



Experimental evidence of the importance of multitrophic structure for species persistence

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Ecological theory predicts that species interactions embedded in multitrophic networks shape the opportunities for species to persist. However, the lack of experimental support of this prediction has limited our understanding of how species interactions occurring within and across trophic levels simultaneously regulate the maintenance of biodiversity. Here, we integrate a mathematical approach and detailed experiments in plant–pollinator communities to demonstrate the need to jointly account for species interactions within and across trophic levels when estimating the ability of species to persist. Within the plant trophic level, we show that the persistence probability of plant species increases when introducing the effects of plant–pollinator interactions. Across trophic levels, we show that the persistence probabilities of both plants and pollinators exhibit idiosyncratic changes when experimentally manipulating the multitrophic structure. Importantly, these idiosyncratic effects are not recovered by traditional simulations. Our work provides tractable experimental and theoretical platforms upon which it is possible to investigate the multitrophic factors affecting species persistence in ecological communities.

species interactions | competition | mutualism | persistence | probability

Understanding how interactions among species modulate their ability to persist is a long-standing topic of research in ecology with direct implications for management, conservation, and restoration of natural systems (1, 2). Over the last decades, ecological theory has revealed important factors associated with the capacity of species to persist when sharing similar biotic and abiotic resources with other species (3–5). On the one hand, the majority of this work has focused on describing species dynamics of small communities within a given trophic level (6). This work has shown that differences between intraspecific and interspecific interactions are key determinants of species persistence (7). While recent studies have started to incorporate information about multitrophic interactions into this framework, the quantitative analyses have remained within single trophic levels (8–13). On the other hand, studies based on a more phenomenological framework of ecological dynamics have revealed how the structure of who interacts with whom across trophic levels affects species persistence (14–16). However, this work has typically ignored potential variation of species interactions and dynamics within each trophic level. Despite the fact that each approach (within and across trophic levels) has provided important insights regarding the factors shaping species persistence, it has remained unclear the extent to which their integration is necessary for a better understanding of how biodiversity is maintained in multitrophic ecological communities (17).

The integration of ecological theory looking at species persistence within and across trophic levels has revealed that the body of classic approaches to study dynamics within trophic levels do not generalize well to multitrophic cases for three main reasons (18–20). First, classic stability metrics for the two-species case cannot be directly extended to larger communities (18, 20).

Second, the processes determining the outcome of species interactions in two-species communities are not independent (19). This is because species interactions are involved in the definition of both stabilizing and fitness differences (see ref. 21 for an empirical example), which makes it difficult to mechanistically understand what combination of interaction strengths allows species to persist. Third, most of the approaches to study species persistence are based on invasibility analysis (see ref. 22 for a review). That is, coexistence predictions are only valid under the assumption that any species can be removed from a community and the rest of the community can persist. This is likely not the case within a multispecies context (18, 20, 23, 24). The severity of these limitations has established a call for new approaches that can investigate species persistence in multitrophic communities (17, 25).

Recently, theoretical work based on a structuralist approach has introduced clear guidelines about the study and integration of species persistence within and across trophic levels (20, 26–29). This has brought the opportunity to investigate the effects of multitrophic structures in determining species persistence in small and large ecological communities. The core of this structuralist approach establishes that species persistence (or more generally, the diversity of forms in nature) is defined by two key factors (30): internal constraints given by a fixed set of biotic conditions (e.g., species interactions within and across trophic levels) and external forces acting as free-changing abiotic conditions (e.g., abiotic conditions determining species intrinsic

Significance

It has been unclear whether understanding how biodiversity is maintained requires us to study species interactions within and across trophic levels simultaneously. Achieving this task remains, however, challenging for practical and theoretical reasons. Here, integrating a simple but detailed experimental plant–pollinator community and a tractable mathematical framework, we show that biodiversity is strongly affected by species competitive interactions among plants and among pollinators, as well as the mutualistic effects between pollinators on plants. Furthermore, we show that experimentally preventing some species to interact can modify the rest of the interactions and affect idiosyncratically the probability of species persistence. These effects are only observable within the empirical evaluation and not with traditional simulation approaches.

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growth rates). Following the structuralist approach, theoretical work has introduced a probabilistic formalism to unify the deterministic nature of mathematical models (the potential rules of design) and the intrinsic variability of real-world systems (driven by unknown and changing external factors) (27–29). Specifically, this theoretical work assumes that the biotic constraints defining the interaction structure of a community establish the possible range of abiotic conditions compatible with the persistence of individual species and of the entire community (27–29). Therefore, the larger the range of compatible abiotic conditions under given biotic constraints, the larger the probability of species persistence. However, these theoretical predictions have not been experimentally tested in multitrophic communities.

To address this gap, here we propose to experimentally assess the effects of multitrophic structure (i.e., within and across trophic levels) on the opportunities for species to persist in a synthetic plant–pollinator community. Specifically, we conduct a detailed controlled experiment to assess the contribution of plant–plant, pollinator–pollinator, and plant–pollinator interactions to species persistence in an experimentally assembled six-species community. Building on our theoretical framework, first we test the theoretical expectation that multitrophic structures can increase the persistence probability of plant communities (14, 16). That is, we investigate the extent to which the average persistence of competing plant species (within a single trophic level) changes when incorporating the effects of plant–pollinator interactions. Second, we investigate to what extent changes to multitrophic structures do not affect equally the persistence of species across trophic levels. That is, we investigate the direction and extent to which the persistence of both plants and pollinators changes when experimentally manipulating a single plant–pollinator interaction. Third, we investigate whether these potential multitrophic effects can be recovered by using traditional simulated manipulations of the multitrophic structure. Finally, we discuss our results in light of the neces-

sity to integrate information within and across trophic levels to increase our understanding of species persistence in multitrophic communities.

Experimental Setup and Theoretical Framework. We conducted an experimental design to study the effect of changes in the multitrophic structure of a synthetic plant–pollinator community on the persistence of species within and across trophic levels. Specifically, we experimentally assembled 17 sealed cages of 3 m³, each with three plant species (radish: *Raphanus raphanistrum*, tomato: *Solanum lycopersicon*, and field bean: *Vicia faba*) and different combinations of three pollinator species (bumblebees: *Bombus terrestris*, mason bees: *Osmia bicornis*, and green bottle flies: *Lucilia sericata*) under contrasting competition regimes within trophic levels and multitrophic structures. To obtain different competition regimes within trophic levels, in all cages we displayed pots of 150 L containing the three plant species growing alone and together both at different relative abundances and densities following a spatial explicit design. Additionally, all cages were divided into different plant treatments with no pollinators, with one pollinator species alone at different densities of plants and pollinators, and with combinations of two pollinator species (*SI Appendix, Fig. S1*). To obtain different multitrophic structures, we selected species with matching and nonmatching morphological traits to create a fully nested multitrophic structure, where generalist species (with more than one interaction) interact with all species but specialist species (with only one interaction) interact only with generalists (Fig. 1A). To change the multitrophic structure, in some cages we physically prevented the bumblebee–radish interaction from the original design (Fig. 1B). This manipulation was achieved using a large mesh size protecting the radish flowers that excluded bumblebees but allowed the visitation of the other two smaller species (see *Materials and Methods* for further details).

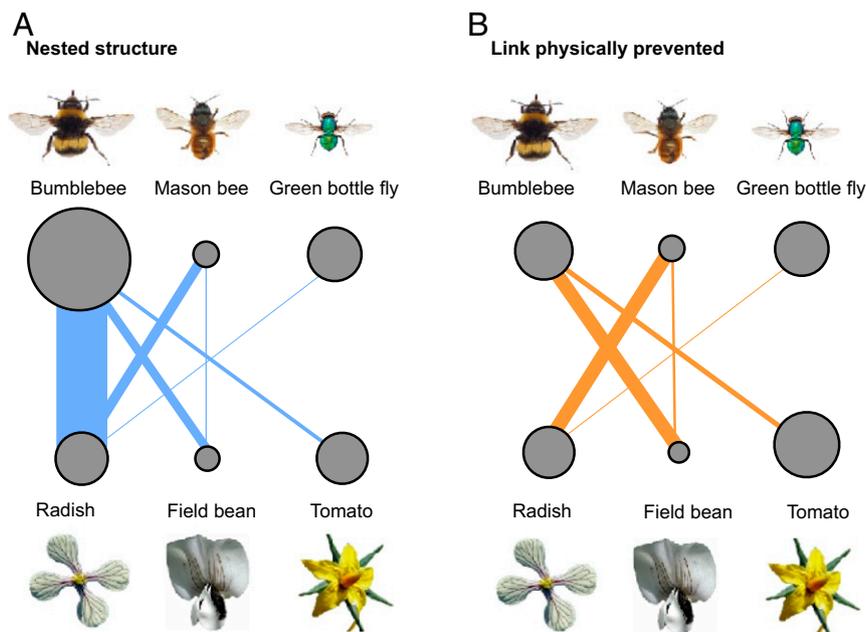


Fig. 1. Scheme of the two network topologies empirically evaluated. The community consisted of three plants (radish: *R. raphanistrum*, tomato: *S. lycopersicon*, field bean: *V. faba*) and three pollinators (bumblebees: *B. terrestris*, mason bees: *O. bicornis*, green bottle flies: *L. sericata*). This figure portrays the number of visits recorded as links (the thickness of the line is proportional to the link strength, i.e., number of pollinator visits) and the observed reproductive success (proportional to circle sizes). For our studied community, we constructed two network topologies of species interactions. (A) A fully nested interaction network. (B) The topology when the interaction between bumblebee and radish is experimentally prevented from the fully nested topology. Note that the thickness of links and size of nodes are different between the two topologies due to the manipulation and subsequent reorganization of the rest of the interaction strengths.

To establish a quantitative analysis, we used our experimental study to field-parameterize a Lotka–Volterra (LV) model describing the annual dynamics of a plant–pollinator community (17) (see *Materials and Methods* for further details). While the LV model is a parsimonious representation of population dynamics without spatial constraints that typically assumes that abiotic dynamics are faster than species dynamics (31), LV models have successfully explained and predicted the dynamics of diverse ecological systems (28, 32–35). In the idealized dynamics of the LV models, the multitrophic structure of a community is phenomenologically captured by the interaction matrix $\mathbf{A} = \{a_{ij}\}^{S \times S}$, where a_{ij} represents the per-capita biotic effect of species j on the per-capita growth rate of species i and S is the number of species in the community. That is, it is assumed that interactions between species do not change across time (any change would be a consequence of an external perturbation outside the established population dynamics). In turn, in the LV model, the vector or intrinsic growth rates $\mathbf{r} = \{r_i\}^S$ represents how species grow in isolation under given abiotic conditions. To parameterize this model, we used linear regression to fit the data from our experimental design into the LV model (see *Materials and Methods* for further details). We experimentally parameterized the LV model for the treatment with plants only; we call these parameters the pair $(\mathbf{A}_p, \mathbf{r}_p)$. We also parameterized the treatment with plants and pollinators together under the original multitrophic structure [what we call the parameter pair $(\mathbf{A}_o, \mathbf{r}_o)$] and the treatment with plants and pollinators together under the experimentally manipulated multitrophic structure [what we call the parameter pair $(\mathbf{A}_m, \mathbf{r}_m)$].

Next, we studied the dynamics generated from a simulated manipulation of the original interaction matrix (what we call \mathbf{A}_z) with the bumblebee–radish interaction set to zero ($a_{ij} = a_{ji} = 0$, where i and j correspond to radish and bumblebee, respectively). This simulation mimics the experimental manipulation without considering a potential reorganization of species interactions across the multitrophic structure. In addition, we studied the dynamics generated by potential reorganizations of species interactions following the simulated removal of the bumblebee–radish interaction (which was set to zero, as mentioned above). Because we have no a priori information that dictates how a reorganization should take place in the community and any assumption would be untested, we simply drew randomly interaction strengths from a normal distribution with same mean as the original interaction strengths (\mathbf{A}_o) and variance equal to the variation in interaction strength observed between \mathbf{A}_o and \mathbf{A}_m (see *Materials and Methods* for further details). This null model provides an expectation of population dynamics considering a naive nondirectional reorganization of species interactions. Note that this reorganization is different from a full reorganization (36) given that we preserved matching traits by keeping all of the original absence of interactions between species.

To estimate the effect of multitrophic structure on species persistence, we calculated the persistence probability of species under each specific treatment following a structuralist approach (20, 28). Specifically, the persistence probability of a species is calculated as the fraction of different directions of \mathbf{r} -vectors that would lead to positive abundances at equilibrium (i.e., $\mathbf{N}^* = \mathbf{A}^{-1}\mathbf{r}$) for a given species i that forms part of an interaction matrix \mathbf{A} . This methodology assumes that we have no a priori knowledge of how exactly external perturbations will impact the intrinsic growth rate and, consequently, the probability is the survival expectation of a species across all abiotic conditions selected with equal likelihood and arbitrary initial conditions (28). Thus, first, to study the effect of multitrophic structures within trophic levels, we compared the persistence probability of an average plant species without $[\omega(\mathbf{A}_p)]$ and with pollina-

tors under the original $[\omega(\mathbf{A}_{op})]$ and manipulated multitrophic structures $[\omega(\mathbf{A}_{mp})]$. Note that the matrix \mathbf{A}_p is a 3×3 matrix (plants only). Hence, to make fair comparisons, we extracted matrices \mathbf{A}_{op} and \mathbf{A}_{mp} as the subsets [the block $\alpha^{(P)}$, plants only] of the matrices \mathbf{A}_o and \mathbf{A}_m , respectively (see *Materials and Methods* for further details). These submatrices represent the structure of plant interactions in the original and experimentally modified communities, respectively. Second, to study the effect of multitrophic structures across trophic levels, we compared the persistence probability $[P(i|\mathbf{A})]$ of each individual plant and pollinator under the original (\mathbf{A}_o) and manipulated (\mathbf{A}_m) multitrophic structures. Third, to study whether potential multitrophic effects can be recovered by simulating the manipulation without considering additional reorganization effects (i.e., strengthening or weakening the original interaction strengths) on the multitrophic structure, we compared the persistence probability $[P(i|\mathbf{A})]$ of each individual plant and pollinator under the experimental (\mathbf{A}_m) and simulated manipulations (\mathbf{A}_z). Finally, we also compared the observed persistence probability $[P(i|\mathbf{A})]$ against our naive null model assuming a nondirectional reorganization of species interactions after the simulated manipulation (see *Materials and Methods* for further details).

Results

In line with previously established theoretical expectations (14, 16), we found that incorporating plant–pollinator interactions increased the persistence probability of plant species (Fig. 2). Specifically, we found that the persistence probability of an average plant species was $\omega(\mathbf{A}_p) = 0.29$ without incorporating the effects of plant–pollinator interactions. Once these multitrophic effects were introduced, the probability increased to $\omega(\mathbf{A}_{op}) = 0.60$ and $\omega(\mathbf{A}_{mp}) = 0.75$ under the original and experimentally manipulated multitrophic structures, respectively. This result agrees with theoretical expectations that certain plant–pollinator structures can increase the overall persistence of a community by both decreasing the effective competition acting within trophic levels and increasing the range of intrinsic growth rates compatible with species coexistence according to the observed species interactions (9, 14). For example, we observed that the field bean had a positive effect on tomato when pollinators are present but negative otherwise. This may reflect the fact that field beans can increase nitrogen availability, but this benefit may be only expressed when pollinators maximize seed production. Importantly, the different average effects on plant persistence generated by the original and manipulated multitrophic structures raised the question of how these benefits are distributed over the individual species across the two trophic levels.

Addressing the question above, we found that the persistence probabilities $[P(i|\mathbf{A})]$ of individual plants and pollinators exhibited idiosyncratic changes when experimentally manipulating the multitrophic structure (Fig. 3). In particular, we found that the persistence probabilities of individual species changed in different directions and strengths under the original $[P(i|\mathbf{A}_o)]$ and manipulated $[P(i|\mathbf{A}_m)]$ multitrophic structures (Fig. 3A). That is, changes were not proportional across all species; that is, $P(i|\mathbf{A}_o)/P(i|\mathbf{A}_m) \neq P(j|\mathbf{A}_o)/P(j|\mathbf{A}_m)$. As expected, the two species linked to the manipulated interaction (radish and bumblebee) decreased their persistence probability [i.e., $P(i|\mathbf{A}_o) > P(i|\mathbf{A}_m)$], while the other four species increased it [i.e., $P(i|\mathbf{A}_o) < P(i|\mathbf{A}_m)$]. Note that the persistence probability of an average species from the plant–pollinator community was similar in both the original $[\omega(\mathbf{A}_o) = 0.24]$ and manipulated $[\omega(\mathbf{A}_m) = 0.25]$ multitrophic structures. This reveals that in our experiment any potential difference in species persistence between the two structures is the result of a redistribution of benefits rather than a single directional change.

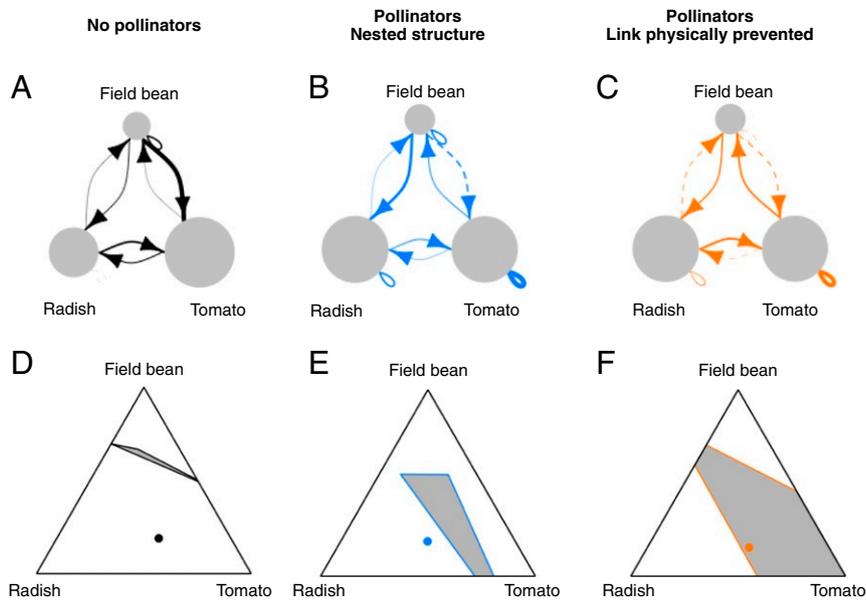


Fig. 2. Effects of multitrophic structure within a trophic level. (A–C) A graphical representation of the community structure within the trophic level formed by plants in each of the empirical scenarios investigated: plants with no pollinators, plants with pollinators, and plants with pollinators with the bumblebee–radish interaction experimentally prevented. In the illustration, plants’ intrinsic growth rates are proportional to circle size and the strength of their interactions is proportional to thickness of arrows. Solid and dashed lines imply negative and positive interactions, respectively. (D–F) The simplex representation of the feasibility domain (the sum of intrinsic growth rates is normalized to one). The size of the feasibility domain (colored area) corresponds to the fraction of directions of intrinsic growth rates compatible with the persistence of the three species. Note that the feasibility domain is a function of the species interactions (shown in A). The solid dot corresponds to the direction of the vector of the observed intrinsic growth rates (also shown in A). Note that this simplex is the normalized projection in an $n - 1$ dimensional space of the original parameter space of intrinsic growth rates. Each vertex then represents a basis vector of this parameter space, that is, $\{1, 0, 0\}$, $\{0, 1, 0\}$, or $\{0, 0, 1\}$. Consequently, each vertex represents the position where one species in particular dominates the entire parameter space.

Importantly, we found that the observed effects of the experimental manipulation were not recovered by traditional simulated manipulations. First, we focused on the simulated manipulation that does not consider any additional reorganization (i.e., just setting to zero the radish–bumblebee interaction). Fig. 3A shows that the persistence probabilities of both plants and pollinators remained almost identical under the original and simu-

lated manipulation. That is, the mean relative change between $P(i|\mathbf{A}_0)$ and $P(i|\mathbf{A}_z)$ was only 2%. By contrast, the probabilities derived from the experimental manipulation displayed a large difference from the original probabilities (Fig. 3A). The mean relative change between $P(i|\mathbf{A}_0)$ and $P(i|\mathbf{A}_m)$ was 27% (paired Wilcoxon test between experimental and simulated results yield a P value of 0.03; *SI Appendix, Fig. S3*). Then, we focused on the

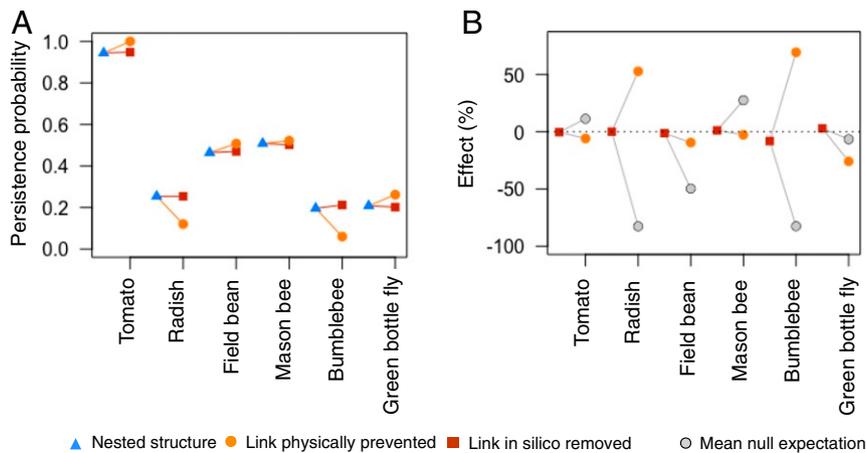


Fig. 3. Effects of multitrophic structure across trophic levels. (A) The probability of persistence for each species estimated from a structural stability analysis. For each species we estimate the probability within each multitrophic structure: original fully nested structure (blue triangles), the in silico-manipulated structure (bumblebee–radish interaction set to zero; red squares), and the physically manipulated structure (bumblebee–radish interaction experimentally prevented from the original structure, orange circles). (B) The relative changes in persistence probability (i.e., effect) of the in silico-modified network structure (red squares), as compared with the relative changes in persistence probability of the experimentally modified network structures (orange circles) and the mean null probability expected under random interaction reorganization. Note that the in silico-modified network barely changes, while the observed experimental changes are often in opposite direction to the expected (mean) values derived from the null model.

simulated manipulation that takes into account a nondirectional reorganization of species interaction strengths. Specifically, we calculated how the direction of the persistence probability in the original community changed after the experimental and simulated manipulations. Fig. 3B shows that the directions (increase or decrease) of change in the experimentally manipulated community were mostly the opposite to the ones expected under the simulated manipulation. These results illustrate that when an interaction is experimentally (or naturally) manipulated by preventing its occurrence (as we did in our study), secondary modifications such as the reorganization of interaction strengths happen across trophic levels (SI Appendix, Fig. S4). Furthermore, these results imply that this reorganization is directional; however, not all species experience it in the same way (i.e., while some species may increase others may decrease their probability of persistence). This result aligns well with the strong evidence found in natural systems that different environmental impacts are modifying the interaction structure of plants and pollinators, such as the increased densities of managed species (37) or phenological mismatches due to climate warming (38, 39).

Discussion

Our findings have shown that the multitrophic structure defined by species interactions occurring simultaneously within and across trophic levels strongly regulates the possibilities for species to persist in ecological communities. That is, the effects occurring within and across trophic levels percolate across the two scales. Thus, work that does not take into account this integration can be underestimating or overestimating species persistence. Moreover, we have shown that traditional simulated changes to the multitrophic structure did not recover the experimental impact on the community. Note that classic simulation studies looking at the robustness of ecological communities (40) have typically not considered these additional and directional modifications observed in empirical studies (36, 41). Indeed, although our results are sensitive to the specific experimental conditions and the idealized model considered, they have highlighted the importance and challenges of parameterizing species interactions and their changes within and across trophic levels simultaneously.

While experimental approaches can be difficult to scale up, the combination of simple models and heuristic processes (42) can facilitate the inference of parameters needed to estimate the expected additional modifications acting across multitrophic structures. Moreover, field observations can be another alternative to experimental approaches in which variation of environmental conditions creates natural changes in species interaction strengths and network structure (12, 43). A third source of analyses can come from extracting information of species interactions based on long-term changes in species abundances or biomass (44). We therefore believe there are available several experimental and observational methods that can be combined with recent theoretical advances in order to adopt a more integrative multitrophic perspective that can increase our understanding about the emergence and robustness of ecological communities.

Finally, it is important to remember that regardless of our ability to obtain experimental information to parameterize population models describing the dynamics of interacting species, ecological dynamics in a broad sense are probabilistic due to the multiple unknown factors affecting species persistence (29, 45, 46). This implies that it is critical to integrate methodologies that can take into account the deterministic and stochastic nature of ecological communities (28). In this line, the structuralist approach that we have used in this study separates the internal rules of a community (given by the assumed population model and by the structure of species interactions both competitive and mutualistic in our case) from the external factors (the chang-

ing abiotic conditions acting on intrinsic species properties such as their population growth rates) contributing to the observed diversity (30). That is, the essence of the structuralist approach is to provide expectations of what is possible and what is not in nature. While details will change depending on the internal and external factors considered in a study, focusing on the degree to which species interactions embedded in multitrophic networks shape opportunities (probabilities) for species to persist can get us closer to a general understanding about the maintenance of ecological communities across a diverse set of contexts.

Materials and Methods

Experimental Design.

The experiments were run in 2017 at Instituto de Recursos Naturales y Agrobiología de Sevilla—Consejo Superior de Investigaciones Científicas facilities (Finca “La Hampa”, <https://www.irnas.csic.es/en/finca-experimental/>). We assembled 17 3-m³ cages sealed with a 1-mm mesh size (Howitt S.L.) to avoid the entrance of other pollinator insects from the outside. All cages had 10 (or five for the low plant abundance cages) 150-L pots, where all three plant species are grown alone and together at different relative abundances and densities following a spatial explicit design. We measured plant reproductive success as the number of seeds per individual in 836 individuals across the different cages. This allows us to estimate the plants’ intrinsic growth rates and the strength of intraspecific and inter-specific interactions (SI Appendix, Fig. S1). Sixteen cages were divided in treatments with no pollinators, with one pollinator species alone at different densities of plants and pollinators, or with combinations of two pollinators together (SI Appendix, Fig. S1). Bumblebees and flies were provided by Koppert and mason bees by WAB-Mauerbiennenzucht. This design also allows estimating the strength of competitive interactions among pollinators as well as the mutualistic effects of pollinators on plants, and vice versa (Inference Analysis). Note, however, that we did not account for the effect of environmental variability on species persistence because interaction strength estimations were done between two time steps (i.e., we account only for a single generation). Prior knowledge on plant and pollinator species allowed building a fully nested plant–pollinator network as follows. Cherry tomato, which only produces pollen and requires buzz pollination to extract the pollen from its anthers, acts as a specialist plant only visited by bumblebees. Field bean has a closed flower, accessible only to big and medium bees, and therefore is visited by both bumblebees and mason bees. Radish shows, in contrast, an open flower attracting all three pollinator species. All three plant species are partially self-compatible, which means that they can produce offspring without pollinators, yet pollinators enhance seed production. Bumblebees were brought in small nest cages (10 workers, which were kept constant through the experiment) and offspring production was measured as the number of new workers produced divided by 3 to estimate the expected number of reproductive queens (47). Mason bee cocoons were kept at low temperatures until the start of the experiment. Nesting sites were provided to allow a natural establishment and foraging following (48) and offspring production per female was measured at the end of the experiment. Finally, while flies are not central-place foragers; adults feed on nectar and lay eggs in rotten meat. Larvae develop quickly and fall to the ground to spend the winter there. We placed plastic trays under small pieces of liver standing in a rack and collected the larva daily. To modify this network structure, we prevented bumblebee visits to radish by using a mesh of 9-mm size over the targeted flowers, which excluded bumblebees but not the other two smaller species. We measured visitation rates of each pollinator to each plant in 3-min periods twice during peak bloom for each cage (total of 576 min of observations). Additionally, pollen deposition per visit for each plant–pollinator combination was measured to ensure all pollinators are effective following (49). Mean pollen deposition per single visit was not different across species ($P > 0.1$).

Inference Analysis.

Our experimental study was designed to field parameterize a time discrete LV model describing annual dynamics of a plant–pollinator system (17):

$$\begin{cases} \log \left(\frac{N_{P_i}(t+1)}{N_{P_i}(t)} \right) = r_i^{(NP)} - \sum_j \alpha_{ij}^{(NP)} N_{P_j}(t) + \sum_j \gamma_{ij}^{(NP)} N_{A_j}(t) \\ \log \left(\frac{N_{A_i}(t+1)}{N_{A_i}(t)} \right) = r_i^{(NA)} - \sum_j \alpha_{ij}^{(NA)} N_{A_j}(t) + \sum_j \gamma_{ij}^{(NA)} N_{P_j}(t) \end{cases} \quad [1]$$

where the variables $N_{P_i}(t)$ and $N_{A_i}(t)$ denote the abundance of plant and animal species i at year t , respectively. The parameters of this mutualistic model correspond to the values describing intrinsic growth

rates (r_i), within-trophic interactions (α_{ij}), and the interaction effects across trophic levels (γ_{ij}). All these interaction strengths can, in turn, be embedded in a two-by-two block matrix $\mathbf{A} = \begin{bmatrix} \alpha^{(P)} & -\gamma^{(P)} \\ -\gamma^{(A)} & \alpha^{(A)} \end{bmatrix}$. Plants and pollinators abundance at year $t=1$, $N_{Pi}(1)$ and $N_{Ai}(1)$, were set by the experimental settings. At year $t=2$, we measure the number of seeds $Y_i^{(P)}$ for the plants and the number of eggs $Y_i^{(A)}$ for the pollinators. These quantities are related to the abundance variables $N_{Pi}(t)$ and $N_{Ai}(t)$. For the plants $N_{Pi}(t) = g_i^{(P)} \cdot s_i^{(P)} \cdot Y_i^{(P)}$, where $g_i^{(P)}$ is the germination rate and $s_i^{(P)}$ the survival rate. Similarly, for the pollinators, $N_{Ai}(t) = g_i^{(A)} \cdot s_i^{(A)} \cdot Y_i^{(A)}$, where $g_i^{(A)}$ is the hatching rate and $s_i^{(A)}$ the survival rate. Therefore, we can infer the parameters using a simple regression: $\log\left(\frac{N_{Pi}(t+1)}{N_{Pi}(t)}\right) = \log\left(Y_i^{(P)}(t+1)\right) - \log\left(N_{Pi}(t)/(g_i^{(P)} \cdot s_i^{(P)})\right)$; we obtain that $\log\left(Y_i^{(P)}(t+1)\right) = \log\left(N_{Pi}(t)/(g_i^{(P)} \cdot s_i^{(P)})\right) + r_i^{(P)} - \sum_j \alpha_{ij}^{(P)} N_{Pj}(t) + \sum_j \gamma_{ij}^{(P)} N_{Aj}(t)$. By performing a regression of $Y_i^{(P)}$ as function of $N_{Pj}(t)$ and $N_{Aj}(t)$, we can estimate the slopes as the interaction strength and the intercept equals $\log\left(N_{Pi}(t)/(g_i^{(P)} \cdot s_i^{(P)})\right) + r_i^{(P)}$ from which we can extract the intrinsic growth rate. This method to estimate the interaction strength is an adaption to multispecies system of the classical approach developed by Laska and Wootton (50), which use two consecutive time steps in order to estimate the interaction strength outside ecological equilibrium. Below we provide details on how the data are used to estimate all parameters.

As explained above, to infer the interaction matrix \mathbf{A} we used linear models (one per species and treatment) in which individual viable seed production was regressed against the number and identity of neighbors within a radius of 7.5 cm and the number and identity of pollinators present in the cage. This number of neighbors varied from zero to six following the spatial pot design (SI Appendix, Fig. S1) with a total of 836 observations across species. Model estimates were used to construct the plant–plant interaction submatrix $\alpha^{(P)}$ and pollinator–plant interaction submatrix $\gamma^{(P)}$. To estimate pollinator–pollinator interactions $\alpha^{(A)}$ and the effect of plants on pollinators $\gamma^{(A)}$, we were limited by having one replicate per treatment due to the large effort of rearing pollinators in cages. Hence, pollinator–pollinator interactions were estimated from the subtraction of the reproductive success of pollinator i in cages with and without the pollinator j present (including intraspecific competition). Interaction effects of plants on pollinators were estimated from subtracting reproductive success in cages with high or low plant abundance (SI Appendix, Fig. S1). To partition the effect per plant we used the observed visitation rates as a proxy of plant contribution to pollinator fitness (51). Note that the intrinsic growth rates correspond to the intercepts of such models (reproductive success in the absence of competitors) and the competitive or mutualistic pairwise effects correspond to the estimated slopes of each species effect on reproductive success. All these matrices were inferred for the different plant treatments without pollinators \mathbf{A}_p , the original multitrophic structure with pollinators \mathbf{A}_o , and the modified multitrophic structure with pollinators \mathbf{A}_m . The in silico-manipulated multitrophic structure \mathbf{A}_z is identical to the original multitrophic structure \mathbf{A}_o except that the radish–bumblebee mutualistic interactions is set to zero, that is, $\gamma_{ij}^{(P)} = \gamma_{ij}^{(A)} = 0$. See Fig. S1 for a detailed schema of the treatments. Note that in \mathbf{A}_p the only inferred submatrix is $\alpha^{(P)}$.

To answer whether the observed reorganization is different from a naive nondirectional random reorganization we built 250 null interaction-strength matrices and compared the mean (expected) persistence probability of each species against the persistence probability estimated from the observed reorganization values. To that end, these null matrices were generated by adding variation only to the nonzero elements of the \mathbf{A}_z matrix. This variation was introduced to each of these nonzero elements by drawing random values from a normal distribution with mean equal to the corresponding value of the matrix element in \mathbf{A}_o and an SD equal to the observed variation of the changes in interaction strengths between \mathbf{A}_o and \mathbf{A}_m . The results were qualitatively similar to building null matrices by drawing

random values from a half-normal distribution with mean and SD equal to those in \mathbf{A}_o , while the sing and zero elements of matrix \mathbf{A}_z are preserved.

Probability Analysis.

Following a structuralist approach (20, 28), we calculate the average probability of persistence of plant species within a community \mathbf{A} of three species as

$$\omega(\mathbf{A}) = \left(\frac{2^3 \text{vol}(D_F(\mathbf{A}) \cap \mathbb{B}^3)}{\text{vol}(\mathbb{B}^3)} \right)^{\frac{1}{3}}, \quad [2]$$

where $\text{vol}(\mathbb{B}^3)$ is the volume of the three-dimensional unit ball representing the parameter space of \mathbf{r} , 2^3 normalizes the unit ball to the positive orthant, and $\text{vol}(D_F(\mathbf{A}) \cap \mathbb{B}^3)$ corresponds to the volume of the intersection of the feasibility domain with the unit ball. The feasibility domain is defined as $D_F(\mathbf{A}) = \{\mathbf{r} = N_1^* \mathbf{v}_1 + N_2^* \mathbf{v}_2 + N_3^* \mathbf{v}_3, \text{ with } N_1^*, N_2^*, N_3^* > 0\}$, where \mathbf{v}_i is the i -th column vector of the interaction matrix \mathbf{A} (26). Note that we assume that plants have positive intrinsic growth rates, that is, $r_i > 0$. Because we are only interested in the direction of positive \mathbf{r} -vectors, we can consider only vectors \mathbf{r} for which $\|\mathbf{r}\| \leq 1$ and normalize the size of the feasibility domain using the positive orthant of the unit ball (i.e., $\mathbb{B}^3 \cap \mathbb{R}_{\geq 0}^3$). Note that we are fixing the magnitude of the \mathbf{r} -vectors to one under the Euclidean norm (i.e., $\|\mathbf{r}\| = 1$); however, the analysis can be done using any norm without altering the conclusions (52). Thus, $\omega(\mathbf{A}) \in [0, 1]$ is a probabilistic measure and can be efficiently computed for even relatively large communities (26, 53). Ecologically, $\omega(\mathbf{A})$ can be interpreted either as the probability of persistence of a randomly chosen species or as the expected fraction of persistent species within community \mathbf{A} .

Following the same approach (28), we estimate the persistence probability of a given species i within a community (interaction matrix) \mathbf{A} as

$$P(i|\mathbf{A}) = \sum_{j=1}^{n_i} P(E_j^{(i)}|\mathbf{A}), \quad [3]$$

where $P(E_j^{(i)}|\mathbf{A})$ corresponds to the probability of observing the j -th species collection containing species i and n_i is the total number of collections that contain species i starting from community \mathbf{A} . This probability is defined as

$$P(E_j^{(i)}|\mathbf{A}) = \frac{\text{vol}(E_j^{(i)} \cap \mathbb{B}^S)}{\text{vol}(\mathbb{B}^S)}, \quad [4]$$

where S is the number of species in community \mathbf{A} (note that in our case $S=6$), $\text{vol}(\mathbb{B}^S)$ is the volume of the S -dimensional unit ball (i.e., the full parameter space of \mathbf{r}), and $\text{vol}(E_j^{(i)} \cap \mathbb{B}^S)$ corresponds to the volume of the intersection of the domain of the collection with the unit ball. To calculate this probability we ran 10^5 simulations of the LV dynamics using a given inferred matrix, random initial conditions, and randomly drawing \mathbf{r} uniformly over the unit sphere $\|\mathbf{r}\|_2 = 1$. We assume that plants are constrained to positive intrinsic growth rates, while pollinators have either positive or negative values. Thus, $P(i|\mathbf{A}) \in [0, 1]$ and is given by the fraction of times that species i was found with positive abundance at equilibrium ($N_i^* > 10^{-5}$).

Data Availability. Data and code to reproduce the analysis have been deposited at Zenodo (<http://doi.org/10.5281/zenodo.4579645>) (54).

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Supplementary Information for
Experimental evidence for the importance of multi-trophic
structure in regulating species persistence

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Contents

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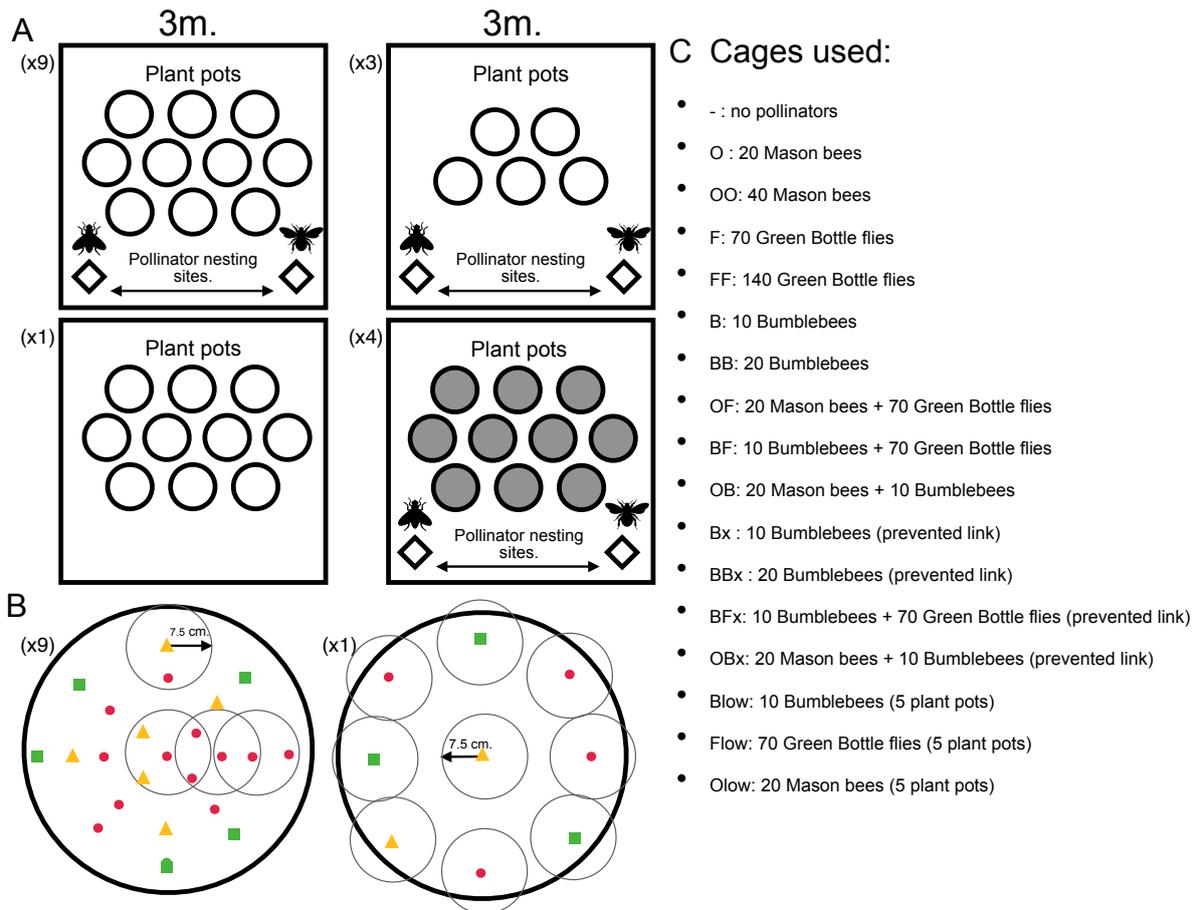


Figure S1: A: Examples of the four experimental cage configurations and the number of cages set up per configuration (in brackets). Cages had 10 or 5 replicas of plant-plant competition pots, and up to two pollinator nesting sites. Grey pots indicates a mesh prevented the bumblebee-radish interaction. All cages except those with low plant resources had 10 pots with all three plants growing in different configurations that create different competition gradients of inter- and intra-specific neighbors at 7.5 cm. B: Example of a plant-plant competition pot where we have different plant individuals (species indicated by different colors and shapes) surrounded by different number of neighbours (from 4 to 1 neighbours). In addition, two pots per cage had plant individuals growing in no competition (no neighbours present in > 15 cm). In each of the 10 pots, the relative densities of the three species varied to create all combinations of inter and intraspecific competition at all densities within a cage. C: The 17 cages had different pollinator treatment levels that allow us to calculate plant-plant competition, pollinator-pollinator pairwise competition, plant-pollinator mutualistic effect, and pollinator-plant mutualistic effect as well as specific growth rates. The number of pollinator individuals is selected as to use the same pollinator biomass for each species (i.e. bumblebees are 7 times bigger and active than flies).

	Radish	Field Bean	Tomato
Bumblebee	-100 % (-116 visits)	44 % (+12 visits)	-33 % (-2 visits)
Mason bee	58 % (+7 visits)	+200% (+2 visits)	
Green bottle fly	+100% (+1 visits)		

Figure S2: Changes (in percentage and raw number of visits) in pollinators visitation rate per plant-pollinator interaction when comparing the structure with and without the radish-bumblebee link. For radish we see that, as expected, bumblebee visitation rate decreases by 100 percent, dropping to zero when the link is prevented, but mason bees and green bottle flies increase visitation rate when bumblebees are prevented from visiting radish. Moreover, visitation rate from bumblebees to field beans increases when the link with radish is prevented.

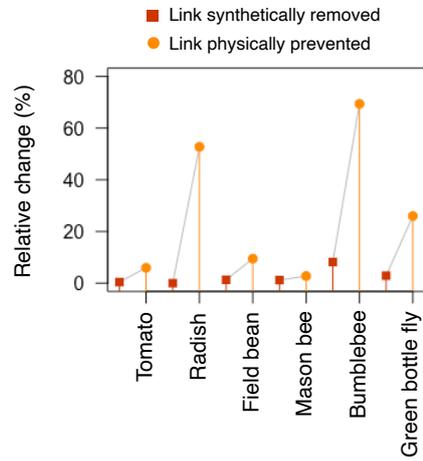


Figure S3: Absolute value of the relative changes in persistence probability of synthetically (red squares) or experimentally (orange circles) modified network structures. We can observe that in the manipulated structure, radishes and bumblebees reduce their probability by more than 50%, while the other species increase it moderately. However, the effects of this experimental manipulation were not recovered (changes in probability are negligible) by the synthetically manipulated structure that does not consider additional effects across the multi-trophic structure.

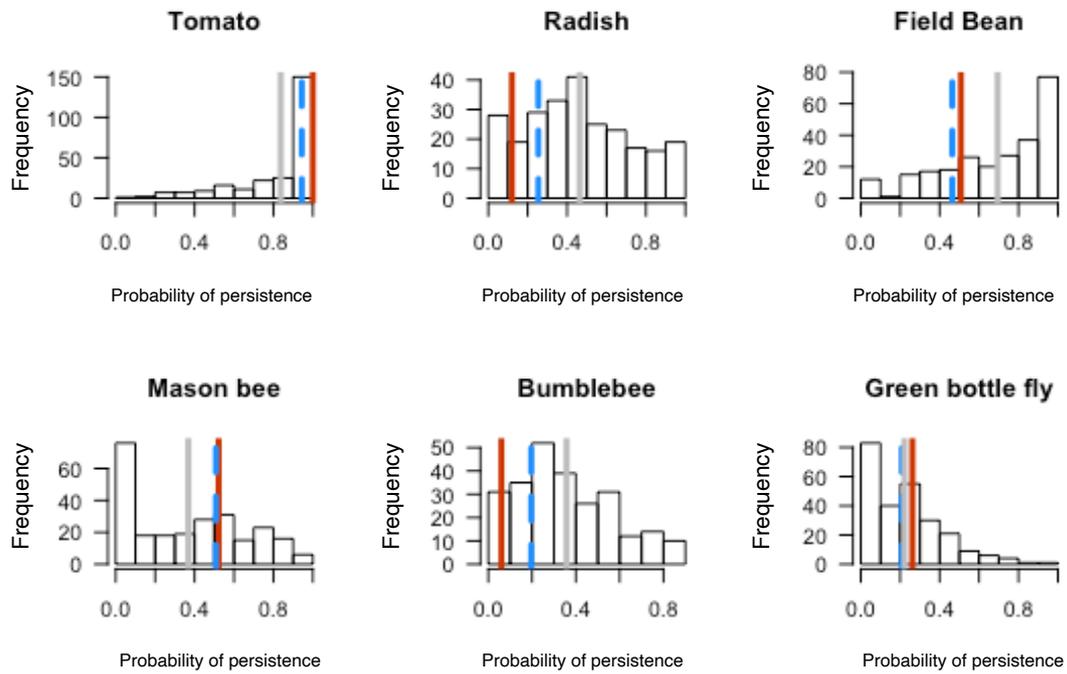


Figure S4: For each species we show the histogram of the persistence probability of all null models calculated. The persistence probability of the original (blue line), the link physically prevented (red line) and the mean null expectation (grey line) are shown. Note that the observed probability of persistence under the experimental link removal goes in opposite direction as the expected under the null model, when we compare them against the original configuration