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

Article type: Letter

Number of words abstract: 242

Number of words main text: 5630

Number of figures: 5

Number of tables: 1

Number of references: 72

**Keywords:** second-growth expanding forest, seed dispersal, seed rain, plant-animal mutualisms, maternal progenies, microhabitat, stand maturity, Mediterranean forests, frugivore’s forging patterns, natural regeneration**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.**Introduction**

Deforestation has led to a global reduction of 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 wellbeing 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., 1996). 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 commences with the formation of the seed rain, i.e., the spatial distribution throughout the landscape of all seeds dispersed in each reproductive event (Harper, 1977; Fig.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 Fig. 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, i.e., whether deposition sites receive seeds from one/few fruiting trees or, instead, they accumulate seeds from many different mother trees (Fig. 1) (Karubian et al., 2010; García & Grivet, 2011; Scofield et al., 2012; Carvalho et al. 2020). 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 to 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 previously suggested (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 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; Carvalho et al., 2020; Carlo & Morales, 2008). 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 (Jordano et al., 2007; Schupp et al., 2010; González-Varo et al., 2023). Larger frugivores exhibit longer seed retention times and move larger distances through space while feeding on fruits and dispersing seeds compared to smaller frugivores. Thus, we expect that medium-sized 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.

**Materials & Methods**

*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).

*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 (Fig. 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 (Fig. 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 under cover 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 Suppl. Mat. 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 Supp. Mat. for sampling details. We collected samples every ten days following the protocol described by González-Varo et al., (2014). We estimated the seed rain density (no. seeds/m2) 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 Fig. 2A) in which we both analyzed the contribution of frugivore species and identified the maternal progeny composition of the seed rain.

*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 Supp. Mat. 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 & Escribano (2016), see *Seed progenies identification* section in Supp. Mat. 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 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, 2016). Then, we assessed three components of the maternal genetic correlations of the seed rain in each deposition site (Fig. 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 & 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.

*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 if 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 if 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, 2012). 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.

**Results**

*Animal-generated seed rain patterns across a heterogeneous expansion gradient*

We collected 3,389 feces (or regurgitated seeds) across all deposition sites (N= 539) that included 7,698 dispersed seeds of *J. phoenicea*. Seed density among deposition sites ranged from 0 seeds/m2 to 620 seeds/m2 (Fig. 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 to 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 (Fig. 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.

*Frugivore contribution to seed rain patterns across a heterogeneous expansion gradient*

We identified ten species of frugivores dispersing *J. phoenicea* seeds through DNA-barcoding of samples collected from focal deposition sites (N deposition sites= 105, N feces = 518, N seeds = 1000, Table 1, Fig. 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*, that 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 (*X2* = 95.3, *d.f* = 18, p < 0.001) and microhabitats (*X2* = 365.3, *d.f* = 36, p < 0.001), (Fig. 3A, 3B). 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 consistenly dispersed at least half of the seeds they mobilized, while only the main dispersers contributed to open area sites (Fig. 3B). As a result, we found a wider range of frugivores contributing to *P. pinea* and *J. phoenicea* microhabitats, attracting the highest number of dispersed seeds, compared to microhabitats with a low number of dispersed seeds (open areas and other fleshy-fruited shrubs), (Fig. 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 (Fig. 3C, Fig. S2).

*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 (Fig. 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 415-830 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 (Fig. 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 (Fig. 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 (Fig. S5).

The maternal relatedness among seeds collected within the same scat was high compared to 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 (Fig. 4, Table S2), but not among stands (Fig. 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 the differential contribution of frugivores (Wilk’s λ = 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 R2 = 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) (Fig. 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 variations in the structure of relatedness among the deposition sites (Fig. 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 multi-dimensional CCA space, sorted by microhabitat type (Fig. 5), but not by stand maturity (Fig. 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* (Fig. 5).

**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 explains 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 (Fig. 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 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 (Bialocyt 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 reveals 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, Fig. 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, Fig. 1). Clustering of progenies results from frugivores favoring 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 & Álvarez-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 (Fig 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 wouldaccount 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 et al., 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 favor the dilution of local genetic structure within plant populations (Pérez-Méndez et al., 2016; Gelmi-Candusso et al., 2017). However, the implications of neighbourhood kinship are complex and pervasive down to small-scale contexts, affecting both plant-plant and plant-animal interactions, e.g., 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; Lloret et al., 2022; Isla et al., 2023) to confirm the fundamental role of biotic interactions in determining the composition and structure of expanding secondary-growth forests in a changing world.

**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.

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.

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

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., 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.

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

de Almeida, A., Marques, M. C. M., Ceccon-Valente, M. de 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 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.

FAO (2016) *State of the world’s forests 2016. Forests and agriculture: Land- use challenges and opportunities.* Rome, Food and Agriculture Organization of the United Nations.

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.

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.

Gandhi, S. R., Korolev, K. S. & Gore, J. (2019) Cooperation mitigates diversity loss in a spatially expanding microbial population. *PNAS,* 116, 23582–23587.

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

Godoy, J. A. & Jordano, P. (2001) Seed dispersal by animals: Exact identification of source trees with endocarp DNA microsatellites. *Molecular Ecology* 10, 2275–2283.

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-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., Albrecht, J., Arroyo, J. M., Bueno, R. S., Burgos, T., Escribano-Ávila, N. F., García D., et al., (2023). Frugivore-mediated seed dispersal in fragmented landscapes: Compositional and functional turnover from forest to matrix. *Proceedings of the National Academy of Sciences.* 120, e2302440120.

González, I., Déjean, S., Martin. P. G. P. & Baccini, A. (2008) CCA: An R package to extend canonical correlation analysis. *Journal of Statistical Software.* 23, 1–14.

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.* London: 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., 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., 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., Rigueiro, C., Arroyo, J. M., Jordano, P., & García, C. (2023) Animal-mediated seed dispersal and the demo-genetic configuration across plant colonization gradients [Data set]. Zenodo. <https://doi.org/10.5281/zenodo.10579387>

Janzen, D.H. (1970) Herbivores and the number of tree species in tropical forests. *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. & Schupp, E.W. (2000) Seed disperser effectiveness: The quantity component and patterns of seed rain for *Prunus mahaleb*. *Ecological Monographs,* 70, 591–615.

Jordano, P. & Godoy, J. A. (2002) Frugivore-generated seed shadows: a landscape view of demographic and genetic effects. *Seed dispersal and frugivory: ecology, evolution and conservation.* (eds. Levey, D., Silva, W. & Galetti, M.), CAB International, Wallingford, UK 305–321.

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*, *104*, 3278–3282.

Jordano, P. (2017). What is long-distance dispersal? And a taxonomy of dispersal events. *Journal of Ecology*, *105*, 75–84.

Jordano, P. (2014) Fruits and frugivory. In *Seeds: the ecology of regeneration in plant communities.*  3rd edition *(*ed. Gallagher, R. S.) CABI, Wallingford, UK.

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., Machon, N., Petit, R. J. & Kremer, A. (1997) Colonization with long-distance seed dispersal and genetic structure of maternally inherited genes in forest trees: a simulation study. *Genetics Research,* 69, 117–125.

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. *Science of Total Environment.* 806.

Paulose, J. & Hallatschek, O. (2020) The impact of long-range dispersal on gene surfing. *PNAS,* 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 SA. (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. *et al.* (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.

Roques, L., Garnier, J., Hamel, F. & Klein, E. K. (2012) Allee effect promotes diversity in traveling waves of colonization. *PNAS,* 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., J. L. Telleria, and E. Virgos. (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. doi: 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. *American Naturalist,* 180, 719–732.

Snow, B. & Snow, D. (1988) *Birds and berries. A study of an ecological interaction.* Poyser. London, UK.

Tewksbury. J. J. *et al.* (2002) Corridors affect plants, animals, and their interactions in fragmented landscapes. *PNAS,* 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 OJ. 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 and Evolution* 17, 379–386.

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

Waters, J. M., Fraser, C. I. & Hewitt, G.M. (2013) Founder takes all: Density-dependent processes structure biodiversity. *Trends in Ecology and Evolution,* 28, 78–85.

Whitham, T.G. *et al.* (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.

**Tables**

**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 color 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 nº scats | Total nº seeds dispersed | Mean nº seeds/scat (±SD) | Nº deposition sites (n=105) | Total nº progenies dispersed | Mean nº 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 |

**Figure Legends**

**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, favoring 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**, i.e., 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 /m2). 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 colored in green tones) and decreases when seeds come from distant trees because adult trees show strong spatial genetic correlations (Vekemans & Hardy, 2004).

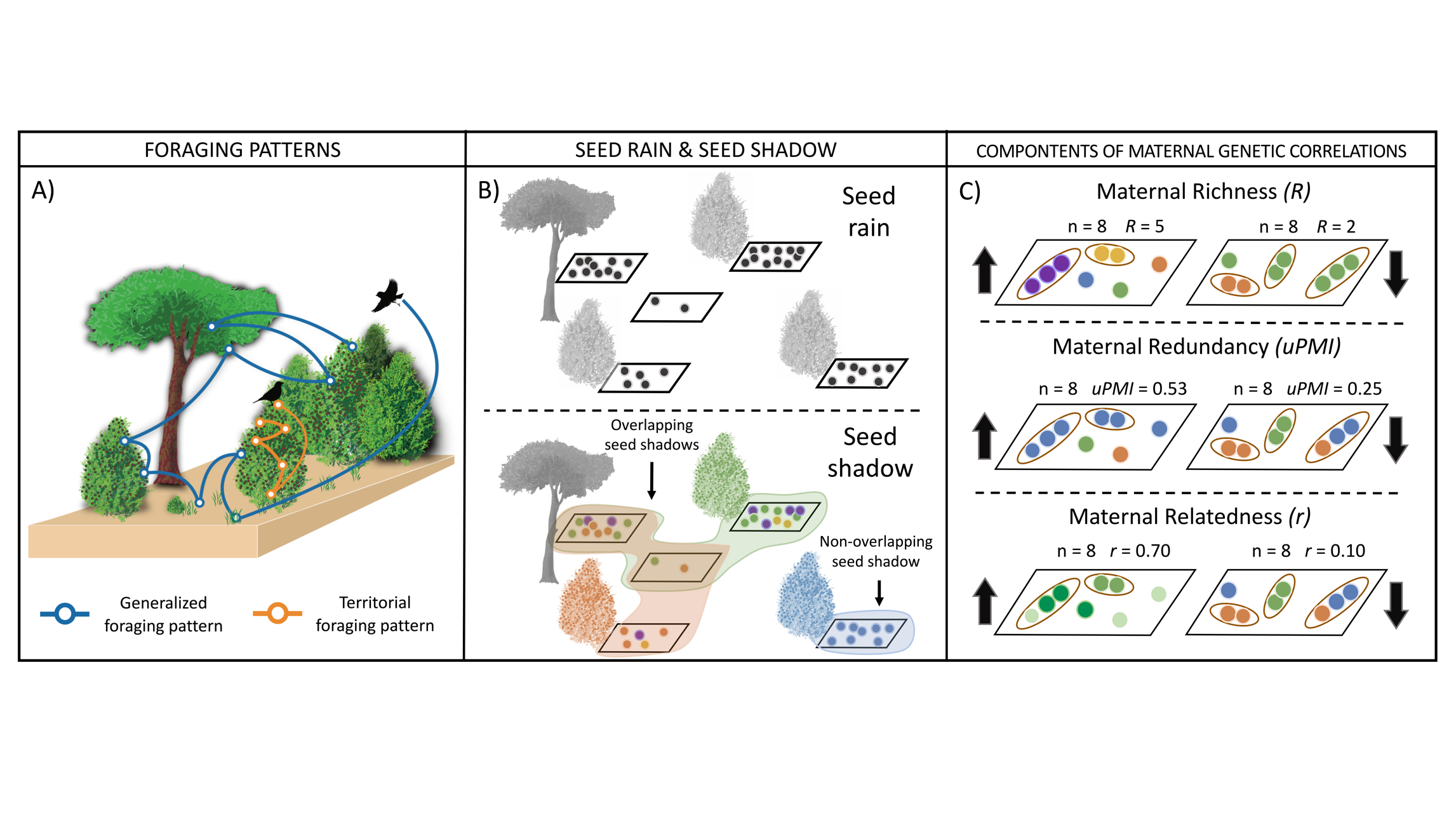
**Figure 2.** Seed rain of *J. phoenicea* across three stands in a population expansion gradient in Doñana National Park. A) Circles represent the locations of deposition sites with the size of the circle proportional to the estimated seed rain density and colored according to the microhabitat type. 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).

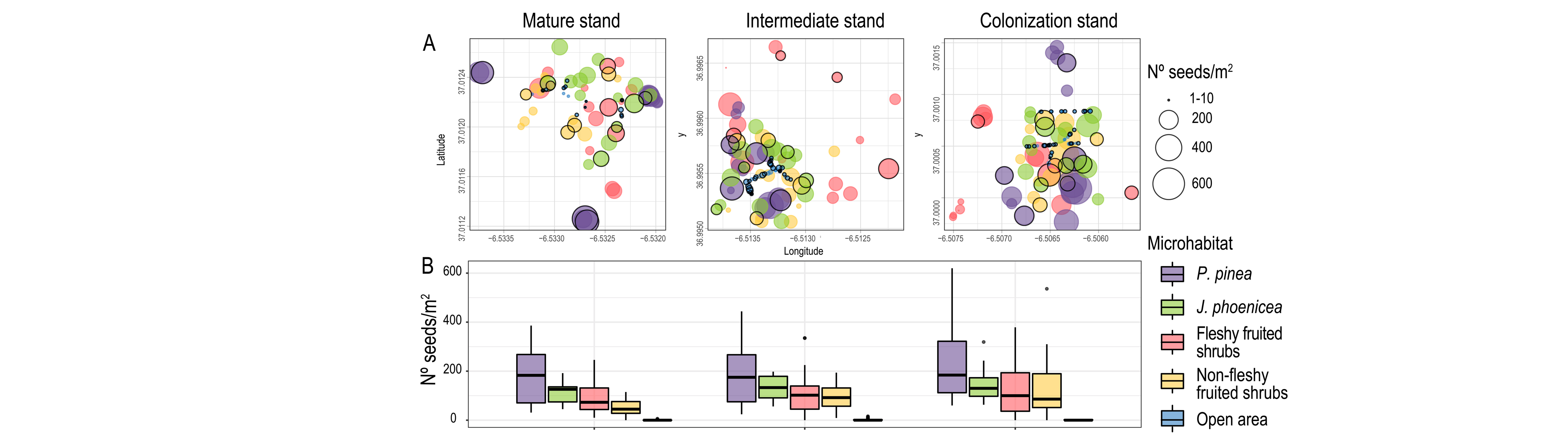
**Figure 3.** Relative contributions of the ten frugivore species to the seed rain of *J. phoenicea* at different spatial scales; (A) among stands, (B) among microhabitats and (C) among deposition sites. The relative contribution of frugivores per microhabitat is weighted by the number of deposition sites sampled. The species names of the 6 ancillary dispersers (that disperse <3% of the seeds) are gray colored. 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 colors 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.

**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.

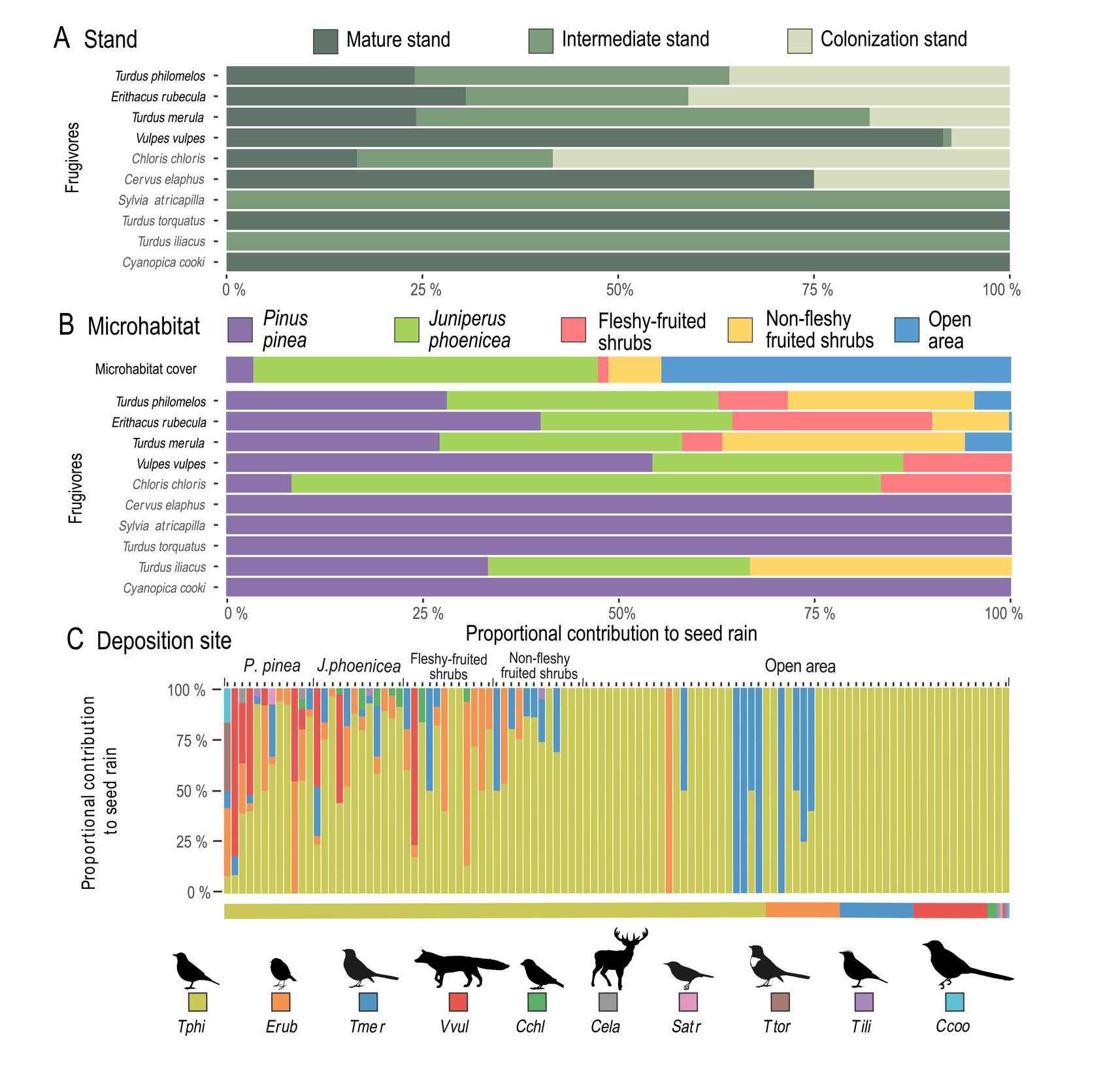
**Figure 5.** Canonical correspondence analysis (CCA) biplot, showing the strength of association of seed contribution per frugivore species (arrows) with values of Maternal richness, relatedness and redundancy (*u*PMI) in deposition sites (dashed arrows) according to the length of the arrows. Animal 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*).

**Fig. 1**

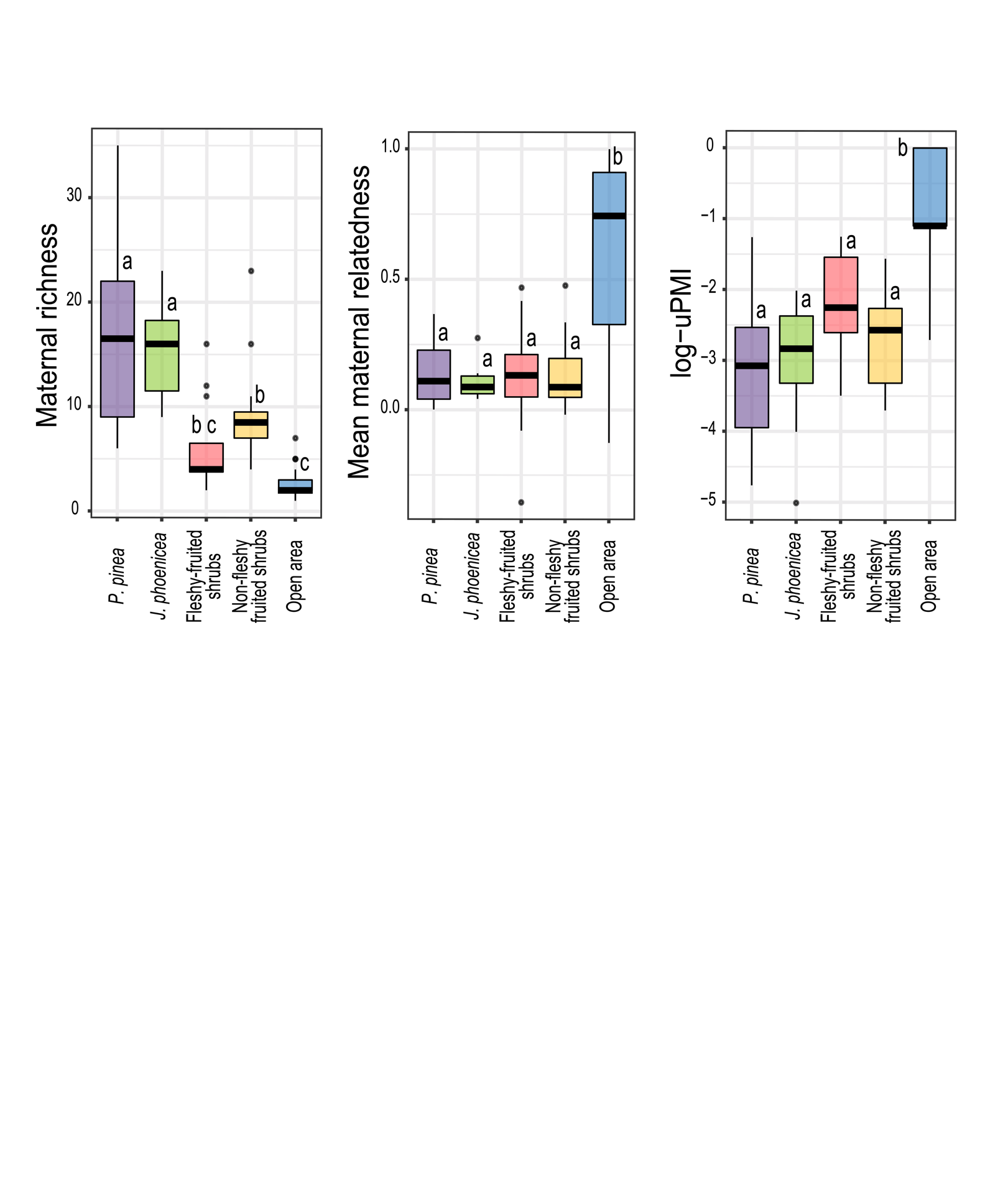
**Fig. 2**

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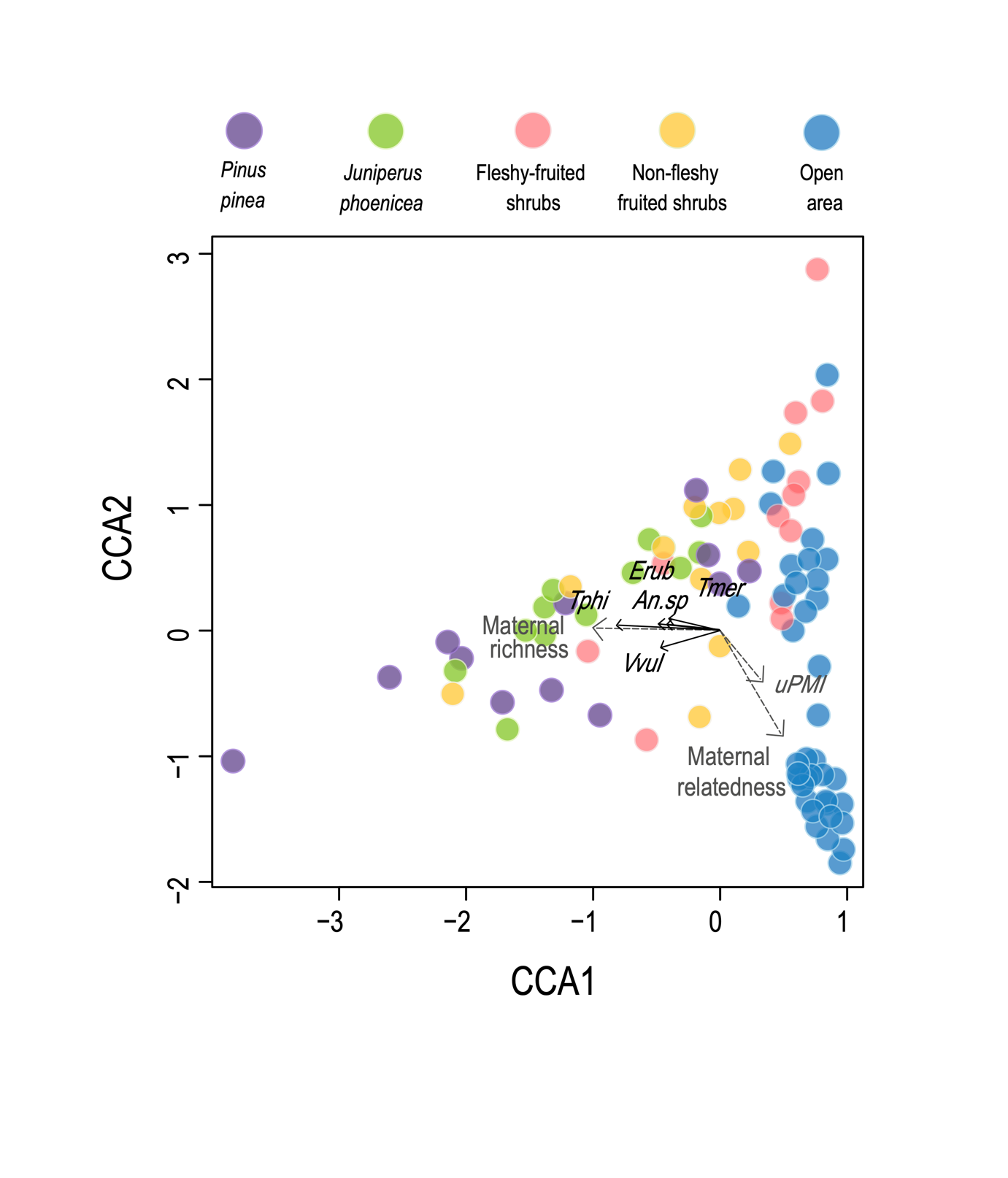
**Fig. 3**

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**Fig. 4**

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**Fig. 5**

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