

ECOGRAPHY

Research

Trophic rewilding benefits a tropical community through direct and indirect network effects

Pedro Mittelman, Anna Rebello Landim, Luísa Genes, Ana Paula A. Assis, Carolina Starling-Manne, Paula V. Leonardo, Fernando A. S. Fernandez, Paulo R. Guimarães Jr. and Alexandra S. Pires

P. Mittelman (<https://orcid.org/0000-0003-0428-463X>) ✉ (uchoa.mitt@gmail.com), Georg-August-Univ. Göttingen, Wildlife Sciences, Göttingen, Germany. – P.M., A. R. Landim, L. Genes, C. Starling-Manne, P. V. Leonardo and F. A. S. Fernandez, Programa de Pós-Graduação em Ecologia, Univ. Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil. LG also at: Dept of Biology, Stanford Univ., Stanford, CA, USA. – A. P. A. Assis and P. R. Guimarães Jr., Depto de Ecologia, Univ. de São Paulo, São Paulo, SP, Brazil. – A. S. Pires, Depto de Ciências Ambientais, Univ. Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil.

Ecography

44: 1–11, 2021

doi: 10.1111/ecog.05838

Subject Editor and
Editor-in-Chief:
Jens-Christian C Svenning
Accepted 14 October 2021



Species reintroductions can be used as a conservation strategy to restore ecological interactions and the functionality of impoverished ecosystems. The ecological effects of reintroductions go beyond restoring pairwise interactions, because reintroductions can change how extant species are indirectly linked to each other in an ecological community. These indirect pathways, in turn, may shape a myriad of ecological and evolutionary processes operating in ecological systems. Here, we investigated how reintroductions may affect the direct and indirect pathways connecting species in ecological networks. We modeled the potential effects of the reintroduction of four frugivore species (channel-billed toucans, red-humped agoutis, brown howler monkeys and yellow-footed tortoises) to the local seed dispersal network in an Atlantic Forest site, the Tijuca National Park (Rio de Janeiro, Brazil). We used a seed dispersal interaction dataset together with data on species occurrences in Tijuca to build network models. Then, we calculated how network structure and the total amount of indirect effects varied across simulated networks with and without the reintroduced species. Using random reintroduction simulations, we tested if the observed network changes were expected merely from the increase in species richness. The reintroduction of the frugivore species increased network connectance, nestedness, robustness, number of pathways and total amount of indirect effects in all simulated networks. The increase in number of pathways with the addition of the four reintroduced species was greater than the sum of isolated effects for each species, because some interaction pathways contained several reintroduced species. These changes in network metrics were significantly greater than if a randomly chosen set of four species was reintroduced. Furthermore, our results indicate that multiple reintroductions in the same area, known as refaunation, may have an even greater restoration effect than single species reintroductions through increased indirect connections in the network.

Keywords: indirect effects, plant–animal interactions, reintroduction, restoration, seed dispersal



www.ecography.org

© 2021 The Authors. Ecography published by John Wiley & Sons Ltd on behalf of Nordic Society Oikos
This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Introduction

Species reintroductions are a conservation strategy with different possible goals ranging from species-focused conservation (Seddon et al. 2014) to ecosystem-level restoration in trophic rewilding (Oliveira-Santos and Fernandez 2010, Svenning et al. 2016). By re-establishing populations, reversing the effects of defaunation (Kurten 2013, Dirzo et al. 2014), reintroductions may alter the structure and dynamics of ecosystems, affecting biodiversity across levels of organization. In this sense, the effects of reintroductions on the structure and dynamics of ecosystems can be explored by measuring structural changes in ecological networks.

Ecological networks can be used to assess the consequences of extinctions on other species in a community (Mommott et al. 2004, Kaiser-Bunbury et al. 2010, Pires et al. 2020). Specifically, local animal extinctions increase functional homogenization of interaction networks (Emer et al. 2020) but decrease absolute functional redundancy. For instance, zoochoric plants will have a smaller number of animal species interacting with them and fewer options to get their seeds dispersed, thus reducing resilience and increasing vulnerability (Schleuning et al. 2015). Likewise, the addition of new species can also change network structure, and thus, network analysis can be used to predict how reintroductions affect ecological communities (Pires 2017). In this sense, we would expect that interaction restoration through species reintroduction would increase ecosystem resilience through the addition of new, and often unique, pairwise interactions (Tylianakis et al. 2010, Genes et al. 2017).

In an ecological network, species are connected not only through direct interactions but also through indirect pathways that occur when, for example, different species share the same interaction partner. Indirect effects generated by such a chain of interactions can cascade through the network (Guimarães 2020). Empirical and theoretical work support the relevance of indirect effects by analyzing 1) simulations of species removal; 2) data of recent extinction of species and species interactions; and 3) removal experiments of a particular species. These studies highlighted that indirect effects can reshape abundance and trait distributions across species (Ohgushi et al. 2012), fuel coextinctions (Pires et al. 2020), change species richness and composition (Paine 1966) and influence ecosystem services (Estes et al. 2011). In this sense, species that have multiple interactions in an ecological community, are fundamental to foster indirect effects in ecological systems (Gilbert and DeLong 2017, García-Callejas et al. 2019). Highly connected species may generate a variety of indirect effects, such as trophic cascades, trait-mediated cascades and apparent competition in ecological communities (Guimarães 2020). In mutualisms, highly connected species may provide stable sets of resources and services (Thompson 2005) which, in turn, may prevent losses due to secondary extinctions (Jordano et al. 2003, Mommott et al. 2004).

Therefore, in mutualistic systems, reintroductions can enhance network robustness to secondary extinctions by restoring key interactions, increasing the number of

interactions in the system, adding redundant interactions and creating indirect pathways that may provide alternative routes to the reorganization of plant–animal assemblages. For instance, in a network of plants and their seed dispersers, the reintroduction of a highly connected seed disperser might affect not only the plant species it disperses but also other frugivores and plants it does not directly interact with, generating both facilitative and competitive interactions (Bastolla et al. 2009).

Here, we aimed to assess how animal reintroductions can affect network structure and the consequences of these changes for plant and animal communities. We specifically aimed to quantify the potential of reintroduction to promote indirect pathways, using seed dispersal by vertebrates in networks. We studied the consequences of reintroducing four seed disperser species into an Atlantic Forest site, the Tijuca National Park (Rio de Janeiro, Brazil). The species reintroduced to restore recently lost important ecological interactions were the chanel-billed toucan *Ramphastos vitellinus arielis*, the red-humped agouti *Dasyprocta leporina*, the brown howler monkey *Alouatta guariba* and the yellow-footed tortoise *Chelonoidis denticulatus*. These species are generalist herbivores/omnivores known for being important dispersers of large seeds (Jerozolinski et al. 2009, Jansen et al. 2012, Galetti et al. 2013, Arroyo-Rodríguez et al. 2015).

The uncertainty of interaction occurrence is always present, even when interactions are locally sampled, due to imperfect detection (Jordano 2016). Many approaches have been suggested to deal with this uncertainty, mostly involving probabilistic networks (Poisot et al. 2015, Kristensen et al. 2019) and predictive ‘coverage-deficit’ models that try to identify missing links (Terry and Lewis 2020). We followed the rationale of these approaches, and we incorporated interaction occurrence’s uncertainty in our analysis by creating four different network models based on different assumptions on how plants and animals would interact. Specifically, we used models based on phylogeny and seed-traits to deal with absent interactions. Additionally, these multiple models allow us to test our hypotheses in different ways, checking the consistency of our results regardless of the underlying assumptions made about which interactions occur by each individual model.

We evaluated how multiple-species reintroductions affect connectance, nestedness, robustness and the number of indirect interactions in this previously defaunated seed dispersal network in the Brazilian Atlantic Forest. We hypothesized that the reintroduced species, by creating multiple interactions in the network, would increase all aforementioned metrics, and also the number of indirect pathways in the network. This is surmised because the four reintroduced species are highly connected generalists frugivores that should increase connectance and the pairwise overlap of interactions among animals in a sparse network (thus also increasing nestedness, Almeida-Neto et al. 2008). Robustness should augment because highly connected species increase the redundancy among frugivores in the network.

Methods

Study system

Tijuca National Park is a 3953 ha Atlantic Forest reserve located within Rio de Janeiro city, Brazil, which was used for farming and charcoal production up to the 19th century, when vegetation was partially restored. Prior to species reintroductions, Tijuca lacked medium and large-sized frugivores, and large apex predators still do not occur in the area. Channel-billed toucans *Ramphastos vitellinus arielis* were reintroduced to Tijuca in 1970s, while red-humped agoutis *Dasyprocta leporina*, brown howler monkeys *Alouatta guariba* and yellow-footed tortoises *Chelonoidis denticulatus* were reintroduced over the past 10 years (Coimbra-Filho et al. 1973, Fernandez et al. 2017). These four species were the reintroduced species we analyzed in our simulations. These last three species were chosen because they are generalist seed dispersers with small home range sizes that had suitable captive stocks and did not represent high health or economic risk to humans (following criteria listed by Galetti et al. 2017). The toucan comes from a previous and independent reintroduction effort but guided by the same principles of ecological restoration and thus also matching the listed criteria (Coimbra-Filho et al. 1973). Previous studies have assessed the role of agouti (Mittelman et al. 2020) and howler (Genes et al. 2019) reintroductions (two of the species analyzed here) at Tijuca on the restoration of plant–animal interactions, but so far, no study explored plant–animal community-scale effects of such reintroductions.

Data

Pairwise seed dispersal interaction data are scarce for Tijuca National Park. Therefore, to build seed dispersal networks, we first gathered plant and animal species occurrence data in Tijuca (ICMBio 2008) and excluded all plant species that were not dispersed by animals based on our natural history knowledge. Our network includes 124 frugivorous species, including the four reintroduced species, and their 472 animal-dispersed plant species. Then, to estimate the pairwise interactions between plants and seed dispersers, we used the Atlantic-Frugivory dataset (Bello et al. 2017), which compiles pairwise seed dispersal interactions across the Atlantic Forest. Because the yellow-footed tortoise is the only seed disperser in our dataset that had no interaction recorded in the Atlantic-Frugivory dataset, we made an extensive literature review on *Chelonoidis* spp. fruit consumption to infer its diet (see details in the Supporting information). Finally, we assembled seed trait data by compiling information available in the Atlantic-Frugivory dataset and conducting a literature search in Google Scholar for “Plant species” (seed OR fruit) (diameter OR length)’ and complemented with publications that we knew of but did not appear in our search. Thus, data about species’ interactions were gathered from the literature a posteriori to the reintroductions.

Interaction networks

We used four different models to infer pairwise interactions in our network. Therefore, we built four types of networks based on different assumptions about which interactions would occur in Tijuca. These four different approaches to network estimation allow us to investigate how sensitive our estimates of network patterns were to different assumptions when assigning interactions, and if they were consistent regardless of how networks were estimated. Thus, these network models take into account the uncertainty about interactions occurrence, reducing the potential biases of each assumption and verifying if our results hold independently of the model used (see Pires et al. 2014 for a similar approach).

For all networks, we assumed that interactions recorded elsewhere in the Atlantic Forest would also occur in Tijuca if the same plant and animal species were present in Tijuca. However, because some interactions might be absent in the literature records, we added interactions based on the following assumptions:

Phylogenetic model. In this network, there are two sets of nodes and each node depicts one species. We assumed that, if an animal species was recorded interacting with a plant of a given genus, this animal would interact with all plant species of the same genus found in Tijuca.

Genus-level model. In this network, there are two sets of nodes. Animal species are depicted as nodes, whereas each node in the second set depicts a plant genus. Thus, plant taxa were collapsed to the genus level, which would reduce the effects of under-sampled interactions within the genus, but leading to a description of network patterns at a different taxonomic level.

Probabilistic phylogenetic model. We assume that there is a probability p that a given animal species i – that interact with a plant species j from the genus k – would also interact with another plant l from the same genus k . We assume that p is fixed to all potential pairwise interactions and we assume $p=0.5$. Thus, in this network, if one interaction between an animal species i and plant genus j occurred, the same animal i ended up interacting with a fraction of plant species of genus j as well. We built 100 pairs (with and without reintroduced species) of these networks to estimate the mean effects of reintroduced species on network structure.

Seed size model. We used plant species’ seed length and seed diameter data to construct a network. We first used empirical data on species interactions to record the maximum and minimum seed length and diameter of fruits consumed by each animal species, with no taxonomic constraints. We then assumed that each animal species interacts with all plant species in the network with seed length and seed diameter values in between the maximum and the minimum observed for plants. We assumed that if one of the seed dimensions was not within the interval formed by the minimum and maximum value of seeds dispersed by the animal, then the animal did not interact with the plant, i.e. the interaction is forbidden by this seed dimension (Allesina et al. 2008, Jordano et al. 2003).

This last network model strongly minimizes the effects of sub-sampling since understudied species with an extremely low number of recorded interactions are connected with a wide range of plants in the network, given that they can consume fruits/seeds of broadly different sizes. Having said that, this model may overestimate the number of interactions and is limited to a subset of plant species ($n = 249$ species) in our database with known values of seed diameter and length. In this network, nine animal species end up having no interaction partners because either they interacted only with plants with unknown values of seed size or there were no plant species that had seed length and diameter simultaneously compatible with the interacting range of the animal species. We ran network analyses with and without these non-interacting species but there were no significant differences in results (Supporting information).

Several networks were built to take into account the uncertainty about interactions occurrence, to reduce the potential biases of each assumption and to check if our results hold independently of the model used.

Network analysis

For each network, we calculated connectance (realized interactions/total number of possible interactions), nestedness (the network pattern in which specialists interact with subsets of species with whom generalists interact, measured by the NODF metric, Almeida-Neto et al. 2008) and robustness (network tolerance to species' extinction, measured here by the area below the curve or 'R', Memmott et al. 2004, Burgos et al. 2007). Computed metric values from networks without reintroduced animals were then compared to values from networks where reintroduced animals and their interactions had been inserted.

To assess if the changes observed in the networks when reintroduced species are present were due to just an increase in the total number of species or if the four reintroduced species were particularly important to change the network metrics, we simulated random reintroductions as if our focal species had never gone locally extinct but another group of four species had been extinct and subsequently reintroduced. For this, we built networks containing our four focal species but without our four randomly drawn animal species (simulating a before-reintroduction scenario) and then we added these same species back into the network (simulating an after-reintroduction scenario). We built 100 pairs of these networks and assessed how adding random animal species changed networks' connectance, nestedness and robustness. Then, we compared these simulated changes to the ones simulated by the four species that were in fact reintroduced in Tijuca. Analyses were done in the R environment (<www.r-project.org>) using the 'bipartite' package (Dormann et al. 2008).

We now turn our attention to the characterization of the indirect pathways that the reintroduced species add to the network. We defined an indirect pathway as a chain of direct interactions (Estes and Palmisano 1974). The length of the

pathway is then measured as the number of (non necessarily unique) interactions that compose the pathway. We estimated the number of indirect pathways of lengths ranging from two through five for each network. We used five as the maximum pathway length recorded because this is the largest pathway, in number of links, between any pair of species in the network that involves unique direct interactions, i.e. the network diameter. The matrix that describes the paths of length two was calculated by multiplying the adjacency matrix **A** by itself, **AA**, and three by multiplying this again by the **A** matrix and so on. To estimate the total number of paths of a given length we just summed across the entire matrix $\sum_i \sum_j a_{i,j}^l$, where the element $a_{i,j}^l$ describes the number of pathways with length (l) connecting species i and species j (Guimarães et al. 2017).

We estimated the total number of pathways in all network models without the reintroduced species. Then, for each network model, we analyzed the effects of adding each of the reintroduced species separately (*Ramphastos*-only, *Dasyprocta*-only, *Alouatta*-only and *Chelonoidis*-only networks). We did so to assess the number of indirect pathways (of length 2 through 5) that each reintroduced species added to their respective networks. Then we repeated indirect pathways analyses with the network containing all the four reintroduced species altogether and estimated how many indirect pathways were added to this network compared to the one without reintroduced species. To do this, we estimated the ratio between the networks with reintroduced species divided by the networks without any reintroductions. We assessed if the network with all four reintroduced species presented indirect pathways that go through more than one of the reintroduced species, contributing to these indirect pathways in a higher ratio than the sum of each reintroduced species separately. Finally, we also compared if the number of indirect pathways the four focal species contributed to the network was higher than if any other random group of four species was reintroduced. To do this, we used the same approach for random reintroductions described above using 100 randomly sampled networks.

Results

Despite the inherent differences in the underlying assumptions and the network structure generated by the network models, simulated results showed that the four reintroduced species affected in consistent ways the structure of the seed dispersal network (Fig. 1, 2). The first impact of reintroducing species in the Tijuca network was to increase both species richness (four seed dispersers) and the total number of interactions. Indeed, in addition to that, some plants species in our network models only interacted with reintroduced species (from 6 to 43 plant species depending on the network model used to assess interactions).

The reintroduced seed dispersers interacted on average with more plant species than the other extant seed dispersers in all our model simulations [Supporting information; (median

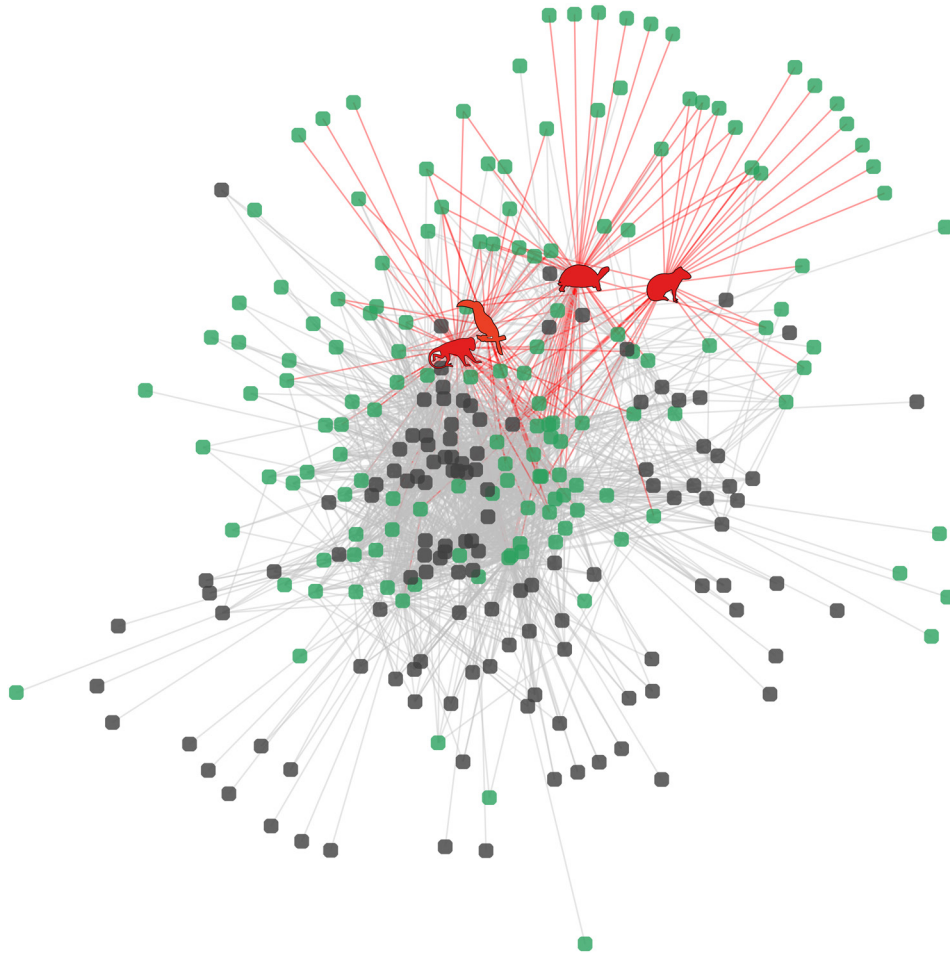


Figure 1. Tijuca National Park's seed dispersal network after trophic rewiring. Reintroduced species and their pairwise interactions are depicted in red, while other animal species are represented by grey dots and plant species by green dots. This network is based in the genus-level model. Animal icons were made by J. Colussi. See the Supporting information for networks using the different models.

and 95% median confidence interval (CI) for 1) phylogenetic model: reintroduced species = 168, CI [109, 202], other seed dispersers = 62.5, CI [42, 73]; 2) genus-level model: reintroduced species = 38, CI [30, 49], other seed dispersers = 11, CI [9, 18]; 3) probabilistic phylogenetic model (average values for the 100 simulations): reintroduced species = 98.42, CI [92.27, 126.21], other seed dispersers = 26.80, CI [14.44, 34.75]; 4) seed size model: reintroduced species = 202.5, CI [174, 246], other seed dispersers = 132.5, CI [63, 160]). For all network models, $\frac{(E+F)}{(A+4)} > \frac{E}{A}$, in which E is the number of interactions recorded without the reintroduced species, F is the number of interactions of reintroduced species and A is the animal species richness, leading to an increase in connectance (mean increase in connectance considering all four network models and 95% mean CI = $4.35 \pm 1.40\%$, Fig. 2a; network metrics for all network models are available in Supporting information). The reintroduced species increase not only the connectance of all networks but also the overlap in patterns of interaction between highly connected and poorly connected species, increasing nestedness (mean

nestedness increase = $12.20 \pm 2.63\%$, Fig. 2b). Because of increasing both the number of interactions and the overlap among seed dispersers and their plant partners, reintroduced species increase the robustness in all simulated scenarios (mean robustness increase = $11.42 \pm 4.02\%$, Fig. 2c). The increased values of connectance, nestedness and robustness were significantly greater than expected if a random set of four species were reintroduced into the networks (Fig. 3). Moreover, network robustness to secondary extinctions increased after reintroductions, regardless of the network model applied (Fig. 4, Supporting information).

The above simulated results indicate how reintroduced species affect the direct patterns of interaction in the Tijuca network. We now focus on the changes reintroduced species made to the indirect pathways in the network. Our analyses show that each reintroduced species increased the number of indirect pathways in the network. Using the seed size model, species reintroduction increased by 8% the number of pathways of length 2, i.e. those in which two seed dispersers are connected by a shared fruiting plant or two plant species are connected by a shared seed disperser (2.741×10^5 pathways

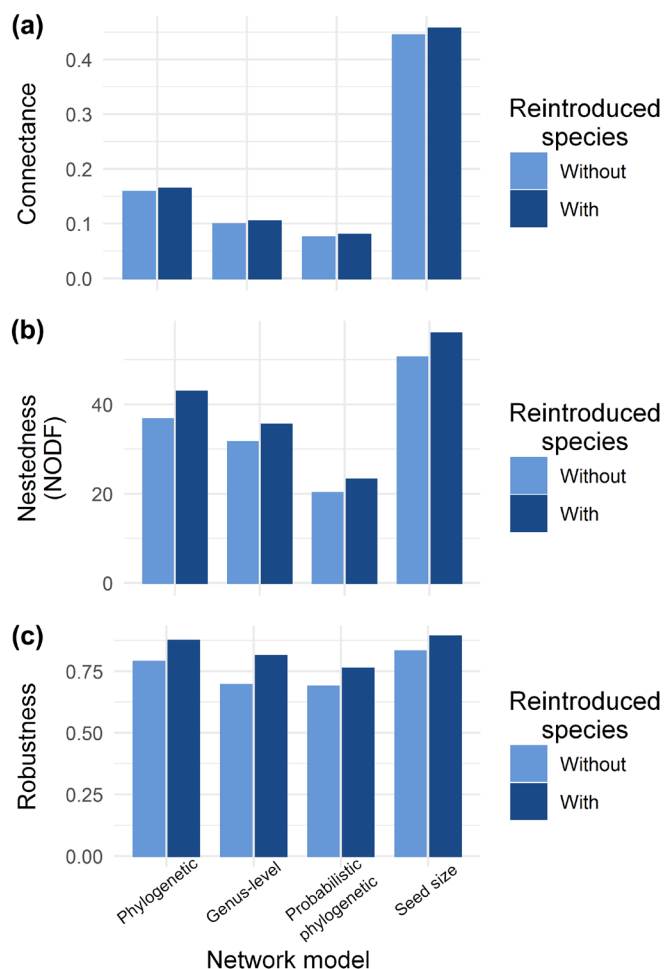


Figure 2. Connectance, nestedness and robustness increase when reintroduced species are present in Tijuca seed-dispersal network regardless of the model used to assess network interactions. Connectance was measured by the total number of realized interactions. Nestedness was measured using NODF metric which is based on overlap and decreasing fill of the interaction matrix. Robustness was measured by the extinction curve inflexion. For the probabilistic phylogenetic model, the values presented are the average of the 100 simulations.

added to the total of 3.251×10^6 pathways in the network without reintroduced species). The increase in the number of pathways with length 3 was higher than 38 million pathways, representing a 12% increase. The overrepresentation of pathways associated with the reintroduced species increased with pathway length. For example, for pathways of size 4, reintroduced species led to a 14% increase (more than 5 billion pathways), and for length 5, an 18% increase (more than 665 billion more pathways). *Dasyprocta leporina* and *Alouatta guariba* are the species that contributed most to the indirect pathways increase. Still, the network including all the four reintroduced species presented a substantial increase in the number of pathways (Fig. 5). When the four reintroduced species were present in the network, the number of indirect paths increased more than the sum of each reintroduced species separately (additional increase represented by the red

bars in Fig. 5). Thus, the reintroduction of multiple species led to a synergistic increase in the number of indirect pathways. Those results were qualitatively similar if we used any of the network models (Supporting information). In addition, the reintroduction of the four focal species contributed to a higher amount of indirect pathways than any other resampled network representing the reintroduction of other four randomly selected species (Fig. 5b).

Discussion

One of the problems in the study of ecological networks is to understand how changes in species composition led to changes in interaction patterns that, in turn, reshape key ecological and evolutionary processes (Solé and Montoya 2001, Tylianakis et al. 2008, Poisot et al. 2015, Ponisio et al. 2017, Guimarães 2020). Here, we show that the reintroduction of channel-billed toucans, red-humped agoutis, brown howler monkeys and yellow-footed tortoises can affect the network structure of the interacting assemblage of frugivores and zoochoric plants at an Atlantic Forest site. Specifically, our study contributes to the understanding of the role of trophic rewinding on ecological networks in three main ways.

First, when reintroduced species are incorporated in the network, the structure shifts in consistent ways, irrespective of the uncertainty of the interaction rules structuring the seed dispersal network. All network metrics analysed (connectance, nestedness and robustness) significantly increased after the four species were added to the network. Structural changes observed in the simulated networks imply an increasing number of interactions (higher connectance) and overlap among interacting patterns (higher nestedness). These changes, in turn, may diminish the vulnerability of the entire system against species loss (Vieira and Almeida-Neto 2015). Indeed, our analyses suggest that robustness to secondary extinctions is higher in the network with the reintroduced seed dispersers. The changes in network metrics such as connectance, nestedness and robustness we observed in our study suggest that reintroduced species can reverse the changes of network structure due to local extinctions. In this sense, our analysis can be viewed as a quantitative exploration of how trophic rewinding can contribute to the recovery of the structure of ecological assemblages.

Second, the impact of the four reintroduced species goes beyond the direct patterns of interaction. Reintroduced species also increased the number of pathways and, potentially, the total amount of indirect effects in the network. Because the number of pathways increases exponentially with path length (Borrett et al. 2007), reintroduced species promote even greater changes in the number of longer pathways. Although it is well known that indirect effects that reshape ecological communities (Paine 1966, Estes et al. 2011) propagate through indirect pathways formed by chains of interactions, we are just beginning to understand the consequences to network patterns of indirect pathways to both evolutionary and ecological processes (Terborgh and Estes

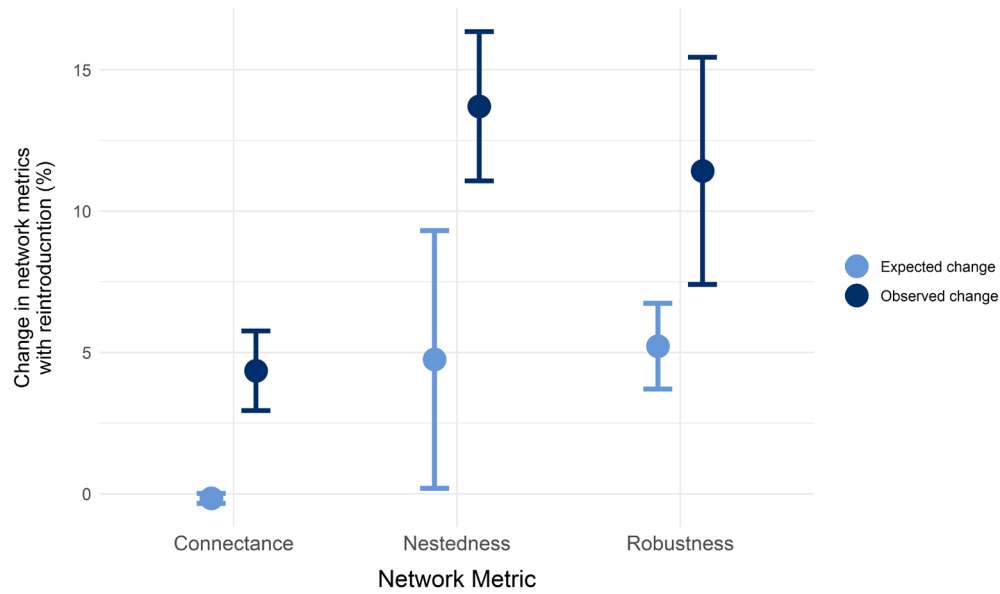


Figure 3. Mean increase in Tijuca network metrics when reintroduced species were present was significantly greater than expected if a random set of four species was inserted into the network. Dots are the mean of the four network models, and error bars represent the 95% CIs.

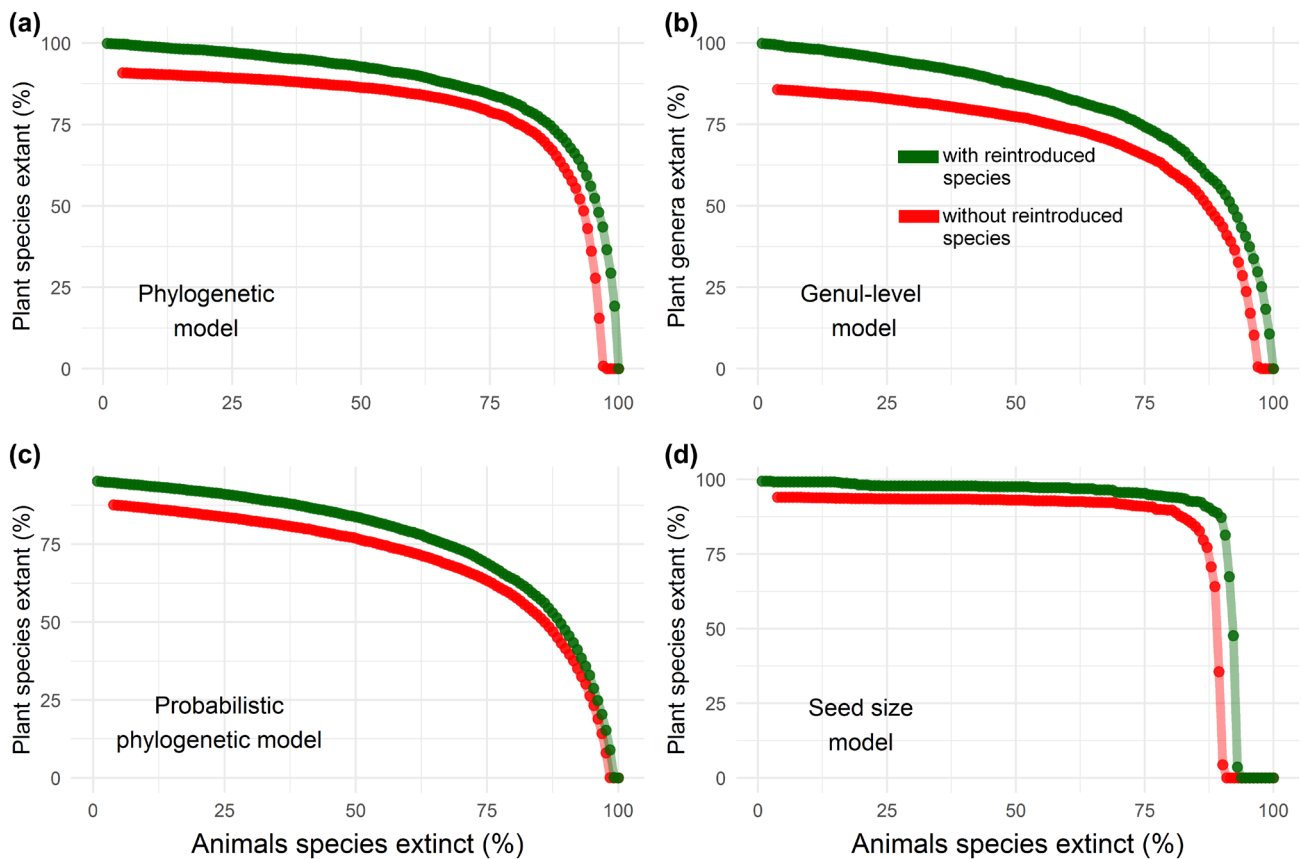


Figure 4. Network tolerance to extinctions (robustness) increases after simulated insertion of the reintroduced species into Tijuca network in all models used to assess network interactions. Robustness models simulate random species extinctions; after extinction events, species without interactions are withdrawn from the network emulating secondary extinctions. Lines show how many plants remain extant after random animal extinctions. Thus, the greater the area under the curve more robust and less prone to secondary extinction a network is. Lines do not start from the same point because networks without reintroduced animals have less animals and some plants already begin extinct because they have no interaction partners besides the reintroduced animals.

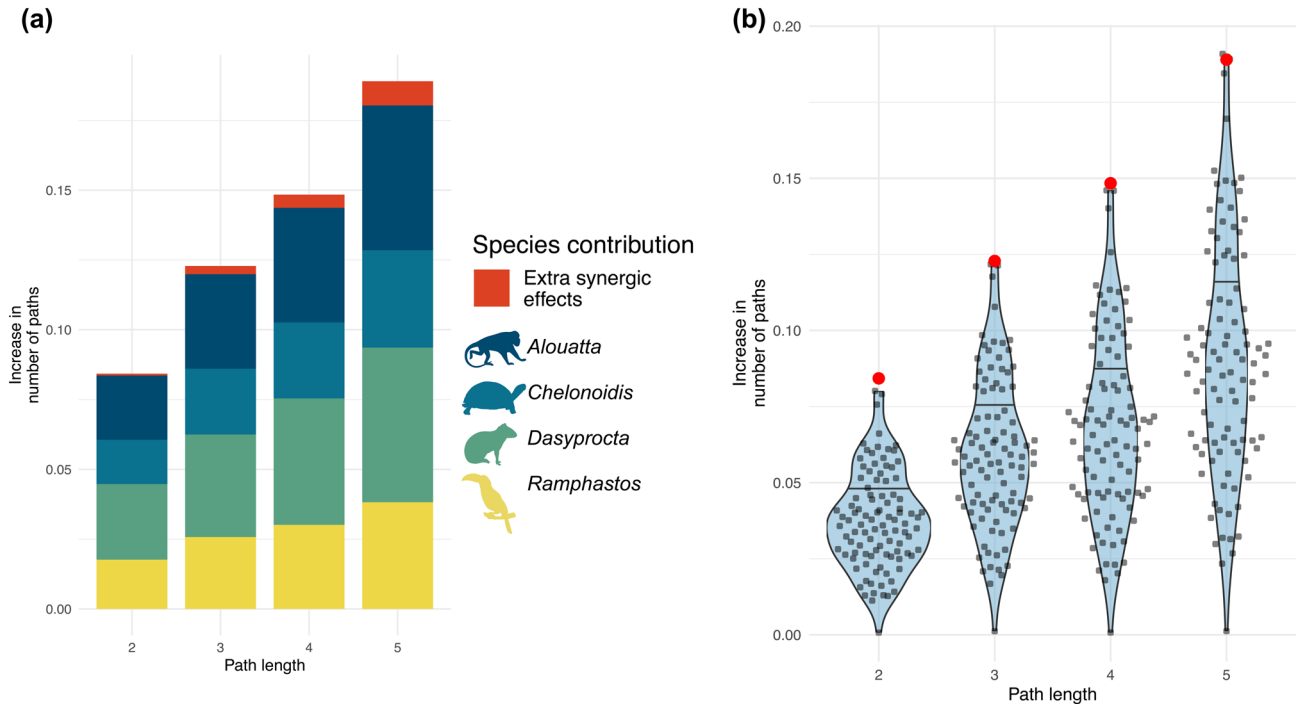


Figure 5. Reinintroduced species increased network indirect effects by creating a greater number of interaction pathways. (A) The four reintroduced species together had a greater effect than the sum of the isolated effects of each species. (B) The four focal species (red points) have a higher contribution to the amount of indirect pathways than other sets of our four randomly sampled species (grey points). Horizontal continuous black line represent 75% lower interval of randomly sampled species, and violin plots show total distribution for each pathway length for the seed traits model. See the Supporting information for the analyses using the other network models. Animal icons by J. Colussi.

2010, Guimarães et al. 2017, Pires et al. 2020). By now, it is possible to infer that reintroduced species foster multiple pathways. In this sense, it is important to emphasize that the effect of the four species added together was greater than the sum of the isolated effects of each of them. This happened because some interaction pathways only occur because more than one reintroduced species are present. Thus, multiple species reintroductions produce a synergistic effect, creating novel interaction pathways that connect more than one reintroduced species.

Third, the observed changes in both direct and indirect patterns of interaction may affect the resilience and vulnerability of ecological assemblages to perturbations. These consequences, however, may depend on the natural history of the system and the type of perturbations. For example, connectance and nestedness create network redundancy and robustness against extinctions (Memmott et al. 2004) and may favor facilitative effects in mutualistic systems (Bastolla et al. 2009). However, by increasing cohesiveness effects in the system, connectance and nestedness may favour the propagation of perturbation across the system's pathways (Gardner and Ashby 1970, Allesina and Tang 2012). Nevertheless, in our study system, the reintroduction of seed dispersers may be essentially beneficial. By increasing the number of direct interactions and the overlap among partners, the network increases its redundancy and robustness against species loss. Thus, reintroductions made the network more robust and

less prone to secondary extinctions. An increase in robustness to secondary extinctions is critical to defaunated ecosystems, given that other sources of disturbance, such as climate change, are already in place (Losapio and Schöb 2017). Theoretical and empirical work also support the notion that highly connected species are key to the propagation of perturbations across systems (Paine 1966, Allesina and Tang 2012). Specifically, the extinction of species involved in more indirect pathways may trigger cascading effects, such as coextinction cascades (Pires et al. 2020). Therefore, reintroducing them may restore indirect effects that may reverse some of the changes caused by their extinction.

The reintroduced species at the studied site are all large- and medium-sized frugivores which disperse large seeds and interact with a wide range of species, and Tijuca is a defaunated tropical forest previously lacking most dispersers of large seeds (Fernandez et al. 2017). The dispersal of large seeds is of particular importance to plant regeneration and forest conservation globally (Neuschulz et al. 2016). It is known that the extinction of large-bodied frugivores had major impacts on a variety of ecological and evolutionary patterns and processes (Galetti et al. 2013, Mello et al. 2015). Indeed, the empirical restoration value of these species at the studied site has been demonstrated for howler monkeys by Genes et al. (2019) and for agoutis by Mittelman et al. (2020) and is being tested for tortoises. However, our analyses provide independent evidence of the importance of these

reintroductions to the conservation of the whole frugivore assemblage in Tijuca, beyond the species that directly interact with the reintroduced species. These and other large-sized species are disproportionately impacted by anthropogenic effects such as hunting, habitat degradation and fragmentation (Peres 2002, Peres and Palacios 2007, Dirzo et al. 2014, Ripple et al. 2019). Consequently, extinct large species are usually precisely those that play an important role in the stability of the community (Vidal et al. 2014). In this sense, the extinction of large-bodied animal species often implies a reduction in the number of interactions (Vidal et al. 2013), leading to some plant species losing all their seed dispersers (Janzen and Martin 1982).

Our results indicate that trophic rewilding could be an effective strategy to restore ecological networks in a way that may mitigate secondary extinctions in the future. This provides further evidence on the role of trophic rewilding in restoring complex ecosystems by increasing trophic complexity, dispersal and resilience in the face of stochastic disturbances (Perino et al. 2019). Moreover, we have found that reintroducing four species has stronger network effects due to the increase in indirect pathways that connect all species, which further highlights the importance of reintroducing whole sets of species, as proposed by trophic rewilding and refaunation (Oliveira-Santos and Fernandez 2010, Svenning et al. 2016), in comparison to single-species reintroductions. In this sense, we hypothesize that frugivore reintroductions in other defaunated areas, where seed dispersal interaction credits are high (Marjakangas et al. 2018), would similarly affect community structure and, potentially, dynamics through the addition of such disproportionately important missing links and indirect pathways.

Previous studies had suggested that reintroductions could potentially benefit communities through changes in the network structure (Genes et al. 2017, Pires 2017). However, to our knowledge, this is the first work to show those effects with *de facto* reintroductions. In this case study, we modeled the effects of species additions on the network after the reintroductions had occurred. Nevertheless, this same type of analysis can be used in the planning stages of reintroductions, in order to delineate priority species for reintroductions, depending on specific management goals and corresponding network metrics. Conservation goals have been giving increasing importance to the maintenance of functions in the face of environmental changes (Cadotte et al. 2011). Assessing the effects of species addition on network structure prior to reintroductions could help inform if the selected species will benefit the local community and thus, ecosystem function and stability.

Acknowledgements – We thank the Refauna Team (especially M. L. Rheingantz), and many volunteers, for their invaluable help in the species reintroductions and in productive discussions.

Funding – This work was partially funded by the following agencies that support the Refauna project: Coordenação de Aperfeiçoamento

de Nível Superior (CAPES), Conselho Nacional de Pesquisa e Desenvolvimento Científico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Fundação Grupo Boticário de Proteção à Natureza (Programa 0010/2014), Instituto Luisa Pinho Sartori (ILPS) and National Geographic Society (NGS-64305C-19). This study was also supported by CNPq (307134/2017-2, 140232/2018-4), and the Royal Society, London (CHL/R1/180156). LG is funded by the Gabilan Stanford Graduate Fellowship. APAA is supported by a postdoctoral fellowship from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, 2016/14277-2) and CAPES. PRG is supported by FAPESP (2018/14809-0).

Author contributions

Pedro Mittelman: Conceptualization (lead); Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Project administration (equal); Writing – original draft (equal); Writing – review and editing (equal). **Anna R. Landim:** Conceptualization (supporting); Data curation (equal); Investigation (equal); Methodology (supporting); Project administration (supporting); Resources (equal); Writing – original draft (equal); Writing – review and editing (equal). **Luísa Genes:** Conceptualization (lead); Investigation (equal); Methodology (supporting); Project administration (equal); Writing – original draft (equal); Writing – review and editing (equal). **Ana Paula A. Assis:** Conceptualization (supporting); Formal analysis (equal); Methodology (equal); Writing – original draft (equal); Writing – review and editing (equal). **Carolina Starling-Manne:** Conceptualization (supporting); Investigation (equal); Project administration (equal); Writing – original draft (equal); Writing – review and editing (equal). **Paula V. Leonardo:** Conceptualization (supporting); Writing – original draft (supporting); Writing – review and editing (supporting). **Fernando A. S. Fernandez:** Conceptualization (supporting); Project administration (equal); Supervision (equal); Writing – original draft (equal); Writing – review and editing (equal). **Paulo R. Guimarães Jr.:** Conceptualization (supporting); Methodology (equal); Project administration (equal); Supervision (equal); Validation (equal); Writing – original draft (equal); Writing – review and editing (equal). **Alexandra S. Pires:** Conceptualization (lead); Project administration (equal); Supervision (equal); Writing – original draft (equal); Writing – review and editing (equal).

Transparent Peer Review

The peer review history for this article is available at <<https://publons.com/publon/10.1111/ecog.05838>>.

Data availability statement

Data are available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.q83bk3j>> (Mittelman et al. 2021).

References

- Allesina, S. and Tang, S. 2012. Stability criteria for complex ecosystems. – *Nature* 483: 205–208.
- Allesina, S. et al. 2008. A general model for food web structure. – *Science* 320: 658–661.
- Almeida-Neto, M. et al. 2008. A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. – *Oikos* 117: 1227–1239.
- Arroyo-Rodríguez, V. et al. 2015. Seed dispersal by howler monkeys: current knowledge, conservation implications and future directions. – In: Kowalewski, M. et al. (eds), *Howler monkeys: behavior, ecology and conservation*. Springer, pp. 111–140.
- Bastolla, U. et al. 2009. The architecture of mutualistic networks minimizes competition and increases biodiversity. – *Nature* 458: 1018–1020.
- Bello, C. et al. 2017. Atlantic frugivory: a plant–frugivore interaction data set for the Atlantic Forest. – *Ecology* 98: 1729.
- Borrett, S. R. et al. 2007. Functional integration of ecological networks through pathway proliferation. – *J. Theor. Biol.* 245: 98–111.
- Burgos, E. et al. 2007. Why nestedness in mutualistic networks?. – *J. Theor. Biol.* 249: 307–313.
- Cadotte, M. W. et al. 2011. Beyond species: functional diversity and the maintenance of ecological processes and services. – *J. Appl. Ecol.* 48: 1079–1087.
- Coimbra-Filho, A. F. et al. 1973. Nova contribuição ao restabelecimento da fauna do Parque Nacional da Tijuca. – *Brasil Florestal* 4: 7–25.
- Dirzo, R. et al. 2014. Defaunation in the Anthropocene. – *Science* 345: 401–406.
- Dormann, C. F. et al. 2008. Introducing the bipartite package: analysing ecological networks. – *R News* 8: 8–11.
- Emer, C. et al. 2020. Seed dispersal networks in tropical forest fragments: area effects, remnant species and interaction diversity. – *Biotropica* 52: 81–89.
- Estes, J. A. and Palmisano, J. F. 1974. Sea otters: their role in structuring nearshore communities. – *Science* 185: 1058–1060.
- Estes, J. A. et al. 2011. Trophic downgrading of planet earth. – *Science* 333: 301–306.
- Fernandez, F. A. S. et al. 2017. Rewilding the Atlantic Forest: restoring the fauna and ecological interactions of a protected area. – *Perspect. Ecol. Conserv.* 15: 308–314.
- Galetti, M. et al. 2013. Functional extinction of birds drives rapid evolutionary changes in seed size. – *Science* 340: 1086–1090.
- Galetti, M. et al. 2017. Reversing defaunation by trophic rewilding in empty forests. – *Biotropica* 49: 5–8.
- García-Callejas, D. et al. 2019. Spatial trophic cascades in communities connected by dispersal and foraging. – *Ecology* 100: e02820.
- Gardner, M. R. and Ashby, W. R. 1970. Connectance of large dynamic (cybernetic) systems: critical values for stability. – *Nature* 228: 784.
- Genes, L. et al. 2017. Credit of ecological interactions: a new conceptual framework to support conservation in a defaunated world. – *Ecol. Evol.* 7: 1892–1897.
- Genes, L. et al. 2019. Effects of howler monkey reintroduction on ecological interactions and processes. – *Conserv. Biol.* 33: 88–98.
- Gilbert, J. P. and DeLong, J. P. 2017. Phenotypic variation explains food web structural patterns. – *Proc. Natl Acad. Sci. USA* 114: 11187–11192.
- Guimarães, P. R. 2020. The structure of ecological networks across levels of organization. – *Annu. Rev. Ecol. Evol. Syst.* 51: 433–460.
- Guimarães, P. R. et al. 2017. Indirect effects drive coevolution in mutualistic networks. – *Nature* 550: 511–514.
- ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade) 2008. Plano de manejo: Parque Nacional da Tijuca. – Inst. Brasileiro de Desenvolvimento Florestal, Brasília.
- Jansen, P. A. et al. 2012. Thieving rodents as substitute dispersers of megafaunal seeds. – *Proc. Natl Acad. Sci. USA* 109: 12610–12615.
- Janzen, D. H. and Martin, P. S. 1982. Neotropical anachronisms: the fruits the gomphotheres ate. – *Science* 215: 19–27.
- Jerozolinski, A. et al. 2016. Are tortoises important seed dispersers in Amazonian forests? – *Oecologia* 161: 517–528.
- Jordano, P. 2016. Sampling networks of ecological interactions. – *Funct. Ecol.* 30: 1883–1893.
- Jordano, P. et al. 2003. Invariant properties in coevolutionary networks of plant–animal interactions. – *Ecol. Lett.* 6: 69–81.
- Kaiser-Bunbury, C. N. et al. 2010. The robustness of pollination networks to the loss of species and interactions: a quantitative approach incorporating pollinator behaviour. – *Ecol. Lett.* 13: 442–452.
- Kristensen, N. P. et al. 2019. Dealing with high uncertainty in qualitative network models using Boolean analysis. – *Methods Ecol. Evol.* 10: 1048–1061.
- Kurten, E. L. 2013. Cascading effects of contemporaneous defaunation on tropical forest communities. – *Biol. Conserv.* 163: 22–32.
- Losapio, G. and Schöb, C. 2017. Resistance of plant–plant networks to biodiversity loss and secondary extinctions following simulated environmental changes. – *Funct. Ecol.* 31: 1145–1152.
- Marjakangas, E. L. et al. 2018. Estimating interaction credit for trophic rewilding in tropical forests. – *Phil. Trans. R. Soc. B* 373: 20170435.
- Mello, M. A. R. et al. 2015. Keystone species in seed dispersal networks are mainly determined by dietary specialization. – *Oikos* 124: 1031–1039.
- Memmott, J. et al. 2004. Tolerance of pollination networks to species extinctions. – *Proc. R. Soc. B* 271: 2605–2611.
- Mittelman, P. et al. 2020. Agouti reintroduction recovers seed dispersal of a large-seeded tropical tree. – *Biotropica* 52: 766–774.
- Mittelman, P. et al. 2021. Data from: Trophic rewilding benefits a tropical community through direct and indirect network effects. – Dryad Digital Repository, <<http://dx.doi.org/10.5061/dryad.q83bk3jrr>>.
- Neuschulz, E. L. et al. 2016. Pollination and seed dispersal are the most threatened processes of plant regeneration. – *Sci. Rep.* 6: 29839.
- Ohgushi, T. et al. 2012. Trait-mediated indirect interactions: ecological and evolutionary perspectives. – Cambridge Univ. Press.
- Oliveira-Santos, L. G. R. and Fernandez, F. A. S. 2010. Pleistocene rewilding, frankenstein ecosystems and an alternative conservation agenda. – *Conserv. Biol.* 24: 2–5.
- Paine, R. T. 1966. Food web complexity and species diversity. – *Am. Nat.* 100: 65–75.
- Peres, C. 2002. Synergistic effects of subsistence hunting and habitat fragmentation on Amazonian forest vertebrates. – *Conserv. Biol.* 15: 1490–1505.

- Peres, C. and Palacios, E. 2007. Basin-wide effects of game harvest on vertebrate population densities in Amazonian forests: implications for animal-mediated seed dispersal. – *Biotropica* 39: 304–315.
- Perino, A. et al. 2019. Rewilding complex ecosystems. – *Science* 364: eaav5570.
- Pires, M. M. 2017. Rewilding ecological communities and rewiring ecological networks. – *Perspect. Ecol. Conserv.* 15: 257–265.
- Pires, M. M. et al. 2014. Reconstructing past ecological networks: the reconfiguration of seed–dispersal interactions after megafaunal extinction. – *Oecologia* 175: 1247–1256.
- Pires, M. M. et al. 2020. The indirect paths to cascading effects of extinctions in mutualistic networks. – *Ecology* 101: e03080.
- Poisot, T. et al. 2015. Beyond species: why ecological interaction networks vary through space and time. – *Oikos* 124: 243–251.
- Ponisio, L. C. et al. 2017. Opportunistic attachment assembles plant–pollinator networks. – *Ecol. Lett.* 20: 1261–1272.
- Ripple, W. J. et al. 2019. Are we eating the world’s megafauna to extinction? – *Conserv. Lett.* 12: e12627.
- Schleuning, M. et al. 2015. Predicting ecosystem functions from biodiversity and mutualistic networks: an extension of trait-based concepts to plant–animal interactions. *Ecography* 38: 380–392.
- Seddon, P. J. et al. 2014. Reversing defaunation: restoring species in a changing world. – *Science* 345: 406–412.
- Solé, R. V. and Montoya, J. M. 2001. Complexity and fragility in ecological networks. – *Proc. R. Soc. B* 268: 2039–2045.
- Svenning, J.-C. et al. 2016. Science for a wilder Anthropocene: synthesis and future directions for trophic rewilding research. – *Proc. Natl Acad. Sci. USA* 113: 898–906.
- Terborgh, J. and Estes, J. A. 2010. *Trophic cascades: predators, prey and the changing dynamics of nature.* – Island Press.
- Terry, J. C. D. and Lewis, O. T. 2020. Finding missing links in interaction networks. – *Ecology* 101: e03047.
- Thompson, J. N. 2005. *The geographic mosaic of coevolution.* – Princeton Univ. Press.
- Tylianakis, J. M. et al. 2008. Global change and species interactions in terrestrial ecosystems. – *Ecol. Lett.* 11: 1351–1363.
- Tylianakis, J. M. et al. 2010. Conservation of species interaction networks. – *Biol. Conserv.* 143: 2270–2279.
- Vidal, M. M. et al. 2013. Large vertebrates as the missing components of seed-dispersal networks. – *Biol. Conserv.* 163: 42–48.
- Vidal, M. M. et al. 2014. Frugivores at higher risk of extinction are the key elements of a mutualistic network. – *Ecology* 95: 3440–3447.
- Vieira, M. C. and Almeida-Neto, M. 2015. A simple stochastic model for complex coextinctions in mutualistic networks: robustness decreases with connectance. – *Ecol. Lett.* 18: 144–152.