Uncovering mechanisms of bird seed dispersal in semiarid environments to help to restore them

V. Martínez-López,[1,2,†] V. Zapata,[2] P. De la Rúa,[1] and F. Robledano[2]

[1]Department of Zoology and Physical Anthropology, Veterinary Faculty, University of Murcia, Murcia 30100 Spain
[2]Department of Ecology and Hydrology, Biology Faculty, University of Murcia, Murcia 30100 Spain

Abstract.   Mediterranean semiarid areas are suffering a growing process of agricultural abandonment that represents a challenge for restoration. Limited seed arrival, drought, and scarce suitable microhabitats in oldfields make plant colonization difficult. Restoration through ecological succession can take advantage of increased seed dispersal through animal vectors, but precise knowledge of the factors involved (e.g., seed disperser behavior, rate of effective recruitment of dispersed seeds, and effectiveness of the applied restoration structures) is critical. Otherwise, efforts to foster the processes of interest may be misdirected. We propose and test a methodological approach based on the combination of ecological (bird censuses and camera trapping) and genetic tools (DNA barcoding), to assess seed dispersal of fleshy-fruited shrub species by birds toward oldfields in one of the driest areas of southeastern Spain. We assisted natural restoration with artificial bird attractors (perches and water troughs) in two abandoned areas with different ages. Furthermore, we provided microenvironments (rocks) and prevented herbivory (by fencing) to enhance plant recruitment. Results revealed strong differences among assessment techniques in the characterization of dispersal interactions. While bird censuses only informed about frugivores occurrence, camera trapping and barcoding enabled the quantification of species responses. Attractors proved to be effective in triggering a quantifiable seed arrival, but no subsequent plant establishment occurred. We highlight the importance of implementing different techniques to characterize and assess avian seed disperser performance. Water provision increased seed arrival, but our design failed to enhance plant recruitment. The next logical step in the restoration of Mediterranean oldfields is the optimization of conditions for seed germination and successful plant establishment.

Key words: bird censuses; camera trapping; DNA barcoding; frugivorous birds; oldfield restoration.

† **E-mail:** vicente.martinez2@um.es

Introduction

Land abandonment is increasing worldwide (Cramer and Hobbs 2007), especially in developed countries of temperate areas such as the Mediterranean Basin, where models predict its increase (Rounsevell et al. 2006). Oldfields represent an opportunity for the recovery of natural vegetation (Schröter et al. 2005) and hence are considered target areas for restoration policies (Navarro and Pereira 2015). Oldfield secondary succession in Mediterranean areas has been widely studied under mesic conditions (see references in Pausas et al. 2006), but much less

under semiarid climates (but see Pausas et al. 2006, Robledano-Aymerich et al. 2014). Mediterranean semiarid environments (with a long drought period and extreme rainfall events) show slow natural recovery (Pugnaire et al. 2006, Rey-Benayas et al. 2015) which can entail severe soil erosion (Romero-Díaz et al. 2017). Therefore, practical restoration strategies for activating and accelerating secondary succession are urgently needed (Méndez et al. 2008).

Frugivorous animals can play a key role in the restoration of oldfields by dispersing seeds from natural vegetation remnants (Méndez et al. 2008) and have been widely studied as drivers of restoration in degraded lands. In Mediterranean oldfields, perching birds tend to deposit seeds beneath isolated remnant trees (Debussche et al. 1982) that also provide better conditions—higher humidity, less insolation—for seedling establishment (facilitation effect, Verdú and García-Fayos 1998). This produces a nucleation effect concentrating seeds in suitable microenvironments (Verdú and García-Fayos 1996, Pausas et al. 2006). Pausas et al. (2006) recommended taking advantage of such effect in management policies of degraded landscapes, enhancing vegetation recovery in oldfields by providing artificial perches. Guidetti et al. (2016) found that artificial perches increase seed arrival to target restoration habitats, being considered one of the cheapest and more effective nucleation techniques. However, they pointed out some research gaps as the lack of studies evaluating plant recruitment, and particularly, of practical studies in temperate areas such as the Mediterranean biome (e.g., Heelemann et al. 2012).

In the absence of perch effect, the role of frugivorous birds in restoration of oldfields can be limited, resulting in a poor contribution to seed dispersal in open areas with low vegetation cover (as many semiarid Mediterranean degraded habitats; Debussche et al. 1982) where other animal vectors can be more effective (e.g., carnivorous mammals, Escribano-Ávila et al. 2015). Furthermore, oldfields are unattractive to frugivorous birds since low vegetation cover implies high predation risk and scarce trophic resources (i.e., few fruits; García et al. 2011). However, many fleshy-fruited plants in Mediterranean habitats depend largely on birds for the dispersal of their seeds (Herrera 1989). Contribution of birds to habitat restoration, as well as their responses to restoration initiatives, has been investigated mostly at community level because of the difficulty of establishing species-specific interactions (bird–plant interactions) through classical methods such as bird censuses or observational studies (Lavabre et al. 2016). The study of such relationships, however, is crucial as different bird species provide complementary seed dispersal services (García et al. 2013, Lavabre et al. 2016, González-Varo et al. 2017). Non-intrusive tracking methods (e.g., camera trapping) can elucidate the composition of animal communities (Burton et al. 2015), their associated services (e.g., seed removal, Mokotjomela et al. 2016), and their response to restoration policies (Boone et al. 2017), whereas molecular techniques can shed light on seed dispersal processes (e.g., seed dispersal distances and spatial patterns of seed rain, Jordano et al. 2007, Lavabre et al. 2016; effects of fragmentation, Martínez-López et al. 2017) and dispersal networks of bird communities (González-Varo et al. 2017). However, these genetic tools have been rarely applied to restoration studies (Galimberti et al. 2016).

Here, we test a methodological approach to measure frugivorous bird contribution to the colonization in semiarid Mediterranean oldfields by fleshy-fruited shrubs, as well as to study species-specific relationships (bird–plant interactions) in relation to restoration activities. We installed artificial perches and water troughs (a limiting resource in arid conditions; Degen et al. 1983) to attract birds to abandoned lands, and we created microenvironments and prevented herbivory to favor seedling survival. Since not all species are expected to respond in the same way to our attractors, we combined ecological and genetic tools to get a comprehensive picture of the bird seed dispersal pattern and its relevance for restoration. First, we studied bird community composition in a "natural restoration unit" involving seed arrival and seed source sites (oldfields and surrounding natural land) through classical methods (bird census) to know all potentially attractable bird species. Then, we used non-intrusive detection methods (camera trapping) in oldfield patches to determine which species were lured by our attractors. Finally, we used molecular techniques (DNA barcoding) to identify which species were dispersing seeds into

oldfields. Our hypotheses are (1) this methodological approach detects differential species-specific responses of the frugivores in the areas under restoration; (2) our restoration framework attracts frugivorous birds providing a seed flow directed to water troughs; and (3) the microenvironments and protection against herbivory provided, promote seedling and subsequent plant establishment.

MATERIALS AND METHODS

Methodological approach

A detailed description of our methodological approach can be found in Appendix S1. It includes three different tools to tackle the key questions arising in frugivorous bird-based restoration projects. Briefly, birds in the study area are recorded by a classical census method (point counts, Blondel et al. 1981). Then, camera trapping assesses how each species from the disperser assemblage reacts to restoration facilities (e.g., water troughs and/or perches). Data are then transformed in an unbounded comparative seed dispersal index used as a proxy of the seed dispersal service provided by frugivores. The index, adapted from Pons et al. (2003) in a previous study (Robledano-Aymerich et al. 2014), makes easier to display differences between study units. However, we have improved it by including additional features shaping the dispersal potential of species (e.g., body mass; details about the index parameters in Appendix S2: Table S1). Finally, DNA barcoding is used to determine which birds dispersed the seeds found (González-Varo et al. 2014). This technique allows the identification of species by the amplification of a specific region of the mitochondrial DNA which shows high specificity among animals (cox1: cytochrome c oxidase subunit I; Hebert et al. 2003). Therefore, the method can be used to amplify remaining avian DNA in seed surfaces after defecation or regurgitation to identify bird species (González-Varo et al. 2014).

Case study

Study area.—This study was conducted in Castillo de Chuecos, a rural area located in a coastal semiarid Mediterranean mountain zone of the southeast of the Iberian Peninsula (37°3′57.25″N,

1°36′2.89″O; 550–600 m a.s.l.; Fig. 1a; description of the area in Appendix S3). Here, we studied seed dispersal from mid-November to late January in 2016–2017 and 2017–2018 (16.2 ± 2.9 and 14.0 ± 0.4 d between sampling dates, respectively; five samplings each year, dates in Data S1: Bird censuses). The sampling period was selected according to the peak of fructification of most Mediterranean fleshy-fruited shrub species (Jordano 1988). In addition, abiotic conditions are better for plant recruitment as temperatures are cooler and precipitation is more likely (rainfall in Mediterranean areas concentrates in autumn and spring).

Restoration design.—We applied a bird-based restoration scheme in two abandoned almond fields devoid of fleshy-fruited shrubs, from September 2015 till January 2018. One field remained unploughed for more than 20 yr (old abandonment, OA, hereafter) showing a shrubland cover over 90%, mostly of *Anthyllis cytisoides* L. The second one, abandoned shortly before the beginning of the experiment (recent abandonment, RA, hereafter), had <10% of vegetation cover (mainly annual species and chamaephytes; Appendix S2: Fig. S1). We established 16 square plots of 64 m^2 (eight in each abandonment type), half of them fenced for preventing from the effect of herbivory on plant recruitment. However, only 15 plots were considered by the end of the study since the water troughs of one were stolen in 2016 (unfenced plot 3 in RA, Fig. 1b). Two plots (fenced and unfenced) were kept as controls in each study area (OA and RA; Fig. 1b). In each of the remaining plots, we established water troughs (three replicates per plot; a drum connected to a cement container) which were surrounded by three artificial crossbars perches (90 cm high with a 20 cm long crossbar; Appendix S2: Fig. S1c) to increase bird use of the plots (Fig. 1c). A plastic tray (44 × 28 cm, 7 cm height) was placed under a perch in each replicate to collect bird-dispersed seeds. Trays were covered with wire mesh (1-cm light) to prevent post-dispersal seed predation, and small holes were made on the bottom (1 mm diameter) to allow water drainage. Other perch was kept in bare soil, and some rocks (around 10) were placed under the third perch to provide better microenvironments (i.e., shade and wind protection) for seedling establishment. Rocks are
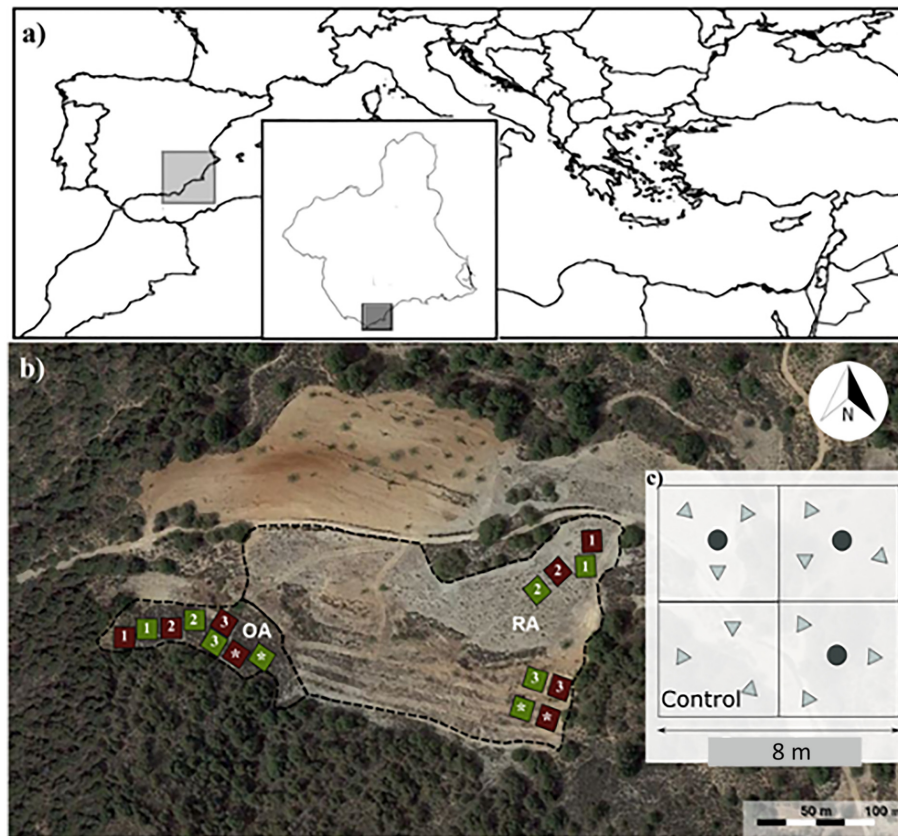
Fig. 1. (a) Study area. (b) Restoration design (OA, old abandonment; RA, recent abandonment). Plots of study are represented with squares (green squares, unfenced; brown squares, fenced; asterisk, control plots). (c) Experiment design inside each plot (circles, water trough; triangles, artificial perches).

recognized as effective facilitators increasing plant recruitment (Fujita and Mizuno 2015). In every plot, we also had a control, which consisted of three perches without water trough (Fig. 1c). Three plastic trays were located in each control plot to assess seed arrival in the absence of perches and water (i.e., perch effect provided by birds would depend on natural vegetation existing in these plots).

Plant sampling.—We sampled data on shrubs both as seed sources and as colonizers (individuals established from seeds germinating in the plots; Appendix S2: Table S2). In 2016 and 2017, we surveyed natural vegetation in the surroundings of the study fields to assess fleshy-fruited species and fruit abundance. Such information is crucial since we did not supply additional propagules (e.g., through seeding or planting); thus, plant colonization depended exclusively on

wild vegetation remnants. We determined the density of fleshy-fruited shrub species (individuals/surface unit) in 16 circular units of 100 m² and assessed fruit abundance visually (total number of fruits per individual recorded). These circular units were located in the first 100 m from the studied oldfields since most seed dispersal events mediated by birds occur in such distances (Jordano et al. 2007). To assess seed arrival effect on vegetation recovery, we fortnightly checked fleshy-fruited plant establishment in eight perches per plot, two per water trough (those without tray), and two in the control point during the three years of study.

Identification of the seed-dispersing birds via censuses and camera trapping.—First, we recorded the presence of birds in the area through nine point counts of ten minutes each considering a radius of 50 m (see Appendices S1, S2: Table S2):

three in the oldfields and six in the surrounding natural vegetation. Data collected (individuals of each species per unit of time, birds/hour) were used to calculate seed dispersal indexes (Appendix S1, index calculation details in Data S1: Bird censuses and seed dispersal indexes bird censuses). The seed dispersal index for each date was calculated from the average bird abundance of the nine point counts. Then, we identified bird species using water troughs by camera trapping. We set six cameras (three in RA and three in OA) which were randomly rotated among plots of each abandoned category (we only used data from five cameras for sampling periods 1 and 5 (2016–2017) and sampling periods 2 and 3 (2017–2018) because one of the cameras failed in each of them, see Data S1: Bird camera trapping, seed dispersal indexes camera trapping, and averaged seed dispersal index camera trapping per period for more details). Cameras were focused on the water trough and programmed to record 10-s. video when activated by movement. We also calculated seed dispersal indexes based on birds using water troughs. Bird abundance was calculated by dividing the number of individuals detected for each species between the number of hours that each camera was functioning (birds/hour, see details in Data S1: Bird camera trapping). The seed dispersal index of each period was calculated as an average of the seed dispersal indexes of cameras in each category (RA and OA). Finally, we checked for seed arrival to the study plots by sampling plastic trays under perches and water troughs (from mid-November to late January 2015–2016, 2016–2017, and 2017–2018).

Identification of seed-dispersing birds via DNA barcoding.—Seeds were directly sampled into Eppendorf plastic tubes avoiding any contact that could contaminate samples before molecular analysis. Once in the laboratory, samples were kept frozen (−20°C) until DNA extraction. Bird dispersers were identified through DNA barcoding following González-Varo et al. (2014; laboratory protocol in Appendix S4). After DNA extraction, seeds were used to identify the dispersed plants by the seed's external morphology. Sequences were edited with Mega7 (Kumar et al. 2016), and identification was done through "BOLD: The Barcode Life of Data System" (Ratnasingham and Hebert 2007). This platform allows researchers to identify species by

uploading sequences and comparing them with those stored in the system.

Data analysis.—Data analyses were performed using R v. 3.2.5 (R Development Core Team 2016). Statistical differences among dispersal indexes in the area (data from point counts) and dispersal indexes in the oldfields with water troughs (data from camera trapping) were checked with Mann–Whitney *U*-tests. For this analysis, we pooled data from the two sampling periods 2016–2017 and 2017–2018. Data from different periods were considered independent as we assumed that bird behavior is affected by spatio-temporal variation of resources abundance (similar approach as in Tellería et al. 2008). We also tested for statistically significant differences between periods. Weighted seed dispersal networks between birds and dispersed seeds were plotted using bipartite R package (Dormann et al. 2009).

RESULTS

Fleshy-fruited shrubs and fruits in the area

Five fleshy-fruited species were detected (individuals/ha ± SE): *Asparagus horridus* L. (18.75 ± 6.25), *Dhapne gnidium* L. (3.12 ± 3.12), *Juniperus oxycedrus* L. (100.0 ± 12.5), *Rhamnus alaternus* L. (6.25 ± 0), and *Rhamnus lycioides* L. (21.87 ± 3.12; Data S1: Shrub density). *Juniperus oxycedrus* was by far the most abundant species. All fruits observed belonged to this species, being more abundant in 2016–2017 than in 2017–2018 (3593.5 vs. 1131.2 fruits/ha; Data S1: Shrub density).

Seed disperser bird community

We detected 24 frugivorous bird species through point counts. Of these, seven were legitimate dispersers (*Erithacus rubecula* L., *Phoenicurus ochruros* S. G. Gmelin, *Sylvia melanocephala* Gmelin, *Sylvia undata* Boddaert, *Turdus merula* L., *Turdus philomelos* Brehm, and *Turdus viscivorus* L.) and five facultative (*Aegithalos caudatus* L., *Alectoris rufa* L., *Columba palumbus* L., *Phylloscopus collybita* Vieillot, and *Pica pica* L.; details of frugivorous bird classification in Appendix S1). Seed dispersal indexes ranged from 0.005 to 0.017 (2016–2017) and from 0.005 to 0.034 (2017–2018; Data S1: Seed dispersal indexes bird censuses). There were no significant differences between both periods (Census *P*-value = 0.841, *n* = 10).

Camera trapping detected 16 bird species using the water troughs (2303 individuals); six were legitimate seed dispersers (*E. rubecula*, *P. ochruros*, *S. melanocephala*, *S. undata*, *T. merula* and *T. viscivorus*) and two facultative (*A. caudatus* and *P. pica*). Seed dispersal indexes ranged from 0.151 ± 0.095 to 2.202 ± 0.947 in 2016–2017 and from 0.148 ± 0.078 to 3.830 ± 1.575 in 2017–2018 (Data S1: Averaged seed dispersal index camera trapping per period). There were no significant differences neither between periods (OA *P*-value = 0.095, *n* = 10; RA *P*-value = 0.841, *n* = 10) nor between categories (i.e., when data from different years were pooled, OA-RA *P*-value = 0.420, *n* = 20, Fig. 2b). Seed dispersal indexes of bird censuses were significantly different from seed dispersal indexes of camera trapping (*P*-value >0.001, *n* = 30, Fig. 2a).

Frugivorous bird seed dispersal to oldfields

We collected 256 seeds during the three sampling campaigns (2015–2016, 2016–2017, and 2017–2018; Data S1: Dispersed seeds and seed dispersers). Seed arrival to study plots was quite heterogeneous among years. No seed was detected in 2015–2016, whereas most (255) were found in 2016–2017, and only one in 2017–2018. No seed was detected in the control plots along the study period.

DNA barcoding allowed us to identify the dispersers of 248 seeds (96.87% of those collected): *E. rubecula* (1.21% of dispersed seeds), *P. ochruros* (6.45% of dispersed seeds), and *T. viscivorus* (92.34% of dispersed seeds; Data S1: Dispersed seeds and seed dispersers). Four plant species were dispersed into oldfields: *A. albus*, *A. horridus*, *J. oxycedrus*, and *Osyris lanceolata* Hochst & Steud (Fig. 3). In 2016–2017, most seeds were deposited in fenced RA plots (Fig. 4). Furthermore, seed arrival to controls inside study plots (i.e., perches without water trough, Fig. 1c), expressed as the number of seeds found in each sampling averaged for the three study plots in fenced RA and two study plots in unfenced RA, was negligible (RA$_{control}$ fenced = 0.33 ± 0.33; RA$_{control}$ unfenced = 0) when compared with seeds found close to the water troughs (RA-fenced = 26.00 ± 10.45; RA-unfenced = 1.67 ± 0.67). Regarding places where seeds arrived, most of them were deposited in the water trough (95.92%) mainly by *T. viscivorus* (Appendix S2: Fig. S2).

We did not detect any recruitment (no seedling emerged from the seeds deposited in the study plots) throughout the sampling period. However, we can confirm the usage of perches by birds as we detected feces on the rocks placed beneath them (Appendix S2: Fig. S3).
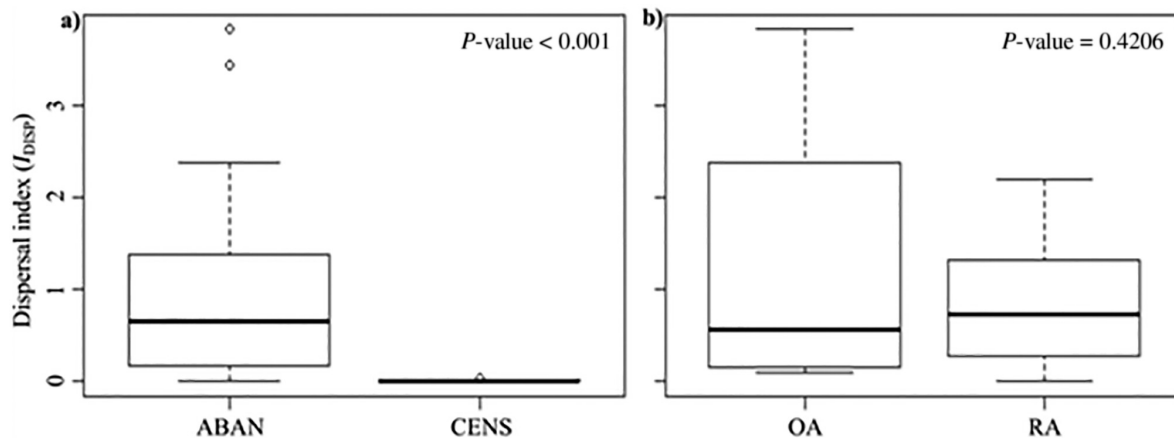

Fig. 2. Seed dispersal indexes in the study area and in the plots where restoration activities have been applied. Data were pooled for the ten sampling periods (five in 2016–2017 and five in 2017–2018). Boxplots show the median, the quartiles, and the outliers (white dots). Significance of Mann–Whitney *U*-tests is shown upper right. (a) Comparison between seed dispersal indexes of camera trapping (ABAN; *n* = 20) and point counts (CENS, *n* = 10). (b) Comparison between seed dispersal indexes of camera trapping in old abandonment (OA, *n* = 10) and recent abandonment (RA, *n* = 10).
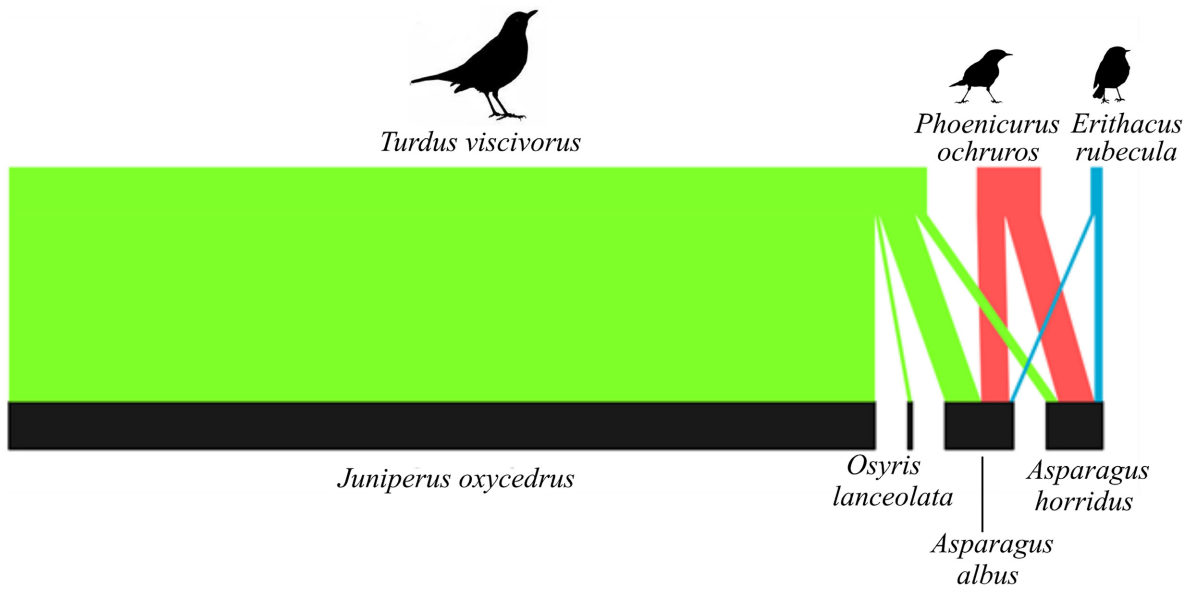
Fig. 3. Seed dispersal networks among frugivorous birds and fleshy-fruited dispersed plants in the target area after applying our restoration design (based on 248 seeds). Bird–plant interactions have been established through DNA barcoding.
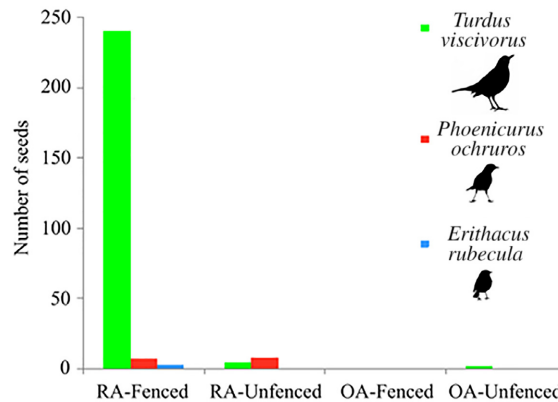

Fig. 4. Seed rain mediated by each identified disperser inside the different sampled plots in oldfields during 2016–2017 campaign (RA, recent abandonment; OA, old abandonment).

DISCUSSION

The proposed methodological approach based on the combination of ecological and genetic tools succeeded in identifying bird species-specific responses to habitat restoration activities. The combination of perches and water troughs proved effective in attracting frugivores, which resulted in an increase in seed arrival to oldfields. However, no positive impact on colonization could be detected, since seedling establishment did not occur.

García et al. (2010) suggested to use fruit availability as a signal of the seed dispersal in a study area, since frugivores show a high capability of tracking such trophic resources in Mediterranean ecosystems (Tellería et al. 2005). Fruit availability in our study area was low and highly variable between years (3,593.5 fruits/ha 2016–2017 and 1,131.2 fruits/ha 2017–2018), given that a single

shrub of *J. oxycedrus* could carry from hundreds to thousands of fruits (authors personal observation). Furthermore, all fruits detected belonged to *J. oxycedrus*, whose fruit diameter is around 10 mm (Adams 2014). Fruits that birds can disperse are restricted to those they can swallow. In other words, it depends on the gape width of each species (Wheelwright 1985). In our system, the gape width of the seed disperser species ranges from 6.48 mm (*S. undata*) to 13.52 mm (*T. merula*) (Data from Pigot et al. 2016), with only three species (*Turdus* spp) exceeding the fruit diameter of *J. oxycedrus*. Thus, only a small fraction of the frugivorous community can use these resources (but see González-Varo and Traveset 2016). Indeed, our results mirror such limitation since only *T. viscivorus* dispersed seeds of *J. oxycedrus* into the oldfield. However, other plant species were also dispersed into the habitat under restoration (*A. albus*, *A. horridus*, and *O. lanceolata*), which highlights the known ability of birds to disperse seeds from more distant source populations than those sampled by us (>100 m, Jordano et al. 2007). Furthermore, species with greater body mass are more important in seed dispersal networks in terms of interaction strength (i.e., number of plant species that depends on each frugivore; Pigot et al. 2016). Results of our seed dispersal networks mirror such trends since *T. viscivorus*, with one of the highest body mass in our system (Appendix S2: Table S1a), dispersed the seeds of all plant species that arrived at the study plots. Other studies highlighted the role of this thrush in seed deposition in degraded landscapes (García et al. 2013).

Seed rain mediated by frugivores determines the chances for fleshy-fruited plant recovery in abandoned lands. However, this represents a challenge for colonization by bird-dispersed shrubs since such open areas are generally avoided by the frugivorous bird community due to scarce trophic resources and low vegetation cover (García et al. 2011). In our case study, most of the seed dispersers in the landscape also occurred in oldfields (six out of seven, with the only exception of *T. philomelos*). Seed dispersal service, measured through seed dispersal indexes, was significantly higher in the study plots (data from camera trapping) than in the surrounding landscape (data from point counts). This fact indicates the effectiveness of our structures (perches and water troughs) directing birds toward oldfields. Therefore, our restoration framework managed to overcome the barrier that open areas constitute for many birds increasing seed dispersal services to target areas. Seed dispersal indexes did not significantly change between study periods (neither in the landscape nor in the oldfield) in spite of fruit availability variation. Frugivores can track changes in fruit abundance within heterogeneous landscapes (Tellería et al. 2005). However, some species (e.g., *T. philomelos*) perform better in such role in the Mediterranean (Tellería et al. 2008). In fact, *T. philomelos* occurrence was low during our study period and did not use the water troughs, which could be related to the low abundance of fruits in the area.

Seed rain in oldfields was highly heterogeneous between years with one sampling accounting for nearly all seeds (255 out of 256 in 2016–2017). This could be ascribed to the observed fluctuation in fruit abundance between years, what is a key predictor of seed dispersal in temperate ecosystems (García et al. 2010). Interannual changes in fruit availability are common in ecosystems even within the same landscape (García et al. 2013). The lack of seed arrival to the control plots evidences the effectiveness of our structures (perches and water troughs) to enhance seed deposition in open areas. Our results are in line with previous studies revealing that artificial perches and water troughs enhance seed dispersal (García-Castellanos et al. 2016, Guidetti et al. 2016). Seed arrival was much greater in perches close to water troughs than in controls only with perches inside each plot, which shows that the attraction of water outperforms the effect of artificial perches used alone. Most seeds were deposited in RA-fenced plots what contrasts with the absence of differences between abandonment categories in terms of seed dispersal service. Such differences may occur due to habitat preferences of different bird species (forest species *vs* open habitat species, González-Varo et al. 2017) as OA presents much higher vegetation cover than RA. *Turdus viscivorus* is by far the most important disperser in our system, and it has been described to use open areas more than other *Turdus* species (García et al. 2013). *Phoenicurus ochruros*, the second species with higher contribution to seed

rain, is also a common disperser in open habitats (González-Varo et al. 2017). Indeed, open habitat species abundance was markedly greater in RA than in OA in 2016–2017 (Appendix S2: Fig. S4). Seed deposition was higher in fenced plots due to *T. viscivorus* disperser activity. This phenomenon could be related to the use of the fence as intermediate perch when birds fly toward water troughs since we observed them using these structures. *Turdus viscivorus* was the largest seed disperser we detected, and it may prefer bigger and/or taller perch sites. This fact points out that the fence could be another restoration structure susceptible to be monitored in future studies. Moreover, we identified dissimilar seed rain in the two monitored restoration structures (i.e., water troughs, 236 seeds; and perches, 12 seeds). These results point out the importance of identifying species-specific interactions with attractors, in order to adapt the restoration design to bird preferences as seed fate can influence the chance of seedling survival (Rey et al. 2004). Our perches were smaller than those used in previous studies (e.g., 6 m high, Holl 1998; 1.8 m high; de Almeida et al. 2016). The idea behind this design was to make their usage easier by future practitioners since their size was felt enough to be used by birds (vegetation in RA was low and plant height in OA was always shorter than the perch). Furthermore, contrary to precedent studies relying only on perches, we combined them with another attractor (water). The fact that perches were used by birds confirms that the design appears suitable.

For activating oldfield secondary succession, seed arrival needs to be translated into plant recruitment. Our restoration measures improved seed arrival, but plant recruitment cannot be granted by this fact (Reid and Holl 2013). Different biotic and abiotic factors can limit plant recruitment. With regard to the biotic ones, post-dispersal seed predation by small mammals and herbivory can represent a barrier for vegetation recovery in fragmented landscapes (Santos and Tellería 1994, MacDougall and Wilson 2007). We did not record any signal of seed predation (e.g., seed coats), and herbivory was controlled by fencing. Thus, recruitment seems limited by abiotic factors (light, water, etc.), in turn determined by the microhabitat where the seeds arrive (Rey et al. 2004). Our microenvironments (rock piles)

were not fully effective in spite of their demonstrated facilitator effect (Fujita and Mizuno 2015). Although they could represent safe sites against seed predation, microclimate amelioration was insufficient to allow plant establishment.

Conclusions and Future Perspectives

Many studies have highlighted the importance of taking advantage of frugivorous animals to restore degraded lands (Pausas et al. 2006, Méndez et al. 2008). However, there is a gap of knowledge regarding practical experiences in Mediterranean environments (but see Rey-Benayas et al. 2015, Fedriani et al. 2017, Castillo-Escrivà et al. 2018), where such information is crucial to develop management policies that promote vegetation recovery of degraded lands threatened by desertification processes such as the Iberian Southeast (Romero-Díaz 2016).

Our measures were mainly directed at increasing frugivore occurrence and seed arrival to target areas, and we supplied only one type of microhabitat (rocks), which proved insufficient for seedling establishment (Reid and Holl 2013). In the future, they could be oriented to the implementation of facilitating elements. Many studies report an enhancement of recruitment when shrub species are located beneath perching sites as seeds arrive to suitable environments with proper conditions for plant establishment (Gómez-Aparicio et al. 2005). Hence, active planting of vegetation under perches could help plant colonization. Furthermore, the characterization of the microhabitat of seed deposition (García-Cervigón et al. 2018) and the performance of germination trials could help to get a comprehensive idea of the contribution of frugivorous animals to the effective colonization of fleshy-fruited plants in oldfields. On the other hand, landscape configuration (e.g., fruit abundance and vegetation cover) can condition restoration success. Restoration frameworks can also increase propagule abundance in target areas by supplying fruits, a solution when extreme landscape degradation eliminates surrounding seed sources. Indeed, an increase in resource availability can prevent the loss of mutualistic interactions in fragmented landscapes (Fontúrbel et al. 2017).

There are other management alternatives such as seeding or planting which could be used in

oldfield restoration. These measures could result in a faster vegetation colonization of oldfields in relation to techniques which rely on seed disperser attraction through artificial structures as our approach. However, promoting regeneration via seed dispersal ensures the use of local resources (seeds from local plant populations) which can be important in terms of preserving the genetic identity of local plant populations. Furthermore, some measures, as providing water troughs, can have additional benefits for the fauna, especially in semiarid areas. With regard to the economical investment, our restoration framework can be considered cheap if we do not install fences (each restoration point —perches plus water troughs—costs around 50€) and it requires a scarce maintenance as we can provide water from October/November till May/June with a 50 L deposit. Active planting or seeding under semiarid conditions would require watering during the first years; otherwise, plant establishment would be null or quite limited. This would be a critical point to restore large areas. Therefore, each restoration framework has intrinsic advantages and drawbacks and the selection of a suitable restoration approach requires a case-by-case detailed analysis which considers the natural ecosystem resilience, the landscape matrix, and the land-use history (Holl and Aide 2011). Combinations of both types of measures, with localized active planting and the enhancing of natural processes of seed dispersal, would be a good option for future applied investigations.

Our methodological approach effectively identified species-specific disperser responses to attractors. The application of such approach makes possible to adapt restoration practices to the target frugivores according to particular conditions (landscape characteristics such as fruit abundance and distribution, or vegetation cover). Our study represents, to our knowledge, the first example of applied research on the use of perches and water troughs for enhancing frugivore-mediated oldfield restoration in semiarid Mediterranean environments. Thereby, our results provide restoration ecologists and practitioners with practical knowledge that can be used as a tool to manage abandoned lands in semiarid environments where habitat restoration remains as a challenge.

Literature Cited

Adams, R. P. 2014. Morphological comparison and key to *J. deltoides* and *J. oxycedrus*. Phytologia 96:58–62.

Blondel, J., C. Ferry, and B. Frochot. 1981. Point counts with unlimited distance. Studies in Avian Biology 6:41–420.

Boone, W. W. IV, R. A. McCleery, and B. E. Reichert. 2017. Fox squirrel response to forest restoration treatments in longleaf pine. Journal of Mammalogy 98:1594–1603.

Burton, A. C., et al. 2015. Wildlife camera trapping: a review and recommendations for linking surveys to ecological processes. Journal of Applied Ecology 52:675–685.

Castillo-Escrivà, A., G. M. López-Iborra, J. Cortina, and J. Tormo. 2018. The use of branch piles to assist in the restoration of degraded semi-arid steppes. Restoration Ecology. https://doi.org/10.1111/rec.12704

Cramer, V. A., and R. J. Hobbs. 2007. Old fields: dynamics and restoration of abandoned farmland. Island Press, Washington, D.C., USA.

de Almeida, A., M. C. Marques, M. de Fátima Ceccon-Valente, J. Vicente-Silva, and S. B. Mikich. 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.

Debussche, M., J. Escarré, and J. Lepart. 1982. Ornithochory and plant succession in Mediterranean abandoned orchards. Vegetatio 48:255–266.

Degen, A. A., B. Pinshow, and P. U. Alkon. 1983. Summer water turnover rates in free-living chukars and sand partridges in the Negev Desert. Condor 85:333–337.

Dormann, C. F., J. Fründ, N. Blüthgen, and B. Gruber. 2009. Indices, graphs and null models: analyzing bipartite ecological networks. Open Ecology Journal 2:7–24.

Escribano-Ávila, G., B. Pías, A. Escudero, and E. Virgós. 2015. Importancia ecológica de los mamíferos frugívoros en la dinámica de regeneración de tierras abandonadas en ambientes mediterráneos. Revista Ecosistemas 24:35–42.

Fedriani, J. M., T. Wiegand, D. Ayllón, F. Palomares, A. Suárez-Esteban, and V. Grimm. 2017. Assisting seed dispersers to restore oldfields: An individual-based model of the interactions among badgers, foxes and Iberian pear trees. Journal of Applied Ecology 55:600–611.

Fontúrbel, F. E., D. A. Salazar, and R. Medel. 2017. Increased resource availability prevents the disruption of key ecological interactions in disturbed habitats. Ecosphere 8:art4.

Fujita, T., and K. Mizuno. 2015. Role of nurse rocks on woody plant establishment in a South African grassland. Tropics 24:57–64.

Galimberti, A., S. Spinelli, A. Bruno, V. Mezzasalma, F. Mattia, P. Cortis, and M. Labra. 2016. Evaluating the efficacy of restoration plantings through DNA barcoding of frugivorous bird diets. Conservation Biology 30:763–773.

García, D., D. Martínez, J. M. Herrera, and J. M. Morales. 2013. Functional heterogeneity in a plant–frugivore assemblage enhances seed dispersal resilience to habitat loss. Ecography 36:197–208.

García, D., R. Zamora, and G. C. Amico. 2010. Birds as suppliers of seed dispersal in temperate ecosystems: conservation guidelines from real-world landscapes. Conservation Biology 24:1070–1079.

García, D., R. Zamora, and G. C. Amico. 2011. The spatial scale of plant-animal interactions: effects of resource availability and habitat structure. Ecological Monographs 81:103–121.

García-Castellanos, F. A., F. Robledano-Aymerich, V. Zapata-Pérez, V. Martínez-López, and G. González-Barberá. 2016. Bebederos de aves: dinámica de uso y función en la dispersión ornitócora. Revista de anillamiento 35:55–63.

García-Cervigón, A. I., M. Żywiec, M. Delibes, A. Suárez-Esteban, R. Perea, and J. M. Fedriani. 2018. Microsites of seed arrival: spatio–temporal variations in complex seed-disperser networks. Oikos 127:1001–1013.

Gómez-Aparicio, L., J. M. Gómez, R. Zamora, and J. L. Boettinger. 2005. Canopy vs. soil effects of shrubs facilitating tree seedlings in Mediterranean montane ecosystems. Journal of Vegetation Science 16:191–198.

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

González-Varo, J. P., and A. Traveset. 2016. The labile limits of forbidden interactions. Trends in Ecology & Evolution 31:700–710.

Guidetti, B. Y., G. C. Amico, S. Dardanelli, and M. A. Rodriguez-Cabal. 2016. Artificial perches promote vegetation restoration. Plant Ecology 217:935–942.

Hebert, P. D., S. Ratnasingham, and J. R. de Waard. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London B: Biological Sciences 270:S96–S99.

Heelemann, S., C. B. Krug, K. J. Esler, C. Reisch, and P. Poschlod. 2012. Pioneers and Perches—Promising Restoration Methods for Degraded Renosterveld Habitats? Restoration Ecology 20:18–23.

Herrera, C. M.. 1989. Frugivory and seed dispersal by carnivorous mammals, and associated fruit characteristics, in undisturbed Mediterranean habitats. Oikos 55:250–262.

Holl, K. D. 1998. Do bird perching structures elevate seed rain and seedling establishment in abandoned tropical pasture? Restoration Ecology 6:253–261.

Holl, K. D., and T. M. Aide. 2011. When and where to actively restore ecosystems? Forest Ecology and Management 261:1558–1563.

Jordano, P. 1988. Diet, fruit choice and variation in body condition of frugivorous warblers in Mediterranean scrubland. Ardea 76:193–209.

Jordano, P., C. García, J. A. Godoy, and J. L. García-Castaño. 2007. Differential contribution of frugivores to complex seed dispersal patterns. Proceedings of the National Academy of Sciences of USA 104:3278–3282.

Kumar, S., G. Stecher, and K. Tamura. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870–1874.

Lavabre, J. E., L. J. Gilarranz, M. A. Fortuna, and J. Bascompte. 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 B Biological Sciences 371:20150280.

MacDougall, A. S., and S. D. Wilson. 2007. Herbivory limits recruitment in an old field seed addition experiment. Ecology 88:1105–1111.

Martínez-López, V., P. De la Rúa, V. M. Zapata, and F. Robledano. 2017. Ecological and genetic consequences of fragmentation in a semiarid Mediterranean urban forest. Urban Ecosystems 20:1161–1168.

Méndez, M., D. García, F. T. Maestre, and A. Escudero. 2008. More ecology is needed to restore Mediterranean ecosystems: a reply to Valladares and Gianoli. Restoration Ecology 16:210–216.

Mokotjomela, T. M., C. F. Musil, and K. J. Esler. 2016. An appraisal of seed enumeration and videographic techniques for determining seed removal rates by birds. African Journal of Ecology 54:281–288.

Navarro, L., and H. Pereira. 2015. Rewilding Abandoned Landscapes in Europe. Pages 3–23 *in* H. Pereira, and L. Navarro, editors. Rewilding European landscapes. Springer, Berlin, Germany.

Pausas, J. G., A. Bonet, F. T. Maestre, and A. Climent. 2006. The role of the perch effect on the nucleation process in Mediterranean semi-arid oldfields. Acta Oecologica 29:346–352.

Pigot, A. L., T. Bregman, C. Sheard, B. Daly, R. S. Etienne, and J. A. Tobias. 2016. Quantifying species contributions to ecosystem processes: a global assessment of functional trait and phylogenetic metrics across avian seed-dispersal networks. Proceedings of the Royal Society B 283:20161597.

Pons, P., B. Lambert, E. Rigolot, and R. Prodon. 2003. The effects of grassland management using fire on habitat occupancy and conservation of birds in a mosaic landscape. Biodiversity & Conservation 12:1843–1860.

Pugnaire, F. I., M. T. Luque, C. Armas, and L. Gutiérrez. 2006. Colonization processes in semiarid Mediterranean old-fields. Journal of Arid Environments 65:591–603.

R Development Core Team. 2016. R: a language an environment for statistical computing. R foundation for Statistical Computing, Vienna, Austria.

Ratnasingham, S., and P. D. Hebert. 2007. BOLD: the Barcode of Life Data System (http://www.barcodinglife.org). Molecular Ecology Resources 7:355–364.

Reid, J. L., and K. D. Holl. 2013. Arrival≠ survival. Restoration Ecology 21:153–155.

Rey, P. J., J. M. Alcántara, F. Sánchez-Lafuente, A. M. Garrido, J. L. Ramírez, and A. J. Manzaneda. 2004. Seedling establishment in *Olea europaea*: seed size and microhabitat affect growth and survival. Ecoscience 11:310–320.

Rey-Benayas, J. M., L. Martínez-Baroja, L. Pérez-Camacho, P. Villar-Salvador, and K. D. Holl. 2015. Predation and aridity slow down the spread of 21-year-old planted woodland islets in restored Mediterranean farmland. New Forests 46:841–853.

Robledano-Aymerich, F., A. Romero-Díaz, F. Belmonte-Serrato, V. M. Zapata-Pérez, C. Martínez-Hernández, and V. Martínez-López. 2014. Ecogeomorphological consequences of land abandonment in semiarid Mediterranean areas: integrated assessment of physical evolution and biodiversity. Agriculture, Ecosystems & Environment 197:222–242.

Romero-Díaz, A. (coord.). 2016. Abandono de cultivos en la Región de Murcia. Consecuencias ecogeomorfológicas. Universidad de Murcia, Servicio de Publicaciones (Editum), Murcia, Spain.

Romero-Díaz, A., J. D. Ruiz-Sinoga, F. Robledano-Aymerich, E. C. Brevik, and A. Cerdà. 2017. Ecosystem responses to land abandonment in Western Mediterranean Mountains. Catena 149:824–835.

Rounsevell, M. D. A., et al. 2006. A coherent set of future land use change scenarios for Europe. Agriculture, Ecosystems & Environment 114:57–68.

Santos, T., and J. Tellería. 1994. Influence of forest fragmentation on seed consumption and dispersal of Spanish juniper *Juniperus thurifera*. Biological Conservation 70:129–134.

Schröter, D., et al. 2005. Ecosystem service supply and vulnerability to global change in Europe. Science 310:1333–1337.

Tellería, J. L., A. Ramirez, and J. Pérez-Tris. 2008. Fruit tracking between sites and years by birds in Mediterranean wintering grounds. Ecography 31:381–388.

Tellería, J. L., A. Ramírez, and J. Pérez-Tris. 2005. Conservation of seed-dispersing migrant birds in Mediterranean habitats: shedding light on patterns to preserve processes. Biological Conservation 124:493–502.

Verdú, M., and P. García-Fayos. 1996. Nucleation processes in a Mediterranean bird-dispersed plant. Functional Ecology 10:275–280.

Verdú, M., and P. García-Fayos. 1998. Old-field colonization by Daphne gnidium: seedling distribution and spatial dependence at different scales. Journal of Vegetation Science 9:713–718.

Wheelwright, N. T. 1985. Fruit–size, gape width, and the diets of fruit–eating birds. Ecology 66:808–818.

Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2673/full