

2 **Unravelling seed dispersal through fragmented landscapes:**
4 **Frugivore species operate unevenly as mobile links**

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Abstract

24 Seed dispersal constitutes a pivotal process in an increasingly fragmented world, promoting
population connectivity, colonization and range shifts in plants. Unveiling how multiple
26 frugivore species disperse seeds through fragmented landscapes, operating as mobile links, has
remained elusive owing to methodological constraints for monitoring seed dispersal events.
28 We combine for the first time DNA barcoding and DNA microsatellites to identify,
respectively, the frugivore species and the source trees of animal-dispersed seeds in forest and
30 matrix of a fragmented landscape. We found a high functional complementarity among
frugivores in terms of seed deposition at different habitats (forest *vs.* matrix), perches (isolated
32 trees *vs.* electricity pylons) and matrix sectors (close *vs.* far from the forest edge), cross-habitat
seed fluxes, dispersal distances, and canopy-cover dependency. Seed rain at the landscape-
34 scale, from forest to distant matrix sectors, was characterized by turnovers in the contribution
of frugivores and source-tree habitats: open-habitat frugivores replaced forest-dependent
36 frugivores, whereas matrix trees replaced forest trees. As a result of such turnovers, the
magnitude of seed rain was evenly distributed between habitats and landscape sectors. We thus
38 uncover key mechanisms behind ‘biodiversity–ecosystem function’ relationships, in this case,
the relationship between frugivore diversity and landscape-scale seed dispersal. Our results
40 reveal the importance of open-habitat frugivores, isolated fruiting trees, and anthropogenic
perching sites (infrastructures) in generating seed dispersal events far from the remnant forest,
42 highlighting their potential to drive regeneration dynamics through the matrix. This study helps
to broaden the ‘mobile link’ concept in seed dispersal studies by providing a comprehensive
44 and integrative view of the way in which multiple frugivore species disseminate seeds through
real-world landscapes.

46

Introduction

48 Currently, most of the Earth's ice-free terrestrial land is anthropogenic, mainly agricultural
fields and urban settlements (Foley *et al.* 2005; Ellis *et al.* 2010). Natural or semi-natural
50 habitats only cover the remaining 45% and a substantial amount of them (~40%) persist as
patches embedded in a matrix of anthropogenic land covers (Ellis *et al.* 2010; Driscoll *et al.*
52 2013; Haddad *et al.* 2015). Under this scenario, dispersal becomes a critical process for
community dynamics (Butaye *et al.* 2002; Damschen *et al.* 2008; Montoya *et al.* 2008).
54 Species must be able to disperse through the matrix for the connectivity of their populations,
the colonization of vacant habitats after disturbance, or to shift their ranges in response to
56 climate change (Trakhtenbrot *et al.* 2005; Corlett & Westcott 2013; González-Varo *et al.*
2017).

58 Frugivorous animals provide seed dispersal services for a substantial proportion of woody
plant species across many vegetation types (> 40%; especially in tropical forests: > 70%),
60 playing a central role in their regeneration (Jordano 2013). Frugivores ingest fleshy fruits,
transport the seeds in their guts, and drop them in conditions that are generally suitable for
62 germination, generating spatial templates for early plant recruitment (Nathan & Muller-Landau
2000; Wang & Smith 2002). The ability of these plants to disperse through the matrix relies
64 therefore on the spatial behaviour of the frugivore species that feed on their fruits (Carlo &
Yang 2011; Morales *et al.* 2013). Organisms that actively move across the landscape and
66 transfer propagules towards and within disturbed habitats are termed 'mobile links' (Lundberg
& Moberg 2003), and are considered essential for ecosystem resilience after disturbance (Folke
68 *et al.* 2004; Kremen *et al.* 2007). Then, how do multiple frugivore species disperse seeds
through the matrix operating as mobile links?

70 Addressing this question deserves an important consideration: the matrix is not an
"ecological desert" (Haila 2002; Driscoll *et al.* 2013). On the one hand, native woody species

72 can in fact occur in the matrix, as isolated single elements (e.g. trees; Guevara & Laborde
1993; Duncan & Chapman 1999; Herrera & García 2009) or as part of unmanaged and
74 regenerating areas, such as hedgerows and abandoned lands (Debussche & Lepart 1992;
Harvey 2000; Escribano-Avila *et al.* 2012). Notably, non-native plants often occur in these
76 areas after colonization from gardens or crops (Deckers *et al.* 2008; Lenda *et al.* 2012). Thus,
the matrix is also a source of plant propagules. On the other hand, frugivores can vary in their
78 response to landscape alteration, a property known as ‘response diversity’ among species
contributing to the same ecosystem function (Elmqvist *et al.* 2003). We know that many
80 frugivore species not only move through anthropogenic land covers (Lenz *et al.* 2011; Pizo &
dos Santos 2011), but also use them regularly (Sekercioglu *et al.* 2007; Albrecht *et al.* 2012).
82 The fine-grained vegetation of the matrix, including isolated trees and hedgerows, can act as
stepping-stones and corridors, or even as usual foraging sites (e.g. Luck & Daily 2003; Pizo &
84 dos Santos 2011), depending on whether frugivores behave as matrix *avoiders* or *frequenters*.
Hence, seed fluxes between habitats are a crucial feature to consider when tackling seed
86 dispersal in anthropogenic landscapes: some frugivore species might foster seed dispersal from
remnant vegetation whereas others might promote seed dispersal from matrix elements, as
88 suggested by studies on seed rain composition (e.g. Guevara & Laborde 1993; Duncan &
Chapman 1999). Moreover, different frugivore species might foster seed dispersal towards
90 natural or artificial sites of the matrix. For instance, birds can drop seeds in deforested areas
beneath different types of perches (Holl 1998), such as isolated trees (Duncan & Chapman
92 1999) and electricity pylons (Kurek *et al.* 2015).

Unravelling how different frugivores contribute to seed fluxes within and between habitats
94 is essential to understand the processes driving plant community dynamics in the
Anthropocene (Gosper *et al.* 2005; McConkey *et al.* 2012). Yet, despite increasing advances in
96 our knowledge on frugivory interactions in fragmented landscapes (i.e. *who eats what?*;

(Schleuning *et al.* 2015), there is still a significant gap of empirical information about the
98 comprehensive seed dispersal process (i.e. *who dispersed the seeds, where, and from where?*),
especially when diverse animal assemblages and large-scale landscapes are considered (Côrtes
100 & Uriarte 2013). Tackling these questions has been mainly hindered by two methodological
constraints that are inherent to the study of animal-mediated seed dispersal: (i) the
102 identification of the frugivore species and (ii) the identification of the source plant involved in
each seed dispersal event (see González-Varo *et al.* 2013; González-Varo *et al.* 2014, and
104 references therein). The first is essential to understand the complementary or redundant roles of
multiple mutualists in the seed dispersal process, therefore, the mechanisms driving
106 ‘biodiversity–ecosystem function’ relationships (García & Martínez 2012; Schleuning *et al.*
2015). The second enables detecting seed fluxes between habitats, measuring contemporary
108 dispersal distances and characterizing landscape features around the dispersal events, therefore,
characterizing multiple functional components that determine the role of different frugivore
110 species as mobile links (Jordano *et al.* 2007; González-Varo *et al.* 2013; González-Varo *et al.*
2017).

112 Here, we address how multiple frugivore species disperse seeds through the matrix acting
as mobile links. We combine for the first time two sets of DNA-based molecular markers to
114 identify the frugivore species (DNA barcoding) and the source tree (DNA microsatellites) of
frugivore-dispersed seeds directly sampled in the field. We focus on a tree species in a
116 fragmented landscape that occurs both in the remnant forest and in the matrix, both as isolated
trees and as a main component of hedgerows. Specifically, we assess whether different
118 frugivore species (i) disperse seeds unevenly through the landscape, in different habitats (forest
vs. matrix), perches (natural *vs.* artificial) and matrix sectors (close *vs.* far from the forest
120 edge); (ii) promote contrasting seed fluxes between habitats; (iii) produce different seed
dispersal distances; and (iv) choose differently tree canopies as stepping-stones or corridors

122 when dispersing seeds through the landscape. According with the ‘biodiversity–ecosystem
function’ relationships reported in plant-animal mutualisms (Klein *et al.* 2003; García &
124 Martínez 2012), we expected to find complementarity among frugivore species across the
multiple functional components analyzed.

126

Materials and methods

128 *The plant-frugivore system*

The plant-frugivore system comprised a widespread fleshy-fruited species that is dispersed by
130 a diverse guild of frugivorous birds. The study plant was the wild olive tree (*Olea europaea*
var. *sylvestris*, Oleaceae), a main component of mature woodlands and forests in warm areas
132 across the Mediterranean Basin. Its fruits are ellipsoidal drupes with a lipid-rich pulp that
ripens during the late autumn (mean diameter = 9.0 mm, mean length = 13.4 mm, $n = 60$ fruits
134 from 12 plants). Each fruit contains a single seed wrapped in a hard endocarp; hereafter, the
whole unit referred as a seed (mean diameter = 5.7 mm, mean length = 11.1 mm). Wild olives
136 are consumed by a diverse guild of small- to medium-sized frugivorous birds belonging to
families Sylviidae, Turdidae, Muscicapidae, Columbidae, Sturnidae and Corvidae (Jordano
138 1987; Rey & Alcántara 2014). Many of these birds are migratory species from Central and
Northern Europe that use Mediterranean woodlands as their main wintering quarter (Tellería *et*
140 *al.* 2005), even those woodlands within highly fragmented landscapes (González-Varo 2010).

142 *Study landscape*

We conducted our study in an anthropogenic landscape located in southern Spain (Cádiz
144 province; 36° 39′ N, 5° 57′ W), in a lowland area (40–60 m a.s.l.) devoted to intensive
agriculture (Fig. S1). The study landscape, which extends over 280 ha (1.4 km in longitude × 2
146 km in latitude), includes a forest remnant embedded in an agricultural matrix (Fig. S1). The

remnant is a Mediterranean lowland forest of ca. 120 ha, 80 of which are within the study
148 landscape. Its vegetation consists of large holm- (*Quercus ilex* subsp. *ballota*) and cork- (*Q.*
suber) oaks, and an understorey dominated by treelets and shrubs, among which wild olive
150 trees, kermes oaks (*Q. coccifera*, Fagaceae), lentiscs (*Pistacia lentiscus*, Anacardiaceae),
evergreen buckthorns (*Rhamnus alaternus*, Rhamnaceae) and rockroses (*Cistus salvifolius*,
152 Cistaceae) are the dominant species. The adjacent matrix is composed of cereal fields where
some isolated trees (mean density = 2.1 trees per ha; mean canopy cover = 2.9%), mainly holm
154 oaks and wild olive trees, have been left after forest destruction during the 20th century (aerial
digital orthophotos dating from 1956 available at
156 <http://www.juntadeandalucia.es/medioambiente/site/rediam>). The landscape also has a large
hedgerow (ca. 1450 m length) along a water channel in the south, and different types of
158 infrastructures, including roads, a semi-urban area in the west, an industrial park in the south,
and two (medium-voltage) power lines with electricity pylons (Fig. S1, S2). The covers of the
160 main land uses within this landscape are as follows: crop fields 52.3%, forest 28.5%,
infrastructures 6.5% and tree orchards 2.1%; the remaining 10.6% is accounted by pastures,
162 field margins, hedgerows, small vegetable orchards and gardens. The wild olive tree is present
in the forest remnant (mean = 41.0 trees per ha, $n = 14$ plots of 0.15–0.34 ha) and also in the
164 matrix, as isolated trees in the crop fields (mean = 0.7 trees per ha, in 86, 1-ha grid cells) and as
a main component of the hedgerow (~8.3 trees per 100-m length).

166

Sampling frugivore-dispersed seeds

168 We sampled wild olive seeds dispersed by birds in the forest and in the matrix of the study
landscape. Sampling was carried out during the whole dispersal period of the wild olive (late
170 October to early April) and for two consecutive fruiting seasons (2013–2014 and 2014–2015).
We used seed traps placed beneath plant canopies (trees and shrubs) to quantify the magnitude

172 of seed deposition (seeds per m²) in each habitat type (details below). Seed traps consisted of
plastic trays (40 cm × 55 cm, 8 cm height) with small holes (1 mm diameter) to allow the
174 drainage of rainwater, and covered with wire mesh (1 cm light) to prevent post-dispersal seed
predation by vertebrates (Fig. S2). We also used fixed transects to quantify the magnitude of
176 seed deposition in (canopy free) open interspaces, where bird-mediated seed-rain is less likely
and post-dispersal seed predation is typically low due the lack of shelters for rodents (see
178 González-Varo *et al.* 2014). Moreover, we used direct searches to increase the total number of
seeds for DNA identification of disperser species and seed sources. We conducted sampling
180 surveys fortnightly during each fruiting season. We sampled each bird-dispersed wild olive
seed (i.e. defecated or regurgitated) putting it with a minimum of handling into a 2.0-mL sterile
182 tube with the aid of the tube cap (Fig. S2). Tubes were labelled and stored in a freezer at –20°C
until DNA extraction (González-Varo *et al.* 2014). Sampling in the forest and in the matrix was
184 as follows.

In the forest, we sampled bird-dispersed seeds beneath the canopy of different
186 vegetation components and in open interspaces. We monitored a total of 37 and 42 seed traps
during the fruiting seasons of 2013–2014 and 2014–2015, respectively, placed beneath
188 different oak trees (11 and 12), treelets/shrubs bearing fleshy fruits (14 and 13) and
treelets/shrubs not bearing fleshy fruits (12 and 17). Distance between seed traps ranged from 5
190 to 530 m. In the 2013–2014 season, we set up six fixed transects (23 to 45-m long and 1-m
wide) to sample in open interspaces. In the 2014–2015 season, we considered the route we
192 fortnightly used to survey the seed traps as a single fixed belt-transect (≈ 1550 m length and 1-
m wide) where we sampled dispersed seeds in open interspaces. Additionally, we also
194 conducted direct searches of dispersed seeds at under-sampled microhabitats. The sampling
area in the forest covered ca. 20 ha in its southwest limit (Fig. S1).

196 In the matrix, we sampled bird-dispersed seeds beneath the canopy of isolated oaks,
beneath electricity pylons and in open areas. We monitored a total of 31 and 35 seed traps
198 during the fruiting seasons of 2013–2014 and 2014–2015, respectively, placed beneath isolated
oaks (one trap per oak). These oaks were located in the south of the landscape (Fig. S1),
200 between the forest and the hedgerow, with distances to the forest edge ranging from 5 to 325
m; distances between the target oaks ranged from 10 to 610 m. We also placed plastic mesh
202 rectangles (1.5 × 2.0 m) beneath the target oaks, where we easily found dispersed seeds in
direct searches during our periodical surveys (Fig. S2). We considered the route we fortnightly
204 used to survey the isolated oaks as a single fixed (1-m wide) transect to sample dispersed seeds
in open interspaces (\approx 1820 and 2250 m length in seasons of 2013–2014 and 2014–2015,
206 respectively). Moreover, we periodically conducted direct searches in the concrete-made base
(0.6 m²) of ten electricity pylons (Fig. S2), five in each of two power lines, one crossing the
208 crop in the north of the landscape and the other parallel to the hedgerow in the south (Fig. S1).

210 *Seed disperser identification through DNA barcoding*

We used DNA barcoding to identify the bird species that dispersed the seeds sampled ($n =$
212 582), both in the forest ($n = 248$) and in the matrix ($n = 334$). DNA of animal origin can be
extracted from the surface of defecated or regurgitated seeds (Fig. 1), allowing the
214 identification of the frugivore species responsible of each dispersal event (González-Varo *et al.*
2014). Briefly, disperser species identification was based on a 464-bp mitochondrial DNA
216 region (COI: cytochrome c oxidase subunit I). For DNA extraction, we used a GuSCN/silica
protocol, incubating each seed directly in extraction buffer (added to the 2.0-mL tube where the
218 seed was sampled in the field). For PCR amplification, we used the primers COI-fsdF and
COI-fsdR following PCR protocol described by González-Varo *et al.* (2014). For a subset of
220 sampled seeds ($n = 42$) that failed to amplify using COI-fsd primer pair (apparently as a

consequence of DNA degradation after strong rains), we tested additional protocols using other
 222 primer sets in order to gain in amplification success for smaller DNA fragments. We designed
 two new primers to amplify our 464-bp COI DNA region in two fragments (228 and 272 bp):
 224 COI-fsd-degR (5'-GTTGTTTATTCGGGGGAATG-3'), to be combined with COI-fsdF, and
 COI-fsd-degF (5'-GGAGCCCCAGACATAGCAT-3'), to be combined with COI-fsdR. We
 226 also tested two primers pairs (BirdF1-AvMiR1 and AWCintF2-AWCintR4; amplicon size 404
 and 314 bp respectively) for avian DNA barcode when working with degraded DNA reported
 228 in Lijtmaer *et al.* (2012). Nested-PCR reactions using COI-fsd-degF and COI-fsdR primer set
 on the AWCintF2-AWCintR4 amplicon as template (following Alcaide *et al.* 2009) provided
 230 successful results for 22 of these 42 seeds.

We only sequenced one strand (forward primer) of the amplified COI fragments
 232 because in most cases the electrophoretic patterns were clear and resulting sequences (length:
 mean = 364 bp; median = 401 bp; range = 95–417 bp) allowed successful discrimination
 234 between species. Sequences (i.e. barcodes) were aligned and edited using SEQUENCHER 4.9,
 and then identified using the 'BARCODE OF LIFE DATA' identification system (BOLD:
 236 <http://www.boldsystems.org>; Ratnasingham & Hebert 2007). BOLD accepts sequences from
 the 5' region of the COI gene and returns species-level identification and assigns a percentage
 238 of similarity to matched sequences (for details, see González-Varo *et al.* 2014). In our study
 system, barcoding is unable to discern between the starlings *Sturnus unicolor* and *S. vulgaris*
 240 owing to the low degree of genetic differentiation (<2%) between these species, which in fact
 are treated as subspecies by some authors (Lovette *et al.* 2008). We assigned our samples to *S.*
 242 *unicolor* based on field observations.

244 *Source tree identification through DNA microsatellites*

We used DNA microsatellites to identify the source tree, and thus the source habitat, of the dispersed seeds sampled in the matrix ($n = 334$). We extracted the endocarp DNA of the seed and analyzed its multilocus genotype since it is a tissue of maternal origin (Fig. 1), with identical DNA copies of its source tree (Godoy & Jordano 2001). We sampled leaves from a total of 283 trees present in the study landscape in order to match their microsatellite genotypes with that of the endocarps. We sampled all adult (>1 m height) wild olive trees present in the study matrix ($n = 201$), including isolated trees in the crop field ($n = 73$), trees from the main hedgerow ($n = 114$) and a few trees growing in the edge of gardens, roads and buildings ($n = 14$). Besides, we sampled leaves from wild olive trees present in the forest, in the area adjacent to the matrix area where we placed the seed traps (see Fig. S1). These trees ($n = 82$) accounted for a small proportion (10%) of the estimated number of trees present within our study plot in the forest (~ 820 trees). However, we targeted our sampling towards very large trees most of which were located along the forest edge (e.g. Fig. S3), aiming to increase the likelihood of detecting ‘forest to matrix’ seed dispersal events (see Fig. S1). Notably, the crop size of these large trees ($\sim 10^5$) can be up to four orders of magnitude greater than that of small- and medium-sized trees (10^1 – 10^4 ; JPGV *unpubl. data*), accounting for a large fraction of the fruits produced in the forest.

For DNA isolation from dried leaves and endocarps, we followed the protocols described by Pérez-Méndez *et al.* (2016); the single exception was that we also used a modified CTAB extraction method for endocarps. We used a set of 11 polymorphic microsatellite markers (out of 16 tested) developed for the olive tree (*O. europaea* var. *europaea*) that successfully amplified from both seed endocarps and leaves: IAS-oli11, IAS-oli17 (Rallo *et al.* 2000), IAS-oli23 (Díaz *et al.* 2006), *ssrOeUA-DCA1*, *ssrOeUA-DCA3*, *ssrOeUA-DCA4*, *ssrOeUA-DCA7*, *ssrOeUA-DCA8*, *ssrOeUA-DCA9*, *ssrOeUA-DCA15*, *ssrOeUA-DCA18* (Sefc *et al.* 2000). Details on PCR protocols can be found in Appendix S1. DNA fragments

270 were sized in ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA)
using GeneScan 500 LIZ size standard (Applied Biosystems), and were scored using
272 GENEMAPER v.4.1 software (Applied Biosystems). Each marker presented between five and 29
alleles with an estimated mean number of 16.4 alleles per locus and a paternity exclusion
274 probability of 0.999. Dispersed seeds were assigned to a mother tree by matching the endocarp
multilocus genotype with the genotype of sampled trees (Godoy & Jordano 2001). All wild
276 olive trees genotyped had a distinct multilocus genotype, thereby unambiguous source tree
assignments can be made. Matches between endocarp and adult genotypes were found using
278 the R package ALLELEMATCH (Galpern *et al.* 2012), which applies a hierarchical clustering
method to robustly infer unique individuals (unique genotype profiles) at an optimal threshold
280 of mismatches. In 97.6% of seeds (249 out of the 255) where source trees were successfully
identified, there was a perfect matching with their adult genotypes; in the remaining 6 samples,
282 we conservatively applied an allowed mismatch of up to two alleles, below the threshold
(*alleleMismatch* = 3) estimated by ALLELEMATCH. The overall missing-data load of our
284 dataset was 1.9%.

Importantly, we discarded that some seeds dispersed in the matrix could come from
286 nearby olive orchards located within and outside the study landscape. We genotyped cultivated
olive trees ($n = 29$) from five different orchards, but found no evidence of such dispersal
288 events, which makes sense considering that these orchards produce very large green olives that
are harvested unripe for local consumption (see details in Appendix S2).

290

Data analyses

292 All analyses were performed using R v. 3.2.3 (R Development Core Team 2015) and QGIS v.
2.14.0 (Quantum GIS Development Team 2015). We used the R package ‘bipartite’ version
294 2.03 (Dormann *et al.* 2009) to plot a weighted seed deposition network between the frugivore

species identified through DNA barcoding and the habitats/microhabitats where they dispersed
 296 the seeds. We considered ‘forest’ and ‘matrix’, differentiating in the latter between seeds
 deposited beneath natural (‘isolated trees’) or artificial perches (‘electricity pylons’).

298 In order to assess spatial trends in seed rain magnitude, frugivore contributions to seed
 rain and seed dispersal fluxes between habitats, we classified the sampling sites to belong to
 300 the forest or to five different 50-m band distance classes from the forest edge in the matrix (i.e.
 0: forest; 1: 0–50 m; 2: 50–100 m; 3: 100–150 m; 4: 150–200 m; 5: > 200 m). Such classes
 302 represent a gradient of landscape sectors from the most natural (0) to the most anthropogenic
 and furthest from the forest (5). Number of seed traps per class were as follows: $n_0 = 43$, $n_1 = 8$,
 304 $n_2 = 9$ (2), $n_3 = 7$, $n_4 = 9$ (3), $n_5 = 5$ (4); numbers in parentheses denote electricity pylons.

We used data from seed traps to assess differences in the magnitude of seed rain in the
 306 forest and the different distance classes from the forest edge in the matrix. We pooled both
 study years (2013–2014 and 2014–2015 fruiting seasons) by averaging data per seed trap, then
 308 calculating the average number of seeds per m^2 (i.e. annual seed density). For this analysis we
 excluded seed traps placed beneath fruiting wild olive trees ($n = 5$) in order to account for
 310 actual dispersal events, that is, involving horizontal movement away from the canopies of
 source trees. We used a Kruskal-Wallis test to assess differences in seed rain density between
 312 distance classes.

We used DNA barcoding identifications to calculate the relative contributions (%) of
 314 different frugivore species to seed rain at different distance classes. We calculated two
 contributions, first considering only natural microhabitats (i.e. trees, shrubs and open ground)
 316 and, secondly, considering all microhabitats, including electricity pylons. We performed χ^2
 contingency tests to assess significant heterogeneity in relative frugivore contributions across
 318 distance classes. We quantified the similarity in frugivore contributions to seed rain between
 distance classes by calculating a proportional similarity index (PS; Hurlbert 1978): $PS_i =$

320 $\sum_{i=1}^n \min(p_{ia}, p_{ib})$; where for n species, p_{ia} is the relative contribution of the species i at
 distance class a , and p_{ib} is the relative contribution of the species i at distance class b . Hence,
 322 the PS ranged from 0 (no overlap in frugivore contributions) to 1 (complete overlap) (e.g.
 Jordano 1994; González-Varo 2010). We used the nonparametric Kendall's rank correlation
 324 coefficient (τ) to test for monotonic associations between the relative contributions of different
 frugivore species to seed rain and increasing distance classes from the forest edge (i.e. distance
 326 classes, 0: forest; 1: 0–50 m; etc.). We hypothesized this relationship to be negative for forest-
 dependent frugivores while positive for open-habitat frugivores.

328 We used DNA microsatellite assignments to calculate the relative contributions (%) of
 different source habitats to seed rain at different distance classes. Seeds were classified into
 330 three categories: 'forest' (when the source tree was located in the forest), 'matrix' (when the
 source tree was located in the matrix) or 'unknown' (when the source tree was not identified).
 332 We performed a χ^2 contingency test to assess significant heterogeneity in the contribution of
 each source habitat to seed rain at different distance classes. We used the nonparametric
 334 Kendall's rank correlation coefficient (τ) to test for monotonic associations between the
 relative contributions of each source habitat and increasing distance classes from the forest
 336 edge. We hypothesized this relationship to be negative for 'forest' while positive for 'matrix'.
 We also assessed these relationships for each of the main frugivore species, in order to assess
 338 whether they mediated distinctive seed flows between habitats.

We calculated dispersal distances of seeds sampled in the matrix using the UTM
 340 coordinates of the microsatellite-identified source trees and the sampling sites (i.e. isolated
 trees and electricity pylons). Besides, we calculated the canopy cover (including the canopy of
 342 both isolated trees and the forest) within a 25-m buffer along each seed dispersal segment (i.e.
 50-m band; Fig. S1). We chose a 25-m buffer not only because it is a spatial scale that has
 344 proved to affect movement patterns of frugivorous birds (e.g. Morales *et al.* 2013), but also

because it provided enough variability to assess frugivores' preferences for specific canopy
 346 cover along their movements (range = 0.7–66.2%). We used Kruskal–Wallis tests to assess
 statistical differences between frugivore species in seed dispersal distances and canopy cover
 348 along the seed dispersal events they mediated. We used *post-hoc* Mann–Whitney *U*-tests to
 assess differences between pairs of species. We also used Mann–Whitney *U*-tests to assess
 350 whether the canopy cover along the seed dispersal events mediated by each frugivore species
 differed from that available in the landscape, within 120, 100 × 100 m cells (see Fig. S1).
 352 These cells were the subset of cells that intersected with the buffers, thus including the area
 within which all seed dispersal events occurred.

354

Results

356 We analyzed a total of 582 seeds, 248 seeds sampled in the forest (191 in seed traps, 48 in
 direct searches and 9 in transects) and 334 seeds sampled in the matrix (114 in seed traps, 137
 358 in direct searches and 83 in electricity pylons). The vast majority of seeds (97.9%) was found
 beneath perches, either natural or anthropogenic; only 12 seeds (2.1%) were sampled from
 360 open interspaces on the ground, all them in the forest. We successfully identified through DNA
 barcoding a total of nine frugivore species from 532 seeds (91.4%), six species from 218 seeds
 362 sampled in the forest and six species from 314 seeds in the matrix (Fig. 2). Three species were
 identified in seeds dispersed in both habitats (*Sylvia atricapilla*, *Turdus philomelos* and
 364 *Columba palumbus*), although their relative contribution varied between habitats (Fig. 2).
 Three species were only identified from seeds sampled in the forest and other three species
 366 from seeds sampled in the matrix (species names in Fig. 2). Yet, only four species accounted
 for 97.4% of frugivore-identified seeds, referred hereafter by their genus name (*Sylvia*, *Turdus*,
 368 *Columba* and *Sturnus*; Fig. 1). Notably, *Sturnus* was the only disperser species identified from
 seeds sampled under electricity pylons (Fig. 2).

370

Seed rain density and frugivore contributions

372 Seed rain in open interspaces was almost negligible in the forest (mean = 0.03 seeds per m²)
 and null in the matrix. We calculated that 99.8% of seeds dispersed per forest hectare were
 374 deposited beneath woody plant canopies, and virtually 100% of seeds dispersed per matrix
 hectare were beneath isolated trees and electricity pylons. Seed rain density beneath natural
 376 perches (woody plants) was not significantly different between forest and matrix (mean = 6.3
 and 7.3 seeds per m², respectively; MW *U* test: $P = 0.283$). Moreover, seed rain density
 378 beneath electricity pylons (mean = 10.8 seeds per m²) did not differ significantly from that
 found beneath isolated trees of the matrix (MW *U* tests: $P = 0.088$).

380 We found non-significant differences in the magnitude of seed rain beneath natural
 perches between the forest and the different distance classes from the forest edge in the matrix
 382 ($\chi^2_5 = 5.53$, $P = 0.355$; Fig. 3A). However, frugivore contributions significantly varied
 between distance classes, both when considering seed deposition in natural microhabitats (χ
 384 $^2_{40} = 241.2$, $P \ll 0.001$; Fig. 3B) and, especially, when considering all microhabitats, including
 electricity pylons ($\chi^2_{40} = 438.5$, $P \ll 0.001$; Fig. 3C). Such differences reflected a significant
 386 decrease in the contribution of *Sylvia* ($\tau = -0.87$, $P = 0.008$) along with a parallel increase in
 the contribution of *Sturnus* ($\tau = 0.83$, $P = 0.011$) with increasing distance from the forest edge
 388 (Fig. 3B, 3C); *Turdus* and *Columba* were identified in all distance classes and their relative
 contribution was not significantly associated with distance from forest ($|\tau| \leq 0.6$, $P > 0.6$; Fig.
 390 3B, 3C). Indeed, *Sylvia* was not identified in seeds sampled in class '> 200 m', whereas
Sturnus was not in seeds sampled in classes 'forest' and '0–50 m' (Fig. 3B, 3C). Consequently,
 392 frugivore contributions gradually and significantly shifted while moving farther from the
 forest, as shown by a significant decrease in proportional similarity (PS index) (see detailed

394 results in Table S1). For example, there was a similarity of 84% in frugivore contribution
 between ‘forest’ and the first distance class ‘0–50 m’, but a similarity of 9–32% between the
 396 forest and the farthest distance class (> 200 m’), depending on whether only considering
 natural microhabitats (32%; Fig. 3B) or all microhabitats, including electricity pylons (9%; Fig.
 398 3C).

400 *Source habitat contributions*

We successfully identified the source tree in 76.3% of the seeds sampled in the matrix (255 out
 402 of 334); the remaining 23.7% seeds (79) were assigned to ‘unknown’ source tree. Among seeds
 with successfully identified source trees, 16.1% (41) came from trees located in the forest and
 404 83.9% (214) from trees located in the matrix. We found significant variation in the contribution
 of different source habitats to seed rain in the matrix at different distance classes from the
 406 forest edge ($\chi^2_8 = 123.2$, $P \ll 0.001$; Fig. 3D). Such differences reflected a significant
 decrease with increasing distance from the forest edge in the contribution of forest trees ($\tau = -$
 408 1.00 , $P = 0.008$; Fig. 3D) along with a parallel increase in the contribution of matrix trees ($\tau =$
 0.80 , $P = 0.042$; Fig. 3D). Source trees located in the forest trees accounted for 56% of seeds
 410 sampled between 0–50 m from the forest edge, for 13–15% between 50–150 m, for 3%
 between 150–200 m and for 0% at distances farther than 200 m (Fig. 3D). In contrast, source
 412 trees located in the matrix accounted for 13% of seeds sampled between 0–50 m from the
 forest edge, for 49–50% between 50–150 m, and for 81–86% at distances farther than 150 m
 414 (Fig. 3D). We found non-significant association between the contribution of unknown sources
 and distance from the forest edge ($\tau = -0.40$, $P = 0.242$).

416 At the frugivore species level, *Sylvia* ($\tau = -0.91$, $P = 0.035$) and *Turdus* ($\tau = -1.00$, P
 $= 0.008$) significantly dispersed less seeds from the forest in the matrix with increasing
 418 distance from the forest edge (Fig. 4). Yet, *Turdus* dispersed forest seeds towards the matrix

twice as far than *Sylvia* (Fig. 4). On the other hand, *Turdus* and *Columba* significant dispersed
 420 more seeds belonging to matrix trees while moving away from the forest ($\tau = 0.80$, $P = 0.042$
 in both species). We also found that *Columba* significantly dispersed a lower proportion of
 422 seeds of unknown source at further distance classes ($\tau = -1.00$, $P = 0.008$) (see details in
 Table S2).

424

Distance and canopy cover along seed dispersal events

426 We successfully identified both the frugivore species and the source tree in 74.3% of the seeds
 sampled in the matrix (248 out of 334). Among them, dispersal distances differed significantly
 428 between the four main frugivore species ($\chi^2_3 = 27.4$, $P \ll 0.001$; Fig. 4). Distances mediated
 by *Sylvia* and *Turdus* were very similar: they deposited most seeds within 300 m from source
 430 trees and very rarely dispersed seeds further (*Sylvia* up to 638 m and *Turdus* up to 1321 m; Fig.
 4). On average, *Sturnus* dispersed most seeds at slightly longer distances (up to 559 m),
 432 whereas *Columba* did it at distances remarkably longer, with several events above 500 m up to
 1224 m (Fig. 4). The two dispersal distances obtained from *Corvus monedula* were 292 m and
 434 942 m, whereas the two from *Phoenicurus ochruros* were 15 m and 63 m.

The canopy cover along these dispersal events also differed significantly between the
 436 four main frugivore species ($\chi^2_3 = 124.3$, $P \ll 0.001$; Fig. 4). Buffer areas along dispersal
 events mediated by *Turdus* and – particularly – *Sylvia* had a high canopy cover of isolated trees
 438 or forest edge (Fig. 4). In contrast, buffers along dispersal events mediated by *Columba* and,
 especially, *Sturnus* showed a low canopy cover. Indeed, *Sturnus* was the only species that
 440 dispersed seeds along areas having canopy covers non-significantly different from those
 available in the landscape (MW *U*-test: $P = 0.209$; in the other three species all $P \leq 0.016$; Fig.
 442 4).

444 **Discussion**

445 Organisms that actively move across the landscape and transfer propagules from remnant to
446 disturbed habitats, and between elements within disturbed habitats, have been defined as
mobile links (Lundberg & Moberg 2003). Here, we reveal seed dispersal across habitats and
448 landscape sectors as a spatially structured process, characterized by turnovers in the
contribution to seed rain of both frugivore species and source-tree habitats. Seed rain in the
450 matrix was mostly mediated by matrix-frequenter frugivores, which include matrix visitors
from the forest and open-habitat species. Moreover, most seeds dispersed in the matrix came
452 from source trees located there; the contribution of forest trees sharply declined with increasing
distances from the forest edge. *Sturnus*, an open-habitat species, provided a unique function by
454 dropping seeds from matrix trees beneath human-made perches. Finally, the most forest-
dependent frugivores dispersing seeds in the matrix (*Sylvia* and *Turdus*) did it predominantly
456 along areas of high canopy cover, which potentially acted as stepping-stones or corridors.
Taken together, our results demonstrate a remarkable functional complementarity among
458 frugivore species operating as mobile links. In fact, the magnitude of seed rain beneath perches
was evenly distributed through the landscape as a result of very unevenly distributed
460 contributions of distinct frugivore species.

462 *Functional complementarity in seed deposition by frugivores through the landscape*

464 We found that seed deposition was virtually confined beneath natural and artificial perches,
which reinforces the documented importance of perching sites for bird-mediated seed dispersal,
466 especially in anthropogenic habitats (Guevara & Laborde 1993; Duncan & Chapman 1999;
Harvey 2000; Graham & Page 2012; Rey & Alcántara 2014). Our results evidenced a clear
468 spatial turnover in frugivore contributions to seed rain between forest and matrix (Fig. 2). Only

three frugivore species out of the nine identified (*Columba*, *Sylvia* and *Turdus*) deposited seeds
 470 in both habitats; the other six species deposited seeds either in the forest or in the matrix.

Independent data on bird abundances lead us to discard that the turnover observed in the five
 472 species with minor contributions reflected under-sampling; i.e. these species were
 predominantly abundant either in the forest or in the matrix (see Appendix S3). The turnover
 474 between forest and matrix became also evident in terms of the relative contribution by those
 species that dispersed seeds in both habitats: *Sylvia* mostly dispersed seeds in the forest
 476 whereas *Turdus* and *Columba* mostly did it in the matrix, yet at different frequencies. These
 results allow to rank the forest-dependence of these species as: *Sylvia* > *Turdus* > *Columba*
 478 (Fig. 2); which is congruent with their abundances in forest and matrix (Appendix S3). Our
 findings are in line with studies documenting changes in frugivore assemblages in
 480 anthropogenic landscapes not only as a result of species loss, but also of species turnover
 (Luck & Daily 2003; Pizo & dos Santos 2011; Albrecht *et al.* 2012; Farwig *et al.* 2017).
 482 Hence, the matrix acts as a filter for some forest species, but it comprises the usual domains of
 matrix-frequenter species (e.g. Sekercioglu *et al.* 2007), which can be either forest species that
 484 regularly visit the matrix (*Columba* > *Turdus* > *Sylvia*) or open-habitat species (here *Sturnus*,
C. monedula and *P. ochruros*).

486 We also found such spatial turnover at a finer grain within the matrix, between different
 distance classes from the forest edge (Fig. 3B-C), and between natural and artificial perches
 488 (i.e. isolated trees and electricity pylons; Fig. 2). First, there was a gradual shift in frugivore
 contributions to seed rain with increasing distance from the forest edge. Secondly, only one
 490 species (*Sturnus*) – out of the six identified in the matrix – deposited seeds beneath electricity
 pylons (Fig. 2). This demonstrates that seed dispersal towards infrastructures can be mediated
 492 by a very reduced subset of open-habitat species. The latter is in accordance with observational
 studies about the use of artificial perches (crossbars) by frugivorous birds in cleared tropical

494 forests (Holl 1998; Graham & Page 2012). This function can be key for community dynamics
since perching infrastructures are very ubiquitous in anthropogenic landscapes and often
496 located in unmanaged lands, where focal plant regeneration is possible (Kurek *et al.* 2015). In
fact, it is common to observe young wild-olive trees growing beneath electricity pylons of the
498 study region (see Fig. S4).

Our study provides a good example of how response diversity among frugivore species
500 can translate into functional complementarity in seed deposition patterns, and thereby into
resilience of the seed dispersal function across a fragmented landscape (Elmqvist *et al.* 2003;
502 García *et al.* 2013). Functional complementarity in our study system became evident through
the similar seed rain densities sampled in the forest and at different distance classes from the
504 forest edge in the matrix (Fig. 3A), beneath natural perches and electricity pylons. Importantly,
such evenly distributed seed rain densities resulted from unevenly distributed frugivore
506 contributions in different habitats, landscape sectors and perching sites. That means that losing
a frugivore species from this system, especially any of the four main species (*Columba*, *Sylvia*,
508 *Turdus* or *Sturnus*), would impact only specific parts of the landscape. Our findings align with
correlational evidence of functional complementarity in seed deposition by thrushes (*Turdus*
510 spp.) in a fragmented landscape (García & Martínez 2012; García *et al.* 2013). In the study
landscape, seedling establishment beneath most isolated trees and electricity pylons is virtually
512 prevented by the current management practices, mainly, ploughing for cropping and livestock
grazing. However, the observed seed dispersal patterns are expected to generate recruitment
514 patterns whenever these perching sites are located in abandoned lands or unmanaged matrix
sectors (Debussche & Lepart 1992; Escribano-Avila *et al.* 2012; Rey & Alcántara 2014).

516

Differential contribution of source habitats to seed deposition through the
518 *landscape*

The evenly distributed seed rain densities through the landscape also resulted from turnovers of source-habitat contributions, illustrating how the landscape-scale seed rain is structured on seed shadows of individual trees located in different habitats. We found that wild olive trees located in the forest were the predominant sources of seeds deposited within the first 50-m of the matrix. However, their contribution declined sharply at further distances from the forest edge, where most seeds came from matrix trees, especially at distances further than 150 m (Fig. 3D). Our results are consistent with previous studies suggesting that most seeds arriving to deforested lands might not come from the forest but rather from nearby disturbed sites (Duncan & Chapman 1999; Pizo & dos Santos 2011; Graham & Page 2012). The fact that the contribution of unknown sources was not associated with the distance from forest edge strongly suggests these non-genotyped trees were located both in the forest and in the matrix, outside the study landscape. Interestingly, these general patterns emerged from frugivore-specific differences in seed dispersal from – and towards – the different habitats. For instance, *Sylvia* and especially *Turdus* dispersed seeds from the forest towards nearby isolated oaks during their incursions into the matrix (Fig. 4), which were much more frequent in the latter (Fig. 3B-C). In contrast, *Sturnus* mainly dispersed seeds from the matrix and towards the furthest sectors from the forest. Thus, the seeds from unknown source trees dispersed by *Sturnus* likely belonged to trees located in anthropogenic habitats outside the study landscape. Finally, the fact that most seeds dispersed by *Columba* came from unknown sources, particularly at closer distances from the forest, along with the long-dispersal distances mediated by this species, suggests that such unknown sources were probably located in the forest (Fig. 4).

It is not difficult to envisage how these seed dispersal patterns might occur under distinct landscape configurations, for example, within a landscape with smaller forest patches at distances of a few hundred meters from each other. Our results suggest that *Turdus* and,

544 especially, *Columba*, would play a major role dispersing seeds between patches (Fig. 4). Yet,
 they also suggest that most immigrant seeds arriving to a particular forest patch would belong
 546 to nearby fruiting trees located in the matrix (Fig. 3D), whenever these are present.

548 *Features of seed dispersal events emerging from frugivore behaviour*

We found a remarkable heterogeneity among frugivore species in dispersal distances for the
 550 seeds they deposited in the matrix as well as in the canopy cover along these dispersal events
 (Fig. 4). *Sylvia* and *Turdus* dispersed most seeds at distances below 300 m and through areas
 552 harbouring high canopy cover of isolated trees and forest edge, which would have acted as
 stepping-stones and corridors (Damschen *et al.* 2008; Herrera & García 2009). In contrast,
 554 *Columba* and *Sturnus* dispersed seeds over longer distances (especially *Columba*) and using
 the most open areas of the matrix. These findings support the idea that seed dispersal events
 556 arise from the interaction between landscape features and frugivore traits, including behaviour
 (Morales *et al.* 2013). First, the larger frugivores (*Columba* \approx 500 g; *Sturnus* \approx 85 g) dispersed
 558 seeds further than smaller ones (*Sylvia* \approx 17 g; *Turdus* \approx 70 g), as found in several systems (e.g.
 Jordano *et al.* 2007; González-Varo *et al.* 2013; Pérez-Méndez *et al.* 2016). On the other hand,
 560 frugivores dispersed the seeds through areas varying in canopy cover, according with their
 forest-dependence (i.e. *Sylvia* > *Turdus* > *Columba*; null in *Sturnus*). This is in line with
 562 observational studies documenting variability in spatial behaviour and response to forest loss
 among frugivorous birds (García *et al.* 2013; Morales *et al.* 2013).

564 But why did forest frugivores enter the matrix? Evidence from the observed seed
 dispersal patterns and frugivore densities (Appendix S3) suggests that *Turdus* and *Columba*
 566 actively left the forest, searching for the large crops of isolated wild olive trees of the matrix,
 on average \sim 5 times larger than crops from trees located in the forest (mean \approx 115,000 and
 568 25,000 fruits per tree, respectively; JPGV *unpubl. data*). Hence, seed dispersal in the matrix by

Turdus and *Columba* appeared to be driven by fruit-resource tracking (see García & Ortiz-Pulido 2004; e.g. Albrecht *et al.* 2012; García *et al.* 2013). In contrast, the patterns observed in *Sylvia* suggest that seed dispersal in the matrix arose mostly from a passive spillover from the forest, during the nomadic displacements of this superabundant wintering bird (see Tellería *et al.* 2005; González-Varo 2010).

Applicability and generalization of the approach

The use of microsatellite markers to identify the source plants has proven to be a milestone in our understanding of seed dispersal patterns generated by animals (Godoy & Jordano 2001; Jordano *et al.* 2007). However, the identification of the animal species that dispersed the seeds has been, until very recently, a pervasive constraint that has hindered a comprehensive characterization of the dispersal events generated by different frugivore species (see González-Varo *et al.* 2014). Here, we combine for the first time DNA barcoding and DNA microsatellites to identify, respectively, the frugivore species (*who*) and the source plant (*from where*) of individual seeds sampled in the field (*to where*), characterizing comprehensively how multiple frugivores disperse seeds through the landscape.

Our approach, based on two distinct DNA sources (Fig. 1), can be applied to many other systems, such as those in which microsatellite markers have already been used to identify source plants (Jordano *et al.* 2007; Pérez-Méndez *et al.* 2016). Yet, source plant identification is not always feasible, particularly in very large populations where thousands of individuals must be genotyped to obtain a decent number of maternal assignments. Two different approaches have dealt with this problem by providing statistical tools to characterize the compositional diversity of seeds within and between sampling sites (e.g. seed traps). Seed clumps within and between sites can be characterized according to their genetic relatedness (reviewed in García & Grivet 2011) or, alternatively, according to diversity indices (alpha, beta

594 and gamma) applied to the composition of seed sources (Scofield *et al.* 2012). Importantly,
both approaches do not require identifying the location of the source trees and have proven
596 useful to disentangle the spatial scale of seed dispersal by animals. Therefore, our approach can
be generalized by combining DNA barcoding with microsatellite genotyping to obtain these
598 statistics of compositional diversity of seed sources.

600 *Concluding remarks*

As far as we know, the patterns reported here constitute the most comprehensive direct
602 empirical evidence (i.e. non-correlational) of how multiple frugivore species disseminate seeds
through an anthropogenic landscape, from and towards different habitat types. Our findings
604 provide novel insights into the role of frugivorous animals as mobile links (Lundberg &
Moberg 2003; Kremen *et al.* 2007), uncovering in an unprecedented way key mechanisms
606 behind ‘biodiversity–ecosystem function’ relationships (García & Martínez 2012; Schleuning
et al. 2015). They also suggest that different (non-mutually exclusive) mechanisms may
608 determine the role of different frugivore species as mobile links, including habitat-specificity,
spatial behaviour and fruit-resource tracking (see also Albrecht *et al.* 2012; Morales *et al.*
610 2013).

Although long distance seed dispersal from forest trees and towards the matrix was
612 infrequent, our study reinforces the importance of frugivores for the connectivity of plant
populations and the colonization of vacant sites far from the forest. However, the fact that most
614 seeds arriving to the matrix came from trees located there not only reveals the pivotal role of
matrix plants on vegetation dynamics, it also suggests the potential of open-habitat frugivores
616 to spread invasive fleshy-fruited species (Gosper *et al.* 2005), which typically occur in
anthropogenic habitats (e.g. Lenda *et al.* 2012). In fact, open-habitat frugivores used landscape
618 areas far from forest and, unlike forest frugivores, dropped seeds beneath infrastructures where

recruitment is possible (Kurek *et al.* 2015). The latter underscores the importance of addressing
620 mobile-link functions between the natural and human-made elements of the matrix. Our study
thus helps to widen the ‘mobile link’ concept in seed dispersal studies by providing a
622 comprehensive and integrative view of how multiple frugivore species disseminate seeds
through fragmented landscapes.

624

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638

References

- 640 Albrecht J, Neuschulz EL, Farwig N (2012) Impact of habitat structure and fruit abundance on avian
seed dispersal and fruit predation. *Basic and Applied Ecology* **13**, 347-354.
- 642 Alcaide M, Rico C, Ruiz S, *et al.* (2009) Disentangling vector-borne transmission networks: a universal
DNA barcoding method to identify vertebrate hosts from arthropod bloodmeals. *PLoS ONE* **4**,
644 e7092.
- Butaye J, Jacquemyn H, Honnay O, Hermy M (2002) The species pool concept applied to forests in a
646 fragmented landscape: dispersal limitation versus habitat limitation. *Journal of Vegetation Science*
13, 27-34.
- 648 Carlo TA, Yang S (2011) Network models of frugivory and seed dispersal: Challenges and
opportunities. *Acta Oecologica* **37**, 619-624.

- 650 Corlett RT, Westcott DA (2013) Will plant movements keep up with climate change? *Trends in Ecology & Evolution* **28**, 482-488.
- 652 Côrtes MC, Uriarte M (2013) Integrating frugivory and animal movement: a review of the evidence and
654 implications for scaling seed dispersal. *Biological Reviews of the Cambridge Philosophical Society* **88**, 255-272.
- 656 Damschen EI, Brudvig LA, Haddad NM, *et al.* (2008) The movement ecology and dynamics of plant communities in fragmented landscapes. *Proc Natl Acad Sci U S A* **105**, 19078-19083.
- 658 Debussche M, Lepart J (1992) Establishment of woody plants in Mediterranean old fields: opportunity in space and time. *Landscape Ecology* **6**, 133-145.
- 660 Deckers B, Verheyen K, Vanhellefont M, *et al.* (2008) Impact of avian frugivores on dispersal and recruitment of the invasive *Prunus serotina* in an agricultural landscape. *Biological Invasions* **10**, 717-727.
- 662 Díaz A, Rosa R, Martín A, Rallo P (2006) Development, characterization and inheritance of new
664 microsatellites in olive (*Olea europaea* L.) and evaluation of their usefulness in cultivar identification and genetic relationship studies. *Tree Genetics & Genomes* **2**, 165-175.
- 666 Dormann CF, Fründ J, Blüthgen N, Gruber B (2009) Indices, graphs and null models: analyzing bipartite ecological networks. *The Open Ecology Journal* **2**, 7-24.
- 668 Driscoll DA, Banks SC, Barton PS, Lindenmayer DB, Smith AL (2013) Conceptual domain of the matrix in fragmented landscapes. *Trends in Ecology & Evolution* **28**, 605-613.
- 670 Duncan RS, Chapman CA (1999) Seed dispersal and potential for forest succession in abandoned agriculture in Tropical Africa. *Ecological Applications* **9**, 998-1008.
- 672 Ellis EC, Klein Goldewijk K, Siebert S, Lightman D, Ramankutty N (2010) Anthropogenic transformation of the biomes, 1700 to 2000. *Global Ecology and Biogeography* **19**, 589-606.
- 674 Elmqvist T, Folke C, Nyström M, *et al.* (2003) Response diversity, ecosystem change, and resilience. *Frontiers in Ecology and the Environment* **1**, 488-494.
- 676 Escribano-Avila G, Sanz-Pérez V, Pías B, *et al.* (2012) Colonization of abandoned land by *Juniperus thurifera* is mediated by the interaction of a diverse dispersal assemblage and environmental heterogeneity. *PLoS ONE* **7**, e46993.
- 678 Farwig N, Schabo DG, Albrecht J (2017) Trait-associated loss of frugivores in fragmented forest does not affect seed removal rates. *Journal of Ecology* **105**, 20-28.
- 680 Foley JA, DeFries R, Asner GP, *et al.* (2005) Global consequences of land use. *Science* **309**, 570-574.
- 682 Folke C, Carpenter S, Walker B, *et al.* (2004) Regime shifts, resilience, and biodiversity in ecosystem management. *Annual Review of Ecology, Evolution, and Systematics* **35**, 557-581.

- 684 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 Ecology Resources* **12**, 771-778.
- 686 García C, Grivet D (2011) Molecular insights into seed dispersal mutualisms driving plant population recruitment. *Acta Oecologica* **37**, 632-640.
- 688 García D, Martínez D (2012) Species richness matters for the quality of ecosystem services: a test using seed dispersal by frugivorous birds. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, doi:10.1098/rspb.2012.0175.
- 690
- 692 García D, Martínez D, Herrera JM, Morales JM (2013) Functional heterogeneity in a plant-frugivore assemblage enhances seed dispersal resilience to habitat loss. *Ecography* **36**, 197-208.
- 694 García D, Ortiz-Pulido R (2004) Patterns of resource tracking by avian frugivores at multiple spatial scales: two case studies on discordance among scales. *Ecography* **27**, 187-196.
- 696 Godoy JA, Jordano P (2001) Seed dispersal by animals: exact identification of source trees with endocarp DNA microsatellites. *Molecular Ecology* **10**, 2275-2283.
- 698 González-Varo JP (2010) Fragmentation, habitat composition and the dispersal/predation balance in interactions between the Mediterranean myrtle and avian frugivores. *Ecography* **33**, 185-197.
- 700 González-Varo JP, Arroyo JM, 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.
- 702 González-Varo JP, López-Bao JV, Guitián J (2013) Functional diversity among seed dispersal kernels generated by carnivorous mammals. *Journal of Animal Ecology* **82**, 562-571.
- 704 González-Varo JP, López-Bao JV, Guitián J (2017) Seed dispersers help plants to escape global warming. *Oikos* **00**, 000-000.
- 706 Gosper CR, Stansbury CD, Vivian-Smith G (2005) Seed dispersal of fleshy-fruited invasive plants by birds: contributing factors and management options. *Diversity and Distributions* **11**, 549-558.
- 708 Graham LLB, Page SE (2012) Artificial bird perches for the regeneration of degraded tropical peat swamp forest: a restoration tool with limited potential. *Restoration Ecology* **20**, 631-637.
- 710 Guevara S, Laborde J (1993) Monitoring seed dispersal at isolated standing trees in tropical pastures: consequences for local species availability. In: *Frugivory and seed dispersal: ecological and evolutionary aspects* (eds. Fleming TH, Estrada A), pp. 319-338. Springer Netherlands, Dordrecht.
- 712 Haddad NM, Brudvig LA, Clobert J, *et al.* (2015) Habitat fragmentation and its lasting impact on Earth's ecosystems. *Science Advances* **1**, e1500052.
- 714 Haila Y (2002) A conceptual genealogy of fragmentation research: from Island Biogeography to Landscape Ecology. *Ecological Applications* **12**, 321-334.

- 716 Harvey CA (2000) Windbreaks enhance seed dispersal into agricultural landscapes in Monteverde, Costa Rica. *Ecological Applications* **10**, 155-173.
- 718 Herrera JM, García D (2009) The role of remnant trees in seed dispersal through the matrix: Being alone is not always so sad. *Biological Conservation* **142**, 149-158.
- 720 Holl KD (1998) Do bird perching structures elevate seed rain and seedling establishment in abandoned tropical pasture? *Restoration Ecology* **6**, 253-261.
- 722 Hurlbert SH (1978) The measurement of niche overlap and some relatives. *Ecology* **59**, 67-77.
- Jordano P (1987) Avian fruit removal: effects of fruit variation, crop size, and insect damage. *Ecology*
724 **68**, 1711-1723.
- Jordano P (1994) Spatial and temporal variation in the avian-frugivore assemblage of *Prunus mahaleb*: patterns and consequences. *Oikos* **71**, 479-491.
- Jordano P (2013) Fruits and frugivory. In: *Seeds: the ecology of regeneration of plant communities* (ed. Gallagher RS), pp. 18-61. CABI, Wallingford, UK.
- 728
- Jordano P, García C, Godoy JA, García-Castaño JL (2007) Differential contribution of frugivores to complex seed dispersal patterns. *Proceedings of the National Academy of Sciences, USA* **104**, 3278-3282.
- 730
- 732 Klein AM, Steffan-Dewenter I, Tschamntke T (2003) Fruit set of highland coffee increases with the diversity of pollinating bees. *Proceedings of the Royal Society of London, Series B: Biological Sciences* **270**, 955-961.
- 734
- Kremen C, Williams NM, Aizen MA, *et al.* (2007) Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. *Ecology Letters* **10**, 299-314.
- 736
- 738 Kurek P, Sparks TH, Tryjanowski P (2015) Electricity pylons may be potential foci for the invasion of black cherry *Prunus serotina* in intensive farmland. *Acta Oecologica* **62**, 40-44.
- 740
- 742 Lenda M, Skorka P, Knops JM, *et al.* (2012) Plant establishment and invasions: an increase in a seed disperser combined with land abandonment causes an invasion of the non-native walnut in Europe. *Proc Biol Sci* **279**, 1491-1497.
- Lenz J, Fiedler W, Caprano T, *et al.* (2011) Seed-dispersal distributions by trumpeter hornbills in fragmented landscapes. *Proc Biol Sci* **278**, 2257-2264.
- 744
- Lijtmaer DA, Kerr KCR, Stoeckle MY, Tubaro PL (2012) DNA Barcoding Birds: From Field Collection to Data Analysis. In: *DNA Barcodes: Methods and Protocols* (eds. Kress JW, Erickson LD), pp. 127-152. Humana Press, Totowa, NJ.
- 746
- 748 Lovette IJ, McCleery BV, Talaba AL, Rubenstein DR (2008) A complete species-level molecular phylogeny for the “Eurasian” starlings (*Sturnidae*: *Sturnus*, *Acridotheres*, and allies): Recent

- 750 diversification in a highly social and dispersive avian group. *Molecular Phylogenetics and Evolution* **47**, 251-260.
- 752 Luck GW, Daily GC (2003) Tropical countryside bird assemblages: richness, composition, and foraging differ by landscape context. *Ecological Applications* **13**, 235-247.
- 754 Lundberg J, Moberg F (2003) Mobile link organisms and ecosystem functioning: implications for ecosystem resilience and management. *Ecosystems* **6**, 87-98.
- 756 McConkey KR, Prasad S, Corlett RT, *et al.* (2012) Seed dispersal in changing landscapes. *Biological Conservation* **146**, 1-13.
- 758 Montoya D, Zavala MA, Rodríguez MA, Purves DW (2008) Animal versus wind dispersal and the robustness of tree species to deforestation. *Science* **320**, 1502-1504.
- 760 Morales JM, García D, Martínez D, Rodríguez-Pérez J, Herrera JM (2013) Frugivore behavioural details matter for seed dispersal: a multi-species model for Cantabrian thrushes and trees. *PLoS ONE* **8**, e65216.
- 762
- Nathan R, Muller-Landau HC (2000) Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology and Evolution* **15**, 278-285.
- 764
- 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**, 24820.
- 766
- Pizo MA, dos Santos BTP (2011) Frugivory, post-feeding flights of frugivorous birds and the movement of seeds in a Brazilian fragmented landscape. *Biotropica* **43**, 335-342.
- 768
- Rallo P, Dorado G, Martín A (2000) Development of simple sequence repeats (SSRs) in olive tree (*Olea europaea* L.). *Theoretical and Applied Genetics* **101**, 984-989.
- 770
- Ratnasingham S, Hebert PDN (2007) bold: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular Ecology Notes* **7**, 355-364.
- 772
- Rey PJ, Alcántara JM (2014) Effects of habitat alteration on the effectiveness of plant-avian seed dispersal mutualisms: Consequences for plant regeneration. *Perspectives in Plant Ecology, Evolution and Systematics* **16**, 21-31.
- 774
- 776 Schleuning M, Fründ J, García D (2015) Predicting ecosystem functions from biodiversity and mutualistic networks: an extension of trait-based concepts to plant-animal interactions. *Ecography* **38**, 380-392.
- 778
- Scofield DG, Smouse PE, Karubian J, Sork VL (2012) Use of alpha, beta, and gamma diversity measures to characterize seed dispersal by animals. *The American Naturalist* **180**, 719-732.
- 780
- 782 Sefc KM, Lopes MS, Mendonça D, *et al.* (2000) Identification of microsatellite loci in olive (*Olea europaea*) and their characterization in Italian and Iberian olive trees. *Molecular Ecology* **9**, 1171-1173.

784 Sekercioglu CH, Loarie SR, Oviedo Brenes F, Ehrlich PR, Daily GC (2007) Persistence of forest birds
in the Costa Rican agricultural countryside. *Conservation Biology* **21**, 482-494.

786 Tellería JL, Ramírez A, Pérez-Tris J (2005) Conservation of seed-dispersing migrant birds in
788 Mediterranean habitats: Shedding light on patterns to preserve processes. *Biological Conservation*
124, 493-502.

Trakhtenbrot A, Nathan R, Perry G, Richardson DM (2005) The importance of long-distance dispersal
790 in biodiversity conservation. *Diversity and Distributions* **11**, 173-181.

Wang BC, Smith TB (2002) Closing the seed dispersal loop. *Trends in Ecology & Evolution* **17**, 379-
792 386.

794 J.P.G.V. conceived the study; J.P.G.V. and P.J. planned the sampling design, J.P.G.V., C.C.
and P.J. collected the data in the field; J.M.A. and C.C. performed lab-work; J.P.G.V.
796 conducted the statistical analyses and wrote the first manuscript draft. All authors contributed
substantially to revisions and approved the final manuscript.

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Data accessibility

800 Data associated to this article (seed rain density, seed-level information, barcoding sequences,
microsatellite genotypes, coordinates of genotyped trees, and fully identified seed dispersal
802 events) are deposited in Dryad (doi:10.5061/dryad.f9320).

804 **Supporting information**

Additional supporting information may be found in the online version of this article.

806 **Fig. S1** Aerial photograph and digitalized map of the study landscape showing the sampling sites, the microsatellite-genotyped trees and some seed dispersal events.

808 **Fig. S2** Photographs illustrating different methodological components of this study.

Fig. S3 Example of a large wild olive tree located in the forest edge, the type of tree from the forest for which we targeted sampling for genotyping.

Fig. S4 Example of regeneration of wild olive trees beneath an electricity pylon.

812 **Appendix S1** PCR protocols for leaf and endocarp genotyping.

Appendix S2 Details about the lack of evidence of seed dispersal from nearby olive orchards.

814 **Appendix S3** Frugivore abundances in forest and matrix.

Table S1 Proportional similarity (PS index) in the contribution of different frugivorous birds to seed rain at different distance classes from the forest edge.

818 **Table S2** Nonparametric Kendall's rank correlations (τ) testing for associations between the relative contribution (%) of different source habitats of wild olive trees to seed rain in the matrix at different distance classes from the forest edge, and the ranks of such distance classes.

820

Fig. 1 Scheme of a transversal section of a wild olive seed dispersed by a frugivore, showing the main DNA sources that can be sampled. Black arrows show the two DNA sources used in this study. The frugivore DNA can be extracted from cell and gut tissue remains present in defecated or regurgitated seeds. The source tree DNA can be extracted from the endocarp, which is the woody and maternally originated tissue surrounding the embryo.

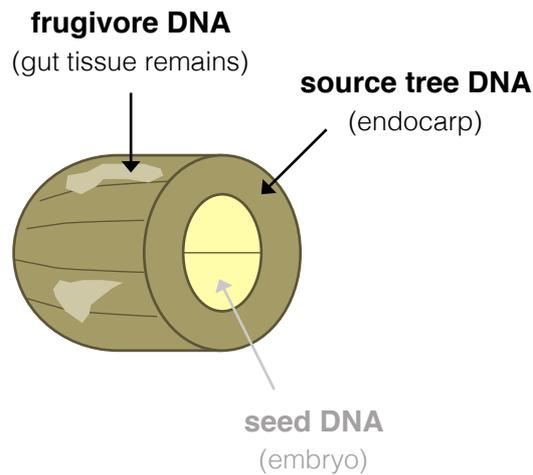


Fig. 2 Seed deposition network connecting frugivore species and the habitat or perch type where they dispersed the seeds ($n = 532$ dispersed seeds with frugivore identified through DNA-barcoding). Horizontal width of the links is proportional to the frequency of seed deposition by each frugivore species in each habitat (forest or matrix) or perch type (isolated trees or electricity pylons). The full species names of less frequent frugivores are *Erithacus rubecula* (*Er*), *Sylvia melanocephala* (*Sm*), *Parus major* (*Pm*), *Corvus monedula* (*Cm*) and *Phoenichuros ochruros* (*Po*).

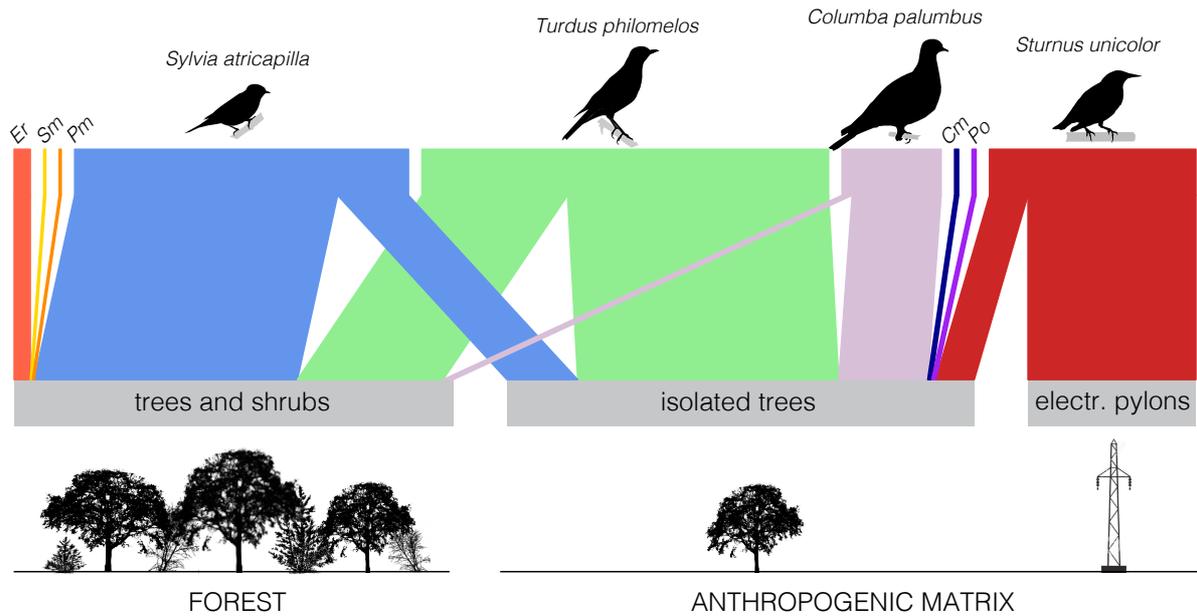


Fig. 3 Seed dispersal patterns (magnitude, vectors and sources) in the forest and at different distance classes from the forest edge. (A) Frugivore-mediated seed rain density measured in seed traps placed under natural perches; boxplot showing median, quartiles, and percentiles 5th and 95th (dots denote mean values). (B) Relative contribution (%) of different frugivore species to seed rain in natural microhabitats (e.g. trees, shrubs). (C) Relative contribution (%) of different frugivore species to seed rain in all microhabitats, including anthropogenic electricity pylons. Colour codes in (B) and (C) as in Fig. 2. (D) Relative contribution (%) of different source habitats (forest, matrix or unknown) to seed rain in the matrix.

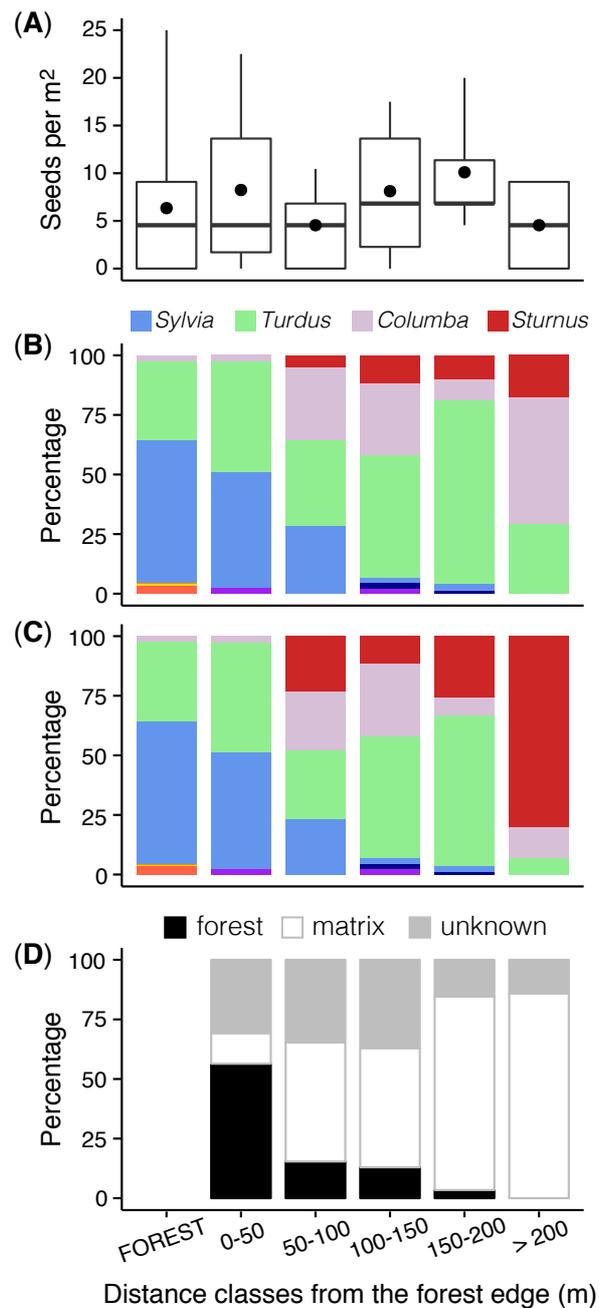


Fig. 4 Seed dispersal patterns in the matrix mediated by different frugivore species (rows). Left panels show the relative contribution (%) of different source habitats (forest, matrix or unknown) to seed rain at different distance classes from the forest edge; n = all seeds dispersed by each species ($n_{Sylvia} = 35$; $n_{Turdus} = 129$; $n_{Columba} = 44$; $n_{Sturnus} = 102$). Central and right panels show, respectively, the relative distribution (%) of seed dispersal distances and canopy cover along the dispersal events (within a 25-m buffer); n = all seeds dispersed by each species with identified source trees ($n_{Sylvia} = 25$; $n_{Turdus} = 116$; $n_{Columba} = 14$; $n_{Sturnus} = 89$); vertical lines denote median values and different letters denote significant differences between frugivores (MW U -tests). Canopy cover along seed dispersal events significantly differed from canopy cover in the landscape in all frugivore species but *Sturnus* (see inset; $n_{landscape} = 120$, 100×100 m cells).

