Using a co-occurrance network

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1 Network Building

My project centered around the creation and analysis of ecological networks of microbiomes. I built networks, in the form of graphs $\mathcal{G} = (\mathcal{V}, \mathcal{E})$, based on co-occurrence of organisms at various taxonomic levels. In these graphs, vertices are labeled by taxa name, and edge sets are defined by co-occurrence or correlation.

1.1 Co-occurrence construction

One method of building a co-occurrence network from a set of samples of microbial communities is to simply count how often various microbes co-occur. I refer to this as the "binning method".

Given a set of samples with abundances of organisms, I map the abundances to discrete levels, as proportions of the maximum abundance *of that organism*. Precisely, let the samples be s_i and raw abundance of organism j in sample i be r_{ji}. I map the abundances according to

$$a(r_{ji}) = \begin{cases} \lfloor \left(\frac{r_{ji}}{\max_{s_k}(r_{jk})} \right) n \rfloor + 1 & \frac{r_{ji}}{\max_{s_k}(r_{jk})} \geq m \\ 0 & \frac{r_{ji}}{\max_{s_k}(r_{jk})} < m \end{cases}$$

where m is some minimum. Then, I create weighted edges between vertices (where 0 weight means no edge exists) where the weights of edges in $\mathcal{E}_1$ are

$$w^1_{jk} = \frac{\|\{i : a(r_{ji}) = a(r_{ki}) \neq 0\}\|}{S}$$

Here, S is the total number of samples. That is, I count the proportion of samples in which the two taxa appear at the same discretized level.

Next, I account for random coincidence of taxa in a sample, following [4]. To do this, I compare to a null model. The null model is defined in the following way.

$$A_j = \sum_{s_i} \mathbf{1}_{a(r_{ji}) \neq 0}$$

and

$$S^l_i = \sum_{v_j} \mathbf{1}_{a(r_{ji}) = l}$$

Then, the null model assumes that if

$$X_{jil} \sim binom\left(A_j, \frac{S^l_i}{\sum_{il} S^l_i} \right)$$

then $P(a^{null}_{ji} = l) = 1 - P(X_{jil} = 0)$. This allows us to calculate the probability of co-occurrence of taxa under the null model. Let w^{null}_{jk} be

$$w^{null}_{jk} = \|\{i : a^{null}_{ji} = a^{null}_{ki} \neq 0\}\|$$

This is the similar to the co-incidence model but now randomized. Then,

$$P(w^N_{jk} = K) = \sum_{\{A \subset \mathcal{V}_2 : |A| = k\}} \prod_{l \in A} a_{jl} a_{kl} \prod_{l \notin A} (1 - a_{jl} a_{kl})$$

Ideally, I would then define $\mathcal{E}$ by the weights

$$w_{jk}^2 = \begin{cases} 1 & P(w_{jk}^{null} \geq w_{jk}^1) \leq t \\ 0 & P(w_{jk}^{null} < w_{jk}^1) > t \end{cases}$$

However, that probability is intractable to compute. Instead, I take

$$\widetilde{P}(w_{jk}^{null} = K) = \sum_{l=0}^{i} \binom{N_1}{l} \binom{N_2}{K-l} p_1^j p_2^{K-l} (1-p_1)^{N-l} (1-p_2)^{N-K+l}$$

where

$$p_1 = p_a - \left(\frac{N_2}{N_1} \frac{N(\mu - \sigma^2) - \mu^2}{N^2} \right)^{1/2}$$

and

$$p_2 = p_a - \left(\frac{N_1}{N_2} \frac{N(\mu - \sigma^2) - \mu^2}{N^2} \right)^{1/2}$$

Finally, N_1, N_2 are to ensure that $p_1, p_2 \in [0, 1]$. These must satisfy:

$$\frac{\mu N(1-p_a) - N\sigma^2}{N(1-p_a) - \sigma^2} \leq N_2 \leq \frac{\mu^2}{\mu - \sigma^2}$$

with μ the mean of the real distribution, σ^2 the variance, and $p_a = \frac{1}{S} \sum_i a_{ji} a_{ki}$. So, I take

$$w_{jk}^2 = \begin{cases} 1 & \widetilde{P}(w_{jk}^{null} \geq w_{jk}^1) \geq t \\ 0 & \widetilde{P}(w_{jk}^{null} < w_{jk}^1) > t \end{cases}$$

1.2 Correlation construction

An alternative to the binning above is sample correlation. With N samples, that is:

$$\rho_{xy} = \frac{1}{N} \frac{(\boldsymbol{x} - \mu_x \mathbf{1}) \cdot (\boldsymbol{y} - \mu_y \mathbf{1})}{\sigma_x \sigma_y}$$

where μ_x is the mean abundance of taxa x, and σ_x is the standard deviation of abundance of taxa x across samples. This can be constructed by modifying the data matrix and then multiplying with it's transpose, making it much faster to compute than the binning method. In expectation, this the covariance divided by the product of the variances. I then construct a graph with weighted edges

$$w_{ij} = \begin{cases} \rho_{\boldsymbol{r}_i \boldsymbol{r}_j} & \rho_{\boldsymbol{r}_i \boldsymbol{r}_j} \geq 0.8 \\ 0 & \rho_{\boldsymbol{r}_i \boldsymbol{r}_j} < 0.8 \end{cases}$$

Alternatively, I can first "thresh-hold" the abundances given, converting to binary presence/absence data. To do this, I find the mean of all non-zero abundance values μ_{nz}, and then

$$r_{ij}^* = \begin{cases} 1 & r_{ij} \geq 0.01 \mu_{nz} \\ 0 & r_{ij} < 0.01 \mu_{nz} \end{cases}$$

The network is then constructed using sample correlation just as before.

Again, I check the significance of each edge, removing edges that have a high probability of occurring in a null model. I check this using Monte-Carlo simulation by estimating $p_{ij} \approx P(\rho_{\boldsymbol{r}_i^{null} \boldsymbol{r}_j^{null}} > \rho_{\boldsymbol{r}_i \boldsymbol{r}_j})$. I take enough Monte-Carlo trials for a 95% confidence interval of length 0.03. I then adjust edges so that

$$p_{ij} > 0.05 \Rightarrow w_{ij} = 0$$

The Null Model

Rather than simply generate an Erdős-Rényi random graph with some chosen parameter, I generate a graph that more closely reflects the idea of random data. That is, I start with random data and then generate a graph. I generate random data in the following way. Let

$$N_j = |\{i : r_{ij} \neq 0\}|$$

and

$$P_i = \frac{|\{j : r_{ij} \neq 0\}|}{|\{(i,j) : r_{ij} \neq 0\}|}$$

then I take

$$\hat{r}_{ij} = binom(N_j, P_i)$$

as randomly generated abundance data. The values N_i are the counts of appearances of taxi i, while the values P_i are the proportions of all appearances which happen within each sample.
Then, I construct our random graph from this random data. If $\hat{\boldsymbol{r}}_i$ is the vector of random "abundances" of taxa i, I have edges

$$w_{ik}^{null} = \frac{1}{N} \frac{(\hat{\boldsymbol{r}}_{\boldsymbol{i}} - \hat{\mu}_i \mathbf{1}) \cdot (\hat{\boldsymbol{r}}_k - \hat{\mu}_k \mathbf{1})}{\hat{\sigma}_i \hat{\sigma}_k}$$

The null model's use of a binolmial random variable can be interpreted in the following way. A "success" for a taxa/sample pair is an appearance of the taxa in the sample. The number of trials is the number of times the taxa appears in the data set. The probability of success is the proportion of all appearances which happen within the sample. This does have some drawbacks: notably that it does not preserve total abundance, or even total appearances. It allows more than one "success" for a single taxa/sample pair, which could be interpreted as higher abundance. Our "random samples" should be scaled by average abundances of a taxa across samples to "look like" real data. This doesn't effect correlation, because $Cov(ax, by) = abCov(x, y)$, and $\sigma_x = Cov(x, x)^{1/2}$, implying that $w_{ik} = Cor(\hat{\boldsymbol{r}}_i, \hat{\boldsymbol{r}}_k) = Cor(a\hat{\boldsymbol{r}}_i, b\hat{\boldsymbol{r}}_k)$. Notice also that the null model is the same as in the binning procedure, with a different construction of a correlation graph.

In [4], this model is called the "binomial model". Interestingly, that paper argues that an "edge swapping" model is more accurate. However, I have chosen the binomial model for speed.

1.3 Significance of the network

I show that the networks connected by the methods above would not occur in randomized data, and so argue for the significance of the networks. I generate random data as in the correlation construction of the network. I then use Monte-Carlo simulation to estimate the following

- $P(\mu_d < \mu_d^{null})$, where μ_d is the mean degree of the nodes of a network
- $P(\sigma_d < \sigma_d^{null})$, where σ_d is the variance of degrees of the nodes of a network
- $P(\lambda_{max} < \lambda_{max}^{null})$, where λ_{max} is the largest eigenvalue of the adjacency matrix
- $P(\lambda_{min} < \lambda_{min}^{null})$, where λ_{min} is the smallest eigenvalue of the adjacency matrix
- $P(|\mathcal{E}| < |\mathcal{E}^{null}|)$

The result with $10,000$ Monte Carlo trials was that it was extremely rare for a network constructed from a null model to have higher mean degree, higher degree variance, larger maximum eigenvalue, smaller minimum eigenvalue, or more edges. This suggests that the networks constructed from real data would be very unexpected according to the null model.

In addition to testing the complete network, I performed the same analysis on networks built from randomly drawn subsets of the data.

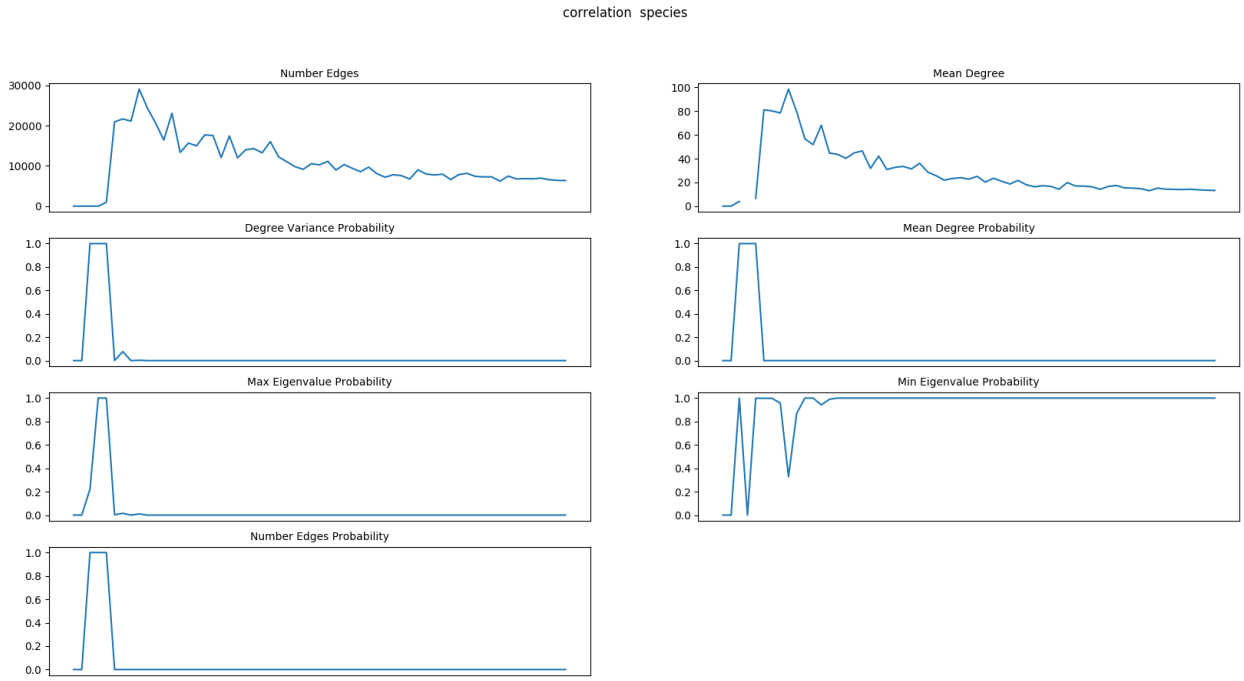


Figure 1: *The result of Monte-Carlo estimation of the likelihood of various network statistitics, as well as the number of edges and mean degree. The networks were made from correlations using randomly drawn subsets of the data of increasing size, from 1 sample to all 60 samples. For each network, 1000 random trials were generated according to the above null model in order to estimate likelihood of statistics.*

2 Network Analysis

We can cluster - community clustering, spectral clustering, comparing to random graphs. Comparison of clusters to grouping by sample type.

Community clustering minimizes a funciton of the graph called modularity [2][5]. That is

$$Q = \frac{1}{2m} \sum_{i,j} \left(w_{ij} + \frac{d_i d_j}{2m} \right) \delta(c_i, c_j)$$

where $m = \sum_{i \sim j} w_{ij}$, d_i is the (weighted) degree of vertex i, c_i is the community containing vertex i, and δ is the Kronecker δ. This done by a kind of gradient search/greedy algorithm.

Spectral clustering performs a k-means clustering on the rows of the matrix whose columns are the k eigenvectors of the graph laplacian corresponding to the smallest k eigenvalues [7] . Morally, this means it clusters points together that are close in the first k (slowest decaying!) modes of the diffusion equation on the graph.

3 Using the network to analyze a sample

The question now is what can we do these networks?

First, assesing GOTTCHA reads. A single GOTTCHA read would produce a network with each connected component complete. I think the co-incidence network is more appropriate. We want to assess the probability that you see this set together. We have the probability that you see any vertex pair (estimated) as the edge weights of $\mathcal{G}_1$. Precisely, the edge weights are

$$w^1_{jk} = P(j \,\&\, k \in S^l_i)$$

where S^l_i is sample i at discrete abundance level l. It might be useful to have the directed weight graph where

$$w^3_{jk} = P(j \in S^l_i | k \in S^l_i)$$

but that wouldn't be hard, because then

$$w^3_{jk} = S\frac{w^1_{jk}}{\|\{i : a(r_{ki}) > 0\}\|}$$

Anyway, let's start with the simplest case of one abundance level. Assume GOTTCHA found taxa $a, b, c, ..., n$. Maybe the first thing we would want is

$$P(a|b, c, d, ..., n),\ P(b|a, c, d, ..., n),\ etc$$

Clearly, we can see directly $P(a|b)$, etc. We can also get a bound for triplets (assuming $P(c, b) \neq 0$):

$$P(a|b, c) \leq \frac{\min_{(i,j)\subset\{a,b,c\}}(P(i, j))}{P(b, c)}$$

but we can't do any better than triplets explicitly, because we don't have any sort of independence (conditional or otherwise) and because our network is not acyclic.

We can probably learn something from asking about the connectivity of the induced subgraph. Notice that if it isn't complete, then one of the $P(a, b)$ is 0 above.

What does the connectivity of the induced subgraph of $\mathcal{G}_2$ tell us? If it is connected, that's good. We could use that network to identify vertices that are connected to many of the vertices in the induced subgraph - this might indicate that node should be in the sample.

I guess $\mathcal{G}_2$ is a markov random field [3]. This might give us a way to calculate the probability you see a group taxa (and maybe others). The main idea of a MRF is that nodes are conditionally independent of nodes they aren't neighbors of (conditioned on ones they are neighbors of). If c are the (maximal) cliques of the graph (complete subgraphs), then the probability of configuration $\boldsymbol{x}$ is

$$P(\boldsymbol{x}) = \frac{1}{Z}\prod_c \psi_c(x_c)$$

where Z is a normalizing constant and ψ_c are potential functions I don't know how to come up with yet. Anyway, if we have a sample that contains (maybe as a subset) s, we can calculate something. Let C_s be the cliques represented in s.

$$P(\boldsymbol{s}) = \frac{1}{Z}\sum_{\{\boldsymbol{x}:s\subset\boldsymbol{x}\}}\prod_{c\in C_s}\psi_c(\boldsymbol{x})$$

And we can ignore cliques not represented in s. We probably have to change Z. I guess we can also use neighbor pairs instead of maximal cliques. Either way we have to figure out what ψ_c are.

3.1 Determining ψ_c

Before figuring out ψ_c, it should be noted that we have a choice of configuration space of the network. We can use a binary $\{1, 0\}^N$ space to denote presence or absence, or we can choose a continuous space to include abundances. To begin, I will only consider presence & absence.

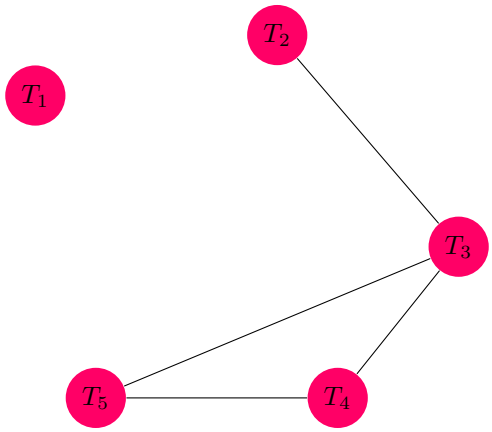


Figure 2: *A small network example*

Snowshoe hairs and Canadian Lynx, CRNT

Now I'm very tempted to think about how this approach relates to mass action dynamical systems. I'm going to think about my very favorite model.

$$\dot{\boldsymbol{x}} = \kappa_1 x \begin{pmatrix} 1 \\ 0 \end{pmatrix} + \kappa_2 xy \begin{pmatrix} -1 \\ 1 \end{pmatrix} + \kappa_3 y \begin{pmatrix} 0 \\ -1 \end{pmatrix}$$

Well. Yes. But there is only one maximal clique so $P(\boldsymbol{x}) = \psi(\boldsymbol{x})$ and that's just whatever it is. We know that it evolves according to the stochastic mass action equations. In fact, the result that the stationary distribution is a product of poissons for a complex balanced system can be thought of as fitting into this framework. I wonder if you could use this theory to re-prove that? Recall that the stationary distribution for a complex balanced system with equilibrium $\boldsymbol{c}$ is

$$\pi(\boldsymbol{x}) = \frac{1}{Z} \prod_{i=1}^{d} \frac{c_i^{x_i}}{x_i!}$$

so here we have that

$$\psi_c = \prod_{i \in c} \frac{c_i^{x_i}}{x_i!}$$

or taking cliques to be just singletons

$$\psi_i = \frac{c_i^{x_i}}{x_i!}$$

I can't think of any way to arrive upon that directly, and so prove the result from this direction. Also, This is even more general than a MRF, as it has self-loops. Self loops play the role of time update. In fact, one might say that a MC is to a MRF as an ODE is to a PDE. Then, self loops in the MRF correspond to time derivatives or forcing appearing in the PDE.

It seems reasonable to use a pairwise RMF. It also seems reasonable to take a log linear function. My initial guess is

$$\psi_{s \sim t}(y_s, y_t) = \exp(\boldsymbol{\theta} \cdot \mathbf{1}_{y_s = i, y_t = j})$$

where $\theta = (0, \log(P(s)), \log(P(t)), \log(1/2(P(s|t) + P(t|s))))$. But that's a guess. It makes sense we have only unconnected nodes. It also also penalizes leaving out nodes that are connected to the nodes we do have. However, this penalizes us too harshly if we include a hub node.

Let's play with a small network to try to get a handle on this. Consider the network shown in fig. 2 I think we

can assume that

$$p(\boldsymbol{x}) = \prod_{s\sim t} \psi_{st}(\boldsymbol{x}) \prod_s \psi_s(\boldsymbol{x})$$

To start, we should think about the distribution of an independent node (T_1). Clearly

$$P(T_1 = x) = \psi_1(x)$$

Similarly, we should have

$$P(T_2 = x) = \psi_2(x) = \int \psi_2(x)\psi_{23}(y)dy = \mathbb{E}(P(T_2 = x|T_3))$$

Maybe a binomial is a good idea:

$$P\left(T_1 = \frac{k}{N}\right) = \binom{N}{k} p^k (1-p)^{N-k}$$

where p is something like the proportion of times T_1 appears in the data, the sum of abundances of T_1 in the data divided by N. N is some scaling parameter, like the largest total abundance or something. But then, it's natural to take N large and get a Gaussian by the CLT. So maybe we do a multivariate Guassian. Then

$$\psi_{s\sim t}(\boldsymbol{x}) = \exp\left(-\frac{1}{2} x_s \Lambda_{st} x_t\right)$$

and

$$\psi_s(\boldsymbol{x}) = \exp\left(\eta_s x_s - \Lambda_{ss} x_s^2\right)$$

[3]. We already have the covariance (normalized by the product of the standard deviations) matrix Σ, in the Pearson coefficient. So, we have $\Lambda = \Sigma^{-1}$, and $\boldsymbol{\eta} = \Sigma^{-1}\boldsymbol{\mu}$. Unfortunately, Σ is singular, it's dimension is bounded by the number of samples I have. This of course leaves us in the wind when it comes to Λ. So, I suppose we have to try to approximate Λ subject to the constraint that if $s \not\sim t$, $\Lambda_{st} = 0$. We can use the SVD:

$$\Sigma = MSN^* \Rightarrow \Sigma^+ = N\tilde{S}M^*$$

where $\tilde{S}$ is diagonal with $1/s_i$ until you get to zero singular values, where you just put a 0. Because Σ is symmetric, we have $M^* = N$. Then,

$$\Sigma^+ = N\tilde{S}N$$

I guess this is used, although it doesn't seem to preserve zeros. There's a function in scipy.

On the other hand, taking N large and p small we get an exponential random variable. So perhaps I want

$$\psi_1(x) = e^{-\lambda} \frac{\lambda^{Nx}}{(Nx)!}$$

or more directly

$$\psi_1(x) = \binom{N}{Nx} p^{Nx} (1-p)^{N-Nx}$$

and using Stirling's formula,

$$\log(\psi_1) \approx N\left[x \log\left(\frac{p}{x}\right) + (1-x)\log\left(\frac{1-p}{1-x}\right)\right]$$

3.2 Comparing configurations without specifying ψ

To compare two configurations $\boldsymbol{x}$ and $\boldsymbol{y}$, we can inspect

$$\frac{P(\boldsymbol{x})}{P(\boldsymbol{y})} > 1$$

without knowing them. For that, I would need to just identify which cliques change, and ask whether they increase or decrease. How exactly to decide that is a question. Probably single nodes we wouldn't change. For a clique, we could assume that ψ_c decreases if we have over half the nodes and reduce and still have at least half, but increase

if the we have less than half and decrease. That is, let c be a clique with n members, and $\psi_c(k)$ be the value of ψ_c with k members "on". Then

$$\frac{\psi_c(k+1)}{\psi_c(k)} \begin{cases} > 1 & k > n/2 \\ < 1 & k \leq n/2 \end{cases}$$

Then, we are basically asserting the cliques like to either be all there or none there. Which seems good.

Here the algorithm for deciding which of two nodes should be on and which should be off would be:

(i) Identify cliques $c_1, ..., c_m$ which contain one or both nodes

(ii) compute $\frac{\psi_{c_i}(k^1)}{\psi_{c_i}(k^2)}$ for each $i \in \{1, ..., m\}$, where k^j is the number of members present in configuration $j \in \{1, 2\}$, and this fraction is computed according to a simple rule of the kind above.

(iii) multiply these ratios: $\frac{P(\text{configuration 1})}{P(\text{configuration 2})} = \prod_{i=1}^{k} \frac{\psi_{c_i}(k^1)}{\psi_{c_i}(k^2)}$

I need to specify that rule. The simplest reasonable thing is probably linear

$$\frac{\psi_c(k+1)}{\psi_c(k)} = \frac{2(1-r_{min})}{n-1}k + r_{min}$$

but one could imagine some kind of sigmoidal rule as well, so that around $n/2$ this ratio is close to 1. This makes sense on a 2 clique, as it implies that

$$\psi(2) > \psi(1) \ \& \ \psi(0) > \psi(1)$$

and in general makes sense for n even. It makes sense for n odd as well. It asserts that it is equally likely to have on $n/2 \pm 1/2$. Finally, if we need to compare k and $k+2$, we simply take

$$\frac{\psi(k+2)}{\psi(k)} = \frac{\psi(k+2)}{\psi(k+1)} \frac{\psi(k+1)}{\psi(k)}$$

That is, a discrete analogue to the chain rule.

To compare $P(v_1 = 1|\xi)$ and $P(v_2 = 1|\xi)$, let X be the configurations with ξ and $v_1 = 1$, and Y the configurations with ξ and $v_2 = 1$. Then,

$$P(v_1 = 1|\xi) = \sum_{x \in X} \frac{P(x)}{P(\xi)}$$

and so

$$P(v_1 = 1|\xi) > P(v_2 = 1|\xi) \Leftrightarrow \sum_{x \in X \setminus Y} P(x) > \sum_{y \in Y \setminus X} P(y)$$

but inspecting X and Y, we see that this is equivalent to

$$\sum_{x \in X \setminus Y} (P(x) - P(\tilde{x})) > 0$$

where $\tilde{x} = x$ for all nodes except v_1 and v_2, while $x(v_1) = \tilde{x}(v_2) = 1$ and $x(v_2) = \tilde{x}(v_1) = 0$. This can be written

$$\sum_{x \in X \setminus Y} \prod_{c: v_1, v_2 \notin c} \psi_c(x) \left(\prod_{c: v_1 \text{ or } v_2 \in c} \psi(x) - \prod_{c: v_1 \text{ or } v_2 \in c} \psi(\tilde{x}) \right) > 0 \tag{1}$$

where c are maximal cliques of the RMF.

3.3 Diffusion based method.

Here's an idea inspired by spectral clustering: Solve the diffusion equation on the graph:

$$\frac{\partial}{\partial t} u(v, t) = Lu(v, t)$$

where v takes values in the vertex set of the graph. Then, we can encode "known" information in three ways: initial values, boundary values, or a forcing vector.

Method 1 (Initial Value Problem)**.** *Let $u_i(t)$ be the solution at node v_i to the discete diffusion problem*

$$\frac{d}{dt}\boldsymbol{u}(t) = -L\boldsymbol{u}$$

where L is the graph laplacian with initial conditions $u_i = 1$ if node v_i is known to be "on", $u_j = 0$ if v_j is known to be "off", and $u_k = 0.5$ (or 0, or perhaps encoded with some confidence in $[0, 1)$) if v_k is unknown. We then normalize the initial vector so that it represents a probability distribution on the nodes.
Then, if $\boldsymbol{K}$ is the information "known" and the values of v_k and v_l are unknown,

$$\int_0^\infty u_k(t)dt - \int_0^\infty u_l(t)dt > 0 \Rightarrow P(v_k = 1|\boldsymbol{K}) > P(v_l = 1|\boldsymbol{K})$$

We can easily compute these comparisons. Solutions to the diffusion equation are of the form

$$\boldsymbol{u} = \sum_{i=1}^{a} c_i \mathbf{1}|_{cc} + \sum_{i=a+1}^{n} c_i e^{-\lambda_i t}\boldsymbol{\xi}_i$$

where a is the number of connected components of the graph, and $\lambda_i, \boldsymbol{\xi}_i$ are eigenvalue, eigenvector pairs of L. Note that each eigenvalue $\lambda_i \geq 0$, with $\lambda_i > 0$ for $i = a + 1, ..., n$ [7]. Then, assuming there is some intial mass on the connected component containing v_1 and v_2,

$$\int_0^\infty u_k(t)dt - \int_0^\infty u_l(t)dt = \sum_{i=a+1}^{n} c_i(\xi_{ki} - \xi_{li}) \int_0^\infty e^{-\lambda_i t}dt = \sum_{i=a+1}^{n} \frac{c_i}{\lambda_i}(\xi_{li} - \xi_{ki})$$

and $\boldsymbol{c} = V^{-1}\boldsymbol{u}(0)$. Therefore, it is straightforward to compute and compare the transitive terms of the solution:

$$\int_0^\infty \left(u_k(t) - \sum_{i=1}^{a} c_i \right) dt = \sum_{i=a+1}^{n} \frac{c_i}{\lambda_i}\xi_{ki}$$

We can regard this method as the Kolmogorov forward equation of a jump process that transitions from taxa to taxa which have an edge between them at a linear rate (see fig. 3). The state space of this process is the affine space $\mathbf{1}^\perp + \boldsymbol{b}_1 \cap \mathbb{Z}^n_{\geq 0}$, where $\boldsymbol{b}_i$ are the standard basis vectors. At any time t, $u_l(t)$ is the probability that the process is in the state $\boldsymbol{e}_l$. This model clearly admits no extinction events and is complex balanced deficiency zero. In the deterministic setting, it has globally attracting equilibrium $\frac{1}{n}\mathbf{1}$. Therefore, according to [1], it has stationary distribution on each connected component

$$\pi(\boldsymbol{x}) = \frac{1}{Z_{cc}} \prod_{i=1}^{n} \frac{\left(\frac{1}{n}\right)^{x_i}}{x_i!} e^{-\frac{1}{n}}$$

The state space of the system is $\{\boldsymbol{b}_j\}$, and we note that for $j = 1, ..., a$

$$c_j = \pi(\boldsymbol{b}_j) = \frac{1}{Z_{cc} n} e^{-\frac{1}{n}}$$

and so the distribution is uniform on each conected component. If it is higher on one connected component than an other, the above integral will be larger on that component. To compare within a connected component of the graph, we must look at the transient behavior, which we take by the integral above, subtracting this stationary distribution. This is going to allow us to compare nodes in the same connected component. We are choosing first the most likely connected components and then ranking within those by transient behavior.

Interestingly, we can regard this as the reaction network directly, deterministically modeled, which is the volume scaling limit of the jump process described above.

This method is the only one of the three that is well suited for judging the entire network, without confidence in the preknowledge encoded in the initial conditions. The other two require at least one (method 2) or two (method 3) confident assertion for each connected component of the graph.

As a sanity check, let's show that the higest rank (rank 0) node is the node given the initial mass.

Let node k be given initial mass, so $u(0) = \boldsymbol{e}_k$. For any $l \neq k$, we must show

$$\sum_{i=a+1}^{n} \frac{c_i}{\lambda_i}\xi_{ki} > \sum_{i=a+1}^{n} \frac{c_i}{\lambda_i}\xi_{li} \tag{2}$$

We can diagonalize L as $L = X\Lambda X^{-1}$. Let $\hat{L}$, $\hat{X}$, and $\hat{\Lambda}$ be the $n-a \times n-a$ matrices formed from L, X, and Λ by taking rows & columns coresponding to non-zero eqigenvalues λ_i. Recall that $\boldsymbol{c} = X^{-1}u(0) = (X^{-1})_k$, and because L is symmetric we have $X^{-1} = X^T$. This implies that $c|_{\lambda>0} = \hat{X}_k^T$. Then, we have that

$$\sum_{i=a+1}^{n} \frac{c_i}{\lambda_i}\xi_{li} = (\hat{\Lambda}^{-1}\hat{X}_k^T)\cdot\hat{X}_l^T \tag{3}$$

Furthermore, X is a unitary matrix. This means that

$$X_k^T \cdot X_l^T = \begin{cases} 0 & k \neq l \\ 1 & k = l \end{cases} \tag{4}$$

Furthermore, $\hat{X}_k^T|_{\lambda=0} \cdot \hat{X}_l^T|_{\lambda=0} = 1/n$. Then,

$$\hat{X}_k^T \cdot \hat{X}_l^T = \begin{cases} -1/n & k \neq l \\ 1 - 1/n & k = l \end{cases} \tag{5}$$

Then, because $\hat{\Lambda}^{-1}$ is clearly positive definite, we can conclude that

$$\sum_{i=a+1}^{n} \frac{c_i}{\lambda_i}\xi_{ki} = (\hat{\Lambda}^{-1}\hat{X}_k^T)\cdot\hat{X}_k^T > 0 > (\hat{\Lambda}^{-1}\hat{X}_k^T)\cdot\hat{X}_l^T = \sum_{i=a+1}^{n} \frac{c_i}{\lambda_i}\xi_{li} \tag{6}$$

This calculation reveals the nature of the relationship between this idea and spectral clustering. In spectral clustering, we ask about "closeness" in the first k eigenmodes of the diffusion process. Here, we ask a similar question, weighting the (transient) eigenmodes by their eigenvalue (and so rate of decay).

Method 2 (Boundary Value Problem)**.** *Let $u_i(t)$ be the solution at node v_i to the discete diffusion problem*

$$\frac{d}{dt}\boldsymbol{u}(t) = -L\boldsymbol{u}$$

where L is the graph laplacian with fixed values (which can be regarded as boundary values) $u_i = 1$ if node v_i is known to be "on", $u_j = 0$ if v_j is known to be "off".
Then, if $\boldsymbol{K}$ is the information "known" and the values of v_k and v_l are unknown, and $\tilde{\boldsymbol{u}}$ is the equilibrium solution to the diffusion problem,

$$\tilde{u}_k dt > \tilde{u}_l \Leftrightarrow P(v_k = 1|\boldsymbol{K}) > P(v_l = 1|\boldsymbol{K})$$

First, we establish conditions under which the boundary value version will always have an equilibrium. A "boundary value" set on the graph is a set of fixed node values. We have seen already that the equilibrium of the diffusion problem is uniform, and so if we only have known "on" nodes $\tilde{\boldsymbol{u}} = \boldsymbol{1}$. If we specify off nodes as well, then there is not an equilibrium solution to the diffusion problem which gives those values at boundary nodes. However, this doesn't mean the boundary value problem (or, more precisely, the equvalent forced problem on the unknown subset) doesn't have an equilibrium solution. Take for example a complete graph on three nodes. If we prescribe one node as 1 and one as 0, the reduced problem is

$$\frac{d}{dt}u = -2u + 1$$

which has an equilibrium at $u = 1/2$. This is not an equilirium to the entire problem, because in that case

$$-L\begin{pmatrix} 1 \\ 1/2 \\ 0 \end{pmatrix} = \begin{pmatrix} -2 & 1 & 1 \\ 1 & -2 & 1 \\ 1 & 1 & -2 \end{pmatrix}\begin{pmatrix} 1 \\ 1/2 \\ 0 \end{pmatrix} = \begin{pmatrix} -3/2 \\ 0 \\ 3/2 \end{pmatrix}$$

The boundary value problem is equivalent to the forced problem on unknown nodes

$$\frac{d}{dt}\boldsymbol{u}|_{\boldsymbol{U}} = L|_{\boldsymbol{U}}\boldsymbol{u} + f_{\boldsymbol{K}}$$

where $\boldsymbol{u}|_{\boldsymbol{U}}$ is the projection of $\boldsymbol{u}$ onto the unknown set of nodes, $L|_{\boldsymbol{U}}$ is L with the rows and columns of the known nodes removed, and $f_{\boldsymbol{K}}$ is the forcing due to the boundary conditions.

The nullity of L is the number of connected components of the graph. Removing a column from a matrix will not lower the rank of that matrix if the column was a linear combination of other columns, which is the case precisely when the column is the first one removed that corresponds to some connected component of the graph. Therefore, as long as we specify at least one "boundary value" from each connected component, $L|_{\boldsymbol{U}}$ is non-singular and there exists a unique equilibrium to the boundary value problem.

The equilibrium is simply computed by solving

$$0 = L|_{\boldsymbol{U}}\tilde{\boldsymbol{u}} + f_{\boldsymbol{K}}$$

The boundary value problem can be interpreted as a population walking around the graph, with the population at some nodes maintained at fixed values. We then assume that probability that a node is "on" is proportional to the size of the population at that node. Perhaps "voters" are a good analogy.

It can also be interpreted as the same reaction network as method 1, but the values of some species fixed.

Method 3 (Forced Problem)**.** *Let $u_i(t)$ be the solution at node v_i to the discete diffusion problem*

$$\frac{d}{dt}\boldsymbol{u}(t) = -L\boldsymbol{u} + f$$

where L is the graph laplacian and f a forcing vector with $f_i = \alpha_{cc}$ if node v_i is known to be "on", $f_j = -\beta_{cc}$ if v_j is known to be "off", where cc denotes a connected component of the graph. We choose α_{cc} and β_{cc} so that on any connected component cc, $\sum \alpha_{cc} = \sum \beta_{cc} = 1$.
Then, if $\boldsymbol{K}$ is the information "known" and the values of v_k and v_l are unknown, and $\tilde{\boldsymbol{u}}$ is the equilibrium solution to the diffusion problem,

$$\tilde{u}_k dt > \tilde{u}_l \Leftrightarrow P(v_k = 1|\boldsymbol{K}) > P(v_l = 1|\boldsymbol{K})$$

Notice that the kernel of L is $span(\{\mathbf{1}|_{cc}\})$, where cc denotes a connected component of the graph. For an equilibrium solution to exist, any connected component with an "on" node must also have an "off" node, so that $f \in span(\{\mathbf{1}|_{cc}\})^{\perp}$, which is the range of L because L is symmetric.

We can easily compute the equilibrium solution by solving, as long as we have guarenteed that $f \in range(L)$. On a practical note, we use numpy's least squares solver because the matrix L is singular. Unfortunately, if we do not specify an off node in a connected component in which we do specify an on node (or vice versa), there is no equilibrium solution.

The third method can be interpreted much like method 2. However, instead of the population being maintained at known nodes, we have a constant inflow or outflow from the graph at these nodes.

This can also be regarded as the same chemical reaction network model as method 1, but now with inflow and outflow.

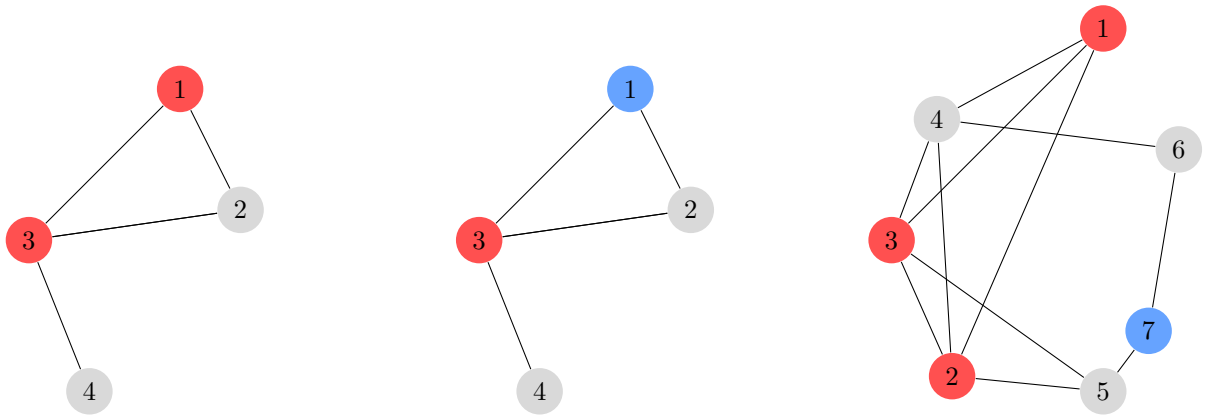
3.3.1 Tests of these methods

I tested these three ideas on two small (unweighted) graphs, shown in fig. 4.

I also tested these methods on a genus level network built from Pearson correlation higher than 0.8 with $p < 0.05$ (determined by Monte Carlo simulation). This network had a number of connected components. I used a column of the sample data used to creat the network as my configuration, with nodes with larger than mean abundance take "on" and no abundance taken "off", while taxa with non-zero but below mean abundance taken as "unknown". By analysing all nodes using method 1 (fig. 5 (a)), positive likelihood can be assigned to nodes assumed "off" if they are connected to nodes assumed "on". In the other two methods, only unkown and nodes assumed on can have positive likelihood (fig. 5 (b)).

(a) A small network example *(b) State transition graph for the IVP*

Figure 3: *Diffusion is equivalent to the Kolmogorov forward equations for the above system.*

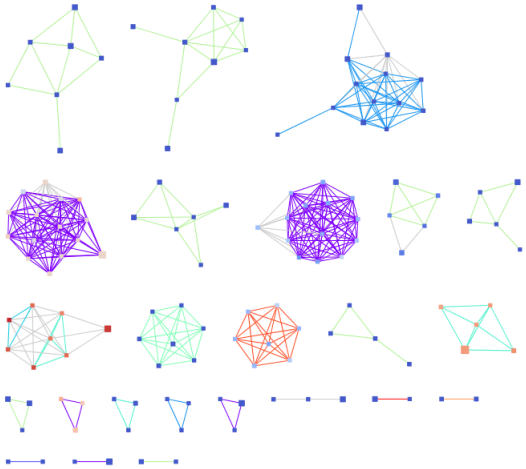


(a) One tested configuration *(b) One tested configuration* *(c) One tested configuration*

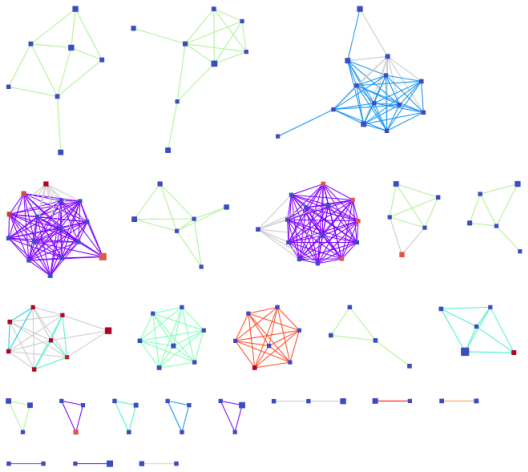
Figure 4: *Test networks. Gray nodes were "unknown", blue nodes "off", and red "on".*

Configuration	Method	Ranking	Ties
fig. 4(a)	IVP	2, 4	none
	BVP	4, 2	4, 2
	Forcing	2, 4	none
fig. 4(b)	IVP	4, 2	none
	BVP	4, 2	none
	Forcing	4, 2	none
fig. 4(c)	IVP	4, 5, 6	none
	BVP	4, 5, 6	none
	Forcing	4, 5, 6	none

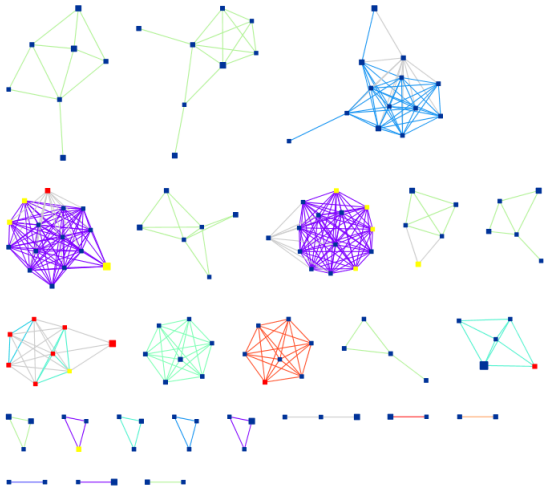
Table 1: *Results of ranking nodes by liklihood of being "on" by the three above methods*



(a) *Result of method 1, with all nodes analyzed. Hotter colors indicate higher likelihood.*



(b) *Result of method 2. Hotter colors indicate higher likelihood, with red indicating assumed "on".*

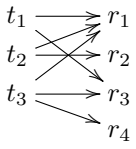


(c) *The "known" set - blue is off and red is on, while yellow is unknown.*

Figure 5: *Result of diffusion methods. Edge color represents the shared sample type of the nodes (i.e. samply type the taxa was found most often in), with gray indicating different types.*

3.4 Read production based analysis

Another way to think about the question of which taxa are present given a sample is to think specifically about the reads, and which taxa produce them. This would give us a "reaction network" of the type



Then, what we observe is the equilibrium condition of the reaction network, and we want to determine the initial condition. Or, perhaps we treat it as an I/O system in which we observe the output and want to know the input.

However, I don't think our taxa genomes get consumed. So $t_i \to r_j$ is probably either

$$t_i \to t_i + r_j$$

or

$$t_i + s \leftrightarrow s_{t_i} \to t_i + s + r_j$$

If it's the second, we can probably do a quasi steady state approximation and just replace t_i with $\lambda(t_i)$.

If we believe that the read production can be abstracted to the above,

$$\boldsymbol{r}(\tau) = \boldsymbol{r}(0) + \sum_{i,j|t_i \to r_j} Y_{ij}\left(\int_0^\tau \lambda(t_i(\sigma))d\sigma\right)\boldsymbol{e}_j$$

($Y_{ij}(\tau)$ is a unit rate Poisson process) or if we take a deterministic version

$$\boldsymbol{r}(\tau) = \boldsymbol{r}(0) + \sum_{j|t_i \to r_j} \lambda(t_i)\tau$$

If we assume that $r(0) = 0$ and we have the deterministic model, then

$$\frac{r_j(\tau)}{r_k(\tau)} = \frac{\sum_{i|t_i \to r_j}\lambda(t_i)}{\sum_{l|t_l \to r_k}\lambda(t_l)}$$

Letting $T_j = \sum_{j|t_i \to r_j}\lambda(t_i)$ and $R_{kj} = \frac{r_j(\tau)}{r_k(\tau)}$, we have then

$$(\boldsymbol{R}T)_j = T_1R_{1j} + T_2R_{2j} + \cdots + T_nR_{nj} = nT_j$$

and so we can get T by solving the eignvalue problem. Notice this only determines T up to multiplicative constant (assuming the multiplicity of n is 1). If we are wrong about what n is, finding the eigenvalues of R could correct us, I guess (of course, that's taking our model as truth). If we know n, then, we can find T up to multiplicative constant c. Finally, we have

$$(cT)_j = c\sum_{j|t_i \to r_j}\lambda(t_i)$$

and so

$$cT = \Gamma c\lambda(\boldsymbol{t})$$

where $\Gamma_{ij} = 1_{t_i \to r_j}$. Then let's say we can solve, so we have $\boldsymbol{\xi} = c\lambda(\boldsymbol{t})$, and if we know and can invert λ we finally have relative abundances $\frac{t_i}{t_j}$.

This is of course full of problems. Right off the bat, deterministic modeling is probably wrong. If we use the stochastic model, we can play a similar game. Let $P_j = \sum_{i|t_i \to r_j} Y_{ij}\left(\int_0^\tau \lambda(t_i(\sigma))d\sigma\right)$. Then, we can still solve for cP just like we did for cT. The issue comes from turning that into the $\lambda(t_i)$. Of course, we still have constant t_i (by construction or some quasi steady-state justification), so we can solve for

$$cP_j = c\sum_{i|t_i \to r_j} Y_{ij}\left(\lambda(t_i)\tau\right)$$

Then, we can solve for relative expected abundances

$$\mathbb{E}(cP_j) = c \sum_{i|t_i \to r_j} \lambda(t_i)\tau$$

so we can solve for $c\lambda(t_i)\tau$ and so get relative abundances, if we think we know $\mathbb{E}(cP)$, meaning we think we have $\mathbb{E}\boldsymbol{R}$ I suppose. More generally, we can write down a distribution for P, but it doesn't do us a whole lot of good. Notice that solving for the expected abundances is the same as solving in the deterministic setting, which makes sense due to the Kurtz scaling limit and LLN.

We might like the model

$$t_i + s \leftrightarrow s_{t_i} \to t_i + s + r_j$$

better. Then, a quasi steady state (i.e., assuming t_i, s, and s_{t_i} bound complex are constant), gives

$$\lambda(t_i) = \frac{s_0 t_i}{1 + t_i}$$

(by solving the system $\dot{t_i} = 0$, $\dot{s} = 0$, $s_{t_i} = s_0 - s$). Then,

$$t_i = \frac{\xi_i}{cs_0 - \xi_i}$$

which is a problem for getting rid of c. We can calculate

$$\frac{\xi_i}{\xi_j} = \frac{t_i(1 + t_j)}{t_j(1 + t_i)}$$

We might instead like something being consumed:

$$t_i + s \to t_i + r_j$$

this leads to the first case with $\lambda(x) = x$ if we look at equilibrium. This is because

$$s(\tau) = s_0 \exp\left(-\tau \sum_p m_p t_p\right)$$

where m_i is the number of distinct reads that can come from taxa i. Then,

$$r_j(t) = s_0 \frac{1 - \exp\left(-\tau \sum_p m_p t_p\right)}{\sum_i m_i t_i} \sum_{i|t_i \to r_j} t_i$$

and so if r_j^* is the value at equilibrium,

$$\frac{r_j^*}{r_k^*} = \frac{\sum_{i|t_i \to r_j} t_i}{\sum_{l|t_l \to r_k} t_l}$$

4 Comparison of Networks

At some point here, I'm going to want to compare networks. Luckily, there's things to do. There are of course the simple and obvious: size, radius, various connectivity and clustering metrics. There are also more interesting things, like the random walk kernel on graphs. Graph kernels are similar to an inner product on two graphs.

A graph kernel $k(G_1, G_2)$ must be symmetric and positive definite (an inner product must also be bilinear) [6]. To compute the most common type (random walk kernels) we need to define the direct product of two graphs G and G'. That is the graph $G_\times$ with vertices

$$(v_i, v'_j) \in V \times V'$$

and edges

$$(v_i, v'_j) \sim (v_k, v'_l) \Leftrightarrow v_i \sim v_k \,\&\, v'_j \sim v'_l$$

We can compute the adjacency matrix of this by computing the Kronecker product of the adjacency matrices of G and G'. The Kronecker product of matrices A and B is

$$A \otimes B = \begin{pmatrix} a_{11}B & a_{12}B & \cdots & a_{1m}B \\ a_{21}B & \ddots & & \\ \vdots & & & \\ a_{n1}B & & & a_{nm}B \end{pmatrix}$$

A random walk on this graph is isomorphic to a random walk on either graph, and so one can consider a random walk on this graph as a simultaneous random walk on both. We can take the adjacency matrices normalized so row sums are 1, call those A', B', and then

$$W_\times = A' \otimes B'$$

This gives a stochastic matrix $W_\times$. Given distributions p and p', define $p_\times = p \otimes p'$. Define the kernel

$$k(G, G') = \sum_{k=0}^{\infty} \mu(k) q_\times^T W_\times^k p_\times$$

where q, q' are stopping probabilities, and p, p' are initial distributions. This counts all the common random walks. The coefficients $\mu(k)$ serve two purposes. One is to make sure the sum is finite. The other allows one to tune the kernel to emphasize walks of certain lengths. I think it's called geometric if $\mu(k) = c^k$. However, this seems like it will overemphasize short walks, of which there will be many in common.

4.1 Matching samples to networks

Suppose we have different networks built from different types of data - i.e. healthy and unhealthy, or different regions, etc. Can we match a sample with the network that makes most the sense? That is, can we say that a sample is more likely to be from one type of data than the other based on the network?

Clearly, the first pass is just asking how many taxa detected in the sample appear in each network. Next, we might ask how many clusters of a network are represented, or similarly how many taxa of the sample are in the same cluster in the network. The diffusion (method 1) idea can do something like this. We should see less nodes ranked highly that aren't in the sample if the sample "fits" a network well. If we imagine a sample is created by performing a random walk on a graph and recording the nodes most often visited, the diffusion idea should identify nodes that the random walk "should" have seen. If there are a bunch of those missing from the sample, we might think the network is not the right one. We could consider the map from rank to abundance, and integrate this against a kernel that weights towards the high ranks. As an example, let $\boldsymbol{s}$ be the sample abundancs (so s_i is the abundance of organism i), and $\boldsymbol{r}$ the ranking (so r_i is the rank of organism i). We might take

$$F(\boldsymbol{s}, \boldsymbol{r}) = \sum_{i=1}^{n} c^{r_i} s_i$$

where $c < 1$. Given a sample, we get ranking from a network N_j. Let $\boldsymbol{r}^j(\boldsymbol{s})$ be the ranking given by diffusion on network j. Then,

$$F_j(\boldsymbol{s}) = F(\boldsymbol{s}, \boldsymbol{r}^j(\boldsymbol{s})) = \sum_{i=1}^{n} c^{r_i^j} s_i$$

and we can attempt to optimize over the networks we have (if there's only a few that's easy). The conjecture is then

Conjecture 1. *Assume that $F_j(\boldsymbol{s}) > F_k(\boldsymbol{s})$. Then $P(\boldsymbol{s}|N_j) > P(\boldsymbol{s}|N_k)$.*

Let's start with a very simple example. Consider the two networks shown in fig. 6. Using "samples" $u_1 = (1/6, 1/2, 1/3, 0, 0, 0)$, $u_2 = (1/6, 1/2, 0, 0, 1/3, 0)$, and $u_3 = (0, 0, 0, 1/6, 1/2, 1/3)$ gave results shown in fig. 7.

I did the same on a column of training data for the species level network, a column of data that was held out of the network building, and a randomly generated sample. The results, shown in fig. 8, have the training data and held out data more likely to "fit" the network than the ramdom data.

(a) *Network A_1* ***(b)*** *Network A_2*

Figure 6: *Test networks.*

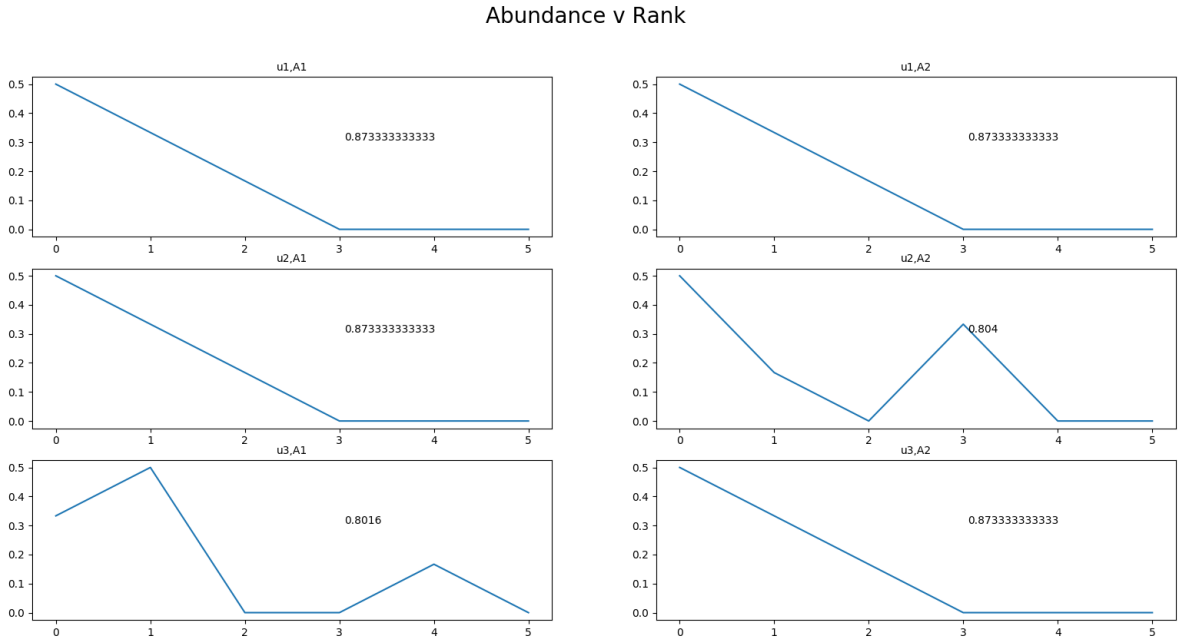
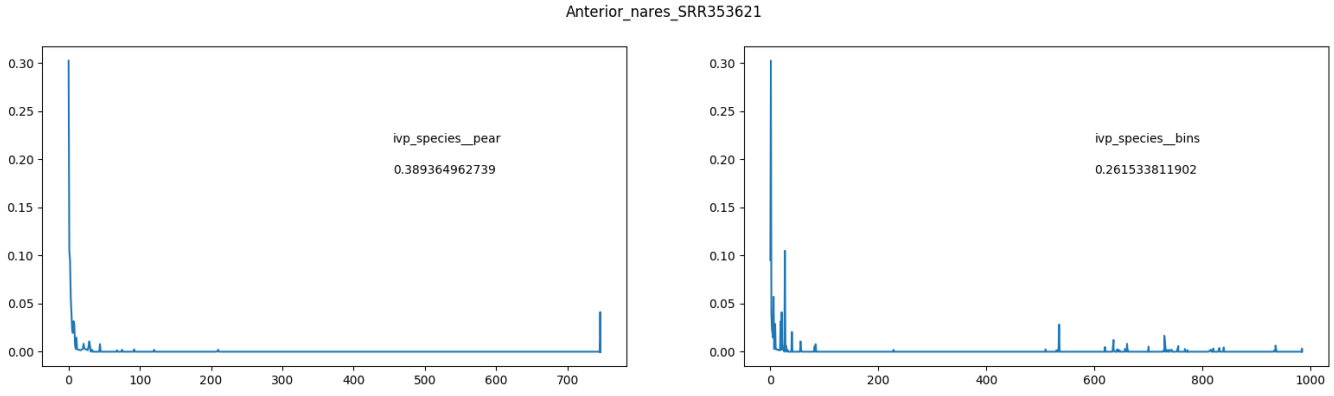
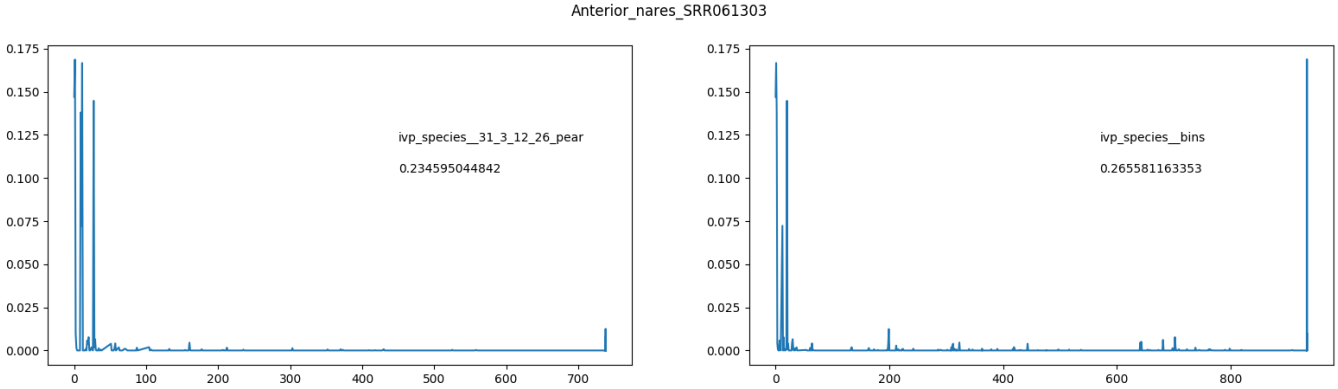


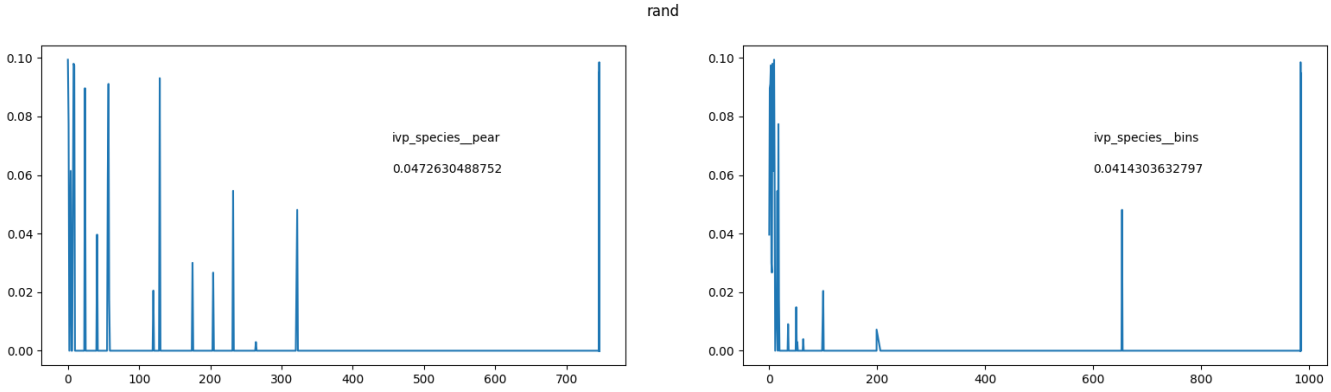
Figure 7: *Results of sample assignment: u_1 cannot be decided, u_2 is assigned to network A_1, and u_3 is assigned to network A_2.*



(a) *Assignment of column of training data to networks built with binning (right) and with correlation (left).*



(b) *Assignment of column of held out data to networks built with binning (right) and with correlation (left).*



(c) *Assignment randomly generated data to networks built with binning (right) and with correlation (left).*

Figure 8: *A training data column fits the network better than a randomly generated sample.*

Formally, we have

$$F_j(\boldsymbol{s}) = \sum_{i=0}^{n-1} c^i s_i$$

where $\boldsymbol{s} = (u_{j_1}(0), u_{j_2}(0), ..., u_{j_n}(0))$ such that if

$$\frac{d}{dt}\boldsymbol{u} = -L\boldsymbol{u}$$

and $U_l = \int_0^\infty u_l(t)dt$ then

$$U_{j_1} \geq U_{j_2} \geq \cdots \geq U_{j_n}$$

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