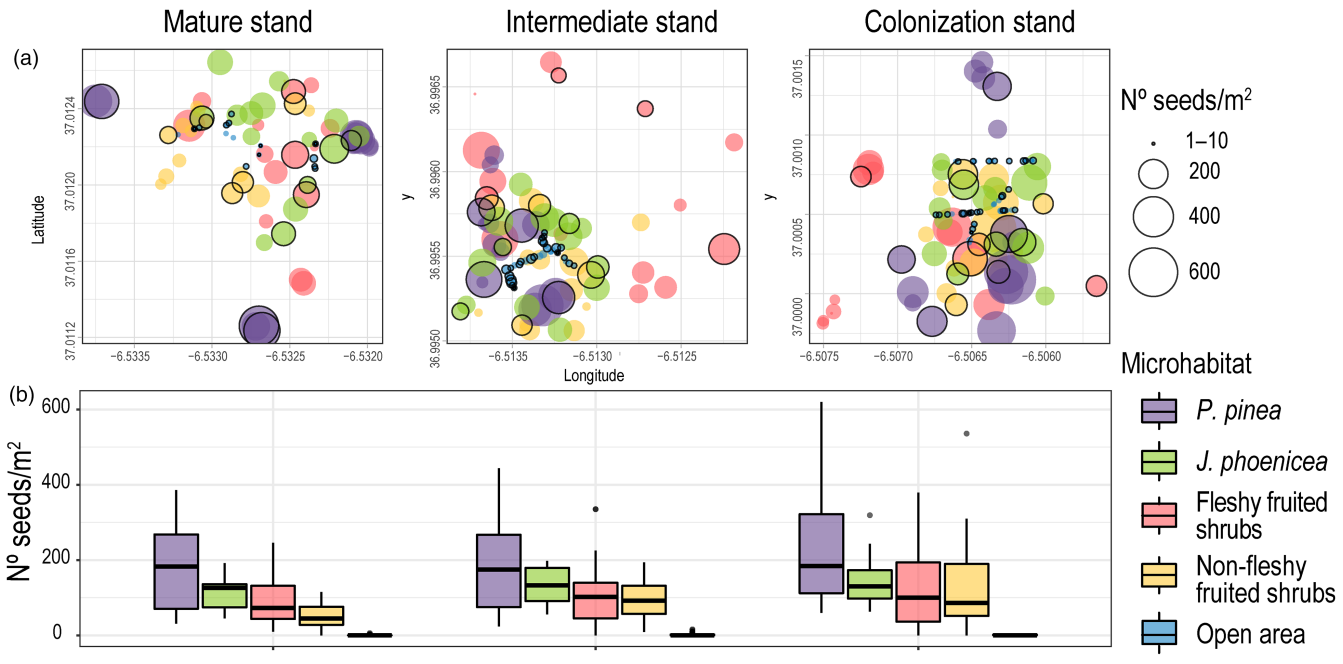

FIGURE 2 Seed rain of *Juniperus phoenicea* across three stands in a population expansion gradient in Doñana National Park. (a) Circles represent the locations of deposition sites with the size of the circle proportional to the estimated seed rain density and coloured according to the microhabitat type. Black-ringed circles represent focal deposition sites in which contributing frugivores and dispersed progenies were identified. (b) Variation in frugivore-mediated seed rain density in deposition sites among microhabitats in the three study stands (boxplots showing median, (50%) quartiles, range and outliers).

fixed an allele mismatch of two alleles (three if one was a null allele) to account for genotyping errors expected in low-quality DNA of juniper-seed endocarps (García & Escribano-Ávila, 2016). Then, we assessed three components of the maternal genetic correlations of the seed rain in each deposition site (Figure 1): (i) Maternal Richness, which is the number of distinct contributing source trees; (ii) Probability of Maternal Identity (uPMI, hereafter) that is the probability that two random seeds from the same deposition site come from the same tree and represents the Maternal Redundancy in the seed rain adjusting for different sample sizes (Grivet et al., 2005); and (iii) Maternal Relatedness, which is the genetic relatedness (allele sharing probability) among contributing trees, following Queller and Goodnight (1989) computed in GenAlEx 6.503 (Peakall & Smouse, 2012). To assess the feeding behaviour of frugivores, we also calculated their Maternal Richness per scat and the mean relatedness between progenies within scat, within deposition sites and among deposition sites.

2.4 | Data analyses

We performed all analyses using R v. 4.1.0 (R Core Team, 2021). To assess the effects of stand maturity and microhabitat type first in seed rain density, and second in the three components of maternal genetic correlations, we performed nested ANOVAs. We fitted a nested ANOVA (microhabitat/stand) using as a response variable the seed rain density found in deposition sites, and as main factors the microhabitat, the stand and the interaction between them. We also performed three nested ANOVAs with the same structure, but using the three components of maternal genetic correlations of the seed rain within focal deposition sites (Maternal Richness, uPMI and Maternal Relatedness) as response variables. A posteriori pairwise contrasts were performed using Tukey post hoc tests. In order to evaluate whether frugivore species disperse seeds independently among stands and microhabitats, we computed two Pearson's chi-square tests. To evaluate whether frugivores dispersed seeds to stands independently, a two-way contingency table was constructed by confronting the frugivore species against the percentage of deposition sites in each stand where they dispersed seeds. Similarly, to evaluate whether frugivores dispersed seeds randomly in the microhabitats or have a preference for certain microhabitats, the contingency table used in the chi-square test was constructed by confronting the frugivore species against the percentage of deposition sites in each microhabitat in which seeds were dispersed by each species. The uneven number of seeds found among stands, microhabitats and contributed by different frugivore species made the direct comparisons of maternal accumulation in the seed rain unreliable. To solve this problem, we performed a rarefaction procedure to compare maternal saturation in accumulation curves among stands, microhabitats and frugivores by *iNEXT* R package (Hsieh et al., 2016). To test to what extent, and how, the seed contribution of frugivore species determined the maternal components in the seed rain, we performed a canonical correlation analysis by CCA R package (González & Déjean, 2021). CCA compared two multivariate data sets with equal number of rows ($n = 86$ deposition sites with more than one seed), in which one set included maternal genetic components of the seed rain while the other data set included the number of seeds that each frugivore species dispersed in each deposition site, generating canonical variables that maximize the correlation between both datasets.

3 | RESULTS

3.1 | Animal-generated seed rain patterns across a heterogeneous expansion gradient

We collected 3389 faeces (or regurgitated seeds) across all deposition sites ($N = 539$) that included 7698 dispersed seeds of *J. phoenicea*. Seed density among deposition sites ranged from 0 seeds/m^2 to 620 seeds/m^2 (Figure 2a). Average seed density differed marginally among stands ($F = 2.8$, d.f. = 2, $p = 0.061$), with slightly higher values in deposition sites located at the intermediate stand compared with those sampled at the mature stand (mean ± SE, 51.4 ± 6.8 vs. 37.3 ± 5.3, respectively, $p = 0.056$). Microhabitat type had a significant effect on seed rain density variability ($F = 191.2$, d.f. = 4, $p < 0.001$). Deposition sites under *P. pinea* microhabitats received a significantly higher number of dispersed seeds than in the other microhabitats. In contrast, open area sites received significantly less, with 272 sites in open areas receiving no seeds (Figure 2b). Stand and microhabitat interacted significantly ($F = 3.4$, d.f. = 8, $p < 0.001$) to impact seed density because sites under non-fleshy fruited shrubs received more seeds in the colonization than in the mature stand.

3.2 | Frugivore contribution to seed rain patterns across a heterogeneous expansion gradient

We identified 10 species of frugivores dispersing *J. phoenicea* seeds through DNA barcoding of samples collected from focal deposition sites (N deposition sites = 105, N faeces = 518, N seeds = 1000, Table 1, Figure 3). *Turdus philomelos* was the species that contributed most to the seed rain, accounting for 68.7% of dispersed seeds, followed by *Erithacus rubecula*, *Turdus merula* and *Vulpes vulpes*, which collectively accounted for 28.4% of dispersed seeds (Table 1). Ancillary dispersers such as other *Turdus* species and non-legitimate dispersers (*Chloris chloris* and *Cervus elaphus*) contributed minimally to the seed rain (<3%). Overall, frugivores contributed unevenly to stands ($\chi^2 = 95.3$, d.f = 18, $p < 0.001$) and microhabitats ($\chi^2 = 365.3$, $d.f = 36$, $p < 0.001$; Figure 3a,b). The main dispersers, *T. philomelos* and *E. rubecula*, contributed evenly to the seed rain across the three stands, while ancillary species dispersed seeds mainly in mature and/or intermediate stands. All frugivores showed strong preferences for *P. pinea* and *J. phoenicea* microhabitats, where they consistently dispersed at least half of the seeds they mobilized, while only the main dispersers contributed to open area sites (Figure 3b). As a result,

TABLE 1 Quantitative characterization of the seed rain and seed rain maternal composition sampled for *Juniperus phoenicea* subsp. *turbinata*. Descriptors are indicated by frugivore species. Species listed in dark colour are core species (dispersing >97% of seeds) and species listed in grey are ancillary species (dispersing <3% of seeds).

	Quantitative characterization of the seed rain				Characterization of the maternal composition seed rain				
Frugivore species	Total n° scats	Total n° seeds dispersed	Mean n° seeds/scat (±SD)	N° deposition sites ($n = 105$)	Total n° progenies dispersed	Mean n° progenies/scat (±SD)	Within scat mean relatedness (±SD)	Within deposition sites mean relatedness (±SD)	Among deposition sites mean relatedness (±SD)
Turdus philomelos	375	687	1.8 ± 1.2	99	397	1.3 ± 0.7	0.74 ± 0.4	0.16 ± 0.38	0.01 ± 0.23
Erithacus rubecula	66	95	1.4 ± 0.9	30	71	1.2 ± 0.5	0.62 ± 0.4	0.17 ± 0.43	0.03 ± 0.27
Turdus merula	48	95	1.9 ± 1.3	30	59	1.4 ± 0.8	0.81 ± 0.3	0.46 ± 0.43	0.03 ± 0.28
Vulpes vulpes	11	94	8.5 ± 4.8	9	74	6.7 ± 4	0.42 ± 0.4	0.36 ± 0.37	0.11 ± 0.20
Chloris chloris	9	12	1.3 ± 0.7	8	11	1.2 ± 0.4	0.54 ± 0.3	0.52 ± 0.26	- 0.06 ± 0.23
Cervus elaphus	2	4	2 ± 1.4	2	4	2 ± 1.4	0.31 ± 0.04	0.31 ± 0.04	NA
Sylvia atricapilla	1	4	4	1	3	3 ± 0	0.29 ± 0.4	0.29 ± 0.36	NA
Turdus torquatus	2	4	2 ± 1.4	1	1	1 ± 0	0.93 ± 0.03	0.93 ± 0.03	NA
Turdus iliacus	3	3	1	3	3	1 ± 0	NA	NA	0.06 ± 0.21
Cyanopica cooki	1	2	2	1	2	2 ± 0	0.52 ± 0	0.52 ± 0	NA

we found a wider range of frugivores contributing to *P. pinea* and *J. phoenicea* microhabitats, attracting the highest number of dispersed seeds, compared with microhabitats with a low number of dispersed seeds (open areas and other fleshy fruited shrubs; Figure 3c). The number of species contributing per deposition site ranged from 1, mostly in open area sites, to 5 under *P. pinea*. *Turdus philomelos* dispersed seeds to most deposition sites (Figure 3c, Figure S2).

3.3 | Diversity and relatedness of maternal progenies in the seed rain

Among 1000 dispersed seeds sampled, we identified 605 distinct maternal trees. Most maternal progenies arrive only at one stand, but two progenies reached both intermediate and colonization stands, proving the existence of LDD events (Figure S3). These documented LDD events included at least two seeds (0.2% of the dispersal events assessed by seed genotyping) mediated by *T. philomelos*, resulting in dispersal distances ranging from 415 to 830 m. Within the three stands, deposition sites collected both seeds from unique progenies (only found at one deposition site) and progenies shared among deposition sites (mother trees that reached their progeny to multiple deposition sites), indicating overlapping of seed shadows (Figure S3). Accumulation curves show that maternal richness increases with increasing sampled seeds across stands, microhabitats and frugivore species, indicating that dispersed seeds come from a wide range of maternal trees (Figure S4). Most maternal progenies were dispersed by *T. philomelos* (66.6%) followed by *V. vulpes*, *E. rubecula*, *T. merula* and ancillary dispersers (12.2, 11.7, 9.7 and 4%, respectively), reflecting the highly variable interaction frequencies of different frugivore species (Figure S5).

The maternal relatedness among seeds collected within the same scat was high compared with relatedness values of seeds sampled within and among deposition sites (Table 1). This result suggests that seeds carried within the same faeces tend to come from the same or nearby source trees and that seeds collected in the same deposition site combine different maternal progenies. The components of maternal genetic correlations of the seed rain differed significantly among microhabitats (Figure 4, Table S2), but not among stands (Figure S6, Table S2). Maternal richness differed significantly among microhabitats ($F = 33.8$, d.f = 4, $p < 0.001$) with *P. pinea* and *J. phoenicea* deposition sites showing the highest values. Mean relatedness and maternal redundancy (*uPMI*) also differed among microhabitats ($F = 19.3$, d.f = 4, $p < 0.001$; $F = 6.1$, d.f = 4, $p < 0.001$) with open area deposition sites showing increased values for both. This result suggests that deposition sites in open area, which receive a low number of seeds, tend to attract seeds from the same or closely related source trees. In contrast, all other deposition sites receive an even number of seeds from various poorly related source trees. CCA confirmed that the distinctive contribution (number of seeds) of this complex assemblage of frugivores explained the composition of maternal progenies in the seed rain. Up to 41% of the variation in diversity and relatedness of maternal progenies was explained by

Journal of Ecology BRITISH ECOLOGICAL SOCIETY

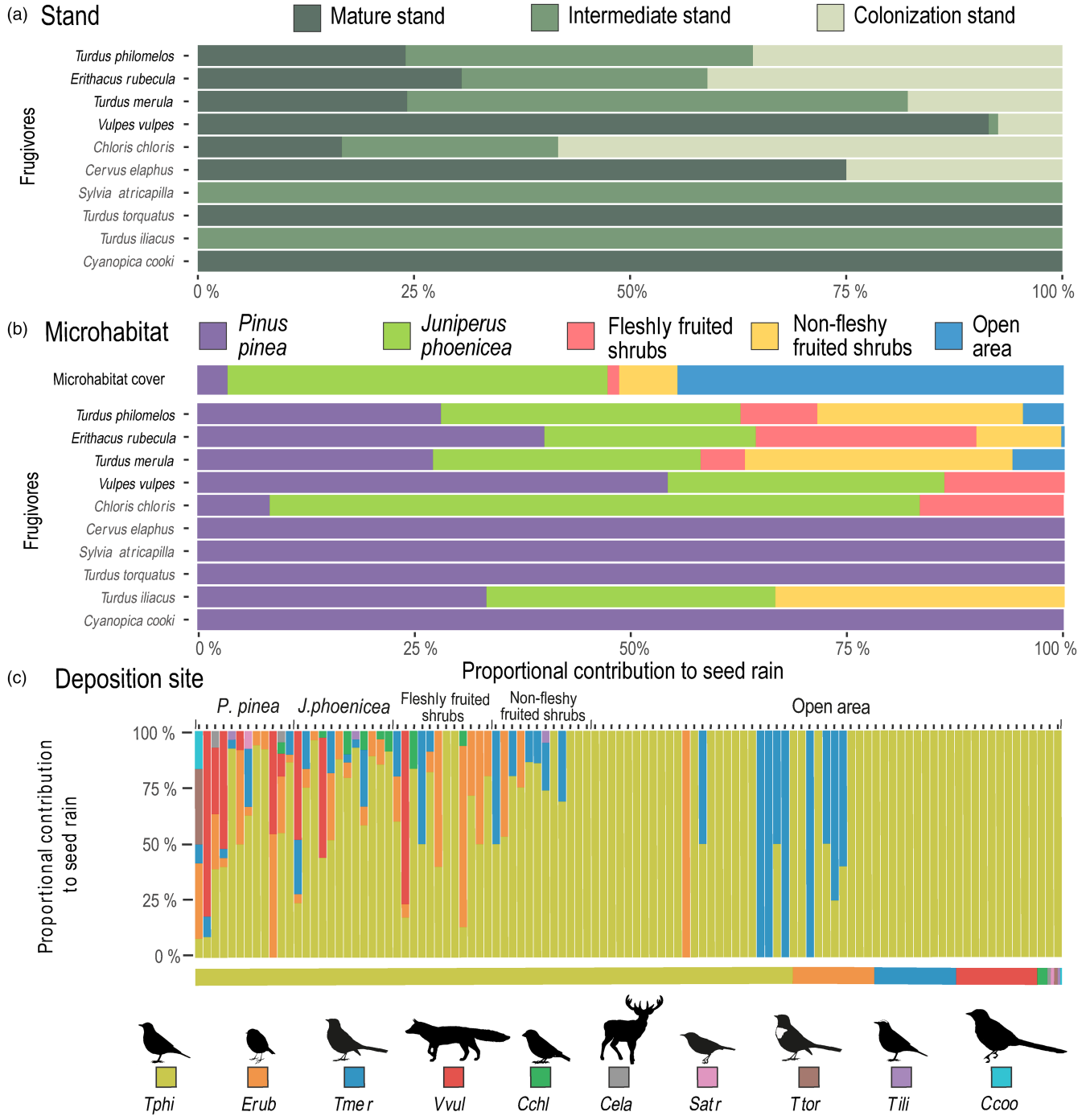

FIGURE 3 Relative contributions of the 10 frugivore species to the seed rain of *Juniperus phoenicea* at different spatial scales; (a) among stands, (b) among microhabitats and (c) among deposition sites. The relative contribution of frugivores per microhabitat is weighted by the number of deposition sites sampled. The species names of the 6 ancillary dispersers (that disperse <3% of the seeds) are grey coloured. The bar microhabitat cover represents the relative cover (%) of the microhabitats in the study area. Each column in C represents a deposition site, and the colours indicate the percentage of seeds found that have been dispersed by each frugivore species. The bottom, horizontal, bar represents the contribution of each frugivore to the overall seed rain.

the differential contribution of frugivores (Wilk's $\lambda = 0.07$, $F = 23.3$, $d.f = 15,215$, $p < 0.001$). The first canonical variable explained 99.5% of the total variance shared between the two original variable sets with a canonical correlation of $R^2 = 0.93$ ($F = 23.3$, $p < 0.001$). Maternal richness was negatively correlated to this canonical variable (−0.99), while relatedness and redundancy were positively correlated (0.48 and 0.34, respectively; Figure 5). The contributions of all frugivores were negatively correlated to this canonical variable with *T. philomelos* showing the highest correlation (−0.79). Although not significant, the second canonical variable was characterized by

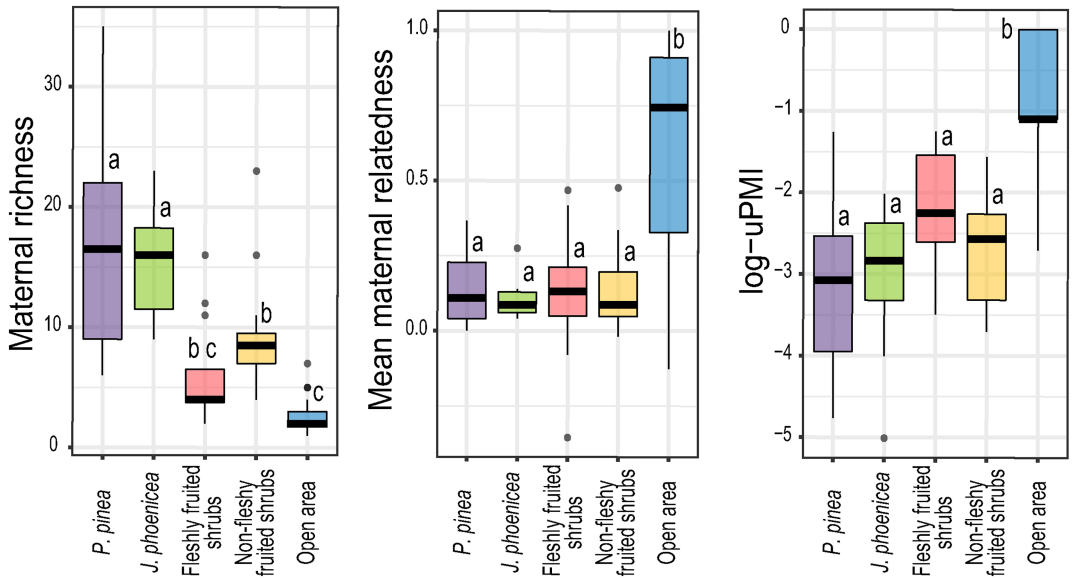

FIGURE 4 Maternal composition of the seed rain (maternal richness, mean genetic relatedness and maternal redundancy *uPMI*) in deposition sites among microhabitats. Note that *uPMI* was log-transformed to improve visualization. Different letters indicate significant differences among microhabitat types. Boxplot showing median, (50%) quartiles, range and outliers.

variations in the structure of relatedness among the deposition sites (Figure 5). These results confirm that species contributing with many seeds tend to increase the number of source trees and decrease relatedness and redundancy among progenies within deposition sites, resulting in extremely diverse seed shadows in terms of contributing maternal trees. The distribution of focal sites in the multidimensional CCA space, sorted by microhabitat type (Figure 5), but not by stand maturity (Figure S7), shows that *J. phoenicea* and *P. pinea* sites tend to correlate with areas highly contributed by frugivores, where high levels of maternal diversity—and low levels of *uPMI*—were found. Deposition sites located under fleshy and non-fleshy fruited shrubs were scattered throughout the multidimensional space. On the contrary, most of the deposition sites in open areas were located in the space defined by low contributions of frugivores and reduced maternal richness, with increased values of relatedness and *uPMI* (Figure 5).

4 | DISCUSSION

Our results show that frugivores generate diverse seed rains regarding the maternal provenance of seeds across a colonization gradient. This is unexpected, according to the long-standing paradigm holding that founder effects, coupled with fast-paced population growth, diminish genetic diversity in the colonization front of expansion (Waters et al., 2013). Our methodological approach allowed us to connect the distribution of maternal progenies in the seed rain to the contributing frugivore species. This establishes a new perspective in the study of dispersal mutualisms, showing that: (1) a diverse assemblage of frugivores, including core and ancillary species, generates maternal-rich seed rains across an expansion gradient, even at the colonization front, and (2) contrary to our expectations, stand maturity does not impact the distribution of maternal progenies in the seed rain. Instead, the strong preferences of most active frugivores for certain microhabitats explain the consistent seed rain patterns regardless of stand maturity. We discuss the fundamental and applied outcomes of our main findings and suggest future research lines to advance our knowledge on the impact of plant–animal interactions underlying the regeneration dynamics and expansion of plant populations.

Our study reveals that the accumulation of seeds and maternal progenies in the seed rain remains consistent across stands, irrespective of their maturity stage. We anticipated limited maternal contributions in areas with few local mother trees. However, contrary to our expectations, we observed a diverse array of maternal trees contributing progenies in the dense seed rain of the colonization front. This suggests thorough cone removal from most local trees, as well as not-so-infrequent long-distance seed dispersal. Our sampling provides evidence for LDD events (seed dispersal to different stands) for at least 0.2% of the 1000 seed dispersal events documented by endocarp genotyping. Most likely this represents an underestimate of the actual percentage of LDD events, given the amount of unique progeny arriving at each stand (Figure S3). Yet, a large sample of adult trees must be genotyped to estimate LDD events frequency with more precision. The recorded LDD events represent seed movements in the distance range of 415–830 m (see Jordano, 2017 for a discussion of within- and among-stand LDD events) and document the existence of among-stand seed dispersal. This includes seeds from external fruiting trees brought by frugivores in recently established stands (Hamrick & Trapnell, 2011; Karubian et al., 2010; Lavabre et al., 2016). These results align with previous findings in the study area which noted a high number of visits by frugivores to fruiting trees at the colonization front (Isla et al., 2023). This process, even with relatively reduced (but not

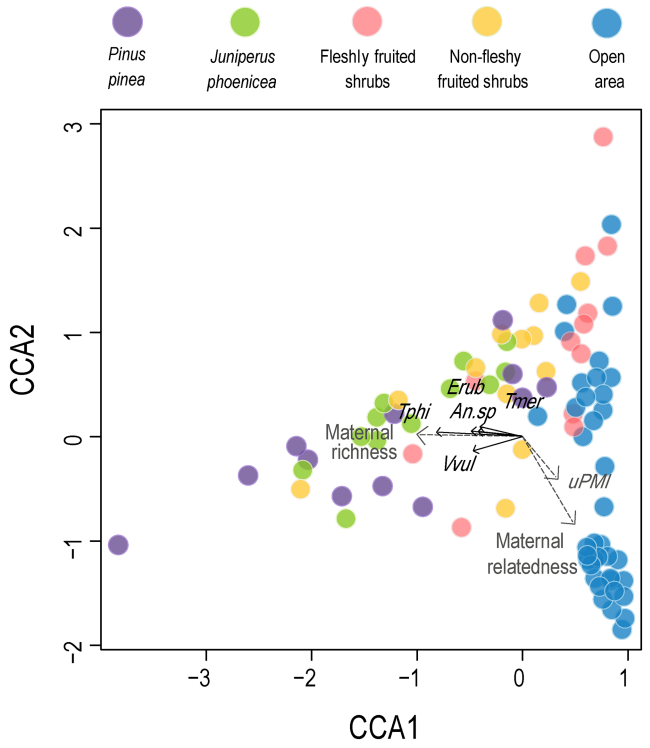

FIGURE 5 Canonical correspondence analysis (CCA) biplot, showing the strength of association of seed contribution per frugivore species (arrows) with values of maternal richness, relatedness and redundancy (*u*PMI) in deposition sites (dashed arrows) according to the length of the arrows. Animal species codes: *Tphi* = *Turdus philomelos*, *Eru.* = *E. rubecula*, *Tmer* = *T. merula*, *Vvul* = *V. vulpes*, *An*.sp. = Ancillary species (*C. chloris*, *C. elaphus*, *S. atricapilla*, *T. torquatus*, *T. iliacus* and *C. cooki*).

very rare) LDD events (Bialocyt et al., 2006), might contribute to maintaining moderate to high genetic diversity throughout the population expansion gradient (Browne & Karubian, 2016; Scofield et al., 2012) mainly through the 'reshuffling' and embolism effect involved in the LDD events (Bialozyt et al., 2006). Our study shows that on top of these non-trivial frequencies of LDD events, animal frugivores also contribute to the seed rain of very high diversity of provenances, even at the colonization front. Overall, these results highlight the potential of frugivores in mitigating the anticipated genetic erosion at the colonization front, which often arises due to founder effects followed by rapid population growth (Waters et al., 2013). This aligns with predictions from range expansion/colonization models where stratified dispersal patterns—combining both local in situ dispersal with LDD events—are expected to yield favourable conditions preserving genetic diversity by swamping the effects of drift and the homogenizing effect of surfing (Bialocyt et al., 2006; Excoffier et al., 2009; Le Corre & Kremer, 1998). Our results show that here, the mutualistic interaction with a diversified frugivore assemblage provides these conditions and that a highly structured seed rain is the primarily outcome of such interaction. This baseline study presents a new perspective on the study of dispersal mutualisms and may prompt further research to explore whether the potential of this interaction for promoting diverse seed rains is sustained in different ecological systems during natural expansion. We found a frugivore assemblage consisting of birds and mammals, resembling those previously documented in juniper populations (Escribano-Ávila et al., 2014; Jordano, 1993; Livingston, 1972; Santos et al., 1999). This assemblage consisted of a core of four primary frugivores, predominantly *T. philomelos* that contributed 68.7% of seeds to the seed rain, along with six ancillary species. The key role of thrushes as seed dispersers is widely acknowledged across various animal-dispersed trees (Escribano-Ávila et al., 2014; Jordano, 1993; Rumeu et al., 2009; Snow & Snow, 1988). Furthermore, our data reveal that *T. philomelos* primarily disperse seeds of *J. phoenicea*, revealing a symmetric interaction between this thrush and juniper trees (Bascompte et al., 2006). This symmetry is likely driven by the phenological alignment of both species in the area, as *J. phoenicea* is the dominant fruiting species during the wintering period of *T. philomelos*. This mutual reliance signifies a reciprocal beneficial relationship (Quintero et al., 2023), that presumably sustains migrant populations of keystone frugivore species in the area, while concurrently enhancing the regeneration and expansion dynamics of the locally abundant juniper tree.

Frugivore preferences for specific microhabitats and their foraging behaviour are the two key elements shaping the density and maternal composition patterns in seed rains (García et al., 2009; Jordano & Schupp, 2000; Tewksbury et al., 2002). The initial determinant entails frugivore preference for distinct microhabitats. These preferred microhabitats are characterized by dense vegetation that facilitates feeding, perching and predator avoidance sites (e.g., *P. pinea*, *J. phoenicea* and fleshy fruited shrubs in our study), contrasting with frugivore elusiveness to low or non-vegetated sites (non-fleshy fruited shrubs and open areas in our study; García et al., 2009; Jordano & Schupp, 2000). These strong microhabitat preferences infuse seed rain patterns, as highly frequented sites amass a broad range of maternal progenies, reflecting the foraging preferences of the most active frugivores (García et al., 2009; Karubian et al., 2010). Corroborating this, we found high seed density and maternal richness in *P. pinea* and *J. phoenicea* deposition sites. Our findings reveal that fewer than 20% of seeds beneath juniper trees are maternal half-sibs (i.e. same mother tree, Figure S3) contrasting with previous studies where 70% of the animal-dispersed seeds were deposited under the mother tree (Jordano & Godoy, 2002). These findings reveal frugivores' transitory foraging behaviours among multiple fruiting trees before dispersing seeds beneath pines and junipers. Less-preferred but vegetated microhabitats like fleshy and non-fleshy fruited shrubs received a lower but sizable number of seeds by the core dispersers (e.g. *T. philomelos* and *T. merula* to non-fleshy fruited shrubs and *E. rubecula* and *V. Vulpes* to fleshy fruited shrubs). Although these microhabitats feature lower seed densities, these seeds emanate from numerous source trees across the landscape. Surprisingly, we did not find differences in seed densities between fleshy and non-fleshy fruited microhabitats, despite fleshy fruited species being highly sought-after by frugivores, typically attracting substantial seed deposition (Carlo & Morales, 2008; García et al., 2009). This is likely attributed to low phenological fruiting overlap between plant species producing fleshy fruits in the area (September–November) and

J. phoenicea (September–May). Open areas were universally least preferred by frugivores (Jordano & Schupp, 2000; Lavabre et al., 2016). Seed rain was scarce in the open areas, resulting in few source trees providing seeds in available sites for colonization. Additionally, the low number of dispersal events led to uneven distribution of progenies from closely related trees. Collectively, these results corroborate the influential role of frugivore microhabitat preferences in shaping the density and maternal composition of the seed rain. Furthermore, we empirically confirmed the potential of certain microhabitats to attract frugivores, generating maternal-rich seed rains evenly across the entire expansion spectrum. The expansion front is therefore a heterogeneous landscape, with a variable number of spatial hotspots that concentrate most of the seed rain available for further population recruitment.

The second factor influencing seed rain patterns is the foraging behaviour of frugivores, encompassing fruit consumption on one or various fruiting trees, feeding rates and subsequent seed dissemination to deposition sites where they (co)dispersed propagules in one or multiple scats. Sequential seed dispersal events to these sites lead to distinctive dissemination patterns, ranging from (i) a strong spatial clustering of maternal progenies dominated by one or few neighbouring trees to (ii) a mixture of progenies from trees scattered across the landscape (i.e. seed shadows overlap, Figure 1). Clustering of progenies results from frugivores favouring few large fruiting trees and/or displaying territorial behaviours that promote co-dispersal of seeds from one or few mother trees to few deposition sites (Epperson & Alvarez-Buylla, 1997; García & Grivet, 2011; Grivet et al., 2005). Conversely, mixtures of progenies in deposition sites arise from frugivores' varied foraging patterns across different trees spread out across the landscape and the dissemination of seeds to multiple deposition sites. Our findings reveal that seeds at deposition sites hail from a broad range of maternal trees, implying a low maternal correlation among dispersal events (i.e. a low frequency of co-dispersal events), particularly in the preferred microhabitats (*P. pinea* and *J. phoenicea*). This suggests an extensive foraging behaviour of all frugivores, where they consume fruits from various source trees across the landscape, mix progenies in their meals and subsequently deposit seeds from multiple progenies in preferred, vegetation-covered, microhabitats. This foraging behaviour aligns with the maternal-diversity accumulation curves (Figure S4), where newly sampled seeds represent novel, distinct progenies rather than augmenting seeds from the same mother trees. While all frugivores disperse seeds from multiple source trees, their scats tend to hold seeds from nearby trees. This is an expected effect because a single scat is the product of a short foraging time lapse in which animals tend to visit neighbouring trees (García et al., 2009; Garrote et al., 2023), even more common for multi-seeded species like *J. phoenicea* (Torimaru et al., 2007). However, at a deposition site scale, successive independent dispersal events (from both large and medium-sized frugivores like *V. vulpes* or *T. philomelos* and small-sized frugivores like *E. rubecula*) culminate in a seed rain composed of progenies from multiple mother trees scattered throughout the landscape. This unexpected result provides important evidence that even small frugivores can promote maternally diverse seed rains through generalized foraging patterns, crucially contributing in situ regeneration (Jordano, 2017). Overall, the generalized foraging tendencies of this frugivore assemblage, which create unsaturated accumulation curves of maternal progenies, establish a close link between frugivore contributions (especially *T. philomelos*) and the maternal composition of the seed rain. Extending the study of maternal progenies from heterogeneous landscapes (García et al., 2009) to a colonization gradient highlights the pivotal roles of frugivore microhabitat preferences and foraging behaviour in shaping that rich maternal seed rains across the entire expansion spectrum.

Our study highlights the critical role of core frugivore species in promoting plant population expansion on abandoned lands, carrying implications for both fundamental and applied research. Amidst the backdrop of ongoing global change, our findings emphasize the necessity of preserving plant–animal mutualistic interactions to ensure the persistence and expansion of natural tree populations, particularly in formerly fragmented landscapes. Furthermore, these results hold relevance for bolstering restoration policies, advocating for the preservation of essential biotic interactions that trigger natural regeneration, rather than solely relying on mass tree planting. Additionally, this study provides further evidence (see de Almeida et al., 2016; La Mantia et al., 2019) supporting the strategic distribution of microhabitats that are highly preferred by frugivores to promote natural regeneration in newly formed or rapidly revegetated sites.

Future studies would benefit from broadening the spatial and temporal scales considered. Temporally, our study demonstrates that two fruiting seasons capture the imprint of frugivores in shaping the distribution of the maternal progenies in the seed rain. However, we encourage studies encompassing multiple fruiting seasons to capture both peak and off-peak fruiting years, particularly for long-lived and masting species, such as *J. phoenicea*. This would account for variations in resource availability, which may alter frugivore foraging behaviour, and subsequently affect the seed rain's maternal composition, as previously found (Grivet et al., 2005; Pesendorfer et al., 2016). Spatially, this approach could be replicated across latitudinal and elevational gradients to evaluate how consistent are the roles of frugivores in generating maternal-rich seed rains and extensive seed shadow overlap, even with shifts in frugivore assemblage composition. Finally, a comprehensive understanding of fragmented forest regeneration and expansion requires an integrated study of pre- and post-dispersal processes across heterogeneous landscapes (Wang & Smith, 2002). In the Mediterranean flora, germination and early survival hinge upon microhabitat specificity (Gómez-Aparicio, 2008). Initial results from ongoing sowing experiments in the area indicate that microhabitats less preferred by frugivores, such as open areas, yield lower recruitment rates after three summers. However, in arid systems, these uncovered sites may also provide an escape from seed predation, creating a conflict between seed and seedling survival probabilities (Schupp, 1995, 2007). Results thus far suggest that frugivores foster tree expansion of animal-dispersed trees not only through efficient dispersal but also by depositing seeds in microhabitats conducive to successful recruitment.

This discovery hints at a frugivore-mediated synergy between dispersal and post-dispersal processes. We already know that at fine scale, maternally rich seed rains may favour the dilution of local genetic structure within plant populations (Gelmi-Candusso et al., 2017; Pérez-Méndez et al., 2016). However, the implications of neighbourhood kinship are complex and pervasive down to small-scale contexts, affecting both plant–plant and plant–animal interactions, for example, interspecific competitive ability depending on whether intraspecific competition is with sibs versus non-sibs (Yamawo, 2015; Yamawo & Mukai, 2020). Next studies should amalgamate insights from plant–plant and plant–animal mutualistic and antagonistic interactions (Isla et al., 2022, 2023; Lloret et al., 2022) to confirm the fundamental role of biotic interactions in determining the composition and structure of expanding secondary-growth forests in a changing world.

AUTHOR CONTRIBUTIONS

Jorge Isla: conceptualization; fieldwork; data curation; formal analysis; investigation; visualization; writing—original draft; writing—reviewing and editing. Miguel Jácome Flores: conceptualization; data curation; formal analysis; visualization; supervision; writing—original draft; writing—reviewing and editing. Cristina Rigueiro and Juan Miguel Arroyo: Investigation; data curation. Pedro Jordano: conceptualization; data curation; formal analysis; project administration; validation; visualization; supervision; writing—original draft; writing—reviewing and editing. Cristina García: conceptualization; data curation; formal analysis; validation; visualization; supervision; writing—original draft; writing—reviewing and editing.

ACKNOWLEDGEMENTS

Discussions with Lucía Acevedo, Elena Quintero, Blanca Arroyo and Gemma Calvo were especially helpful in the development and analysis of the manuscript. We appreciate the help of Enrico Bazzicalupo in programming the reading of genetic sequences in a computerized way. We thank the logistic and facilities support from ICTS-RBD Doñana and the Doñana National Park administrators for onsite access authorizations. The 'Molecular Ecology Laboratory' (LEM-EBD-CSIC; ISO9001:2015 and ISO14002:3026 certifications) provided logistical support for molecular analyses. We thank two anonymous reviewers and editors for their helpful comments.

FUNDING INFORMATION

Our work was funded by grants CGL2017-82847-P and PID2022-136812NB-I00, Spanish Ministry of Science and Innovation and grant SUMHAL, LIFEWATCH-2019-09-CSIC-13, POPE 2014–2020, MICINN through the European Regional Development Fund, as well as funding from the VI Plan Propio de Investigación of the Universidad de Sevilla (VI PPIT-US) (PJ). JI was supported by an FPI grant PRE2018-085916 from the Spanish Ministry of Science and Innovation. MJF was supported by Investigadores por México-CONAHCYT (grant number: 73215). CG was supported by the Research Application Support Programme (RHUL) and the Royal Society (Research Application Support Programme) RaSP.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14280.

DATA AVAILABILITY STATEMENT

Data and code for the analyses are available at the Zenodo Digital Repository: https://doi.org/10.5281/zenodo.10579387 (Isla et al., 2024) and the GitHub repository https://github.com/PJordano-Lab/MS_JuniperusProgeny.

ORCID

Jorge Isla https://orcid.org/0000-0002-2307-9730
Miguel Jácome-Flores https://orcid.org/0000-0003-1504-7920
Pedro Jordano https://orcid.org/0000-0003-2142-9116
Cristina García https://orcid.org/0000-0001-7970-1245

REFERENCES

Armesto, J. J., Diaz, I., Papic, C., & Willson, M. F. (2001). Seed rain of fleshy and dry propagules in different habitats in the temperate rainforests of Chiloé Island, Chile. *Austral Ecology*, *26*, 311–320.

Bascompte, J., Jordano, P., & Olesen, J. M. (2006). Asymmetric coevolutionary networks facilitate biodiversity maintenance. *Science*, *312*, 431–433.

Bialozyt, R., Ziegenhagen, B., & Petit, R. J. (2006). Contrasting effects of long distance seed dispersal on genetic diversity during range expansion. *Journal of Evolutionary Biology*, *19*, 12–20.

Browne, L., & Karubian, J. (2016). Frequency-dependent selection for rare genotypes promotes genetic diversity of a tropical palm. *Ecology Letters*, *19*, 1439–1447.

Butler, B. J., & Chazdon, R. L. (1998). Species richness, spatial variation, and abundance of the soil seed bank of a secondary tropical rain forest. *Biotropica*, *30*, 214–222.

Carlo, T. A., & Morales, J. M. (2008). Inequalities in fruit-removal and seed dispersal: Consequences of bird behaviour, neighbourhood density and landscape aggregation. *Journal of Ecology*, *96*, 609–618.

Carvalho, C. S., García, C., Lucas, M. S., Jordano, P., & Côrtes, M. C. (2020). Extant fruit-eating birds promote genetically diverse seed rain, but disperse to fewer sites in defaunated tropical forests. *Journal of Ecology*, *109*, 1055–1067.

Chazdon, R. L. (2014). *Second growth: The promise of tropical forest regeneration in an age of deforestation*. The University of Chicago Press.

Chazdon, R. L., Lindenmayer, D., Guariguata, M. R., Crouzeilles, R., Rey-Benayas, J. M., & Lazos-Chavero, E. (2020). Fostering natural forest regeneration on former agricultural land through economic and policy interventions. *Environmental Research Letters*, *15*, 043002.

de Almeida, A., Marques, M. C. M., Ceccon-Valente, M. D. F., Vicente-Silva, J., & Mikich, S. B. (2016). Limited effectiveness of artificial bird perches for the establishment of seedlings and the restoration of Brazil's Atlantic Forest. *Journal for Nature Conservation*, *34*, 24–32.

Epperson, B. K., & Alvarez-Buylla, E. R. (1997). Limited seed dispersal and genetic structure in life stages of *Cecropia obtusifolia*. *Evolution*, *51*, 275–282.

Escribano-Ávila, G., Calviño-Cancela, M., Pías, B., Virgós, E., Valladares, F., & Escudero, A. (2014). Diverse guilds provide complementary

dispersal services in a woodland expansion process after land abandonment. *Journal of Applied Ecology*, *51*, 1701–1711.
Espelta, J. M., Cruz-Alonso, V., Alfaro-Sánchez, R., Hampe, A., Messier, C., & Pino, J. (2020). Functional diversity enhances tree growth and reduces herbivory damage in secondary broadleaf forests, but does not influence resilience to drought. *Journal of Applied Ecology*, *57*, 2362–2372.
Excoffier, L., Foll, M., & Petit, R. J. (2009). Genetic consequences of range expansions. *Annual Review of Ecology, Evolution, and Systematics*, *40*, 481–501.
Galpern, P., Manseau, M., Hettinga, P., Smith, K., & Wilson, P. (2012). Allelematch: An R package for identifying unique multilocus genotypes where genotyping error and missing data may be present. *Molecular Ecological Resources*, *12*, 771–778.
Gandhi, S. R., Korolev, K. S., & Gore, J. (2019). Cooperation mitigates diversity loss in a spatially expanding microbial population. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 23582–23587.
García, C., & Escribano-Ávila, G. (2016). An optimized protocol to isolate high-quality genomic DNA from seed tissues streamlines the workflow to obtain direct estimates of seed dispersal distances in gymnosperms. *Journal of Plant Research*, *129*, 559–563.
García, C., Espelta, J. M., & Hampe, A. (2020). Managing forest regeneration and expansion at a time of unprecedented global change. *Journal of Applied Ecology*, *57*, 2310–2315.
García, C., & Grivet, D. (2011). Molecular insights into seed dispersal mutualisms driving plant population recruitment. *Acta Oecologica*, *37*, 632–640.
García, C., Jordano, P., Arroyo, J. M., & Godoy, J. A. (2009). Maternal genetic correlations in the seed rain: Effects of frugivore activity in heterogeneous landscapes. *Journal of Ecology*, *97*, 1424–1435.
García, C., Moracho, E., Díaz-Delgado, R., & Jordano, P. (2014). Long-term expansion of juniper populations in managed landscapes: Patterns in space and time. *Journal of Ecology*, *102*, 1562–1571.
Garrote, P. J., Castilla, A. R., Picó, F. X., & Fedriani, J. M. (2023). Examining the spatiotemporal variation of genetic diversity and genetic rarity in the natural plant recolonization of human-altered areas. *Conservation Genetics*, *24*, 315–330.
Gelmi-Candusso, T. A., Heymann, E. W., & Heer, K. (2017). Effects of zoochory on the spatial genetic structure of plant populations. *Molecular Ecology*, *26*, 5896–5910.
Gómez-Aparicio, L. (2008). Spatial patterns of recruitment in Mediterranean plant species: Linking the fate of seeds, seedlings and saplings in heterogeneous landscapes at different scales. *Journal of Ecology*, *96*, 1128–1140.
González, I., & Déjean, S. (2021). CCA: Canonical correlation analysis. https://cran.r-project.org/web/packages/CCA/index.html
González-Varo, J. P., Albrecht, J., Arroyo, J. M., Bueno, R. S., Burgos, T., Escribano-Ávila, N. F., García, D., Illera, J. C., Jordano, P., Kurek, P., Rösner, S., Virgós, E., & Sutherland, W. J. (2023). Frugivore-mediated seed dispersal in fragmented landscapes: Compositional and functional turnover from forest to matrix. *Proceedings of the National Academy of Sciences of the United States of America*, *120*, e2302440120.
González-Varo, J. P., Arroyo, J. M., & Jordano, P. (2014). Who dispersed the seeds? The use of DNA barcoding in frugivory and seed dispersal studies. *Methods in Ecology and Evolution*, *5*, 806–814.
Grivet, D., Smouse, P. E., & Sork, V. L. (2005). A novel approach to an old problem: Tracking dispersed seeds. *Molecular Ecology*, *14*, 3585–3595.
Hamrick, J. L., & Trapnell, D. W. (2011). Using population genetic analyses to understand seed dispersal patterns. *Acta Oecologica*, *37*, 641–649.
Harper, J. L. (1977). *Population biology of plants*. Academic Press.
Hsieh, T. C., Ma, K. H., & Chao, A. (2016). iNEXT: An R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution*, *7*, 1451–1456.
Isla, J., Jácome-Flores, M., Arroyo, J. M., & Jordano, P. (2023). The turnover of plant—Frugivore interactions along plant range expansion: Consequences for natural colonization processes. *Proceedings of the Royal Society B: Biological Sciences*, *290*, 20222547.
Isla, J., Jácome-Flores, M., Pareja, D., & Jordano, P. (2022). Drivers of individual-based, antagonistic interaction networks during plant range expansion. *Journal of Ecology*, *110*, 2190–2204.
Isla, J., Jácome-Flores, M., Rigueiro, C., Arroyo, J. M., Jordano, P., & García, C. (2024). Data from: Animal-mediated seed dispersal and the demo-genetic configuration across plant colonization gradients. *Zenodo*. https://doi.org/10.5281/zenodo.10579387
Janzen, D. H. (1970). Herbivores and the number of tree species in tropical forests. *The American Naturalist*, *104*, 501–528.
Jordano, P. (1993). Geographical ecology and variation of plant-seed disperser interactions: Southern Spanish junipers and frugivorous thrushes. *Vegetatio*, *108*, 85–104.
Jordano, P. (2014). Fruits and frugivory. In R. S. Gallagher (Ed.), *Seeds: The ecology of regeneration in plant communities* (3rd ed.). CABI, Wallingford.
Jordano, P. (2017). What is long-distance dispersal? And a taxonomy of dispersal events. *Journal of Ecology*, *105*, 75–84.
Jordano, P., García, C., Godoy, J. A., & García-Castaño, J. L. (2007). Differential contribution of frugivores to complex seed dispersal patterns. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 3278–3282.
Jordano, P., & Godoy, J. A. (2002). Frugivore-generated seed shadows: A landscape view of demographic and genetic effects. In D. Levey, W. Silva, & M. Galetti (Eds.), *Seed dispersal and frugivory: Ecology, evolution and conservation* (pp. 305–321). CAB International.
Jordano, P., & Schupp, E. W. (2000). Seed disperser effectiveness: The quantity component and patterns of seed rain for *Prunus mahaleb*. *Ecological Monographs*, *70*, 591–615.
Karubian, J., Sork, V. L., Roorda, T., Durães, R., & Smith, T. B. (2010). Destination-based seed dispersal homogenizes genetic structure of a tropical palm. *Molecular Ecology*, *19*, 1745–1753.
La Mantia, T., Rühl, J., Massa, B., Pipitone, S., Lo Verde, G., & Bueno, R. S. (2019). Vertebrate-mediated seed rain and artificial perches contribute to overcome seed dispersal limitation in a Mediterranean old field. *Restoration Ecology*, *27*(6), 1393–1400.
Lavabre, J. E., Gilarranz, L. J., Fortuna, M. A., & Bascompte, J. (2016). How does the functional diversity of frugivorous birds shape the spatial pattern of seed dispersal: A case study in a relict plant species. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *371*, 20150280.
Le Corre, V., & Kremer, A. (1998). Cumulative effects of founding events during colonisation on genetic diversity and differentiation in an Island and stepping-stone model. *Journal of Evolutionary Biology*, *11*, 495–512.
Livingston, R. B. (1972). Influence of birds, stones and soil on the establishment of pasture juniper, *Juniperus communis*, and red cedar, *J. virginiana* in New England pastures. *Ecology*, *53*, 1141–1147.
Lloret, F., Jaime, L. A., Margalef-Marrase, J., Pérez-Navarro, M. A., & Batllori, E. (2022). Short-term forest resilience after drought-induced die-off in Southwestern European forests. *The Science of the Total Environment*, *806*, 150940.
Paulose, J., & Hallatschek, O. (2020). The impact of long-range dispersal on gene surfing. *Proceedings of the National Academy of Sciences of the United States of America*, *117*, 7584–7593.
Peakall, R., & Smouse, P. E. (2012). GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, *28*, 2537–2539.
Pérez-Méndez, N., Jordano, P., García, C., & Valido, A. (2016). The signatures of Anthropocene defaunation: Cascading effects of the seed dispersal collapse. *Scientific Reports*, *6*, 1–9.
Pesendorfer, M. B., Sillett, T. S., Koenig, W. D., & Morrison, S. A. (2016). Scatter-hoarding corvids as seed dispersers for oaks and pines: A

review of a widely distributed mutualism and its utility to habitat restoration. *Condor*, *118*, 215–237.
Poorter, L., Bongers, F., Aide, T. M., Almeyda Zambrano, A. M., Balvanera, P., Becknell, J. M., Boukili, V., Brancalion, P. H. S., Broadbent, E. N., Chazdon, R. L., Craven, D., de Almeida-Cortez, J. S., Cabral, G. A. L., de Jong, B. H. J., Denslow, J. S., Dent, D. H., DeWalt, S. J., Dupuy, J. M., Durán, S. M., … Rozendaal, D. M. A. (2016). Biomass resilience of Neotropical secondary forests. *Nature*, *530*, 211–214.
Queller, D. C., & Goodnight, K. F. (1989). Estimating relatedness using genetic markers. *Evolution*, *43*, 258–275.
Quintero, E., Rodríguez-Sánchez, F., & Jordano, P. (2023). Reciprocity and interaction effectiveness in generalised mutualisms among free-living species. *Ecology Letters*, *26*, 132–146.
R Core Team. (2021). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.r-project.org/
Roques, L., Garnier, J., Hamel, F., & Klein, E. K. (2012). Allee effect promotes diversity in traveling waves of colonization. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 8828–8833.
Rumeu, B., Padilla, D. P., & Nogales, M. (2009). The key role of a ring ouzel *Turdus torquatus* wintering population in seed dispersal of the endangered endemic *Juniperus cedrus* in an insular environment. *Acta Ornithologica*, *44*, 199–204.
Santos, T., Telleria, J. L., & Virgos, E. (1999). Dispersal of Spanish juniper *Juniperus thurifera* by birds and mammals in a fragmented landscape. *Ecography*, *22*, 193–204.
Schupp, E. W. (1995). Seed-seedling conflicts, habitat choice, and patterns of plant recruitment. *American Journal of Botany*, *82*, 399–409.
Schupp, E. W. (2007). The suitability of a site for seed dispersal is context-dependent. In *Seed dispersal: Theory and its application in a changing world* (pp. 445–462). CAB International.
Schupp, E. W., Jordano, P., & Gómez, J. M. (2010). Seed dispersal effectiveness revisited: A conceptual review. *New Phytologist*, *188*(2), 333–353. https://doi.org/10.1111/j.1469-8137.2010.03402.x
Scofield, D. G., Smouse, P. E., Karubian, J., & Sork, V. L. (2012). Use of alpha, beta, and gamma diversity measures to characterize seed dispersal by animals. *The American Naturalist*, *180*, 719–732.
Snow, B., & Snow, D. (1988). *Birds and berries. A study of an ecological interaction*, Poyser.
Tewksbury, J. J., Levey, D. J., Haddad, N. M., Sargent, S., Orrock, J. L., Weldon, A., Danielson, B. J., Brinkerhoff, J., Damschen, E. I., & Townsend, P. (2002). Corridors affect plants, animals, and their interactions in fragmented landscapes. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 12923–12926.
Torimaru, T., Tani, N., Tsumura, Y., Nishimura, N., & Tomaru, N. (2007). Effects of kin-structured seed dispersal on the genetic structure of the clonal dioecious shrub *Ilex leucoclada*. *Evolution*, *61*, 1289–1300.
Vekemans, X., & Hardy, O. J. (2004). New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology*, *13*, 921–935.
Wang, B. C., & Smith, T. B. (2002). Closing the seed dispersal loop. *Trends in Ecology & Evolution*, *17*, 379–386.
Waters, J. M., Fraser, C. I., & Hewitt, G. M. (2013). Founder takes all: Density-dependent processes structure biodiversity. *Trends in Ecology & Evolution*, *28*, 78–85.
Wenny, D. G., & Levey, D. J. (1998). Directed seed dispersal by bellbirds in a tropical cloud forest. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 6204–6207.
Whitham, T. G., Bailey, J. K., Schweitzer, J. A., Shuster, S. M., Bangert, R. K., LeRoy, C. J., Lonsdorf, E. V., Allan, G. J., DiFazio, S. P., Potts, B. M., Fischer, D. G., Gehring, C. A., Lindroth, R. L., Marks, J. C., Hart, S. C., Wimp, G. M., & Wooley, S. C. (2006). A framework for community and ecosystem genetics: From genes to ecosystems. *Nature Reviews Genetics*, *7*, 510–523.
Yamawo, A. (2015). Relatedness of neighboring plants alters the expression of indirect defense traits in an extrafloral nectary-bearing plant. *Evolutionary Biology*, *42*, 12–19.
Yamawo, A., & Mukai, H. (2020). Outcome of interspecific competition depends on genotype of conspecific neighbours. *Oecologia*, *193*, 415–423.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. In the top, a detail of the top canopy of the mature stand (*Sabinar del Marqués*).

Figure S2. Seed rain of *Juniperus phoenicea* generated by the core (*Turdus philomelos*, *Turdus merula*, *Vulpes vulpes*, *Erithacus rubecula*) and ancillary seed dispersers (*Chloris chloris*, *Cervus elaphus*, *Sylvia atricapilla*, *Turdus torquatus*, *Turdus iliacus* and *Cyanopica cyanus*) across three stands in a population expansion gradient in Doñana National Park.

Figure S3. The relative representation of seed progenies in the seed rain for all the deposition sites under fleshy-fruited shrubs (FF), non-fleshy fruited shrubs (NF), *P. Pinea* (PP) and *J. Phoenicea* (JP) microhabitats for the three stands in which we genotyped all the dispersed seeds (4 columns per microhabitat in each stand separated by the black lines).

Figure S4. Maternal richness accumulation curves as a function of increased sampling effort (number of seeds genotyped per stand) for different stands, microhabitat types, and frugivore species sampled.

Figure S5. Cumulative maternal richness (number of distinct maternal source trees identified) in each stand as a function of increasing contributions to the seed rain (from left to right) of individual vertebrate species in the frugivore assemblage, sorted from lowest to highest contribution.

Figure S6. Maternal components of the seed rain (maternal richness, mean genetic relatedness and maternal redundancy-*u*PMI) in deposition sites (receiving >1 seed) among stands.

Figure S7. Canonical correspondence analysis (CCA) biplot, showing the strength of association (proportional to arrow length) of the seed contribution per frugivore species (arrows) with values of Maternal richness, Maternal relatedness and Maternal Redundancy (uPMI) (dashed arrows) in sampled deposition sites (points).

Table S1. Summary of the main characteristics of the three study stands.

Table S2. Nested ANOVAs results for the effect of stand maturity and microhabitat type (and their interaction) on the maternal components of the seed rain (Maternal richness, Maternal relatedness and Maternal Redundancy (*uPMI*)).

How to cite this article: Isla, J., Jácome-Flores, M., Rigueiro, C., Arroyo, J. M., Jordano, P., & García, C. (2024). Animal-mediated seed dispersal and the demo-genetic configuration across plant colonization gradients. *Journal of Ecology*, *00*, 1–13. https://doi.org/10.1111/1365-2745.14280

Animal-mediated seed dispersal and the demo-genetic configuration across plant colonization gradients

Supplementary material

Seed rain survey protocol

We stratified our sampling within each stand in five different types of microhabitats defined mostly by vegetation cover type. These included microhabitats: under *Juniperus phoenicea*, under *Pinus pinea*, under other fleshy-fruited shrubs, under non-fleshy fruited shrubs and sandy open areas. We selected 15 replicates for each microhabitat (except for the open area microhabitat, see below), evenly distributed within the stands, where we sampled the seed rain. When insufficient replicates were found within the plot, we selected replicates as close as possible (see Figure 2A). To sample birds and mammal scats and regurgitated seeds, we used seed traps installed beneath cover in each microhabitat replicated sampling point. Under *J. phoenicea*, the traps were plastic trays (40 cm x 55 cm x 8 cm) protected with a 1 cm light mesh to protect the seeds against post-dispersal predation by rodents. Under *P. pinea* and fleshy-fruited shrubs, two aluminium trays (37 cm x 31 cm x 8 cm) protected with 1cm light mesh were installed at each site. No trays were used to sample seed rain under non-fleshy fruited shrubs; rather, a fixed area was delimited under each deposition site (Mean area ± SD, 0.25 ± 0.03 m^2), where we collected the samples. This kind of non-tray sampling in a delimited area was also fixed adjacent to the trays under *J. phoenicea*, *P. pinea* and other fleshy-fruited shrubs deposition sites, measuring the size of the sampling surface, to increase the sampled area. To sample the seed rain in open area, we used transects of variable length (Mean ± SD = 38 ± 10 m, n = 12) with a width of one-meter, where we collected and georeferenced all the scats and regurgitated seeds located. We chose this heterogeneous sampling design to maximize the sample collection and to adjust the methodology to the unique characteristics of each microhabitat. We measured the sampled area in square meters in all the deposition sites to allow comparable estimates of seed rain density among them. We conducted visits to each deposition site every ten days during two fruiting seasons (2018-2019 and 2019-2020), following the protocol described by González-Varo et al. (2014) for sample collection. Seed rain density was calculated for all the sampled points (n = 539; *J. phoenicea* = 45, *P. pinea* = 45, Fleshy-fruited shrubs = 45, Non-fleshy fruited shrubs = 45, Open area = 359). We selected a representative subset of these points (four replicates/microhabitat/stand and all the points containing samples in open area, n = 105) to: (i) identify frugivore species behind

each dispersal event (DNA-Barcoding), and (ii) determine the seed maternal progenies (seed endocarp genotyping).

DNA-Barcoding

We used DNA-barcoding to identify the bird and mammal species by collecting scats (or regurgitated seeds) in seed-traps or soil in the 105 focal deposition sites (Figure 1A). After collection in the field, samples were stored at -20°C and processed following protocols described in González-Varo et al. (2014). Animal DNA was extracted from the surface of the scat or regurgitated seeds, allowing the identification of the frugivore species. In the case of mammal samples, visual identification in the field was also cross-checked with DNA-barcoding identification. Frugivore species identification was based on a 272-bp mitochondrial DNA region (COI: cytochrome c oxidase subunit I). All bird samples were amplified by PCR using the COI-fsd-degF and COI-fsdR primers (González-Varo et al. 2017). Mammal samples were amplified following protocols and primers of Alcaide et al. (2009). The PCR product was sequenced and verified for its matching with COI sequences from Barcode of Life Data (BOLD) and Nucleotide Basic Local Alignment Search Tool (BLAST from NCBI) databases. For those samples without successful amplification, we performed nested PCR where we used the same primers on the amplicon of AWCintF2/AWCintR4 (González-Varo et al. 2017; Lijtmaer et al. 2012; Avian DNA barcodes). We considered as a successful identification, of all the sequences, at least 100-bp, with a similarity percentage >98%.

Identification of seed maternal progenies

We used DNA microsatellites to identify the maternal progeny composition of the seed rain (Maternal Richness, Relatedness and Redundancy) in terms of the maternal source trees contributing seeds to a given microsite. To analyze the maternal multilocus genotype, we extracted the seed endocarp/coat DNA (maternally inherited) following the sample extraction and laboratory protocols described in García & Escribano (2016).

We used a modified commercial DNA isolation kit (Isolate II Plant DNA Kit Bioline meridian Bioscience). We introduced two modifications to the standard protocol: (1) we added lysis buffer and incubated this lysate for two hours in an orbital shaker (Engiro genie, Scientific Industries) and (2) we did not do the second wash of the pellet with 200 µl of PAW2 wash buffer. We eluted in a volume of 80µl with the elution buffer heated to 65ºC, and we incubated for 10 min at 65ºC. Our modified protocol yielded an average of 2 to 8 ng μl^{-1} for the seed coats contrasting with the 8.7 ± 0.8 ng μl^{-1} DNA quantity obtained by García & Escribano (2016). This DNA reduction was possibly because we analyzed seeds that had passed through frugivore digestive tracts, thus, reducing the thickness of the coat, while García & Escribano (2016) used manually depulped seeds and, therefore, they had not undergone digestion in the digestive system of frugivores.

We used twelve microsatellite markers (out of 58 tested), presenting between three and 23 alleles with an estimated mean number of 8.4 alleles per locus. We performed two multiplex PCR (Tables A and B) in 10 µl final volume containing 2x Type-it Multiplex PCR Master Mix (Qiagen), Primer mix (10 µM of each primer), 0.01% BSA (Roche Diagnostics), and 2.5µl (2ng/µl) of genomic DNA. Note that PCR product Junpho_127300 was labelled using Vic (Applied Biosystems Foster City, CA) dye on an additional 19 bp M13 primer (5'-CACGACGACGTTGTAAAACGAC-3') according to the methods of Boutin-Ganache et al. (2001), so that it did not overlap in size with Junpho_015521. In addition, a palindromic sequence tail (5'-GTGTCTT-3') was added to the 5' end of all reverse primers to enhance adenylation and facilitate genotyping (Brownstein et al. 1996).

TABLE A

SSR	Sequence	Dye	Repeat type	Alleles	Size (bp)	Mix volume [c]=10µM
Junpho_084596	F:GGGAGCTCTAAGCCAACATC	FAM	ct	23	235-284	20
	R:AGGCTGACTTGTGGTCATAC	None				20
Junpho_037740	F:AACAACGCATACCATTGTCTTG	NED	ac	3	132-134	5
	R:GTGCAGACGTAGTTTGTCTAGTG	None				5
Junpho_015521	F:AGCCTCATTCACGAGGTCTG	VIC	ct	9	170-200	5
	R:CACCTATGCAGAAAATCGAAAGC	None				5
Junpho_DLXB1	F:CAACATTGCAAGGAGCAGAG	PET	ca	3	136-140	10
	R:TACTTGTCCGAAGGGGTGAC	None				10
Junpho_001229	F:GCACCCATATCTTCTCTTTGTCC	PET	atgt	6	203-213	15
	R:AGCAAAATGCAAACTAGGGTAGG	None				15
Junpho_058859	F:CCCAAAAGCTCTCGTACTTTACC	PET	ga	6	236-246	10
	R:AAGGATCACTCCCCATGCTG	None				10

TABLE B

SSR	Sequence	Dye	Repeat type	Alleles	Size (bp)	Mix volume [c]=10µM
Junpho_DFA6A	F:AGATAAGTTGCAAACCAAGACACA	FAM	ag	4	89-95	20
	R:GCATCAACGTTTCTGGTGAG	None				20
Junpho_022206	F:TGACAGCAATTTATCATGTTGAAGC	FAM	ca	5	169-187	10
	R:CCGTTCCCGAATCCAAACTG	None				10
Junpho_DCRW4	F:TCCATTCATCCATACCTACCTAA	NED	ca	3	94-98	20
	R:TGGAGCTAATGTTTGTCATGC	None				20
Junpho_ESGND	F:TAAACATCTAATATCAAGTGGGCA	NED	tg	9	151-175	15
	R:TGAGCTACTTGGTCAATAAATATGC	None				15
Junpho_DMX65	F:TGTAATTATGGGAAATGGATTGG	VIC	gt	16	107-155	20
	R:CATTCACATGCTTCCTTTCA	None				20
Junpho_127300	F:TGCTAGTGTACCATTCTCCATC	None	ac	14	224-260	3.5
	R:GAGCCATATTTGGTTGTTACTTGG	None				25
	F: M13	VIC				40

Samples were incubated in a PCR in a Bio-Rad DNA Engenier Peltier Thermal Cycler, with an initial 15 min of denaturation at 95ºC; 38 cycles at 95ªC for 30 s, annealing at 57ºC for 45 s and 72ºC for 45 s; and a final extension for 30 min at 72ºC. See Table C for a detailed description of the reaction mixture for multiplex PCR. We mixed 2 µl of PCR product mix 1 with 2 µl of PCR product mix 2 and diluted 20 times before mixing with 10 µl (Formamide + Liz500 size marker). Amplified fragments were analyzed on ABI 3130XL. Genetic Analyser and sized using GeneMapper 4.0 (Applied Biosystems) and LIZ 500 size standard. We repeated the multiplex PCR once to cope with missing data.

TABLE C

Component	**10 µl**
RNase-free water	2.5 µl
2x Type-it Multiplex PCR Master Mix	2.5 µl
Primer mix (10 µM of each primer)	2.25 µl
BSA (0.01%)	0.25 µl
DNA (2ng/µl)	2.5 µl

Figure S1. In the top, a detail of the top canopy of the mature stand (*Sabinar del Marqués*). In the bottom, the stand in the colonization front (*Sabinar de Colonización*) grows in an area dominated by low *monte blanco* woody shrubland. Photographs by Pedro Jordano (above) and Jorge Isla (below).

Figure S2. Seed rain of *Juniperus phoenicea* generated by the core (*Turdus philomelos*, *Turdus merula*, *Vulpes vulpes*, *Erithacus rubecula*) and ancillary seed dispersers (*Chloris chloris, Cervus elaphus, Sylvia atricapilla, Turdus torquatus, Turdus iliacus* and *Cyanopica cyanus*) across three stands in a population expansion gradient in Doñana National Park. Each of the squares represents the plot sampled at each stand, approximately one hectare (100 x 100 m). Circles represent the location of each deposition site and the size of the circle is proportional to the number of seeds that each frugivore species disperses in each deposition site, color-coded according to microhabitat type.

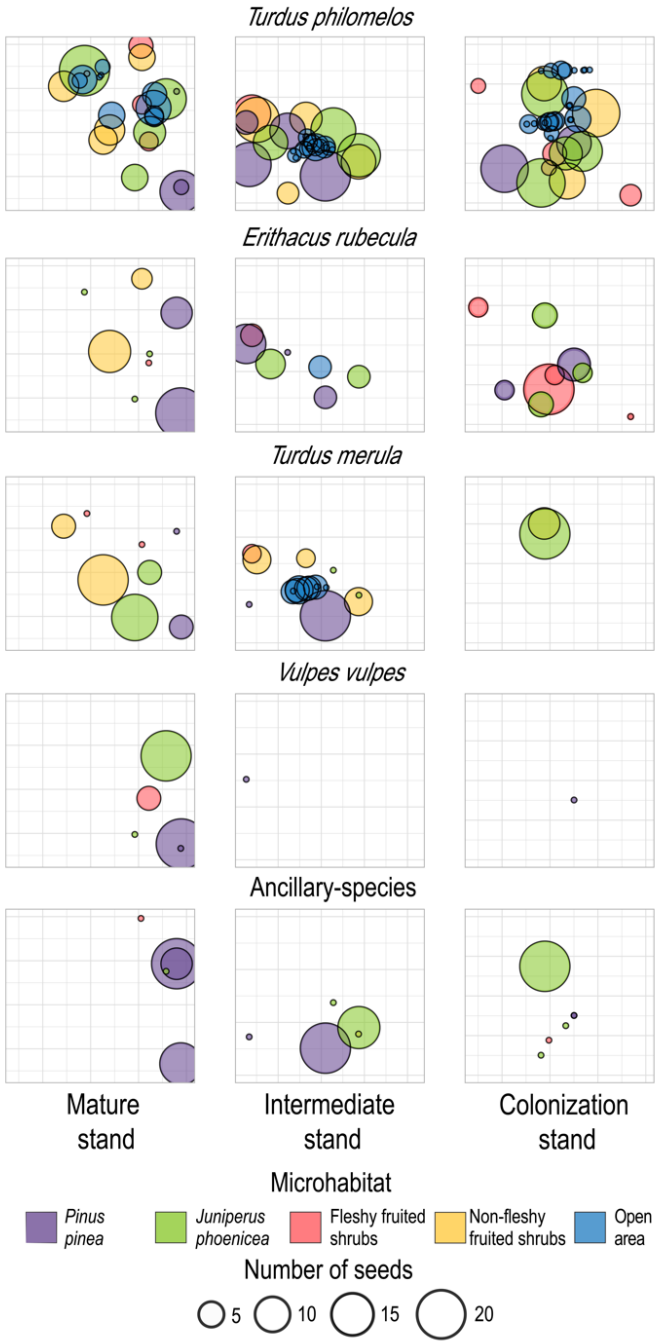

Figure S3. The relative representation of seed progenies in the seed rain for all the deposition sites under fleshy-fruited shrubs (FF), non-fleshy fruited shrubs (NF), *P. Pinea* (PP) and *J. Phoenicea* (JP) microhabitats for the three stands in which we genotyped all the dispersed seeds (4 columns per microhabitat in each stand separated by the black lines). Colored bars indicate the proportion of seeds belonging to specific, identified, distinct seeds dispersed to various deposition sites within each stand (i.e., seeds from a progeny that has been found in two or more deposition sites). For example, progeny #272, was found in the mature stand dispersed in a deposition site under fleshy-fruited shrub microhabitat and in a deposition site under *P. pinea*). Grey boxes indicate distinct progenies (proportion) only found at one deposition site (with one or more seeds, but only at one deposition site). Note that most of the dispersed seeds recorded correspond to progenies that only appeared in one deposition site (grey bars). The two progenies shared between stands, indicative of long-distance dispersal events by frugivores, are shown by coloured stars. Thus, 23 seeds from progeny #600 were found in six deposition sites of the colonization stand and one seed in one deposition site of the intermediate stand. The detection of numerous seeds from a single progeny, with unidentified maternal tree, across multiple deposition sites (red asterisks) in the colonization stand strongly suggests that the source tree is part of this stand. Therefore, the seed found in the intermediate stand, assigned to this same progeny, likely resulted from a long-distance dispersal event (LDD) of 830 m, mediated by *Turdus philomelos* in this particular case. The progeny indicated by the black asterisk also appeared under *J. phoenicea* microhabitat both in the intermediate and the colonization stands. In the case of the progeny indicated with the black star, we found two seeds in the colonization stand and one in the intermediate stand, therefore we cannot infer the location of the source tree, although we can attribute this to an LDD event of at least 415 m, also mediated by *T. philomelos.*

Figure S3

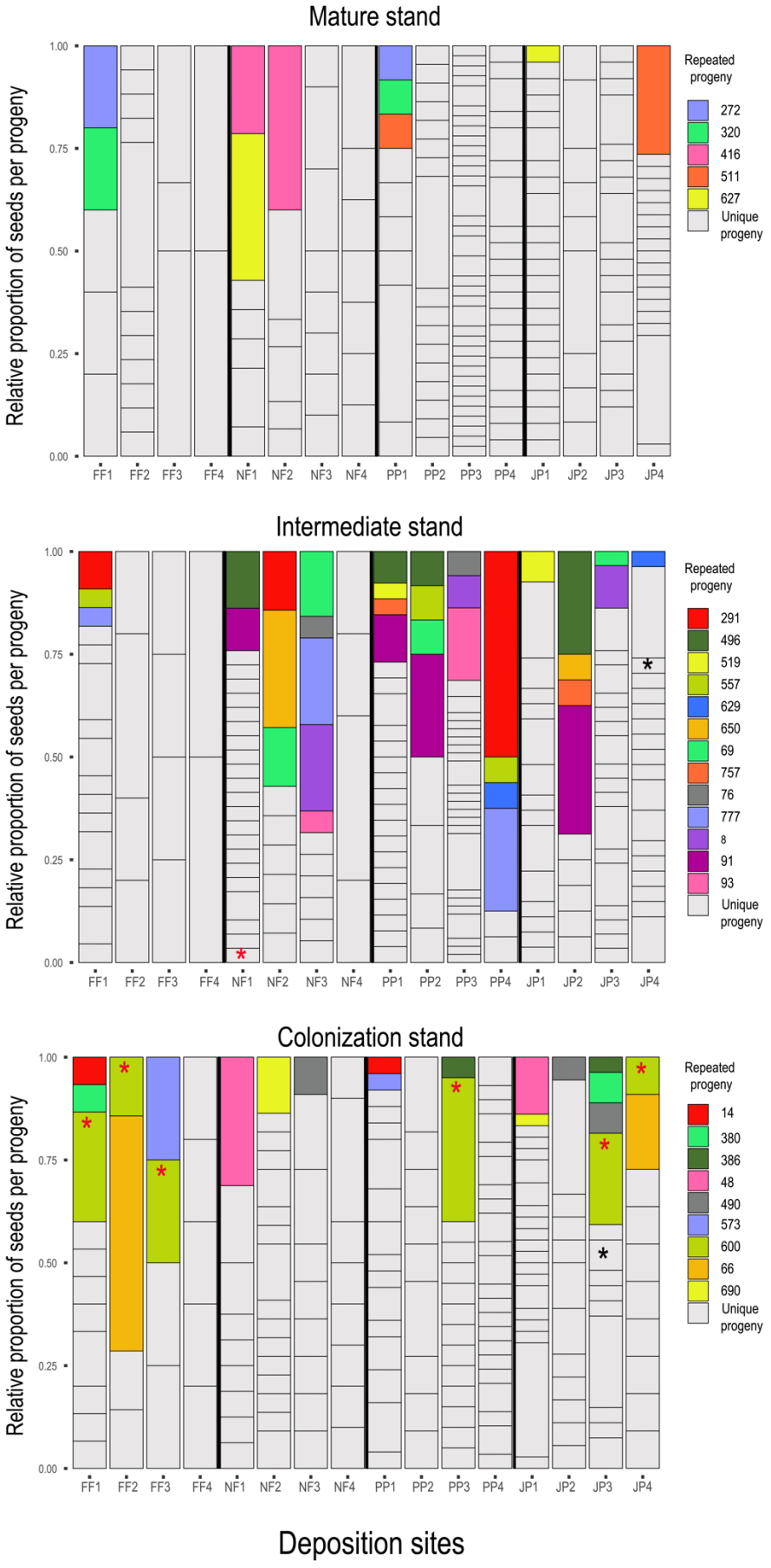
Mature stand
Relative proportion of seeds per progeny
1.00
0.75
0.50
0.25
0.00
FF1 FF2 FF3 FF4 NF1 NF2 NF3 NF4 PP1 PP2 PP3 PP4 JP1 JP2 JP3 JP4
Repeated progeny
272
320
416
511
627
Unique progeny
Intermediate stand
Relative proportion of seeds per progeny
1.00
0.75
0.50
0.25
0.00
FF1 FF2 FF3 FF4 NF1 NF2 NF3 NF4 PP1 PP2 PP3 PP4 JP1 JP2 JP3 JP4
Repeated progeny
291
496
519
557
629
650
69
757
76
777
8
91
93
Unique progeny
Colonization stand
Relative proportion of seeds per progeny
1.00
0.75
0.50
0.25
0.00
FF1 FF2 FF3 FF4 NF1 NF2 NF3 NF4 PP1 PP2 PP3 PP4 JP1 JP2 JP3 JP4
Repeated progeny
14
380
386
48
490
573
600
66
690
Unique progeny
Deposition sites

Figure S4. Maternal richness accumulation curves as a function of increased sampling effort (number of seeds genotyped per stand) for different stands, microhabitat types, and frugivore species sampled. The solid lines represent the asymptotic rarefaction-based estimate sorted by stand, microhabitat or frugivore species for which the fewest genotyped seeds were available, except for the ancillary species group where the dotted line represents the extrapolation up to 95 seeds that were genotyped from *E. rubecula* and *T. merula*. Note that in all three cases, the overlap between the confidence intervals indicates a lack of difference in the richness of maternal progeny, neither among stands, microhabitats, nor frugivore species.

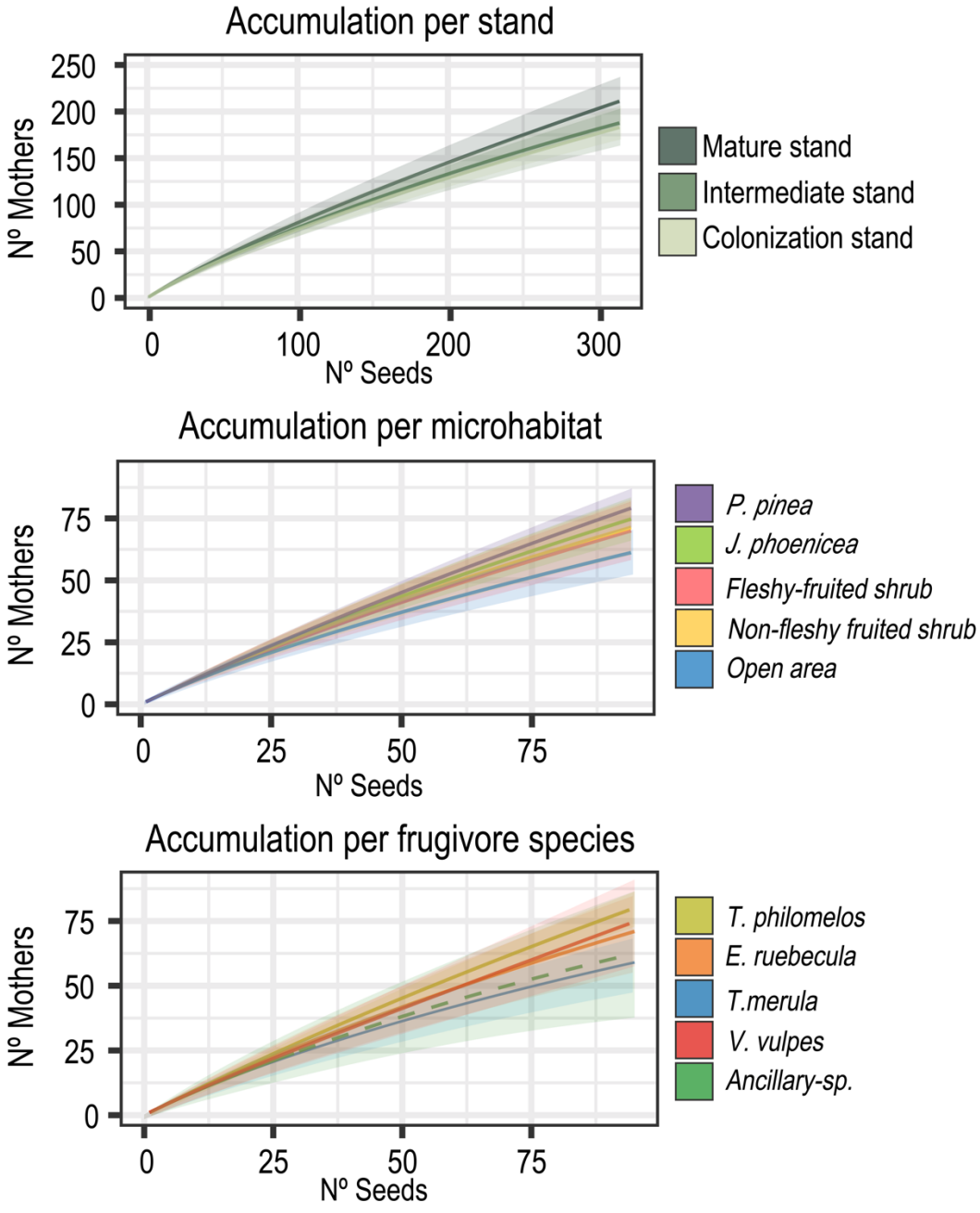

Figure S5. Cumulative maternal richness (number of distinct maternal source trees identified) in each stand as a function of increasing contributions to the seed rain (from left to right) of individual vertebrate species in the frugivore assemblage, sorted from lowest to highest contribution. Note that virtually only the four core frugivores contribute by adding new progenies to the seed rain (particularly *T. philomelos*), in a similar way among stands along the expansion gradient.

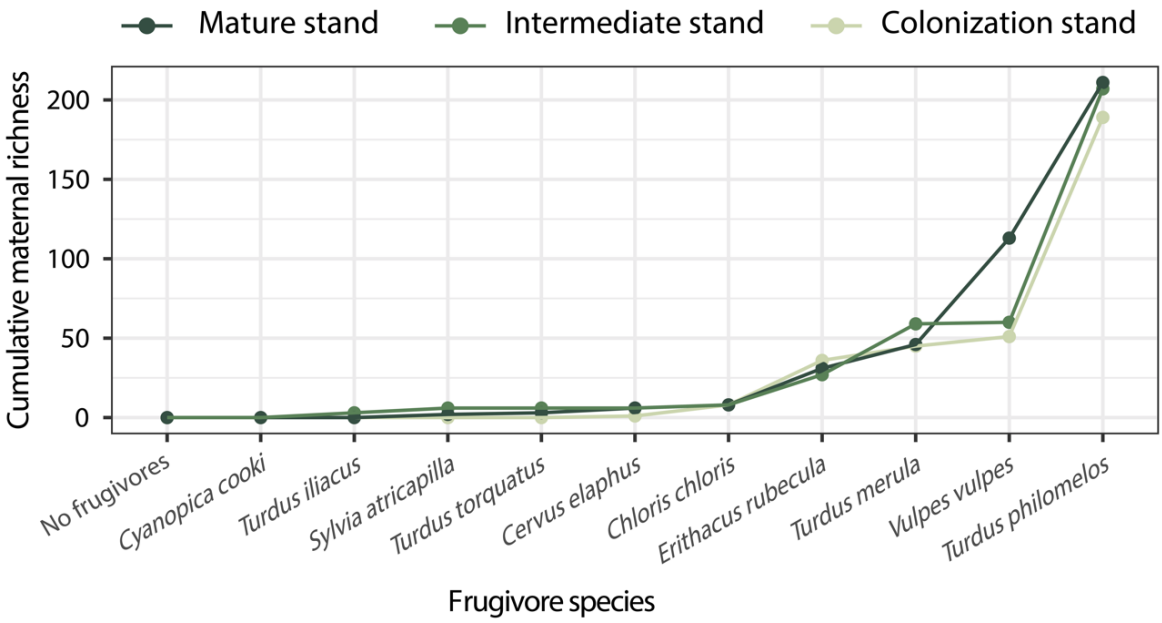

Figure S6. Maternal components of the seed rain (maternal richness, mean genetic relatedness and maternal redundancy-*u*PMI) in deposition sites (receiving >1 seed) among stands. Note that *uPMI* was log-transformed to improve visualization. Boxplot showing median, (50 %) quartiles, range, and outliers.

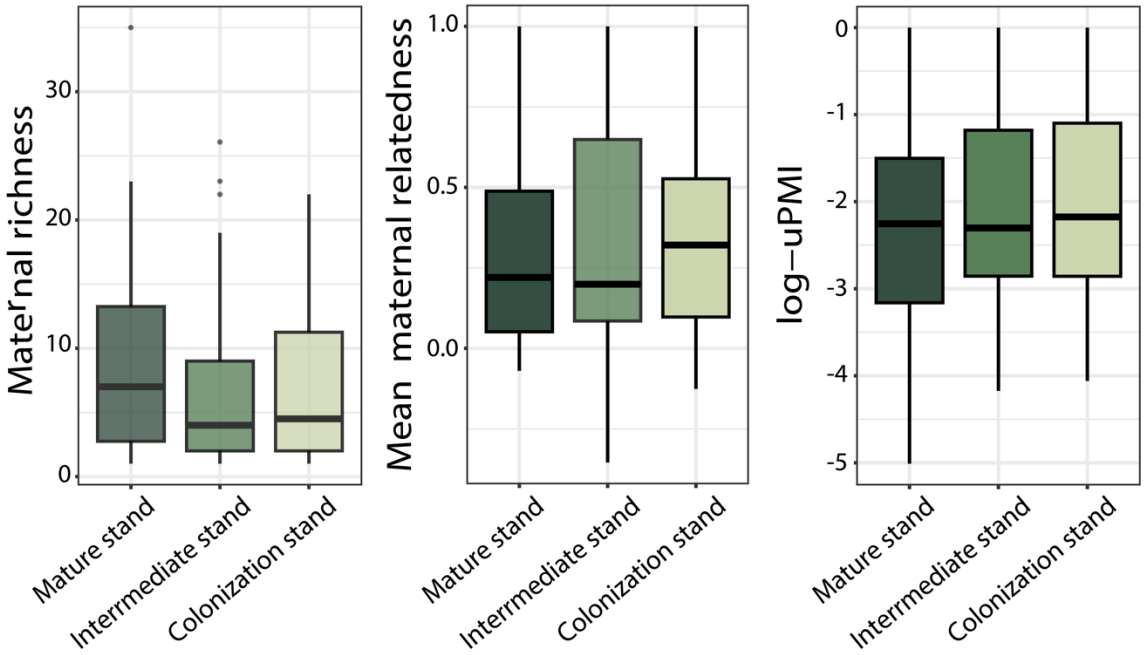

Figure S7. Canonical correspondence analysis (CCA) biplot, showing the strength of association (proportional to arrow length) of the seed contribution per frugivore species (arrows) with values of Maternal richness, Maternal relatedness and Maternal Redundancy (uPMI) (dashed arrows) in sampled deposition sites (points). Animal species codes: Tphi = *Turdus philomelos*, Erub = *E. rubecula*, Tmer = *T. merula*, Vvul = *V. vulpes*, Ansp = Ancillary-species = (*C. chloris, C. elaphus, S. atricapilla, T. torquatus, T. iliacus* and *C. cyanus*). Deposition site colors indicate the stand category.

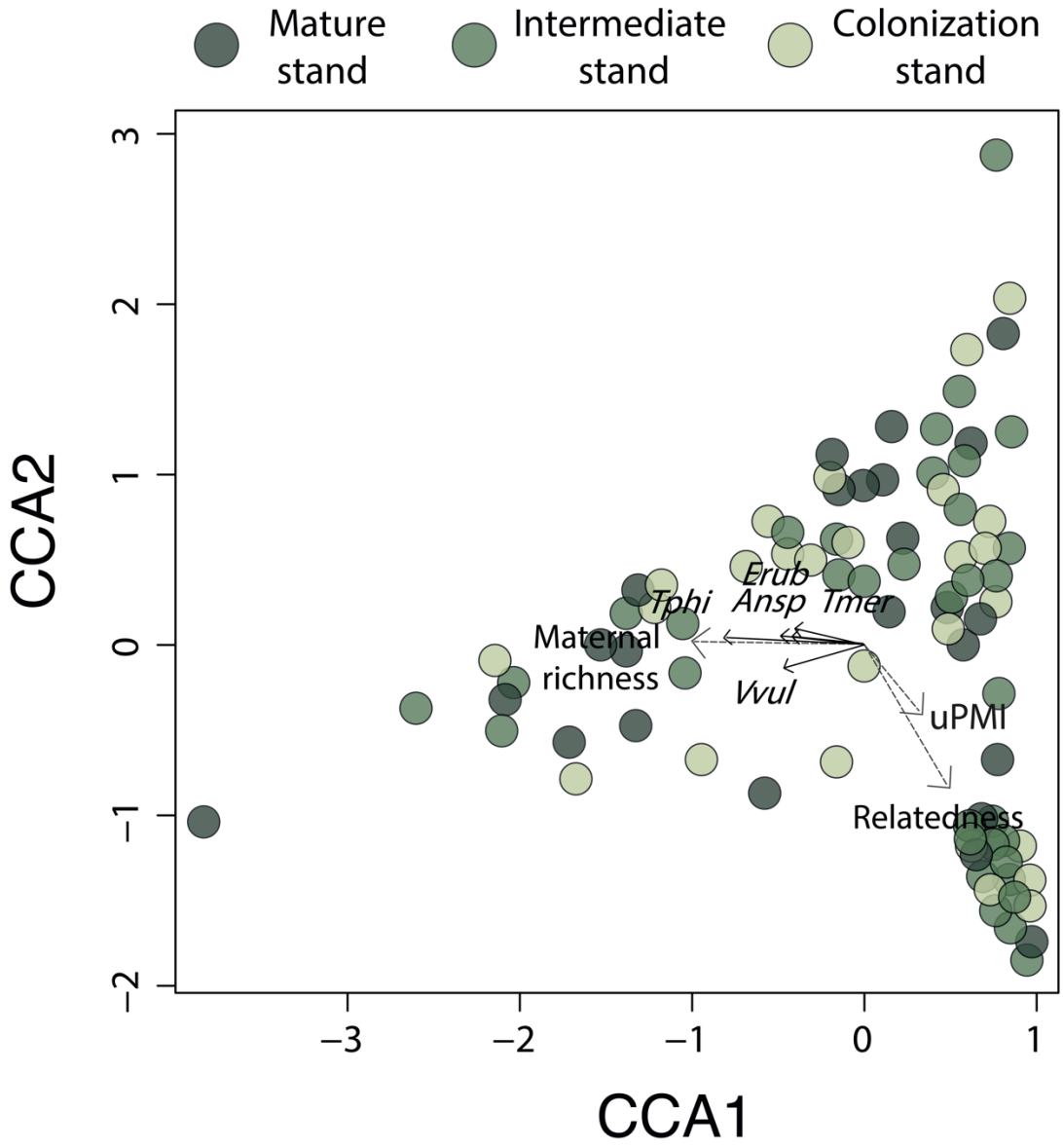

Table S1. Summary of the main characteristics of the three study stands.

	Mature stand	**Intermediate stand**	**Colonization stand**
Site name	*Sabinar del Marqués*	*Sabinar del Ojillo*	*Sabinar de Colonización*
Location	37° 0'44.15"N 6°31'55.38"O	36°59'43.86"N 6°30'48.16"O	37° 0'0.64"N 6°30'23.66"O
Altitude (m)	30	20	19
Juniper density	882 (ind/ha)	700 (ind/ha)	126 (ind/ha)
Mean Juniper height (cm) ± SD (n = 35)	304 ± 83	389 ± 87	493 ± 132
Mean Juniper cover (m^2) mean ± SD (n = 35)	10 ± 6	18 ± 11	24 ± 20
Mean Juniper crop size mean ± SD (n = 35)	2836 ± 2161	2865 ± 1804	7828 ± 6991
Microhabitat cover (%)			
Pinus pinea	0.6	1.9	4.8
Juniperus phoenicea	61	62	33.7
Fleshy-fruited shrubs	6.5	0.7	0.5
Non-fleshy fruited shrubs	9.2	6	6.5
Open areas	22.7	29.4	54.5
Fleshy-fruited plant species	*Phillyrea angustifolia, Osyris lanceolata, Corema album*	*Phillyrea angustifolia, Pistacia lentiscus, Rhamnus lycioides*	*Phillyrea angustifolia, Osyris alba, Osyris lanceolata, Asparagus spp.*
Non-fleshy fruited plant species	*Calluna vulgaris, Cistus libanotis, C. salvifolius, Halimium halimifolium, Salvia rosmarinus*	*Cistus libanotis, C. salvifolius, Halimium halimifolium, Salvia rosmarinus*	*Armeria pungens, Calluna vulgaris, Cistus crispus, C. libanotis, Halimium calycinum, H. halimifolium, Lavandula stoechas, Salvia rosmarinus, Thymus mastichina, Ulex minor, Ulex parvifloruss*

Table S2. Nested ANOVAs results for the effect of stand maturity and microhabitat type (and their interaction) on the maternal components of the seed rain (Maternal richness, Maternal relatedness and Maternal Redundancy (*uPMI*)).

		df	F	p
Maternal richness	Area	2	*1.62*	0.195
	Microhabitat	4	33.87	**8.4 x 10-16**
	Area*Microhabitat	8	0.73	0.657
Mean relatedness	Area	2	0.228	0.797
	Microhabitat	4	19.3	**8.73 x 10-11**
	Area*Microhabitat	8	0.264	0.975
Maternal redundancy *(uPMI)*	Area	2	1.14	0.32
	Microhabitat	4	6.11	**0.0002**
	Area*Microhabitat	8	0.54	0.815

References

Boutin-Ganache, I., Raposo, M., Raymond, M., & Deschepper, C. F. (2001). M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *BioTechniques*, 31, 25-28.

Brownstein, M. J., Carpten, J. D., & Smith, J. R. (1996). Modulation of Non-Templated Nucleotide addition by TAQ DNA polymerase: primer modifications that facilitate genotyping. *BioTechniques*, 20, 1004-1010.

García, C. & Escribano-Ávila, G. (2016) An optimised protocol to isolate high-quality genomic DNA from seed tissues streamlines the workflow to obtain direct estimates of seed dispersal distances in gymnosperms. *Journal of Plant Research,* 129, 559–563.

González-Varo, J. P., Arroyo, J. M. & Jordano, P. (2014) Who dispersed the seeds? The use of DNA barcoding in frugivory and seed dispersal studies. *Methods in Ecology and Evolution* 5, 806–814.

González-Varo, J. P., Carvalho, C. S., Arroyo, J. M. & Jordano, P. (2017) Unravelling seed dispersal through fragmented landscapes: Frugivore species operate unevenly as mobile links. *Molecular Ecology,* 26, 4309–4321.

Lijtmaer, D. A., Kerr, K. C. R., Stoeckle, M. Y. & Tubaro PL. (2012) *DNA Barcoding Birds: From Field Collection to Data Analysis. DNA Barcodes: Methods and Protocols*. Totowa, NJ: Humana Press.

R Packages References

We used R version 4.1.0 (R Core Team 2021) and the following R packages: *allelematch* v. 2.5.1 (Galpern et al. 2012), *candisc* v. 0.8.6 (Friendly and Fox 2021), *CCA* v. 1.2.1 (González and Déjean 2021), *corrplot* v. 0.89 (Wei and Simko 2021), *iNEXT* v. 3.0.0 (Chao et al. 2014; Hsieh, Ma, and Chao 2022), *janitor* v. 2.1.0 (Firke 2021), *knitr* v. 1.36 (Xie 2014, 2015, 2021), *dplyr* v. 1.8.8 (Wickham 2011), *RColorBrewer* v. 1.1.3 (Neuwirth 2022), *reshape2* v. 1.4.4 (Wickham 2007), *rmarkdown* v. 2.8 (Xie, Allaire, and Grolemund 2018; Xie, Dervieux, and Riederer 2020; Allaire et al. 2021), *spatstat* v. 3.0.3 (Baddeley and Turner 2005; Baddeley et al. 2013; Baddeley, Rubak, and Turner 2015), *tidyverse* v. 1.3.1 (Wickham et al. 2019), *vegan* v. 2.5.7 (Oksanen et al. 2020), *VIF* v. 1.0 (Lin 2012).

Allaire, JJ, Yihui Xie, Jonathan McPherson, Javier Luraschi, Kevin Ushey, Aron Atkins, Hadley Wickham, Joe Cheng, Winston Chang, and Richard Iannone. 2021. *rmarkdown: Dynamic Documents for r*. https://github.com/rstudio/rmarkdown.

Baddeley, Adrian, Ege Rubak, and Rolf Turner. 2015. *Spatial Point Patterns: Methodology and Applications with R*. London: Chapman; Hall/CRC Press. https://www.routledge.com/Spatial-Point-Patterns-Methodology-and-Applications-with-R/Baddeley-Rubak-Turner/p/book/9781482210200/.

Baddeley, Adrian, and Rolf Turner. 2005. "spatstat: An R Package for Analyzing Spatial Point Patterns." *Journal of Statistical Software* 12 (6): 1–42. https://doi.org/10.18637/jss.v012.i06.

Baddeley, Adrian, Rolf Turner, Jorge Mateu, and Andrew Bevan. 2013. "Hybrids of Gibbs Point Process Models and Their Implementation."*Journal of Statistical Software* 55 (11): 1–43. https://doi.org/10.18637/jss.v055.i11.

Chao, Anne, Nicholas J. Gotelli, T. C. Hsieh, Elizabeth L. Sande, K. H. Ma, Robert K. Colwell, and Aaron M. Ellison. 2014. “Rarefaction and Extrapolation with Hill Numbers: A Framework for Sampling and Estimation in Species Diversity Studies.” *Ecological Monographs* 84: 45–67.

Firke, Sam. 2021. *janitor: Simple Tools for Examining and Cleaning Dirty Data*. https://CRAN.R-project.org/package=janitor.

Friendly, Michael, and John Fox. 2021. *candisc: Visualizing Generalized Canonical Discriminant and Canonical Correlation Analysis*. https://CRAN.R-project.org/package=heplots.

Galpern, Paul, Micheline Manseau, Pete Hettinga, Karen Smith, and Paul Wilson. 2012. “allelematch: An r Package for Identifying Unique Multilocus Genotypes Where Genotyping Error and Missing Data May Be Present.” *Molecular Ecology Resources* 12:771-778.

González, Ignacio, and Sébastien Déjean. 2021. *CCA: Canonical Correlation Analysis*. https://CRAN.R-project.org/package=CCA.

Hsieh, T. C., K. H. Ma, and Anne Chao. 2022. *iNEXT: Interpolation and Extrapolation for Species Diversity*. http://chao.stat.nthu.edu.tw/wordpress/software_download/.

Lin, Dongyu. 2012. *VIF: VIF Regression: A Fast Regression Algorithm for Large Data*. https://CRAN.R-project.org/package=VIF.

Neuwirth, Erich. 2022. *RColorBrewer: ColorBrewer Palettes*. https://CRAN.R-project.org/package=RColorBrewer.

Oksanen, Jari, F. Guillaume Blanchet, Michael Friendly, Roeland Kindt, Pierre Legendre, Dan McGlinn, Peter R. Minchin, et al. 2020. *vegan: Community Ecology Package*. https://CRAN.R-project.org/package=vegan.

R Core Team. 2021. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Wei, Taiyun, and Viliam Simko. 2021. *R Package "corrplot": Visualization of a Correlation Matrix*. https://github.com/taiyun/corrplot.

Wickham, Hadley. 2007. "Reshaping Data with the reshape Package." *Journal of Statistical Software* 21 (12): 1–20. http://www.jstatsoft.org/v21/i12/.

Wickham, Hadley. 2011. "The Split-Apply-Combine Strategy for Data Analysis." *Journal of Statistical Software* 40 (1): 1–29. https://www.jstatsoft.org/v40/i01/.

Wickham, Hadley, Mara Averick, Jennifer Bryan, Winston Chang, Lucy D'Agostino McGowan, Romain François, Garrett Grolemund, et al. 2019. "Welcome to the tidyverse." *Journal of Open Source Software* 4 (43): 1686. https://doi.org/10.21105/joss.01686.

Xie, Yihui. 2014. "knitr: A Comprehensive Tool for Reproducible Research in R." In *Implementing Reproducible Computational Research*, edited by Victoria Stodden, Friedrich Leisch, and Roger D. Peng. Chapman; Hall/CRC. http://www.crcpress.com/product/isbn/9781466561595.

Xie, Yihui. 2015. *Dynamic Documents with R and Knitr*. 2nd ed. Boca Raton, Florida: Chapman; Hall/CRC. https://yihui.org/knitr/.

Xie, Yihui. 2021. *knitr: A General-Purpose Package for Dynamic Report Generation in r*. https://yihui.org/knitr/.

Xie, Yihui, J. J. Allaire, and Garrett Grolemund. 2018. *R Markdown: The Definitive Guide*. Boca Raton, Florida: Chapman; Hall/CRC. https://bookdown.org/yihui/rmarkdown.