

# Incorporating seed fate into plant–frugivore networks increases interaction diversity across plant regeneration stages

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Plant–animal mutualistic interactions, such as pollination and seed dispersal, affect ecosystem functioning by driving plant population dynamics. However, little is known of how the diversity of interactions in these mutualistic networks determines plant regeneration dynamics. To fill this gap, interaction networks should not only account for the number of seeds dispersed by animals, but also for seed fate after dispersal. Here, we compare plant–animal networks at both the seed dispersal and seedling recruitment stage to evaluate how interaction diversity, represented by different network metrics, changes throughout the process of plant regeneration. We focused on a system with six species of frugivorous birds and three species of fleshy-fruited trees in the temperate secondary forest of the Cantabrian Range (northern Iberian Peninsula). We considered two plant cohorts corresponding to two fruiting years showing strong differences in fruit and frugivore abundance. Seed dispersal interactions were estimated from a spatially-explicit, field-validated model predicting tree and bird species-specific seed deposition in different microhabitats. These interactions were further transformed into interactions at the seedling recruitment stage by accounting for plant- and microhabitat-specific seed fates estimated from field sampling. We found that network interaction diversity varied across plant regeneration stages and cohorts, both in terms of the evenness and the number of paired interactions. Tree–bird interactions were more evenly distributed across species pairs at the recruitment stage than at the seed deposition stage, although some interactions disappeared in the seed-to-seedling transition for one plant cohort. The variations in interaction diversity were explained by between-plant differences in post-dispersal seed fate and in inter-annual fruit production, rather than by differences between frugivores in seed deposition patterns. These results highlight the need for integrating plant traits and disperser quality to predict the functional outcome of plant–animal mutualistic networks.

Plant–animal mutualistic networks (e.g. flowering plants and pollinators; fruiting plants and frugivores providing seed dispersal) are assumed to affect ecosystem functioning by controlling vegetation dynamics (Blüthgen and Klein 2011, Schleuning et al. 2015). Despite the recent interest in the structure of such networks (Bascompte and Jordano 2007, Schleuning et al. 2012), we are still far from understanding their functional effects. This is because the quantitative descriptions provided by interaction frequencies (e.g. number of pollen grains or seeds transported by the animals) may tell little about the concomitant demographic expectancies of animals and plants (but see Vázquez et al. 2007, 2012). This constraint is especially challenging in plant–frugivore networks, where post-dispersal seed fate filters any quantitative effect of frugivores on plants (Wang and Smith 2002, Schupp et al. 2010). Seed fate may be under the control of animals, and frugivore species may differ in their quality as dispersers because, for example, they differentially modify seed germination due to gut passage, or they drop seeds in microhabitats with different conditions for seed and seedling survival (Schupp et al. 2010, Mello et al. 2015).

In fact, these qualitative differences may actually equalize the role of frugivores in interactions networks, for example, when a rare disperser species drops seeds in microhabitats optimal for plant recruitment (Carlo and Yang 2011, Schleuning et al. 2015). Nevertheless, the variability in plant traits affecting seed fate may also drive the functional prominence of plants in networks. For example, plants consumed in small quantities by frugivores but with high probabilities of survival after dispersal (e.g. due to their seed size, low susceptibility to predators or shade tolerance; García et al. 2005a, Xiao et al. 2015), will be more represented in networks, irrespective of the disperser. In sum, we could expect changes in the frequencies of each frugivore and plant species from seed dispersal to recruitment stages when accounting for seed fate which, in turn, could be determined by both disperser quality and plant traits. When seen as a whole, for example by means of interaction–diversity network metrics (Plein et al. 2013, Chama et al. 2013), these global changes in interaction frequencies may be highly informative regarding the final distribution of the effects of frugivores on plant communities. Nevertheless, the prevalence and the

mechanisms of changes in interaction diversity across plant regeneration stages remain empirically undemonstrated (but see Schleuning et al. 2015 for a conceptual model).

Here, we focus on the plant–frugivore system composed of three fleshy-fruited tree species and six avian seed dispersers in the temperate forest of the Cantabrian Range (northern Iberian Peninsula). This assemblage is suitable for evaluating changes in seed-dispersal networks because the plants differ in their post-dispersal seed fate (e.g. susceptibility to seed predators, García et al. 2005a), and dispersers are expected to differ in quality proved that they differed in spatial behaviors (García et al. 2013, Morales et al. 2013). Our general objective was to assess the global patterns of plant recruitment by evaluating the structure of plant–frugivore networks after incorporating plant demography (i.e. seed and seedling fate). We first estimated the seed deposition of fleshy-fruited trees by bird species, in different microhabitats, as predicted by a spatially-explicit, field-validated mechanistic model. We assumed that the quality of seed dispersers was mostly determined by the deposition microhabitat. Then, we transformed seed deposition into seedling recruitment by accounting for species and microhabitat-specific seed fate, estimated from field sampling. Specifically, we compared the structure of networks across the seed dispersal and the seedling recruitment stages, by means of metrics representing interaction diversity. Given that the study system can show strong inter-annual differences in the abundance of both birds and fruits (García et al. 2013), we also compare the networks from two years, representing two different plant demographic cohorts, as well as two landscape scenarios of fruit availability.

## Material and methods

### Study system

This study is focused on a plant–frugivore system composed of fleshy-fruited trees and birds in the temperate secondary forest of the Cantabrian Range (northern Iberian Peninsula). This is a common habitat showing low cover and a high degree of fragmentation due to anthropic pressure (García et al. 2005b). It is dominated by hawthorn *Crataegus monogyna*, holly *Ilex aquifolium* and yew *Taxus baccata*, which are the tree species selected for this study. Their fruits are sugar-rich red berries (arillated seeds in the case of yew); they present similar morphology, size and coloring and contain either a single seed (hawthorn, yew) or 1–4 seeds (holly). The three tree species ripe in autumn (September to November).

The main seed dispersers of these trees are thrushes: black-bird *Turdus merula*, song thrush *T. philomelos*, mistle thrush *T. viscivorus*, fieldfare *T. pilaris*, redwing *T. iliacus* and ring-ouzel *T. torquatus*. All these thrushes are mostly frugivores during fall and winter, and show a non-selective diet (i.e. the consumption of the fruiting species is proportional to the yearly abundance; García et al. 2013). All thrushes have similar fruit-handling behavior, swallowing the entire fruits after picking them and expelling the intact seeds in their feces. Although some size-based differences in gut retention time are expected between species of thrushes, we assumed these differences to be negligible in terms of effects on seed germination ability. Conversely, each thrush species may

vary in their response to landscape structure, a fact that leads to complementary spatial patterns of seed dispersal (Morales et al. 2013).

Previous studies in the same system have revealed that most seeds of the study species are deposited beneath tree canopies, with few reaching uncovered, open areas (García et al. 2005c). Post-dispersal seed predation by rodents (*Apodemus* spp.) varies markedly between microhabitats (under trees >> open areas; García et al. 2005c), and between tree species (*T. baccata* > *I. aquifolium* > *C. monogyna*; García et al. 2005a). Germination of dispersed seeds occurs after 18 months, i.e. in the second spring after seed dispersal, with slight differences between tree species and between microhabitat (Supplementary material Appendix 1 Fig. A3). Seedlings suffer high mortality after emergence due to grazing and trampling by ungulates, but survival increases when seedlings grow under nurse woody plants (García and Obeso 2003, Martínez 2014).

### Study area and field sampling

Field sampling was conducted in the Sierra de Peña Mayor (43°18'00"N, 5°30'29"W, 1000 m a.s.l., Asturias, Spain) where secondary forests occur as edging patches next to deciduous forests of beech *Fagus sylvatica* or as variable-sized fragments (from remnant trees to areas of several hectares) interspersed with a historically deforested matrix of pastures and heathland (Herrera et al. 2011). A rectangular plot of 400 × 440 m (17.6 ha) was set up, in order to cover a gradient of forest loss, from dense forest patches to isolated trees scattered through pastures, so the plot was subdivided into 440 sampling cells of 20 × 20 m (Supplementary material Appendix 1 Fig. A1.B). Likewise, a geographical information system (GIS, ArcGIS ver. 9.3) was developed in order to estimate the percentage of tree cover per cell (in m<sup>2</sup>) by incorporating a grid and a digitized forest cover layer. Additionally, in October 2009 and 2010 we estimated, in the field, the position of all individual trees and the fruit crop of each individual tree of the studied fleshy-fruited species within each plot cell (see Supplementary material Appendix 1 for methodological details). For each year, we incorporated the data on fruit production into the GIS platform in order to quantify the number of trees and the total fruit production per cell.

From October to February of 2008–2009, 2009–2010 and 2010–2011, we studied the spatially-explicit foraging patterns of each thrush species, quantifying their movements, flight distances and perching habitats, as well as the number of fruits consumed from each tree species. Data collection was based on direct observation sequences of individual birds, made from elevated positions located along the central axis of the plot. During field surveys, we also recorded the presence of individual birds across the plot cells, in order to provide a measure of bird species abundance (Supplementary material Appendix 1).

In fall-winter 2009–2010 and 2010–2011 (2009 and 2010 hereafter) we assessed seed deposition by birds in a subset of 220 cells following a checkered pattern (Supplementary material Appendix 1 Fig. A1.C). Within each cell, and in two separate surveys (November and January) of each sampling season, we counted the number of seeds

of fleshy-fruited trees found in bird feces in ten sampling stations (open-ground 50 × 50 cm quadrats) each separated from the others by 2 m (Supplementary material Appendix 1 Fig. A1.C). We assigned each seed sampling station to one of the following five microhabitats depending on the type of fine-scale cover: (a) beneath *C. monogyna*, (b) beneath *I. aquifolium*, (c) beneath *T. baccata*, (d) beneath non-fleshy-fruited trees (e.g. *Corylus avellana*) and (e) in the open (i.e. uncovered by tree canopy, e.g. pastures). For each year, in each sampling station, we calculated the number of dispersed seeds per tree species as the sum of seeds found in the two consecutive surveys.

From April to late August of 2011 and 2012, in the subset of cells for measuring seed deposition, we recorded the number of emerged seedlings of the tree species under study. For each cell, we established five seedling sampling stations (open-ground 50 × 50 cm quadrats), separated from each other by four meters but adjacent to a seed sampling station (Supplementary material Appendix 1 Fig. A1.D). All emerged seedlings were specifically and individually identified, and their survival was monitored monthly throughout the season. We considered a seedling to be established when it survived to the end of the summer, as previous surveys had revealed that the summer period was when seedling mortality was highest (Martínez 2014). The seed dormancy period of all three fleshy-fruited trees lasts 18 months. Thus, we assigned the seedlings emerging in 2010 and in 2011 to the cohorts of seeds dispersed in 2009 and in 2010, respectively. Comprehensive details of field data collection are shown in Supplementary material Appendix 1.

### Seed-dispersal interaction matrices at two regeneration stages

Our analytical goal was to compare the structure of plant-seed disperser (tree–bird) networks across two stages of plant regeneration. This requires estimating quantitative matrices of paired tree–bird interactions at seed deposition and seedling recruitment. Thus, interactions should be based on determining which species of bird was likely to have, respectively, deposited a given seed, and have recruited a given seedling. Due to the methodological constraints in obtaining this kind of information in the field (González-Varo et al. 2014), we opted for an approach based on three principal steps (Fig. 1): 1) estimation of tree–bird and tree–microhabitat matrices of seed deposition (Fig. 1D), based on a mechanistic model of seed dispersal (Fig. 1B); 2) validation of the simulated seed dispersal patterns with field data on the tree-specific seed distributions between microhabitats (Fig. 1D and Fig. 1A); and 3) estimation of the tree–bird matrices of seedling recruitment from the simulated seed deposition matrices (Fig. 1E), taking into account microhabitat-dependent seed fates quantified from field surveys (Fig. 1C).

### Seed deposition matrices from a mechanistic model of seed dispersal

We implemented a model that predicts, through stochastic simulations, the deposition of seeds of different species of trees by birds according to mechanistic rules. These rules combined mathematical functions representing the performance of bird species depending on the movement and foraging

behavior of each species under a realistic scenario (that of our study site and period). The values of model parameters that determined the shape of the functions varied between species, and were estimated from field data of both the relative abundances of fruits and birds and the foraging activity of bird species. The model used here expands the previous versions developed by Morales and Carlo (2006), Carlo and Morales (2008), and Morales et al. (2013), in the sense that it now predicts seed deposition in the five microhabitats distinguished in our field study (i.e. beneath *C. monogyna*, *I. aquifolium*, *T. baccata* and non-fleshy-fruited tree species, and in the open). This therefore enabled us to incorporate a quality component to each seed dispersal event, as seed fate is expected to be mostly driven by microhabitat features. A detailed description of the structure of the model and functions fitted is presented in Supplementary material Appendix 2 (see also Morales et al. 2013).

Basically, the model simulated individual bird tracks (i.e. the displacement of an individual bird, able to consume fruits and expel seeds while moving) within a grid-based, modeling landscape that replicates the spatial extent and the environmental variability of our 440-cells study plot. Globally, the path of tracks varied depending on bird response to landscape heterogeneity (measured by forest cover and fruit abundance); the outcome of tracks (in terms of fruit consumption and microhabitat-dependent seed deposition) depended on which fruiting species were encountered by birds, gut retention time, and microhabitat-dependent perching probabilities (Supplementary material Appendix 2 Fig. A4). The movement of a bird from one cell to another was predicted by a combination of functions that took into account (Fig. 1A): a) the distance to the cell where the movement starts, b) the proportion of forest cover in the destination cell, c) the number of fruits in the destination cell, and d) the distance to the edge of the plot (which allowed birds to leave the modeling landscape). The consumption of fruits by a given bird along a track depended on e) fruit availability in the cell (updated after each track and fruit removal). Gut retention time depended on f) the body size of each bird species. Finally, the probability of seed deposition in a given microhabitat within a cell depended on the destination perch, a combined function of: g) the number of fruits of each tree species in that cell, h) the number of trees of each species in that cell, and i) the species of the ingested seed (as the probability of deposition beneath a conspecific perching tree has been demonstrated to be higher than beneath other tree species; García et al. 2007). We fitted seed deposition probability in the open microhabitat according to the proportion of forested area within each cell.

We obtained each model output (i.e. seed dispersal data) as a spatially-explicit (cell- and microhabitat-based) prediction of seed deposition for each tree species and by each bird species, that is, a multi-specific seed rain across the modeled landscape (Fig. 1B). Each model output was the result of a simulation accounting for 5000 bird tracks, and the simulations were replicated 30 times (i.e. 30 independent model outputs), for each of the two year scenarios (2009 and 2010). These year scenarios accounted for the field-based values of fruit availability and abundance of bird species in the respective years. We finally selected the seed deposition output corresponding to a subset of the 220 cells of the modeling

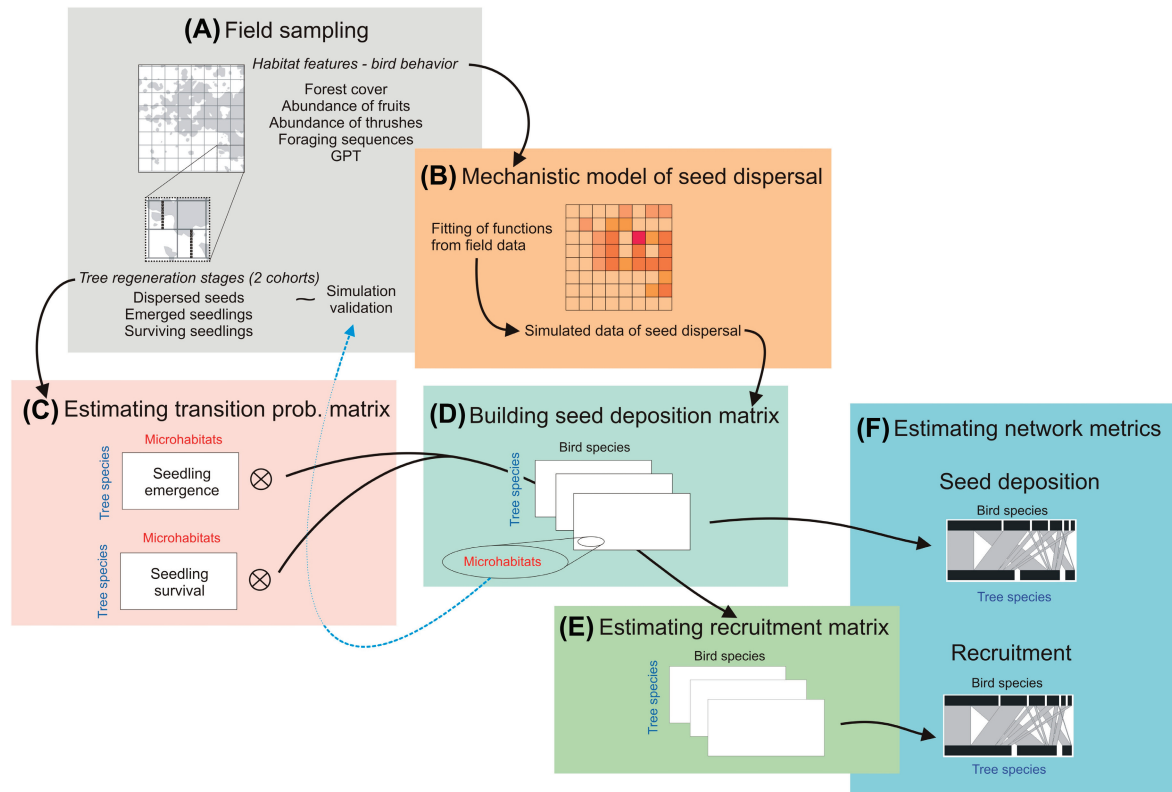


Figure 1. Flow diagram representing the overall procedure to obtain networks of the two plant regeneration stages (i.e. seed deposition and seedling recruitment). Each step is represented by different colors and how the information is used across these steps is specified by black arrows (but see the blue dashed line arrow for the model validation procedure). (A) Field data sampling took place during two years (2009–2010 and 2010–2011) representing two plant cohorts. (B) This information was used to parameterize the movement and foraging behavior rules of a spatially-explicit mechanistic model. (C) Empirical data on tree regeneration stages was used to estimate transition probability matrices of seedling emergence and seedling survival with different microhabitats as columns (in red) and tree species as rows (in blue). (D) We estimated the seed deposition matrices given the simulated data of the number of seeds dispersed of each plant species (as rows, in blue) by each bird species (as columns, in black) in each of the five microhabitats (in red), coming from the mechanistic model after 30 replicates. Afterwards, we built the seed deposition matrices by pooling the seeds of each plant species dispersed by each bird species across microhabitats. For each cohort, the mean simulated seed deposition matrix was validated with those obtained from field data each year (dashed line arrow). (E) Then, seed deposition matrices were transformed into seedling recruitment by taking into account tree- and microhabitat-specific seed fate (i.e. emergence and survival probabilities) estimated from field sampling and pooling again the seeds of each plant species across microhabitats so as to get the recruitment matrices with bird species as columns (in black), and tree species as rows (in blue). (F) Finally, we compared the structure of seed deposition and seedling recruitment networks by calculating global metrics.

landscape in equivalent positions to those containing seed deposition and seedling establishment sampling stations in the field (Fig. 1C; Supplementary material Appendix 1 Fig. A1C).

The data of each seed deposition output, accounting for tree–bird and tree–microhabitat specific information, were pooled across microhabitats. In this way, we obtained a seed deposition matrix that accounted for the number of seeds of each tree species that were dispersed by each bird species. For each year scenario, we thus obtained 30 matrices of simulated seed deposition (Fig. 1D).

#### Validation of model-predicted seed deposition

In order to validate the seed deposition patterns predicted by the mechanistic model, we first re-organized the data of seed deposition outputs by pooling the data from all six species of birds. That is, we generated 30 matrices for each study year, with the tree species as rows, the microhabitats as columns and the number of deposited seeds as matrix-cell

counts (Fig. 1D). Each year, we then calculated a single simulated seed deposition matrix (the average of the 30 replicates) which was correlated, by means of a Mantel test, with a matrix obtained from seed deposition field data for the corresponding year and using the same tree–microhabitat structure (i.e. the total number of seeds of each tree species collected in each microhabitat; Fig. 1A). We performed the Mantel test using the *ecodist* library in R ver. 3.0.2 (<[www.r-project.org](http://www.r-project.org)>).

#### Seedling recruitment matrices: incorporating seed fate into simulated seed deposition

Based on the simulated seed deposition raw outputs (that is, those accounting for microhabitat, tree and bird species), we calculated seedling recruitment matrices as the number of established seedlings attributable to each tree and bird species in each microhabitat (Fig. 1E). For each year (i.e. seeds corresponding to the same yearly fruiting cohorts), we multiplied each simulated seed deposition output by two



matrices of transition probabilities: a) a seedling emergence rate (i.e. the proportion of deposited seeds from which a seedling emerged after an 18-months post-dispersal period), and b) a seedling survival rate (i.e. the proportion of emerged seedlings which survived to the end of the summer season). All transition probabilities were estimated from field data for each tree species, microhabitat and year (seed cohort). Namely, seedling emergence of a given tree species in a given microhabitat was calculated by matching the total number of emerged seedlings of that species, in the sampling stations of that microhabitat, with the total number of seeds of the corresponding cohort deposited in the adjacent seed sampling stations (Supplementary material Appendix 1 Fig. A1.C). Seedling survival was also calculated in each microhabitat, for each species and year, by dividing the total number of established seedlings in the sampling stations of a given microhabitat by the total number of emerged seedlings in those stations.

Similar to the simulated seed deposition matrices, we further re-organized seedling recruitment matrices by pooling the number of established seedlings across microhabitats in order to produce matrices with tree species as rows, birds species as columns, and the number of established seedlings as cell counts, for each year (Fig. 1E).

## Network analyses

A quantitative network approach was used to evaluate the structure of interactions between fleshy-fruited trees and frugivorous birds, considering separately the regeneration stages of seed deposition and seedling recruitment and two cohorts (2009 and 2010; Fig. 1). For each cohort, we applied network analyses to the 30 replicates of our simulated seed deposition and seed recruitment matrices.

As would be suggested by previous conceptual models (Carlo and Yang 2011, Schleuning et al. 2015), we were expecting that incorporating seed fate into plant–frugivore networks would lead to changes in the relative frequencies (interaction weights) and the number of paired interactions (links) within the network. In view of this, we exclusively focused on two complementary metrics representing different aspects of the diversity of interactions in the global network: interaction evenness and linkage density. Interaction evenness is calculated from the Shannon's evenness index. It is a measure of the heterogeneity of interaction frequencies in the whole network (e.g. a more heterogeneous network is expected when few strong tree–bird interactions dominate seed deposition or seedling recruitment). In other words, it provides additional information about the relative allocation of the contributions of all the frugivores for seed dispersal and seedling recruitment. It ranges from 0 (uneven networks) to 1 (uniform network) and the change in this metric would reflect changes in the distribution of interaction weights in the whole network, even with no modifications in the number of interacting species. Linkage density is a measure of the mean number of links per species, weighted by the number of interactions. Thus, it reflects the average richness of links per species at the global network level, and its variability quantifies interaction gains or losses. In weighted networks, changes in this metric also represent the variability in the distribution of interaction weights within specific

species. For more detailed definitions of the parameters used see Dormann et al. (2009).

These two topological parameters were calculated using the 'networklevel' function from the 'bipartite' package (ver. 2.05, Dormann et al. 2009). Likewise, network graphs were represented with the 'plotweb' function. Finally, we compared the values of network metrics between years for both seed deposition and recruitment by means of two sample t-tests, and between regeneration stages from a given cohort, by means of paired t-tests. All statistical analyses were performed in the R statistical software ver. 3.0.2.

## Results

### Overview of field results

Field sampling evidenced strong inter-annual variability in the total abundance of fruits, seeds and seedlings of tree species from 2009 to 2010, as well as of the species of birds (Fig. 2). All six species of birds were observed in both study years, but *Turdus pilaris* and *T. torquatus* accounted for less than 2% of bird observations in each year. However, inter-annual variability was found for the remaining bird species. Namely, *T. iliacus* was the dominant bird in 2009, while *T. philomelos* showed the highest relative abundance in 2010 (Fig. 2A). *T. merula* and *T. viscivorus* always showed intermediate values of relative abundance.

The total abundance of fleshy fruits increased from 2009 to 2010 (Fig. 2B). While in 2009 *Ilex aquifolium* was the dominant species with almost 84% of the total fruit crop, in 2010 it was *Crataegus monogyna* with 65%. Moreover, *Taxus baccata* accounted for less than 10% of fruits in both years. Hence, the fruiting landscape changed between years (Supplementary material Appendix 1 Fig. A2) as a result of the differences in the relative abundance of species between years and their spatial distribution.

As regards seed deposition, *I. aquifolium* was always the most abundant species, even in 2010, when the higher number of seeds per fruit partially compensated for its lower fruit production, compared with *C. monogyna* (Fig. 2C).

Concerning dispersed seeds across microhabitats, both years more than 70% of *I. aquifolium* seeds were deposited beneath conspecific trees, whereas more than 45% of *C. monogyna* seeds were dropped beneath heterospecific, fleshy-fruited trees (Supplementary material Appendix 3 Table A3). For *T. baccata*, conspecific canopy received the largest proportion of seeds. The percentage of seeds found in open areas was always lower than 12%, with *C. monogyna* being the species with most seeds reaching this microhabitat.

The relative abundance of emerged and surviving seedlings was always higher for *I. aquifolium*, most notably in the 2009 cohort (Fig. 2D–E). Nevertheless, *C. monogyna* showed higher relative abundances of emerged and surviving seedlings than expected from its relative abundances at seed deposition. Indeed, in both years the latter was the species with the highest seedling emergence rates in all microhabitats, especially in open areas (Supplementary material Appendix 3 Table A4). Seedling survival rates were lower for *C. monogyna* than for *I. aquifolium* or *T. baccata*

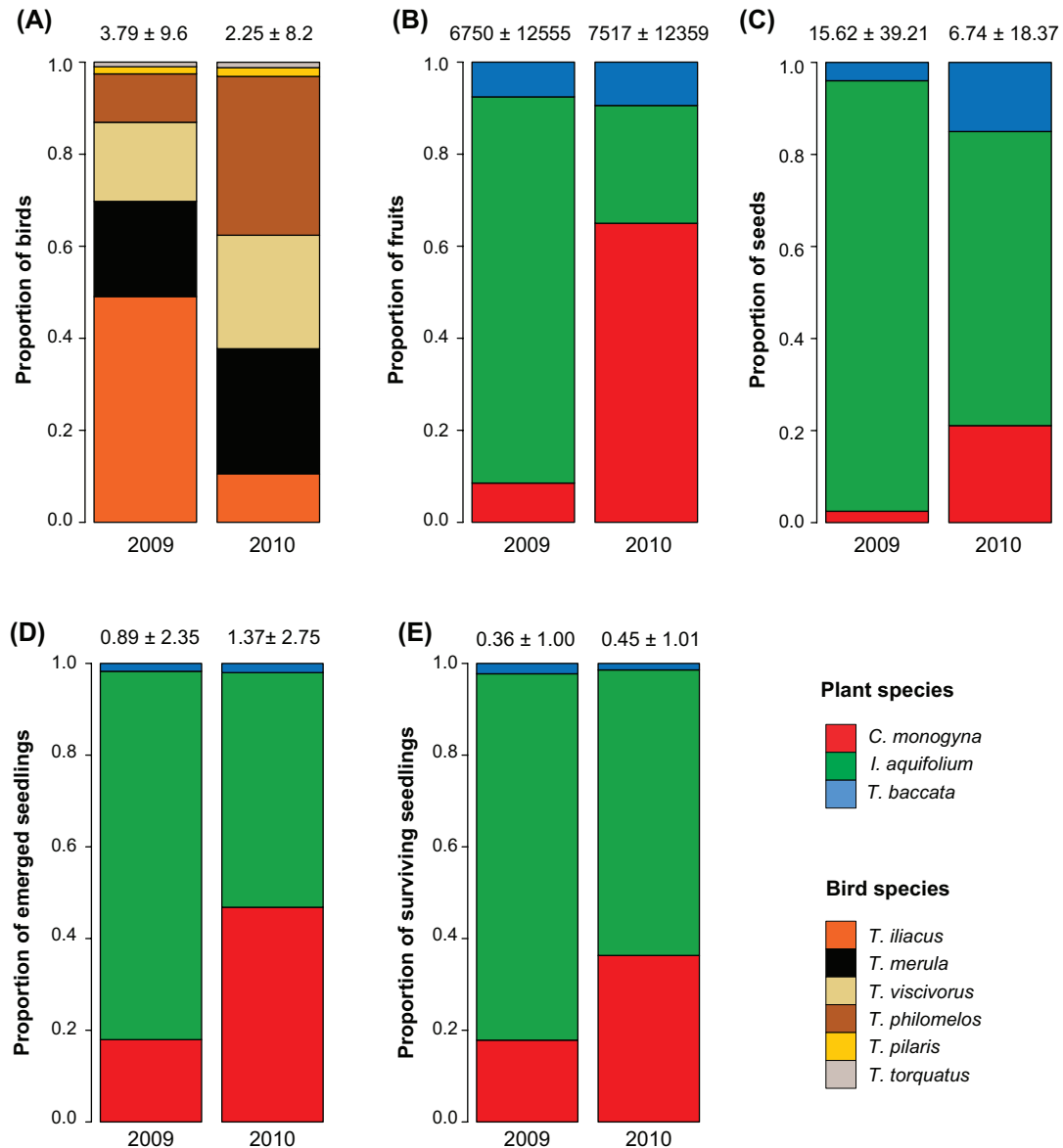


Figure 2. (A) Abundances of bird species relative to total bird abundance, (B) proportions of fruits of each tree species, (C) proportion of seeds of each tree species with respect to the total seed rain collected, (D) proportions of emerged seedlings of each tree species, and (E) proportions of surviving seedlings of each tree species, for two years (plant demographic cohorts). Above the bar of each year: mean  $\pm$  standard deviation of (A) birds per 10 h per cell; (B) fruits per cell; (C) seeds per sampling station per cell; (D) emerged seedlings per sampling station per cell; and (E) surviving seedlings per sampling station per cell (A–B:  $n = 440$  cells; C–E:  $n = 220$  cells).

beneath cover microhabitats, but the reverse occurred in open areas.

### Seed deposition model prediction and validation

Simulations showed that seeds of all three tree species were mainly dispersed beneath their conspecifics, and the percentage of seeds found in open areas was always lower than 12% for all tree species and years with *C. monogyna* being, comparatively, the species with the highest numbers of seeds arriving in this kind of microhabitat (Supplementary material Appendix 3 Table A6). In both years, these simulation results agreed with field data, as suggested by the positive and significant correlations between the observed and

the simulated proportion of each species of seeds found in each microhabitat (Mantel test:  $r \geq 0.889$ ,  $p \leq 0.015$ , for both years; Supplementary material Appendix 3 Table A3, A6). Our mechanistic model was, thus, able to explain a high proportion of the observed variability in seed dispersal.

Most bird species (particularly *T. iliacus*) dispersed the majority of the simulated seeds beneath canopies of fleshy-fruited tree species, but *T. viscivorus* and *T. pilaris* displaced a comparatively higher proportion of seeds into open areas (Supplementary material Appendix 3 Table A8). These differences between bird species were accentuated in the transition from seed deposition to seedling recruitment (Supplementary material Appendix 3 Table A9).

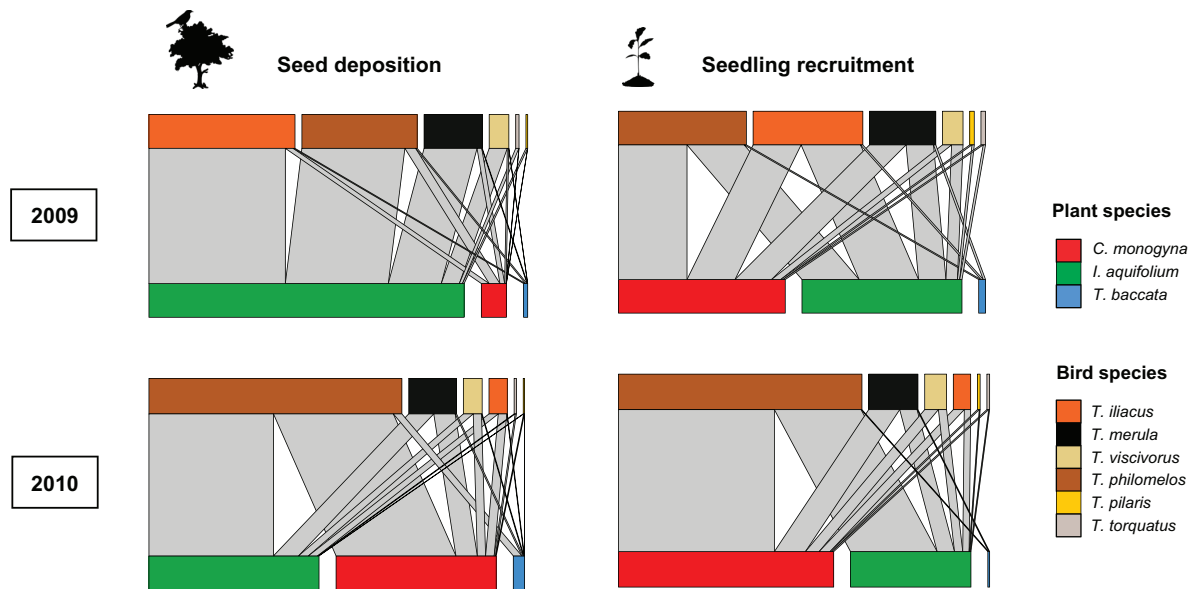


Figure 3. Bipartite graphs representing the interaction networks between species of birds and trees at different tree regeneration stages (left: seed deposition; right: seedling recruitment) and years (2009 and 2010 seed-to-seedling cohorts). They represent the proportion of dispersed seeds and recruited seedlings of fleshy-fruited trees (bottom rows), those dispersed or recruited by birds (top rows) and the proportion of dispersed seeds or recruited seedlings per tree and bird (gray links).

### Interaction networks for different regeneration stages and years

Bipartite graphs revealed that the interaction frequencies of the six birds and the three trees changed between regeneration stages and years. In 2009, *T. iliacus*, together with *T. philomelos* and *T. merula*, accounted for 93% of seed deposition interactions and 89% of seedling recruitment, whereas, it was *T. philomelos* and *T. merula* that dominated both networks in 2010. With respect to trees, the interaction frequency of *C. monogyna* increased from seed deposition to recruitment networks, especially in 2009 (Fig. 3).

Regarding the network metrics, we found a significant increase in interaction evenness from seed deposition to seedling recruitment for both cohorts (Fig. 4), in 2009

(paired- $t = 21.49$ ;  $p < 0.0001$ ) and in 2010 (paired- $t = 3.37$ ;  $p = 0.002$ ). That is, in both cases the homogeneity of interaction weights within the whole network was higher at the seedling recruitment stage than at the seed deposition one, especially in the cohort of 2009. Similarly, linkage density increased from seed deposition to the recruitment stage in the 2009 cohort (paired- $t = 19.43$ ;  $p < 0.0001$ ). This latter structural change was probably related to a modification in the distribution of the interaction weights when looking at the specific species, namely the decrease of dominance of *I. aquifolium* within the main bird species (*T. iliacus*, *T. philomelos*, Fig. 3). However, we found an opposite trend for the 2010 cohort, with a decrease in the linkage density across regeneration stages (paired- $t = -8.66$ ;  $p < 0.0001$ ). The increase in the dominant role of *C. monogyna* from seed

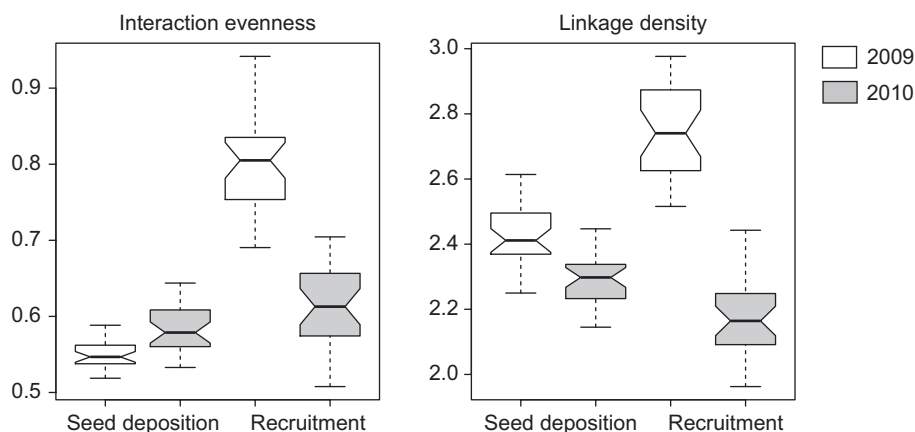


Figure 4. Boxplots representing the distribution of values of two network metrics (interaction evenness and linkage density) corresponding to interaction matrices ( $n = 30$ ) for different tree regeneration stages (seed deposition, seedling recruitment) and cohorts (2009, 2010). Bottom and top of boxes correspond to lower and upper quartiles respectively; notches indicate the 95% confidence intervals around the median (black band). Note that the y-axis varies between indices.

deposition to recruitment and, more importantly, the loss of interactions within the networks, such as the ones between three species of birds when recruiting *T. baccata* probably underpinned the decrease in linkage density (Fig. 3).

Inter-annual differences between networks corresponding to a given regeneration stage were also found (Fig. 4). The distribution of interactions for seed deposition networks was more homogeneous in 2010 than in 2009 (interaction evenness;  $t = 4.78$ ;  $p < 0.0001$ ), apparently due to the decreased dominance of *I. aquifolium* (Fig. 3). At the same time, and probably derived from the increased weighting of *T. philomelos* and *C. monogyna*, the whole recruitment networks presented the opposite trend, being less even in 2010 than in 2009 ( $t = -12.46$ ;  $p < 0.0001$ ). However, the values of linkage density decreased between cohorts for both regeneration stages (seed deposition:  $t = -5.75$ ;  $p < 0.0001$ ; recruitment:  $t = -18.71$ ;  $p < 0.0001$ ), also probably due to the increase in the dominance of a few birds within the main tree species (*I. aquifolium* and *C. monogyna*).

## Discussion

Plant–seed disperser networks have been widely explored in previous studies (Donatti et al. 2011, Mello et al. 2015) in order to identify the topological generalities of these ecological assemblages as well as the consequences of these generalities in terms of stability or evolutionary potential. These studies have usually represented the complexity of plant–frugivore interactions only at the beginning of the plant regeneration process, being blind to the final functional effect of these interactions (Carlo and Yang 2011). In this study we overcome this partial view of interaction diversity in plant–animal assemblages, by incorporating seed fate into simulated plant–seed disperser networks. By using a mechanistic model parameterized with field data, we were able to estimate frugivore-specific seed deposition in different microhabitats. Seed dispersal interactions were later translated into interactions between frugivores and plants at the seedling stage by accounting for field-measured, microhabitat-dependent recruitment expectancies. Overall, we show that the diversity of interactions may increase across plant regeneration stages, and also change between successive plant cohorts.

### Variability in interaction diversity across plant regeneration stages

Here, we took into account the transition between plant regeneration stages, seen as the result of filtering agents (e.g. frugivores, seed predators, abiotic factors causing seedling mortality; Zamora and Matías 2014) operating on successive demographic processes (fruit removal and seed dispersal, seedling establishment; Wang and Smith 2002). We assumed that these filters may modify the global distribution of interaction frequencies between plant and bird species from the stage of seed dispersal to that of seedling recruitment, and thus that these distribution changes are well represented by network metrics related to interaction diversity (Schleuning et al. 2015).

We first detected changes in the dominance of interactions, as reflected by the increase in interaction evenness, from seed dispersal to recruitment (most notably in the cohort of 2009). Thus, tree and bird species made a more even contribution to the whole interaction network after accounting for post-dispersal fate. A negative correlation between the quantitative and qualitative roles of seed dispersers (with the most frequent disperser having the lowest quality and vice versa) could explain such an increase in interaction evenness (Schleuning et al. 2015). However, no strong differences between species of thrushes were expected in seed gut treatment, and no relationship between the abundance of the different thrushes and their contribution to the seed rain was apparent (Fig. 2; Supplementary material Appendix 3 Table A8). We thus need to search for alternative arguments to explain changes of evenness. In this sense, these modifications accord with *C. monogyna* having a stronger, and far more equitable role in the networks, compared to *I. aquifolium*. This probably derived from the higher emergence rates of *C. monogyna* compared to the other trees (Supplementary material Appendix 3 Table A4), and as a consequence of its generally lower seed predation rate (due to its thicker seed coat; García et al. 2005a). These differences in emergence may be accentuated by subtle differences between trees in their spatial patterns of seed dispersal. Namely, compared to the other trees, *C. monogyna* showed a higher proportion of seeds reaching open areas (where seed predation is lower and germination slightly higher; García et al. 2005c), as well as a higher proportion of seeds dispersed below heterospecific canopies (where co-deposition with other species further decreases the predation rate; García et al. 2007).

Concerning linkage density, a decrease in the value of this metric is expected when demographic filtering leads to the disappearance of some links from the network of seed deposition (e.g. when plant species reduce their coteries of dispersers, thereby losing links with bad-quality dispersers; Carlo and Yang 2011). In our case, microhabitat differences in seed fate, and bird differences in seed deposition patterns, seemed to determine a weak-to-moderate gradient in seed disperser quality, with probably weak effects on the loss of links. Nevertheless, we would expect a decrease in linkage density along the demographic process if some links could be lost just owing to sampling effects, when all the seeds of rare plants, dispersed by rare frugivores, disappear after heavy post-dispersal losses. This is what we found for the 2010 cohort, a decrease in the number of links between *T. baccata* and the species of birds responsible for its recruitment (Fig. 3). The high predation rate suffered by seeds of *T. baccata* in the Cantabrian range (García et al. 2005a, 2007), and the concomitant low establishment probability of this species, may also underpin the loss of interactions concerning this tree across regeneration stages. On the other hand, as the number of links could not become larger from seed dispersal to recruitment, higher values found for the 2009 cohort reveals that these changes were due to the relative weight of each plant species within each frugivore species (for example as a consequence of the increase in the relative role of *C. monogyna* in the recruitment network).



## Inter-annual variability in seed deposition and recruitment networks

Our comparison of two plant cohorts evidenced strong differences in interaction frequencies in terms of the distribution across tree and bird species, as well as with regards to the global structure of networks. Changes in interaction frequencies at the seed deposition stage seemed to mirror between-year variation in the relative abundances of both fruits and birds. As for birds, most of the inter-annual variability was accounted for by the opposing trends of *T. iliacus* and *T. philomelos*. Variations in the number of migrant individuals reaching and wintering in the Cantabrian Range each year probably supported these differences (Tellería et al. 2014). As *T. iliacus* decreased in abundance from 2009 to 2010, the seed dispersal and recruitment networks were dominated by *T. philomelos*, with a concomitant decrease in linkage density. With respect to trees, the variability in fruit crop composition provoked by the alternating masting events of *I. aquifolium* and *C. monogyna* cascaded into strong changes in the relative dominance of each species, affecting interaction evenness. In sum, our study evidences that the functional effect of seed dispersal networks depends on the, typically strong, inter-annual dynamism of the fruit–frugivore interaction (see also Carnicer et al. 2009).

Post-dispersal processes may, however, buffer the weight of relative abundances at the seed dispersal stage when driving inter-annual differences in recruitment networks. For example, for the 2010 cohort, the proportion of seeds in the open and beneath non-fleshy trees (microhabitats allowing higher rates of seedling emergence) was comparatively higher, irrespective of the tree and the bird species. This was probably the result of all birds using landscape sectors rich in these types of microhabitats more frequently in 2010 in response to the fruit crop being more widely distributed across the whole study plot (Supplementary material Appendix 1 Fig. A2; Martínez and García 2015).

## Concluding remarks

We show here that the interaction diversity in plant–seed disperser networks increases when considering the functional effect of birds on plants. The process of demographic filtering on plants could neutrally provoke these changes by itself, as the random loss of plant propagules would make those depending on rare interactions become even rarer. Nonetheless, we pinpoint two deterministic forces equalizing the interactions across plant regeneration: disperser quality, here dependent on how the spatial pattern of seed deposition matches that of seed fate; and plant species traits, as each species may make their recruitment more dependent on specific traits (here, for example, seed hardness driving differences in seed predation) than on the deposition microhabitat imposed by dispersers. Interactions between disperser quality and plant traits are, nevertheless, possible, as, on the one hand, a seed not favored for frugivore removal may still recruit if deposited in a particular microhabitat (García et al. 2007), and, on the other hand, the effect of some plant traits may differentially emerge depending on the microhabitat (e.g. seed hardness becoming innocuous when there are very few seed predators; García et al. 2005c). In

sum, this work strongly recommends the development of an integrative framework to predict the balanced and interactive effects of plant and animal traits in the functional outcome of plant–animal mutualistic networks (Schleuning et al. 2015). Further studies should corroborate the present results in species-rich plant–seed disperser assemblages, such as tropical ones (Donatti et al. 2011), by incorporating the demographic data needed to assign specific fates to the seeds of different plants dispersed by different animals.

*Acknowledgements* – We thank J. Rodríguez, C. Guardado and R. Pérez for technical support during fieldwork. J. Fründ and J. M. Morales for statistical advice and R. Lendrum for linguistic review. Dries Bonte provided useful suggestions to improve an early version of the manuscript.

*Funding* – This research was supported by the Spanish National Program of Research and Development (MICINN CGL2011-28430 grant to DG) and the FPI Program - European Social Fund (BES2009-25093 and BES2012-052863 grants to DM and ID, respectively).

*Permits* – Fieldwork was conducted according to Spanish environmental legislation and with the permission of the Regional Government of Asturias.

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Supplementary material (Appendix oik.02509 at <[www.oikosjournal.org/appendix/oik-02509](http://www.oikosjournal.org/appendix/oik-02509)>). Appendix 1–3.