Fluctuating ecological networks: a synthesis of maximum entropy approaches for pattern and perturbation detection

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Authors’ contribution statement

TC and DG developed the concept of the paper, analysed the data and equally contributed to writing. MR critically reviewed the concepts and results of the work, and substantially contributed to writing.
Abstract

Ecological networks such as plant-pollinator systems vary systematically in space and time. This variability includes fluctuations in global network properties such as total number and intensity of interactions in the network, but also in the local properties of individual nodes, such as the number and intensity of species-level interactions. Fluctuations of local properties can significantly affect higher-order network features, e.g. robustness and nestedness. These fluctuations should therefore be controlled for in applications that rely on null models, including pattern detection, perturbation experiments and network reconstruction from limited observations. By contrast, most randomization methods used by ecologists treat node-level local properties as “hard” constraints that cannot fluctuate. Here we synthesise a set of methods based on the statistical mechanics of networks, which we illustrate with some practical examples. We illustrate how this approach can be used by experimental ecologists to study the statistical significance of network patterns and the rewiring of networks under simulated perturbations. Modelling species heterogeneity, while allowing for local fluctuations around a theoretically grounded notion of structural equilibrium, will offer a new generation of models and experiments to understand the assembly and resilience of ecological networks.

key words: ecological networks; null models; network fluctuations; network pattern detection; network reconstruction; network stability; maximum entropy
1. Introduction

Ecologists use network models to understand complex interactions between different individuals, populations and species in communities (Pascual & Dunne, 2006). The nodes of the network can, for example, represent species. The edges linking nodes will then symbolise interactions observed between species. One of the most typical examples of these interactions are the mutualistic interactions between plants and their pollinators (Ings et al., 2009). In the ecological literature, there has been increasing recognition of the spatial and temporal variability of ecological networks, for example in terms of network rewiring (i.e. changes in who is connected to whom) in response to seasonality and perturbations (CaraDonna et al., 2017; Evans et al., 2013). At the same time, there are certain network features that remain rather stable. For example, plant-animal mutualistic networks can be highly variable in terms of which species is connected to which other species (i.e. their topology), while maintaining a clear core-periphery structure where a small number of generalist species, the core, interact with a large number of specialised species, the periphery (Miele et al., 2020). This means that species in the core (periphery) tend to keep a higher (lower) number of connections (the degree), despite the changes in other overall network properties.

The question of how ecological networks are assembled and what controls their stability has been intensely investigated in recent years (Aizen et al., 2016; Allesina & Pascual, 2008; Evans et al., 2013; Fortuna et al., 2010; James et al., 2012; Säterberg et al., 2013; Valdovinos, 2019; Valverde et al., 2020). To answer questions around network assembly processes and stability, network models are typically assembled from data that describe who is connected to whom (adjacency matrix) and possibly the intensity of interactions, i.e. link weights (interaction or weight matrix). The modelling usually starts from the observed or measured network interaction matrix and mostly focuses on extracting salient features from it. These features can be either static structural patterns such as nestedness or the abundance of specific subgraphs/motifs, or simulated dynamical changes in the observed matrices to infer the effects of perturbations. Since
such changes may also be assessed via a comparison with a null model, sometimes there is effectively no fundamental distinction between the two types of structural patterns (Evans et al., 2013; Pascual & Dunne, 2006; Valdovinos, 2019).

In any case, the analysis of ecological matrices is typically based on the formulation of null models. Tailored null models based on constrained permutations of the values observed in the data matrix have become central to hypothesis testing and pattern detection in studies of ecological networks, one goal being testing whether basic observed properties can explain higher-order properties such as nestedness (Bruno et al., 2020; Dormann et al., 2009; Dormann & Strauss, 2014; Payrató-Borras et al., 2019; Strona et al., 2014). These models generally permute, randomize, or sample otherwise, the entries of ecological matrices by keeping certain values fixed. These values can for instance be total sums over the entire matrix (e.g. total interaction strengths, or total number of links), or local sums along each row and/or column (e.g. the number of links of each node, known as the degree). In any case, the selected values are generally measured from the empirical matrix and in the majority of cases (see specific references and exceptions in the next sections) kept strictly fixed, i.e. treated as “hard” constraints, in the construction of the random null model.

In this essay, however, we review and illustrate a different approach and propose that the intrinsic variability of ecological networks can be best modelled by allowing for the chosen constraints to fluctuate, i.e. be assigned values that can vary around the measured ones, while at the same time remaining characteristic for each node (species) in the network. The reason why we put emphasis on fluctuating constraints is twofold. On the one hand, the intrinsic variability of species-level activity, diet or behaviour implies that the interactions observed in a specific moment or experiment are a particular snapshot of a larger set of possible realizations. On the other hand, experimental observations are necessarily subject to measurement errors such as spurious associations (false positives), missing data (false negatives), or noise (incorrect values). Whatever the source of uncertainty, a cautious approach should interpret the measured
entries of ecological matrices as particular realizations from a set of possible ones. Consequently, the properties that are chosen as constraints in ecological null models should themselves fluctuate. This perspective, while poorly explored in ecology (but see references we review in the next sections), is actually well established in statistical mechanics through the formulation of maximum-entropy ensembles of networks (Squartini & Garlaschelli, 2017). In the last two decades, ensembles of networks with fluctuating constraints have been first developed theoretically in the general context of the statistical physics of complex networks (Cimini et al., 2019; Newman, 2018; Park & Newman, 2004), with applications mainly to large social, economic and financial networks. Some seminal applications to ecological networks have been recently proposed in the specific context of the statistical significance of nestedness (Bruno et al., 2020; Payrató-Borras et al., 2019; Payrató-Borràs et al., 2020) but a more general framework for the analysis of ecological systems, possibly encompassing multiple properties (e.g., binary and weighted links) and/or constraints, is now possible.

A network that features no patterns when compared with a null model under certain constraints is also a network that can be reconstructed by the null model. In other words, some of the properties of the real network do not deviate from the statistical expectation of the null model. This fact is useful whenever there is only partial and/or very uncertain information available on the real network and the researcher wishes to reconstruct the network from this uncertain information, which is typical of ecological networks. This problem is well known in the case of financial networks, where due to privacy, there is only aggregate information available on the total amount of financial transactions per node (Cimini et al., 2019). But this problem is also very relevant to ecological networks. For example, there is often only partial information on the number of partners (neighbours) for each node (a plant or root symbiont species for example). Ecologists in some case may have an estimate of the number of nodes interacting with any other node or the total amount of interactions necessary to support the node (e.g. total pollination service to a plant) but there remains uncertain knowledge of which node
is interacting with which other node or the intensity of the interaction between nodes. This is often the case for molecular data, derived from high-throughput sequencing, linking microbes to plants (e.g. Caruso et al. 2012). The question then becomes whether the network can be reconstructed, at least to some extent, from available partial information.

Network reconstruction starts from the assumption that, given some knowledge on the network, there is a potentially very large number of network configurations consistent with this knowledge. All the reconstructed configurations must therefore respect some constraints. The chosen constraints should be informative and capture some fundamental ecological properties of the measured network (Dormann & Strauss, 2014; Gotelli, 2000), typically at the level of individual species. This is why the focus is on constraints that are also local (node-specific) in nature.

In the set of methods we here synthesise and interpret ecologically, pattern detection is intimately connected to network reconstruction because reconstructed networks can be used as null models, and vice versa. This means that, if a class of networks is systematically found to display no particular pattern compared to a null model, then that model can be used as a network reconstruction technique for other networks in the same class. The general approach we review here uses the observable properties of the network to derive an ensemble of networks having those properties, with all the other properties being maximally random (Cimini et al., 2019; Squartini & Garlaschelli, 2017). As we mentioned, this is also the logic of constrained null models in ecology (Dormann & Strauss, 2014; Gotelli, 2000; Strona et al., 2014), although our emphasis here is on approaches that allow for fluctuating constraints.

We remark that the approach we review here have important practical consequences for empirical analyses. In statistical physics, for very large matrices, treating null constraints as fixed (or “hard”), as done in most ecological null models, or fluctuating (“soft”) makes little difference when constraints represent global quantities such as the total number of links or the total interaction strength measured all over the
matrix. This property is known as ensemble equivalence (Touchette, 2015). When ensemble equivalence holds, one is justified to use whichever ensemble, based on mathematical or computational convenience. However, a series of relatively recent findings showed that ensemble equivalence does not hold when constraints are local (i.e. node or species specific), which is central to ecology as it applies both to binary and weighted network matrices, possibly including core-periphery structure, communities, or other forms of modular organization in networks (Garlaschelli et al., 2016; Squartini et al., 2015; Zhang & Garlaschelli, 2020). Why is this important to the experimentalist? Because statistical analyses based on models with hard local constraints (e.g. row and/or column sums that are kept exactly fixed), as mostly implemented by ecologists, can lead to different and even statistically opposite results than those based on fluctuating constraints, as shown by a recent analysis of a large dataset of mutualistic plant-pollinator networks (Bruno et al. 2020).

In this paper we illustrate the construction of network statistical ensembles with fluctuating constraints by presenting the general theoretical framework as well as some examples of application to real networks involving plants. Our discussion will try to emphasize that the approach suits ecological networks very well as it captures both their fundamental variability and experimental uncertainties associated with the measurement of the null model constraints themselves. Moreover, it is also computationally much more efficient than other currently available approaches, which is becoming increasingly important for molecular datasets linking microbes to plants. Also, and differently from current ecological null models, the approach we propose offers unbiased null models not only for presence/absence network data but also for networks with weighted links, which is a very important, new development. Finally, we also propose several future research lines that we argue are needed to shed light on key questions in the processes that structure and control ecological networks and, more generally, any biological system that can be modelled as a network.
**Maximum-entropy ensembles of networks vs. alternative ecological null models**

The starting, and essential, point of the network statistical mechanics approach is that an observed network, which is quantitatively described by an interaction matrix (topology + link weights), is one observed state from an ensemble of possible states (Figure 1 and 2). One state is a particular matrix realization and is assigned a probability of occurrence. All the network realizations, each with its probability, form a statistical ensemble; but how to derive these defining probabilities? The first step is the choice of the constraints, which in this context are based on properties measured on the empirical ecological network(s). One property could for example be the number of pollinator species any plant species possesses (and vice versa). Constraints should generally apply to the local properties of the nodes and not just to aggregated properties such as the total number of links in the network. As we mentioned, this is important because real-world networks, including ecological ones, are not homogeneous (Caldarelli, 2007) and not symmetric upon arbitrary permutations of species (Miele et al., 2020). Some of the signatures of this heterogeneity include the intrinsically hierarchical structure of ecological networks, e.g. the trophic hierarchy and allometric scaling of food webs (Garlaschelli et al., 2003), and the fact that most networks have broad distributions of the number of links per node (Bascompte, 2010). For example, most networks consist of a very high number of species having very few connections and a few species having a very large number of connections. The constraints thus often take the form of a vector with an element for every species or node. Such a local (node-specific) choice of the constraints suits ecological applications as it preserves the identity of every species across empirical and randomized instances of the network.

We stress that the “randomised” networks that form the null model in statistical mechanics should not be confused with generic sets of random matrices generated computationally through any permutation rule or randomization algorithm (Camacho et al., 2007; Dormann et al., 2009; Gotelli, 2000). Often, ensuring that locally exact constraints, such as an exact degree sequence (e.g. numbers of pollinator species...
associated with each plant species), are met by randomly generated matrices, while also ensuring an unbiased sampling of these matrices for constructing null models computationally and efficiently, becomes an unfeasible combinatorics problem for increasingly large real networks. Local rewiring algorithms have in the past been used to generate such ensembles computationally including applications to food webs (Camacho et al., 2007; Stouffer et al., 2007). There are also important and statistically robust approaches developed for binary matrices (Carstens, 2015; Strona et al., 2014, 2018; Ulrich & Gotelli, 2012) allowing unbiased sampling of the random matrices set. One issue is that producing a large number of randomly rewired matrices using these algorithms can be very time consuming for large matrices. This might not apply to a small plant-pollinator network but can become an issue for large plant-microbial networks assembled from molecular sequence data. Even more fundamentally, samples from randomly rewired matrices have been shown to be statistically biased for heterogenous networks (Artzy-Randrup & Stone, 2005; Roberts & Coolen, 2012) unless particular types of data structures and randomization algorithms are considered (Carstens, 2015; Strona et al., 2014, 2018; Ulrich & Gotelli, 2012). This means that, given the heterogeneity of real-world networks, local randomization algorithms carry a risk of not sampling the ensemble uniformly. That means that any quantity averaged over randomizations of the network (e.g. indices of nestedness in mutualistic network) is not guaranteed to correspond to the correct theoretical expectation of that quantity in the null model. Solutions to the issues of local rewiring algorithms are computationally intensive and apply only to specific conditions (Roberts & Coolen, 2012). Potentially, all these issues could be resolved in the future as computational power increases and more efficient algorithms are developed, which definitely applies to some types of typically not particularly large (i.e. rarely over a few hundreds of nodes) but very common ecological networks (Carstens, 2015; Strona et al., 2014, 2018; Ulrich & Gotelli, 2012). But ecological dataset size is increasingly becoming large with molecular sequence datasets, which is particularly central to plant-microbe interactions; and the available
randomization models apply only to topology (binary data) and cannot be generalised in such a way that constraints can include information about link weights, or a combination of topology and link weights, or complex constraints in topology and weight distribution (Squartini & Garlaschelli, 2017). Also very important, as we mentioned earlier, is the fact that ensembles with soft local constraints are not equivalent to ensembles with hard local constraints (Bruno et al. 2020), which implies that even unbiased and efficient algorithms dealing with hard constraints are not a substitute for ensembles with soft constraints.

The statistical mechanics solution

A general solution to all these issues of null model formulation is offered by statistical mechanics (Cimini et al., 2019; Park & Newman, 2004) especially the canonical ensembles as compared to microcanonical ensembles, which we define below in this section. The key quantity and concept to create a statistical mechanics ensemble is entropy. Entropy quantifies the uncertainty encoded in a probability distribution. The best-known expressions of entropy are Shannon entropy and Renyi’s generalization through the so-called Hill numbers. In ecology, these expressions have been applied to various statistical distributions, e.g. in order to measure community diversity in terms of the balance between species richness and evenness (Hill, 1973; Magurran, 2013). There is also the maximum-entropy theory of ecology to explain patterns such as relative species abundance in communities (Harte, 2011; Harte & Newman, 2014). In the context of networks and statistical mechanics, Shannon (or equivalently Gibbs) entropy is applied to the probability distribution of a whole graph in an ensemble of possible ones. The graph probability distribution that maximizes the entropy under certain constraints reflects maximal ignorance of all network properties but those used to set the constraints themselves. This maximisation of entropy corresponds to the construction of networks that are maximally random, apart from the imposed constraints.

In practice there are two fundamentally different ways in which constraints can be applied to derive a statistical mechanics ensemble. One way is the microcanonical
ensemble, which enforces the constraint exactly on each allowed realization. In this case, the maximum-entropy probability is uniform over the compatible configurations and the maximized entropy reduces to Boltzmann’s definition of entropy, i.e. it equals the logarithm of the number of allowed configurations. For example, if the constraint was the degree sequence as observed in a real-world network, all the networks in the ensemble will have exactly the same degree sequence as the observed one. This model is known as the *microcanonical* (or “hard”) *configuration model*, and its entropy quantifies the number of graphs with given degree sequence, which is a combinatorically challenging enumeration problem (Squartini & Garlaschelli, 2017). The other way is the canonical ensemble, which we embrace here. In the canonical ensemble, the constraint is respected by the ensemble only on average and the investigator is looking at a system that fluctuates “at equilibrium” around a set of “typical” configurations, which are collectively the most likely. Following the same example where the constraint is the degree sequence, the corresponding model is known as the *canonical* (or “soft”) *configuration model*. For example, plant A might have five known pollinators, that is a degree of five. Individual networks in the canonical ensemble might have the same species A with three or six, or any other number of pollinators, but such that the ensemble average of the degree of species A is exactly five. Plant A will thus have a theoretical average degree (i.e. weighted by the probability of all possible matrices in the ensemble matrix) exactly equal to five. If the ensemble is instead sampled numerically and the average is performed as an arithmetic average over independently sampled matrices (i.e. using their frequency), the sample average of the degree of plant A will converge to five as the number of sampled matrices increases and the graph probability distribution is sampled progressively more.

**The approach proposed here: canonical ensembles to model ecological networks**

*Matrices*

We now formalize quantitatively the above notions and definitions. Let’s call \( O \)
(observed) the matrix that describes the observed network with $S$ species or nodes. The ensemble we are looking for consists of a large number of matrices, call each of them $E_i$ ("ensemble" matrices). Each $E_i$ has the same size, i.e. the same number $S$ of nodes, as $O$. Moreover, across the entire ensemble, we preserve the identity of all nodes by attaching a unique label $i$ to each of them, i.e. the corresponding networks or matrices are labelled. In general, the difference between each $E_i$ and $O$ is in terms of who is connected to whom and/or the strength of these connections. The set of the matrices $E_i$ are all possible states of the network. Among them, only one is exactly $O$. What characterizes the ensemble is the probability $P(E)$ over the entire set of matrices, and we want this probability to depend on some structural properties of $O$. In particular, we choose a set of constraining properties using their values as empirically observed in $O$. As we want each constraining property to apply locally to each species, our constraint has the form of a vector, i.e. a vector having at least as many elements as the number of species in $O$. We say "at least" because we may want to have multiple constraints for each species, e.g. the number of incoming links and separately the number of outgoing links for each node in a directed network such as a food web. We denote the vector of constraints by $C$. We denote the value of property $C$ attained on a generic network $E_i$ as $C(E_i)$, and so the empirical value of the constraint is denoted as $C^* = C(O)$, where the star means the special value of the constraining property as measured in the observed network $O$. For example, if $O$ represents an undirected network and $C$ is the degree sequence, then $C^*$ will be the degree sequence of matrix $O$, i.e. the list of empirical degrees of all species, which would look something like $C^* = [k_1, k_2, \ldots k_S]$, where $k_i$ is the degree (number of interacting species) of each species $i$ (for all $i=1,S$).

**Entropy Maximization**

The main objective of statistical mechanics is finding the probability distribution $P(E)$ realizing the constraint while assuming maximum randomness of all other
properties. With this probability distribution, we can formulate a statistical expectation of all observable quantities. Mathematically, finding the distribution \( P(E) \) that maximizes the randomness, given the constraints \( C^* \) (plus the additional constraint that \( P(E) \) has to be normalized), requires a quantitative definition of the randomness (i.e. uncertainty) encoded in \( P(E) \) in the first place. The statistical mechanics definition is Shannon (or Gibbs) entropy, defined as

\[
S(P) \equiv -\sum_E P(E) \ln P(E) \quad \text{eq. 1}
\]

which is familiar to ecologists as a diversity index. It is obvious from eq. 1 that entropy would just count the number of allowed states if \( P(E) \) were uniform. This is the same reason why the Shannon diversity index equals just species richness when all species are equally frequent and so the community has maximum evenness. Now, maximizing \( S(P) \) given the constraints, requires that we rigorously clarify what we mean by the fact that we want the matrices \( E_i \) to "realize" the constraint \( C^* \), which was measured in the observed matrix \( O \). There are at least two alternatives, corresponding to the aforementioned microcanonical and canonical ensembles respectively. The microcanonical ensemble is discussed in detail in the Supporting Information.

In the canonical ensemble the constraints are enforced "only" as ensemble averages, i.e. as

\[
\langle C \rangle = \sum_E C(E) P(E) = C^* \quad \text{eq. 2}
\]

where \( \langle C \rangle \equiv \sum_E C(E) P(E) \) denotes the ensemble average (expectation value) of the property \( C \) across all matrices \( E \). Now one has to find the form of \( P(E) \) that maximizes the entropy \( S \) in eq.1 subject to the constraint in eq.2, plus the additional normalization condition \( \sum_E P(E) = 1 \). The result is well known and is obtained with the method of Lagrange multipliers (Park & Newman, 2004; Squartini & Garlaschelli, 2017). The maximum-entropy probability distribution depends on a vector \( \theta \) of Lagrange multipliers and takes the form

\[
P(E|\theta) = \frac{e^{-H(E,\theta)}}{Z(\theta)}, \quad \text{eq. 3}
\]
where

\[ H(E, \theta) \equiv \sum_i \theta_i C_i = \theta C(E) \]

is the so-called Hamiltonian of the network, and

\[ Z(\theta) = \sum_E e^{-H(E, \theta)} \]

is the normalization constant, also known as partition function. Statistical physicists recognize the form of \( P(E|\theta) \) in eq.3 as the Boltzmann-Gibbs distribution over the graphs in the ensemble, where the Hamiltonian generalizes the energy (Park & Newman 2004), while statisticians and social scientists recognize it as the probability for the so-called Exponential Random Graph (ERG) models (Wasserman & Faust, 1994). The exact details of the derivation leading to eq.3 can be found elsewhere (Squartini & Garlaschelli 2017 and technical references therein) but what matters here is that we have introduced the new vector \( \theta \), the Lagrange multipliers, whose values have to be determined in order to calculate the probability of each matrix \( E_i \) in the ensemble. Details on this calculation and estimates of \( \theta \) from the data using maximum likelihood are given in the Supporting Information.

**Null models and pattern detection**

**Using the ensemble as a null model**

Once confirmed that the canonical ensemble respects the critical assumption set by the constraints (Supporting Information, Figure S1), we compare the observed network to the canonical ensemble for properties that were not used as constraints (Figure 2, 3 and 4).

We use the logic of null models and calculate the z-score of a network metric \( X(E) \) as:

\[ z_X = \frac{X(O) - \langle X \rangle}{\sigma(X)} = \frac{X^* - \langle X \rangle}{\sigma(X)} \]

Where (again) \( O \) is the observed matrix, \( X^* = X(O) \) is the observed value of the metric
\( X(E) \), while \( \langle X \rangle \) and \( \sigma(X) \) are the expected value and standard deviation, calculated under the probability \( P(E|\theta^*) \) given by the null model, respectively. In principle, \( \langle X \rangle \) and \( \sigma(X) \) can be calculated analytically from \( P(E|\theta^*) \); however, depending on the choice of \( X(E) \), it may be more convenient to compute them as a sample average and a sample standard deviation respectively, over a large set of random matrices sampled numerically from \( P(E|\theta^*) \). In either case, the z-score is simply the number of standard deviations by which the value of the metric in the observed network differs from its ensemble average. A positive score means that “observed \( X > \) expected \( X \)”. If the distribution of the metric \( X \) in the null model is normal, then the probability of observing a difference beyond two standard deviations just by chance would be roughly 0.05. Alternatively, if the ensemble metric is not normally distributed, a p-value can be defined following standard ecological null models (Gotelli 2000).

**A simple binary example**

As an example, we show the canonical ensemble for undirected binary networks, briefly explain the extension of this model to the case of direct binary networks, and finally show an application to an ecological bipartite network from the literature. For a complete description, see Squartini & Garlaschelli (2017), which we have here adapted to an ecological network to provide a specific example. For binary networks, the matrix \( E \) reduces to the \( S \times S \) binary adjacency matrix \( A \), where \( S \) is the number of species and the elements \( a_{ij} \) are either 1 or 0, depending on whether species \( i \) and \( j \) are connected or not. As constraint, we choose the degree sequence \( k(A) \) with the index \( i \) indicating species \( i \). The Hamiltonian of the graph is therefore \( H(A, \theta) = \sum_i \theta_i k_i(A) = \sum_i \sum_{j<i} (\theta_i + \theta_j) a_{ij} \), which basically associates Lagrange multipliers with each link between any species \( i \) and \( j \). Through the partition function \( Z(\theta) \), which can be calculated explicitly, one can obtain the graph probability as

\[
P(A|\theta) = \prod_i \prod_{j<i} p_{ij}^{a_{ij}} (1 - p_{ij})^{1-a_{ij}} \quad \text{Eq. 4}
\]
Where \( p_{ij} = \frac{x_i x_j}{1 + x_i x_j} \) is the connection probability between nodes \( i \) and \( j \), while \( x_i \equiv e^{-\theta_i} \) is a conveniently transformed Lagrange multiplier.

The knowledge of \( P(A|\theta) \) allows the calculation of the expected degree \( \langle k_i \rangle \) as a function of the transformed Lagrange multipliers. Equating the latter to the empirical degree \( k_i(O) \), as prescribed in general by eq. 2, gives the following set of \( S \) nonlinear coupled equations:

\[
\langle k_i \rangle = \sum_{i \neq j} \frac{x_i x_j}{1 + x_i x_j} = k_i(O) \quad i = 1, S.
\]

The values of \( x_i \) solving the above equations coincide with the values that maximize the log-likelihood \( L(\theta) \). They can be found efficiently using, for example, the routines Max&Sam for MATLAB (Mastrandrea 2014, Squartini et al. 2015) or NEMtropy for Python (Vallarano et al., 2021). Especially NEMtropy comes with a step by step set of instructions and illustrative codes that are relatively easy to implement even for the vast majority of ecologists who predominantly work in the R environment and may be approaching Python for the first time. This is useful, because experimental ecologists do not need to derive the most important models. Here, we rederived one important model just for the purpose of illustration but major models have already been derived and efficient computer routines are already available to fit the models also to ecological networks. At the same time, the theory is flexible enough to accommodate new, future model ecologists will need in order to implement specific constraints and so test specific hypotheses. For the ecological modellers, the implementation of specific constraints equates to find out the form of the graph Hamiltonian (eq. 3) that corresponds to the tested hypothesis or chosen set of constraints. For the ecological experimentalist, the task reduces to fit a routine that corresponds to that Hamiltonian. This is another important advantage of the statistical mechanics approach over ad hoc randomization algorithms. Once inserted back into \( p_{ij} \), the \( x_i \) values allows the calculation of the probability \( P(A|\theta^*) \) of observing a particular configuration \( A \). As clear from eq.4, this probability can be factorised in the probability of observing the links of the network, which
are therefore mutually independent. Thus, the total entropy can be calculated as the sum of the entropies for individual pairs of nodes:

\[
S(A|\theta^*) = -\sum_i \sum_{j<i} [p_{ij} \ln(p_{ij}) + (1-p_{ij}) \ln(1-p_{ij})] = -\ln P(O|\theta^*).
\]

The extension of this model to the bipartite case is straightforward and involves a reparameterization that considers the two sets of nodes in the two layers (e.g., plants and pollinators) of the bipartite graph (Saracco et al., 2015), which is particularly relevant to plant ecology but not only. As an example, we applied the binary bipartite model to a publicly available database of a plant-pollinator network (https://orcid.org/0000-0002-3449-5748), which has been recently proposed and analysed in the context of core-periphery models (Miele et al., 2020). This dataset is particularly interesting because it consists of 6 years of data with three sampling time points each year. The dataset can thus show temporal fluctuations of the same pollinator network. For the purpose of illustrating the general feature of network canonical ensembles, we limited the analysis to the binary version of the dataset using the binary configuration model (Squartini & Garlaschelli, 2017). We estimated the ensemble using the degree sequence as constraint. The ensemble was estimated for each matrix from each of the 18 samplings (thus 18 ensembles for each of the 18 observed matrices). We sampled 999 matrices from each of the 18 ensembles (so, in Figure 2, “n” equals 999; see section “Null models and patterns detection” and Supp. Info).

After fitting the model, we preliminarily checked that the main assumption of eq.2 is fully met by the ensemble, that is the average degree of each node in the ensemble is identical to the observed degree of each species, which is clearly the case for our example (Figure S1 in Supporting Information).

For this example, we calculated four network metrics (Figure 3): the canonical ensemble provides an excellent estimate of functional complementary and the so-called motif 7 (Figure 3b and d) as defined in (Dormann & Strauss, 2014; Simmons et al., 2019) That means that the local properties of the network, which in this case are just described by the degree sequence, are sufficient to describe these higher-order properties. However,
other properties such as niche overlap and NODF (a metric of nestedness) deviate significantly from the ensemble average (Figure 3a and c), meaning that the processes that structure the local properties of the constraint (in this case the degree sequence) are not sufficient to explain these network level properties. This is an extremely useful fact, which allows exploring in specific detail the processes that structure the network. Overall, the method is very general as it can impose multiple constraints, either topological and quantitative, or with different types of topological and quantitative constraints. The model accounts for network fluctuations around an equilibrium and it is computationally effective and statistically unbiased.

Extending and innovating ecological null models

Pattern detection in ecological null models requires randomizing observed data by fixing all the factors that can confound the search for patterns. The ambition is that the detected patterns can be informative of the processes that generate the patterns, which is traditionally very difficult to achieve (Dormann & Strauss, 2014; Gotelli, 2000; Gotelli & Ulrich, 2012). Network reconstruction, instead, primarily aims at reconstructing the network using some of its fundamental features to describe, as accurately and unbiasedly as possible, other observable features of the network. To clarify the difference between traditional null models and network reconstruction, assume the investigators acquire some knowledge on the processes that assemble the network or have hypotheses on these processes. More specifically, the investigators have hypotheses on how these processes structure some fundamental properties of the network. They then measure these properties and use these measurements as constraints to reconstruct the network using the ensemble approach. Now, the investigator can test the quality of their network reconstruction using other (unconstrained) properties to test their hypothesis using the null model approach as described here.

As shown by our simple example and by other examples in ecology (Bruno et al., 2020; Payrató-Borras et al., 2019; Payrató-Borràs et al., 2020) as well as in several other
disciplines (Cimini et al., 2019; Squartini & Garlaschelli, 2017), careful choices of the constraints can lead to canonical ensembles that replicate various other network properties. Network models based on the canonical ensemble thus introduce very useful new tools for the study of ecological networks. Firstly, the canonical ensemble is constructed having in mind two key ideas: the constraints are based on observable properties of the network and, even at equilibrium, networks display natural variability around these properties. The canonical ensemble models such network variability in analogy with thermodynamic equilibrium. This null model allows ecologists to test empirically whether the measured network is at equilibrium, which is a widely made assumption, more or less explicitly, in most ecological works. Note that the postulated equilibrium does not need to be global. It just needs to be local, at a certain scale and within a certain time interval. A corresponding choice of the constraints allows testing these hypotheses by modelling the equilibrium network as continually fluctuating from state to state around the typical configurations. If we then interpret the ensemble as a reconstruction of the network, the processes that control the shape of constraints also control the probability distribution of the network configuration and its equilibrium.

Secondly, a perturbation that changes the processes and factors that control network structure may move the network away from the equilibrium (Squartini et al., 2013). This means that, given a set of constraints, the reconstruction is sometimes successful at predicting non-constrained properties but sometimes it is not. When it is not, the comparison between the empirical evolving network and the canonical null model can reveal and quantify the ongoing departure of the system from equilibrium. To show this, we implemented a sequence of extinctions in the pollinators of the plant-pollinator network of our example (based on (Miele et al., 2020), using classical methods (Memmott et al., 2004). Just to show how the canonical ensemble tracks structural changes and deviation from a starting equilibrium, we removed pollinators starting from the species with the highest number of connections but, of course, many other sequences of extinction could be more appropriate, including purely random ones.
(Memmott et al., 2004). Here, we just wanted to show an example, and not to exhaustively address the specific network nor the general issue of species extinction in networks. The removal in order of decreasing species degree is interesting because the overall effect is that the number of possible configurations will increase as highly connected species are removed. We indeed observe a systematic increase in the entropy (Fig. 4b). This is a deep structural change and shows that the metric of entropy itself, which in most applications is just used as a construct to derive other quantities (see e.g. (Harte & Newman, 2014), could be interpreted as a measure of rewirability of the network because it is logarithmically related to the number of possible alternative configurations. Clearly, the nature of the constraints will have major implications on how to interpret changes in the entropy of the network and an important related aspect is that in ecological networks not all possible reconfigurations are actually permitted. For example, certain plant species are pollinated only by some pollinators and not others. This reduces the number of possible configurations and should be carefully represented in the constraints used to generate the network ensemble. Rewirability is a central aspect of ecological networks and can be essential to the ability of networks to respond to perturbations (CaraDonna et al., 2017; Evans et al., 2013). and we propose that statistical mechanics offers a direct way of measuring rewirability on the condition that the derived canonical ensemble reconstructs the observed network to the level of accuracy needed to predict the network properties under investigation.

Finally, a novel feature we highlight in this paper is that multiple observed properties of a network can be used to constrain the construction of the null model ensemble. The properties chosen as constraints can be enforced one at a time or all at the same time, making the ensemble a more or less tightly dependent on the original network. The choice of the constraints is hypothesis-driven; enforcing different properties one at a time or at the same time can reveal the relative roles of the factors that contribute to the formation of the network. The baseline and null models offered by network statistical mechanics (especially canonical ensembles) can also help detect when the
network moves away from an equilibrium state in response to perturbations.

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Figure 1. Construction of a statistical mechanics ensemble of a network, from the observed network (top) to the final ensemble (bottom). Starting from the observed network, node-level properties such as the node degree (number of connections to the node) are enforced to construct the ensemble. The illustration is based on animal pollinators (light blue) and their plants (light green). The constraint is used to find the probability distribution $P(E)$ that maximises the entropy $S$. In the canonical ensemble, the constraint is enforced only on average. In this case, the maximization of the log-likelihood function defined through $P(E)$ is used to find the $P(E)$ parameterization that also maximises entropy given the constraint. The log-likelihood of the observed network equals the ensemble entropy (but a minus sign), and unbiased sampling of the ensemble is possible through the parameterized $P(E)$. See Figure 2 for the features of this sample.
Many matrices can be sampled from the ensemble to create a null distribution (histogram) for any network property. For example, metrics such as NODF (a measure of nestedness) can be computed for each of the $n$ matrices sampled from the ensemble and on the single matrix of the observed network (blue vertical line). A z-score can also be calculated.

$$z_{score} = \frac{O_{metric} - \langle E_{metric} \rangle}{\sigma(E_{metric})}$$
Figure 3. Four network metrics calculated for the canonical ensemble of the same network of Figure S1, from Miele et al. (2020). The observed metrics in panels b and d falls in the middle of the ensemble distribution, indicating the ensemble reproduces the observed property. The other two metrics in panels a and c, instead, diverge significantly from the ensemble average, meaning that the local properties used to reconstruct the ensemble are not sufficient to predict these network properties.
Figure 4. The green data points show a) the Z-scores of motif 7, and b) the entropy (standardised by network size) of all the matrices in the dataset of Miele et al. (2020). The data show how the network fluctuate around a z-score of about 1 (apart from two samplings diverging from their ensemble average). The orange data points are simulations of a progressive extinction of pollinators, starting from the species with the highest number of connections (see Memmott et al. 2004). As extinctions progress, the divergence of the network from its ensemble for motif 7 increases progressively. Also, the entropy increases, as with the loss of the most connected species, the average number of possible reconfigurations per species also increases, under a simplistic assumption that a species in one layer can connect to any other species in the other layer (ipso facto a neutrality assumption).
Supporting Information

Details on the microcanonical ensemble

In the microcanonical ensemble the constraint is enforced as \( C(E_i) = C^* \) for all \( i \), meaning that each individual matrix \( E_i \) accepted with nonzero probability in the microcanonical ensemble must have the same local property as observed in the constraint (in our example above, exactly the same degree sequence). Maximizing eq. 1 under these “hard” constraints, plus the additional normalization condition \( \sum E P(E) = 1 \), leads to a form of \( P(E) \) that is uniform over the subset of “compatible” matrices \( E_i \) such that \( C(E_i) = C^* \) and zero over the remaining “incompatible” matrices. Let us denote the number of compatible matrices by \( M(C^*) \). Despite the conceptual simplicity of the fact that \( P(E) \) is uniform over the compatible matrices, i.e. equal to the constant value \( 1/M(C^*) \) resulting from the normalization condition, its actual computation is extremely challenging, as calculating \( M(C^*) \) requires the enumeration of all matrices realizing the hard constraint. For instance, for simultaneous constraints on the rows and columns of matrices, only complicated asymptotic enumeration formulas are available (Barvinok 2012), and no closed-form expression exists. Similarly, the (maximized) entropy corresponding to this uniform \( P(E) \) is equal to \( S(P) = \log M(C^*) \) and its value is equally challenging to compute. These mathematical complications are reflected also in the aforementioned computational difficulty (i.e. sampling bias) associated with the numerical generation of individual matrices from the ensemble (Artzy-Randrup & Stone 2005; Roberts & Coolen 2012). Both the mathematical and the numerical construction of the microcanonical ensemble are thus highly complicated combinatorically. Do ecologists need microcanonical ensembles? The literature shows that ecological networks are highly variable in space and time, including evolutionary dynamics (Aizen et al. 2016; Segar et al. 2020). There is also uncertainty even in establishing their topology, and so properties apparently as simple as the number of connections to any species, which vary in space and time (Caruso et al. 2012; Blanchet et al. 2020).
very same fact that ecologists need to measure networks from multiple observations replicated in space and time already implies a degree of measurement uncertainty in the network matrix, which reflects the natural variability of networks. In the short term, under relatively stable conditions where evolutionary dynamics can be ignored, ecologists generally, and often implicitly, assume a condition of equilibrium and interpret the effect of perturbations as a departure from this equilibrium (Pascual & Dunne 2006). In the longer term, where evolutionary dynamics become relevant, as for example observable in microbial networks, the network can depart from a given equilibrium to settle onto a new equilibrium (Segar et al. 2020). The implication is that, even under a temporary short-term equilibrium condition, there is no such thing as a deterministic, fixed set of constraining properties. So, for instance, ecologists would experimentally collect multiple (say, $m$) observations of the same system at various sites and times, i.e. multiple observed matrices $O_1, \ldots, O_m$, resulting in multiple values $C(O_1), \ldots, C(O_m)$ of the constraints. Multiple observations should generally improve statistical analysis, but how can they be effectively combined to achieve a better construction of the ensemble in this case? Unfortunately, in the microcanonical ensemble this is in general not possible. Assume for instance that the $m$ measurements are averaged to produce the single value $C^*=[C(O_1) + \ldots + C(O_m)]/m$. Paradoxically, the value of $C^*$ may even be impossible to realize in a single matrix (in the language of graph theory, it may be non-graphical), for instance because it may now represent an averaged degree sequence containing non-integer values. How to enforce non-integer degrees, or in general non-graphical values of $C^*$, as hard constraints in the ensemble being constructed? This is technically impossible, since when $M(C^*)=0$ the microcanonical ensemble is undefined.

**Estimation of Lagrange multipliers and Maximum Likelihood for the Canonical Ensemble**

For each (e.g. species-level) constraint in the vector $C$ (for example, the degree of a node), there is one Lagrange multiplier. The partition function can be seen as the
additional multiplier required to enforce the normalization of $P(E|\theta)$. Before discussing how to determine the value of $\theta$, let us highlight two key properties of the canonical ensemble. First, $P(E|\theta)$ depends on $E$ only through the constraint $C(E)$, which means that any two matrices $E_1$ and $E_2$ with the same value of $C$, i.e. such that $C(E_1)=C(E_2)$, also have the same probability, i.e. $P(E_1|\theta) = P(E_2|\theta)$. This desirable result follows from entropy maximization: the ensemble is unbiased in the sense that it does not give any unjustified preference (in terms of probability) to configurations that have the same value of $C$, and moreover it gives a specific relative importance to configurations that have different values of $C$. This property also means that, in order to calculate the probability of a matrix configuration $E$, we just need to know $C(E)$. Second, it turns out that, for all the constraints of interest in this paper, i.e. local constraints obtained as sums along rows and/or columns of the observed matrix $O$, $P(E|\theta)$ can be factorised into the contribution ('occupation probability') of each matrix cell. In the case of networks, these contributions represent the probabilities of existence of individual links. The factorization of $P(E|\theta)$ implies that, after the preliminary determination of the Lagrange multipliers, we can sample an arbitrary number of networks from the ensemble by simply going over all pairs of nodes and sampling links independently of one another. This property is extremely useful as it drastically reduces the computational complexity of the problem and efficiently allows for several applications, as we discuss in the next section. Another consequence of the factorization of $P(E|\theta)$ is that the ensemble average of various properties of interest can be calculated analytically from the occupation probabilities of individual matrix cells.

We now come to the problem of the estimation of the value of $\theta$ from actual data. The canonical ensemble has a third important property that facilitates this estimation: the special value $\theta^*$ that realizes the observed numerical value $C^*$ of the constraint as prescribed by eq. 2, i.e. $\sum_{E} C(E) P(E|\theta^*) = C^*$, is unique and coincides with the global maximum of the log-likelihood $L(\theta) \equiv lnP(O|\theta)$ of the observed matrix $O$ (Squartini & Garlaschelli 2017). It should be noted that this very useful property is not true in general
for any network model, and it thus makes the canonical ensemble completely consistent, i.e. maximizing the likelihood implied by a given constraint is the same as enforcing that constraint as an ensemble property (Garlaschelli & Loffredo 2008). Besides being desirable for theoretical consistency, this property has useful practical consequences. First, since the dependence of $P(O|\theta)$, and hence $L(\theta)$, on the observed matrix $O$ is only through $C(O) = C^*$, in order to determine $\theta^*$ we do not need to observe the entirety of the matrix $O$, but just the constraint $C^*$. So, for example, if we were to reconstruct a binary matrix ensemble from the degree sequence, we would need just the degree sequence vector, not the entire adjacency matrix. In ecological practice, we would just need to know how many species are connected to each species, and not which species are connected to which other species. This idea is at the core of maximum-entropy methods for network reconstruction from partial information (Squartini et al. 2018). Second, algorithms that look for the global maximum of the smooth function $L(\theta)$ are more efficient than algorithms that look for the solution of the coupled nonlinear equations represented by eq.2. This is because, even though the two problems are equivalent mathematically, the knowledge of the log-likelihood allows for the calculation of its gradient, which guides and speeds up the numerical search for the maximum (Squartini et al. 2015).

Once $\theta^*$ is determined, it is inserted into eq.3 to obtain the probability $P(E|\theta^*)$, which specifies the ensemble completely. This approach has been systematized for networks of different types including undirected binary and weighted networks, directed binary and weighted networks, bipartite directed and weighted networks, and enhanced models where multiple constraints are simultaneously specified, for example to combine topological and link weight information into the same model (Squartini et al. 2015, Squartini & Garlaschelli 2017). Ecologically, this means that the canonical ensemble can be applied to anything ranging from food webs with directed energy fluxes, to host-parasite and mutualistic networks, to social networks, either in terms of just topology (binary data) or weighted links (e.g. a food web with energy fluxes). Again, the only
information needed is the specification of the constraint, not even the full network matrix (Figure 1). Various Python, MATLAB and C++ computer codes are also available to compute all the different models of canonical ensembles (Squartini et al. 2015, Vallarano et al. 2021) - see https://meh.imtlucca.it/ for an up-to-date overview of these methods. These codes can output any desired number of matrices $E_i$ from the ensemble (ecologists often use 999 matrices for null models) and the vector of (transformed) Lagrange multipliers, which allow calculating the probability of each cell in a general matrix $E$ and ultimately the full probability $P(E|\theta^*)$. These quantities allow calculating the (maximized) entropy

$$S(\theta^*) = - \sum_E P(E|\theta^*) \ln P(E|\theta^*) = \langle H \rangle + \ln Z(\theta^*) = \theta^* C^* + \ln Z(\theta^*)$$

and the maximized log-likelihood

$$L(\theta^*) = \ln P(O|\theta^*) = -\theta^* C^* - \ln Z(\theta^*) = -S(\theta^*),$$

which is simply minus the entropy.
Figure S1 The node degree of the observed network is used as a constraint to derive the canonical ensemble of the network. If the ensemble is estimated correctly, by construction the average node degree of the ensemble should be exactly equal to the observed one for a large number of sampled matrices. In the figure, the star symbol corresponds to the average of 999 matrices from the canonical ensemble of the 8th matrix of the general plant-pollinator dataset of (Miele et al. 2020). It is evident that these averages fall exactly on the identity line, meaning the ensemble respects the key assumption. The coloured dots correspond to the values of 8 particular ensemble matrices. These data show the interesting fact that individual matrices in the ensemble do not always have the same node degree as observed in the network, although the observed value is the most probable. The ensemble thus represents observed node level properties as a number of states around an equilibrium state, with a mean (the equilibrium) and a variance (small fluctuations).