Frugivore biodiversity and complementarity in interaction networks enhance landscape-scale seed dispersal function

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Abstract

1. Animal biodiversity matters for the provision of ecosystem functions derived from trophic activity. However, the mechanisms underlying this pattern remain elusive since animal abundance and diversity, which are the components commonly used for representing biodiversity, provide poor information about ecological complementarity in species assemblages. An approach based on species interaction networks may overcome this constraint.

2. Here, we relate frugivore biodiversity and frugivore-plant network structure with landscape-scale seed dispersal function. We sampled, for two years, and at fourteen plots with variable assemblages of frugivores and plants in the Cantabrian Range (N Spain), data on the abundance and diversity of frugivorous birds, the consumption of fleshy fruits of woody plants, and the landscape-scale patterns of avian seed deposition. As a measure of interaction complementarity in seed dispersal networks, we estimated the degree to which frugivore and plant species specialize in their interacting partners.

3. Specialization varied strongly across the seed dispersal networks of the different plots, being higher in networks harboring smaller bird species that dispersed mostly small-fruited plants, and also in networks with late-ripening, dominant fruiting species dispersed mostly by wintering birds.

4. Bird abundance markedly affected seed deposition. Plots harboring more birds received a higher density of dispersed seeds, and showed higher probabilities of seed arrival and seed deposition in open microhabitats. Bird diversity also had a positive effect on the density of dispersed seed and, to a lesser extent, seed arrival probability. Independently of frugivore abundance and diversity, the density of dispersed seeds increased in plots where seed dispersal networks showed a higher degree of specialization.

5. This study considers the structure of interaction networks to re-address the relationship between biodiversity and ecosystem functionality, evidencing that specialization in frugivore-plant networks drives the large-scale process of seed dispersal. These results encourage the

consideration of interaction complementarity as an underlying mechanism linking animal biodiversity and trophic-related functions.

Keywords: avian seed dispersal, bird abundance, bird diversity, fleshy fruits, frugivorous birds, plant-animal interactions, specialization.

Second Language abstract (ES)

1. La biodiversidad animal es importante para la provisión de funciones ecosistémicas derivadas de la actividad trófica. No obstante, los mecanismos que determinan este hecho son poco conocidos, ya que abundancia y diversidad, los componentes utilizados usualmente para representar la biodiversidad animal, apenas informan sobre la complementariedad ecológica en los conjuntos de especies. Una aproximación basada en redes de interacciones inter-específicas puede superar esta limitación.

2. En este estudio, relacionamos la biodiversidad de frugívoros y la estructura de las redes frugívoro-planta con la función de dispersión de semillas a escala paisajística. Durante dos años, y en catorce parcelas con conjuntos variados de frugívoros y plantas en la Cordillera Cantábrica (N España), muestreamos la abundancia y la diversidad de aves frugívoras, el consumo de frutos carnosos de plantas leñosas, y la deposición de semillas por aves. Como medida de complementariedad de interacción en las redes de dispersión de semillas, estimamos el grado de especialización recíproca de frugívoros y plantas en sus interacciones.

3. La especialización difirió fuertemente entre las redes de dispersión de distintas parcelas, siendo mayor en las redes con especies de aves más pequeñas que dispersaron principalmente plantas de fruto pequeño. Así mismo, las redes con especies de plantas que fructificaron de forma tardía y dominante en número, y dispersadas sobre todo por aves invernantes, mostraron una alta especialización.

4. La abundancia de aves afectó notablemente a la deposición de semillas. Las parcelas con más aves recibieron mayor densidad de semillas dispersadas, y mostraron mayores probabilidades de llegada de semillas y de deposición en los microhábitats abiertos. La diversidad aviar también influyó positivamente en la densidad de semillas y, en menor grado, en la probabilidad de llegada. Independientemente de la abundancia y la diversidad de frugívoros, la densidad de semillas dispersadas fue mayor en las parcelas con redes de dispersión más especializadas.

5. Este estudio tiene en cuenta la estructura de las redes de interacciones para reevaluar la relación entre biodiversidad y funcionalidad ecosistémica, evidenciando que la especialización en las redes frugívoro-planta modela el proceso de dispersión de semillas a escala amplia. Estos resultados animan a considerar la complementariedad de interacción como un mecanismo subyacente al vínculo entre biodiversidad animal y funciones tróficas.

Introduction

The link between animal biodiversity and the ecosystem functions derived from animals' trophic activity is now widely accepted (Cardinale et al., 2006; Reiss, Bridle, Montoya & Woodward, 2009). Species-richer and more complex animal assemblages result in stronger and more stable functions than those shrunk by ecological filtering or impoverished by defaunation (Duffy, 2003; Worm et al., 2006). These patterns involve a variety of trophic functions, from plant pollination by flower visitors (e.g. Fründ, Dormann, Holzschuh & Tscharntke, 2013) to organic matter recycling by detritivores (e.g. Jonsson & Malmqvist, 2000). Although the positive effects of animal biodiversity on ecosystem functions are common, their underpinning mechanisms remain elusive (Reiss et al., 2009; Thompson et al., 2012). Among these mechanisms is niche complementarity through resource partitioning, whereby various species in a given assemblage have complementary manners of exploiting a set of trophic resources (Cardinale, 2011). Both theoretical modeling (e.g. Poisot, Mouquet & Gravel, 2013) and experimental small-scale studies (e.g. Finke & Snyder, 2008) have evidenced the effects of resource partitioning on trophic functions. However, it is unclear whether these effects are prevalent in real-world ecosystems, as niche complementarity may be contingent on interspecific interactions (e.g. competition; Albrecht et al., 2013; Fründ et al., 2013), and on environmental filtering (Cardinale, 2011).

Using networks of interspecific interactions has been highlighted as a powerful tool for understanding the effects of animal biodiversity on trophic functions (Thompson et al., 2012). Ecological networks, like those representing animal-plant relationships (e.g pollinators or seed dispersers and plants), assess the actual contribution of individual species to ecosystem functions (pollination and seed dispersal; Schleuning, Fründ & García, 2015). More importantly, some measures of network complexity, like the degree of specialization which measures how species specialize in terms of their interacting partners, represent resource partitioning and functional complementarity (Blüthgen & Klein, 2010). Despite these advantages, the effects of interaction complementarity on the magnitude of ecosystem functions in the wild remain unclear (e.g. Macfadyen, Gibson, Symondson & Memmott., 2008; Theodorou et al., 2017; but see Peralta, Frost, Rand, Didham & Tylianakis, 2014).

The trophic interactions between frugivorous animals and fleshy-fruited plants are suitable systems for evaluating the relevance of biodiversity in ecosystem functioning (Schleuning et al., 2015). By consuming fruits, frugivores shape plant communities through both antagonistic effects (e.g. when preying on pulp and seeds) and mutualistic ones (e.g. when acting as legitimate seed dispersers by regurgitating or defecating intact seeds; Jordano, 2014; Traveset, Heleno & Nogales, 2014). Alongside plants, frugivores build ecological networks that vary greatly in specialization along environmental gradients at different scales (from landscape to latitudinal), depending mostly on changes in the abundance, richness and composition of animal and fruit resource assemblages (e.g. Schleuning et al., 2012; Albrecht et al., 2013; Vollstädt et al., 2018). Moreover, the abundance and the diversity of frugivores acting as legitimate seed dispersers have been identified as biodiversity components driving fine-scale patterns of seed dispersal (García & Martínez, 2012). Nevertheless, it is unclear whether these effects eventually scale-up to landscapes at regional extents. More importantly, it is not known if complementarity in frugivore-plant networks shapes variability in seed dispersal function.

Here we focus on the assemblages of frugivorous birds and fleshy-fruited trees in the Cantabrian Range to assess the link between frugivore biodiversity and seed dispersal function, explicitly taking into account the structure of seed-dispersal networks. By implementing a large-scale sampling of bird abundance and diversity, their frugivore activity, and their derived seed deposition, we seek to: (i) estimate the degree of complementarity within seed dispersal networks and its local ecological determinants; (ii) evaluate the relationship between frugivore abundance and diversity and seed dispersal function, in terms of quantitative and qualitative large-scale patterns of community-wide seed deposition, and (iii) assess the importance of interaction complementarity in the provision of the seed dispersal function relative to that of frugivore biodiversity. As a general prediction, we expect positive effects of both frugivore biodiversity and network complementarity on seed dispersal.

Methods

Study design

This study was conducted in mid-elevation woodland pastures of the Cantabrian Range in northern Spain (for a comprehensive description of study system, design and all methodologies, see Appendix S1). The woodlands contain variable-sized patches of primary and secondary forest, embedded in an extensive matrix of stony meadows and heathland. The secondary forest is typically dominated by fleshy-fruited trees and shrubs with ripening periods from late summer to early winter. The main frugivores are passerine birds (García, 2016); with most species (e.g. thrushes) performing as legitimate seed dispersers, although some are pulp-eaters and seed-predators with a negligible contribution to the seed rain (Simmons et al., 2018). As the goal of this study was to relate the trophic activity of frugivorous birds with seed deposition, we used a conservative approach and focused exclusively on bird species considered as legitimate seed dispersers, discarding observations of not only non-frugivorous species, but also of antagonistic frugivores. Classification of bird species was based on previous observations of fruit handling and fecal content (e.g. Martínez, García & Obeso, 2008; see also Simmons et al., 2018).

In August 2012, we delimited fourteen 2.25 ha plots (150 x 150 m) in two sites (Sierra de Peña Mayor and Bandujo-Puertos de Marabio) in Asturias Province, Spain (Fig. 1a; Table S1; Fig. S1). All plots had similar vegetation types (forest stands embedded in a matrix of pastures and heathland), geomorphology (slope ≤25%, limestone substrate, altitude 990-1250 m asl) and anthropic management (extensive livestock raising), but were chosen to incorporate wide variability in forest availability (3-69% of forest cover; Table S1; Fig. S1). We assumed that differences in forest cover would lead to high variability in the abundance and richness of fleshy fruits and frugivorous birds (as previously found in the same system; García & Martínez, 2012). Sampling was conducted across two consecutive fruit production and seed dispersal yearly events, from September to March in both 2012-2013 and 2013-2014 (sampling years, hereafter).

Bird abundance and richness

Point-count bird censuses were carried out consecutively at nine regularly distributed points within each plot. To facilitate bird recording from these points, each plot was subdivided into 36 cells measuring 25 x 25 m, each point being in the center of a set of four adjacent cells (Fig. 1a). Censuses were performed from 09.00 to 15.00, avoiding days of heavy rain and wind. In each census, all birds heard or seen within the set of four cells over a 5 min period were registered. Bird records were summed across points in each plot (total sampling effort of 45 min per plot per census) and nine census rounds were performed per sampling year and plot (1-2 censuses/month from September to February). For frugivore species (legitimate seed dispersers only), we estimated absolute and relative abundances per year per plot. Frugivore richness was estimated as the number of species detected across all censuses, per year per plot. Frugivore diversity was estimated from reciprocal Simpson index ($D = 1/\sum p_i^2$; where p_i is the relative abundance of species i; Loreau & Kinne, 2010).

Fruit consumption

Bird fruit consumption was recorded in 17 rounds of 1-hour-per-plot observations throughout the period, though independent, of bird censuses (September-February): 8 rounds in 2012-13 and 9 in 2013-2014. In each round, a given observer visited 3-4 vantage points (Fig. 1a), chosen to ensure that the full extent of the plot was covered (i.e. including the nine points for bird censuses) as well as to focus on the different fruiting species present. Observers recorded every fruit consumption event (i.e. an individual bird consuming fruits) and every feeding bout (i.e. a single bird swallowing a single fruit) detected during the observation round.

Fruit resource abundance

The production of fleshy fruits by woody plants was surveyed at the beginning of the fruiting season, providing an estimate of the yearly, community-wide, fruit resource base available for frugivorous birds. Depending on the different ripening peaks of the various species, we monitored fruit abundance in early September (early-season species) or mid-October (remaining species; Appendix S1) mapping all fruiting trees and shrubs in each plot (Fig. 1b). For each individual tree or shrub, we visually estimated the number of fruits using a semi-logarithmic scale later extrapolated to a natural value of crop size (Appendix S1). For each plant species in each plot, we estimated production, both absolute (number of ripe fruits) and relative (number of fruits of the species/total number of fruits of all species). For each fleshy-fruited species, we measured fruit and seed traits from a sample of 25 ripe fruits (five from each of five individuals) collected in 2012-2013 (Appendix S1).

Seed dispersal function

Avian seed dispersal was studied by identifying and counting the seeds deposited by frugivores, after regurgitation or defecation, during autumn and winter. This was based on data from sampling stations distributed in a grid scheme (108 stations per plot; Fig. 1c) across three types of microhabitat (tree cover, fleshy-fruited shrub cover, and open area not covered by trees or shrubs) each involving a different type of device for seed collection, adapted to the physiognomy of the vegetation and the danger of trampling by ungulates: hanging plastic pots for tree cover, plastic trays under shrub cover, and flag-labelled quadrats on the ground for open area (Fig. 1c). Thus number of stations per microhabitat differed in relation to relative cover of each microhabitat in each plot. All stations were set up in August 2012. Seed traps were checked for seed collection in February-March of 2013 and 2014, and open quadrats in late November and late February of 2012-2013 and 2013-2014. Collected seeds were identified (species are easily identifiable from external morphology) and counted in the lab, after oven-drying for one week at 70ºC. The density of seeds (seeds/m^2) deposited by birds at each sampling station was estimated taking into account the various surfaces of the different devices (0.07, 0.08 and 0.10 m^2 for, respectively, pots, trays and open quadrats).

Three different metrics (components) were used to represent community-wide seed dispersal function. To represent seed rain in quantitative terms we estimated *seed density* (average density of dispersed seeds per sampling station per plot), and *seed arrival rate* (proportion of sampling stations per plot receiving dispersed seeds). As a qualitative metric, we used *seed arrival rate in open* (proportion of open-microhabitat sampling stations per plot receiving bird-dispersed seeds), which represents the potential for tree recolonization of deforested land (García & Martínez, 2012).

Data analysis

Building seed dispersal networks

Seed dispersal networks were based on frugivore-plant interaction matrices built from fruit consumption data. Although restrictive in terms of obtaining large samples of observed interactions (it requires large, often logistically unviable, efforts), this methodology was chosen because of its species unbiased character (García, 2016).

First, we pooled, for each plot, fruit consumption data from the different rounds and both sampling years, in order to reach suitable sampling effort (17 sampling rounds per site) and interaction sample size (number of consumption events and number of fruits consumed per plot ranging from 19 to 153 and 45 to 520, respectively) to ensure accurate estimates of the global metrics of interaction networks. To evaluate the sampling completeness of interaction networks, we constructed plot-level accumulation curves of the number of both bird and plant interacting species, and of the paired links between birds and plants, in relation to the number of sampling rounds and the number of fruit consumption events sampled; these completeness analyses suggested that our sampling effort was adequate to detect the richness of bird and plant interacting species and of bird-plant paired interactions (Appendix S2; Fig. S2.1-6).

Second, we created plot-based matrices representing frugivore-plant interactions in terms of the number of seeds of each plant species dispersed by each bird species. For this we estimated the number of dispersed seeds, for each plant species, by multiplying the cumulative number of fruits consumed by each bird species by the average number of seeds per fruit. The total number of interactions per plot, estimated from the total number of dispersed seeds, averaged 370.6 (±58.6 SE; min-max 92-786).

Complementarity in seed dispersal networks and its ecological determinants

We quantified interaction complementarity by means of *complementary specialization* (sensu Blüthgen, 2010; Blüthgen & Klein, 2011), a measure of the degree of specialization among interacting species. In our case, it represents the degree to which frugivorous birds specialize in fruiting plant species as trophic resources as well as that to which plants specialize in bird species as their of seed dispersal agents. It is thus a direct measure of segregation of the functional niche (and an inverse measure of functional niche overlap; Blüthgen, 2010). We used the index H_2' (standardized two-dimensional Shannon entropy; Blüthgen, Menzel & Blüthgen, 2006), based on the deviation of a species' realized number of interactions from that expected from each species' total number of interactions, and which ranges from 0 (maximum generalization) to 1 (perfect specialization).

Compared to other network metrics, H_2' is demonstrably robust to biases induced by differences in sampling effort and network size (Blüthgen et al., 2006; Fründ, McCann & Williams, 2016). Specifically, one study of bird-plant pollination networks evidenced already stable H_2' values already after 10 h of sampling effort (Vinzentin-Bugoni et al., 2016; see also Costa, da Silva, Ramos & Heleno, 2016). Nevetheless, we standardized the raw values of specialization by estimating Δ-transformed H_2', based on null models ($\Delta H_2' = H_2'$ observed – H_2' null models mean; Patefield model with 1000 replicates; Dormann, Fründ, Blüthgen & Gruber, 2009), as null-model standardization corrects potential overestimations in local specialization patterns due to small observation numbers (Fründ et al., 2016). Raw and standardized network metrics were estimated with the *Bipartite* package (Dormann et al., 2009), in the R 3.01.2 environment (R Development Core Team 2014).

As local ecological determinants of complementarity in seed dispersal networks, we considered the abundance, richness and composition of frugivorous birds and fruiting plants, along with their matching traits (bird body mass and fruit size; Appendix S3), features known as intrinsic drivers of mutualistic networks (Dormann, Fründ & Schaefer, 2017). The composition of both bird and fruiting plant communities was assessed using Principal Components Analysis (PCA, *stats* package in R) of the relative abundance (fruit production in plants) of the different species across plots. Local composition values were estimated from the scores of the three first rotated factors in PCA. Bird body mass was used to estimate a plot-level, community-wide measure of bird size, by means of the Community Weighted Mean ($\text{CWM}=\sum p_i \cdot d_i$; where p_i is the relative abundance of the bird species i and d_i its body mass; Lavorel et al., 2008). A similar procedure, based on fruit diameter and relative fruit production, was applied to estimate CWM fruit size. Additionally, as potential determinants of complementarity, we also included a consumer-resource ratio, estimated from a plot-level quotient of abundance of birds:abundance of fruits (asuming that low ratios may relax competition among frugivores leading to increased specialization; Albrecht et al., 2013), and forest cover (as a major environmental gradient; Chama, Berens, Downs & Farwig , 2013). Relationships between standardized specialization and determinants were tested using Pearson’s correlation tests.

Frugivore biodiversity, interaction complementarity and seed dispersal

Our first goal was to explain the large-scale spatio-temporal variability of the different components of seed dispersal as a function of the abundance and diversity of frugivorous birds. For this, we considered the data from the various plots in each sampling year. We used generalized linear mixed models (GLMMs) with the components of seed dispersal as different response variables (log- or arcsin sqrt transformed when needed), considering Gaussian error distributions and identity links. All models incorporated, as fixed effects, both abundance and diversity of birds, standardized before inclusion. Simpson index was preferred to bird richness to represent diversity because of the significant collinearity between abundance and richness (Pearson's correlation coefficient, $r = 0.426$, $p = 0.024$, $n = 28$), but not between abundance and diversity ($r = 0.293$, $p = 0.130$, $n = 28$). Simpson index thus accounted for both evenness and richness (correlation diversity - richness; $r = 0.515$, $p = 0.005$, $n = 28$), and represented an "effective number of species" in the community (the number of species expected in a situation of equal species abundances; Loreau & Kinne, 2010). Plot identity (nested within site) was included in all models as a random effect, whereas year (2012-2013; 2013-2014) and site (Sierra de Peña Mayor, Bandujo-Puertos de Marabio) were included as fixed effects (due to low number of levels). Year and site factors were excluded from final models when proven initially non-significant ($p > 0.05$). Degree of variance explained by the final complete model and by fixed effects was estimated fromconditional and marginal R^2 values, respectively (Nakagawa & Schielzeth, 2013).

The second step sought to test the simultaneous effects of interaction complementarity and frugivore biodiversity on the provision of seed dispersal function. Given that network metrics were based on interaction matrices that pooled data from both sampling years, we recalculated the local values of frugivore biodiversity and seed dispersal function per plot also from data pooled across years. We used generalized linear models (GLMs) to check for effects of abundance and diversity of frugivorous birds and of standardized specialization (predictors) on each component of seed dispersal (response variables). Predictors were not correlated between themselves ($|r| < 0.40$, $p > 0.15$, $n = 14$; for all paired combinations). We built models with different combinations of predictors, setting the number of predictors per model at two in order to avoid model overparametrization. Inferences about the relative effects of the different predictors were based on effect size and significance (predictors were standardized before inclusion), and Akaike's Information Criterion (with correction for small sample sizes, AICc) value was used to identify the most informative model (i.e. that with the lowest AICc value; Quinn & Keough, 2002). All response variables met normality requirements (after log or arcsin-sqrt transformation, when needed) so models considered Gaussian error distributions and identity links. GLMM and GLM analyses were performed with *lme4* package in R.

Results

General overview

Bird censuses provided 17,732 observations, of which 68.58% corresponded to ten species of legitimate seed dispersers; blackbird *Turdus merula*, redwing *T. iliacus*, and European robin *Erithacus rubecula* being the most frequent and abundant (Appendix S3; Table S3). Ten fruiting woody plant species were recorded (Table S3), the most frequent and abundant being holly *Ilex aquifolium* and hawthorn *Crataegus momogyna* (Table S3). Due to differences in species occurrence and abundances, the composition of bird and fruiting plant assemblages varied markedly across plots (Appendix S4; Table S4, Fig. S4).

Ten bird species and seven fleshy-fruited plants were detected in fruit consumption observations. Plot-based interaction matrices varied in size, with the number of species of frugivorous birds, fruiting plants, and both birds and plants ranging, respectively, 4-7, 2-5, and 6-11 (Appendix S5; Fig. S5). The proportion of observed links between birds and plants, from all potential paired bird-plant combinations per plot, averaged 0.56 (±0.04SE; min-max: 0.35-0.88; Fig. S5). In terms of seed dispersal (i.e. number of dispersed seeds estimated from plot-based interaction matrices), holly and hawthorn -mostly by blackbird and redwing- accounted for 67.11% of all observed interactions.

We recorded seed deposition of fleshy fruited plants in 52.38% of sampling stations (n = 1512 stations/year), mean density being 222.46 (±11.47SE) seeds/m^2 per station per year. Eight plant species were recorded in the sampling of the seed rain, which was heavily dominated by holly, hawthorn, and bramble *Rubus fruticosus/ulmifolius* seeds (Appendix S3; Table S3).

Complementarity in seed dispersal networks

The degree of specialization in seed dispersal networks varied markedly across plots, with raw H_2' values ranging from 0.164 to 0.965 (Fig. 2; Appendix S5; Fig. S5). Poorly specialized networks showed species interacting with many and equally frequent partners, on both the frugivore and the plant side (Fig. 2). Conversely, highly specialized networks showed frequent segregation, especially on the bird side, as species seldom overlapped in terms of the plants with which they interacted (Fig. 2). H_2' raw values significantly differed from null model means in all plots ($t < -29.66$, $p < 0.001$, in all cases) and were positively correlated with $\Delta H_2'$ standardized values ($r = 0.997$, $p < 0.001$, $n = 14$). H_2' was independent of the number of interacting species of birds or plants per plot ($|r| < 0.442$, $p > 0.113$, $n = 14$), the proportion of realized links ($r = 0.300$, $p = 0.296$, $n = 14$), and the number of interactions (i.e. dispersed seeds; $r = -0.076$, $p = 0.794$, $n = 14$).

Concerning the ecological determinants of interaction complementarity, standardized specialization degree was unaffected by local abundance or richness of either frugivorous birds ($|r| < 0.128$, $p > 0.666$, $n = 14$; in both cases) or fruiting plants ($|r| < 0.368$, $p > 0.196$, $n = 14$; in both cases). Specialization was higher in plots with larger relative abundances of blackcap *Sylvia atricapilla*, chiffchaff *Phylloscopus collybita/ibericus* and European jay *Garrulus glandarius* (as represented by the bird PCA3 factor; $r = 0.712$; $p = 0.004$, $n = 14$; Fig. 3; Appendix S4; Fig. S4), as well as in plots with smaller CWM bird body size ($r = -0.552$; $p = 0.041$, $n = 14$; Fig. 3), but it was unaffected by other bird composition trends (PCA1 and PCA2 factors: $|r| < 0.194$, $p > 0.507$, $n = 14$, in both cases). Specialization was also higher in plots with fruit crops dominated by holly (fruiting plants PCA1: $r = 0.535$; $p = 0.048$, $n = 14$; Fig. 3; Fig.

S4), but was unaffected by other fruit composition trends (fruiting plants PCA2 and PCA3) or CWM fruit size ($|r| < 0.368$, $p > 0.196$, $n = 14$, in all cases). Neither consumer-resource ratio nor forest cover affected specialization ($|r| < 0.448$, $p > 0.10$, $n = 14$, in both cases).

Frugivore biodiversity, interaction complementarity and seed dispersal

When analyzed by years using GLMMs, both abundance and diversity of frugivorous birds had an independent positive effect on seed dispersal function, although their respective effects were contingent on the specific seed dispersal component. The density of dispersed seeds was higher in plots with higher values for abundance and diversity of frugivores (Table 1; Fig. 4). Seed arrival rate followed a similar pattern, although in this case bird abundance showed a stronger effect than that of frugivore diversity (Table 1; Fig. 4). Rate of seed arrival in open microhabitats was exclusively affected by abundance of frugivorous birds, with a greater proportion of sampling stations in the open receiving seeds in those plots hosting higher numbers of birds (Table 1).

When applied to the data of the pooled sampling years, GLMs evidenced that interaction complementarity positively affected seed dispersal function, even after accounting for the effects of abundance and/or diversity of frugivorous birds (Table 2; Fig. 5). Namely, seed density was best explained by the combined positive effects of frugivore abundance and specialization (Table 2; Appendix S6; Table S6). Concerning seed arrival rate and seed arrival in the open, GLMs for pooled data suggested effects of frugivore abundance and diversity similar to those evidenced by previous yearly models (Table 2; Table S6).

Discussion

This study addresses the link between frugivore biodiversity and the ecosystem function of seed dispersal, by considering the structure of interaction networks. Although in the long term, by driving plant community dynamics (e.g. Martínez & García, 2017), seed dispersal affects frugivore and fruit assemblages and hence their interaction networks (i.e. the seed dispersal loop; Wang & Smith, 2002), here we focus on the assumed causal relationship between frugivore-plant community attributes and their short-term ecological consequence, seed deposition. As such, we applied an observational approach that incorporates a set of local landscapes harbouring independent gradients of frugivore abundance and diversity, and also matches these community attributes with the fine-scale processes of fruit consumption and seed deposition. We evidence positive responses of seed dispersal process to increases in abundance and diversity of frugivores across landscapes. We also found strong differences in interaction complementarity in frugivore-plant networks, mostly determined by the composition of frugivore and plant assemblages and frugivore size. Importantly, interaction complementarity positively affected the local magnitude of seed dispersal, independent of frugivore abundance and diversity.

Complementary in seed-dispersal networks

Our results reveal large-scale variability in interaction complementarity, estimated from specialization, as shown by other studies over regional extents (e.g. Chama et al., 2013). Local specialization values differed significantly from those expected by the random association of interacting frugivores and plants, suggesting that interaction complementarity resulted from ecological determinants (Dormann et al. 2017). In fact, specialization varied mostly as a response to local differences in bird composition and traits: more specialized networks occurring where common and small-sized bird species, such as blackcap and European robin, feed mostly on small-fruit plants like bramble and elder *Sambucus nigra* (Fig. 2). These specialized interactions may derive from morphological matching; small birds avoiding large fruits due to gape width constraints (González-Castro, Yang, Nogales & Carlo, 2015). Some kind of phenological coupling (González-Castro et al., 2015) may also occur, as bramble and elder are typically late-summer ripening species, a time when blackcap is still abundant in Cantabrian uplands (Santos et al., 2014). Conversely, the occurrence of abundant and medium-to-large size birds -like blackbird and song thrush *T. philomelos*- with diversified fruit diets (thanks to their larger gape width relative to fruit diameter [González-Castro et al., 2015]), would lead to highly generalized networks (Fig. 2). Thus, specialization in networks would mostly reflect frugivore trophic complementarity (less overlap in the trophic niche determined by the quantity and the species of fruits consumed; Blüthgen & Klein, 2011). Indeed, in our case, network complementarity was negatively correlated with niche overlap among bird species (r = -0.81, $p <$ 0.001, n = 14; estimated from *bipartite*). Some variability in specialization was also attributable to the composition of fruiting plants, the presence of holly, a crop-dominant but late-ripening species, leading to interactions almost monopolized, at least in some plots, by wintering frugivores like redwing (Fig. 2; see also Martínez et al., 2008).

Frugivore biodiversity, interaction complementarity, and seed dispersal

Our yearly assessment showed the positive, independent, and consistent relationships of both abundance and diversity of frugivorous birds with different components of the seed dispersal function. Namely, those landscapes harbouring more frugivores from a greater number of species received a denser and wider seed rain of fleshy-fruited woody plants. We thus evidence how the patterns already seen at finer scales in the study system (García & Martínez, 2012) also scale-up at the regional extent and prevailed across years.

We found, on the one hand, that the effects of frugivore abundance on seed dispersal were stronger than those of frugivore diversity, suggesting that this ecosystem function is more dependent on the number of individual animals providing it than on the qualitative functional differences between animals (see also, for pollination by insects, Winfree, Fox, Williams, Reilly & Cariveau, 2015). On the other hand, frugivore biodiversity effects varied across components of seed dispersal, which may be partly associated with the occurrence of complex interactions between frugivore abundance and diversity (e.g. stronger effects of diversity at small abundances, Rumeu et al., 2017), though the present data do not facilitate testing this (but see Rodríguez-Pérez, García, Martínez & Morales, 2017). Even so, our results showed positive effects of diversity irrespective of abundance, probably related to sampling effects (the incorporation of functionally dominant frugivores, Schleuning et al., 2015), spatial complementarity (with a greater diversity meaning a higher variety of spatial behaviors after fruit consumption; Morales; García, Martínez, Rodríguez-Pérez & Herrera, 2013), or even interspecific interactions (with a greater diversity leading to facilitation in, for example, fruit resource tracking; Donoso, García, Martínez, Tylianakis & Stouffer, 2017).

Notably, by incorporating the effects of interaction complementarity the present study goes beyond previous evaluations of the role of frugivore biodiversity on seed dispersal. In line with our expectation, we found that specialization also drove seed dispersal function and, when controlling for the effects of frugivore abundance, it explained the regional variability in the density of dispersed seeds better than frugivore diversity. We would suggest that trophic (diet) complementarity between frugivorous birds underpins these effects. Different birds specializing in different plants would faciliate more effective access to available plant resources, increasing the average number of seeds per deposition point (for such a case with pollinators, see Fontaine et al., 2006). This seems particularly likely to occur when specialization leads to the inclusion of big-crop plant species (like bramble, Table S2). Moreover, increased seed density is also expected when frugivores differ in their spatial patterns of foraging, because they feed selectively on fruiting species that occur in different sectors of the landscape (e.g. bramble shrubs mostly occurs at forest fringes in the study plots, Fig. 1a).

Concluding remarks

Our study re-addresses the relationship between biodiversity and ecosystem functions by explicitly considering the structure of trophic networks. We show, under unmanipulated conditions and at the large scale, a positive link between the degree of network-wide specialization and the magnitude of a function derived from the animal-plant mutualism (for small-scale, microcosm experiments with pollination see Fontaine et al., 2006; Fründ et al., 2013). These results thus reinforce previous observational findings concerning other types of food webs (e.g. host-parasitoids, Peralta et al., 2014), encouraging the consideration of interaction complementarity as an underlying mechanism of the causal link between animal biodiversity and trophic-related ecosystem functions and services.

Authors contributions

DG conceived and designed the study. DG, JRP and ID collected the data. DG and ID analyzed the data. DG wrote the manuscript. All authors revised the manuscript and approved the final version.

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Data accessibility

Original data associated with this article are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.h748c88 (García et al. 2018).

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

Table 1. Generalized linear mixed models for the effects of abundance and diversity of frugivorous birds (fixed effects) on different components of seed dispersal, with site and year included as fixed effects (removed from final models when $p > 0.05$), and plot identity (nested within site) included as a random effect. Values of marginal and conditional (in brackets) R^2 are also shown.

Seed density ($\log_{10}$) R^2 = 0.817 (0.923)				
Fixed effects	Estimate	Std. Error	*t-value*	*p-value*
Abundance of birds	0.135	0.025	5.325	<0.0001
Diversity of birds	0.137	0.027	4.951	<0.0001
Year	0.426	0.038	11.02	<0.0001
Random effects	Variance	Std. Dev.		
Plot [site]	0.009	0.099		
Seed arrival rate R^2 = 0.705 (0.884)				
Fixed effects	Estimate	Std. Error	*t-value*	*p-value*
Abundance of birds	0.105	0.018	5.820	<0.0001
Diversity of birds	0.073	0.019	3.712	0.0035
Year	0.076	0.027	2.84	0.015
Random effects	Variance	Std. Dev.		
Plot [site]	0.005	0.075		
Seed arrival rate in open (arcsin sqrt) R^2 = 0.211 (0.699)				
Fixed effects	Estimate	Std. Error	*t-value*	*p-value*
Abundance of birds	0.064	0.022	2.860	0.0135
Diversity of birds	-0.022	0.021	-1.015	0.5360
Random effects	Variance	Std. Dev.		
Plot [site]	0.009	0.094		

Table 2. Generalized linear models for the effects of frugivore biodiversity and interaction complementarity on seed dispersal. For each seed dispersal component, the combination of predictors with the lowest AICc value is shown. Model and null deviance values are also shown.

Seed density ($\log_{10}$)				
	AICc	Model Dev.	Null Dev.	
	-13.54	0.619	0.767	
Predictors	Estimate	Std. Error	*t-value*	*p-value*
Abundance of birds	0.174	0.032	5.37	0.0002
Specialization ($\Delta H_2'$)	0.110	0.032	3.42	0.0057
Seed arrival rate				
	AICc	Model Dev.	Null Dev.	
	-20.46	0.377	0.468	
	Estimate	Std. Error	*t-value*	*p-value*
Abundance of birds	0.128	0.027	4.66	0.0007
Diversity of birds	0.072	0.028	2.61	0.0240
Seed arrival rate in open (arcsin sqrt)				
	AICc	Model Dev.	Null Dev.	
	-12.46	0.110	0.270	
	Estimate	Std. Error	*t-value*	*p-value*
Abundance of birds	0.074	0.033	2.25	0.0457
Specialization ($\Delta H_2'$)	-0.059	0.034	-1.75	0.1100

Figure 1. Representation of study design. (a) Plot including 25 x 25 m grid cells, forest (pale blue) and bramble (brown) cover, bird-count points (1-9 numbered red circles) and fruit-consumption observation points (yellow dots). (b) Detail of individual trees (green circles: holly; red circles: hawthorn; black dots: other tree species). (c) Detail of stations for sampling dispersed seeds (different figures for different devices in pictures; green squares: open quadrats; red circles: pots in trees; yellow triangles: trays under bramble).

Figure 2. Examples of local seed dispersal networks with increasing levels of interaction complementarity (values of standardized degree of specialization $\Delta H_2'$ are shown). Bipartite graphs show the proportion of seeds (left column) dispersed by frugivorous birds (right column), and the proportion of seeds of each plant consumed by each bird species (gray links). Species codes are based on abbreviated scientific names (Appendix 3; Table S3). Bird species in right panel are scaled to highlight size differences (artwork: Daniel García).

Figure 3. Responses of interaction complementarity (standardized degree of specialization $\Delta H_2'$) to the composition of the communities of frugivorous birds and fruiting plants (axes of Principal Component Analysis) and frugivore traits (community-weighted mean of body mass). Dots represent different plots (data from both years pooled).

Figure 4. Effects of abundance (cumulative number of individuals per plot) and diversity (Simpson Index) of frugivorous birds on the density of dispersed seeds (no. dispersed seeds per square meter) and the probability of seed arrival (proportion of sampling stations receiving dispersed seeds) for different plots and years.

Figure 5. Effects of abundance (cumulative number of individuals per plot) of frugivorous birds and interaction complementarity (standardized degree of specialization $\Delta H_2'$) on the density of dispersed seeds. Dots represent different plots (data from both years pooled).

Figure 1

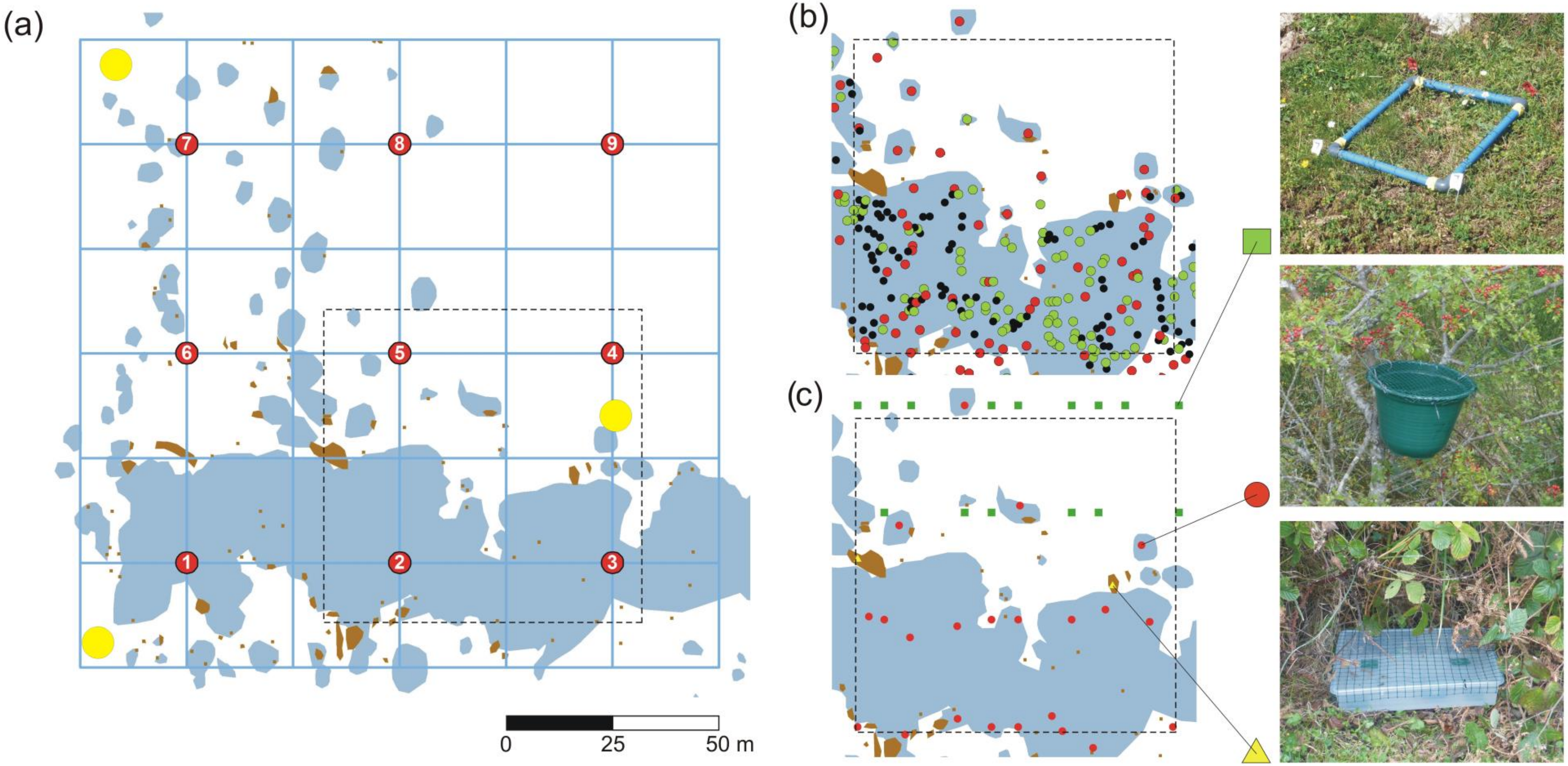
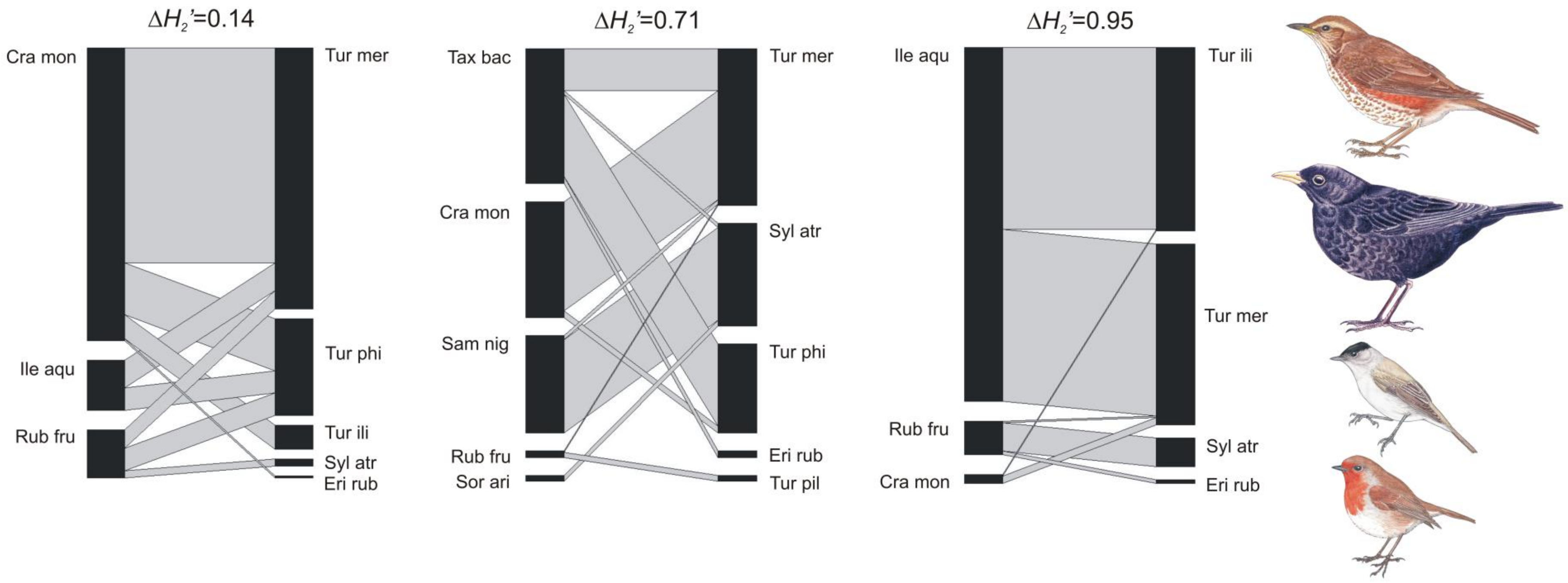


Figure 2


$\Delta H_2'$=0.14
Cra mon
Ile aqu
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$\Delta H_2'$=0.71
Tax bac
Cra mon
Sam nig
Rub fru
Sor ari
Tur mer
Syl atr
Tur phi
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$\Delta H_2'$=0.95
Ile aqu
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Tur ili
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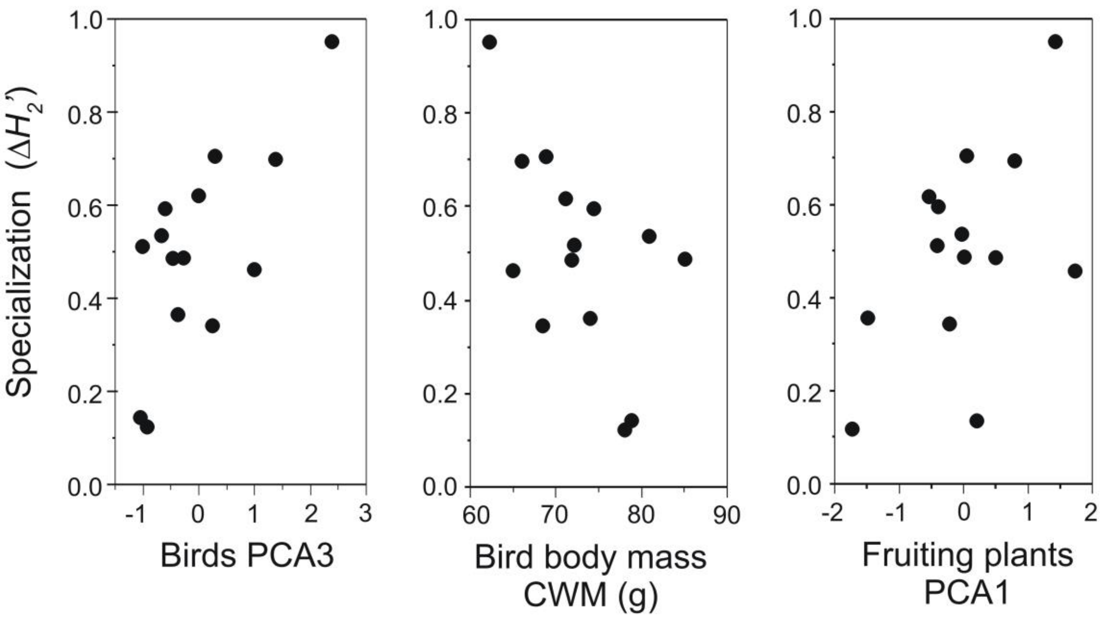
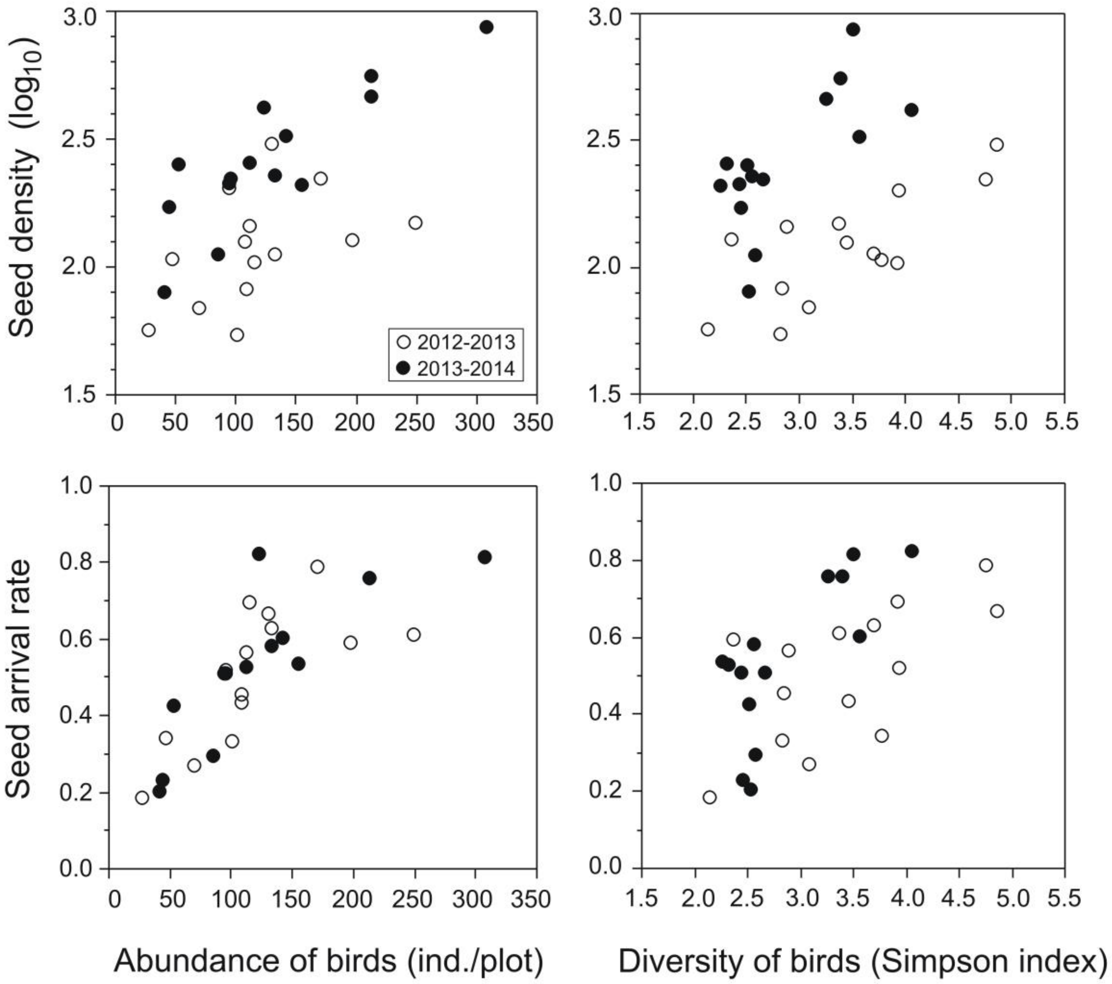
Syl atr
Eri rub

Figure 3


Specialization ($\Delta H_2'$)
1.0
0.8
0.6
0.4
0.2
0.0
-1
0
1
2
3
Birds PCA3
60
70
80
90
Bird body mass CWM (g)
-2
-1
0
1
2
Fruiting plants PCA1

683 Figure 4



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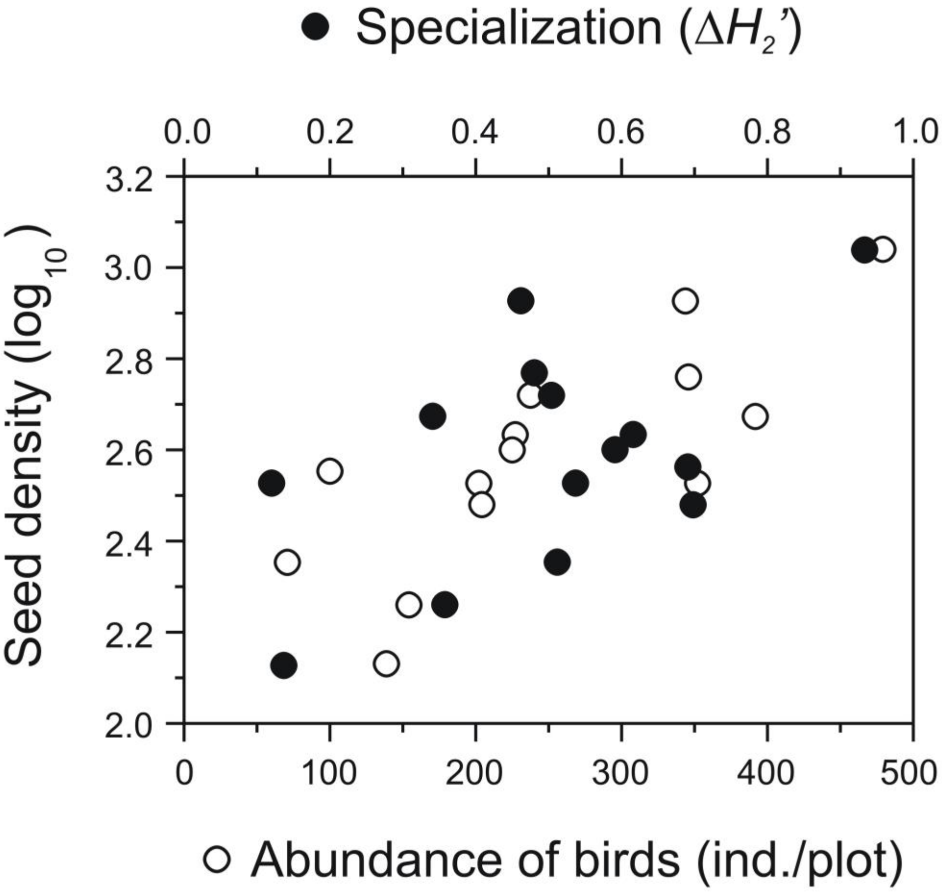
687 Figure 5



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