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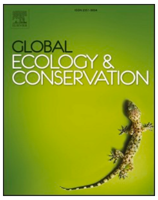

Evaluating drivers shaping the structure of bird-plant pollination and seed dispersal mutualistic networks in a subtropical evergreen broadleaf forest

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ABSTRACT

Animal and plant mutualistic interactions can be shaped by various simultaneous processes, including neutral-based processes that emphasize species relative abundance, as well as forbidden links that reflect phenotypic complementarity in terms of species phenology, geographic distribution, or morphology. A thorough comprehension of various factors that shape the architecture of different mutualistic networks is essential for understanding ecological stability of natural communities. However, the variation in the relative importance of underlying processes across different types of mutualistic networks within the same research system remains unclear. In this study, we investigated the relative importance of species relative abundance, phenological overlap, and morphological constraint/matching in explaining the architecture of both nectarivorous bird-plant (pollination) and frugivorous bird-plant (seed dispersal) mutualistic networks within a subtropical evergreen broadleaf forest. Furthermore, we assessed the relationship between fruit nutrients (water, lipid, protein, fiber, carbohydrate, and energy) and species degree, pairwise interaction frequency, interaction strength, and Z-score (species contribution to nestedness) in the seed dispersal networks. Among processes shaping the two mutualistic network structures, phenological overlap was most important in explaining the frequency of pairwise interactions in the pollination network, while a combination of morphological constraint and species relative abundance best explained the frequency of pairwise interactions in the seed dispersal network. Furthermore, morphological constraint among species within the pollination network predicted nestedness. In addition, the combination of species relative abundance, phenological overlap and morphological constraint could explain the nestedness of the seed dispersal network. Our results showed that the water and fiber content of fruits influences both the species degree and interaction strength within the seed dispersal network. Overall, our findings provide evidence regarding the difference of underlying processes that shape the structure of two types of mutualistic networks within a research system.

1. Introduction

Mutualistic interactions, including those between flowering plants and their pollinators, as well as between fruiting plants and their

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seed dispersers, play crucial roles in ecological functions and ecosystem services (Tylianakis et al., 2010; Schleuning et al., 2015). Research on this has seen a significant increase since the beginning of this century (Vázquez et al., 2009a; Bascompte and Jordano, 2014.). Mutualistic networks typically exhibit distinct structural properties, such as low connectance, significant nestedness and modularity, as well as strong asymmetry in the relationships among interacting species (Jordano, 1987; Bascompte et al., 2003; Bastolla et al., 2009; Vázquez et al., 2009a). The structure of mutualistic networks could affect the stability of natural communities (Bastolla et al., 2009; Thébault and Fontaine, 2010). However, a comprehensive understanding on the various factors shaping the structure of mutualistic networks remains a significant challenge (Burns, 2006; Bascompte and Jordano, 2007; Ebeling et al., 2011; Gonzalez and Loiselle, 2016).

Two processes primarily drive mutualistic network structures: neutrality and the so-called "forbidden links". The neutral-based processes postulates that species are ecologically equivalent, meaning that relatively abundant species interact more frequently and with a greater number of species than rarer species do, thus determining network patterns (Dupont et al., 2003; Vázquez et al., 2007). Conversely, the concept of forbidden links propose that interactions between species are limited by constraint and/or mismatching in their morphology, or the complementarity of their phenologies and spatial distributions (Jordano et al., 2003; Santamaría and Rodríguez-Gironés; 2007; Olesen et al., 2011; Vitorino et al., 2021). Recent empirical and theoretical work suggest that both processes may simultaneously contribute to mutualistic network structures (Bascompte and Jordano, 2007; Vizentin-Bugoni et al., 2018), but their relative importance may vary and could be affected by environmental factors (Sáyago et al., 2013; Maruyama et al., 2014; Olito and Fox, 2015; Sonne et al., 2020; Zhao et al., 2022). Moreover, additional factors such as species nutritional and habitat preference, or phylogenetic relationships among species, may also extert an influence on the structure of mutualistic networks (Rezende et al., 2007; Schleuning et al., 2011; González-Castro et al., 2015).

Previous studies have assessed the relative importance of these underlying processes in pollination (e.g. Vázquez et al., 2009b; Vizentin-Bugoni et al., 2014; Olito and Fox, 2015; Gonzalez and Loiselle, 2016; Vitorino et al., 2021), seed dispersal (e.g. Machado-de-Souza et al., 2019; Pires and de Melo, 2020; Vitorino et al., 2022; Vizentin-Bugoni et al., 2022), epiphyte-phorophyte interactions (e.g. Sáyago et al., 2013), and plant-ectomycorrhizal fungal networks (e.g. Zhu et al., 2022). However, these studies primarily concentrated on a single type of network at a time, which complicates any comparisons between different types of networks. Documenting the variation in the relative importance of underlying processes across different network types within the same research system is essential. For example, mutualistic networks involving bird-plant pollination and seed dispersal that are constructed using similar sampling techniques and efforts.

Apart from species phenotypes which are related to animal and plant morphological and phenological properties in networks, the nutritional content of plant fruits could also shape seed-dispersal networks in local ecosystems (Jordano, 2000; González-Castro et al., 2015; Pizo et al., 2021; Vizentin-Bugoni et al., 2021). It is expected that frugivorous consumers prefer specific fruits that optimize nutrition acquisition relative to the cost associated with foraging for them (Sobral et al., 2010). Previously, it was thought that energy content of ripe fruit was the most important attribute in efficient seed dispersal (Peters et al., 2010). However, relationship between fruit nutrient composition and species' role (e.g. centrality) in networks indicates that protein, water, and fiber also seem to matter (González-Castro et al., 2015; Sebastián-González, 2017; Lei et al., 2021; Wang et al., 2024). As there is no consensus yet, more empirical studies are needed to elucidate the effect of fruit nutrient content on plant species' roles in maintaining the structure of ecological interactions.

In this study, we studied the bird-plant pollination and seed dispersal mutualistic networks within a subtropical evergreen broadleaf forest in southwest China. We assessed relative importance of species relative abundance, phenological overlap, and morphological constraint/matching on the frequency of pairwise interactions, as well as their relative importance on five aggregate network metrics for both networks. Additionally, we examined the relationship between six fruit nutrient content (water, lipid, protein, fiber, carbohydrate, and energy) and four species-level properties of the seed dispersal network. We hypothesize that: (i) forbidden links, particularly the overlap in phenology, might be the most efficient predictor of interaction frequency for both mutualistic networks; (ii) abundance may also play a significant role in determining interaction frequency between frugivorous birds and fruiting plants, due to the high abundance of fruit-eating birds such as Leiotrichidae and Timaliidae in this evergreen broadleaf forest; (iii) a combination of neutrality and forbidden links could account for most aggregate network metrics, especially nestedness, but it may not explain network specialization and connectance, according to previous researches (e.g. Gonzalez and Loiselle, 2016; Machado-de-Souza et al., 2019; Pires and de Melo, 2020); and (iv) fruit nutrient content could be significantly related to some species-level network properties (Sebastián-González, 2017), for example, fruits with higher energy content could be visited by more bird species.

2. Methods

2.1. Study area and bird-plant interaction

This study was conducted in a subtropical evergreen broadleaf forest from 2300 m to 2600 m elevation (Fig. S1), on the west slope of the north Ailao Mountains, central Yunnan Province, China (E 100°54′-101° 30′, N 23°44′-24°44′). The area has an annual mean temperature of 11.3 °C, with a monthly mean ranging from 5.4 to 23.5 °C. The annual mean precipitation is 1840 mm. Rainy season is from May to October and dry season from November to April (Tan et al., 2011).

Plant-bird interaction data were collected from May 2021 to July 2022. We conducted flowering and fruiting plant surveys 20 m on both sides of five uncrossed transects ranging from 2.1 to 4.4 km within an area of ~35 km^2. Focal observation and infrared camera traps were used to record plant-bird interactions. In our previous surveys, no hornbills and fruit pigeons (*Ducula* and *Ptilinopus*) were recorded in the study area. Therefore, the target fruiting plant species selected were those that produce fruits of an equatorial diameter of less than 30 mm (Corlett, 1998, 2017). Only fruit swallowed was considered as a seed-dispersal interaction (Schleuning et al., 2011). In the study region, there is a scarcity of documented plant species producing large fleshy fruits (>13 mm equatorial diameter), which are typically too large for most birds to swallow whole (Corlett, 1998). Consequently, the equatorial diameter threshold for the fruit observed in this study was 15 mm for *Smilax lebrunii*. According to our prior field observations, the flowers of herbaceous plants in this area are barely visited by birds, with the majority of visits from insects. Consequently, all flowering tree, shrub, epiphyte, and liana species were selected for interaction observation. A total of 31 flowering and 42 fruiting plant species were observed in this study area. Further information can be found in Appendix B: 1.1.

2.2. Interaction probability matrices

To determine the influence of drivers on pollination and seed dispersal network structures, we followed the framework developed by Vázquez et al. (2009b). Given the high mobility of birds and the limited size of the study area (35 km^2), we assumed there were no spatial restrictions to interactions between birds and plants within the local community. Species abundance, phenology and morphological traits of birds and plants were collected in the field and database (details in Appendix B: 1.2). Specifically, the phenology of bird species was assessed based on monthly bird transect surveys, with scores indicating either the presence (1) or absence (0) of each bird species. Similarly, plant phenology was characterized by the presence (1) or absence (0) of ripe fruits or open flowers for each plant species during each month (details in Appendix B: 1.2). For morphological interaction probability matrices, we consider both the constraint model and the matching model. In morphological constraint model (Mor1), we assume that birds tend to have higher probability to interact with flowers with corolla length shorter than beak length, and fruit with equatorial diameter smaller than their gape width (Olito and Fox, 2015; Pires and de Melo, 2020). In the morphological matching model (Mor2), we assumed that birds are more likely to interact with flowers and fruits that exhibit similar corolla and fruit sizes, corresponding to their beak lengths and gape widths, respectively (Sazatornil et al., 2016). A total of eleven interaction probability matrices were generated base on species relative abundance (Abu), phenological overlap (Phe), the morphological constraint model (Mor1), the morphological matching model (Mor2), and all possible combination of these matrices ($n = 7$). These combined matrices represented the anticipated interaction probability under the simultaneous influence of multiple factors. They are Abu*Phe, Abu*Mor1, Abu*Mor2, Mor1 *Phe, Mor2 *Phe, Abu*Mor1 *Phe, and Abu*Mor2 *Phe (details in Appendix B: 1.3). We also constructed a null matrix (Null) in which all plant and bird species had the same interaction probability as 1/ij, where i and j are the number of plant and bird species in the network.

2.3. Probability matrices ability to predict the frequency of pairwise interactions

We used the likelihood approach to test whether relative abundance, phenological overlap or morphological constraint/matching can predict the observed frequency of pairwise interactions (the number of times a pair of species was recorded interacting in the field) (Vázquez et al., 2009b; Vizentin-Bugoni et al., 2014). We assumed that the pairwise probability of interaction between a given plant and a given bird followed a multinomial distribution. We calculated Akaike information criteria (AIC) as $AIC = -2\ln(L) + 2k$, where ln (L) is the log-likelihood of the model, and k is the number of parameters involved in generating the probability matrix. AIC calculation parameters were the total number of bird and plant species, using 54 and 82 parameters for one model (Abundance, Phenology, and Morphology), 108 and 164 for double models (Abu*Phe, Abu*Mor, and Mor*Phe), and 162 and 246 for the full model (Abu*Mor*Phe) for pollination and seed dispersal networks, respectively. As the null matrix was not properly based on a matrix, we assumed it had one parameter (Maruyama et al., 2014; Vizentin-Bugoni et al., 2014). We then calculated the AIC for each model and subtracted each model AIC from the best-fitted AIC model (the lowest AIC) to have an ΔAIC value for each model. The model with the lowest ΔAIC indicated that it provided the best fit to the observed data (Burnham and Anderson, 2004). We calculated likelihood and AIC using the function “dmultinom” in R (R core team, 2024).

2.4. Probability matrices ability to predict aggregate network metrics

We considered five frequently used aggregate network metrics in the analysis of plant-animal mutualistic networks: connectance, nestedness, interaction evenness, interaction asymmetry, and specialization (details in Appendix B: 1.4). A randomization algorithm was used to assess how well the hypotheses predicted the five network metrics for both pollination and seed dispersal networks. We generated 1000 randomized networks for each of the twelve probabilistic matrices maintaining the same interaction numbers as their respective observation (1979 for pollination network and 2051 for seed dispersal) (Vázquez et al., 2009b). We used function “mgen” in R package bipartite, constraining all species to at least one interaction. We calculated mean values and 95 % confidence intervals of the randomized metrics with the function “confint” in R package bipartite. We considered that the predictors could explain the aggregate network metrics whenever we found an overlap between observed values and 95 % confidence intervals.

2.5. Fruit nutrient content and the relationship between nutrients to species-level network properties

Ripe fruits of plant species were collected based on color and softness. According to China national standards, including GB 5009.3–2016, GB 5009.5–2016, GB 5009.6–2016, GB 5009.88–2014, and GB/Z 21922–2008, fruit nutrient content analyses were performed by Yunnan Institute of Product Quality Supervision and Inspection (https://www.ynqsi.org/index.aspx). Nutrient content of each 100 g fruit was measured using different methods: direct drying for water; acid hydrolysis for lipid; Kjeldahl for proteins; and the enzymatic gravimetric method for fiber. Carbohydrate was calculated as 100 minus total water, protein, lipid, ash, and fiber content. Fruit energy content was measured in kilojoules (kJ) and calculated based on the energy derived from protein, lipid, carbohydrate, and fiber. As nearly half of the plant species did not meet the required sample weight threshold of 500 g, finally nutrient data for only 19 species were obtained (Table S15). We investigated the relationships between the six nutrients with Pearson's product-moment correlation coefficients and principal component analysis (PCA) in R. The four selected species-level network properties were species degree, interaction frequency of pairwise interactions, species strength, and species contribution to nestedness. Detailed information can be found in Appendix B:1.5.

To analyze the relationships between fruit nutrient content and species-level network properties, we used R package ggtrendline to build different linear and nonlinear models. The fruit nutrients were set as explanatory variables and species-level network properties as response variables. The optimal model was chosen based on the lowest AIC values (Mei and Montanari, 2022).

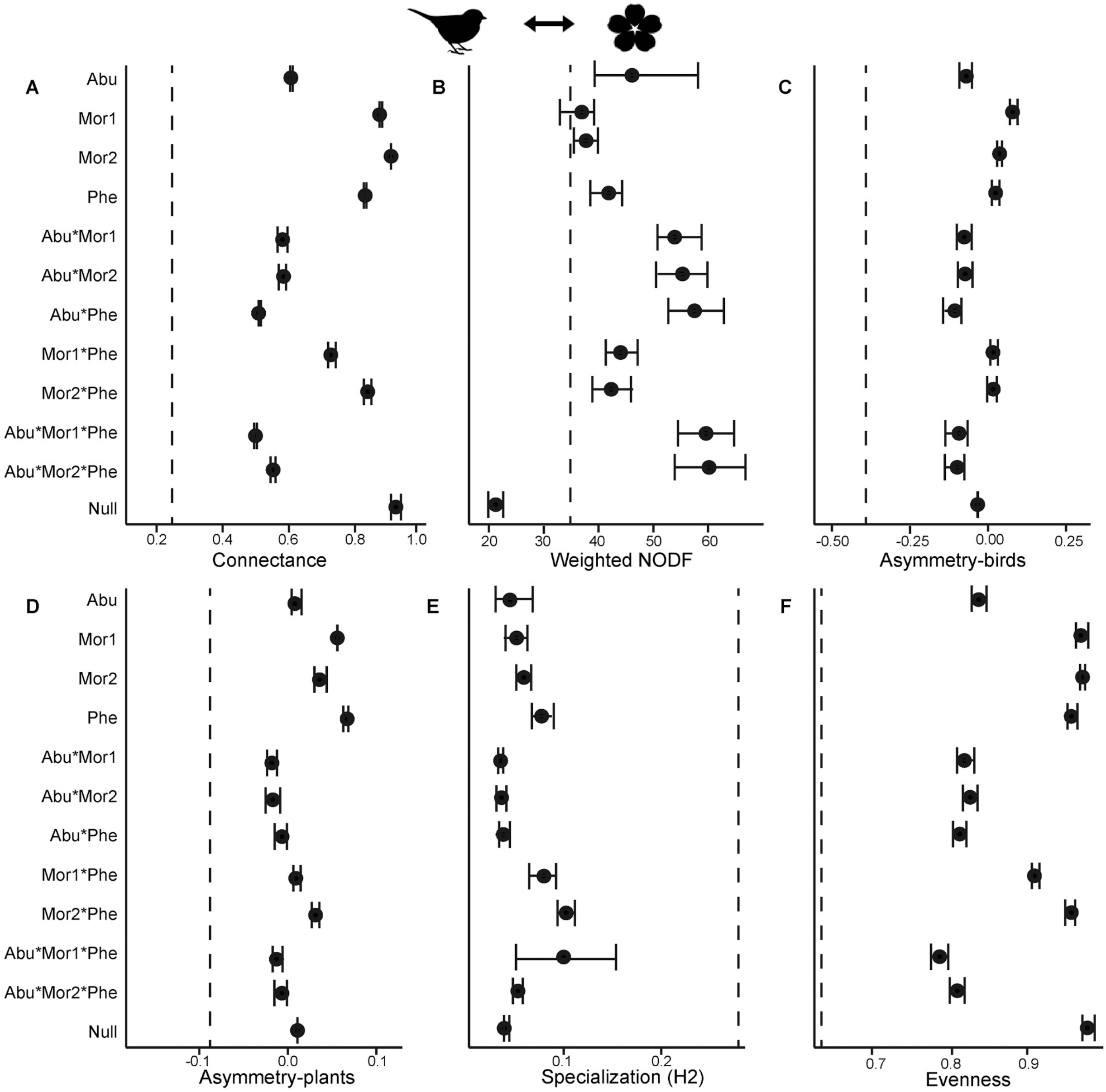

Fig. 1. Network metrics comparisons produced by probability matrices at the mean and 95 % confidence interval and the actual values for the pollination network (dashed vertical lines) of the Ailao Mountains. The probabilistic matrices were constructed based on species abundance, phenology, morphology, and all combinations among them. Mor1 was referred to the constraint model of morphology and Mor2 referred to matching model of morphology (details in Appendix B). Null model was constructed as all plant and bird species had the same interaction probability.

3. Results

3.1. Bird-plant interaction networks

We did not record any interaction events for seven out of 31 flowering plant species, and five out of 42 fruiting plant species (the species name can be found in Appendix B: 2.1). Among plant species that interacted with birds, the number of individuals ranged from four to 41 for flowering plants and from three to 25 for fruiting plants, excluding *Ficus neriifolia*. It was excluded from all analyses because there was only one individual that produced nearly 40 fruits.

In total, 24 flowering plant species belonging to 19 genera, 15 families, and 9 orders were visited by 30 bird species belonging to 22 genera, 13 families, and 1 order. Observations included 1979 pairwise interactions with 169 unique interactions (Table S1, Fig. S2 and Fig. S4). *Aethopyga gouldiae, Yuhina occipitalis*, and *Heterophasia melanoleuca* were the top three nectarivorous bird species in

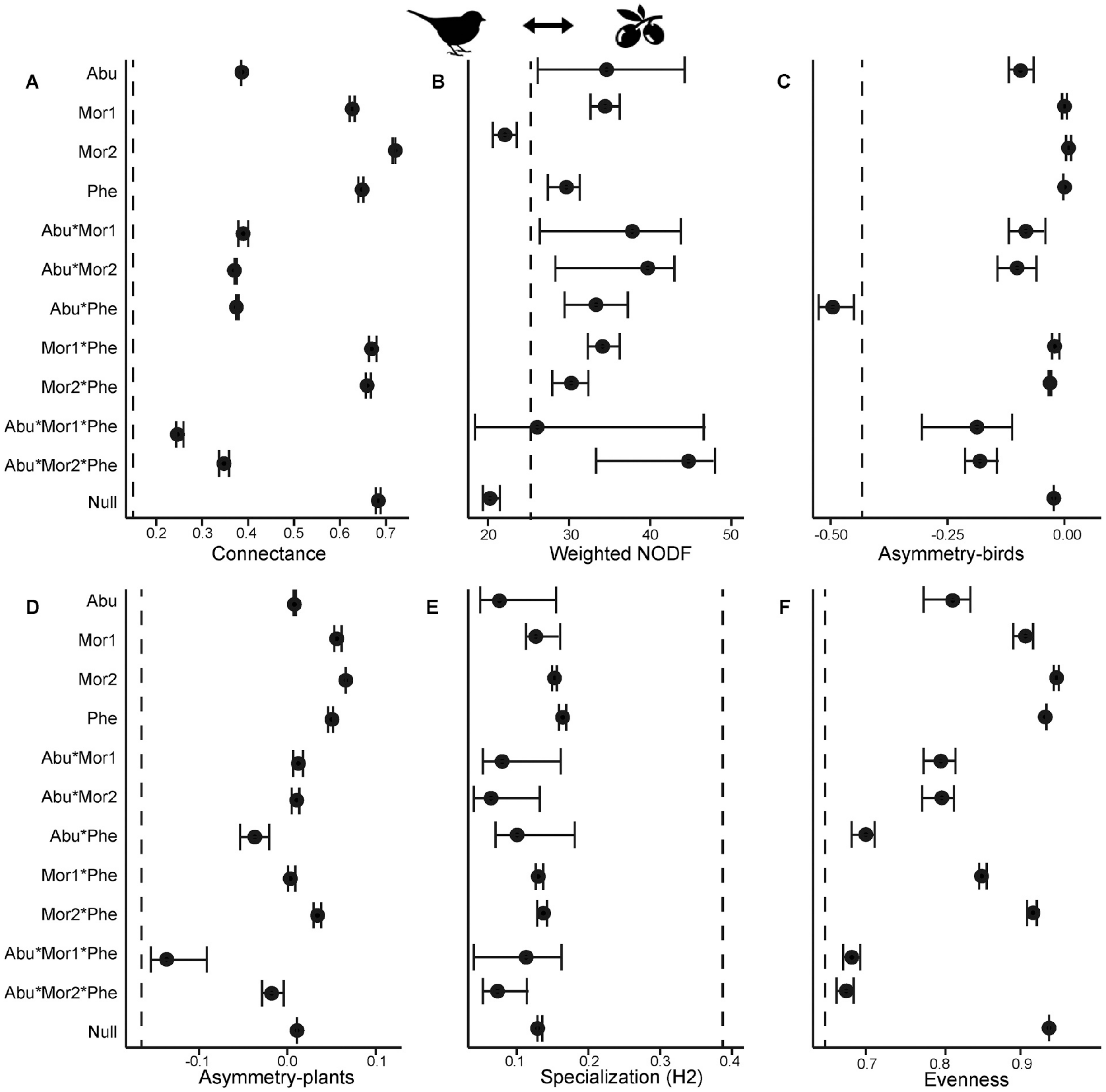

Fig. 2. Network metrics comparisons produced by probability matrices at the mean and 95 % confidence interval and the actual values for the seed dispersal network (dashed vertical lines) of the Ailao Mountains. The probabilistic matrices were constructed based on species abundance, phenology, morphology, and all combinations among them. Mor1 was referred to the constraint model of morphology and Mor2 referred to matching model of morphology (details in Appendix B). Null model was constructed as all plant and bird species had the same interaction probability.

interaction frequency that interacted with 20 (87.5 %), 18 (79.2 %), and 12 (50 %) flowering plant species, respectively. The top three flowering plants were *Cerasus cerasoides*, *Sympoclos sumuntia*, and *Leucosceptrum canum*, which received visits from 16, 12, and 5 bird species, respectively.

We recorded 36 fruiting plant species from 29 genera, 19 families, and 13 orders that were visited by 46 bird species from 33 genera, 13 families, and 2 orders. Observations included 2051 pairwise interactions with 232 unique interactions (Table S2, Fig. S2 and Fig. S3). Of the 46 bird species recorded, *H. melanoleuca*, *Yuhina gularis*, and *Y. occipitalis* were the top three in interaction frequency, engaging with 27 (75.0 %), 24 (66.7 %), and 16 (44.4 %) plant species, respectively. The top three plant species were *Padus grayana*, *Polygonum molle*, and *Eurya jintungensis*, which were visited by 17, 15, and 15 bird species, respectively. In addition, double mutualism (DM) was observed between four pairs of birds and plants (Fuster et al., 2019). They are *Y. occipitalis* and *Gaultheria fragrantissima, Hypsipetes mcclellandii* and *G. fragrantissima*, *Heterophasia melanoleuca* and *Taxillus sutchuenensis*, *Y. occipitalis* and *T. sutchuenensis*.

Both the observed pollination and seed dispersal networks showed similar patterns to those expected. Compared with the null models, they were less connected and had less evenly distributed interactions, more nested, specialized, and had greater asymmetry among bird or plant species (Fig. 1 and Fig. 2, Table S16). Additionally, both networks were well sampled, with their accumulation curves approximating an asymptote, and the observed number of birds and unique interactions approaching expected values (Fig. S5 and Fig. S6).

3.2. Determinants of the frequency of pairwise interactions and aggregate network metrics

The best predictor of pairwise interaction frequency between nectivorous birds and flowering plants was phenological overlap. Conversely, the model that combined species abundance and morphological constraint (Abu*Mor1) had the lowest AIC among the twelve considered models for the seed dispersal network (Fig. 3).

Morphological constraint model of pollination could predict network nestedness (Fig. 1). In addition, morphological matching model was close to the observed nestedness. For the seed dispersal network, the model that combined species abundance, morphological constraint and phenological overlap (Abu*Mor1 *Phe) could predict the network nestedness. Though models considering species abundance or morphological constraint or matching alone, or their combinations with phenological overlap, closely approximated the remaining aggregate network metrics observed except specialization, none of these models were able to predict them (Fig. 2). All probabilistic models are unable to predict network specialization in both pollination and seed dispersal networks.

3.3. Effect of nutrients on species properties in seed dispersal networks

Fruit water content was negatively related to all other nutrients (Fig. S7). The four network metrics were significantly positively

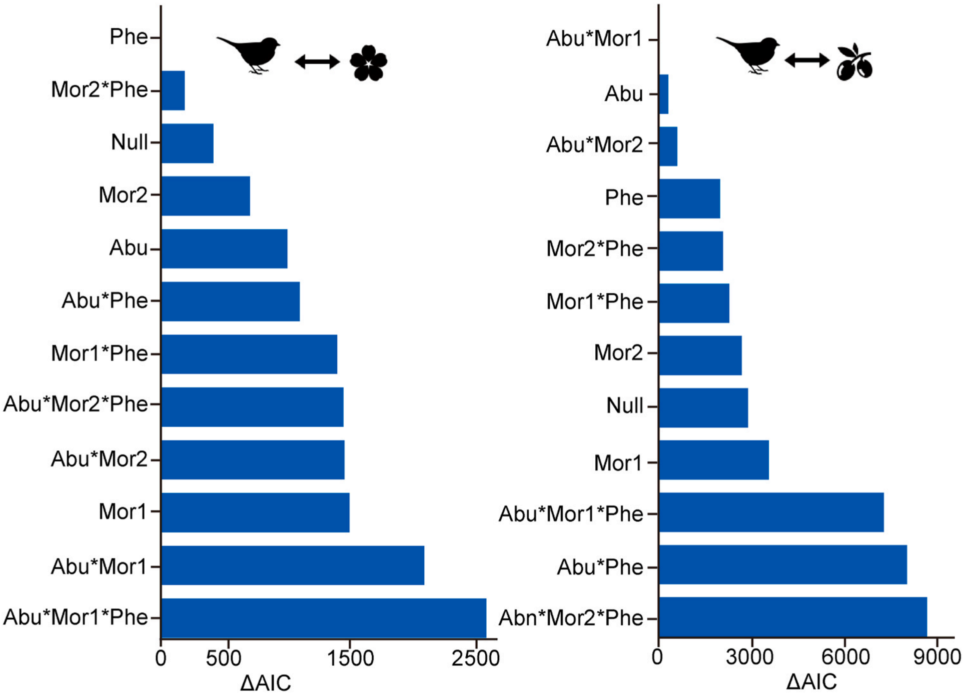

Fig. 3. ΔAIC values of the probabilistic matrices derived from models of species abundance (Abu), phenology overlap (Phe), morphological constraint and morphological matching (Mor1 and Mor2), as well as all conceivable combinations of these factors. Null is a model with all pairwise interactions having the same probability. The best-fitting model was set at ΔAIC = 0.

related to each other (Pearson's correlation coefficient≧0.69) (Fig. S8). Species degree and species strength showed similar relationship patterns with nutrients. They were significantly negatively correlated with nutrition PC1 (Fig. 4). As water had large, positive loadings whereas energy had large negative loadings on PC1 (>0.50) (Fig. S9 and Table S17), we further investigated the relationship between species degree/strength and nutrition PC1 without water. There was no significant relationship between them (Fig. S10 and Table S18). Nutrition PC2 was not correlated with any of the four species-level network properties (Fig. S11).

Among the six nutrients, water exhibited a significant negative correlation with both species degree and interaction strength, while fiber demonstrated a significant positive relationship with both (Figs. 5A and 5D; Fig. S12A and S12D). No significant relationship was observed between nutrient contents and frequency of pairwise interactions, nor between nutrient contents and Z-score (Fig. S13 and Fig. S14).

4. Discussion

Phenological overlap was the most significant driver explaining interaction frequency for the pollination network in the subtropical evergreen broadleaf forest, whereas the combination of neutral processes and morphological constraint could account for the frequency of pairwise interactions for the seed dispersal network. Consequently, our first hypothesis was supported, while the second partially supported. The combination of neutrality, phenological overlap and morphological constraint predict nestedness for seed dispersal network. Furthermore, none of the three metrics, network connectance, evenness or specialization for both types of networks could be predicted by any models considered. As a result, hypothesis three was generally supported, with the exception of the pollination network in which morphological constraint between interacting species could predict nestedness. Additionally, our results supported the fourth hypothesis, which posits that the nutrient content of fruits influence the role or position of plant species within seed dispersal network.

4.1. *Determinants of pairwise interaction frequency*

In this study, phenological overlap emerged as a significant predictor of pairwise interaction frequency in the pollination network, while the combination of relative abundance and morphological constraint could explain the interaction frequency in the seed dispersal network. Neutral-based processes posit that species are ecologically equivalent and have an equal probability of interaction (Vázquez et al., 2007). In the study area, the highest interaction frequency in both pollination and seed dispersal networks was

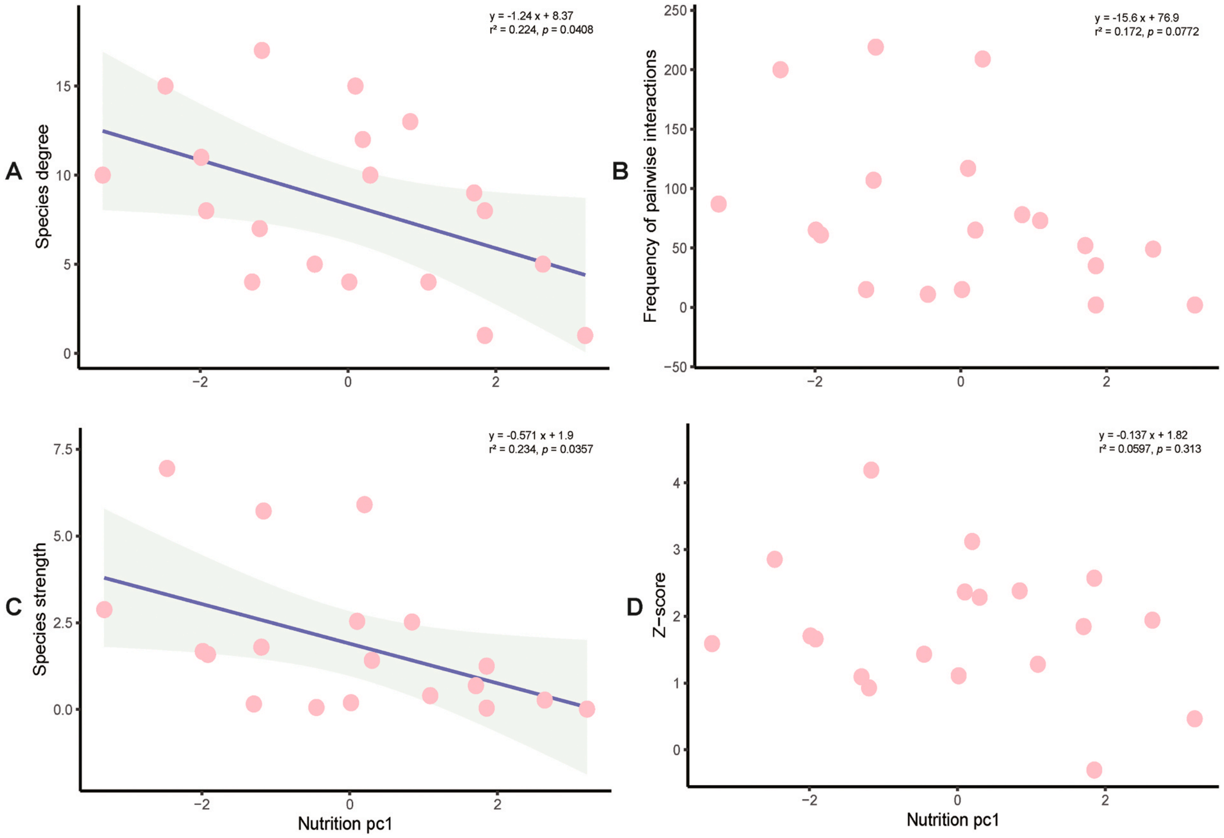

Fig. 4. Correlations between nutrition PC1 and species degree (A), frequency of pairwise interactions (B), species strength (C), and Z-score (D).

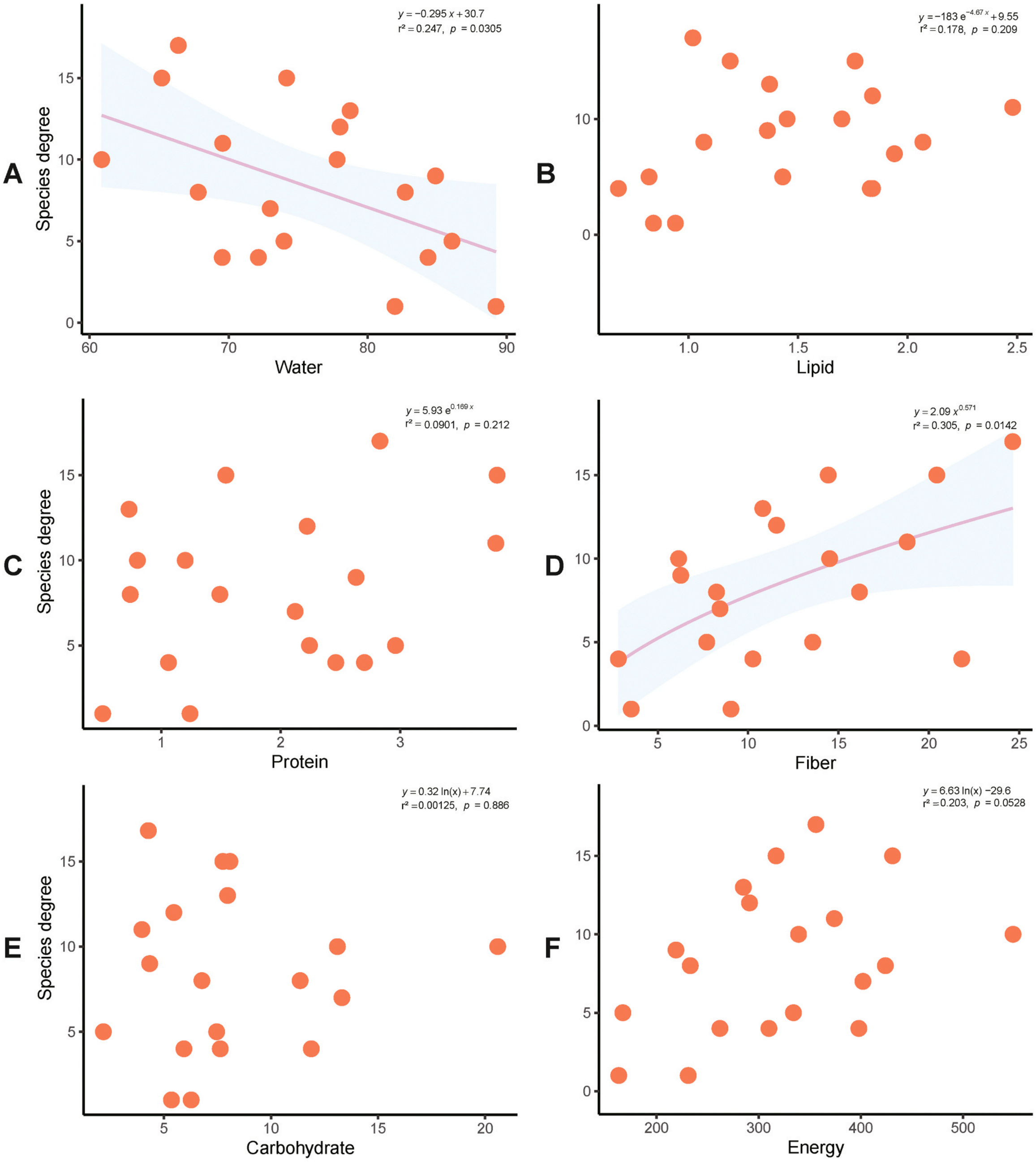

Fig. 5. Relationships between each fruit nutrient and species degree. (A) water, (B) lipid, (C) protein, (D) fiber, (E) carbohydrate, and (F) energy.

observed for the most abundant *Y. occipitalis*. However, species relative abundance was apparently unable to predict pairwise interaction frequency for some species. For instance, although *Aeschynanthus buxifolius* was the third most abundant plant in the pollination network, its interaction frequency with birds ranked below that of 21 other plant species. This may explain why the abundance model was not the best fit for predicting pairwise interaction frequency.

Phenological overlap has been identified as a crucial factor influencing the structure of plant-pollinator networks (Vázquez et al., 2009b; Vizentin-Bugoni et al., 2014). In the study area, most nectarivorous birds recorded within the pollination network were residents, but the flowering season of plants lasted from one to four months. As a result, the importance of phenological overlap could be explained primarily by plants' flowering duration. Phenological shifts, especially in flowering plants, have the potential to alter the structure of the pollination network. Consequently, these changes may impact the fitness of interaction partners (e.g. Schenk et al., 2018).

As previous studies have demonstrated, the morphological constraint or matching could explain pairwise interaction frequency within the seed dispersal network (González-Castro et al., 2015; Peña et al., 2023). Fruit size is influenced by disperser-driven selection (Mack, 1993; Jordano, 1995; Lord, 2004; Eklöf et al., 2013). In the current seed dispersal network, the maximum equatorial diameter of fruits was 10.00 mm (*Sorbus caloneura*). Four out of the five plant species that did not interact with birds produced larger fruits (e.g. *Machilus gamblei*, 14.29 mm). The remaining one had a crop size of less than 50, this means that birds in this subtropical evergreen forests tend to disperse seed from smaller size fruits (Corlett, 1998). What is noteworthy is that morphological constraint, compared with morphological matching, was more important to pairwise interaction frequency. This finding suggests that, at least for the current network, birds are more likely to interact with fruits that have an equatorial diameter smaller than their gape width (Burns, 2013).

4.2. *Prediction of network aggregated metrics*

High nestedness is a prominent structural characteristic of bird-plant mutualistic networks (Bascompte and Jordano, 2014). Both seed dispersal and pollination networks exhibited higher nestedness levels compared with the null model. In pollination networks, morphological constraint model can predict nestedness, meanwhile models considering morphological matching closely align with observed nestedness, highlighting the importance of morphological traits among interacting species. This supports previous research suggesting that forbidden links may explain network nestedness in bird-plant pollination (Gonzalez and Loiselle, 2016). For the seed dispersal network, our study provide further evidence for the importance of both neutrality and forbidden links in shaping the structure of seed dispersal networks, particularly with regard to nestedness (Machado-de-Souza et al., 2019).

Interaction strength asymmetry, especially for birds, can be predicted by a combination of neutrality and forbidden links in insect pollinator-plant (Stang et al., 2007; Vázquez et al., 2009b), hummingbird-plant (Vizentin-Bugoni et al., 2014), and bird-seed dispersal (Pires and de Melo, 2020) networks. In our study, no probability matrix could predict asymmetry for both birds and plants in seed dispersal and pollination networks. Nevertheless, predictions from Abu*Mor1 *Phe probability matrices and Abu*Phe were closest to the observed asymmetry of plants and birds respectively for seed dispersal. This suggests that interaction strength asymmetry could be driven by both neutrality and forbidden links (Stang et al., 2007). Additionally, asymmetry in birds was higher than in plants for both network types, which was consistent with former research (Vázquez et al., 2007; Vizentin-Bugoni et al., 2014; Gonzalez and Loiselle, 2016; Pires and de Melo, 2020).

None of the considered models managed to adequately explain connectance, evenness, and specialization for either network, which is consistent with previous research (Vizentin-Bugoni et al., 2014; Machado-de-Souza et al., 2019). The observed networks exhibited lower connectance, lower evenness, and higher specialization than those in any of the models, suggesting that there might be other underlying drivers for the observed characteristics of the overall network (Olito and Fox, 2015; Machado-de-Souza et al., 2019). For instance, phylogenetic relationships among species (Ives and Godfray, 2006; Rezende et al., 2007; Benadi et al., 2022), bird forest strata preference (Schleuning et al., 2011), or fruit nutritional content (Blendinger et al., 2016; González-Castro et al., 2015;) may influence mutualistic networks structure. Additionally, network characteristics such as specialization (H2) are not necessarily ideal indices for examining underlying network processes (Vizentin-Bugoni et al., 2014; Machado-de-Souza et al., 2019; Pires and de Melo, 2020).

4.3. *Fruit nutrient preference and species role in networks*

Fruit nutrient contents may influence plant species position (importance) in seed dispersal networks (e.g. Sebastián-González, 2017). However, they could not be incorporated into the probability matrix framework as there is no widely accepted relationship between them. Consequently, we investigated the relationship between nutrients and four species-level network properties separated from the probability matrix method. Among the six nutrients, water and fiber were significantly related to species degree and species strength. The negative relationship between PC1 and both species degree and strength can primarily be attributed to the influence of water.

Species with a large species degree may play an important role due to their extensive interactions with a wide range of partners (Bascompte and Jordano, 2007). In our study, as water content decreased and fiber content increased, plants were visited by more bird species, suggesting that frugivorous species preferred fleshy fruits with lower water and higher fiber content. Similarly, plant species strength was negatively correlated with fruit water content within plant-frugivore interaction networks in a seasonal tropical forest (Ramos-Robles et al., 2018). Our findings provide more evidence that water content was related to the importance of plants in terms of frugivory, and the maintenance of network structure. Although water is crucial for frugivores, higher water content can lower the proportion of other nutrients (Herrera, 1982; Jordano, 2000; Lei et al., 2021). For instance, fiber was identified as an important nutrient affecting pairwise interaction frequency within a seed dispersal network in a small Mediterranean scrubland (González-Castro et al., 2015). Previous studies in poultry have shown that fiber is essential for regular digestive organ functioning, including nutrient digestion, fermentation, and absorption processes (González-Alvarado et al., 2008; Mateos et al., 2012). Therefore, fiber might also contribute to gastrointestinal tract development in wild frugivorous birds. Further research is needed to provide additional evidence.

Frugivores may seek fruits that best satisfy their nutritional demands, as the consumption of highly energetic fruits could maximize bird species' fitness (Peters et al., 2010). Fruit energy content was identified as the most significant characteristic determining the role of plants among nutrient content and morphological traits in 23 seed-dispersal networks (Sebastián-González et al.; 2017). In addition, lipid was found to be positively associated with species' role of seed dispersal network in O'ahu, Hawaiian Archipelago (Vizentin-Bugoni et al., 2021). Fruit energy content was positively correlated with lipids and carbohydrates, as indicated by previous (e.g. Cazetta et al., 2008). However, neither energy content nor lipid and carbohydrate levels demonstrated a correlation with the selected four species-level network properties. It is noteworthy that the role of species in seed dispersal can be represented by various indices, including within-module and between-module connectivity (Olesen et al., 2007), which were not taken into account in the current study. As a result, further investigation is necessary.

5. Conclusions

This study is the first to evaluate the processes driving pairwise interaction frequency and network structure in both bird pollination and seed dispersal networks within a subtropical evergreen broadleaf forest. We revealed different processes shaping the structure of these two mutualistic networks. Specifically, phenological overlap was the most important explanation for pairwise interaction frequency in pollination network, whereas a combination of neutrality and morphological constraint could explain pairwise interaction frequency in the seed dispersal network. Morphological constraint among species in the pollination network could predict nestedness. The combination of species neutrality, phenological overlap and morphological constraint could explain the nestedness of the seed dispersal network. Network interaction asymmetry, connectance, evenness, and specialization of both network types could not be predicted by any of our models. We also provided evidence that fruit water and fiber content are important nutrients affecting both the species degree and interaction strength in seed dispersal networks, highlighting the importance of birds' preference for nutrient profiles in defining species interactions. Our findings should encourage further research on the variation of the relative importance of driving forces for different mutualistic network types along spatial and temporal scales.

Ethics statement

This manuscript does not include human or animal research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2025.e03523.

Data availability

Data will be made available on request.

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