Role of avian seed dispersers in tree recruitment in woodland pastures

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Electronic Supplementary Material

Appendix A. Additional information on sampling methodologies

Classification of big trees

We classified under the category of "big tree" any individual tree simultaneously meeting two requirements:

A) Its top outgrew the average forest canopy by at least 1.5 m, this fact being visually assessed in the field. We considered that this height difference led these trees to act as milestones for frugivorous birds, as the forest canopy height is rather homogeneous in the study area (authors' personal observation; see Martínez and García 2015 for similar procedure).

B) The diameter of its crown was wider than 7 m, assessed from the orthophotomap. We considered that this diameter size differentiated an individual tree from the surrounding trees, as the mean tree crown diameter in the study plot was 3.88 ± 0.04 m (N = 2407 trees; authors' unpublished data).

Methodology of bird censuses

We performed bird censuses in the study plot to quantify the abundance of frugivorous birds (thrushes: *Turdus merula, T. iliacus, T. philomelos, T. pilaris, T. torquatus, T. viscivorus*) during the fruiting season. Direct observations of thrushes in different sampling cells were made from five different stations selected located at vantage positions in elevated outcrops, distributed along the central axis of the plot (Fig. A1). Observations were made from October to late January of each year, with a cumulative observation time of 105 and 156 h for 2009 and 2010, respectively. Observation time was allocated in a balanced number of 1-h observation periods between stations throughout each season. In each observation period, the observer, with the help of 8 × 30 binoculars, counted all thrushes seen (or heard) in the area surveyed. Bird sightings were assigned to the different geo-referenced sampling cells covered from each vantage position, with the help of printed maps. In some cases, the consecutive sightings of a given species could have corresponded to the same individuals remaining within, or successively entering a given cell. In these doubtful cases, we considered as independent those sightings separated by at least five minutes. Also, the sightings potentially corresponding to a given individual bird in different cells – or in the same cell on different days – were considered to be as valid as those from different individuals.

Due to the elevated location of vantage positions (ca. 70 m of elevation gradient) and the patchy and sparse structure of forest cover, a high visual and/or acoustic detectability of thrushes was achieved across almost the entire plot, even in those cells at a considerable distance away (ca. 200 m). However, due to the denser forest canopy and topographical features, bird detectability was lower in some of the easternmost cells of the plot (Fig. 1B in the main text) and therefore, we supplemented our observations in forest with point-count data for positions within the forest in these areas (see García and Martínez 2012; Martínez and García 2015 for similar procedure). Twelve forest point-count positions were established, each corresponding to the center of a group of four cells. Observations were made in 10 min periods, recording any thrush heard or seen within the four surrounding cells. Total observation time from each point-count was 110 and 195 min for 2009 and 2010, respectively. Rather than assessing the actual size of bird populations, our goal was to provide a measure of bird abundance in functional terms, i.e. an estimation of the total activity of frugivorous thrushes throughout the season in the study plot. For this, we calculated the abundance of birds per cell as the cumulative number of birds heard or seen in each cell through the season. We divided the cumulative number of birds by the total observation time for each cell, thereby calculating the number of birds per 10-h of observation. Weighting by total observation time per cell enabled the comparison of abundance between cells, correcting for overestimation in those cells observed from different positions and thus accounting for longer observation times, and also between years with different observation efforts.

Methodology for sampling the regeneration stages of fleshy-fruited trees

We assessed the availability of seeds in sampling stations across the whole plot in 2009 and 2010. Ten sampling stations, separated from each other by 2 meters, were placed along the central north-south axis of each cell in a subset of 220 cells following a chess-board design (Fig. 1C in the main text). In each station, we set a permanently labeled 50 × 50 cm quadrat on the ground, from which all fleshy fruits fallen from trees, and all fleshy-fruited tree seeds deposited by birds which were found were collected and counted in two consecutive surveys (late November and early January). We estimated the contribution of fallen fruits to seed availability by extrapolating the number of seeds inside fruits to the quadrat surface area. *Crataegus monogyna* fruits and *Taxus baccata* arils are single seeded. *Ilex aquifolium* fruits contain 2-4 seeds, thus, we considered three seeds as the average value. Bird-dispersed seeds are unequivocally identifiable:

they are clean of pulp remains, unlike seeds in fruits fallen beneath trees, and occur in small clusters easily distinguishable from those occurring in mammal feces, and they can be almost exclusively attributable to thrushes (Martínez and others 2008). We estimated the total number of seeds per sampling station as the sum of seed from fallen fruits and bird dispersed seeds, and further expressed this density as the number of seeds per square meter. Previous works had demonstrated that the removal of dispersed seeds or fallen fruits by predators like rodents from quadrats is low, especially in open microhabitats, and it mostly happens in winter, much later in the year than the monitoring dates used here (García and others 2005).

We assessed seedling emergence and establishment in sampling stations across the whole plot in 2011 and 2012. Five sampling stations were located in each of the chess-board cells (Fig. 1D in the main text). Seedling sampling stations, separated from each other by 4 meters, were located adjacent to seed availability quadrats. In each station, we set a permanently labeled 50 × 50 cm quadrat on the ground, and checked the emergence of all fleshy-fruited tree seedlings during spring (April-May). Emerged seedlings are distinguishable by the presence of cotyledons but no leaves, knots or lignifications of the stem. Seedlings of the three tree species studied were recognized by the different shape and coloration of the cotyledons and the coloration of the stem. For each sampling station, we individually distinguished each emerged seedling by positioning it within the sampling quadrat with x, y coordinates, mapping it on a drawing template. Survival of emerged seedlings was monitored monthly through spring and summer, until late August. We considered that a seedling was established if it continued to survive by the last summer survey, as preliminary sampling had shown that seedling mortality through the whole year is concentrated in high summer (authors' personal observation).

In each of the 220 chess-board cells, we surveyed the entire surface counting all saplings of the studied tree species. We considered as a sapling any pre-reproductive individual $\geq$ 10 cm tall or with a stem basal diameter $\geq$ 0.5 cm. For the oldest individuals, we verified the absence of flowers and fruits in the following spring and autumn respectively. As this sapling category included individuals of different ages, we performed a single year sampling to determine sapling density, in the summer of 2011. During that sampling we labeled 386 individuals equally distributed between microhabitats (covered, pasture and heather) and species (holly, hawthorn and yew), and covering the whole extent of the study plot. We checked the survival of labeled saplings in late summer (September) 2012. Sapling survival was very high, and only 11 individuals died. Mortality was not concentrated in any particular sector of the plot but rather distributed through its whole extent. Of the dead individuals, six appeared under tree cover, four in pastures and one in heather. Mortality was also widespread across species, affecting three holly, four hawthorn and four yew saplings. Based in these results, we considered that the magnitude and spatial template of sapling establishment represented a suitable surrogate of long-term tree recruitment.

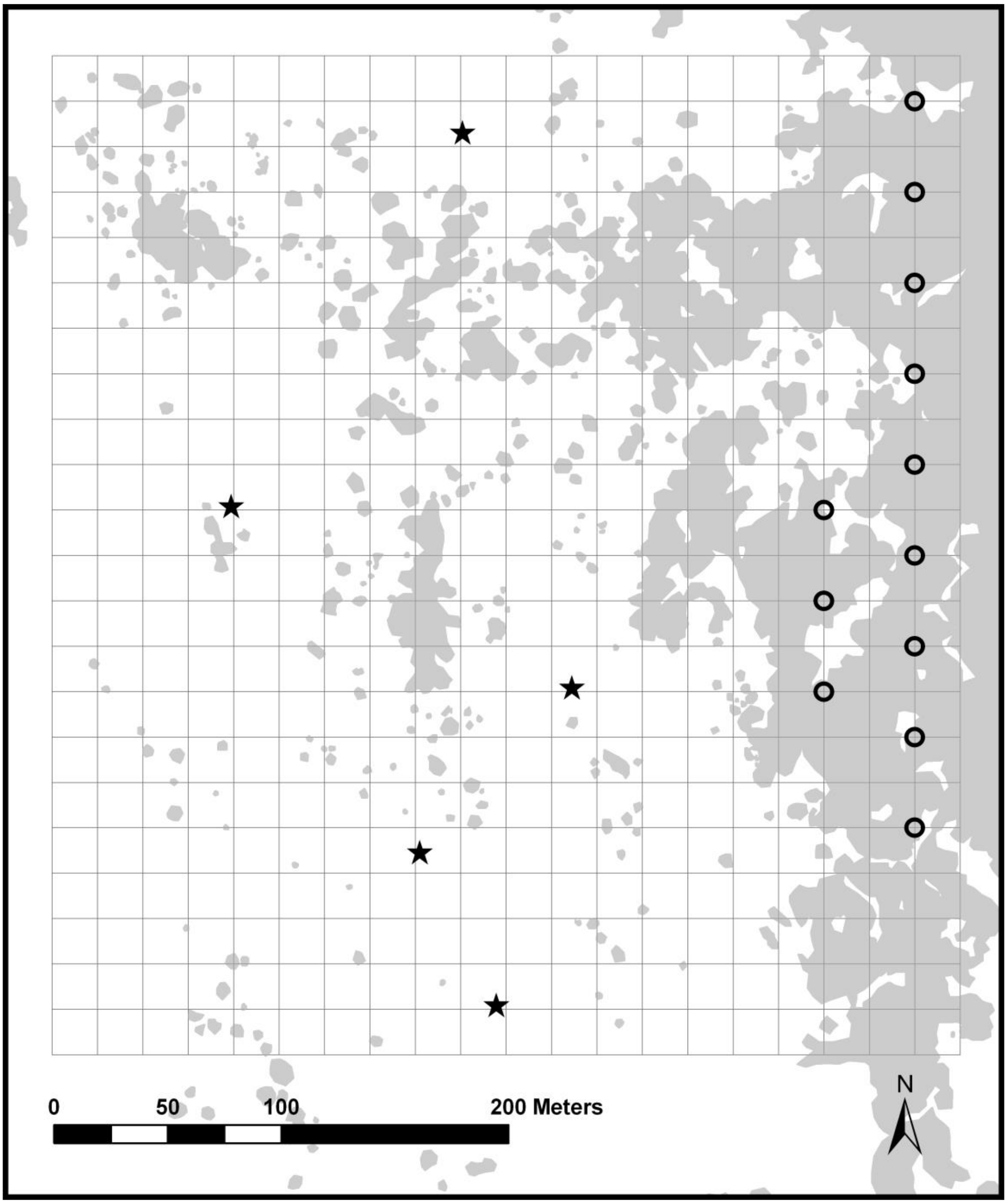

Figure A1. Map of the study plot, subdivided into 20 × 20 m cells, showing the extent of forest cover (gray area), and the vantage (black stars) and point-count (circles) positions for bird observation.

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Appendix B. Spatial Simultaneous Autoregressive models

As the sampling design of our study is based on different adjacent cells of the study plot (main text, Fig. 1), our analyses may be influenced by spatial trends existing in the studied variables (Legendre 1993). Because of this, the effects of habitat characteristics and bird abundance on the different tree regeneration stages may have been estimated incorrectly in the Structural Equation Models (SEM) due to the presence of spatial autocorrelation in the variables considered (Keitt and others 2002). Thus, to check for consequences of spatial constraints in these models, we fitted simultaneous autoregressive models (SARs; Keitt and others 2002). We considered that the partial regression coefficients provided by SAR models represented the direct effects of predictor variables on the different regeneration stages, free of autocorrelation constraints (see García and others 2010 for similar procedure). We performed independent SAR models for the different tree regeneration stages. In each model, we considered as predictor variables those included in the corresponding SEM and which might potentially be determining direct effects on the regeneration stage considered. SAR models were performed with SAM 3.0 software (Rangel and others 2006).

Table B1. Results of spatial simultaneous autoregressive (SAR) models verifying the effects of habitat characteristics and abundance of thrushes on the different tree regeneration stages (seeds, emerged seedlings, established seedlings and established saplings), for the forest regeneration process (N = 220 cells). In the case of density of seeds and emerged seedlings, independent SAR models were performed for each study year. The values of the partial regression coefficients, their standard errors, the values of the *t* statistic and *p* values are shown, together with the proportion of variance explained (R^2) for each model.

Seeds

2009				$R^2 = 0.41$
	Coefficient	Std. Error	t	*p*
Big trees	-78.914	48.344	-1.632	0.104
Forest cover	114.862	18.628	6.166	<0.001
Fruits	40.658	14.227	2.858	0.005
Thrushes	-3.131	12.385	-0.253	0.801

2010				$R^2 = 0.45$
	Coefficient	Std. Error	t	*p*
Big trees	-11.023	25.482	-0.433	0.666
Forest cover	47.850	9.568	5.001	<0.001
Fruits	24.001	7.006	3.426	<0.001
Thrushes	12.520	6.261	2.000	0.047

Emerged seedlings

2009				$R^2 = 0.58$
	Coefficient	Std. Error	t	*p*
Forest cover	1.184	0.627	1.889	0.060
Seeds	6.319	0.539	11.726	<0.001

2010				$R^2 = 0.39$
	Coefficient	Std. Error	t	*p*
Forest cover	3.116	0.887	3.511	<0.001
Seeds	4.340	0.753	5.762	<0.001

Established seedlings				$R^2 = 0.89$
	Coefficient	Std. Error	t	*p*
Forest cover	0.032	0.139	0.230	0.818
Scrub cover	0.051	0.102	0.498	0.619
Emerged seedlings	3.744	0.114	32.862	<0.001

Established saplings				$R^2 = 0.35$
	Coefficient	Std. Error	t	*p*
Forest cover	0.026	0.005	5.365	<0.001
Scrub cover	0.018	0.004	5.041	<0.001
Established seedlings	0.003	0.004	0.827	0.409

Table B2. Results of spatial simultaneous autoregressive (SAR) models verifying the effects of habitat characteristics and abundance of thrushes on tree regeneration stages (seeds, emerged seedlings, established seedlings and established saplings), for the forest recolonization process (N = 183 cells). In the case of density of seeds and emerged seedlings independent SAR models were performed for each study year. The values of the partial regression coefficients, their standard errors, the values of the *t* statistic and *p* values are shown, together with the proportion of variance explained (R^2) for each model.

Seeds

2009				$R^2 = 0.34$
	Coefficient	Std. Error	t	*p*
Forest cover	3.873	1.412	2.743	0.007
Fruits	2.608	1.281	2.035	0.043
Thrushes	3.361	1.037	3.362	0.001

2010				$R^2 = 0.40$
	Coefficient	Std. Error	t	*p*
Forest cover	-0.224	1.388	-0.161	0.872
Fruits	6.158	1.184	5.201	<0.001
Thrushes	4.798	1.032	4.650	<0.001

Emerged seedlings

2009				$R^2 = 0.25$
	Coefficient	Std. Error	t	*p*
Forest cover	-0.027	0.113	-0.235	0.815
Seeds	0.516	0.102	5.039	<0.001

2010				$R^2 = 0.10$
	Coefficient	Std. Error	t	*p*
Forest cover	3.116	0.887	3.511	<0.001
Seeds	4.340	0.753	5.762	<0.001

Established seedlings				$R^2 = 0.28$
	Coefficient	Std. Error	t	*p*
Forest cover	0.058	0.071	0.818	0.415
Scrub cover	-0.062	0.055	-1.119	0.265
Emerged seedlings	0.349	0.062	5.669	<0.001

Established saplings				$R^2 = 0.29$
	Coefficient	Std. Error	t	*p*
Forest cover	0.019	0.004	4.901	<0.001
Scrub cover	0.020	0.003	5.971	<0.001
Established seedlings	<0.001	0.003	0.106	0.916

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Appendix C. Direct, indirect and total effects estimated in Structural Equation Models

Table C1. Direct, indirect and total effects in the SEMs for the forest regeneration model. Direct effects are standardized path coefficients (see Fig. 4). Indirect effects are computed as the sum of products of the coefficients along all of the possible routes from the predictor to the response variable. Total effects are the sum of direct and indirect effects. A) Effects of habitat characteristics, abundance of thrushes and seed availability in the density of emerged seedlings, for 2009 and 2010 cohorts. B) Effects of habitat characteristics and the density of emerged seedlings on the densities of established seedlings and established saplings. All effects are significant at $p \leq 0.05$, except for the effect in gray ($p = 0.1$).

A) Seedling emergence model

		2009			2010		
Response	Predictor	Direct	Indirect	Total	Direct	Indirect	Total
Seeds	Big trees	-0.15	0.41	0.26	—	0.03	0.03
	Forest cover	0.57	0.12	0.69	0.46	0.21	0.67
	Fruits	0.19	—	0.19	0.25	—	0.25
	Thrushes	—	—	—	—	—	—
Emerged seedlings	Big trees	—	0.19	0.19	—	0.28	2.28
	Forest cover	0.09	0.48	0.57	0.24	0.29	0.53
	Fruits		0.13	0.13	—	0.11	0.11
	Thrushes	—	—	—	—	—	—
	Seeds	0.69	—	0.69	0.44	—	0.44

B) Sapling establishment model

Response	Predictor	Direct	Indirect	Total
Established seedlings	Big trees	—	0.30	0.30
	Forest cover	—	0.51	0.51
	Scrub cover	—	—	—
	Emerged seedlings	0.94	—	0.94
Established saplings	Big trees	—	0.26	0.26
	Forest cover	0.57	-0.13	0.44
	Scrub cover	0.34	—	0.34
	Emerged seedlings	—	—	—
	Established seedlings	—	—	—

Table C2. Direct, indirect and total effects in the SEMs for the forest recolonization model (containing only data from regeneration in open microhabitats). Direct effects are standardized path coefficients (see Fig. 5 in the main text). Indirect effects are computed as the sum of products of the coefficients along all of the possible routes from the predictor to the response variable. Total effects are the sum of direct and indirect effects. A) Effects of habitat characteristics, abundance of thrushes and seed availability on the density of emerged seedlings, for 2009 and 2010 cohorts. B) Effects of habitat characteristics and the density of emerged seedlings in the densities of established seedlings and established saplings. All effects are significant at $p \leq 0.05$.

A) Seedling emergence model

		2009			2010		
Response	Predictor	Direct	Indirect	Total	Direct	Indirect	Total
Seeds	Forest cover	0.30	0.27	0.57	—	0.33	0.33
	Fruits	0.20	0.05	0.25	0.39	—	0.39
	Thrushes	0.23	—	0.23	0.31	—	0.31
Emerged seedlings	Forest cover	—	0.24	0.24	—	0.09	0.09
	Fruits	—	0.11	0.11	—	0.10	0.10
	Thrushes	—	0.10	0.10	—	0.08	0.08
	Seeds	0.43	—	0.43	0.25	—	0.25

B) Sapling establishment model

Response	Predictor	Direct	Indirect	Total
Established seedlings	Forest cover	—	0.25	0.25
	Scrub cover	—	—	—
	Emerged seedlings	0.48	—	0.48
Established saplings	Forest cover	0.37	-0.11	0.26
	Scrub cover	0.44	—	0.44
	Emerged seedlings	—	—	—
	Established saplings	—	—	—