Title: Merging theory and experiments provides insights into co-extinctions

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Abstract

In an era of mass extinction, predicting the consequences of species loss has become a priority for ecologists. Extinction of one species can trigger the loss of dependent species, sometimes leading to cascades of extinctions. Simulations predict that cascading extinctions should be common, but empirical observations of extinction cascades rarely match ones predicted by simulation. In contrast, species-removal field experiments have yielded surprises, such as novel interactions following removals. Thus, given this mismatch, the true predictive value of extinction simulation studies is unknown. Here, we explore the value of ground-truthing extinction simulations with observational and experimental studies. We propose a new framework that unites both approaches to studying extinction cascades, which reveals new opportunities to couple theory and data.

Robustness in a time of mass extinction

The consequences of species losses for the persistence and stability of ecosystems is both a fundamental question in ecology and challenging to resolve (e.g.[1–5]). One challenge is that the complexity of ecosystems can obscure the consequences of species losses. Indeed, species threatened with extinction often provide resources and habitat for other species in multiple types of **ecological networks** (see **Glossary**). As a result, losing even one species can alter persistence of other species through a variety of interactions – and, in some cases, trigger **secondary extinctions (co-extinctions)** [6,7] that can accumulate as **extinction cascades** [8–10] in food webs and mutualistic networks. For instance, the majority of known local extinctions from climate change were not due to direct effects of climate, but rather were secondary extinctions due to losses of resources, symbionts, or biotic habitat [11]. In response, researchers have asked:

to what extent will species loss trigger cascading extinctions? To address this question, **robustness analyses** are a useful and common tool to study the potential for species losses to propagate through ecological networks and cause co-extinctions (Box 1 and 2). In ecological networks, robustness quantifies the tolerance of an ecosystem to species losses by measuring secondary extinctions [12,13] when consumers or mutualists lose their resources (Box 1), and complements the large literature focused on trophic cascades and top-down effects (e.g. [9,14–19]). In complex systems, robustness analysis provides a baseline for examining how an ecological network responds to the loss of species [20] (*reviewed in* **Box 1** and **2**).

Many studies performing robustness analyses – which simulate the consequences of species losses based on modeled or real-world ecological networks (Boxes 1, 2) – predict that loss of highly **connected species** should trigger waves of co-extinctions (e.g. in food webs [13,20–23], seed dispersal networks [24], and plant-pollinator networks [25,26]). While some instances of extinction cascades have been observed, particularly in cases of foundation species loss (e.g. [27,28]) and loss of important prey species (e.g. [29]), observations of cascading extinctions are more scarce than theory would indicate. This difference between common theoretical predictions and observations in nature begs the question: *Are extinction cascades less common in nature than predicted by theory because the ecosystems in question are understudied, because confounding mechanisms are missed in common theoretical frameworks (e.g.,* ***interaction rewiring****), or because time lags and data limitations obscure our empirical findings?* Addressing these questions is challenged by the fact that while robustness analysis is fundamentally conducted via computer simulations (“***in silico***”, Box 1) [20], primary extinctions are frequently observed or manipulated via experimental removals ***in situ*** (Figure 1). Each study type has unique advantages for addressing the consequences of species losses in interaction networks: *in silico* species loss can examine the extinction dynamics of many more species than we can manipulate *in situ*, while field studies may capture processes not typically accounted for in ecological networks or in robustness analysis (Box 2). To date, however, these lines of research have progressed largely in parallel.

We orient current work along two axes that describe the nature of the network data (theoretical vs. empirical) and the nature of the extinction (simulated vs. observed), and by doing so highlight understudied areas. We propose a forward-looking research agenda that could validate predicted ecosystem-wide consequences of the loss of threatened species, improve predictions about the types of systems most vulnerable to cascading extinctions, and identify mechanisms that allow ecological networks to buffer species losses.

Synthesizing robustness research to understand cascading extinctions

Combining strengths of theoretical and empirical lines of work on robustness can shed new light on the consequences of species losses in complex systems of interacting species (Figure 1). We classify robustness studies based on the two components which define the research approach: where the network data come from, and the approach to studying **primary** and **secondary extinctions**. First, ecological network data can be generated from models ("theoretical networks"), such as the niche model [30], or based on observed interactions among species ("empirical networks", e.g. [31]) (Figure 1, x-axis). Second, robustness studies use computer simulations or direct observations of real-world systems to track the remaining species and interactions following primary species extinction (Figure 1, y-axis). Simulation-based studies remove species according to a specified **extinction sequence** and track hypothetical secondary extinctions *in silico* (Box 1). In contrast, empirical studies can either mimic an extinction through species-removal experiments [32–34], or through observing a naturally-occurring species loss (e.g. [35,36]), and then observe subsequent changes in remaining species *in situ*. By considering these two elements as axes, we can place prior robustness studies on a continuum between theoretical vs. empirical network data and *in silico* vs. *in situ* observations of species losses and co-extinctions (Figure 1).

These axes reveal four quadrants that describe active research areas on robustness and key gaps in current approaches (Figure 1). Quadrant I contains theoretical studies that simulate network data using static (topological) or dynamic models, and simulate species loss using robustness analysis to predict secondary extinctions and changes to network structure (e.g. [13,37,38]). Quadrant II includes studies that collect empirical data to build the ecological network, and then simulate species loss to predict secondary extinctions and changes to network structure (e.g. [7,39,40]). The data requirements of dynamic models can be limiting, so static models are often used in studies of complex natural systems. Quadrant III contains studies that collect empirical data to build the ecological network, observe a naturally occurring species loss (e.g. [35,36]) or experimentally remove species (e.g. [32]), and monitor the network *in situ* for changes in species abundance, interactions, or biodiversity. Studies in this quadrant must consider background extinction rate, ethical and logistical concerns with species removals, and often require a long time scale of observation. Quadrant IV would include studies that simulate network data using models and recreate the network in an experimental setting. Then, species would be experimentally removed to track *in situ* secondary extinctions. As opposed to studies in

Quadrant III that leverage existing networks in nature, studies in Quadrant IV could, for example, generate theory-based hypotheses about the relationship between specific network structures and robustness and then test those hypotheses *in situ*. These types of studies could utilize static or dynamic models but are likely limited to small or simple networks. Overall, the act of comparing predicted secondary extinctions from simulations to observed secondary extinctions connects quadrants that fall above and below the x-axis, and provides a method of validating predictions from simulations and contextualizing empirical findings with theory (Figure 1, V).

Most robustness studies fall in Quadrants I and II, simulating species losses *in silico* in either theoretical or empirical networks (Figure 1) (e.g. [7,13,37,39–41]). *In situ* species-removal experiments in Quadrant III are also common (e.g. [34,42–44]), but it is more rare for these studies to conduct **sequential species removals** due to logistical and ethical limitations (but see [32]). To our knowledge, few studies fall in Quadrant IV (but see [33]). For example, Firkowski et al. [45] demonstrate how food webs can be assembled with particular structures in experimental mesocosms, but the study did not examine robustness.

Studies face several challenges in attempting to connect multiple quadrants by pairing *in silico* robustness analysis of empirical networks with *in situ* observations of species loss (Figure 1V). First, relating simulated extinctions to real systems poses a challenge because simulations tend to represent longer time scales than most experimental studies [46]. Thus, secondary extinctions may take longer than the time-scale of a typical experiment to occur and, therefore, are difficult to observe or to tease apart from local extinctions due to natural variability in communities from year to year (Outstanding Question 1). Second, species-removal experiments typically remove one to several species simultaneously (e.g. [32,34,42–44]), while *in silico* approaches often remove species sequentially from the network until extinction cascades occur (Box 1). Perspectives from metapopulation and metacommunity stability research (e.g.[47–51]) may prove useful in solving these cross-scale challenges, as experimental species removals are often similar to metapopulation studies in that the species is only removed from a small area for a short time scale. Despite these challenges, recent studies have made strides in bridging these gaps between theory and field observations (e.g. [32,33,35,36]). These types of studies can maximize the strengths of each approach ultimately helping to bridge theory and empirical work on co-extinctions.

Insights from robustness analysis provide testable predictions

Theoretical studies have generated a wealth of predictions about the factors that lead to robust ecological networks (*reviewed in [52]*), but to date, most of these predictions remain untested in the field (Table 1). Further, prior theoretical studies have also generated conflicting predictions about the types (i.e., **structures**) of networks that influence robustness (Table 1) that could serve as alternative hypotheses in field studies. Readily available field data present an opportunity to advance our understanding through tests of theory.

Testing predictions about network structure and robustness

In silico studies of species loss suggest that robustness can depend on the **network structure –** particularly network **complexity** (e.g., how connected it is) and **architecture** (the arrangement of species and connections, Figure 2). For instance, the overall complexity of ecological networks is determined by the number of species present and the connections among them, prompting questions such as: *Are species-rich networks more vulnerable to collapse than species-poor ones [13,37,53]? Do abundant connections among species provide redundant pathways, or do they increase the likelihood that an extinction will affect the entire network [41,53,54]?* Despite being widely analyzed *in silico*, the relationship between network structure and robustness remains nuanced, with few broad generalities (Outstanding Question 2). Though beyond the scope of this review, we list areas of active research in Table 1.

By pairing predictions of species loss *in silico* with *in situ* experiments and observations, further progress can be made on the longstanding question of how network structure relates to robustness (Table 1). Experiments could leverage natural variation in network structure among sites or along abiotic gradients. Replicated removal experiments could then be conducted among sites to assess the relationship between network structure and *in situ* robustness to species removal. In addition, networks could be directly assembled or manipulated to generate particular structural variation of interest, and then be subjected to species removals (e.g. [29]). Potential mechanisms for observed patterns can be confirmed with network simulations, demonstrating the benefit of combining these two approaches (e.g. [29]).

Testing predictions about secondary extinctions

Fundamentally, robustness analysis can generate questions about the order, identity, and cause of secondary extinctions (Box 1) that can be tested *in situ* (Figure 1, V). Notable studies have taken information gained from robustness analyses into the field to test these questions and exemplify the types of insights that can be gained using a synergistic approach. First, Pearse &

Altermatt [36] compared simulated secondary extinctions to observed regional extinctions in a plant-Lepidoptera network, and correctly predicted the number of observed butterfly extinctions due to plant loss given the rate of plant extinction [36]. However, simulations greatly underestimated total extinctions [36], demonstrating the need to consider other sources of species loss (e.g. habitat disturbance) and background extinction rates (Outstanding Question 3) in further work (Box 2). Second, Biella et al. [32] sequentially removed the four most-connected plant species in a large field experiment, tracked pollinator and interaction extinctions, and compared observed extinctions to modeled secondary extinctions. Overall, predictions from robustness analysis underestimated observed extinctions, and did not accurately predict the identity of species or interactions that went extinct [32]. Importantly, both of these studies [32,36] found that *in silico* predictions of species loss did not correctly identify the species that were lost *in situ* and underestimated total extinctions, emphasizing the importance of pairing *in silico* and *in situ* approaches synergistically.

Opportunities to test theory with available field data

Field data from species-removal experiments and long-term observations of communities can be paired with ecological network information to test theory and predictions about robustness. In some cases, studies have paired single-species removal experiments with ecological network data, which enables a comparison between the result of *in situ* and *in silico* loss of one species (Figure 1, Quadrant IV). Secondary extinctions were not observed in single-species removal experiments for plant-pollinator networks [42,43], parasitoid-host networks [55], or simple food webs [44], but empirical observations of changes to network structure can inform robustness theory. For example, the experimental removal of the most connected species in a pollination network [42] and seed dispersal network [34,56] led to changes in network structure that suggested rewiring. These studies demonstrate that removing only one species can provide insight into processes that relate to robustness, such as prey switching, interaction rewiring, or interaction turnover.

Robustness simulations can also be used to make explicit predictions that could be tested against long-term community time-series data. Several studies have used historical datasets of community composition (e.g. [57]), as well as paleontological and archeological evidence (e.g. [58]), to reconstruct networks from periods prior to known extinction events and examine extinction over time. These pre-extinction networks can be used to compare predicted changes in network structure and/or species' abundance from simulations to the observed network structure in time-series data following the known extinction (e.g. [35,36]). Many ecological monitoring programs are ongoing (such as the International Long Term Ecological Research Network and Forest Global Earth Observatory) and linking long-term community data with network information and robustness simulations can inform our expectations about the time scales of network response after species loss and potential **secondary extinction debts.** A network-approach could also help to tease apart mechanisms underlying background extinction rates (Outstanding Question 3). Overall, existing data sources and ecological methods can feasibly be brought into a network robustness framework.

Knowledge gaps for an integrated robustness research program

Our understanding of secondary extinction can be advanced even further if theory and data are not only explicitly paired, but studied iteratively and in conjunction. For example, *in* *silico* species loss results can suggest the identity of species to remove in an experiment, and the results of the *in-situ* loss experiment can be compared with, and ultimately improve, the simulated loss scenario. In this section, we highlight several areas of robustness research that could greatly benefit from an approach that fundamentally integrates theory and data throughout the research process.

Realism of species removal sequences

The order that species that are lost in primary extinctions (known as the **'extinction sequence'**) can greatly influence robustness [23,24,59]. *In silico* studies predict that multiple types of networks are less robust to the loss of highly-connected species than to the loss of less-connected species or random removals [7,24,31,60–69]. However, extinctions in the real-world do not necessarily occur in order from most-connected to least-connected. Species are instead lost based on species traits such body size [64,70,71], life history [24,26,71,72], and sensitivity to global change drivers [6,7,59,71,73,74]. These traits may be related to connectedness, as the most-connected species are often abundant and less likely to go extinct [6,75], but loss of threatened species from bipartite networks may reduce robustness more than random species loss [7]. Further, *in silico* studies of mutualistic plant-animal networks [59], aquatic food webs [74], and host-parasite networks [7] found that species with low connectedness were also the most sensitive to environmental change, but specialized parasites also tend to use hosts with few natural enemies, which could reduce risk of co-extinction [76]. *In situ* observations of which species are lost in an ecosystem following a disturbance could inform realistic extinction sequences for *in silico* robustness analysis to investigate network-level impacts (e.g. [77]). We lack more general data on whether vulnerable species tend to correspond to highly or poorly connected species in ecological networks (Outstanding Question 4). Analyses are needed that explicitly pair network data, species identity, traits, and global change responses.

Network rewiring

In contrast to common model assumptions (Box 2), species can adapt in response to loss of their resources, potentially leading to interaction rewiring in networks. The magnitude of rewiring and its effect on secondary extinctions is a source of uncertainty in robustness analyses (Outstanding Questions 5, 6). From *in silico* studies of species loss, it is generally predicted that rewiring should increase robustness [25,39,78–82] but there is not complete consensus on this prediction among network types or modeling approaches (e.g.[80,83]). Empirical evidence for rewiring has been observed in single-species removal experiments in plant-pollinator [42,84] and seed dispersal [34,56] networks. However, the level of rewiring to include in simulations is an open question, as rewiring may vary by system and through time. Where studies have paired *in situ* species-removal experiments with simulations including rewiring, simulations overestimated the impacts of species loss, suggesting that rewiring was even more important than specified in the model, but different levels of rewiring were not compared [34]. Notably, empirical secondary extinctions were underestimated by models without rewiring in studies where multiple species were sequentially removed or lost naturally [32,36], whereas single species-removal studies have found models with rewiring either performed better [56] or overestimated extinctions [34]. This variability strongly suggests that *in situ* observations should be used to further inform the rules for when and how species rewire in existing simulation approaches, and how thresholds of primary extinction interact with rewiring [25,39,80]. Regardless, failure to consider rewiring in models can contribute to mismatches between predictions from robustness analyses and observations in real-world systems.

Learning from predicted-observed mismatch

When the theory fails to predict observed secondary extinctions, this mismatch can provide important insights into where strategic extensions or revisions to robustness and ecological network theory are needed. For example, consider a food web or mutualistic network that experiences more secondary extinctions in an experiment than predicted by robustness simulations. This may suggest that species subject to primary extinction were interacting more strongly with the network than represented in the model and/or that interactions not included in the network are important (such as positive interactions that are not captured by the food web, or trophic interactions not captured by the mutualistic network). These patterns could also occur if **functional extinctions** contributed to cascading extinctions [85] and/or if the cause of species loss (e.g. environmental change) affected additional aspects of the network. Alternatively, if the network experienced fewer secondary extinctions than predicted, this also suggests that interactions not included could be important, that interaction rewiring is important, or that species interactions were previously under-sampled. In this way, identifying mismatches is key to making progress towards predicting the impact of primary extinction on the broader ecosystem.

Future robustness studies can bridge theory and data by focusing on mechanisms behind potential mismatches *a priori*, via model design and experimental design. Past studies that compared predicted and observed secondary extinctions tended to explain mismatches *post hoc*, attributing them to factors such as habitat loss [36] or rewiring [34]. By anticipating possible mismatches based on past work, future studies could test predictions generated by multiple models to identify the mechanism for the mismatch. For example, one could generate predictions about secondary extinction from topological robustness analysis (Box 2), a dynamical model with top-down effects [23], or an empirical network that includes trophic and non-trophic interactions, such as habitat associations[86] and mutualisms [87]. Models could also be specified to include different levels of rewiring [80] and background primary extinction rates. Then, multiple models could be compared to observed patterns from a species-removal experiment or observational studies. These models could then be extended to include additional features of ecosystems, such as ecosystem services [88], to explore the impacts of cascading extinctions beyond ecological networks themselves (Outstanding Question 7). By leveraging the wealth of alternative hypotheses generated by both theoretical and empirical work, future studies can greatly improve our understanding of the effects of extinction on ecosystems and provide more specific recommendations for conservation applications.

Concluding Remarks

Explicitly linking *in situ* and *in silico* approaches could present a powerful tool to address Outstanding Questions regarding the role of network structure and robustness (Table 1, Figure 2). Linking approaches could also help develop and test predictions on cascading extinctions and the order of species loss in networks [32,35,36]. Of the few empirical studies that tested theoretical robustness predictions, observed extinctions tended to be underestimated by simulations [32,36]. However, we highlight ways to leverage existing data from species-removal experiments and long-term community monitoring that could be readily used to test the generality of these predictions across a range of ecosystems. In addition, we identify ways in which ongoing field research could develop *a priori* hypotheses that directly link possible empirical outcomes to robustness theory, while still addressing questions relevant in applied contexts. Each approach offers valuable insight into the impact of species losses and cascading extinctions in real-world ecosystems. By combining simulations of ecological networks with long-term community data and species-removal experiments, our framework will improve our ability to predict network responses to species losses and environmental change.

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Box 1. Defining and measuring robustness

Robustness of ecological networks quantifies the extent to which species losses spread, leading to secondary, or 'cascading' species losses. In this context, robustness analysis typically entails the simulation of primary species losses (i.e., removing species in a designated order, or randomly), and tracking the secondary loss of other species. Secondary species losses can occur under a variety of assumptions. Most often, secondary losses occur when a species loses all of its resources. In dynamic models that account for bottom-up, top-down, and sometimes competitive effects, secondary species losses generally occur when a species' biomass or abundance drops below a specified threshold (e.g., increased predation leads to a drop in primary producer biomass) [89,90]. Most models do not include recolonization from a metacommunity, and are simulating the total global loss of a resource or mutualist (though see [50,51]).

While there are many ways that robustness can be measured (e.g.,[54,66,91,92]), we highlight two common metrics used to assess ecological network robustness. The first metric is known as 'R50', or the fraction of species that need to be removed in order to lose 50% or more of the original network's species richness [8]. R50 tracks the cumulative fraction of secondary extinctions—where the denominator is initial richness—in relation to the fraction of species removed (Fig. I). Robustness values fall between 0 and 0.5, where lower values indicate a less robust network. This metric can be adjusted to assess different thresholds of network collapse [93].

The second most common metric used to measure robustness is known as 'AUC', or Area Under the Curve [94]. Unlike R50, AUC tracks the fraction of species *remaining* in the network in relation to the fraction of species removed; and calculates the area under the curve created by these values (Fig. I). Often, studies will divide the AUC by the fraction of species removed (e.g., [94]). Scaled this way, robustness values fall between 0 and 1. Similar to R50, low values indicate less robust networks.

Box 1 Figure I. Caption

(A) Robustness can be calculated as R50, the fraction of species that must be removed to cause 50% of the species in the network to go extinct. This is calculated by tracking the cumulative fraction of secondary extinctions (y-axis) as species are removed through primary extinctions (x-axis). The accumulation of secondary extinctions (shown here as the fraction of total species) is shown by the dotted line. The points at which total species losses would equal 50% is shown by the dashed line. R50 is indicated by a red cross at the intersection of the two lines and is reported as the x-value for this intercept. A less robust network would experience many secondary extinctions after relatively few primary extinctions, so the slope of the dotted line would steeply increase and R50 would be a smaller value. A network with the highest robustness possible would experience no secondary extinctions and R50 would equal 0.5. (B) Robustness can also be calculated using the Area Under the Curve (R_{AUC}) measured as $\sum y(x)$ where x is the fraction of species removed (x-axis) and y is the fraction of susceptible species (such as parasites in a host-parasite network) remaining (y-axis). This approach does not require all species to be removed or secondarily lost and should be scaled to the length of the sequence (i.e., $\frac{\sum y(x)}{\max(x)}$).

<u>Box 2- Caveats of robustness analysis</u>

There are both shortcomings and advantages to the two most commonly used approaches for robustness: dynamic species interaction models or **topological networks**. Dynamic species interaction models involve modelling population and interaction dynamics (e.g., Lotka-Volterra models [33,95,96], bioenergetic consumer-resource models [90]). While dynamic models can account for a variety of ecological processes, including functional extinctions [82,89] and metapopulation stability [97], there are many challenges associated with parameterizing dynamic models. For instance, the choice of parameters and functional forms tend to influence the dynamics [98] and are often highly uncertain given existing data. Robustness studies that incorporate dynamics largely use simulated data [13,23,63,99].

Topological networks are based on empirical data and simulations omit dynamics (e.g.,[8,100,101]) This approach has been widely implemented in the study of food webs for the past 20 years, and is the dominant approach used to study stability in empirically-derived ecological networks [8,100,101]. A benefit of this approach is that it does not require the strong assumptions about many largely unknown parameters that dynamic models do [98]. Further, one can analyze larger networks than feasible with dynamic approaches. However, this approach has been critiqued for only considering "bottom-up" extinctions, where species go secondarily extinct when they lose all of their resources or mutualists—ignoring top-down and competitive effects[70]. The omission of dynamics (including changing abundance, functional extinction, and varied extinction risk) can lead to an overestimation of robustness compared to dynamic models[23]. Furthermore, topological approaches frequently focus on binary interactions (i.e., the presence/absence of an interaction; but see, [25,72]). This assumption omits the effects of interaction strength, which can increase robustness [102]. Including interaction weights (which measure the frequency or importance of interactions) offers a middle ground between topological and dynamic approaches.

A critique that applies to both approaches is the inclusion of only one interaction type. The majority of robustness studies, regardless of approach, consider either trophic (e.g. [71,77,103,104]) or mutualistic (e.g. [39,80,84,102]) interactions only. However, ecosystems (including the associated dynamics, structure, etc.) are shaped by multiple interaction types (e.g., mutualistic, trophic, competitive; [105]). Thus, the tendency for robustness studies to consider one interaction type results in skewed conclusions about ecological networks' tolerance to species losses [52].

Glossary:

Ascendancy: The extent to which energy flows are confined to interactions among specialists in a food web.

Co-extinction (or Secondary Extinction): The loss of a species in response to extinction of a necessary resource species, such as the extinction of a predator when its only prey is lost, or the extinction of a plant when its only pollinator is lost.

Connectance: The proportion of interactions in a network that are realized out of the maximum number of interactions possible.

Connectedness: The number of direct interactions a species has with other species.

Degree distribution: The frequency distribution of direct interactions among species.

Ecological network: A matrix that describes interactions between nodes (species, life stages, or aggregations of species). Interactions may be binary (present/absent) or weighted (representing interaction strength) and may indicate trophic or mutualistic interactions (networks of competitive interactions are possible but not considered here).

Expansibility: Describes the degree to which a network can be divided into at least two parts by removing only a few species or links (known as "bottlenecks").

Extinction Cascade: A sequence of extinctions that occurs when secondary extinctions or functional extinctions lead to additional co-extinctions. Extinction cascades may occur over long time scales, and may not necessarily occur in step-wise fashion due to delayed secondary extinctions.

Extinction sequence: The order in which species go extinct in or are lost from an ecosystem.

Functional extinction: When a species' abundance is reduced to the extent that it is no longer serving the same function in the ecosystem, which may lead to secondary extinctions of dependent species (e.g. a pollinator is not abundant enough to have a measurable impact on pollination of host plants).

Functional redundancy: When two or more species in an ecological network have the same relationship with a mutual interaction partner.

Generalization: The mean number of resource species a consumer interacts with.

In silico: Conducted in a computer simulation.

In situ: Conducted "in place", such as in a field experiment, mesocosm, or observations of a natural system.

Interaction Rewiring: Formation of a novel link between two species. For the purposes of this review, we do not consider not temporal variability in linkages as rewiring.

Modularity: The extent to which species within network are grouped into subsystems (compartments) of species which they interact with more strongly than outside species.

Nestedness: The extent to which specialist interactions are subsets of generalist interactions.

Network Structure: The complexity and architecture of species and interactions in a network.

Network Complexity: The relationship between the number of species and interactions in a network.

Network Architecture: The pattern of organization of species and interactions within a network, such as subsets of closely interacting species.

Robustness: A measure of network response to primary extinction; tracks secondary extinction and the proportion of species losses that lead to network collapse.

Primary extinction: The loss of a species from an ecological network due to either experimental removal, a simulated species removal, or in natural systems, anthropogenic or stochastic impacts..

Secondary Extinction Debt: The decline of a species due to the loss of a required interaction, but the species has not yet gone extinct.

Secondary Extinction: <u>See Co-extinction.</u>

Sequential species removal: An experiment where a set of species are removed from an ecological network one at a time. The network is monitored for secondary extinctions between each species removal.

Species Sensitivity: The tolerance of a species to environmental conditions, based on the species life history and biological traits.

Topological network: A representation of an ecological network where interactions and species presence are binary (present or absent); interaction strengths and species abundances are not included.

<u>*Tables*</u>

Table 1 Caption: ***Robustness predictions and opportunities for empirical examination***

Examples of network properties that have been predicted to affect robustness by *in silico* studies and whether they have been tested empirically. References in support or not in support are examples of *in silico* studies addressing each prediction, not an all-inclusive list of references. Unless indicated as a property of food webs, properties apply to multiple network types. Also note that many network properties are related (e.g. nestedness and modularity).

Table 1.

Property relates to:	Prediction	Ex. studies in support	Ex. studies not in support	Empirical validation?
Network complexity	Robustness increases with free-living **species richness**.	[13]	[37,53]	no
	Robustness increases with **connectance**.	[54,106]	[41,53]	no
	Networks with uniform **degree distributions** are more robust.	[41]		no
	Robustness increases with **generalization**.	[41,103]		no
	Robustness increases with **functional redundancy.**	[96]		[33]
	Parasitism reduces robustness.	[107–109]		no
Network architecture	Robustness increases with **nestedness**.	[38,77,108,110]	[109]	no
	Robustness decreases with **ascendancy**.	[41]		no
	Robustness increases with **modularity**.	[31,39]		no
	Robustness is higher in networks with good **expansion** (few bottlenecks).	[60,106]		no
	Robustness decreases as **maximum trophic level** increases in food webs.	[53]		no
	Interaction rewiring increases network robustness.	[39,80]	[83]	[34,111], partial
Extinction sequence	Ecological networks are less robust to loss of most **connected** species.	[7,31,64]		[32]
	Food webs are less robust to loss of lower **trophic level** nodes.	[23,71]		no
	Ecological networks are more robust to loss of less **prevalent**/more **sensitive** species than common species.	[7,59,71]		no
	Food webs are less robust to loss of species with small-medium **body size**.	[64]		no

Figure Captions

Figure 1. ***Classifying robustness studies reveals opportunities to bridge knowledge gaps***

Robustness studies can be classified based on the origin of network data (represented on the x-axis) and the method of studying extinctions (y-axis). Here we categorize ecological network data that is generated from models as "theoretical", shown to the left of the origin, while networks based on observed interactions among species are "empirical", and fall to the right of the origin. Studies can then track primary and secondary extinctions through computer simulations (*in silico)*, above the origin, or through observing experimental species removals or natural species losses *in situ,* below the origin. Blue indicates theoretical approaches (modeled networks and *in silico* extinctions). Green indicates empirically networks or *in situ* extinction observations. Dashed arrows and ovals indicate methodological steps that are either rarely done or have not been done, but that have the potential to help validate predictions from simulations, and support empirical findings with theory. These axes create four quadrants that classify areas of active research: I. studies that simulate network data with different structures, and simulate species loss using robustness analysis to predict secondary extinctions using either static or dynamic models [13,37]; II. studies that collect empirical data to build the ecological network, and then simulate species loss to predict secondary extinctions. In Quadrant II, use of static models is most common due to complexity of natural systems and data requirements of dynamic models [39,71]; III. studies that collect empirical data to build the ecological network, observe a naturally occurring species loss or experimentally remove species and monitor the network *in situ* for changes in species abundance, interactions, or biodiversity. In Quadrant III, static models are again most common, studies require a long time scale of observation and methods to distinguish secondary extinctions from background extinction rate, and species removal experiments can be logistically difficult or unethical [32,35,36]; IV. studies that simulate network data using models, recreate the network in an experimental setting, and experimentally remove species to track *in situ* secondary extinctions. Studies in Quadrant IV are rarely or not done, and are logistically constrained to simple networks, but could use static or dynamic models; V. studies that compare the predictions from simulations with *in situ* observations of secondary extinctions connect quadrants. For V, most studies compare empirical species loss to predictions from static models because comparisons with dynamic models are logistically difficult due to data requirements.

Figure 2. ***Network structures that may influence robustness***

Examples of network structural attributes that have been linked to robustness. Note that the metrics below are not arranged to show correlation with each other. *C*= directed connectance, S= number of species or nodes, cbar = compartmentalization. Network graphs and histograms were generated using the niche model ("trophic" package) or a random model ("tnet" package) in R version 3.6.2[112]

Highlights

- We are facing many local and global extinction events as part of the world's 6th mass extinction.
- Many studies focus on the extinction risk of individual species, but what are the knock-on effects when the prey of a specialist predator goes extinct or a plant loses its only pollinator?
- The potential loss of species and their interactions through co-extinction remains difficult to predict, which obscures our ability to predict extinctions from global change and impacts on ecosystems.
- Experimental and theoretical studies of co-extinction have progressed largely in parallel. Many studies simulate species loss using robustness analysis, while relatively few monitor co-extinctions *in situ* following natural species loss or experimental species removal.
- Theoretical and empirical studies on co-extinctions could provide new predictive insight in complex ecosystems if combined.

Outstanding Questions

1. Theoretical studies often consider global extinction over long time scales, while an experimental species removal is necessarily smaller in temporal and spatial scale. What are the appropriate scales of extinction to simulate?

2. What is the relationship between network structure and *in situ* (experimental) robustness to species removal? Many theoretical studies have focused on the relationship between network structure and robustness, but generally, predictions have not been tested empirically.

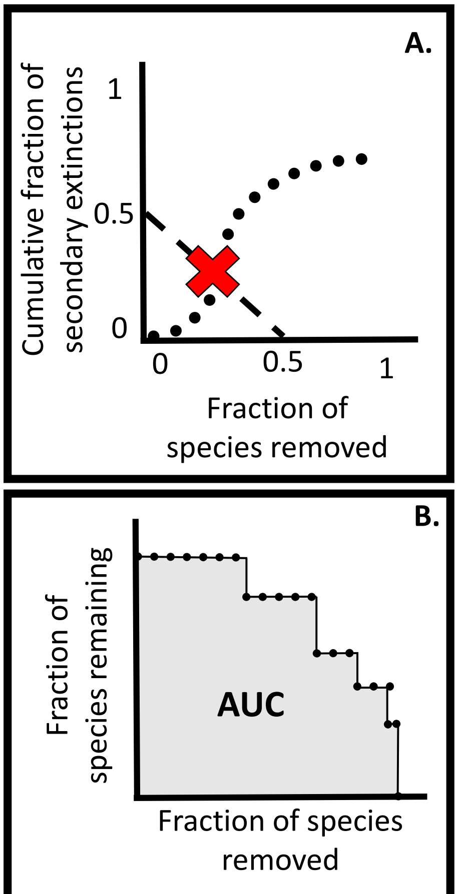
3. In natural systems, secondary extinctions can be difficult to tease apart primary and stochastically occurring extinctions. How can studies introduce controls in models to differentiate background extinctions from secondary extinctions?

4. What is the relationship between species extinction vulnerability and likelihood of triggering an extinction cascade? Are the species that are the most vulnerable to extinction also the most important to network robustness? Species vary in their ecological roles in networks as well as their susceptibility to threats.

5. There is empirical evidence for interaction rewiring, but it remains difficult to predict. How common is interaction rewiring in real ecosystems, and how much does it vary among systems? How should this be incorporated into simulations?

6. If rewiring occurs in an ecological network, to what extent does rewiring stabilize real networks in the face of species losses? Does the nature of this relationship change with different levels of rewiring?

7. Co-extinction is predicted to impact ecosystem functions and services, yet most insights from robustness analysis have not been tested empirically. How does co-extinction impact ecosystem functions and services *in situ*, beyond the effects of primary species losses? Are certain ecosystem services more vulnerable to co-extinctions and indirect effects than others?



$$R_{AUC} = \frac{1}{\max(x)} \sum y(x)$$

Box 1 Figure 1

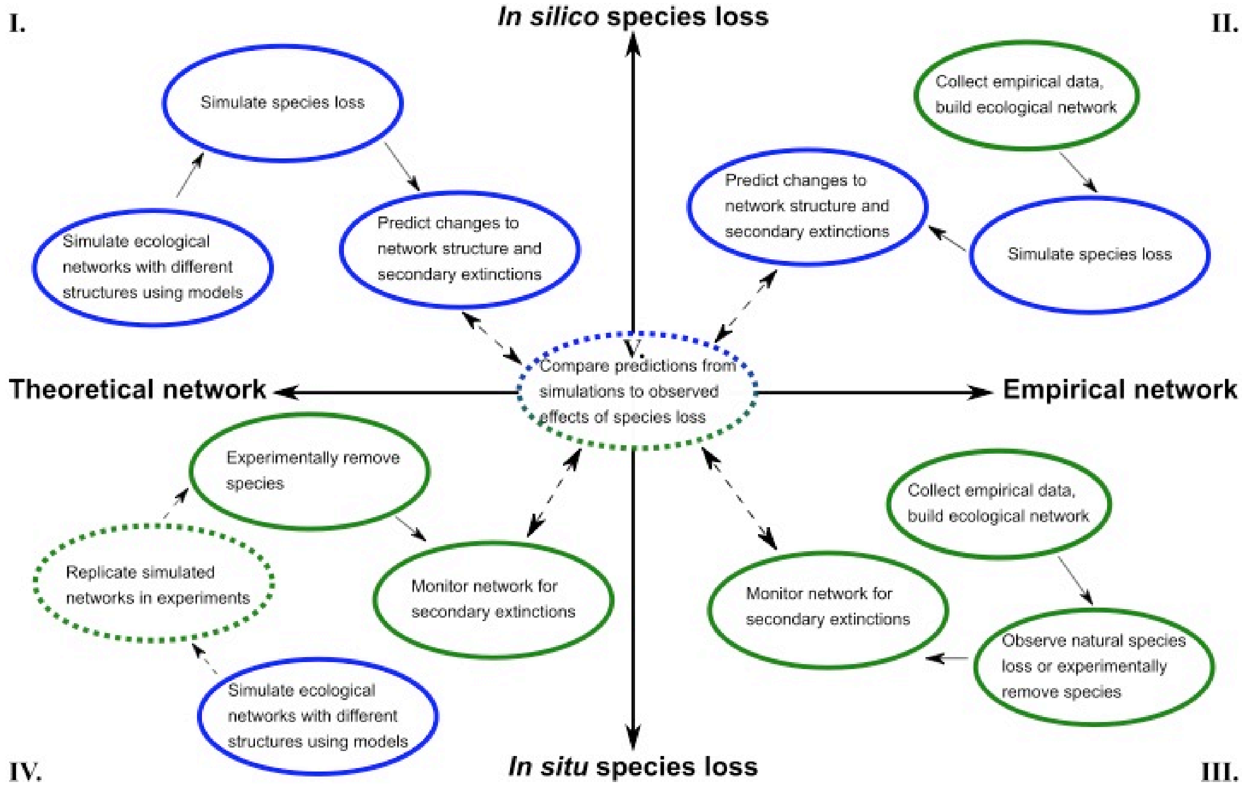

I.
In silico species loss
II.
Simulate species loss
Simulate ecological networks with different structures using models
Predict changes to network structure and secondary extinctions
Collect empirical data, build ecological network
Predict changes to network structure and secondary extinctions
Simulate species loss
V.
Compare predictions from simulations to observed effects of species loss
Theoretical network
Empirical network
Experimentally remove species
Replicate simulated networks in experiments
Monitor network for secondary extinctions
Simulate ecological networks with different structures using models
Collect empirical data, build ecological network
Monitor network for secondary extinctions
Observe natural species loss or experimentally remove species
IV.
In situ species loss
III.

Figure 1.

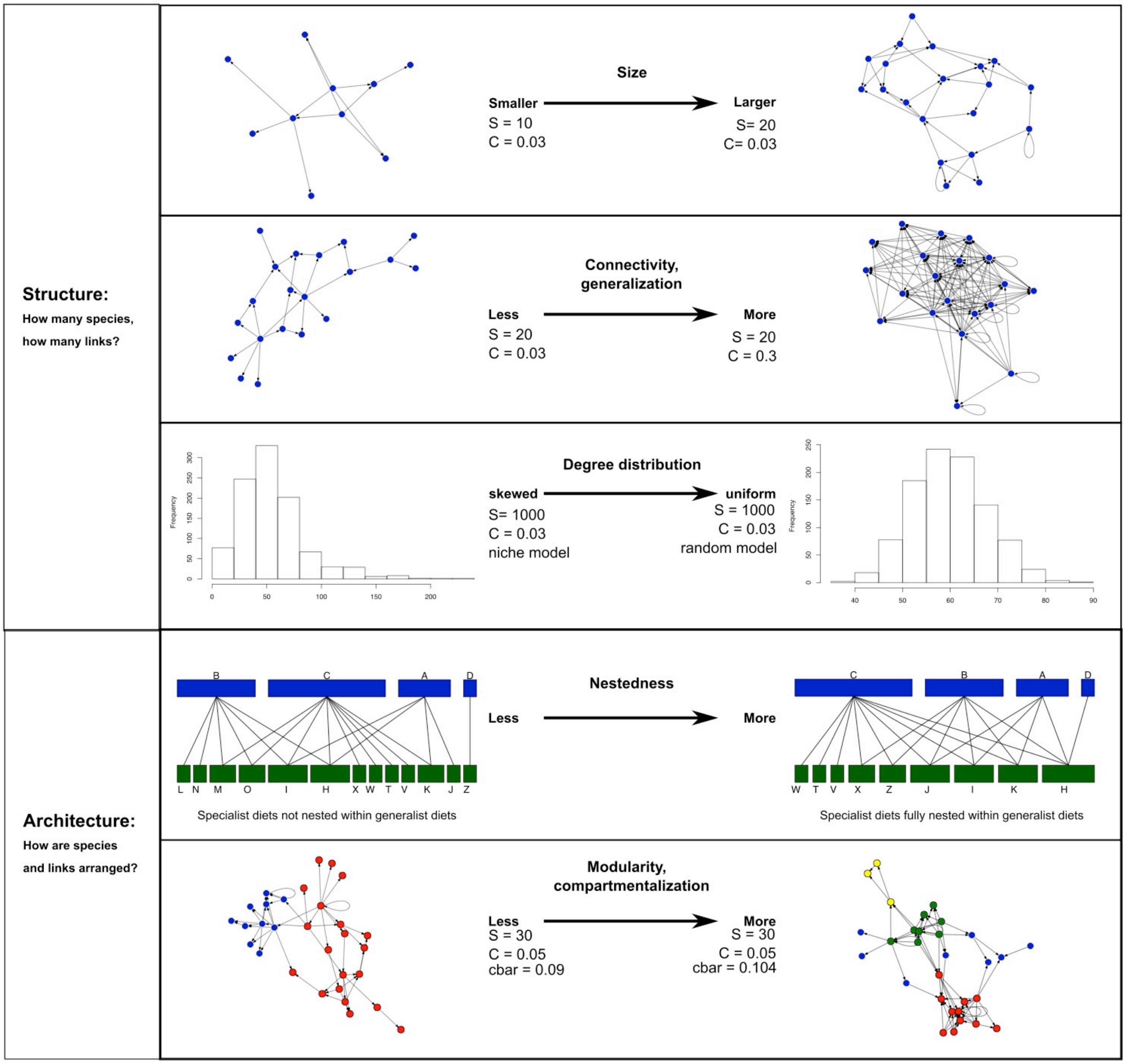

Structure:
How many species,
how many links?
Size
Smaller
Larger
S = 10
C = 0.03
S= 20
C= 0.03
Connectivity,
generalization
Less
More
S = 20
C = 0.03
S = 20
C = 0.3
Degree distribution
skewed
uniform
S= 1000
C = 0.03
niche model
S = 1000
C = 0.03
random model
Frequency
Architecture:
How are species
and links arranged?
Nestedness
Less
More
B C A D
L N M O I H X W T V K J Z
C B A D
W T V X Z J I K H
Specialist diets not nested within generalist diets
Specialist diets fully nested within generalist diets
Modularity,
compartmentalization
Less
More
S = 30
C = 0.05
cbar = 0.09
S = 30
C = 0.05
cbar = 0.104

Figure 2