How to use MENAP?

http://ieg4.rccc.ou.edu/mena/

Version 20251202

Institute for Environmental Genomics (IEG)
University of Oklahoma, Norman, OK, USA

Daliang Ning (ningdaliang@ou.edu)

DODGE FAMILY COLLEGE OF ARTS AND SCIENCES
INSTITUTE FOR
ENVIRONMENTAL GENOMICS
The UNIVERSITY of OKLAHOMA

Outline

- **Requirements** | **Registration** | **Log in**
- **Upload your dataset** | **Construct the network** | **Download matrixes**
- **Analyze the networks**
  - **Global Network Properties** | **Individual Nodes Centrality**
  - **Module Separation and Modularity**
  - **Simple Network Plot** | **Output for the Cytoscape**
  - **Use Cytoscape to Visualize Network**
    - Prepare files | Import files | Select style | Layout | Manual adjustment
    - Node color | Node size | Node label | Edge color | Edge size
    - Edge transparency | Edge label | Export image | Save session
  - **Fit Power-Law Models** | **Randomize the network**
  - **Relationship with Environmental traits** | **Module-EigenGene Analysis**

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Data requirements

- **Sample number: better ≥ 8**
  - **Remember fewer samples lead to less reliable correlation coefficients.**
- **Taxa number: qualified taxa > 50 and < 2000**
  - **‘Qualified taxa’ mean taxa detected in more than a certain number of samples**
    - MENAP default setting is 50% of samples (see ‘majority’ in ‘construct network’ step)
  - **If too few, not recommend to use MENAP.**
  - **If too many, consider denoising the data or ask the maintainer for help.**

Current maintainers: Daliang Ning (ningdaliang@ou.edu)
Yajiao Wang (Yajiao.Wang-1@ou.edu)

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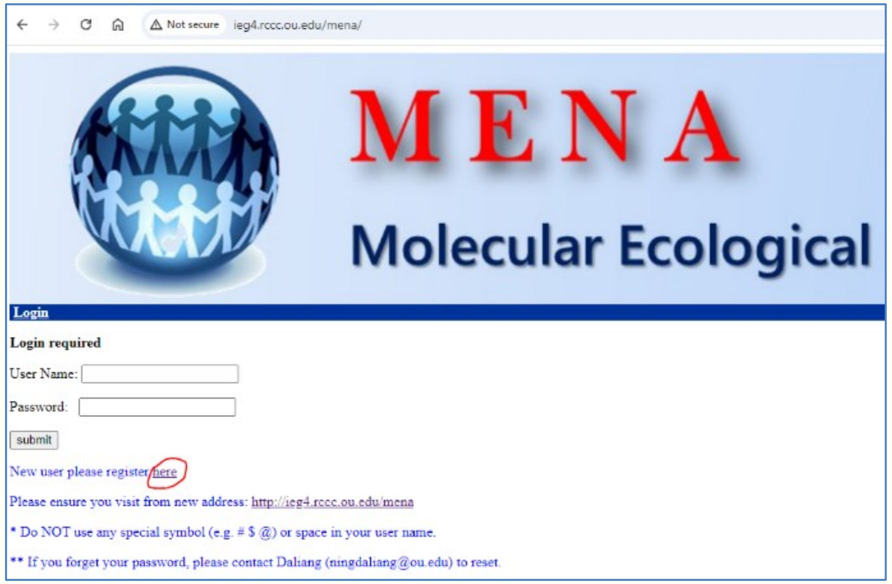
Registration

- http://ieg4.rccc.ou.edu/mena/register.cgi



Login required

User Name:

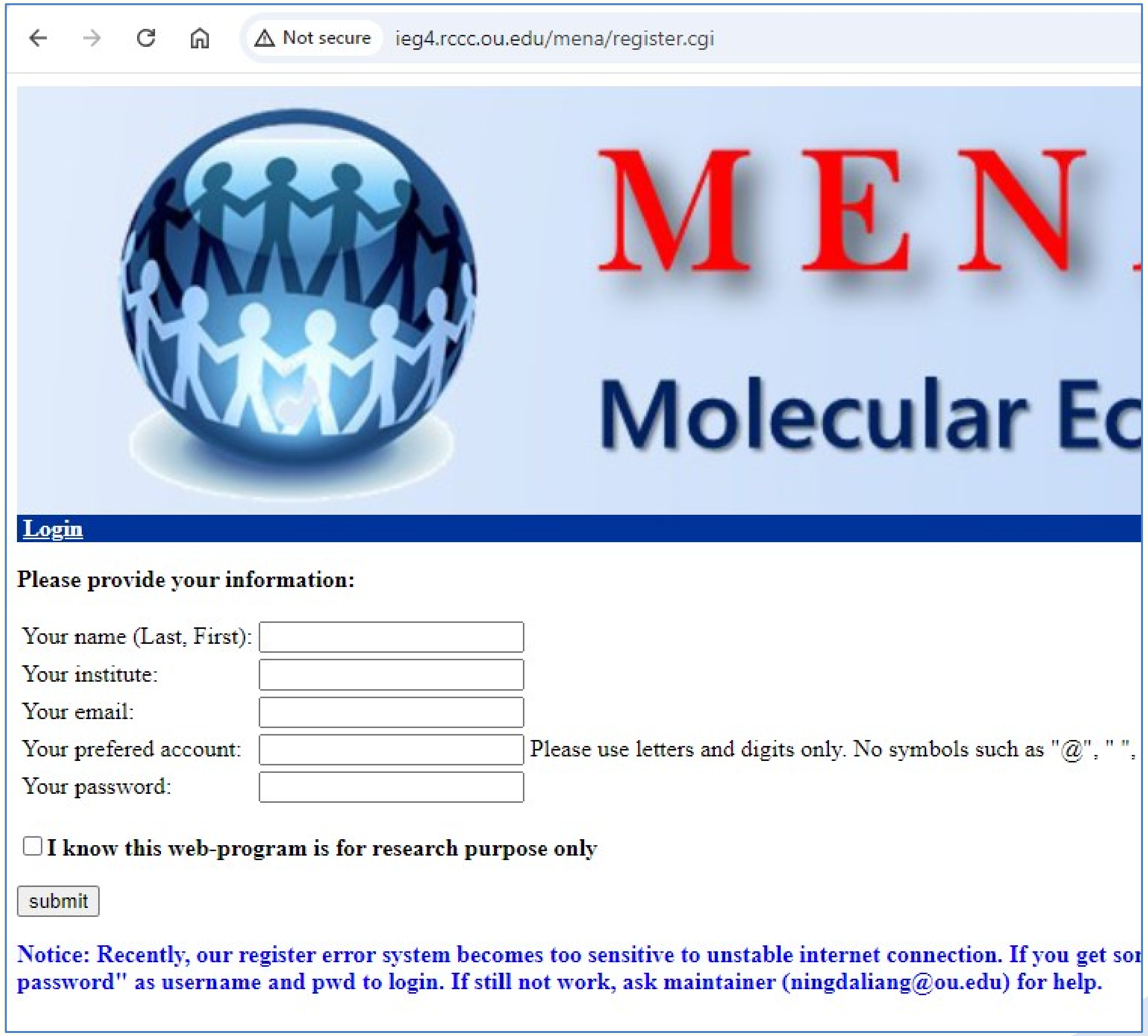
Password:

New user please register here

Please ensure you visit from new address: http://ieg4.rccc.ou.edu/mena

* Do NOT use any special symbol (e.g. # $ @) or space in your user name.

** If you forget your password, please contact Daliang (ningdaliang@ou.edu) to reset.



Please provide your information:

Your name (Last, First):

Your institute:

Your email:

Your prefered account: Please use letters and digits only. No symbols such as "@", " ",

Your password:

☐ **I know this web-program is for research purpose only**

Notice: Recently, our register error system becomes too sensitive to unstable internet connection. If you get so password" as username and pwd to login. If still not work, ask maintainer (ningdaliang@ou.edu) for help.

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Registration

- http://ieg4.rccc.ou.edu/mena/register.cgi
- ‘Your preferred account’ will be your ‘User Name’
    - Please use letters and digits only. No symbols such as "@", " ", etc. Please do not use your email address.
    - To ensure no conflict with other users, you may need to use a relatively complex account name. Adding time code like 202410061815 can be a good option.
    - Example username: MUS202410061815a
- Unexpected Error message
    - If you get some warning like "this email or username has been used" but you are sure it has not, just go ahead to use what you input to "Your prefered account" and "Your password" as username and password to login http://ieg4.rccc.ou.edu/mena/
- For any problem, you can always ask the maintainer for help.

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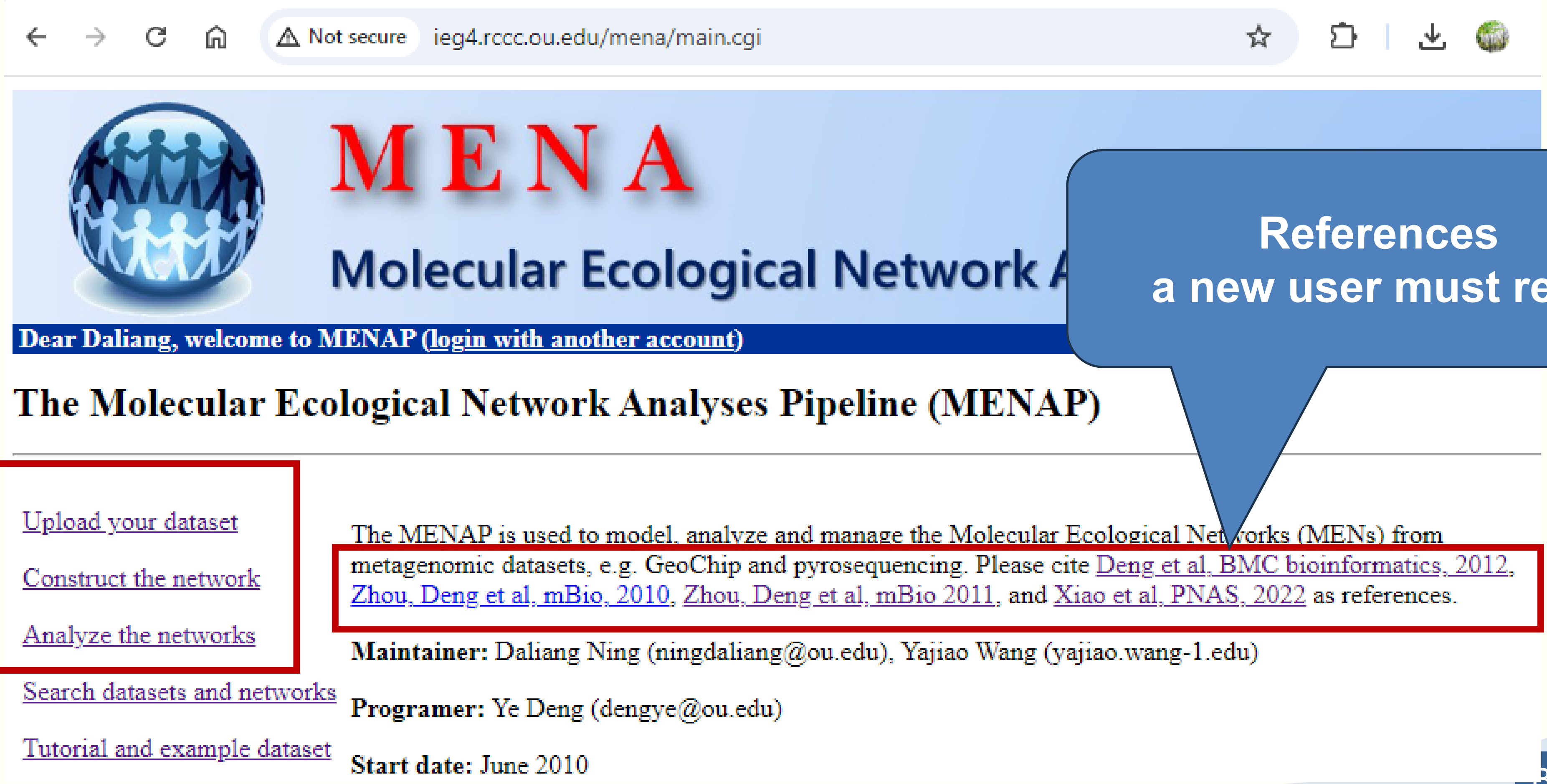
Log in main page

- http://ieg4.rccc.ou.edu/mena
- Once log in, you can see the Three Steps and Recommended References



Dear Daliang, welcome to MENAP (login with another account)

The Molecular Ecological Network Analyses Pipeline (MENAP)

Upload your dataset

Construct the network

Analyze the networks

Search datasets and networks

Tutorial and example dataset

The MENAP is used to model, analyze and manage the Molecular Ecological Networks (MENs) from metagenomic datasets, e.g. GeoChip and pyrosequencing. Please cite Deng et al, BMC bioinformatics, 2012, Zhou, Deng et al, mBio, 2010, Zhou, Deng et al, mBio 2011, and Xiao et al, PNAS, 2022 as references.

Maintainer: Daliang Ning (ningdaliang@ou.edu), Yajiao Wang (yajiao.wang-1.edu)

Programer: Ye Deng (dengye@ou.edu)

Start date: June 2010

The Three Steps

References
a new user must read

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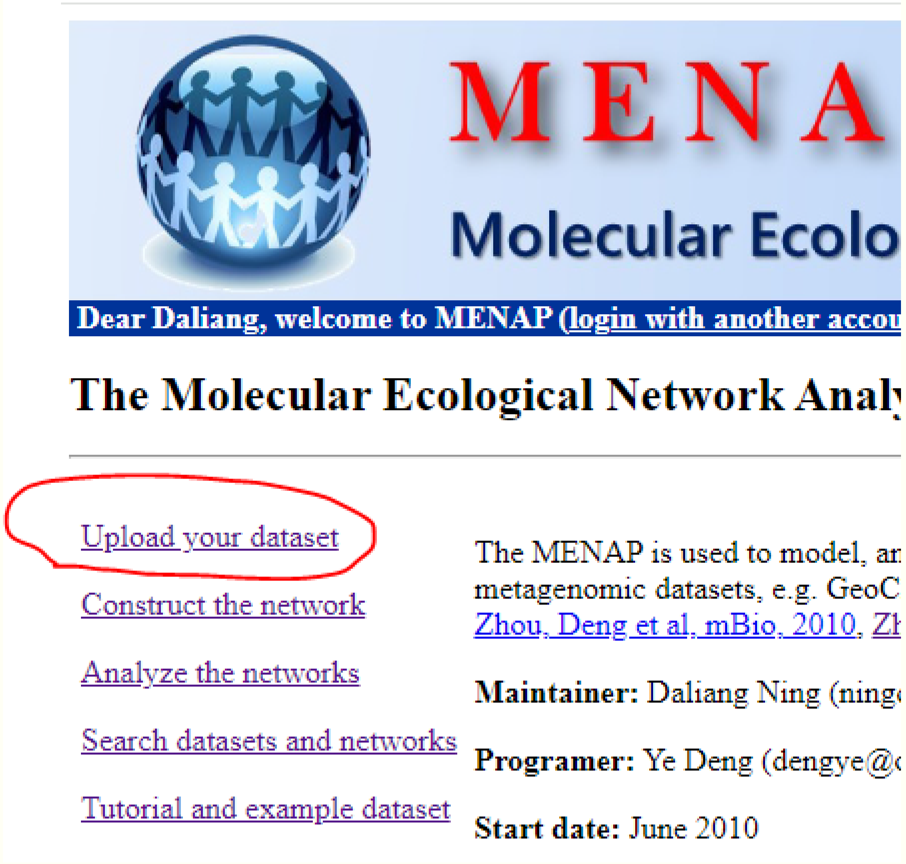
Upload your dataset (1)



Dear Daliang, welcome to MENAP (login with another accou

The Molecular Ecological Network Analy

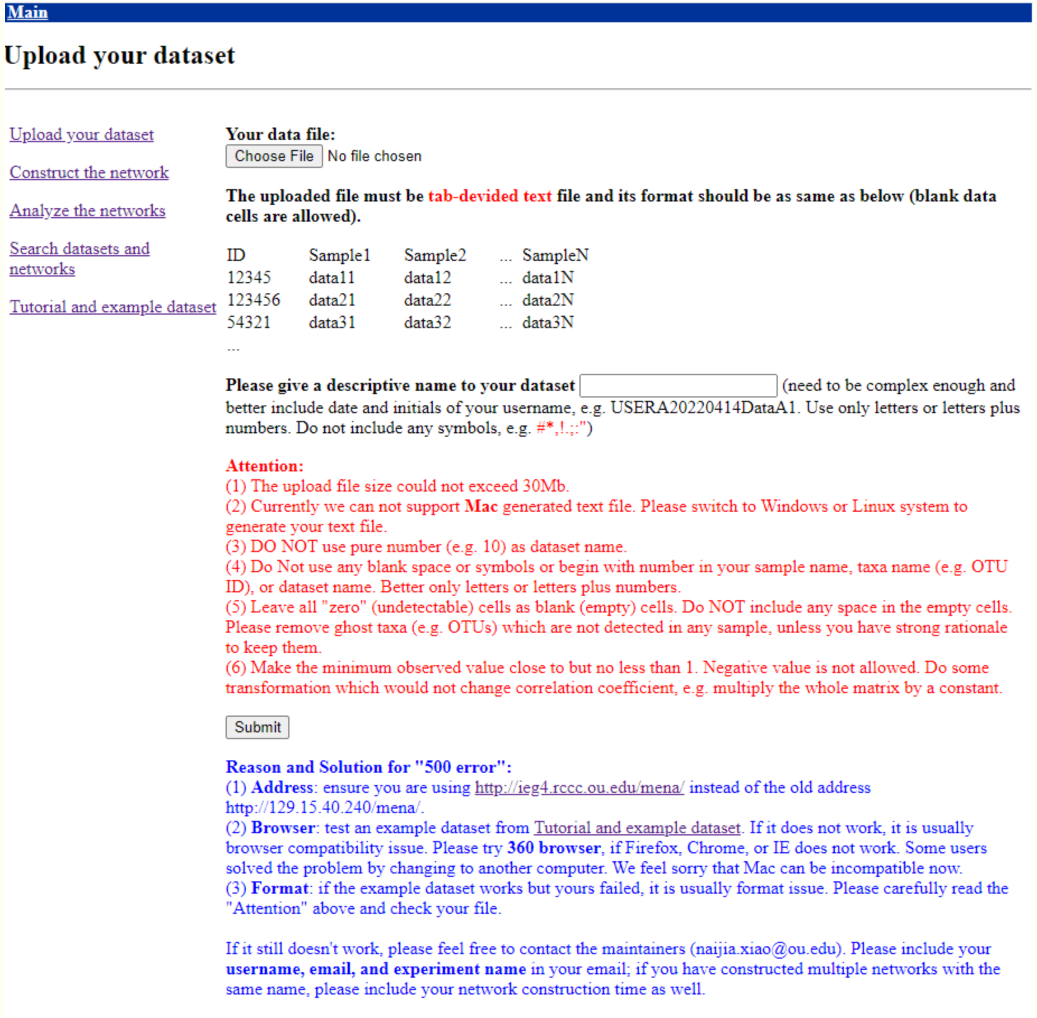
- Upload your dataset
- Construct the network
- Analyze the networks
- Search datasets and networks
- Tutorial and example dataset

The MENAP is used to model, ar metagenomic datasets, e.g. GeoC Zhou, Deng et al, mBio, 2010, Zl

Maintainer: Daliang Ning (ning

Programer: Ye Deng (dengye@

Start date: June 2010



Upload your dataset

- Upload your dataset
- Construct the network
- Analyze the networks
- Search datasets and networks
- Tutorial and example dataset

Your data file:

No file chosen

The uploaded file must be tab-devided text file and its format should be as same as below (blank data cells are allowed).

ID	Sample1	Sample2	...	SampleN
12345	data11	data12	...	data1N
123456	data21	data22	...	data2N
54321	data31	data32	...	data3N
...				

Please give a descriptive name to your dataset (need to be complex enough and better include date and initials of your username, e.g. USERA20220414DataA1. Use only letters or letters plus numbers. Do not include any symbols, e.g. #*,!.;:")

Attention:

(1) The upload file size could not exceed 30Mb.
(2) Currently we can not support **Mac** generated text file. Please switch to Windows or Linux system to generate your text file.
(3) DO NOT use pure number (e.g. 10) as dataset name.
(4) Do Not use any blank space or symbols or begin with number in your sample name, taxa name (e.g. OTU ID), or dataset name. Better only letters or letters plus numbers.
(5) Leave all "zero" (undetectable) cells as blank (empty) cells. Do NOT include any space in the empty cells. Please remove ghost taxa (e.g. OTUs) which are not detected in any sample, unless you have strong rationale to keep them.
(6) Make the minimum observed value close to but no less than 1. Negative value is not allowed. Do some transformation which would not change correlation coefficient, e.g. multiply the whole matrix by a constant.

Reason and Solution for "500 error":

(1) **Address**: ensure you are using http://ieg4.rccc.ou.edu/mena/ instead of the old address http://129.15.40.240/mena/.
(2) **Browser**: test an example dataset from Tutorial and example dataset. If it does not work, it is usually browser compatibility issue. Please try **360 browser**, if Firefox, Chrome, or IE does not work. Some users solved the problem by changing to another computer. We feel sorry that Mac can be incompatible now.
(3) **Format**: if the example dataset works but yours failed, it is usually format issue. Please carefully read the "Attention" above and check your file.

If it still doesn't work, please feel free to contact the maintainers (naijia.xiao@ou.edu). Please include your **username, email, and experiment name** in your email; if you have constructed multiple networks with the same name, please include your network construction time as well.

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Upload your dataset (2)

- **OTU or ASV table or Gene (normalized) abundance table**
- **Tab delimited txt file**
- **'Please give a descriptive name to your dataset' the name needs to be complex enough to ensure uniqueness**
  - **e.g., UserA20241006DataC**
- **Do Not use any blank space or symbols or begin with number in your sample name, taxa name (e.g. OTU ID), or dataset name. Better only letters or letters plus numbers.**
  - **Good ID examples: OTU1246 SampA453**
  - **Bad ID examples: 1246 OTU_1246 453A 453_A Samp A 453**
- **Leave all "zero" (undetectable) cells as blank (empty) cells. Do NOT include any space in the empty cells.**
- **Please remove ghost taxa (e.g. OTUs) which are not detected in any sample, unless you have strong rationale to keep them.**
- **Make the minimum observed value close to but no less than 1. Negative value is not allowed.**
  - **If your data have value <1 or negative values, do some linear transformation which would not change correlation coefficient, e.g., multiply by a constant to bring all observed numbers ≥1**

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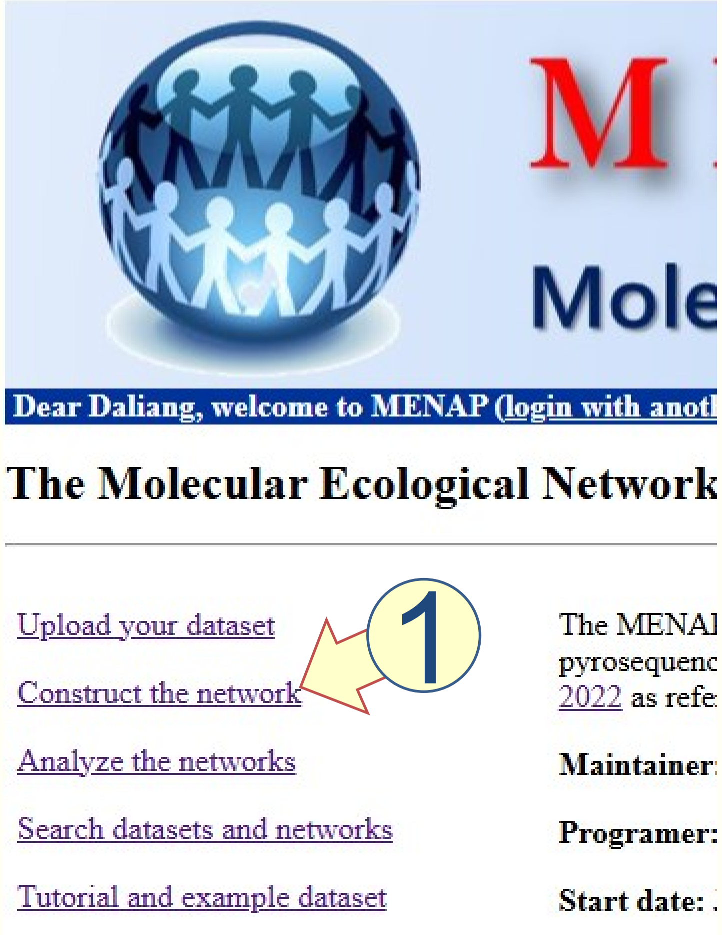
Construct the network (1)



Dear Daliang, welcome to MENAP (login with anot

The Molecular Ecological Network

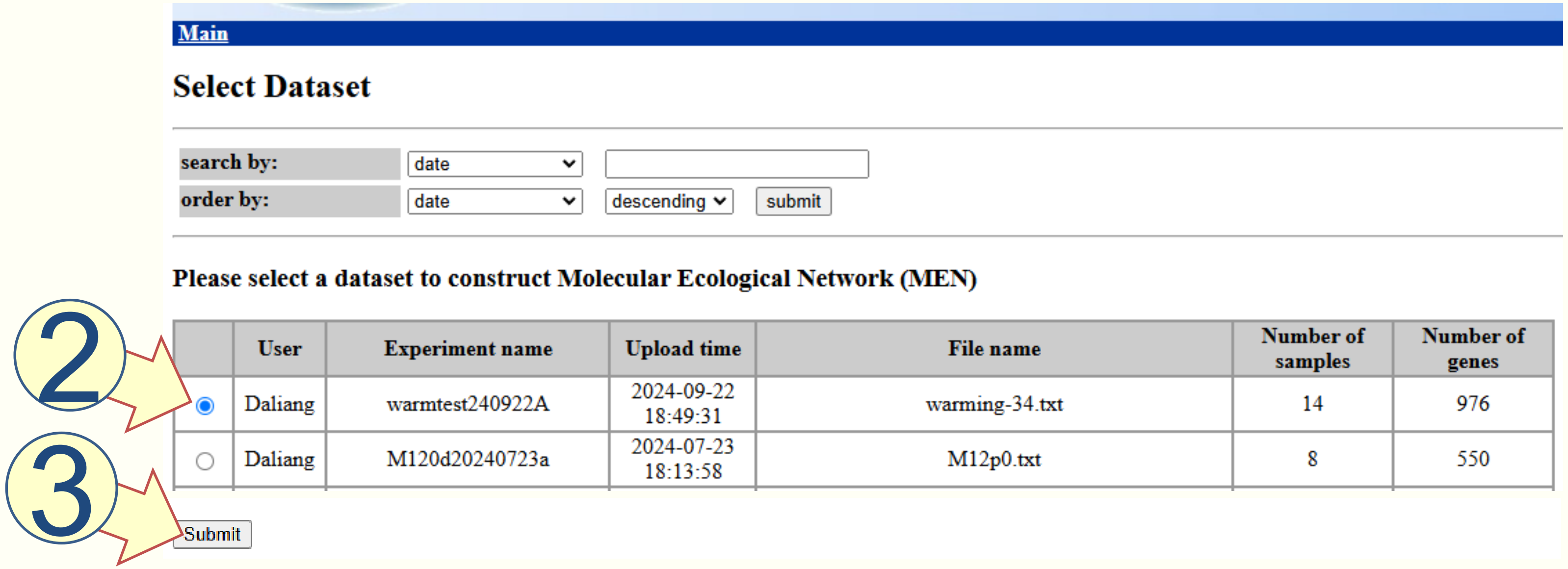
- Upload your dataset
- Construct the network
- Analyze the networks
- Search datasets and networks
- Tutorial and example dataset

The MENAI
pyrosequenc
2022 as refe

Maintainer:

Programer:

Start date:



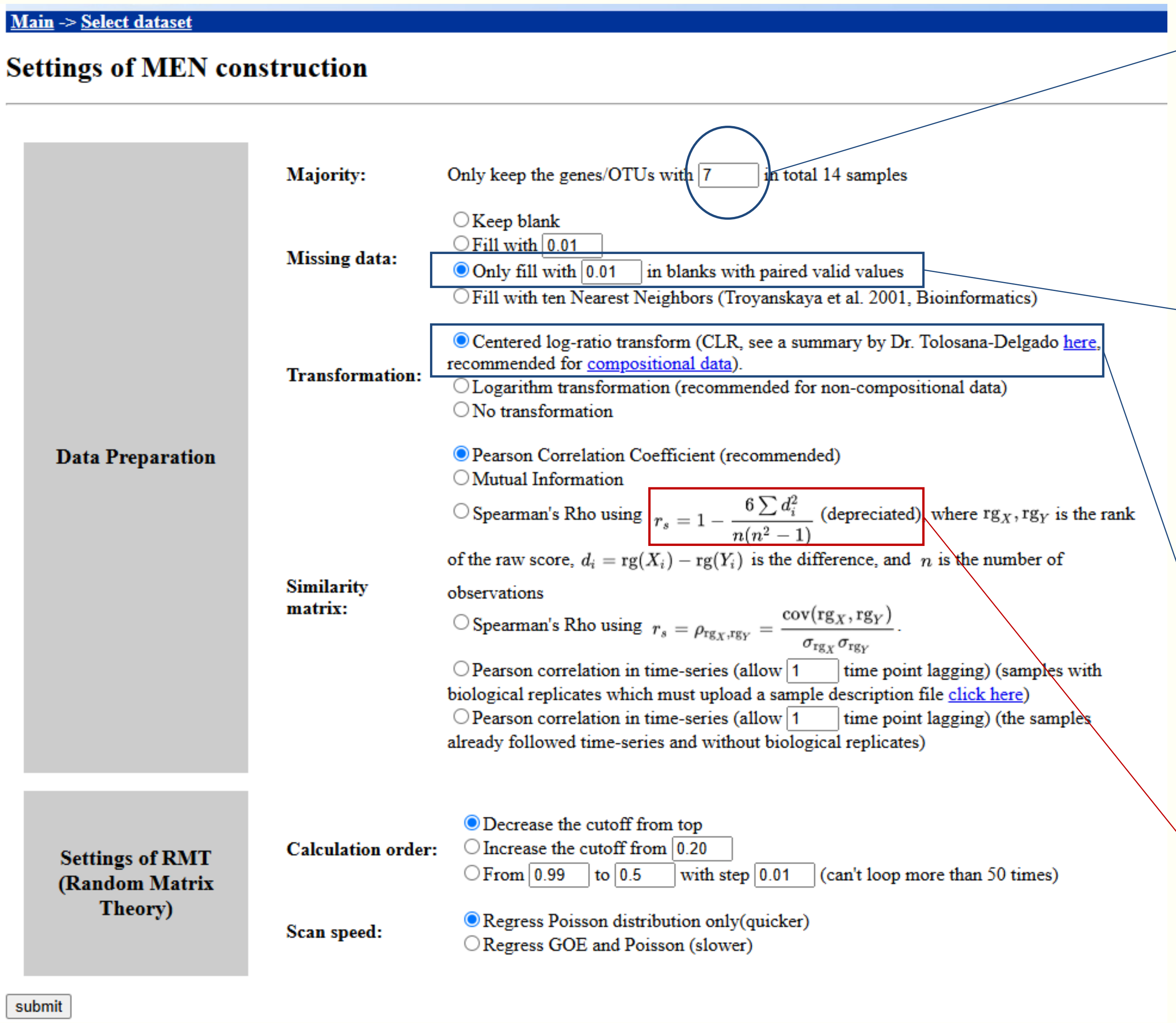
Select Dataset

search by: date
order by: date descending submit

Please select a dataset to construct Molecular Ecological Network (MEN)

	User	Experiment name	Upload time	File name	Number of samples	Number of genes
●	Daliang	warmtest240922A	2024-09-22 18:49:31	warming-34.txt	14	976
○	Daliang	M120d20240723a	2024-07-23 18:13:58	M12p0.txt	8	550

Construct the network (2)



Main -> Select dataset

Settings of MEN construction

Data Preparation

Majority: Only keep the genes/OTUs with 7 in total 14 samples

Missing data:
- ○ Keep blank
- ○ Fill with 0.01
- ● Only fill with 0.01 in blanks with paired valid values
- ○ Fill with ten Nearest Neighbors (Troyanskaya et al. 2001, Bioinformatics)

Transformation:
- ● Centered log-ratio transform (CLR, see a summary by Dr. Tolosana-Delgado here, recommended for compositional data).
- ○ Logarithm transformation (recommended for non-compositional data)
- ○ No transformation

Similarity matrix:
- ● Pearson Correlation Coefficient (recommended)
- ○ Mutual Information
- ○ Spearman's Rho using $r_s = 1 - \frac{6\sum d_i^2}{n(n^2-1)}$ (depreciated) where $\mathrm{rg}_X, \mathrm{rg}_Y$ is the rank of the raw score, $d_i = \mathrm{rg}(X_i) - \mathrm{rg}(Y_i)$ is the difference, and n is the number of observations
- ○ Spearman's Rho using $r_s = \rho_{\mathrm{rg}_X,\mathrm{rg}_Y} = \frac{\mathrm{cov}(\mathrm{rg}_X, \mathrm{rg}_Y)}{\sigma_{\mathrm{rg}_X}\sigma_{\mathrm{rg}_Y}}$.
- ○ Pearson correlation in time-series (allow 1 time point lagging) (samples with biological replicates which must upload a sample description file click here)
- ○ Pearson correlation in time-series (allow 1 time point lagging) (the samples already followed time-series and without biological replicates)

Settings of RMT (Random Matrix Theory)

Calculation order:
- ● Decrease the cutoff from top
- ○ Increase the cutoff from 0.20
- ○ From 0.99 to 0.5 with step 0.01 (can't loop more than 50 times)

Scan speed:
- ● Regress Poisson distribution only(quicker)
- ○ Regress GOE and Poisson (slower)

You can change this number. Recommend to use 50% - 80% of the total sample number, or a number you can well justify.

Recommend to use this option. You may change the 'fill-with' number, but it must be lower than the minimum observed number, but not so low that it can lead to false correlation. Default is 0.01, assuming the minimum observed number is 1.

This is recommended, to mitigate the 'compositional data' effect on correlation estimation. If your data is not compositional, e.g., absolute abundance data, try other options.

DO NOT use this Spearman option. Use the next one if you want to use Spearman.

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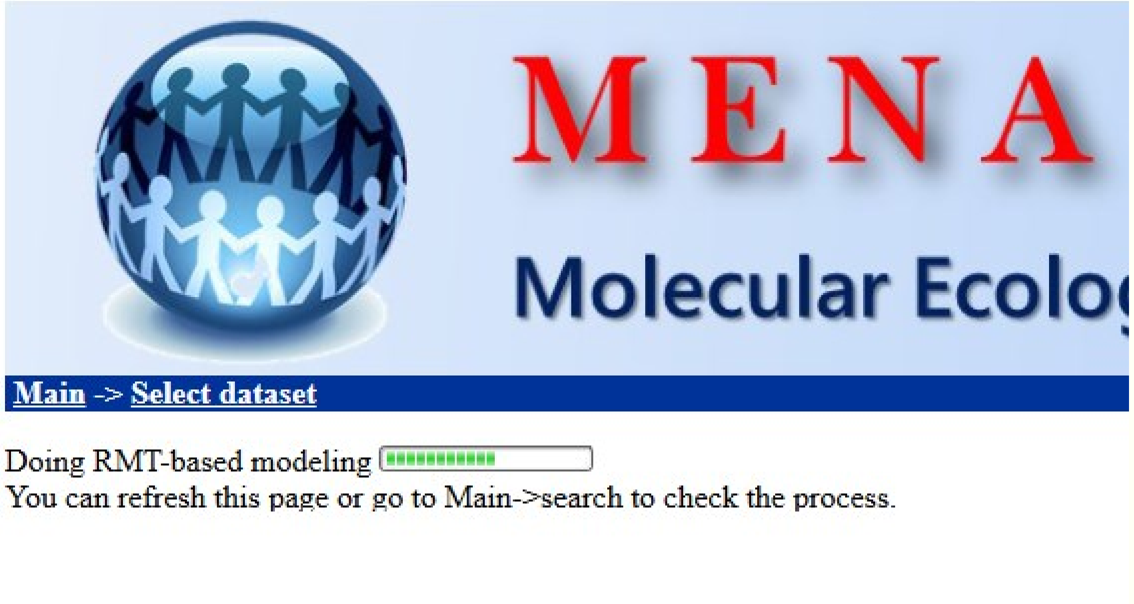
Construct the network (3)

When the page does not show ‘You can refresh this page …’, DO NOT close or refresh the page, but wait.

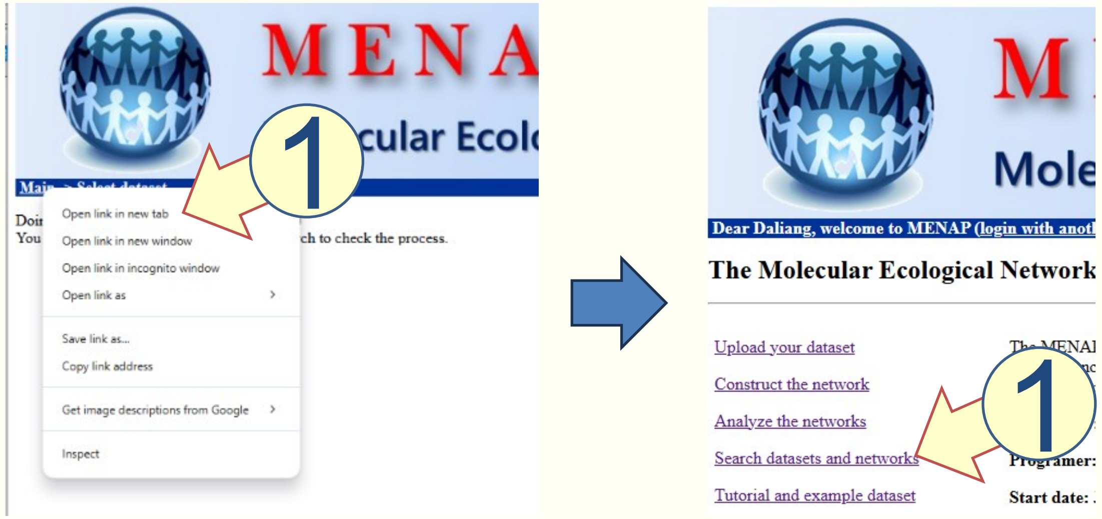


Main -> Select dataset

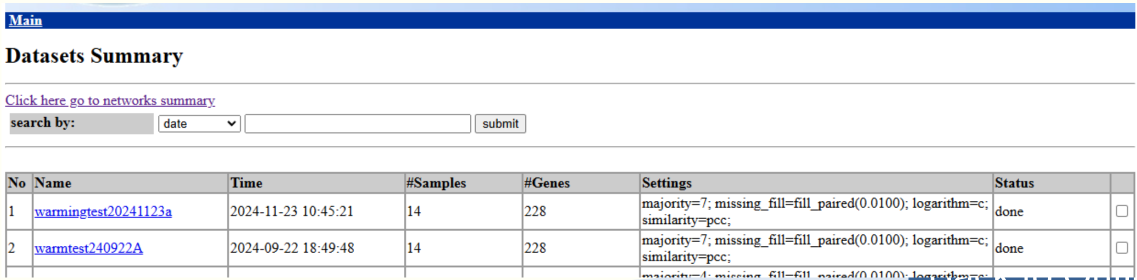
Doing RMT-based modeling

You can refresh this page or go to Main->search to check the process.

When waiting, you can right-click ‘Main’ → ‘open link in new Tab’, then in a new Tab, click ‘Search datasets and networks’ to check progress



Once the ‘Status’ shows ‘done’, you may refresh the original page, or click on the ‘Name’ of the dataset.



Main

Datasets Summary

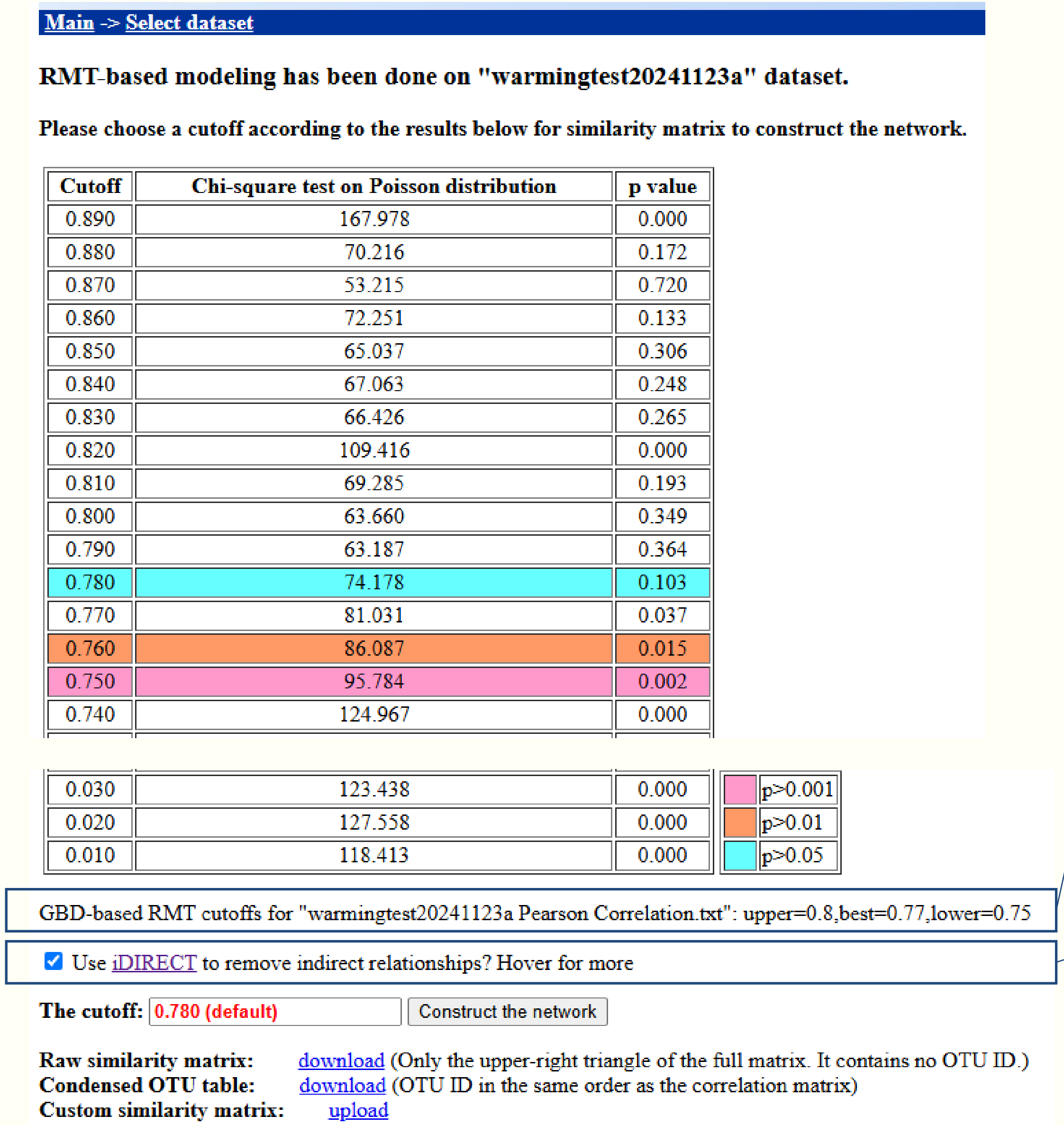
Click here go to networks summary

search by: date ▾ submit

No	Name	Time	#Samples	#Genes	Settings	Status	
1	warmingtest20241123a	2024-11-23 10:45:21	14	228	majority=7; missing_fill=fill_paired(0.0100); logarithm=c; similarity=pcc;	done	☐
2	warmtest240922A	2024-09-22 18:49:48	14	228	majority=7; missing_fill=fill_paired(0.0100); logarithm=c; similarity=pcc;	done	☐

Back to Outline

Construct the network (4)



Main -> Select dataset

RMT-based modeling has been done on "warmingtest20241123a" dataset.

Please choose a cutoff according to the results below for similarity matrix to construct the network.

Cutoff	Chi-square test on Poisson distribution	p value
0.890	167.978	0.000
0.880	70.216	0.172
0.870	53.215	0.720
0.860	72.251	0.133
0.850	65.037	0.306
0.840	67.063	0.248
0.830	66.426	0.265
0.820	109.416	0.000
0.810	69.285	0.193
0.800	63.660	0.349
0.790	63.187	0.364
0.780	74.178	0.103
0.770	81.031	0.037
0.760	86.087	0.015
0.750	95.784	0.002
0.740	124.967	0.000
0.030	123.438	0.000
0.020	127.558	0.000
0.010	118.413	0.000

🟪	p>0.001
🟧	p>0.01
🟦	p>0.05

GBD-based RMT cutoffs for "warmingtest20241123a Pearson Correlation.txt": upper=0.8,best=0.77,lower=0.75

☑ Use iDIRECT to remove indirect relationships? Hover for more

The cutoff: 0.780 (default)

Raw similarity matrix: download (Only the upper-right triangle of the full matrix. It contains no OTU ID.)
Condensed OTU table: download (OTU ID in the same order as the correlation matrix)
Custom similarity matrix: upload

'RMT cutoffs based on GBD function' is more recommended now. Theoretically, any number between the 'upper' and 'lower' values can be used as a good cutoff.

- If you only construct one network for your project, please use the 'best' value;
- If you will compare several different networks, you better get this range ('upper' to 'lower') for each network first, then use a cutoff value which can fall into this range for every network. Then, by using the same cutoff value, those networks can be more comparable.

'iDIRECT' is recommended to remove the 'indirect' associations, to make the result network only include 'direct' associations.

- If you selected 'iDIRECT' once but want to try not to use 'iDIRECT', you need to upload your file again with another name.

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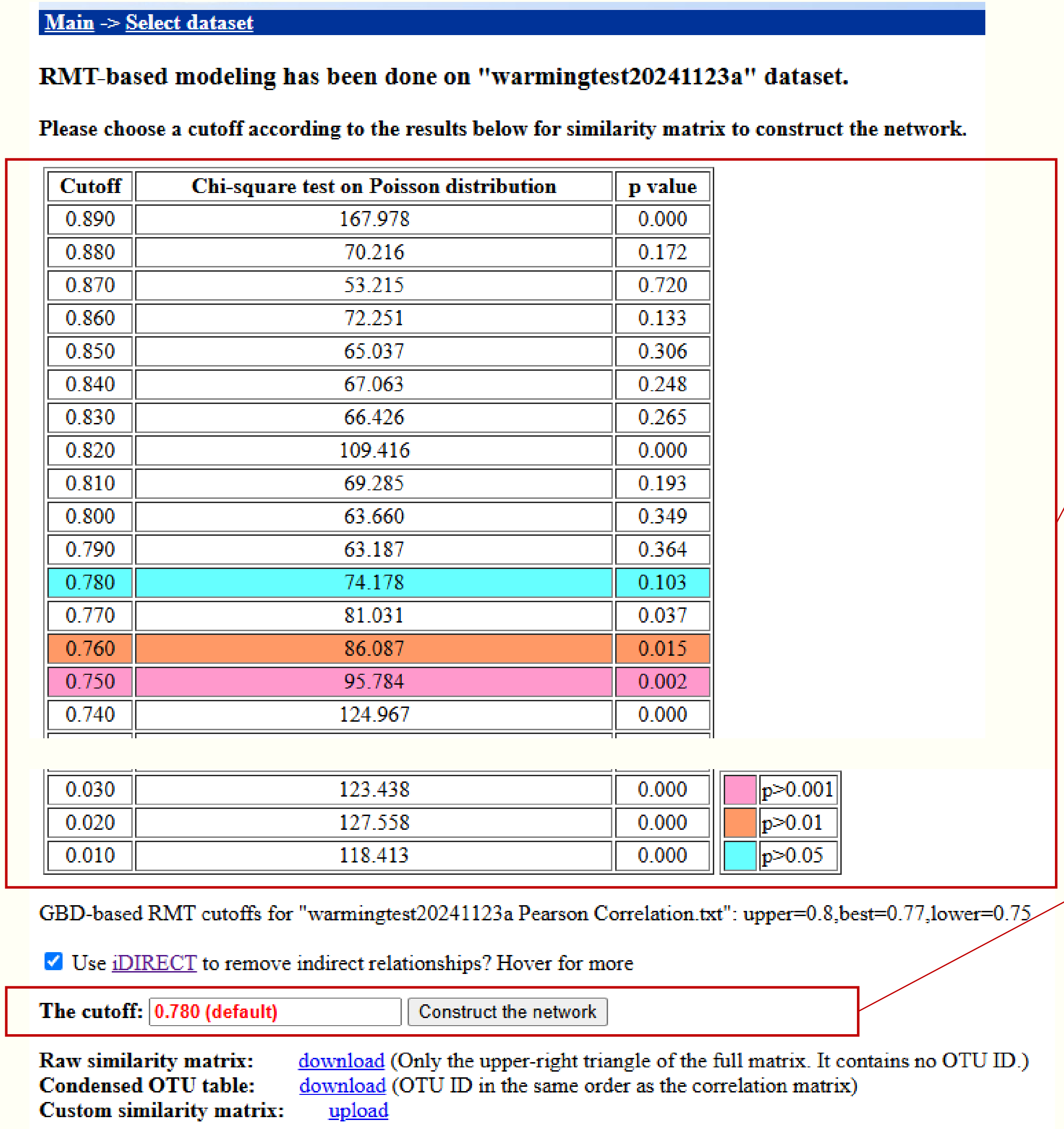
Construct the network (5)



Main -> **Select dataset**

RMT-based modeling has been done on "warmingtest20241123a" dataset.

Please choose a cutoff according to the results below for similarity matrix to construct the network.

Cutoff	Chi-square test on Poisson distribution	p value	
0.890	167.978	0.000	
0.880	70.216	0.172	
0.870	53.215	0.720	
0.860	72.251	0.133	
0.850	65.037	0.306	
0.840	67.063	0.248	
0.830	66.426	0.265	
0.820	109.416	0.000	
0.810	69.285	0.193	
0.800	63.660	0.349	
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0.770	81.031	0.037	
0.760	86.087	0.015	
0.750	95.784	0.002	
0.740	124.967	0.000	
0.030	123.438	0.000	🟪 p>0.001
0.020	127.558	0.000	🟧 p>0.01
0.010	118.413	0.000	🟦 p>0.05

GBD-based RMT cutoffs for "warmingtest20241123a Pearson Correlation.txt": upper=0.8,best=0.77,lower=0.75

☑ Use iDIRECT to remove indirect relationships? Hover for more

The cutoff: 0.780 (default) Construct the network

Raw similarity matrix: download (Only the upper-right triangle of the full matrix. It contains no OTU ID.)
Condensed OTU table: download (OTU ID in the same order as the correlation matrix)
Custom similarity matrix: upload

This is from our old algorithm, NOT recommended any more, which did not work for a few datasets.

Here, the default value is from our old algorithm, NOT recommended any more. Please consider using the GBD-based RMT cutoffs as described on previous page.

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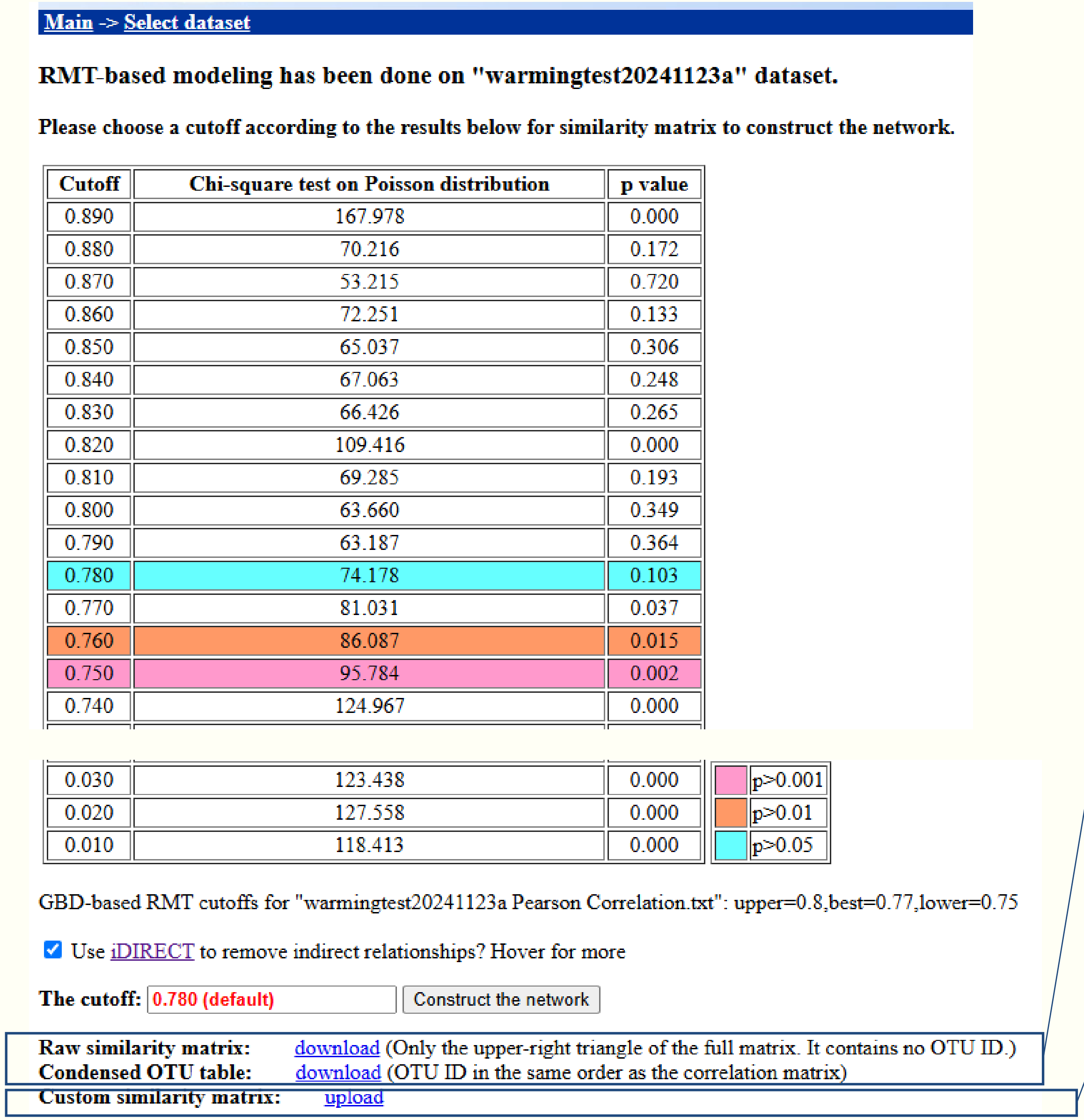
Construct the network (6)



RMT-based modeling has been done on "warmingtest20241123a" dataset.

Please choose a cutoff according to the results below for similarity matrix to construct the network.

Cutoff	Chi-square test on Poisson distribution	p value
0.890	167.978	0.000
0.880	70.216	0.172
0.870	53.215	0.720
0.860	72.251	0.133
0.850	65.037	0.306
0.840	67.063	0.248
0.830	66.426	0.265
0.820	109.416	0.000
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🟪	p>0.001
🟧	p>0.01
🟦	p>0.05

GBD-based RMT cutoffs for "warmingtest20241123a Pearson Correlation.txt": upper=0.8,best=0.77,lower=0.75

☑ Use iDIRECT to remove indirect relationships? Hover for more

The cutoff: 0.780 (default)

Raw similarity matrix: download (Only the upper-right triangle of the full matrix. It contains no OTU ID.)
Condensed OTU table: download (OTU ID in the same order as the correlation matrix)
Custom similarity matrix: upload

Raw similarity matrix: the upper-right triangle of the ‘association matrix’ before applying any cutoff, where the taxa IDs are not included but in the exact order as the taxa IDs in the ‘condensed OTU table’.

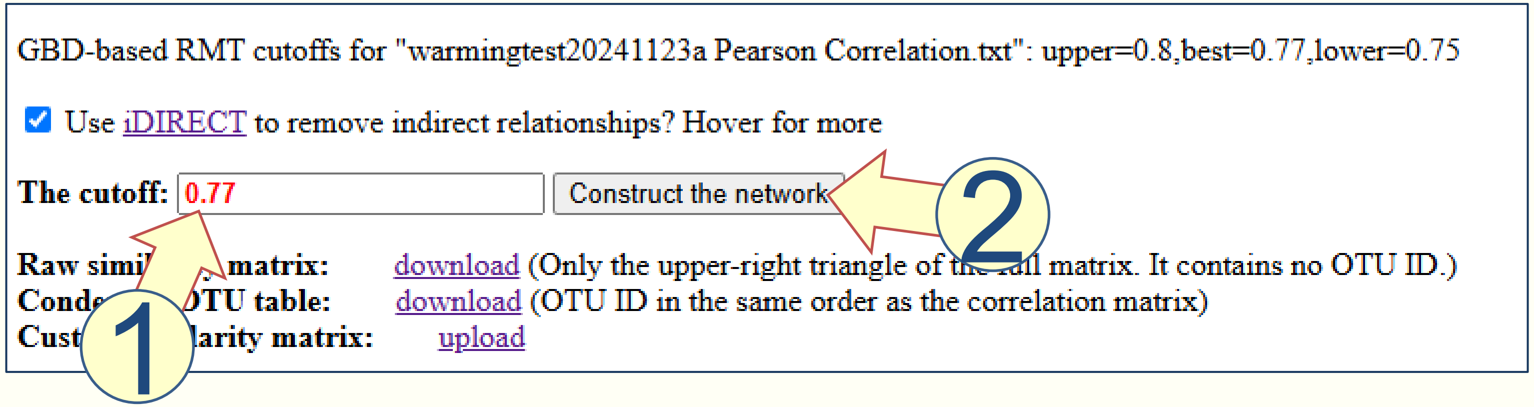
Condensed OTU table: the community composition matrix, each row is a taxon, each column is a sample, after applying the ‘majority’ filter and data transformation.

This is where you may upload a similarity matrix (association matrix) which results from your own algorithm that is not available in MENAP.

- Remember to follow the format requirement.

Construct the network (7)

Revise the cutoff according to GBD-based RMT cutoffs. See notes in the previous slide .

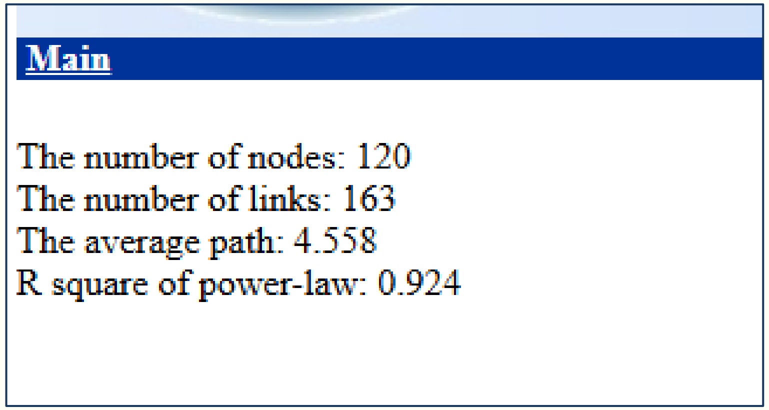


GBD-based RMT cutoffs for "warmingtest20241123a Pearson Correlation.txt": upper=0.8,best=0.77,lower=0.75

☑ Use iDIRECT to remove indirect relationships? Hover for more

The cutoff: 0.77

Raw simi matrix: download (Only the upper-right triangle of the full matrix. It contains no OTU ID.)
Conde TU table: download (OTU ID in the same order as the correlation matrix)
Cust larity matrix: upload

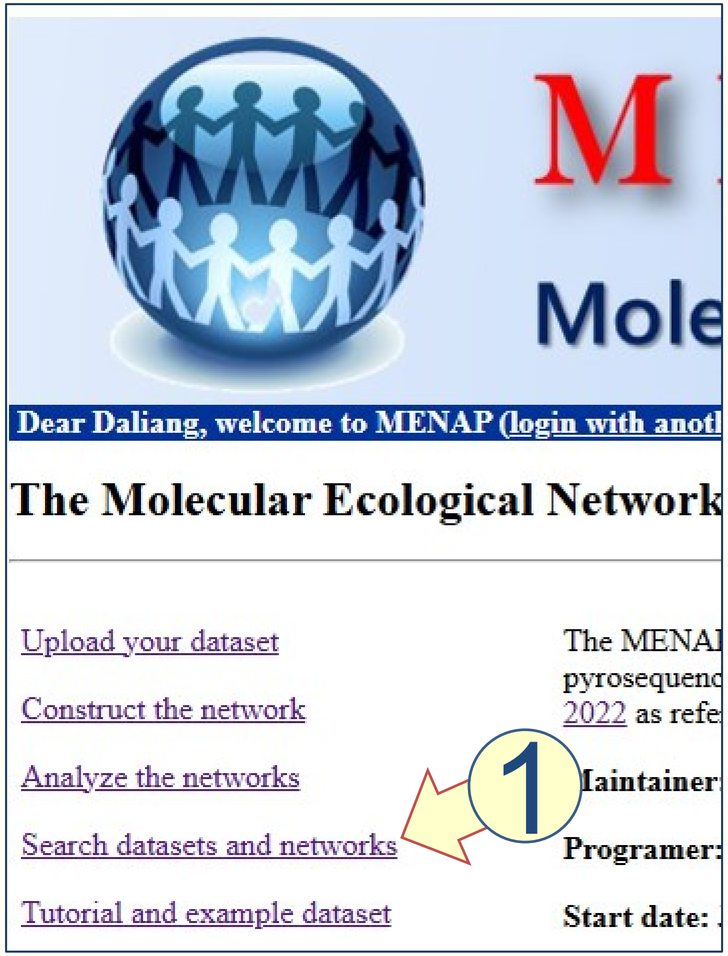


The number of nodes: 120
The number of links: 163
The average path: 4.558
R square of power-law: 0.924

A rough summary will show, it is good to go to the Main page and start next step.

Download matrixes (1)

- Download correlation/similarity matrix and condensed OTU table
  - First, ‘Search datasets and networks’ -> select the dataset



Dear Daliang, welcome to MENAP (login with anot

The Molecular Ecological Network

Upload your dataset

Construct the network

Analyze the networks

Search datasets and networks

Tutorial and example dataset

The MENAI
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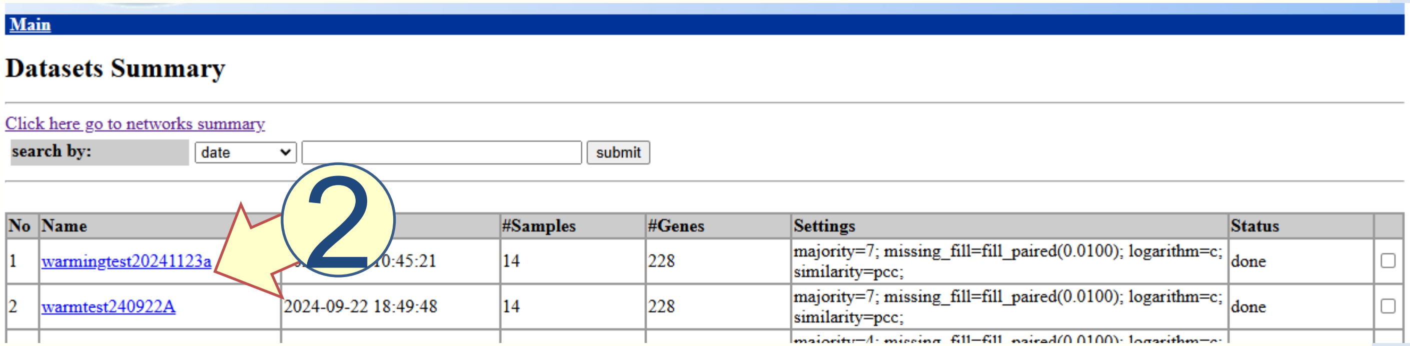
2022 as refe
laintainer
Programer:
Start date:



Datasets Summary

Click here go to networks summary

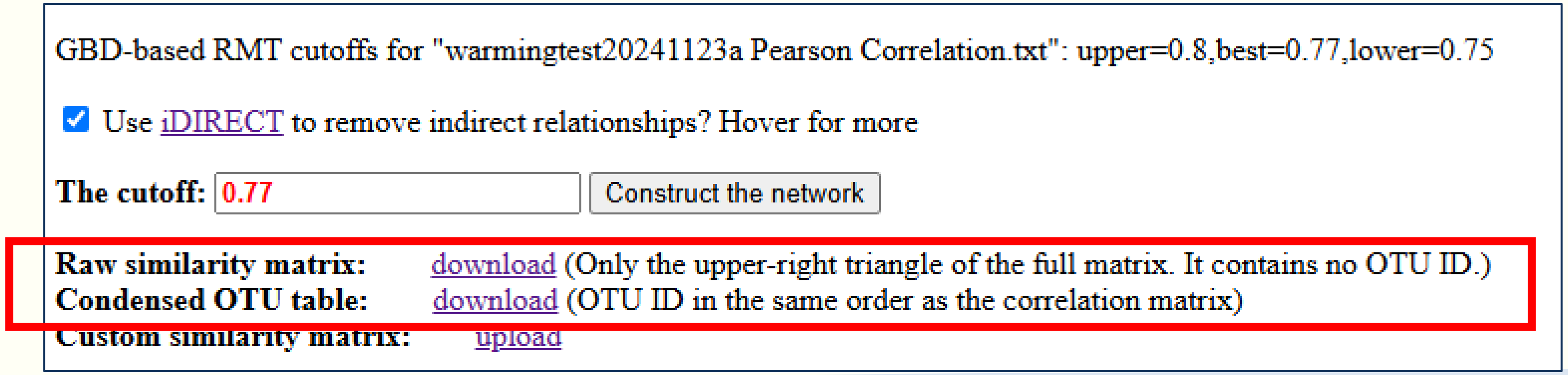
search by:

No	Name		#Samples	#Genes	Settings	Status	
1	warmingtest20241123a	0:45:21	14	228	majority=7; missing_fill=fill_paired(0.0100); logarithm=c; similarity=pcc;	done	☐
2	warmtest240922A	2024-09-22 18:49:48	14	228	majority=7; missing_fill=fill_paired(0.0100); logarithm=c; similarity=pcc;	done	☐

Back to Outline

Download matrixes (2)

- Download the similarity matrix and condensed OTU table
  - ‘Condensed OTU table’
    - the OTU/ASV/gene table after applying ‘majority’ rule, i.e., without taxa detected in too few samples.
  - ‘Raw similarity matrix’
    - the pairwise correlation coefficients, but those with absolute values lower than the default cutoff are set to zero. If you need all the values, contact the administrator and provide your user name and the dataset name.
    - The file has only upper-right triangle values of the full matrix and no ID (but the rows are in the same order as the condensed OTU table)
      - An example R code to transform the data file to a full matrix http://ieg4.rccc.ou.edu/MENA/download/MENA.similarity.matrix.convert.r.txt



GBD-based RMT cutoffs for "warmingtest20241123a Pearson Correlation.txt": upper=0.8,best=0.77,lower=0.75

☑ Use iDIRECT to remove indirect relationships? Hover for more

The cutoff: 0.77

Raw similarity matrix: download (Only the upper-right triangle of the full matrix. It contains no OTU ID.)

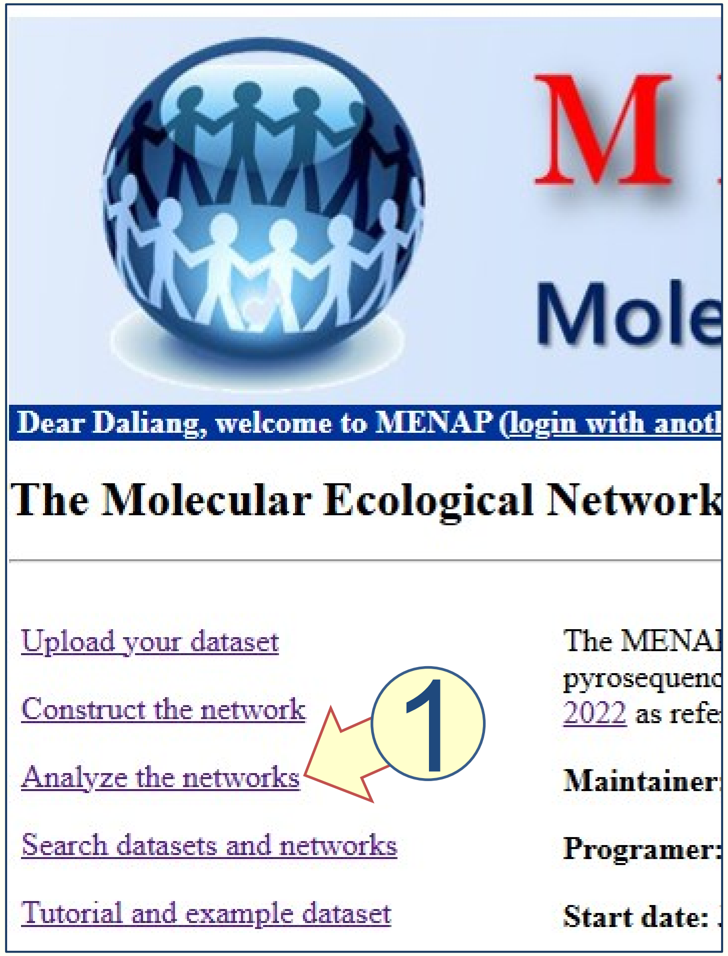
Condensed OTU table: download (OTU ID in the same order as the correlation matrix)

Custom similarity matrix: upload

Right-click ‘*download*’ and ‘Save link as …’ to download the tables.

Back to Outline

Analyze the networks



Dear Daliang, welcome to MENAP (login with anot

The Molecular Ecological Network

Upload your dataset

Construct the network

Analyze the networks

Search datasets and networks

Tutorial and example dataset

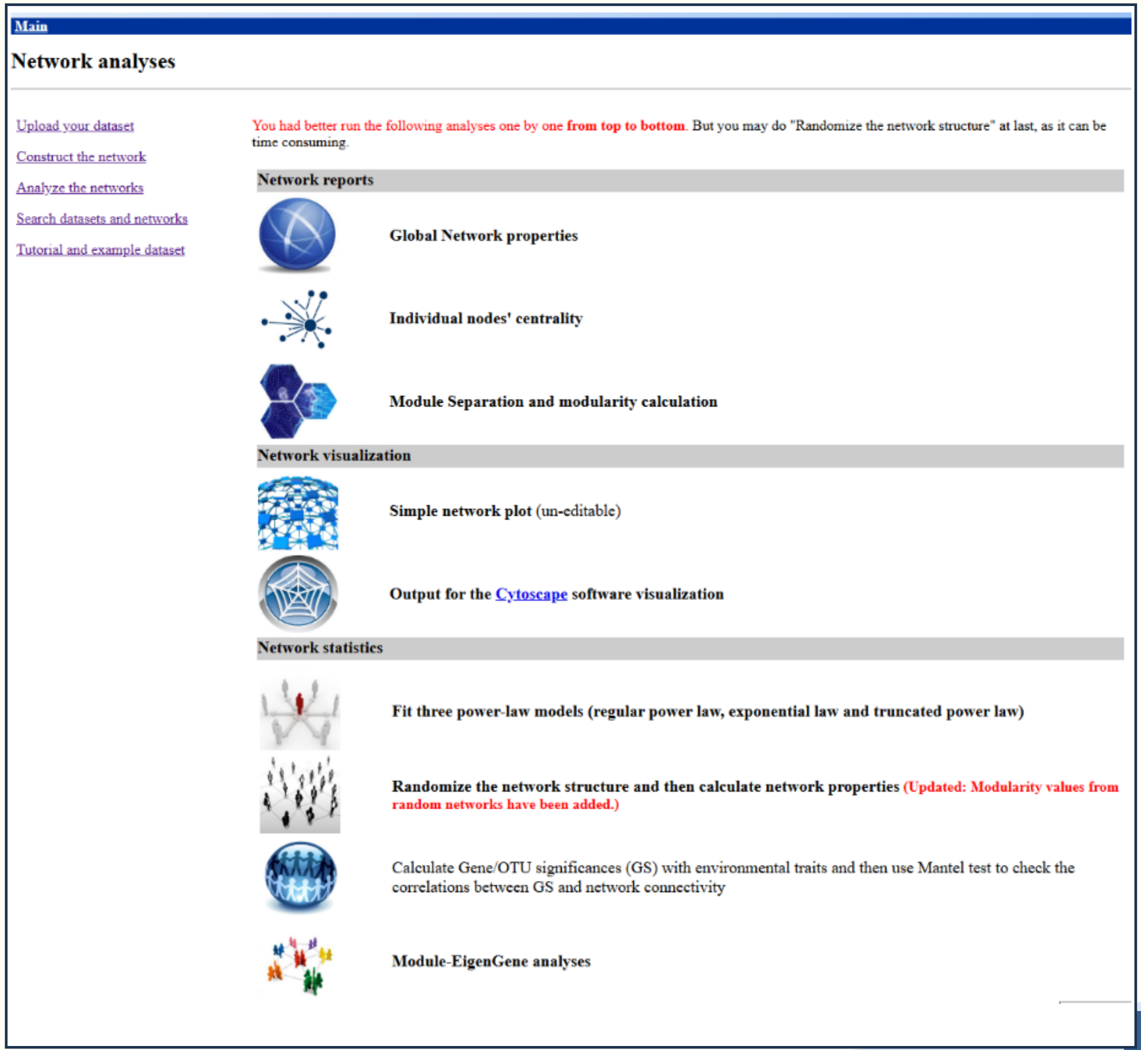
The MENAI
pyrosequenc
2022 as refe

Maintainer:

Programer:

Start date:

Please run the tools one by one from top to bottom, except the ‘randomize the network …’ which is time consuming and can be run at the last.



Network analyses

- Upload your dataset
- Construct the network
- Analyze the networks
- Search datasets and networks
- Tutorial and example dataset

You had better run the following analyses one by one **from top to bottom**. But you may do "Randomize the network structure" at last, as it can be time consuming.

Network reports

Global Network properties

Individual nodes' centrality

Module Separation and modularity calculation

Network visualization

Simple network plot (un-editable)

Output for the Cytoscape software visualization

Network statistics

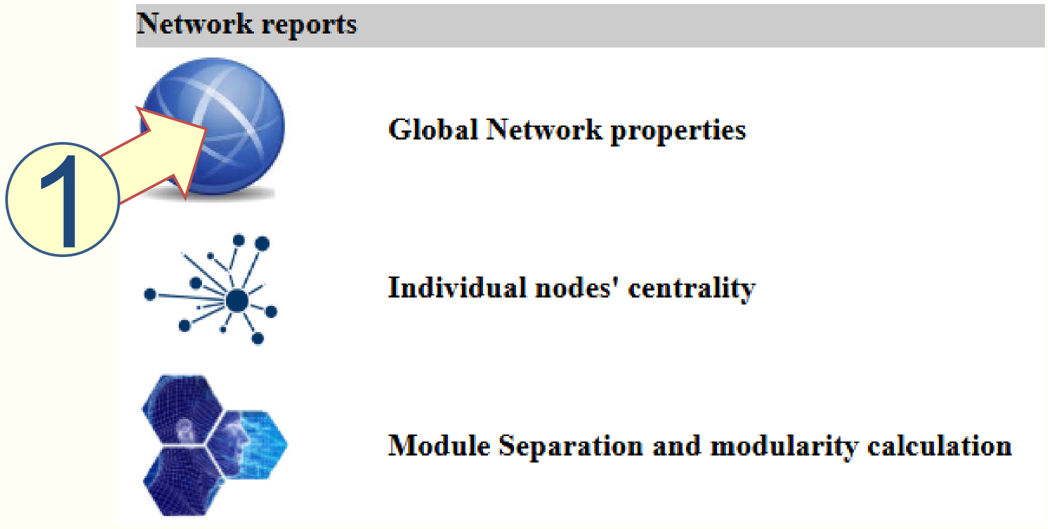
Fit three power-law models (regular power law, exponential law and truncated power law)

Randomize the network structure and then calculate network properties (Updated: Modularity values from random networks have been added.)

Calculate Gene/OTU significances (GS) with environmental traits and then use Mantel test to check the correlations between GS and network connectivity

Module-EigenGene analyses

Global Network Properties

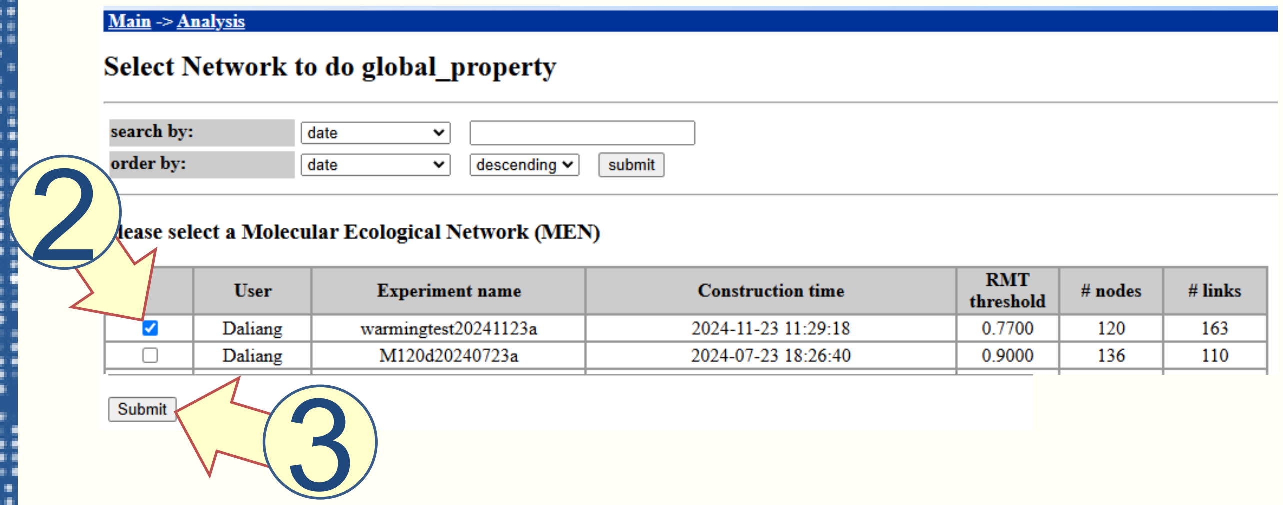


Network reports

Global Network properties

Individual nodes' centrality

Module Separation and modularity calculation



Main -> Analysis

Select Network to do global_property

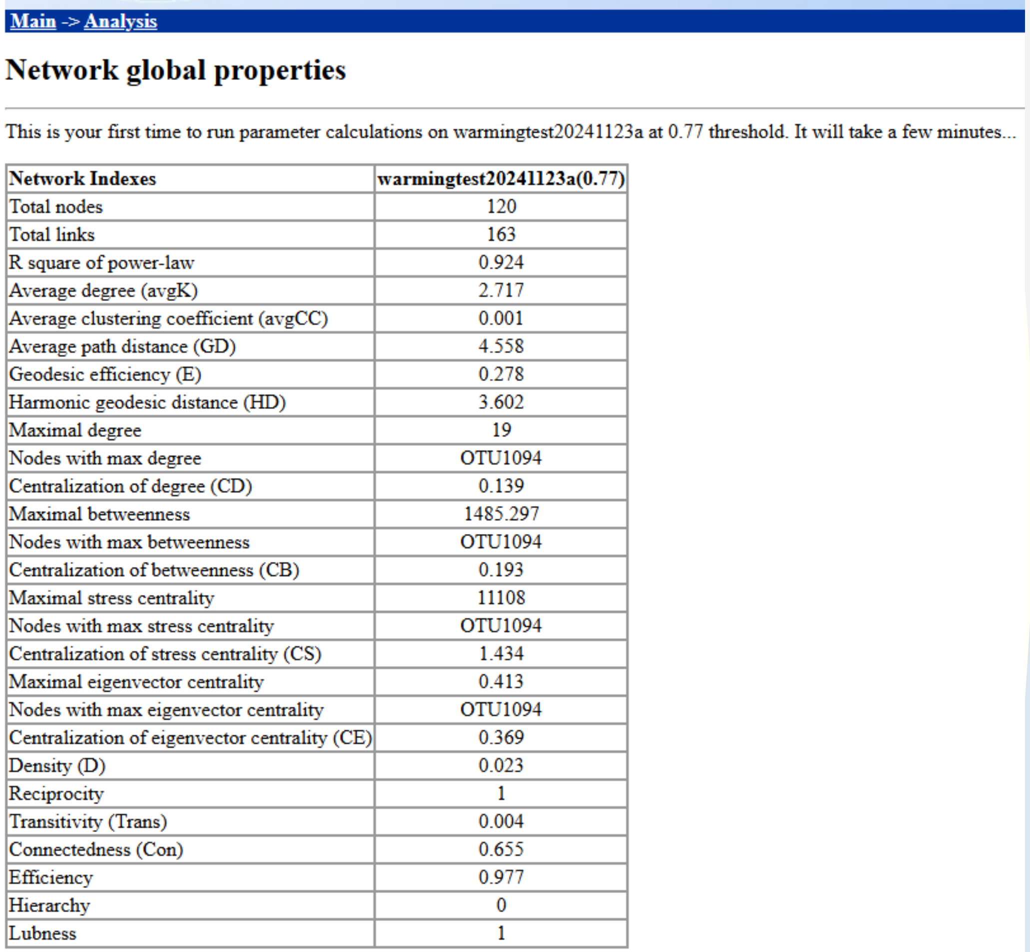
search by:	date		
order by:	date	descending	submit

Please select a Molecular Ecological Network (MEN)

	User	Experiment name	Construction time	RMT threshold	# nodes	# links
☑	Daliang	warmingtest20241123a	2024-11-23 11:29:18	0.7700	120	163
☐	Daliang	M120d20240723a	2024-07-23 18:26:40	0.9000	136	110

Submit

If the dataset is very big, you may need to wait for some hours.



Main -> Analysis

Network global properties

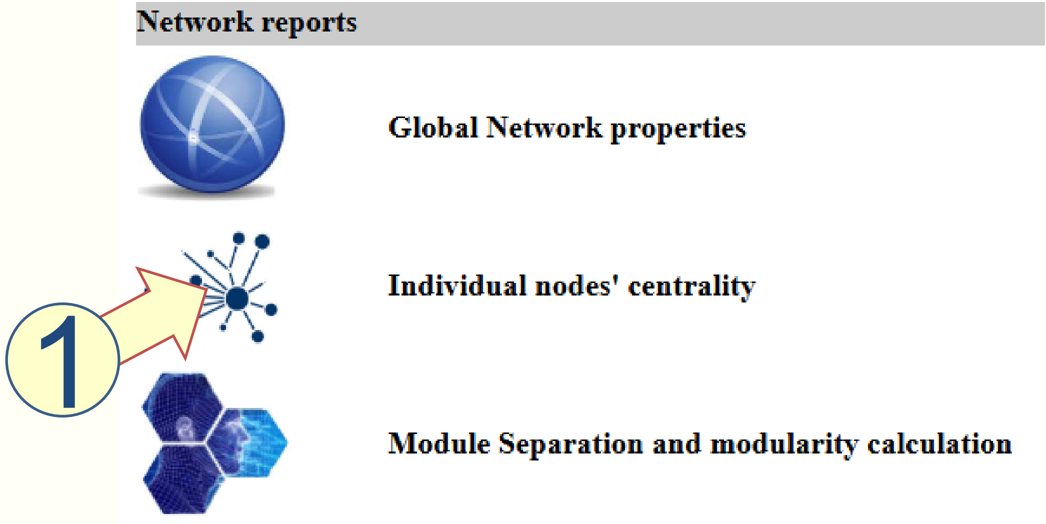
This is your first time to run parameter calculations on warmingtest20241123a at 0.77 threshold. It will take a few minutes...

Network Indexes	warmingtest20241123a(0.77)
Total nodes	120
Total links	163
R square of power-law	0.924
Average degree (avgK)	2.717
Average clustering coefficient (avgCC)	0.001
Average path distance (GD)	4.558
Geodesic efficiency (E)	0.278
Harmonic geodesic distance (HD)	3.602
Maximal degree	19
Nodes with max degree	OTU1094
Centralization of degree (CD)	0.139
Maximal betweenness	1485.297
Nodes with max betweenness	OTU1094
Centralization of betweenness (CB)	0.193
Maximal stress centrality	11108
Nodes with max stress centrality	OTU1094
Centralization of stress centrality (CS)	1.434
Maximal eigenvector centrality	0.413
Nodes with max eigenvector centrality	OTU1094
Centralization of eigenvector centrality (CE)	0.369
Density (D)	0.023
Reciprocity	1
Transitivity (Trans)	0.004
Connectedness (Con)	0.655
Efficiency	0.977
Hierarchy	0
Lubness	1

Copy paste the table to a spreadsheet

Back to Outline

Individual Nodes Centrality (node attributes)

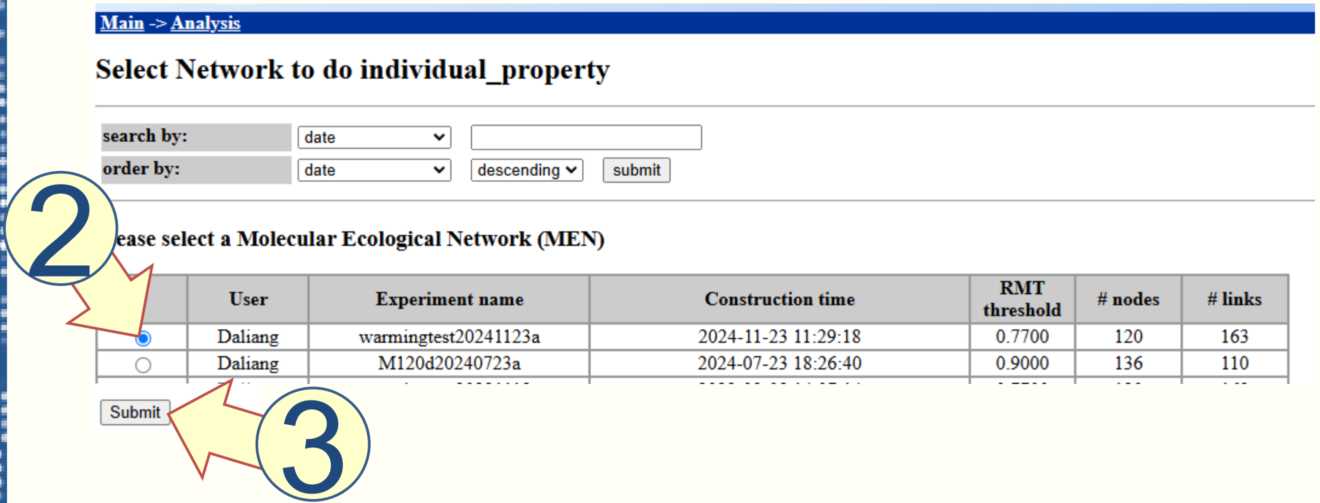


Network reports

Global Network properties

Individual nodes' centrality

Module Separation and modularity calculation

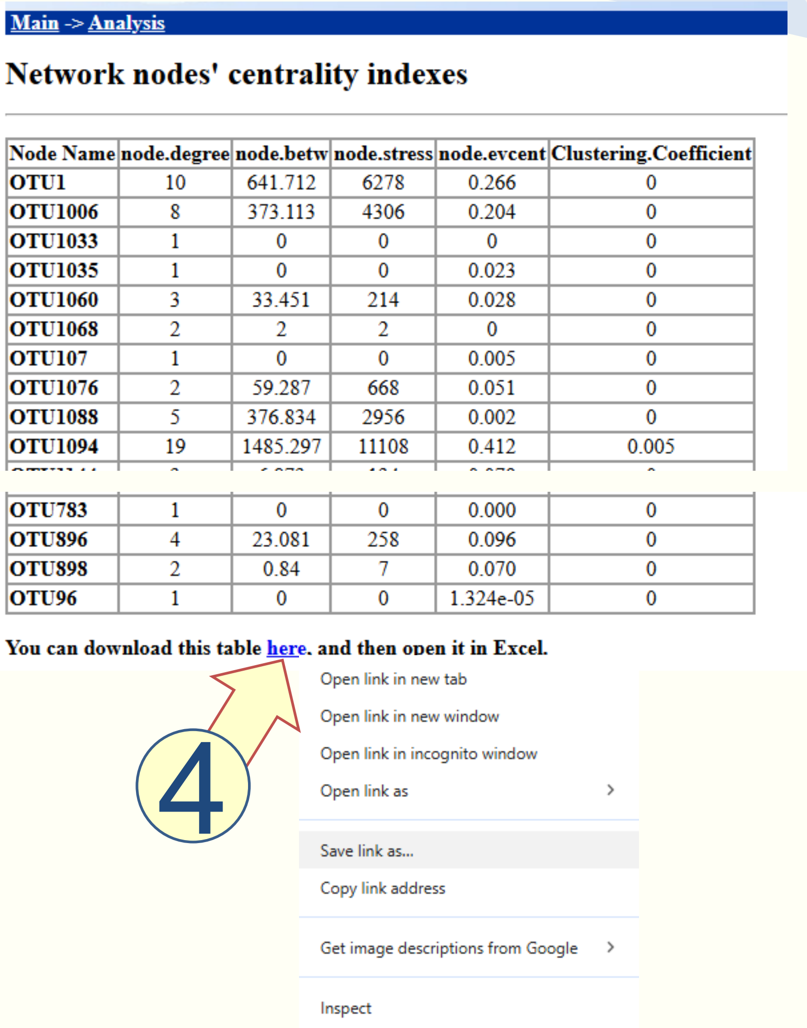


Select Network to do individual_property

search by:	date		
order by:	date	descending	submit

ease select a Molecular Ecological Network (MEN)

	User	Experiment name	Construction time	RMT threshold	# nodes	# links
●	Daliang	warmingtest20241123a	2024-11-23 11:29:18	0.7700	120	163
○	Daliang	M120d20240723a	2024-07-23 18:26:40	0.9000	136	110



Network nodes' centrality indexes

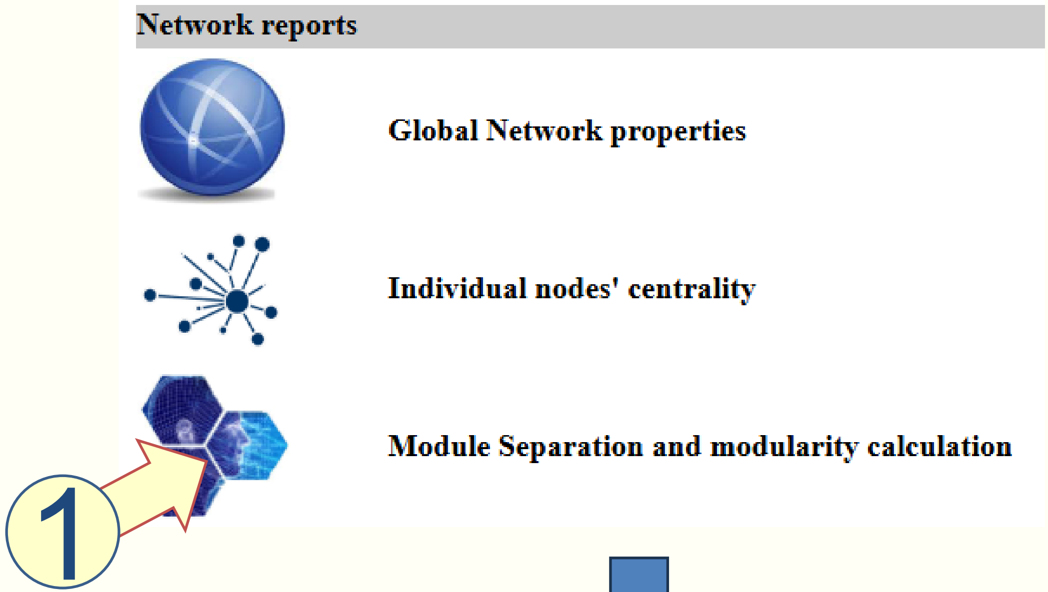
Node Name	node.degree	node.betw	node.stress	node.evcent	Clustering.Coefficient
OTU1	10	641.712	6278	0.266	0
OTU1006	8	373.113	4306	0.204	0
OTU1033	1	0	0	0	0
OTU1035	1	0	0	0.023	0
OTU1060	3	33.451	214	0.028	0
OTU1068	2	2	2	0	0
OTU107	1	0	0	0.005	0
OTU1076	2	59.287	668	0.051	0
OTU1088	5	376.834	2956	0.002	0
OTU1094	19	1485.297	11108	0.412	0.005
OTU783	1	0	0	0.000	0
OTU896	4	23.081	258	0.096	0
OTU898	2	0.84	7	0.070	0
OTU96	1	0	0	1.324e-05	0

You can download this table here, and then open it in Excel.

Right-click ‘*here*’ and ‘Save link as …’ to download the table.

Back to Outline

Module Separation and Modularity

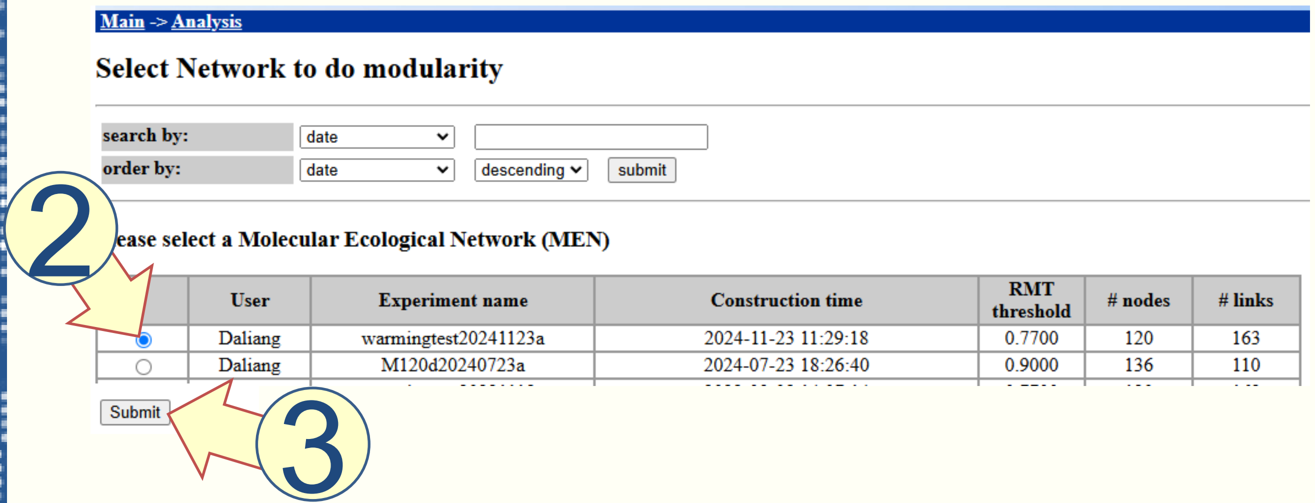


Network reports

Global Network properties

Individual nodes' centrality

Module Separation and modularity calculation



Main -> Analysis

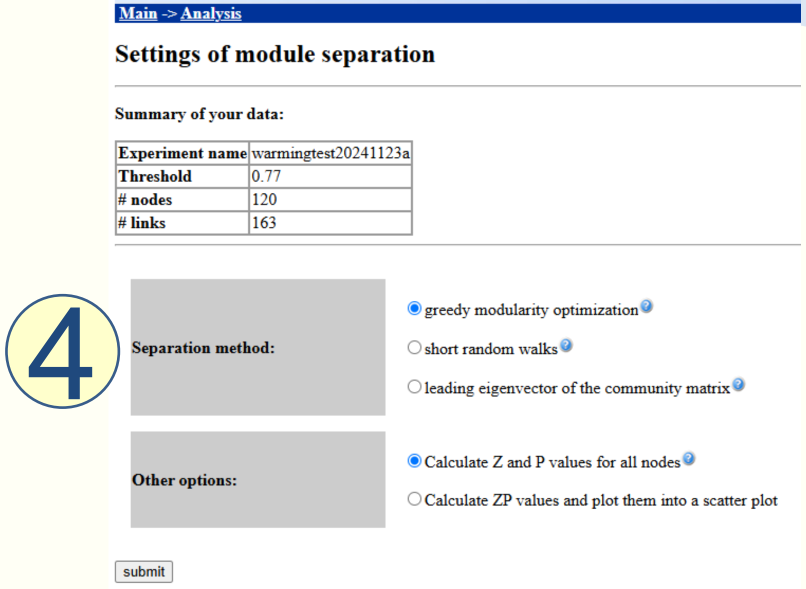
Select Network to do modularity

search by:	date		
order by:	date	descending	submit

ease select a Molecular Ecological Network (MEN)

	User	Experiment name	Construction time	RMT threshold	# nodes	# links
●	Daliang	warmingtest20241123a	2024-11-23 11:29:18	0.7700	120	163
○	Daliang	M120d20240723a	2024-07-23 18:26:40	0.9000	136	110

Submit



Main -> Analysis

Settings of module separation

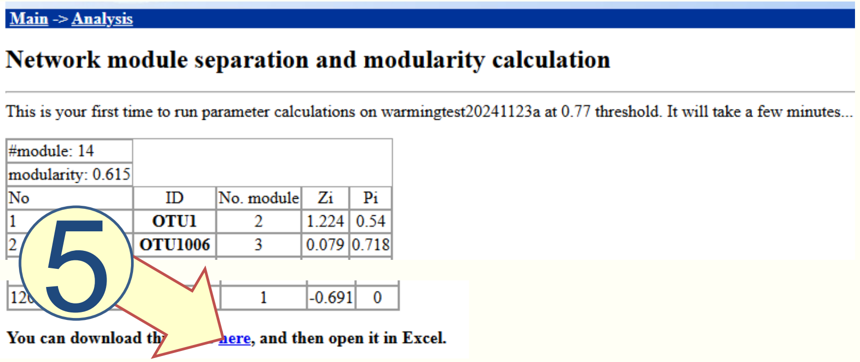
Summary of your data:

Experiment name	warmingtest20241123a
Threshold	0.77
# nodes	120
# links	163

Separation method:
- ● greedy modularity optimization
- ○ short random walks
- ○ leading eigenvector of the community matrix

Other options:
- ● Calculate Z and P values for all nodes
- ○ Calculate ZP values and plot them into a scatter plot

submit



Main -> Analysis

Network module separation and modularity calculation

This is your first time to run parameter calculations on warmingtest20241123a at 0.77 threshold. It will take a few minutes...

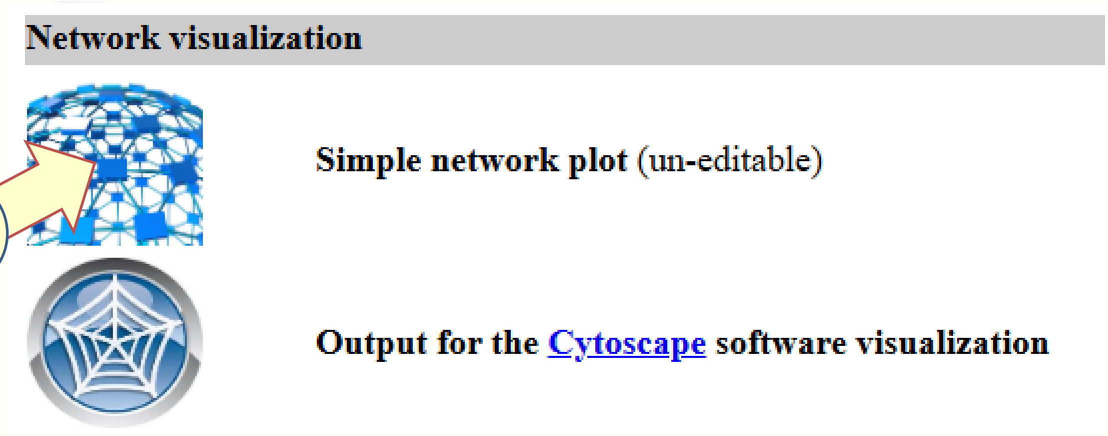
#module: 14				
modularity: 0.615				
No	ID	No. module	Zi	Pi
1	**OTU1**	2	1.224	0.54
2	**OTU1006**	3	0.079	0.718
120		1	-0.691	0

You can download th[illegible] here, and then open it in Excel.

Right-click ‘*here*’ and ‘Save link as …’ to download the table.

Back to Outline

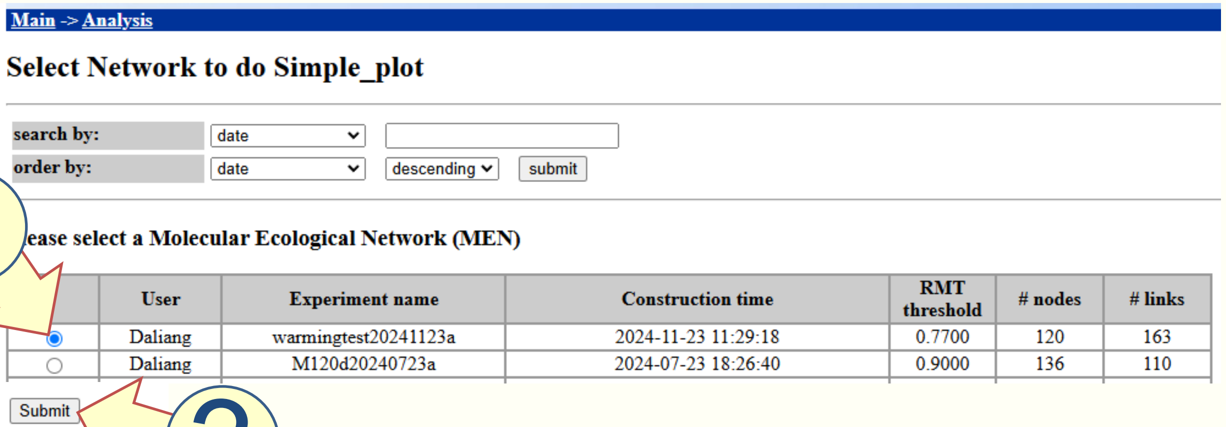
Simple network plot (un-editable)



Network visualization

Simple network plot (un-editable)

Output for the Cytoscape software visualization



Main -> Analysis

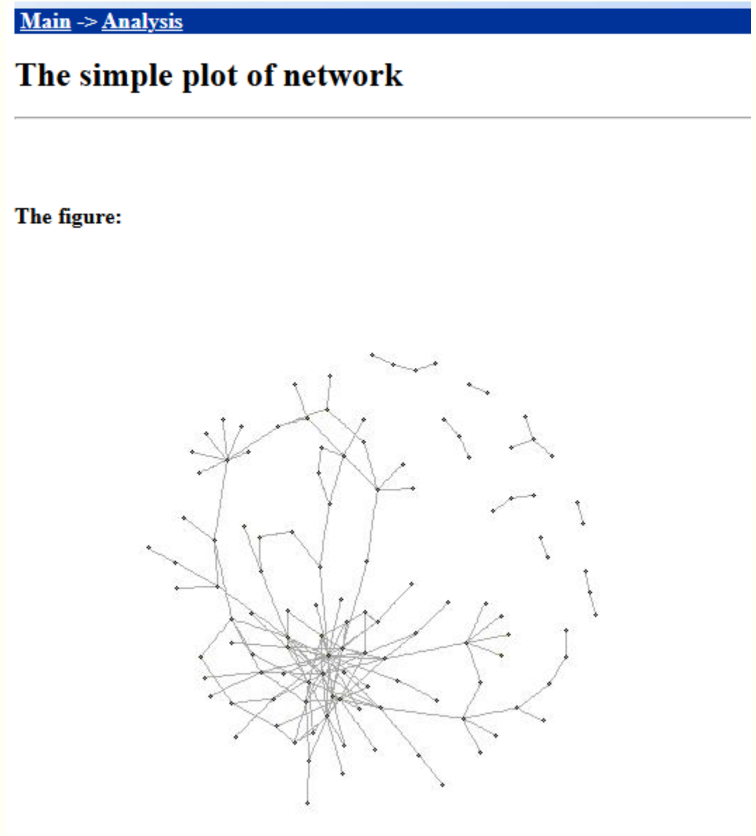
Select Network to do Simple_plot

search by: date

order by: date descending submit

ease select a Molecular Ecological Network (MEN)

	User	Experiment name	Construction time	RMT threshold	# nodes	# links
●	Daliang	warmingtest20241123a	2024-11-23 11:29:18	0.7700	120	163
○	Daliang	M120d20240723a	2024-07-23 18:26:40	0.9000	136	110



Main -> Analysis

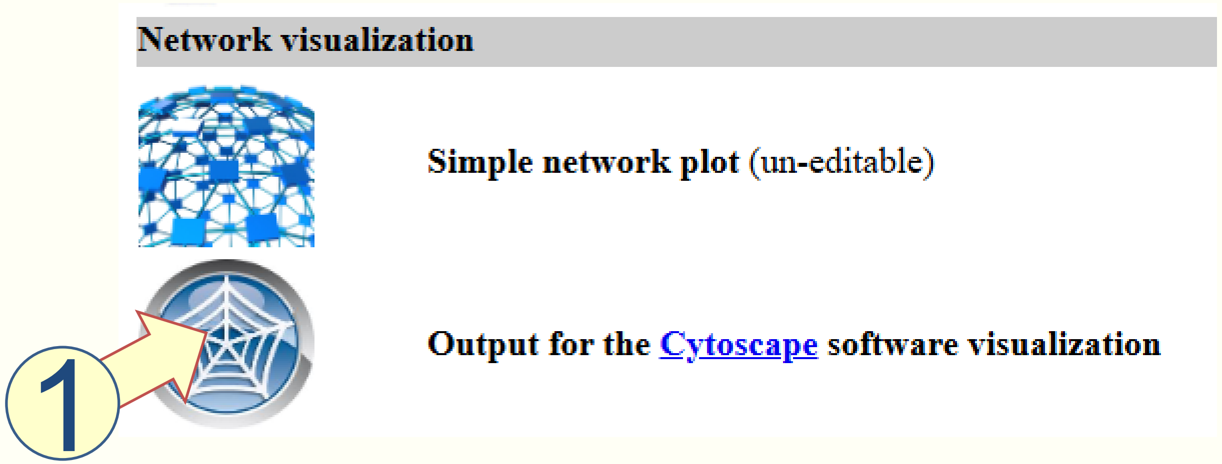
The simple plot of network

The figure:

This is a quick overview of the network.

Back to Outline

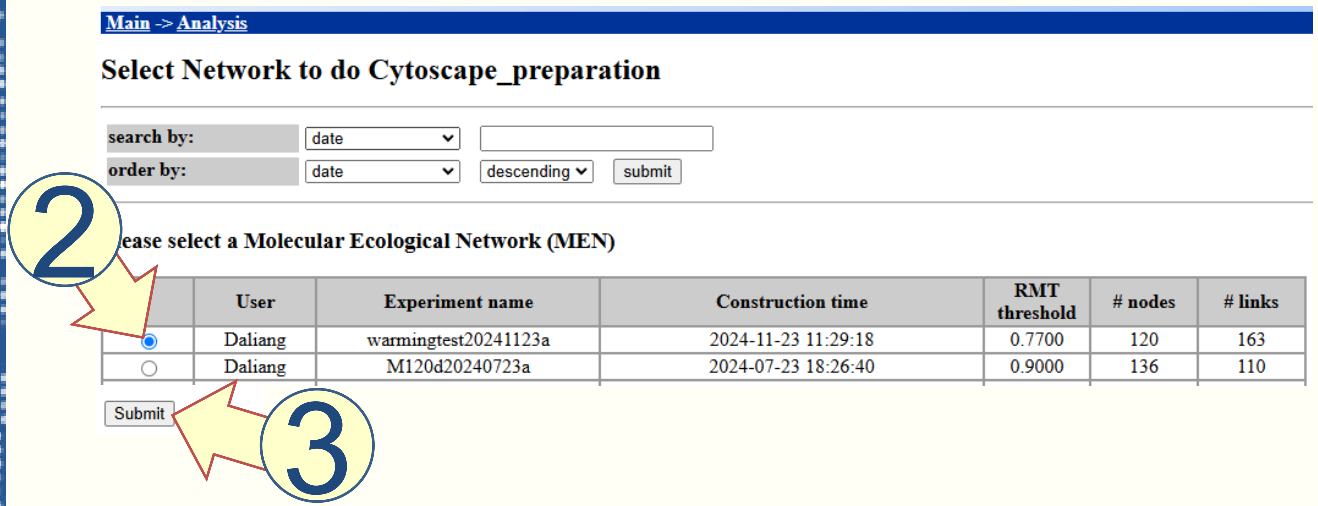
Output for the Cytoscape software visualization



Network visualization

Simple network plot (un-editable)

Output for the Cytoscape software visualization



Main -> Analysis

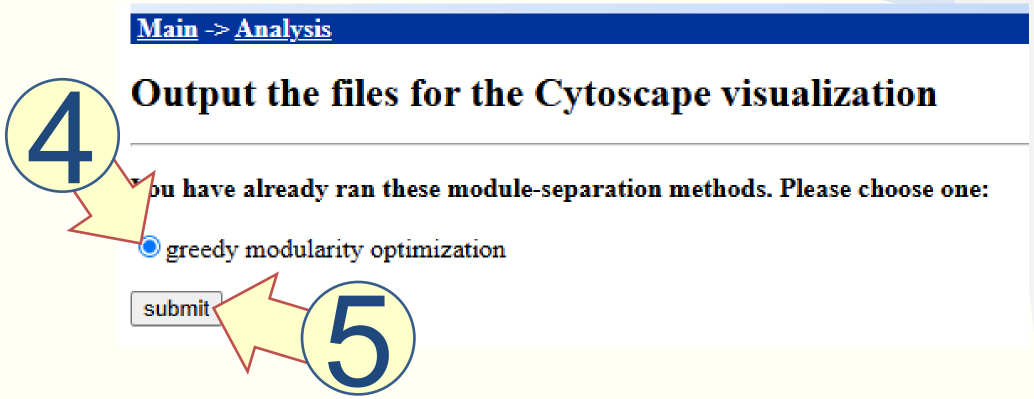
Select Network to do Cytoscape_preparation

search by: date

order by: date descending

ease select a Molecular Ecological Network (MEN)

	User	Experiment name	Construction time	RMT threshold	# nodes	# links
●	Daliang	warmingtest20241123a	2024-11-23 11:29:18	0.7700	120	163
○	Daliang	M120d20240723a	2024-07-23 18:26:40	0.9000	136	110

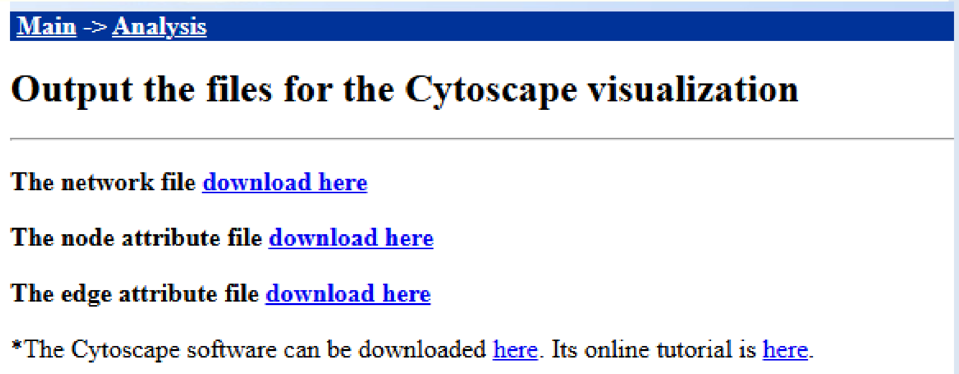


Main -> Analysis

Output the files for the Cytoscape visualization

u have already ran these module-separation methods. Please choose one:

● greedy modularity optimization



Main -> Analysis

Output the files for the Cytoscape visualization

The network file download here

The node attribute file download here

The edge attribute file download here

*The Cytoscape software can be downloaded here. Its online tutorial is here.

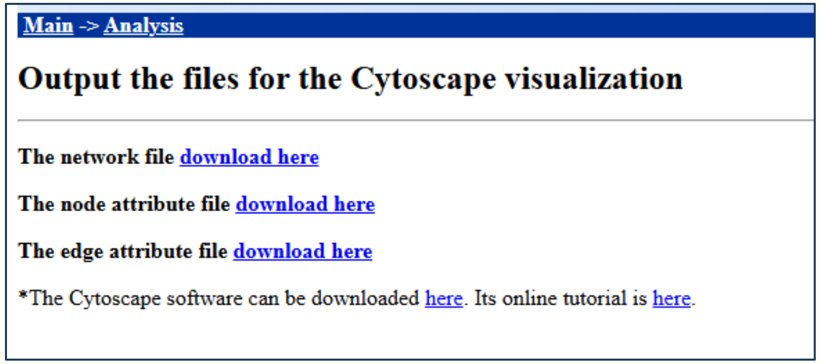
Right-click each file link and ‘Save link as …’ to download the table.

Use Cytoscape to visualize your network

- Download and install Cytoscape
  - https://cytoscape.org/
- Check the online tutorial from Cytoscape
  - https://github.com/cytoscape/cytoscape-tutorials/wiki
- Below is a simple example
  - Using Cytoscape 3.10.3

Use Cytoscape: prepare your files

- Download files from MENAP function ‘Output for the Cytoscape software visualization’



Main -> Analysis

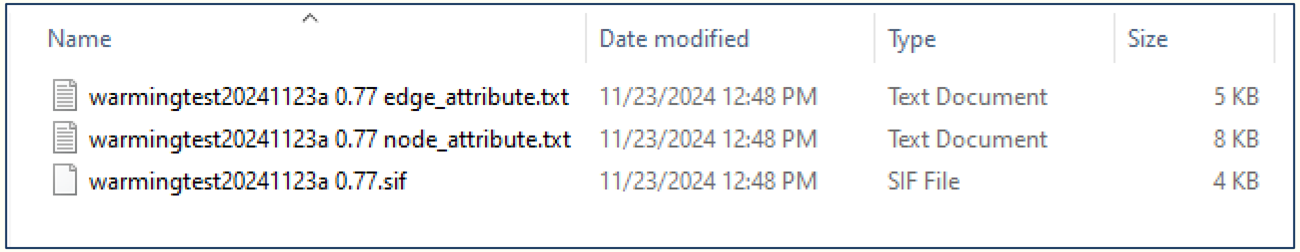
Output the files for the Cytoscape visualization

The network file download here

The node attribute file download here

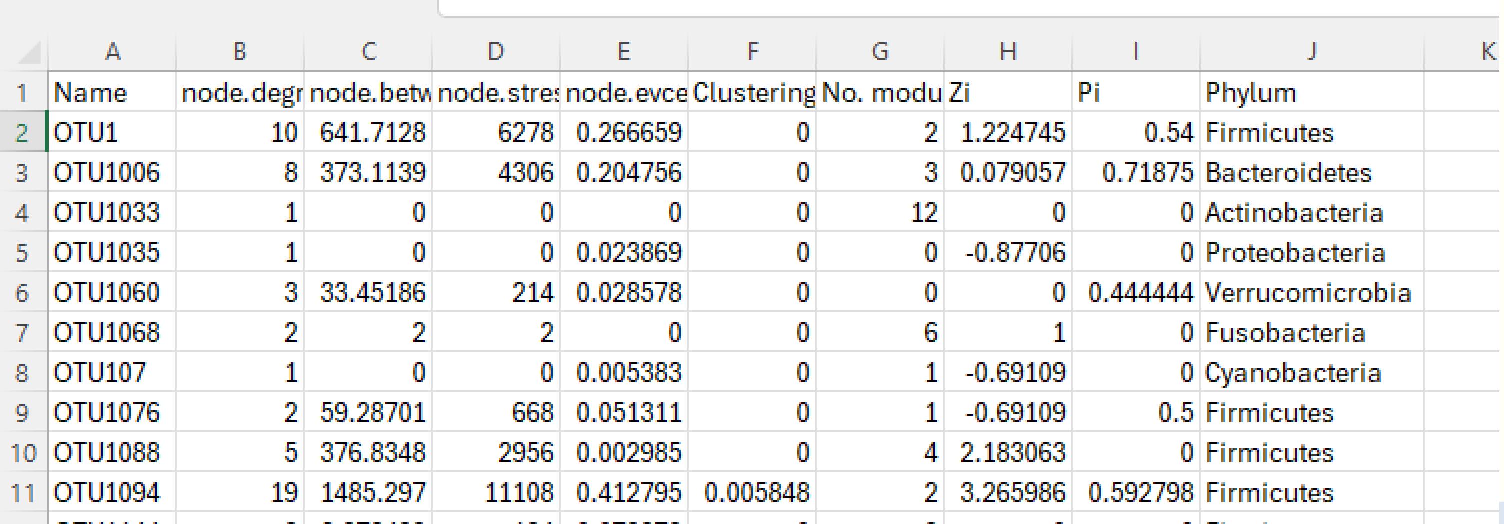
The edge attribute file download here

*The Cytoscape software can be downloaded here. Its online tutorial is here.



Name	Date modified	Type	Size
warmingtest20241123a 0.77 edge_attribute.txt	11/23/2024 12:48 PM	Text Document	5 KB
warmingtest20241123a 0.77 node_attribute.txt	11/23/2024 12:48 PM	Text Document	8 KB
warmingtest20241123a 0.77.sif	11/23/2024 12:48 PM	SIF File	4 KB

- Use Excel to edit the ‘…node_attribute.txt’ or ‘…edge_attribute.txt’ if you want to add more attributes to show in the network figure.
  - For example, add the phylum information



	A	B	C	D	E	F	G	H	I	J	K
1	Name	node.degr	node.betw	node.stres	node.evce	Clustering	No. modu	Zi	Pi	Phylum	
2	OTU1	10	641.7128	6278	0.266659	0	2	1.224745	0.54	Firmicutes	
3	OTU1006	8	373.1139	4306	0.204756	0	3	0.079057	0.71875	Bacteroidetes	
4	OTU1033	1	0	0	0	0	12	0	0	Actinobacteria	
5	OTU1035	1	0	0	0.023869	0	0	-0.87706	0	Proteobacteria	
6	OTU1060	3	33.45186	214	0.028578	0	0	0	0.444444	Verrucomicrobia	
7	OTU1068	2	2	2	0	0	6	1	0	Fusobacteria	
8	OTU107	1	0	0	0.005383	0	1	-0.69109	0	Cyanobacteria	
9	OTU1076	2	59.28701	668	0.051311	0	1	-0.69109	0.5	Firmicutes	
10	OTU1088	5	376.8348	2956	0.002985	0	4	2.183063	0	Firmicutes	
11	OTU1094	19	1485.297	11108	0.412795	0.005848	2	3.265986	0.592798	Firmicutes	

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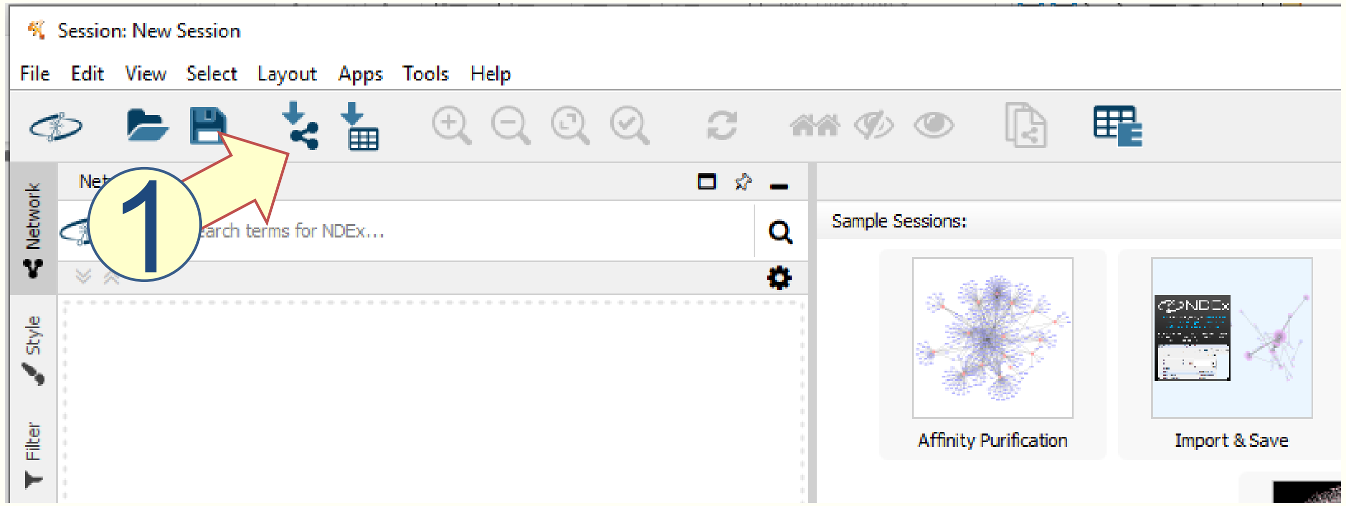
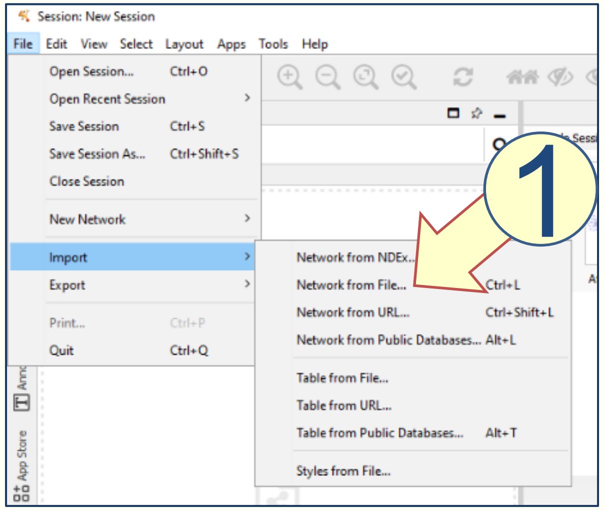
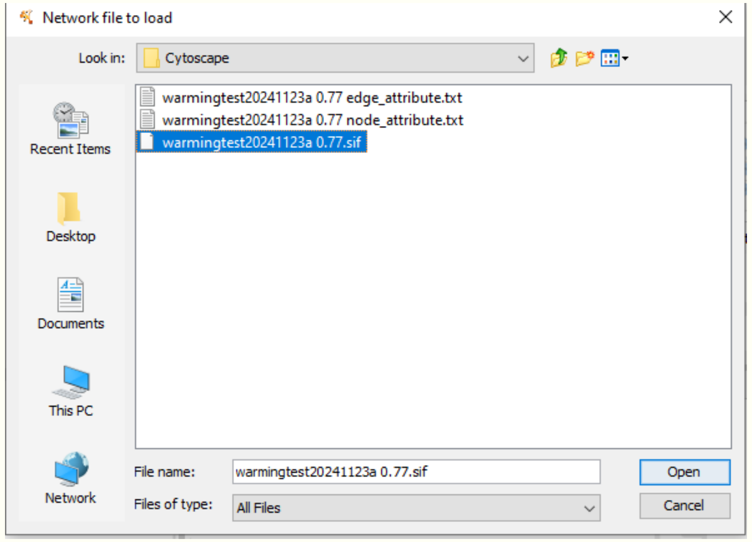
Use Cytoscape: import data files (1)

- Import the network file '…sif'



OR





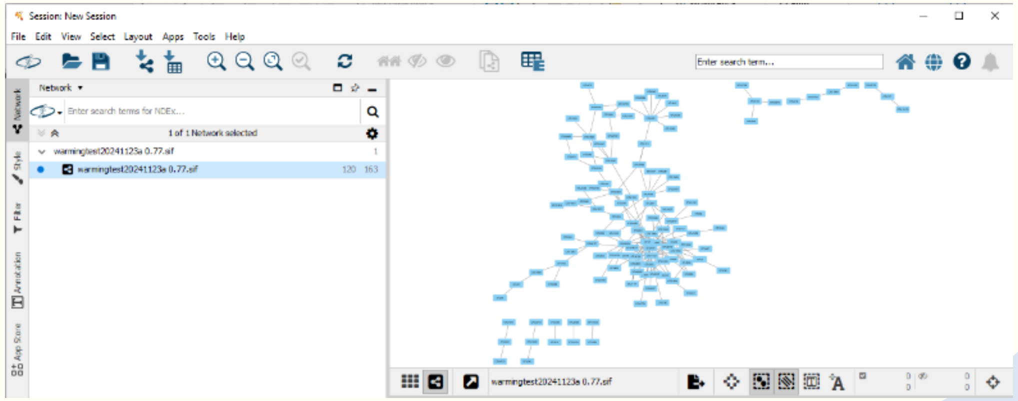
Network file to load

Look in: Cytoscape

warmingtest20241123a 0.77 edge_attribute.txt
warmingtest20241123a 0.77 node_attribute.txt
warmingtest20241123a 0.77.sif

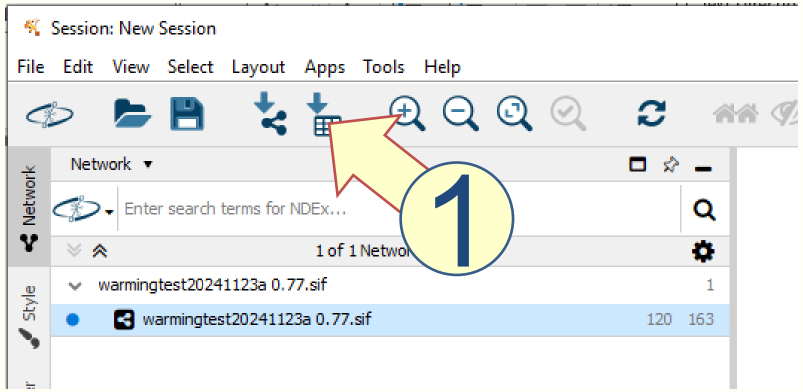
File name: warmingtest20241123a 0.77.sif

Files of type: All Files

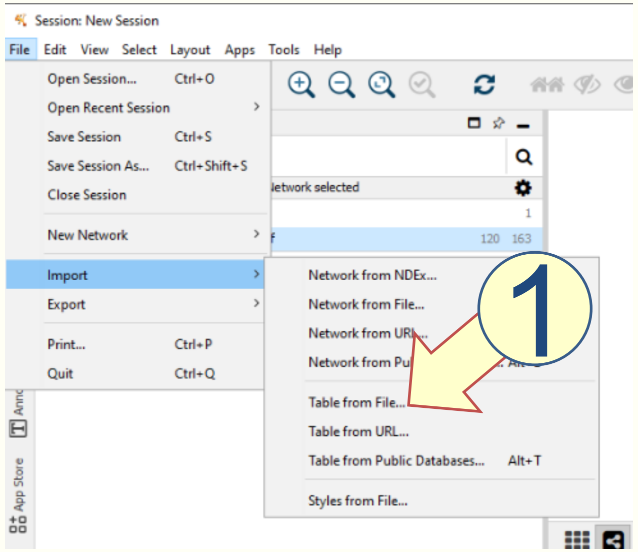


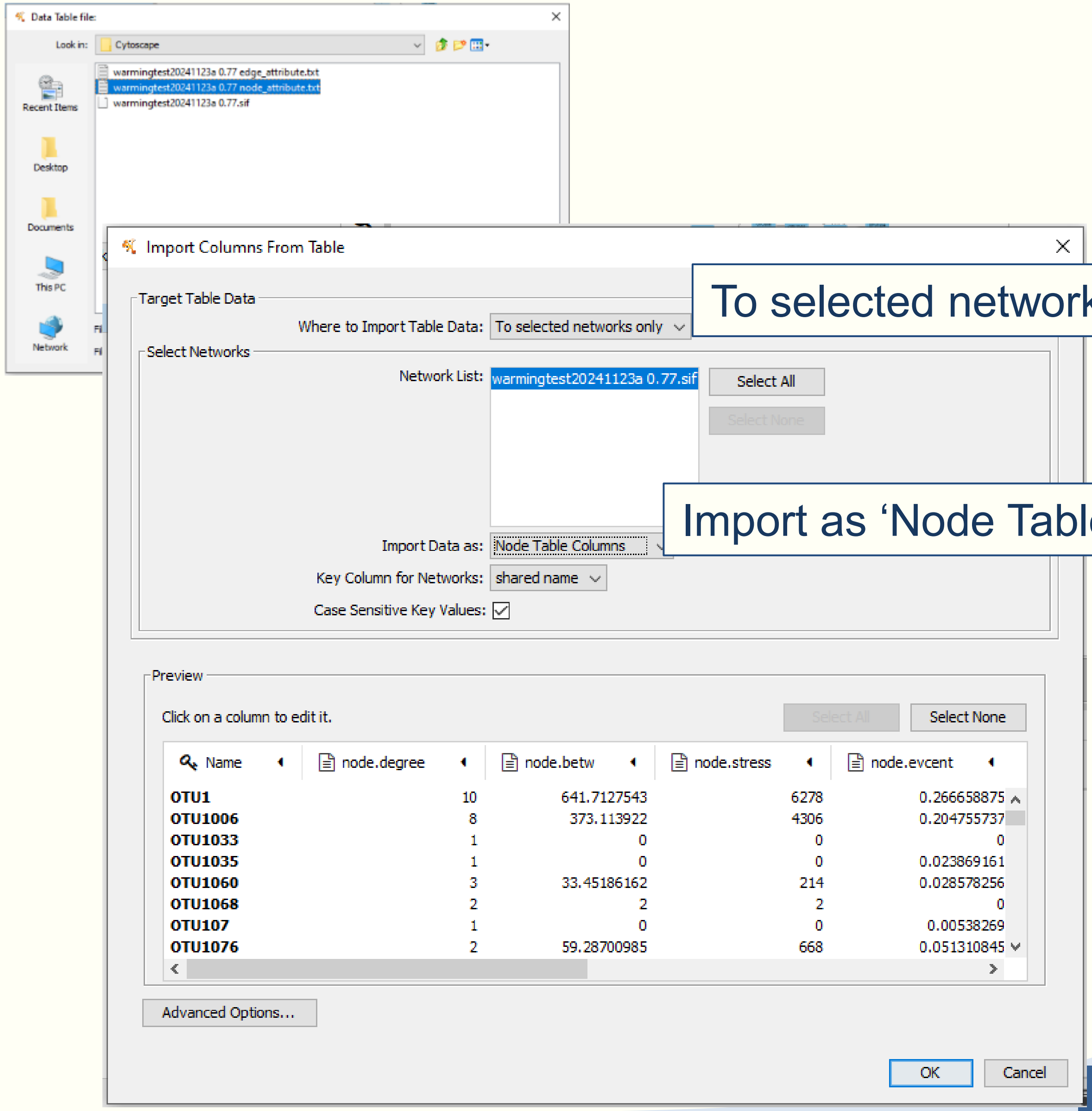
Use Cytoscape: import data files (2)

- Import the '…node_attribute.txt' file



OR





Look in: Cytoscape

warmingtest20241123a 0.77 edge_attribute.txt
warmingtest20241123a 0.77 node_attribute.txt
warmingtest20241123a 0.77.sif

Import Columns From Table

Target Table Data

Where to Import Table Data: To selected networks only

Select Networks

Network List: warmingtest20241123a 0.77.sif

Import Data as: Node Table Columns

Key Column for Networks: shared name

Case Sensitive Key Values: ☑

Preview

Click on a column to edit it.

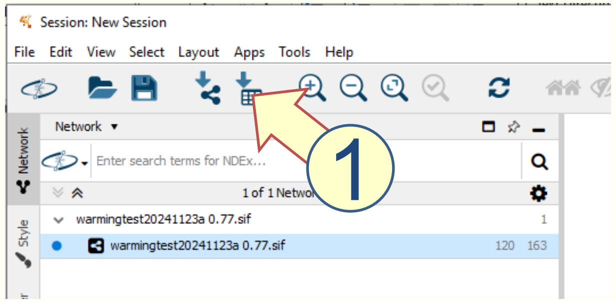
Name	node.degree	node.betw	node.stress	node.evcent
OTU1	10	641.7127543	6278	0.266658875
OTU1006	8	373.113922	4306	0.204755737
OTU1033	1	0	0	0
OTU1035	1	0	0	0.023869161
OTU1060	3	33.45186162	214	0.028578256
OTU1068	2	2	2	0
OTU107	1	0	0	0.00538269
OTU1076	2	59.28700985	668	0.051310845

To selected networks only

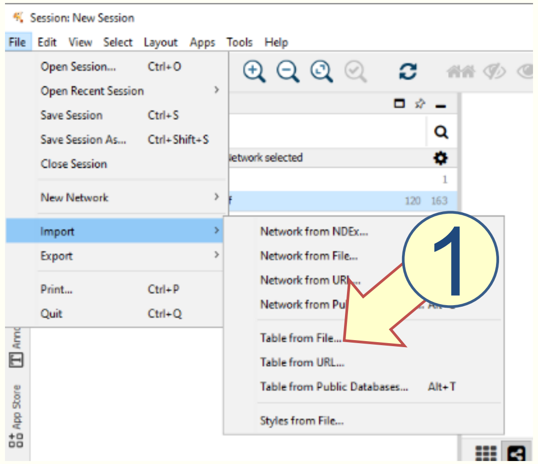
Import as 'Node Table Columns'

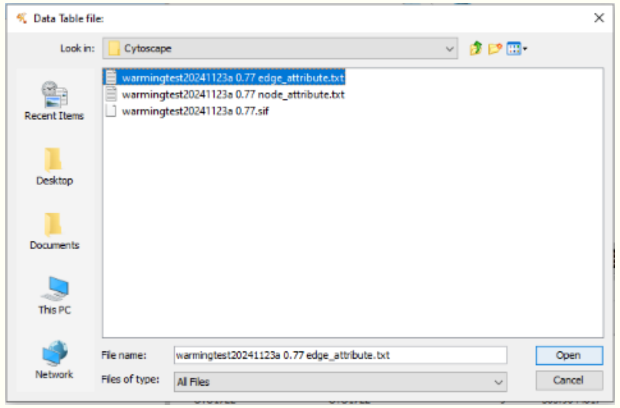
Use Cytoscape: import data files (3)

- Import the '…edge_attribute.txt' file



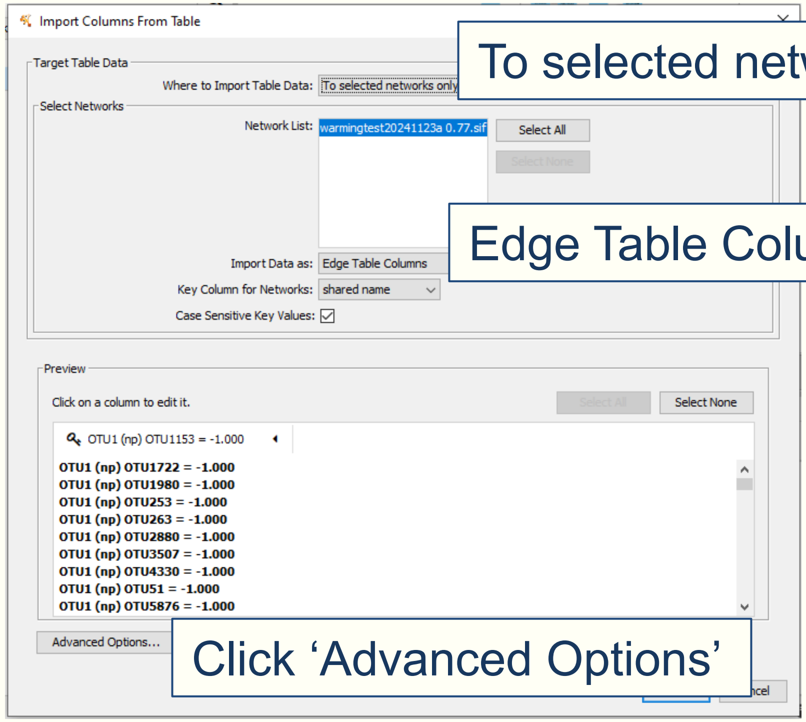
OR





File name: warmingtest20241123a 0.77 edge_attribute.txt

Files of type: All Files



Where to Import Table Data: To selected networks only

Network List: warmingtest20241123a 0.77.sif

Import Data as: Edge Table Columns

Key Column for Networks: shared name

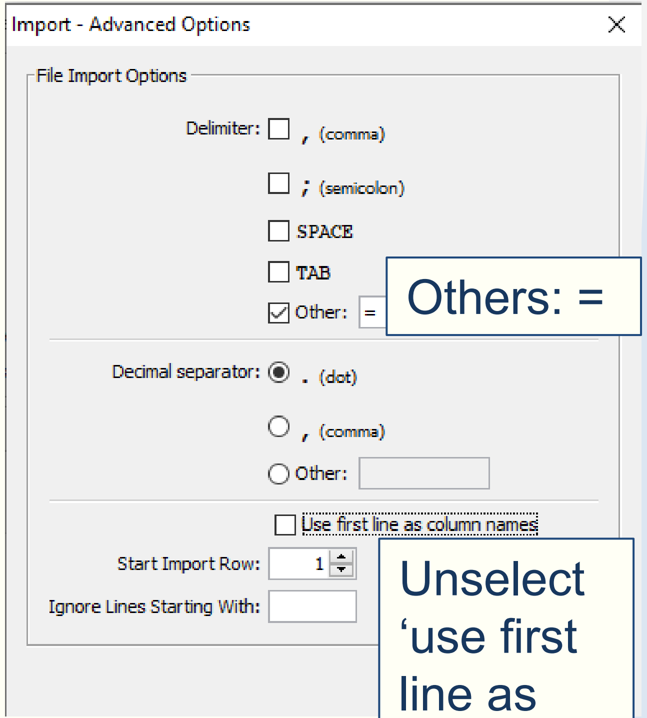
Case Sensitive Key Values: ☑

```
OTU1 (np) OTU1153 = -1.000
OTU1 (np) OTU1722 = -1.000
OTU1 (np) OTU1980 = -1.000
OTU1 (np) OTU253 = -1.000
OTU1 (np) OTU263 = -1.000
OTU1 (np) OTU2880 = -1.000
OTU1 (np) OTU3507 = -1.000
OTU1 (np) OTU4330 = -1.000
OTU1 (np) OTU51 = -1.000
OTU1 (np) OTU5876 = -1.000
```

To selected networks only

Edge Table Columns

Click 'Advanced Options'



Delimiter: ☐ , (comma)

☐ ; (semicolon)

☐ SPACE

☐ TAB

☑ Other: =

Decimal separator: ● . (dot)

○ , (comma)

○ Other:

☐ Use first line as column names

Start Import Row: 1

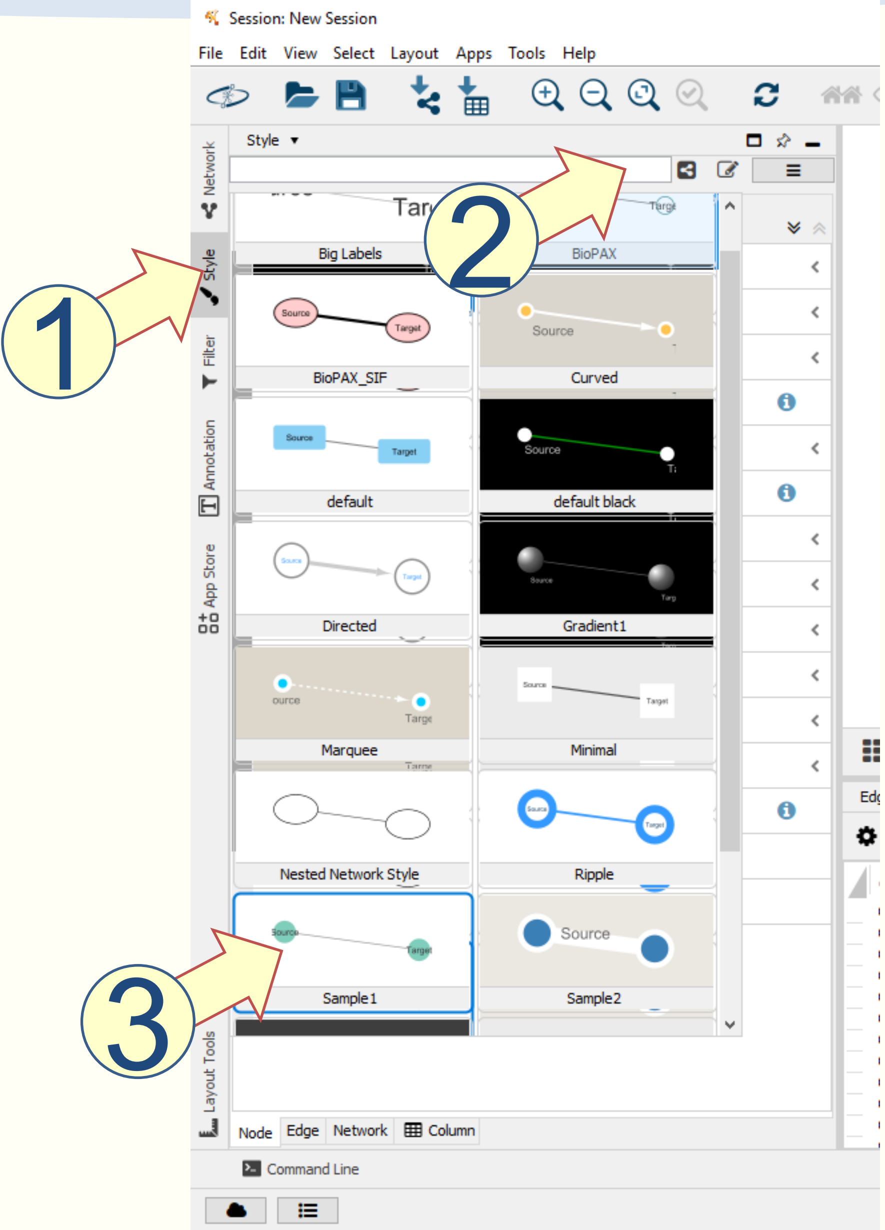
Ignore Lines Starting With:

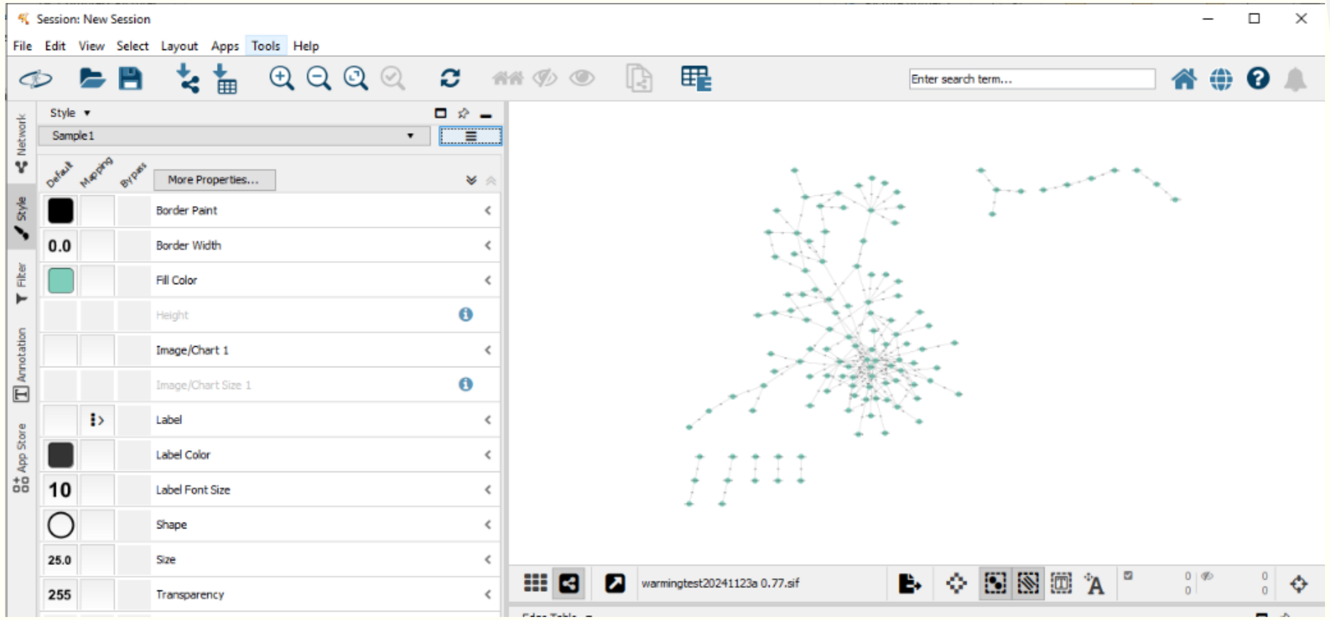
Others: =

Unselect 'use first line as column names'

Use Cytoscape: select style

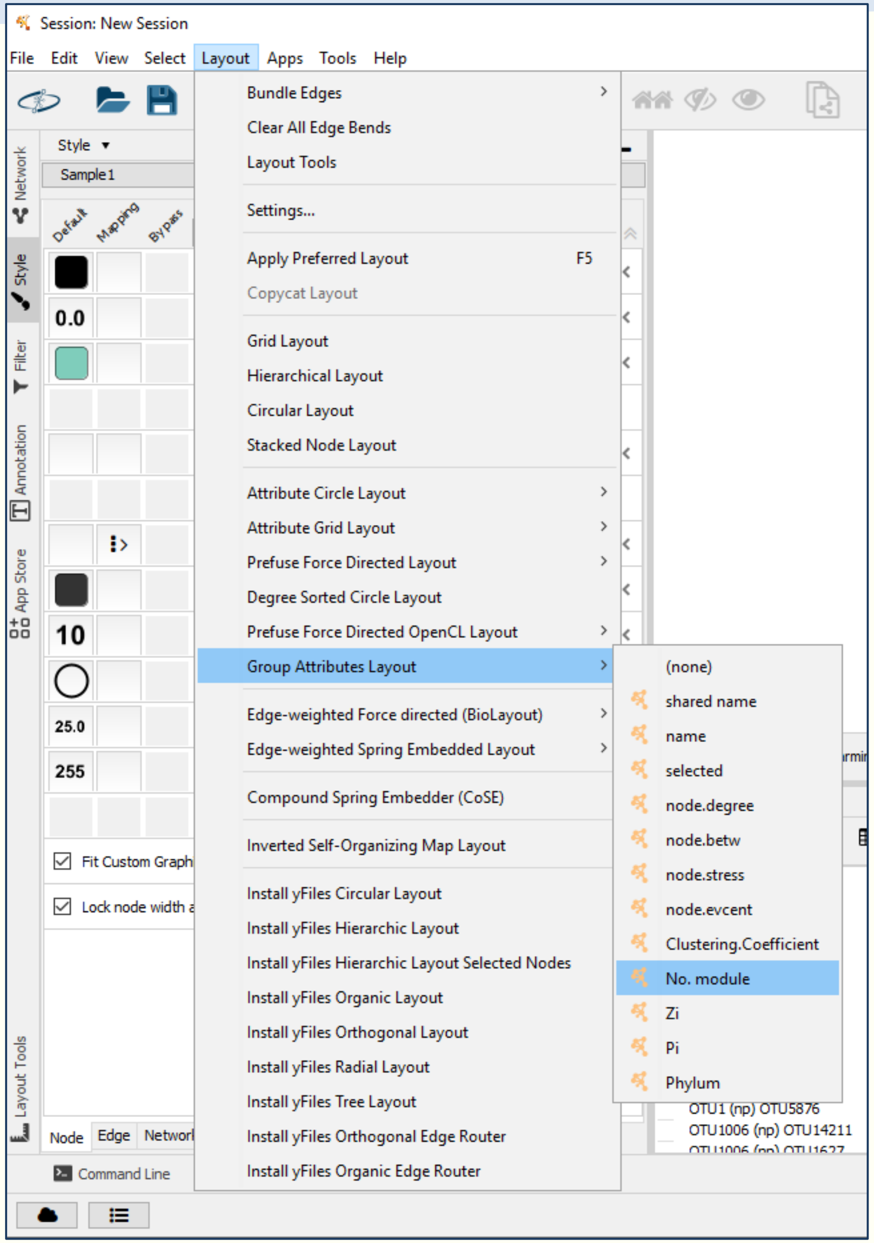
- Select ‘Style’ → ‘Sample 1’



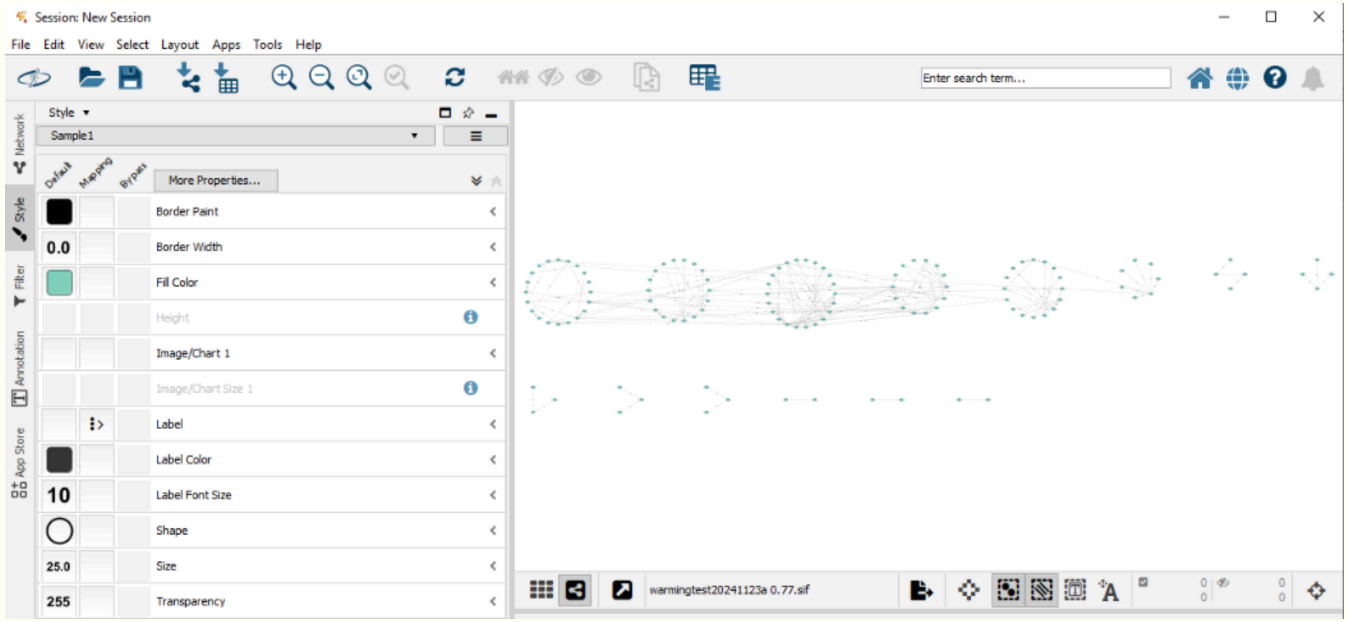


Back to Outline

Use Cytoscape: layout

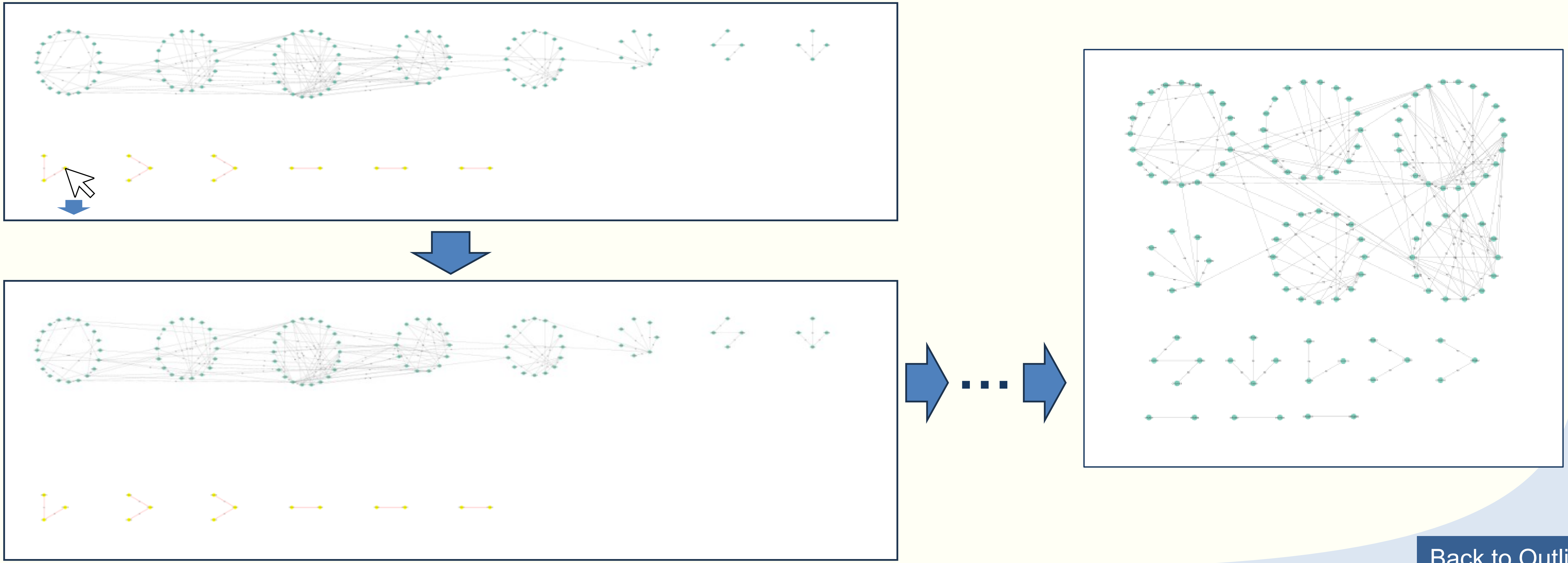


- You may explore different layout options
- Here the example shows ‘group by module’



Use Cytoscape: manual adjustment of layout

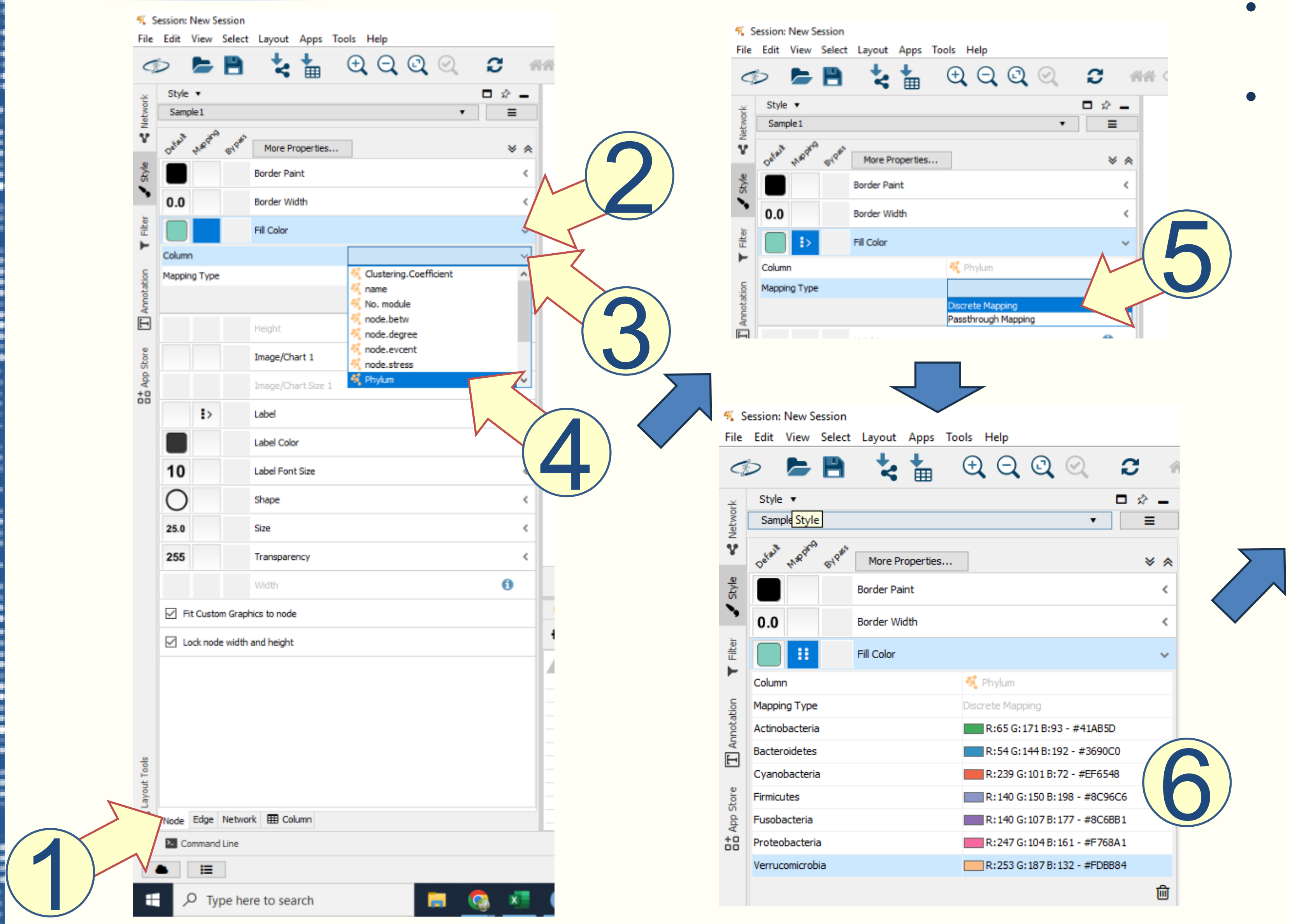
- To select nodes and edges, hold ‘Ctrl’ and use mouse drag (hold down left mouse button and drag) to select. Selected nodes will become yellow, and selected edges will become red.
- Move mouse above one of the nodes you want to move, then hold down left mouse and move.
- Click any other position will deselect the nodes and edges.
- Repeatedly select nodes/edges and move to adjust the layout.



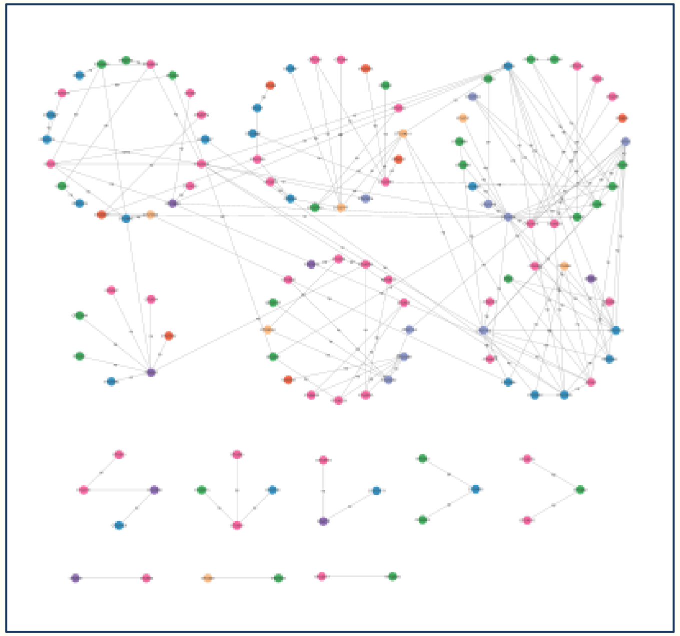
Back to Outline

Use Cytoscape: Node color

- You may explore node color by other different attributes.
- Here the example shows node color by phylum information.

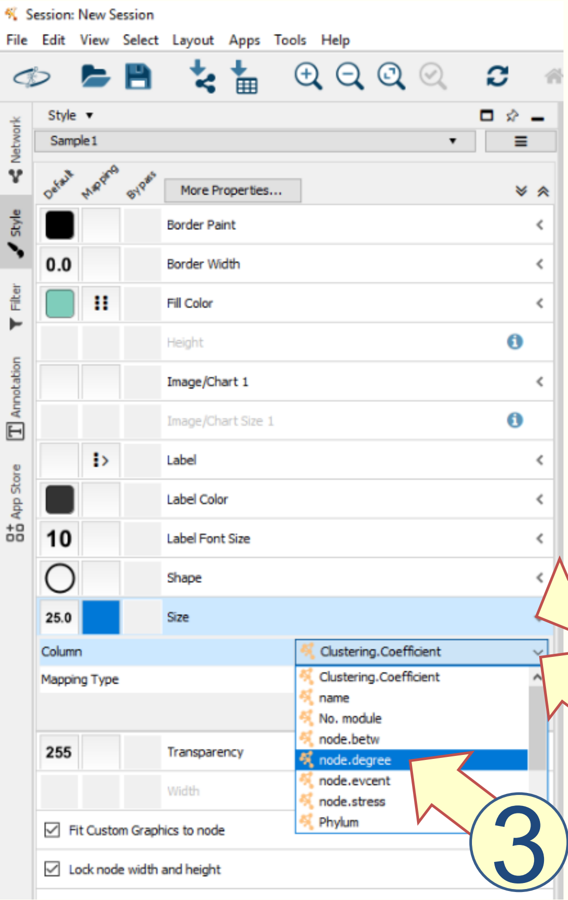


Column	Phylum
Mapping Type	Discrete Mapping
Actinobacteria	R:65 G:171 B:93 - #41AB5D
Bacteroidetes	R:54 G:144 B:192 - #3690C0
Cyanobacteria	R:239 G:101 B:72 - #EF6548
Firmicutes	R:140 G:150 B:198 - #8C96C6
Fusobacteria	R:140 G:107 B:177 - #8C6BB1
Proteobacteria	R:247 G:104 B:161 - #F768A1
Verrucomicrobia	R:253 G:187 B:132 - #FDBB84

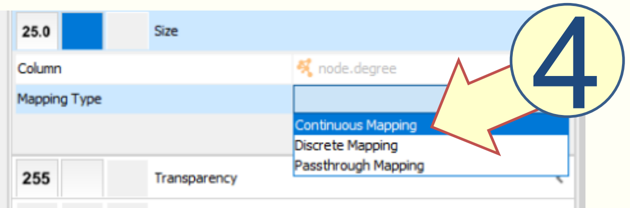


Use Cytoscape: Node size

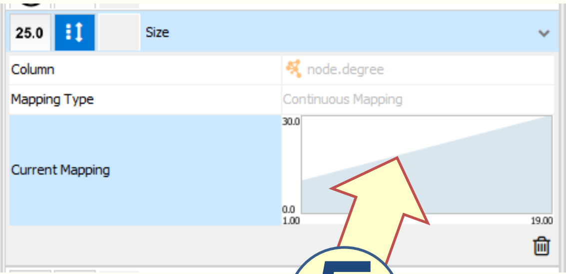
- You may explore node size by other attributes.
- Here the example shows node size by node degree.



①
②
③

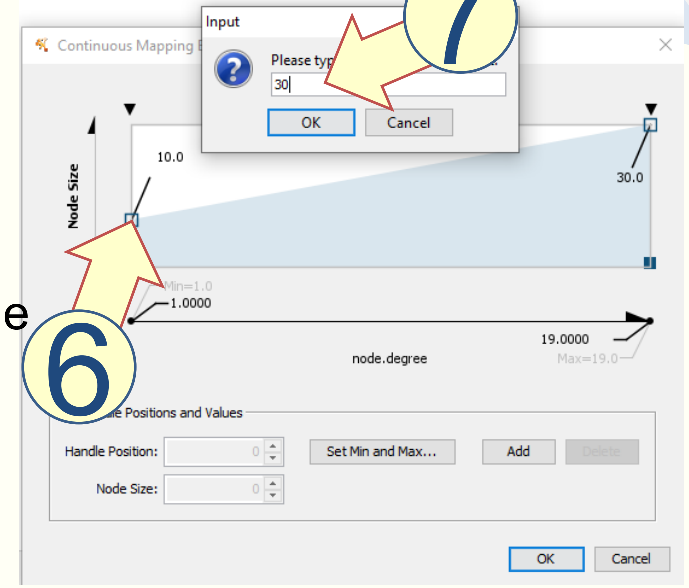


④



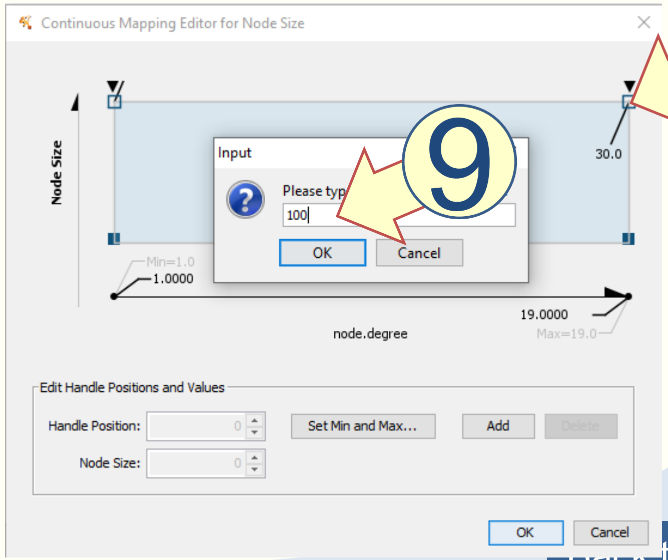
⑤

Double click



Double click

⑥
⑦

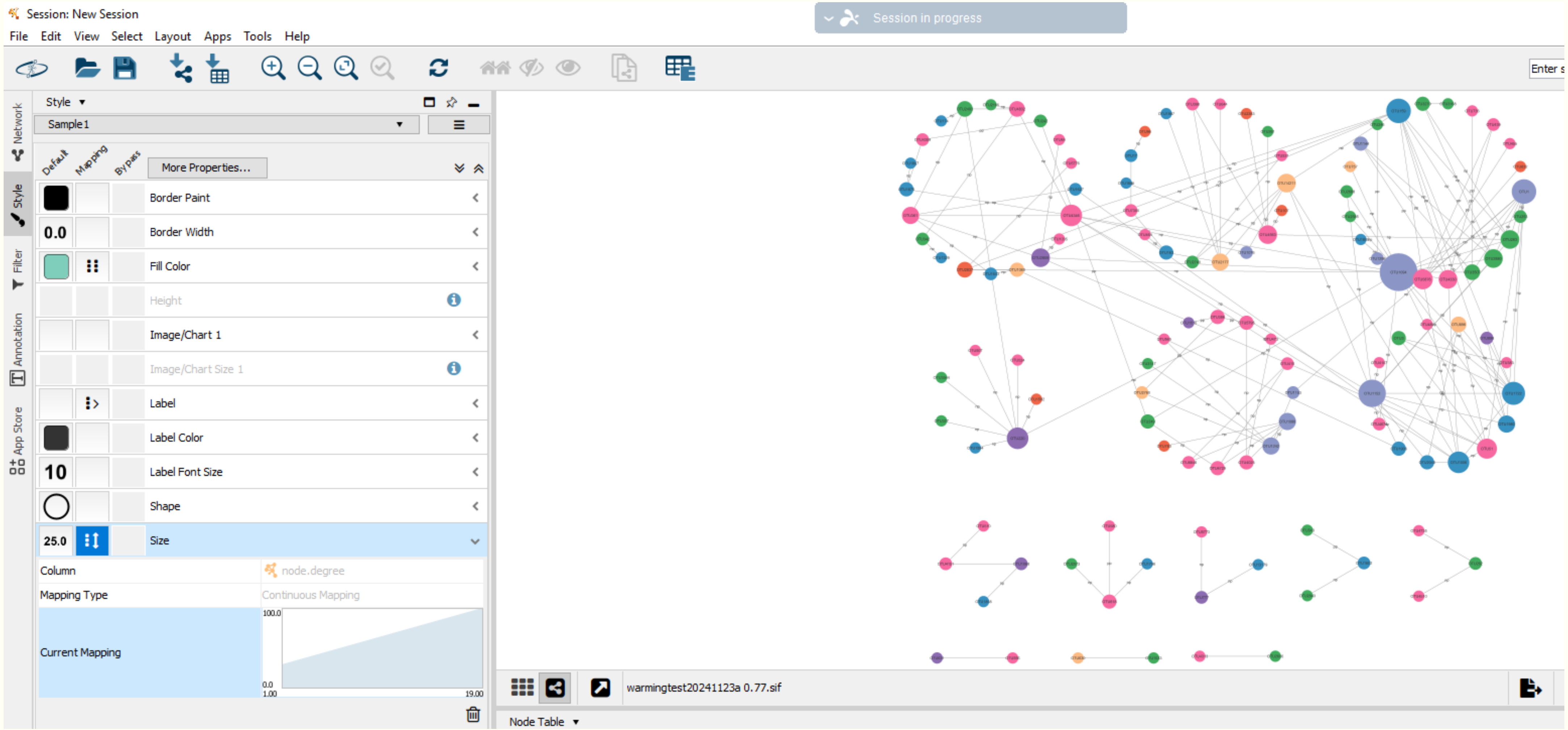


⑧
⑨

Double click

Use Cytoscape: Node size

Node size setting result



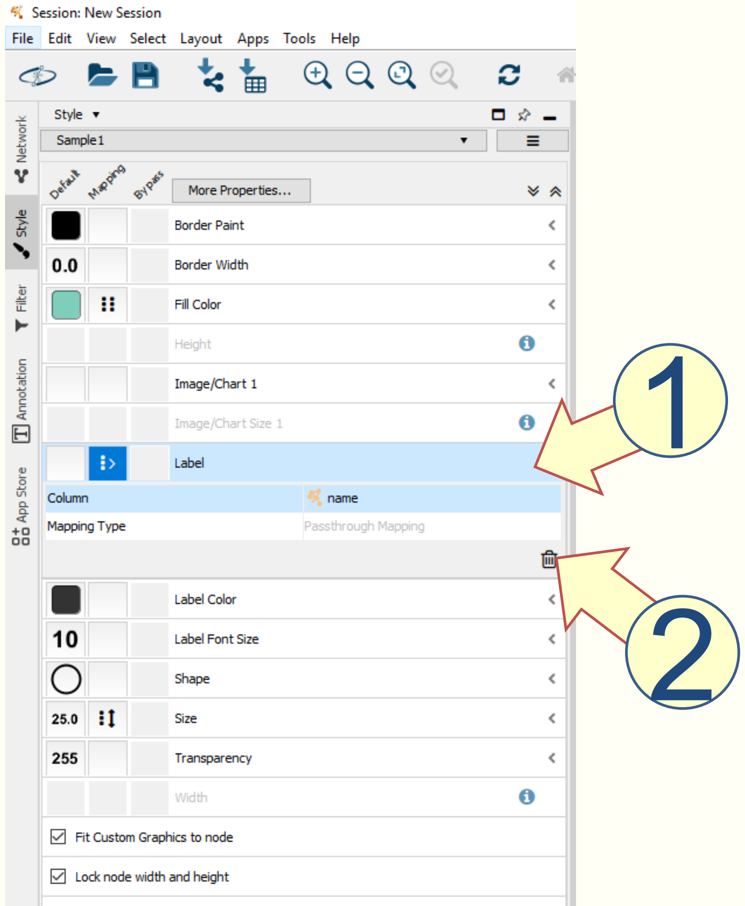
Default	Mapping	Property
■		Border Paint
0.0		Border Width
■	⠿	Fill Color
		Height
		Image/Chart 1
		Image/Chart Size 1
	⠿>	Label
■		Label Color
10		Label Font Size
○		Shape
25.0	⠿	Size

Column: node.degree
Mapping Type: Continuous Mapping
Current Mapping: 100.0, 0.0, 1.00, 19.00

warmingtest20241123a 0.77.sif

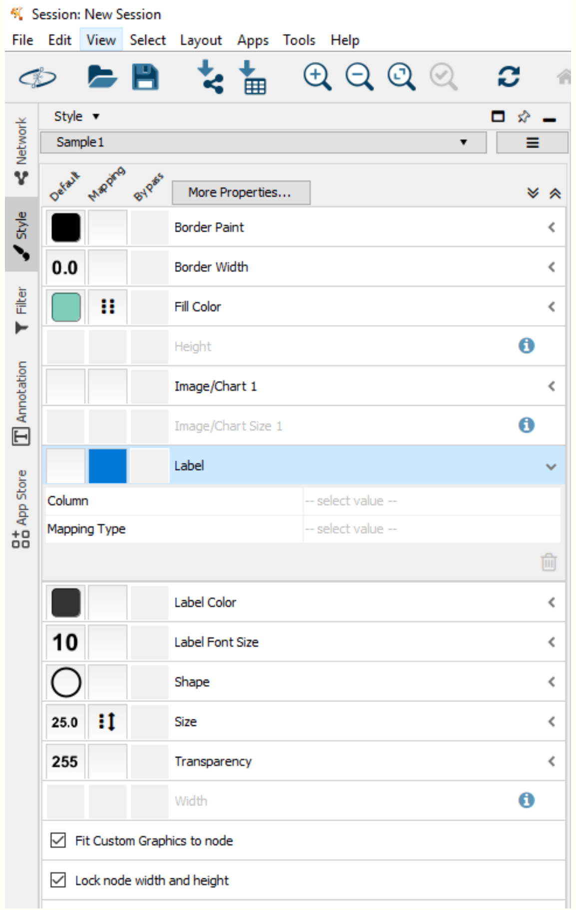
Use Cytoscape: Remove node labels

- Usually, the node labels are not necessary.



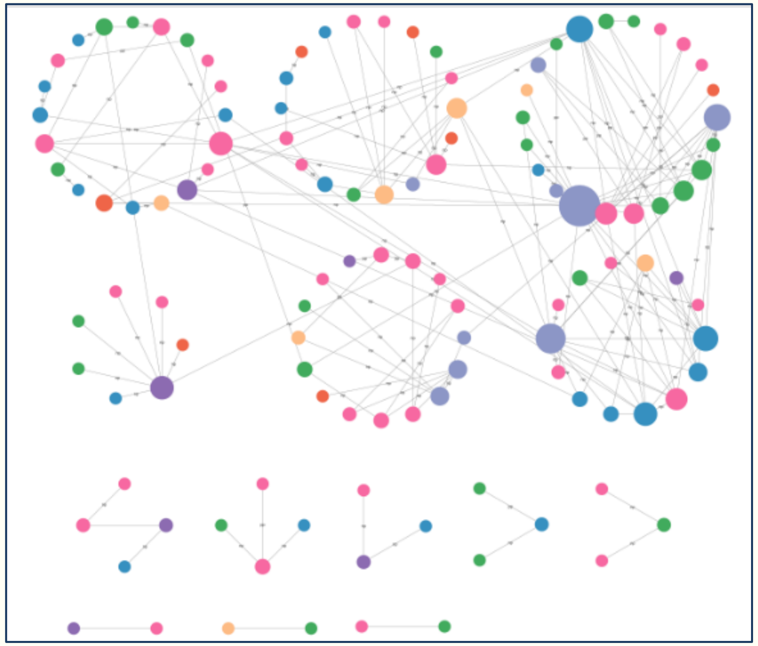
Style ▾
Sample1

0.0 Border Paint
Border Width
Fill Color
Height
Image/Chart 1
Image/Chart Size 1
Label
Column: name
Mapping Type: Passthrough Mapping
Label Color
10 Label Font Size
Shape
25.0 Size
255 Transparency
Width
☑ Fit Custom Graphics to node
☑ Lock node width and height



Style ▾
Sample1

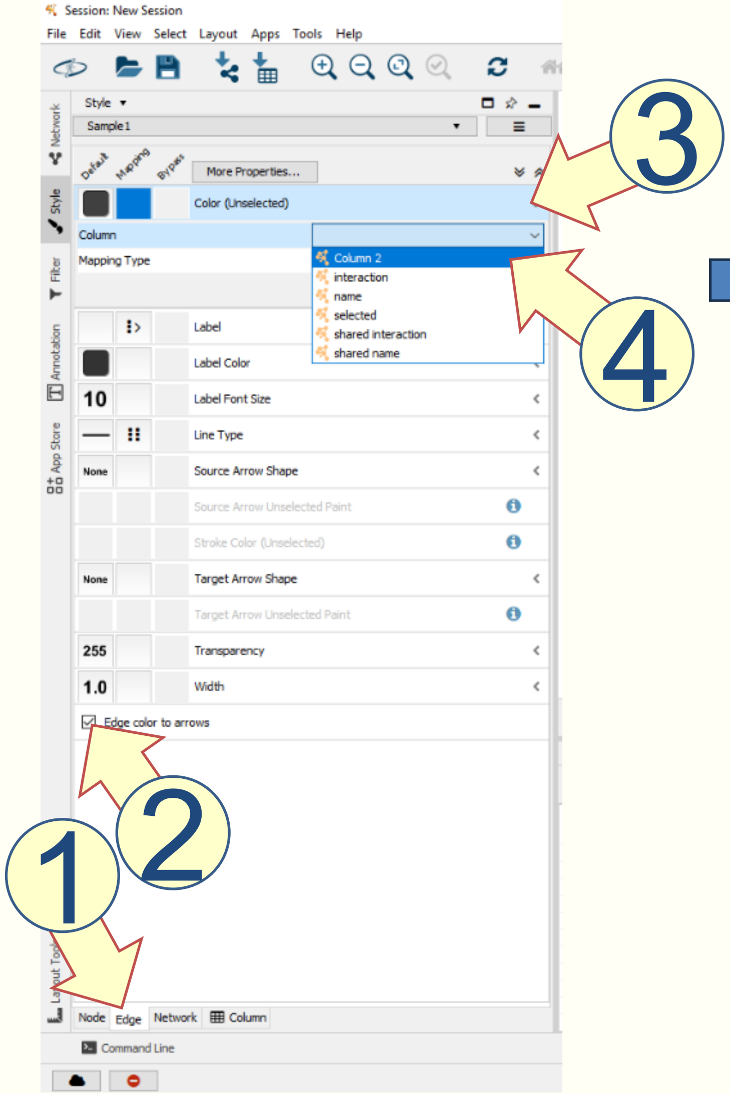
Border Paint
0.0 Border Width
Fill Color
Height
Image/Chart 1
Image/Chart Size 1
Label
Column: -- select value --
Mapping Type: -- select value --
Label Color
10 Label Font Size
Shape
25.0 Size
255 Transparency
Width
☑ Fit Custom Graphics to node
☑ Lock node width and height



Back to Outline

Use Cytoscape: Edge color

- You may explore edge color by other attributes.
- Here the example shows edge color by positive vs negative correlation.



Style: Sample1

Color (Unselected)

Column: Column 2

- Column 2
- interaction
- name
- selected
- shared interaction
- shared name

Mapping Type

Label Font Size: 10

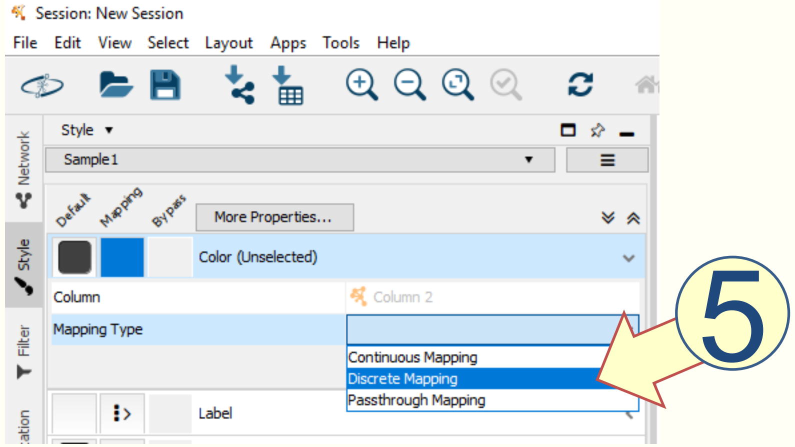
Source Arrow Shape: None

Target Arrow Shape: None

Transparency: 255

Width: 1.0

☑ Edge color to arrows



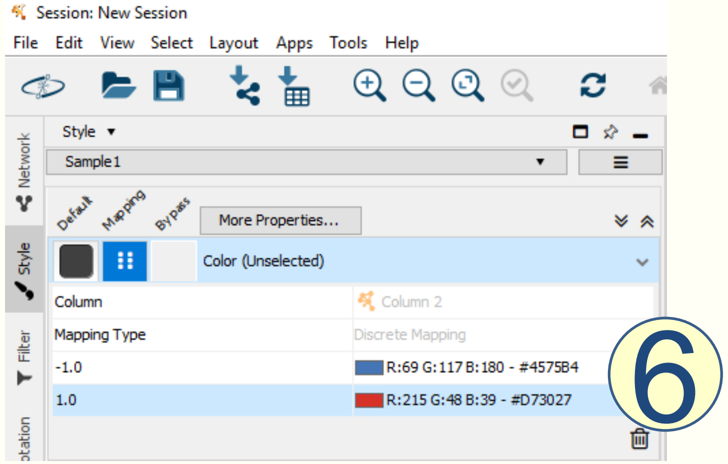
Style: Sample1

Color (Unselected)

Column: Column 2

Mapping Type

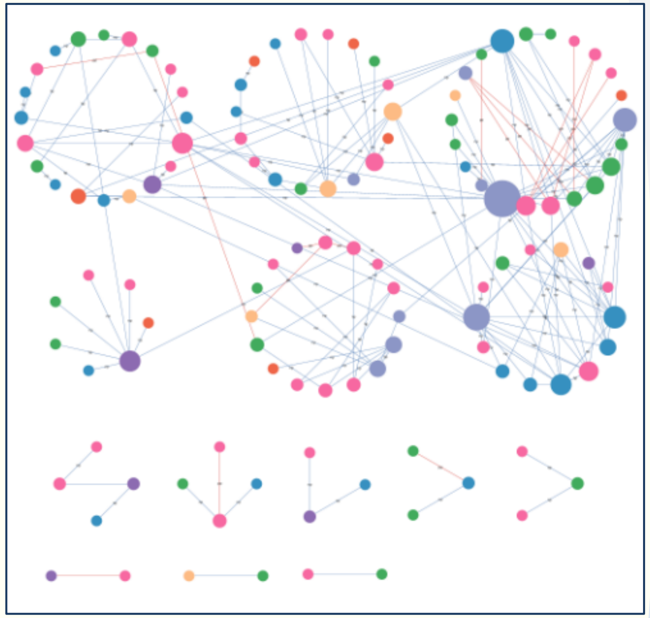
- Continuous Mapping
- Discrete Mapping
- Passthrough Mapping



Style: Sample1

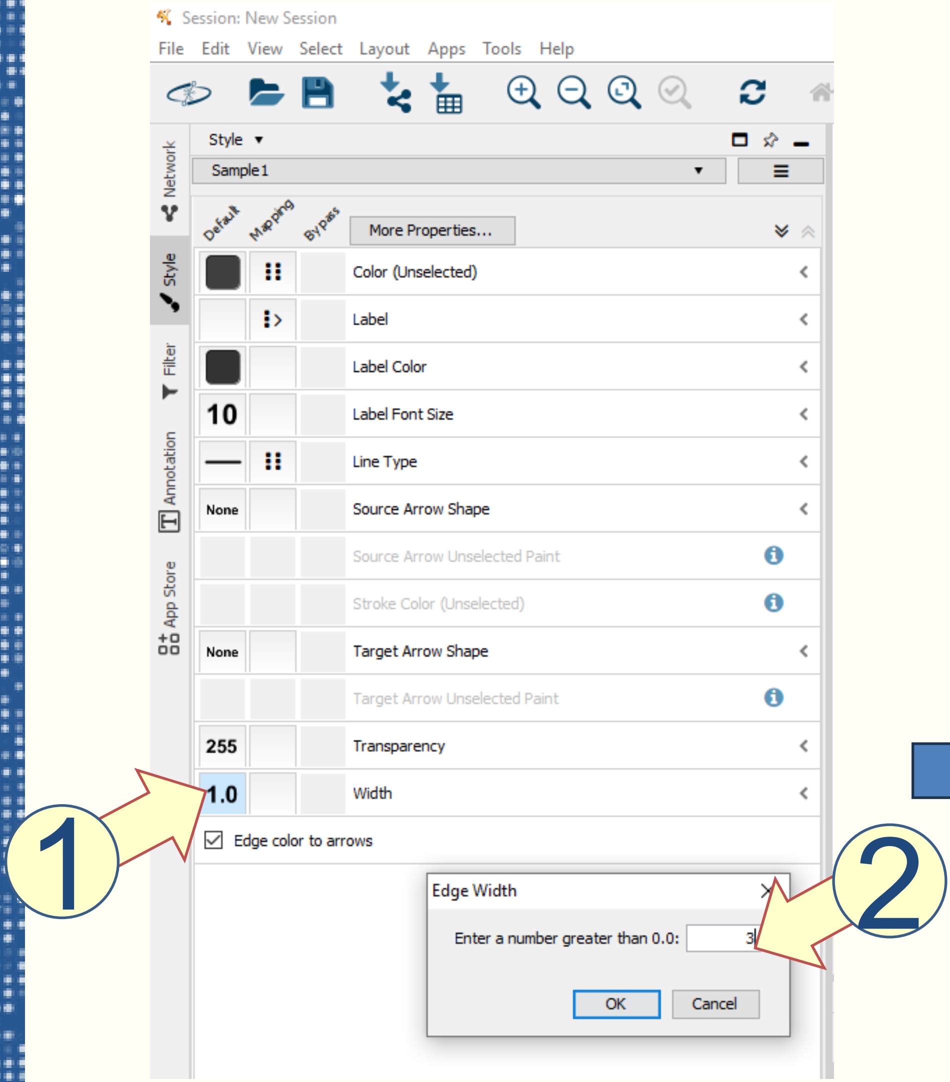
Color (Unselected)

Column	Column 2
Mapping Type	Discrete Mapping
-1.0	R:69 G:117 B:180 - #4575B4
1.0	R:215 G:48 B:39 - #D73027



Back to Outline

Use Cytoscape: Edge size



Sample1

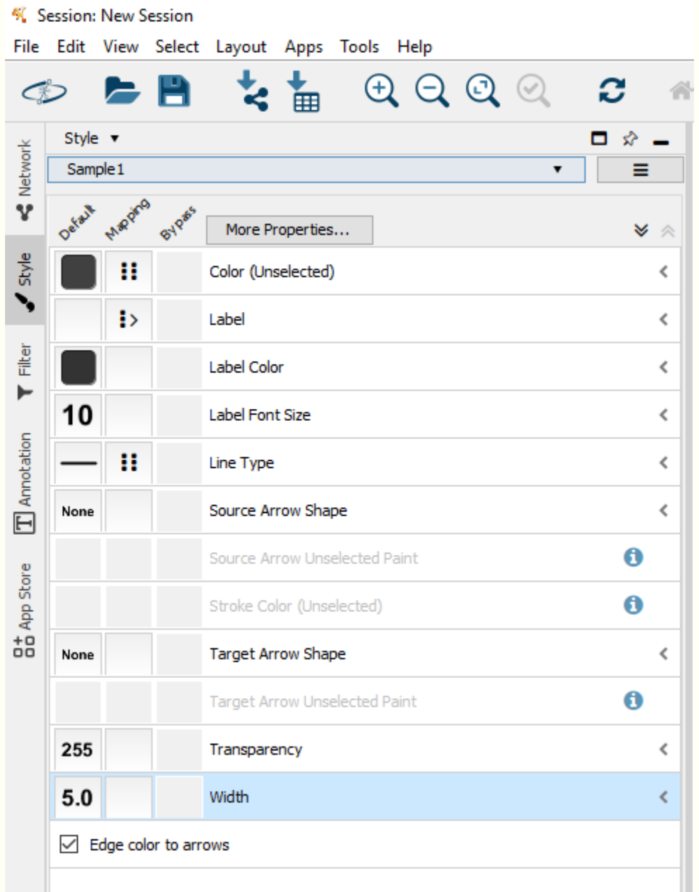
Default	Property
	Color (Unselected)
	Label
	Label Color
10	Label Font Size
	Line Type
None	Source Arrow Shape
	Source Arrow Unselected Paint
	Stroke Color (Unselected)
None	Target Arrow Shape
	Target Arrow Unselected Paint
255	Transparency
1.0	Width

☑ Edge color to arrows

Edge Width

Enter a number greater than 0.0: 3

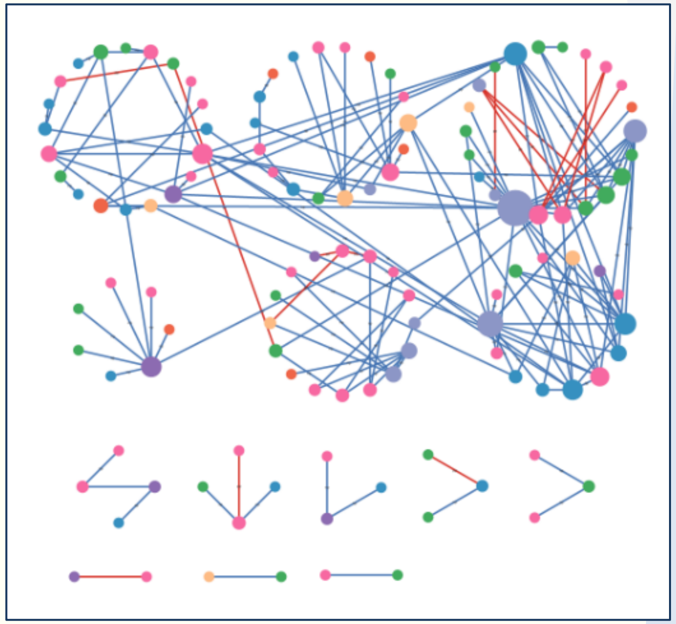
OK Cancel



Sample1

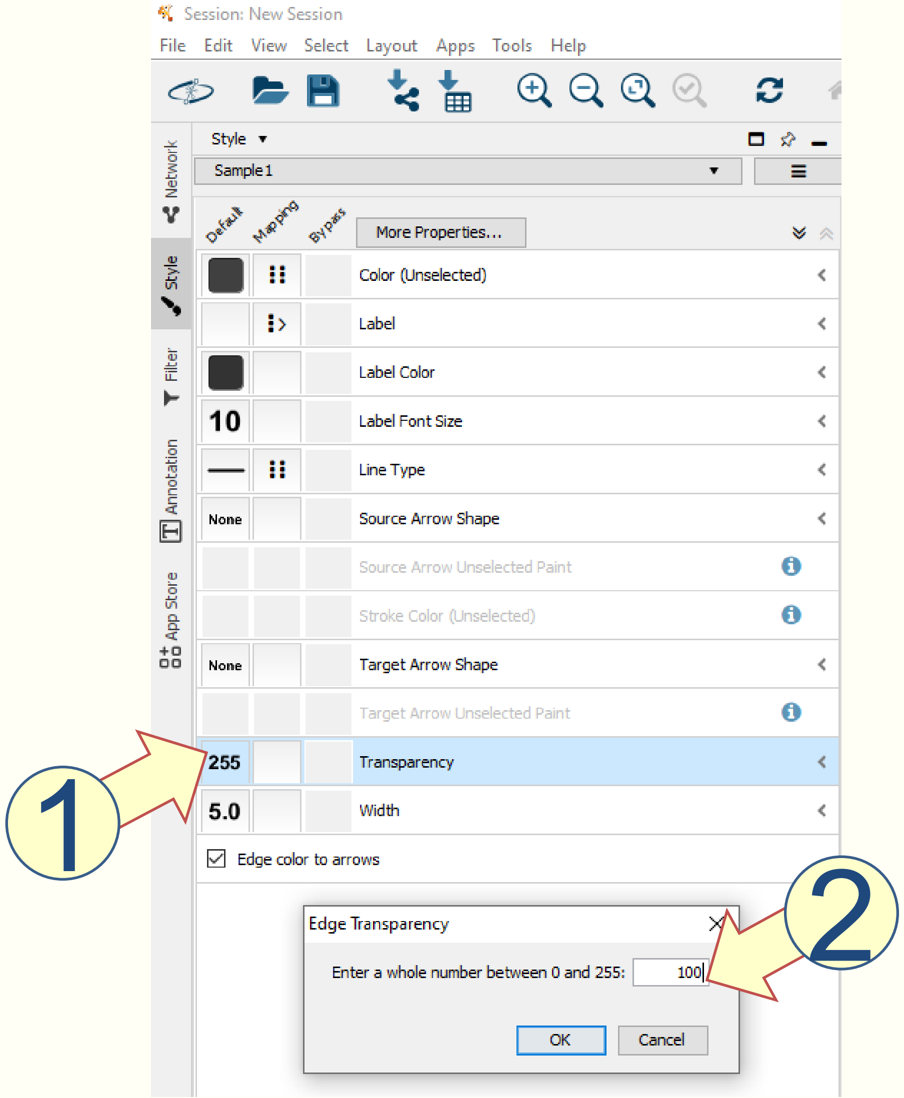
Default	Property
	Color (Unselected)
	Label
	Label Color
10	Label Font Size
	Line Type
None	Source Arrow Shape
	Source Arrow Unselected Paint
	Stroke Color (Unselected)
None	Target Arrow Shape
	Target Arrow Unselected Paint
255	Transparency
5.0	Width

☑ Edge color to arrows



Use Cytoscape: Edge transparency

- If you want to make the edge color lighter, you can decrease the value for transparency



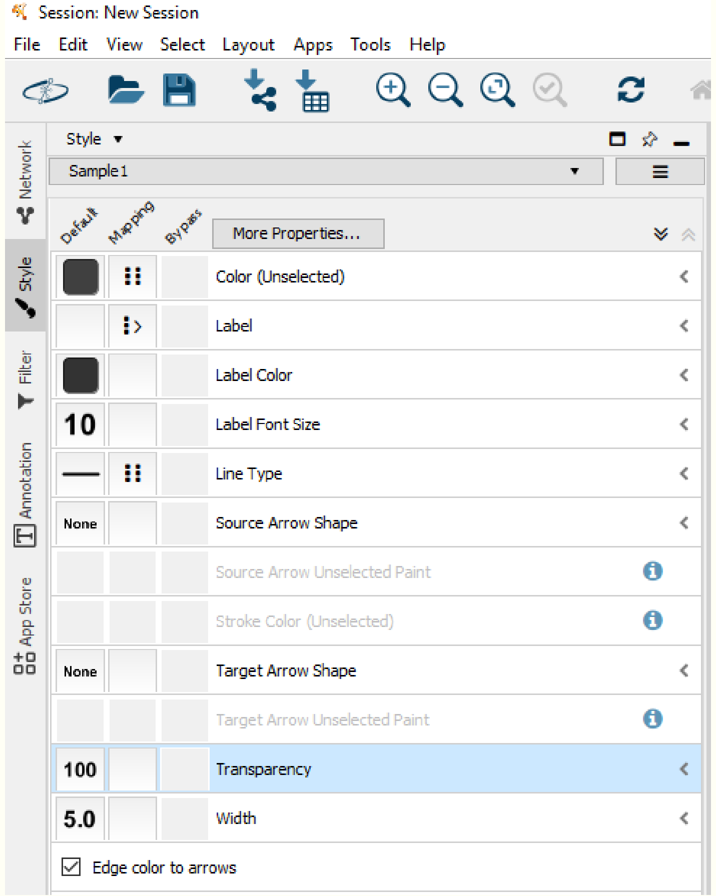
Style: Sample1

Default	Property
■	Color (Unselected)
	Label
■	Label Color
10	Label Font Size
—	Line Type
None	Source Arrow Shape
	Source Arrow Unselected Paint
	Stroke Color (Unselected)
None	Target Arrow Shape
	Target Arrow Unselected Paint
255	Transparency
5.0	Width

☑ Edge color to arrows

Edge Transparency

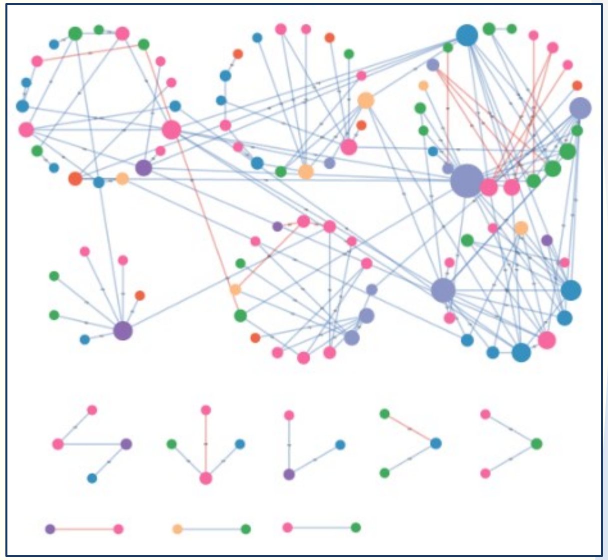
Enter a whole number between 0 and 255: 100



Style: Sample1

Default	Property
■	Color (Unselected)
	Label
■	Label Color
10	Label Font Size
—	Line Type
None	Source Arrow Shape
	Source Arrow Unselected Paint
	Stroke Color (Unselected)
None	Target Arrow Shape
	Target Arrow Unselected Paint
100	Transparency
5.0	Width

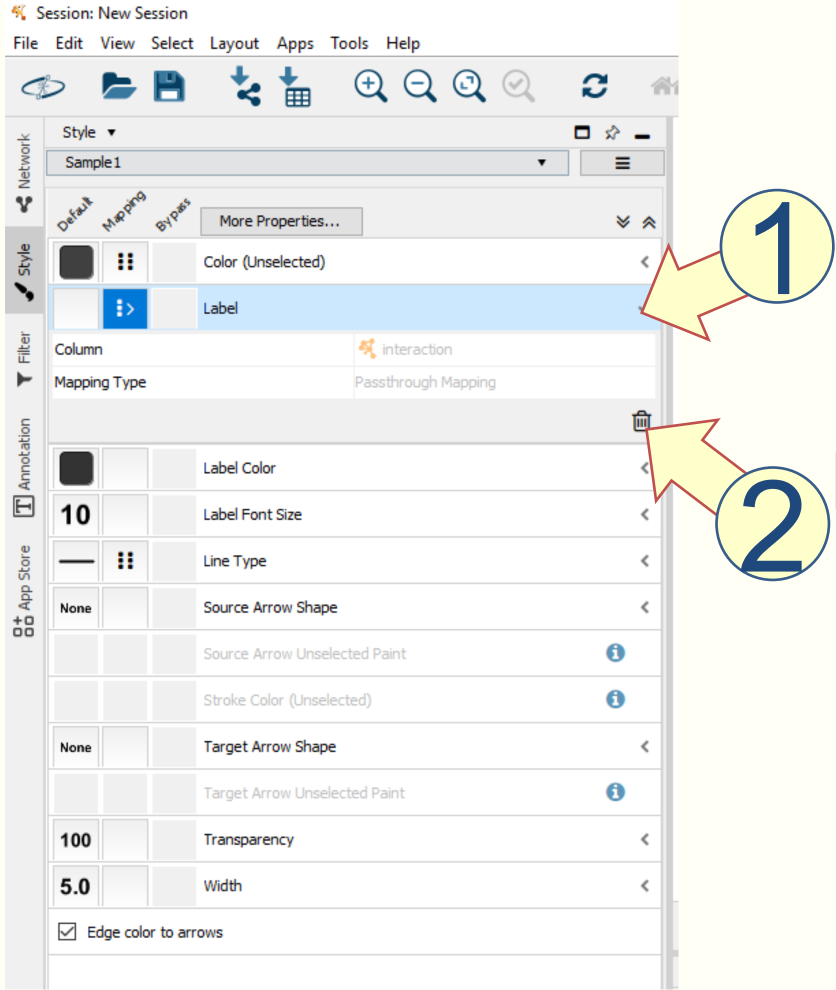
☑ Edge color to arrows



Back to Outline

Use Cytoscape: Remove edge label

- Usually, the edge labels are not necessary.



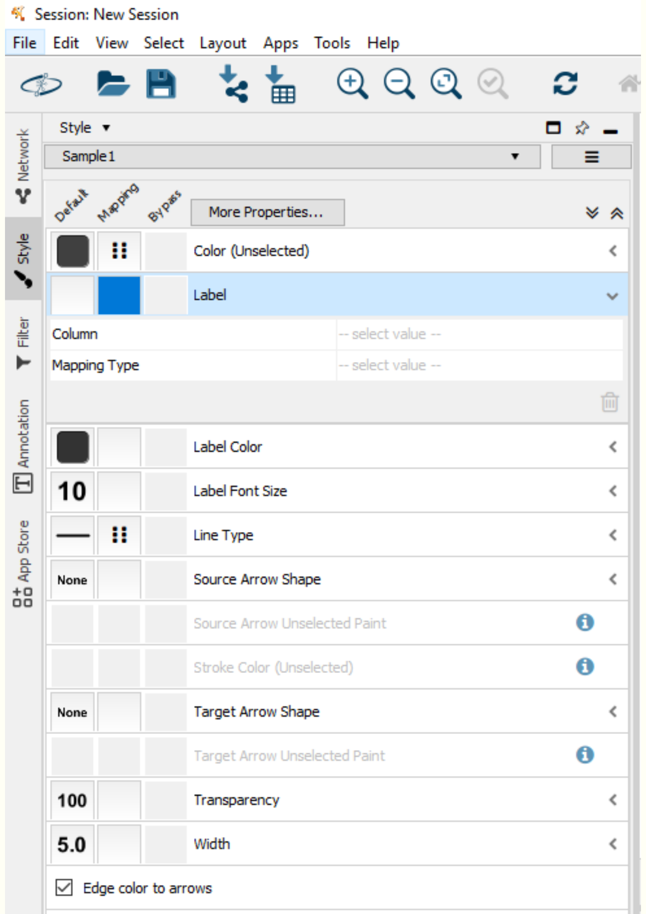
Sample1

Default	Mapping	Bypass	Property
			Color (Unselected)
			Label

Column: interaction
Mapping Type: Passthrough Mapping

Default	Mapping	Bypass	Property
			Label Color
10			Label Font Size
—			Line Type
None			Source Arrow Shape
			Source Arrow Unselected Paint
			Stroke Color (Unselected)
None			Target Arrow Shape
			Target Arrow Unselected Paint
100			Transparency
5.0			Width

☑ Edge color to arrows



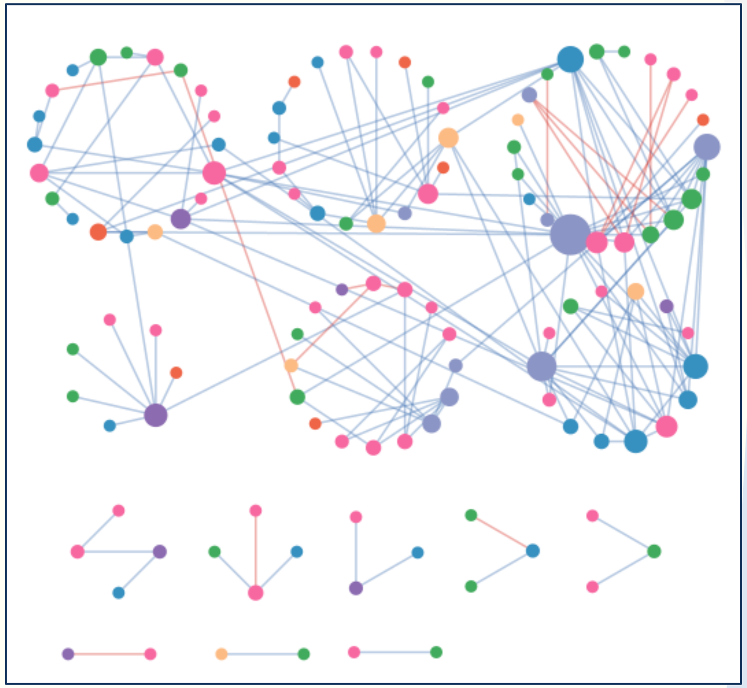
Sample1

Default	Mapping	Bypass	Property
			Color (Unselected)
			Label

Column: -- select value --
Mapping Type: -- select value --

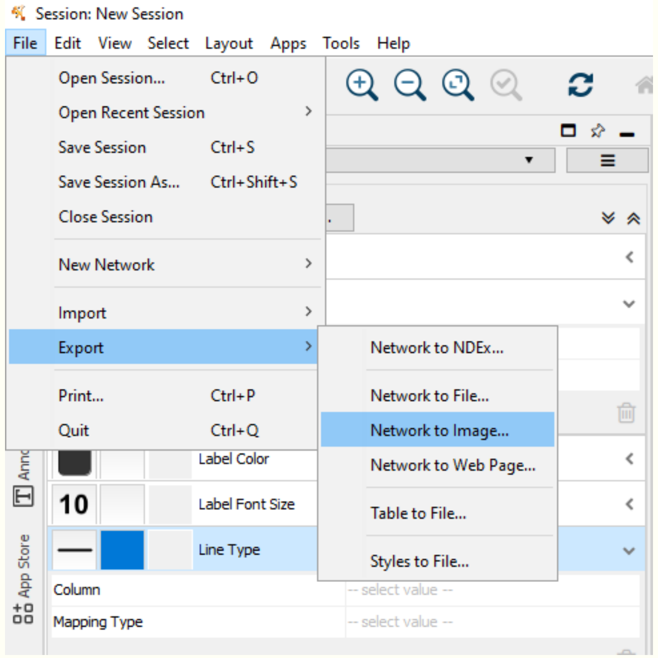
Default	Mapping	Bypass	Property
			Label Color
10			Label Font Size
—			Line Type
None			Source Arrow Shape
			Source Arrow Unselected Paint
			Stroke Color (Unselected)
None			Target Arrow Shape
			Target Arrow Unselected Paint
100			Transparency
5.0			Width

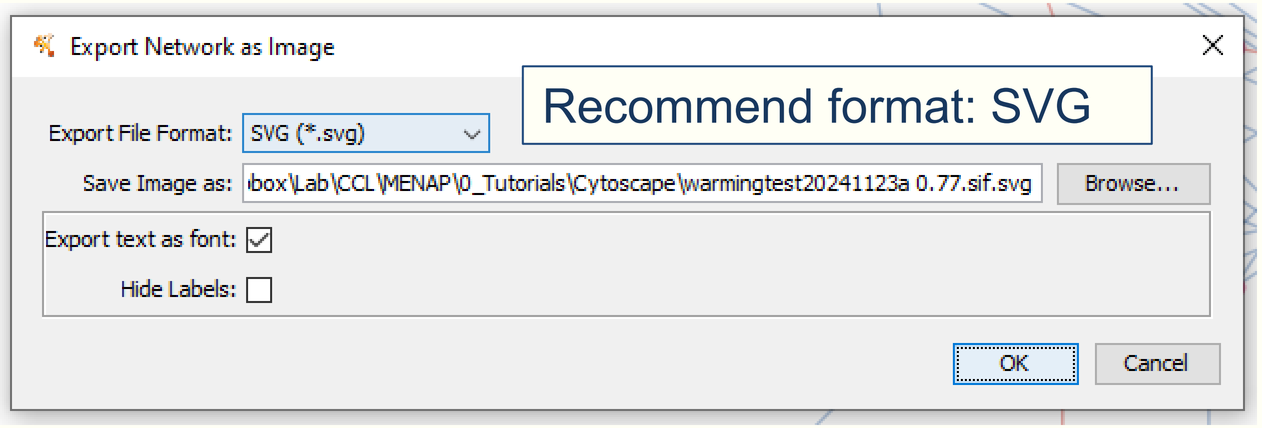
☑ Edge color to arrows



Back to Outline

Use Cytoscape: Export image




Export Network as Image

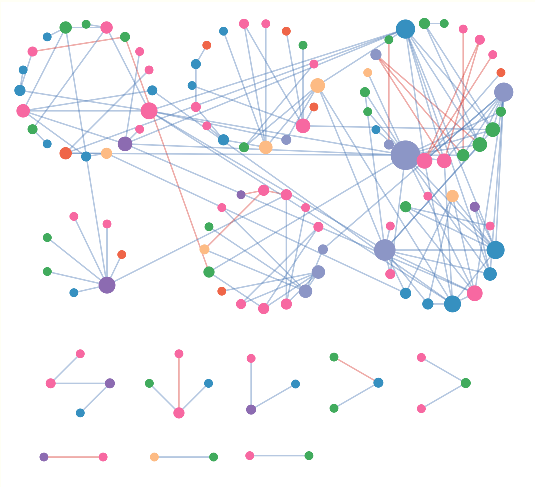
Recommend format: SVG

Export File Format: SVG (*.svg)

Save Image as: ıbox\Lab\CCL\MENAP\0_Tutorials\Cytoscape\warmingtest20241123a 0.77.sif.svg

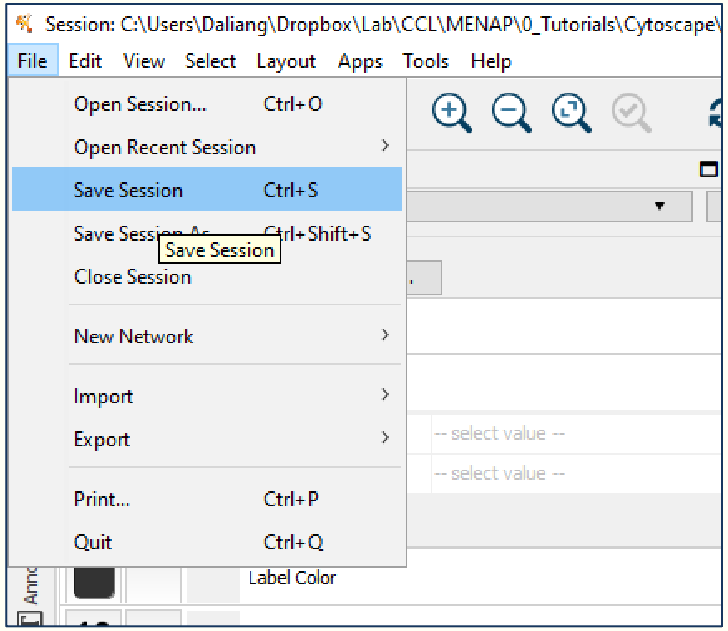
Export text as font: ☑

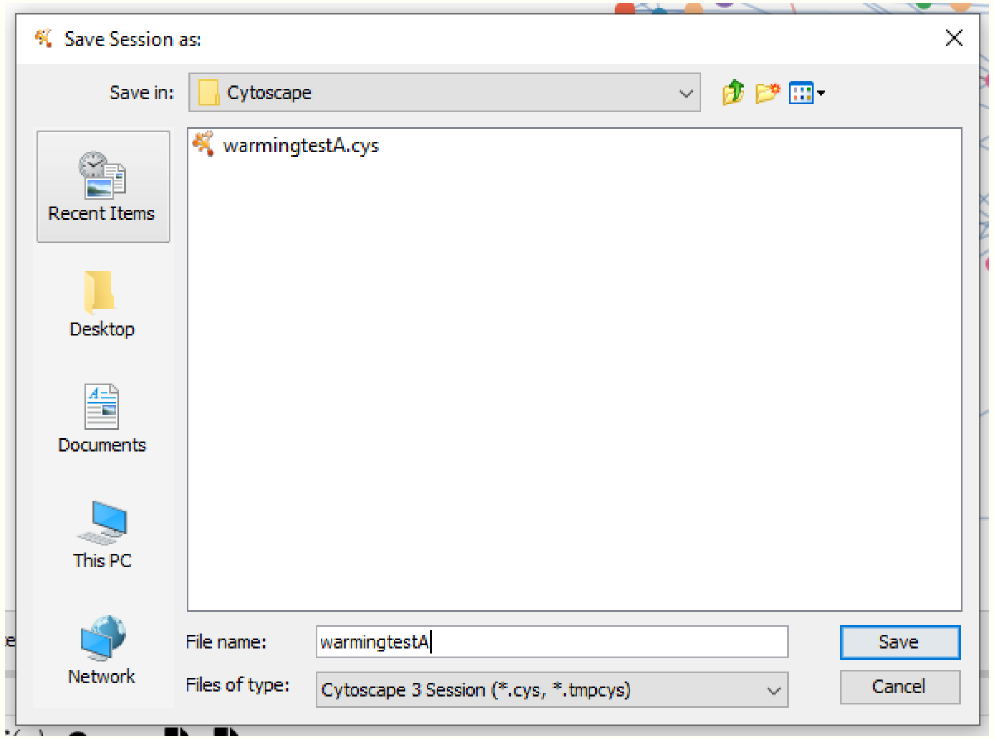
Hide Labels: ☐



Back to Outline

Use Cytoscape: Save session





Save in: Cytoscape

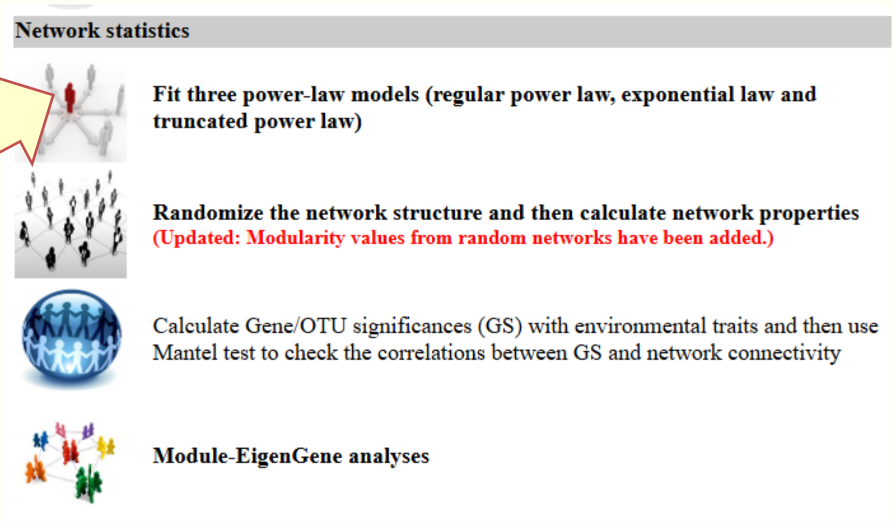
File name: warmingtestA

Files of type: Cytoscape 3 Session (*.cys, *.tmpcys)

Back to Outline

Fit three power-law models

1



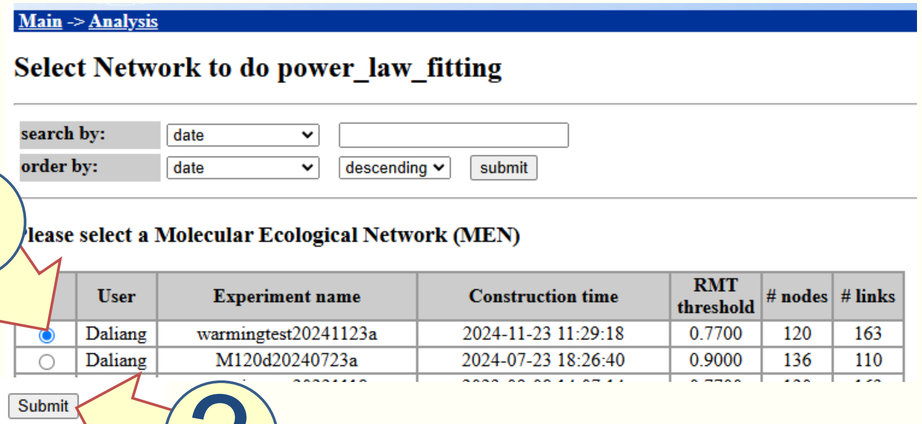
Network statistics

Fit three power-law models (regular power law, exponential law and truncated power law)

Randomize the network structure and then calculate network properties
(Updated: Modularity values from random networks have been added.)

Calculate Gene/OTU significances (GS) with environmental traits and then use Mantel test to check the correlations between GS and network connectivity

Module-EigenGene analyses



Main -> Analysis

Select Network to do power_law_fitting

search by: date

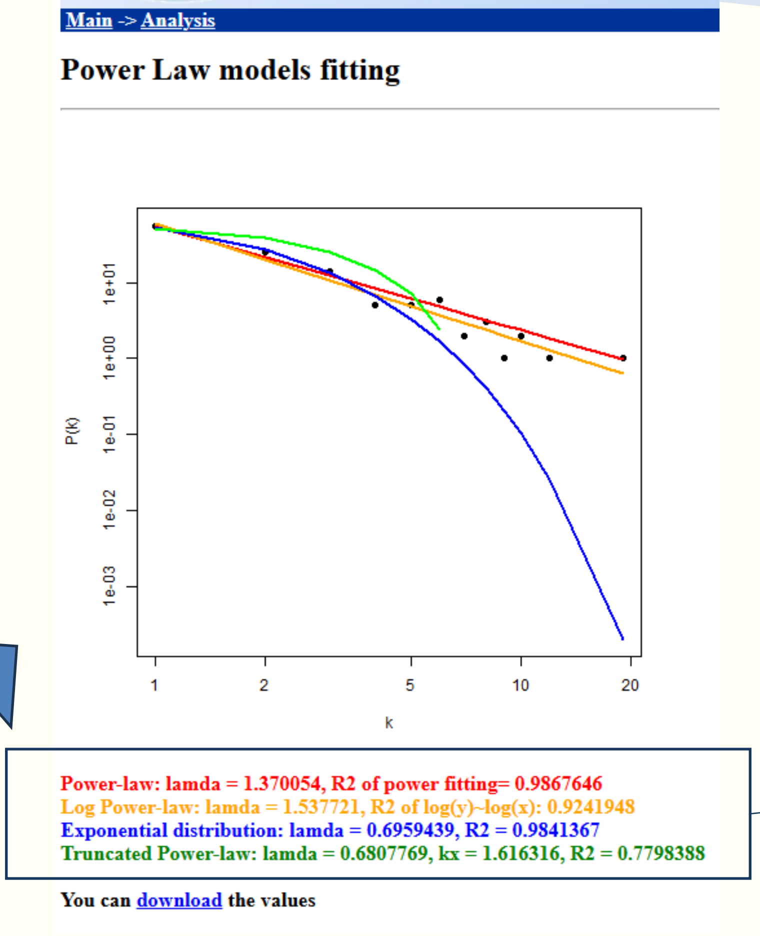
order by: date descending

Please select a Molecular Ecological Network (MEN)

	User	Experiment name	Construction time	RMT threshold	# nodes	# links
●	Daliang	warmingtest20241123a	2024-11-23 11:29:18	0.7700	120	163
○	Daliang	M120d20240723a	2024-07-23 18:26:40	0.9000	136	110

2

3



Main -> Analysis

Power Law models fitting

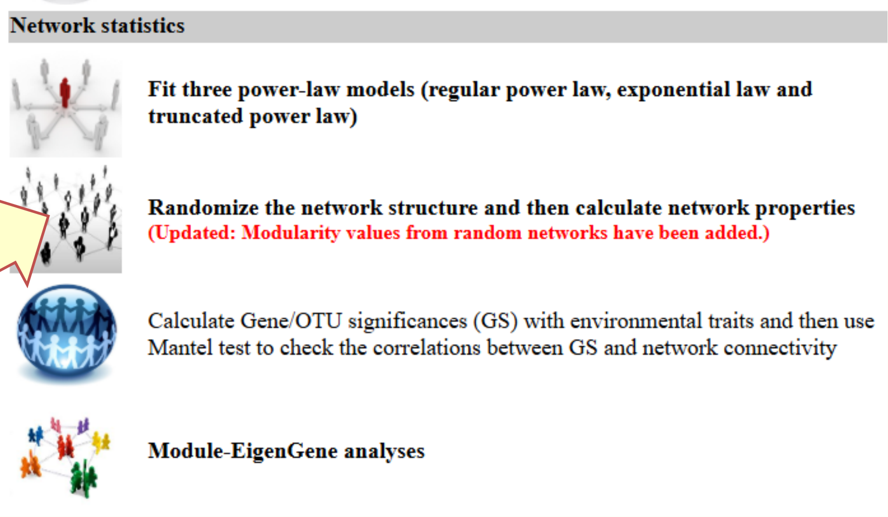
Power-law: lamda = 1.370054, R2 of power fitting= 0.9867646
Log Power-law: lamda = 1.537721, R2 of log(y)~log(x): 0.9241948
Exponential distribution: lamda = 0.6959439, R2 = 0.9841367
Truncated Power-law: lamda = 0.6807769, kx = 1.616316, R2 = 0.7798388

You can download the values

Copy paste this part for your record.

Right-click '*download*' and 'Save link as …' to download the table.

Randomize the network



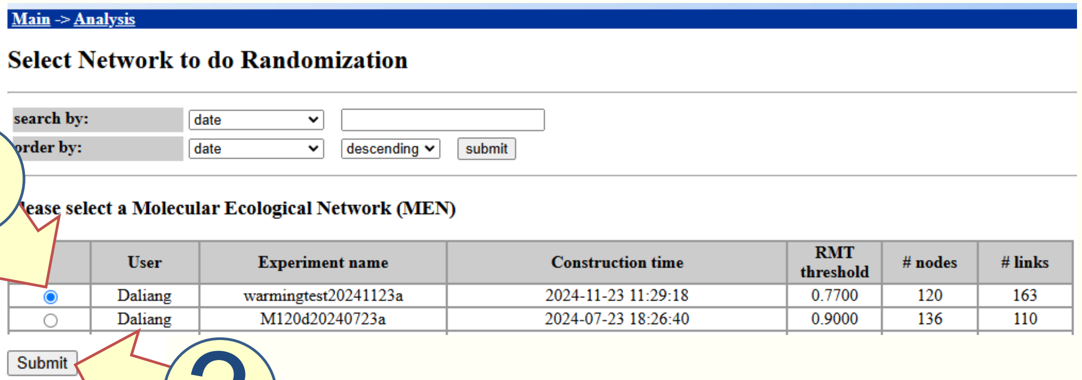
Network statistics

Fit three power-law models (regular power law, exponential law and truncated power law)

Randomize the network structure and then calculate network properties
(Updated: Modularity values from random networks have been added.)

Calculate Gene/OTU significances (GS) with environmental traits and then use Mantel test to check the correlations between GS and network connectivity

Module-EigenGene analyses



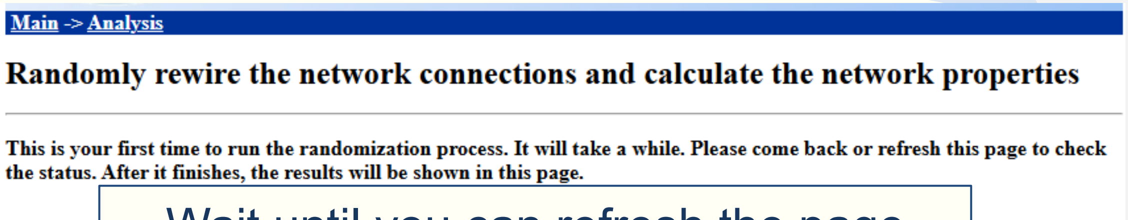
Main -> Analysis

Select Network to do Randomization

search by: date
order by: date descending submit

lease select a Molecular Ecological Network (MEN)

	User	Experiment name	Construction time	RMT threshold	# nodes	# links
●	Daliang	warmingtest20241123a	2024-11-23 11:29:18	0.7700	120	163
○	Daliang	M120d20240723a	2024-07-23 18:26:40	0.9000	136	110

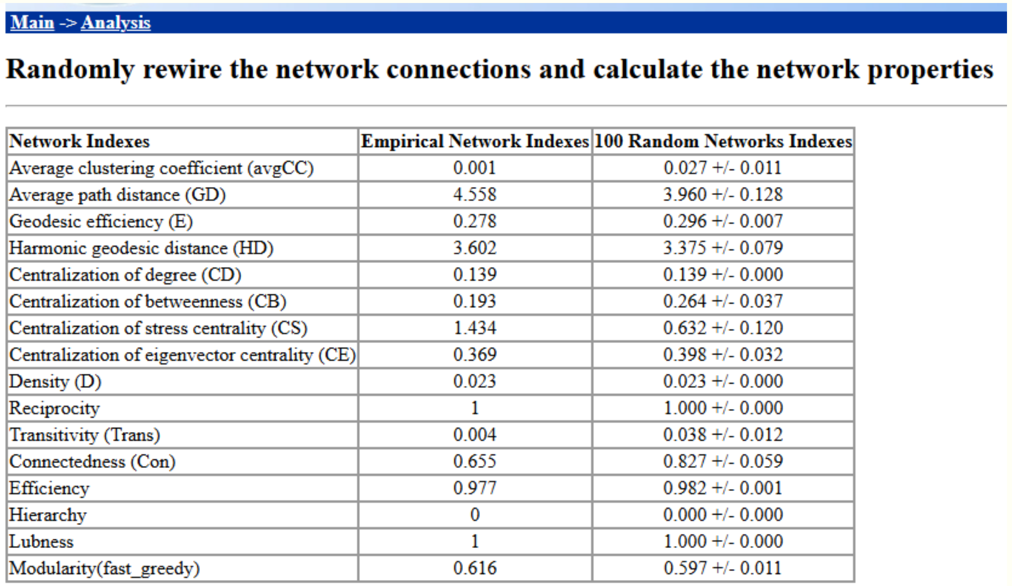


Main -> Analysis

Randomly rewire the network connections and calculate the network properties

This is your first time to run the randomization process. It will take a while. Please come back or refresh this page to check the status. After it finishes, the results will be shown in this page.

Wait until you can refresh the page



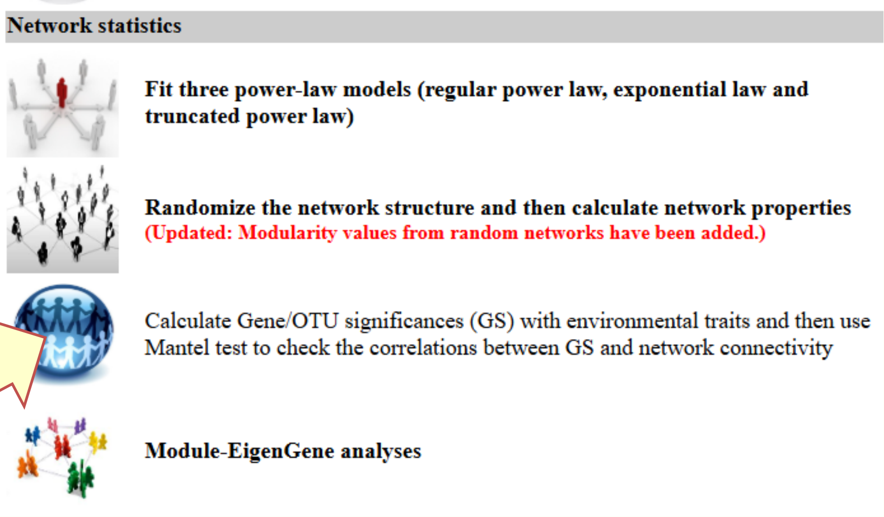
Main -> Analysis

Randomly rewire the network connections and calculate the network properties

Network Indexes	Empirical Network Indexes	100 Random Networks Indexes
Average clustering coefficient (avgCC)	0.001	0.027 +/- 0.011
Average path distance (GD)	4.558	3.960 +/- 0.128
Geodesic efficiency (E)	0.278	0.296 +/- 0.007
Harmonic geodesic distance (HD)	3.602	3.375 +/- 0.079
Centralization of degree (CD)	0.139	0.139 +/- 0.000
Centralization of betweenness (CB)	0.193	0.264 +/- 0.037
Centralization of stress centrality (CS)	1.434	0.632 +/- 0.120
Centralization of eigenvector centrality (CE)	0.369	0.398 +/- 0.032
Density (D)	0.023	0.023 +/- 0.000
Reciprocity	1	1.000 +/- 0.000
Transitivity (Trans)	0.004	0.038 +/- 0.012
Connectedness (Con)	0.655	0.827 +/- 0.059
Efficiency	0.977	0.982 +/- 0.001
Hierarchy	0	0.000 +/- 0.000
Lubness	1	1.000 +/- 0.000
Modularity(fast_greedy)	0.616	0.597 +/- 0.011

Copy paste the table to a spreadsheet

Relationship with environmental traits (1)



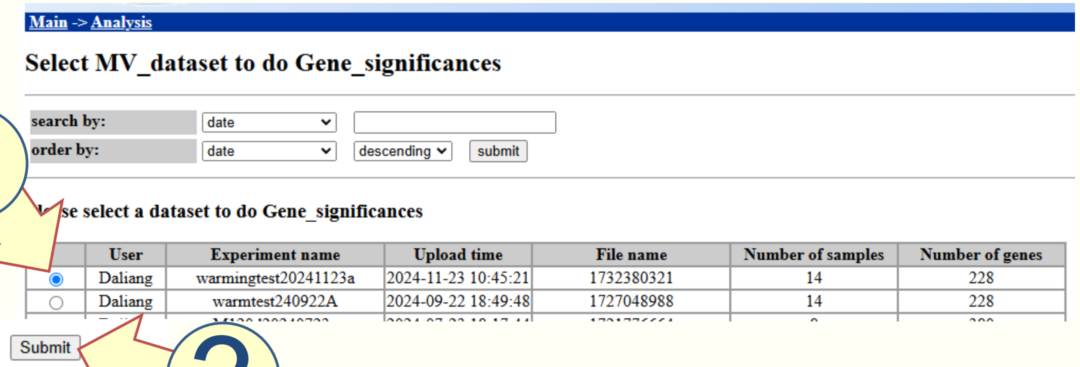
Network statistics

Fit three power-law models (regular power law, exponential law and truncated power law)

Randomize the network structure and then calculate network properties
(Updated: Modularity values from random networks have been added.)

Calculate Gene/OTU significances (GS) with environmental traits and then use Mantel test to check the correlations between GS and network connectivity

Module-EigenGene analyses



Main -> Analysis

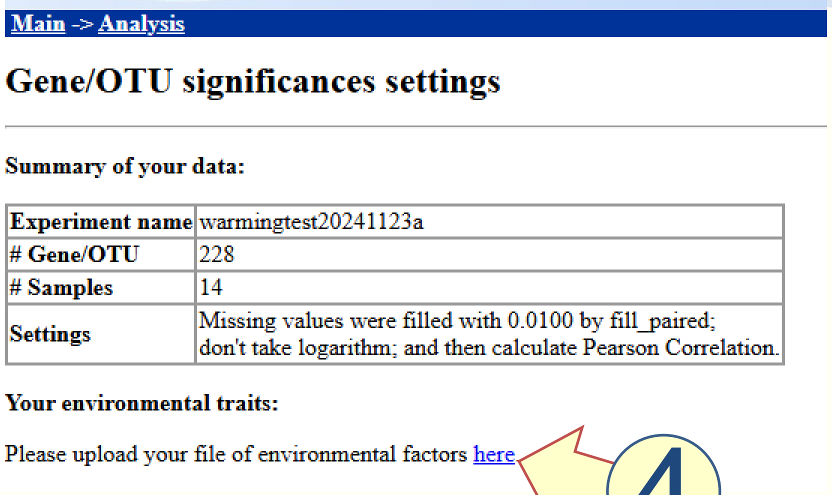
Select MV_dataset to do Gene_significances

search by: date
order by: date descending submit

se select a dataset to do Gene_significances

	User	Experiment name	Upload time	File name	Number of samples	Number of genes
●	Daliang	warmingtest20241123a	2024-11-23 10:45:21	1732380321	14	228
○	Daliang	warmtest240922A	2024-09-22 18:49:48	1727048988	14	228

Submit



Main -> Analysis

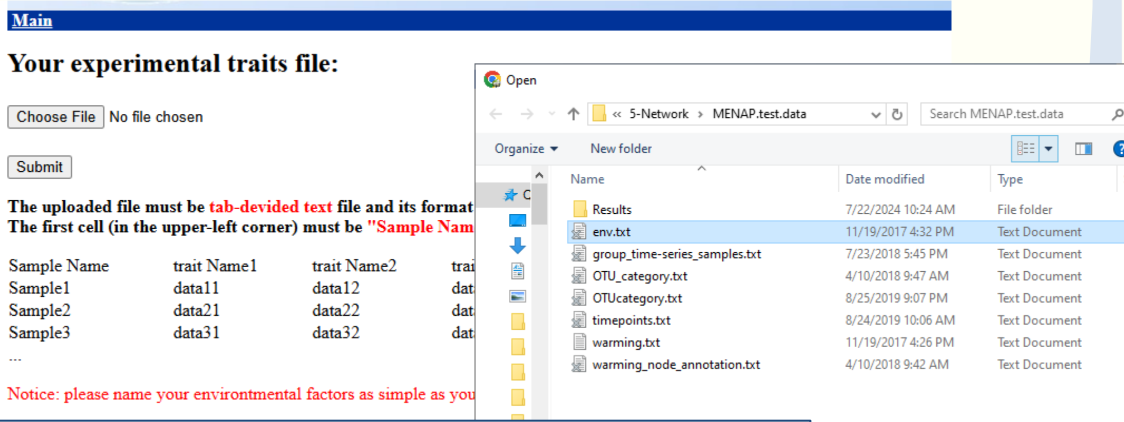
Gene/OTU significances settings

Summary of your data:

Experiment name	warmingtest20241123a
# Gene/OTU	228
# Samples	14
Settings	Missing values were filled with 0.0100 by fill_paired; don't take logarithm; and then calculate Pearson Correlation.

Your environmental traits:

Please upload your file of environmental factors here



Main

Your experimental traits file:

Choose File No file chosen

Submit

The uploaded file must be tab-devided text file and its format
The first cell (in the upper-left corner) must be "Sample Nam

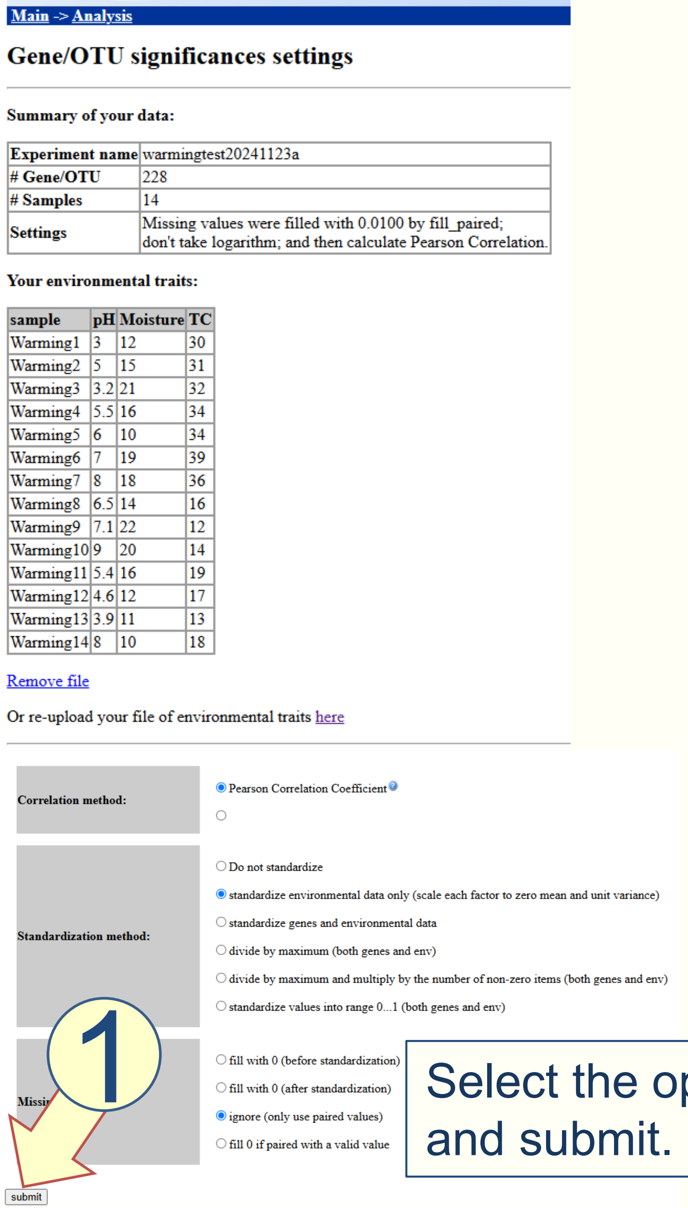
Sample Name	trait Name1	trait Name2	trai
Sample1	data11	data12	dat
Sample2	data21	data22	dat
Sample3	data31	data32	dat
...			

Notice: please name your environtmental factors as simple as you

Name	Date modified	Type
Results	7/22/2024 10:24 AM	File folder
env.txt	11/19/2017 4:32 PM	Text Document
group_time-series_samples.txt	7/23/2018 5:45 PM	Text Document
OTU_category.txt	4/10/2018 9:47 AM	Text Document
OTUcategory.txt	8/25/2019 9:07 PM	Text Document
timepoints.txt	8/24/2019 10:06 AM	Text Document
warming.txt	11/19/2017 4:26 PM	Text Document
warming_node_annotation.txt	4/10/2018 9:42 AM	Text Document

Prepare environmental factor table according to the format requirements, then upload.

Relationship with environmental traits (2)



Main -> Analysis

Gene/OTU significances settings

Summary of your data:

Experiment name	warmingtest20241123a
# Gene/OTU	228
# Samples	14
Settings	Missing values were filled with 0.0100 by fill_paired; don't take logarithm; and then calculate Pearson Correlation.

Your environmental traits:

sample	pH	Moisture	TC
Warming1	3	12	30
Warming2	5	15	31
Warming3	3.2	21	32
Warming4	5.5	16	34
Warming5	6	10	34
Warming6	7	19	39
Warming7	8	18	36
Warming8	6.5	14	16
Warming9	7.1	22	12
Warming10	9	20	14
Warming11	5.4	16	19
Warming12	4.6	12	17
Warming13	3.9	11	13
Warming14	8	10	18

Remove file

Or re-upload your file of environmental traits here

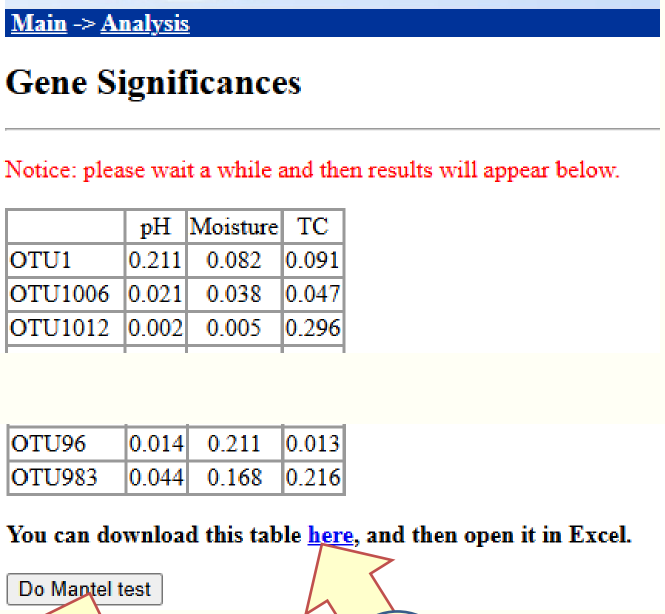
Correlation method:
- ● Pearson Correlation Coefficient
- ○

Standardization method:
- ○ Do not standardize
- ● standardize environmental data only (scale each factor to zero mean and unit variance)
- ○ standardize genes and environmental data
- ○ divide by maximum (both genes and env)
- ○ divide by maximum and multiply by the number of non-zero items (both genes and env)
- ○ standardize values into range 0...1 (both genes and env)

Missi
- ○ fill with 0 (before standardization)
- ○ fill with 0 (after standardization)
- ● ignore (only use paired values)
- ○ fill 0 if paired with a valid value

1

Select the options and submit.



Main -> Analysis

Gene Significances

Notice: please wait a while and then results will appear below.

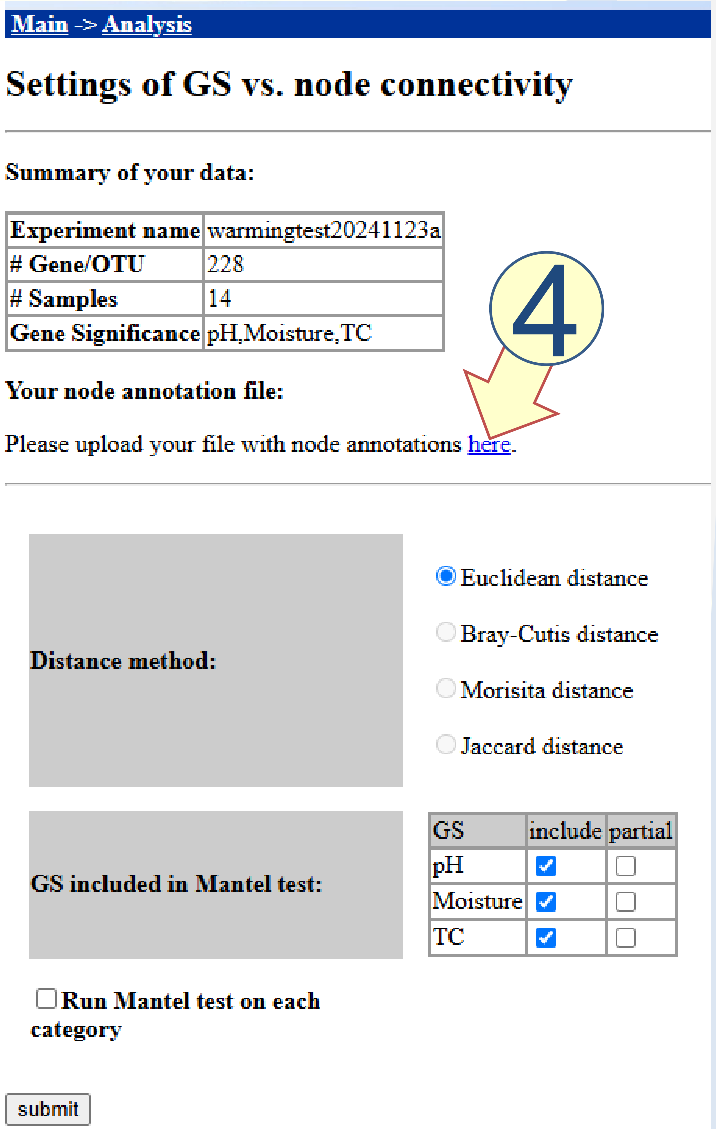
	pH	Moisture	TC
OTU1	0.211	0.082	0.091
OTU1006	0.021	0.038	0.047
OTU1012	0.002	0.005	0.296
OTU96	0.014	0.211	0.013
OTU983	0.044	0.168	0.216

You can download this table here, and then open it in Excel.

3

2

Right-click ‘*here*’ and ‘Save link as …’ to download the table.



Main -> Analysis

Settings of GS vs. node connectivity

Summary of your data:

Experiment name	warmingtest20241123a
# Gene/OTU	228
# Samples	14
Gene Significance	pH,Moisture,TC

Your node annotation file:

Please upload your file with node annotations here.

Distance method:
- ● Euclidean distance
- ○ Bray-Cutis distance
- ○ Morisita distance
- ○ Jaccard distance

GS included in Mantel test:

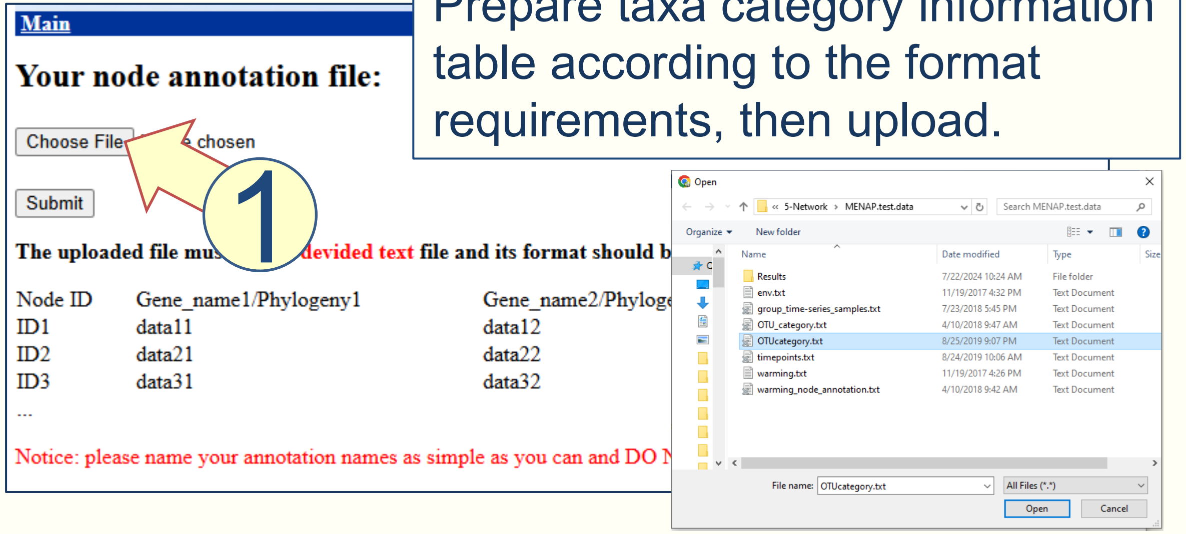
GS	include	partial
pH	☑	☐
Moisture	☑	☐
TC	☑	☐

☐ **Run Mantel test on each category**

4

Relationship with environmental traits (3)

Prepare taxa category information table according to the format requirements, then upload.



Main

Your node annotation file:

e chosen

The uploaded file mus devided text **file and its format should b**

Node ID	Gene_name1/Phylogeny1	Gene_name2/Phyloge
ID1	data11	data12
ID2	data21	data22
ID3	data31	data32
...		

Notice: please name your annotation names as simple as you can and DO N

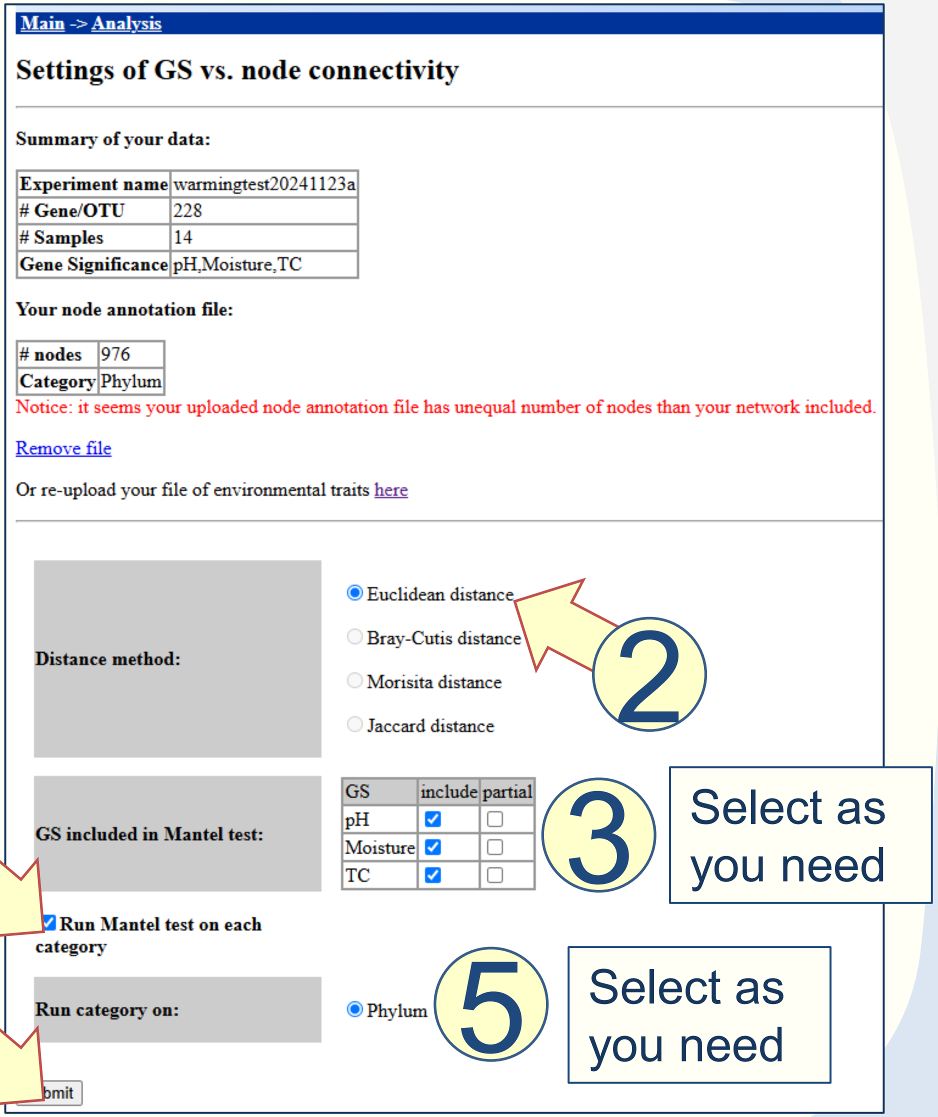
« 5-Network › MENAP.test.data

Search MENAP.test.data

Name	Date modified	Type	Size
Results	7/22/2024 10:24 AM	File folder	
env.txt	11/19/2017 4:32 PM	Text Document	
group_time-series_samples.txt	7/23/2018 5:45 PM	Text Document	
OTU_category.txt	4/10/2018 9:47 AM	Text Document	
OTUcategory.txt	8/25/2019 9:07 PM	Text Document	
timepoints.txt	8/24/2019 10:06 AM	Text Document	
warming.txt	11/19/2017 4:26 PM	Text Document	
warming_node_annotation.txt	4/10/2018 9:42 AM	Text Document	

File name: OTUcategory.txt

All Files (*.*)



Main -> Analysis

Settings of GS vs. node connectivity

Summary of your data:

Experiment name	warmingtest20241123a
# Gene/OTU	228
# Samples	14
Gene Significance	pH,Moisture,TC

Your node annotation file:

# nodes	976
Category	Phylum

Notice: it seems your uploaded node annotation file has unequal number of nodes than your network included.

Remove file

Or re-upload your file of environmental traits here

Distance method:
● Euclidean distance
○ Bray-Cutis distance
○ Morisita distance
○ Jaccard distance

GS included in Mantel test:

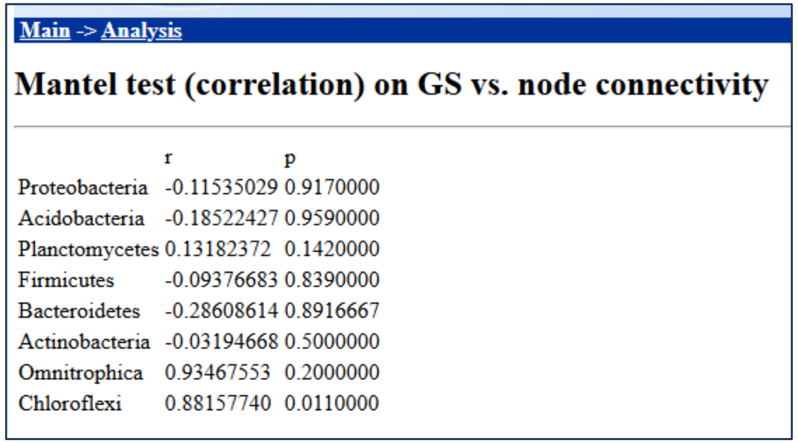
GS	include	partial
pH	☑	☐
Moisture	☑	☐
TC	☑	☐

Select as you need

☑ **Run Mantel test on each category**

Run category on: ● Phylum

Select as you need



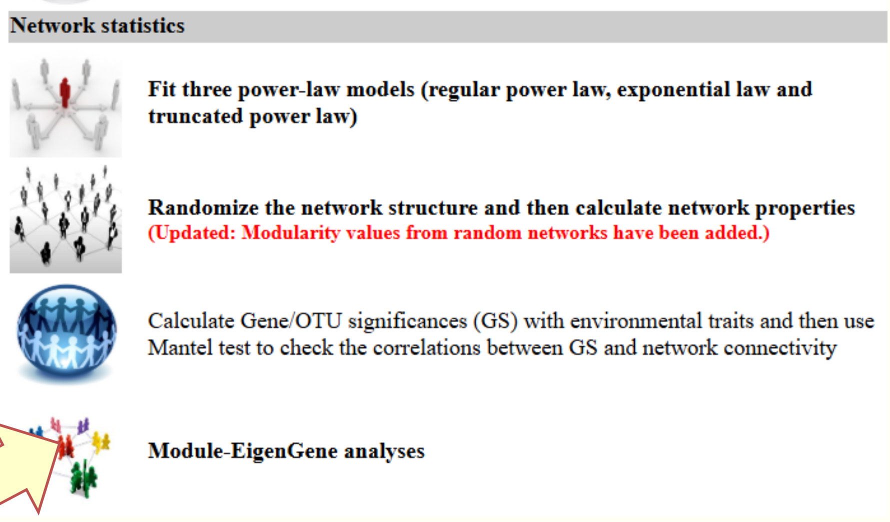
Main -> Analysis

Mantel test (correlation) on GS vs. node connectivity

	r	p
Proteobacteria	-0.11535029	0.9170000
Acidobacteria	-0.18522427	0.9590000
Planctomycetes	0.13182372	0.1420000
Firmicutes	-0.09376683	0.8390000
Bacteroidetes	-0.28608614	0.8916667
Actinobacteria	-0.03194668	0.5000000
Omnitrophica	0.93467553	0.2000000
Chloroflexi	0.88157740	0.0110000

Back to Outline

Module-EigenGene analyses (1)



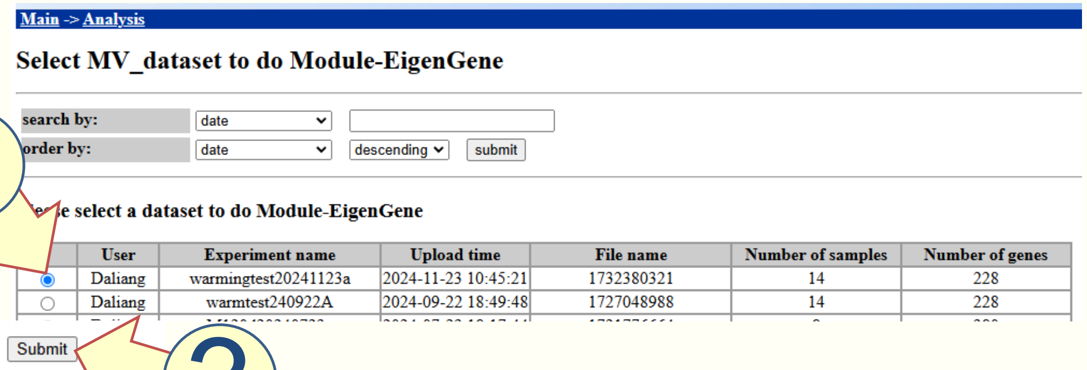
Network statistics

Fit three power-law models (regular power law, exponential law and truncated power law)

Randomize the network structure and then calculate network properties
(Updated: Modularity values from random networks have been added.)

Calculate Gene/OTU significances (GS) with environmental traits and then use Mantel test to check the correlations between GS and network connectivity

Module-EigenGene analyses



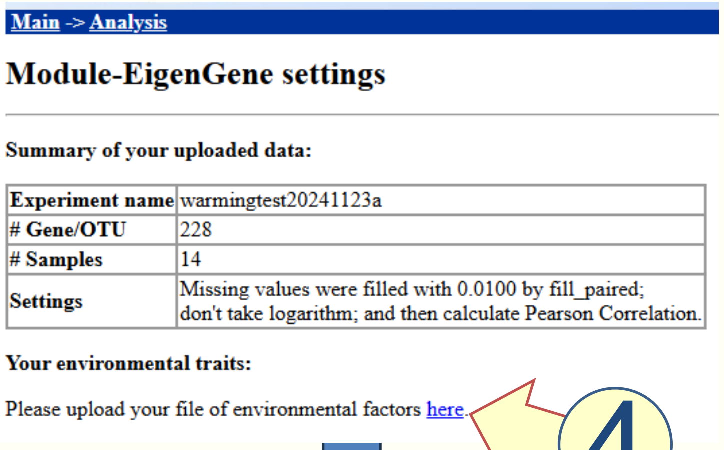
Main -> Analysis

Select MV_dataset to do Module-EigenGene

search by: date
order by: date descending

…e select a dataset to do Module-EigenGene

	User	Experiment name	Upload time	File name	Number of samples	Number of genes
●	Daliang	warmingtest20241123a	2024-11-23 10:45:21	1732380321	14	228
○	Daliang	warmtest240922A	2024-09-22 18:49:48	1727048988	14	228



Main -> Analysis

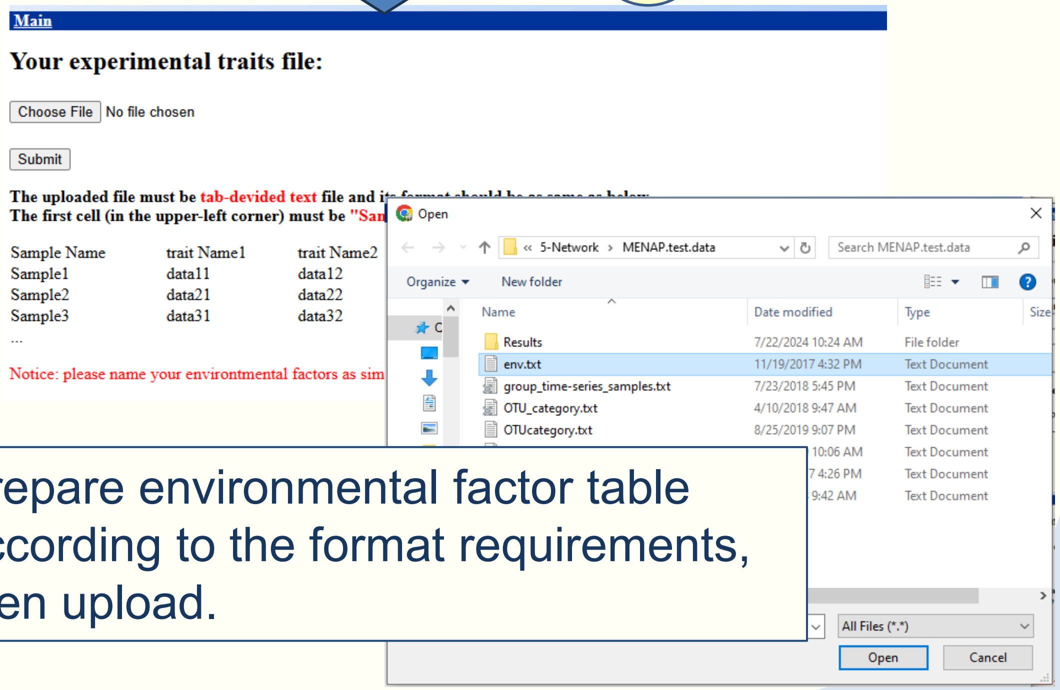
Module-EigenGene settings

Summary of your uploaded data:

Experiment name	warmingtest20241123a
# Gene/OTU	228
# Samples	14
Settings	Missing values were filled with 0.0100 by fill_paired; don't take logarithm; and then calculate Pearson Correlation.

Your environmental traits:

Please upload your file of environmental factors here.



Main

Your experimental traits file:

The uploaded file must be tab-devided text file and it…
The first cell (in the upper-left corner) must be "San…

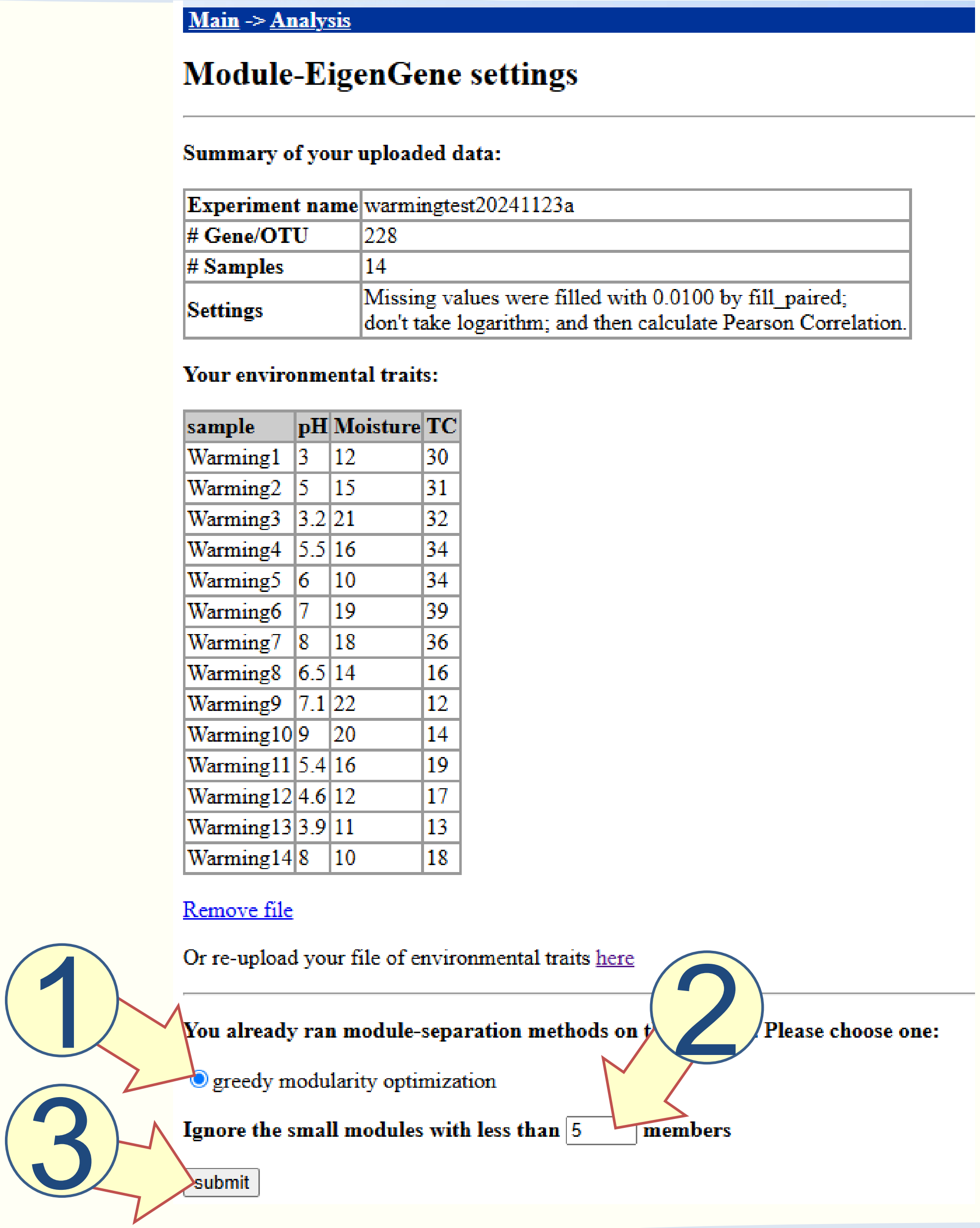
Sample Name	trait Name1	trait Name2
Sample1	data11	data12
Sample2	data21	data22
Sample3	data31	data32
...		

Notice: please name your environtmental factors as sim…

Name	Date modified	Type	Size
Results	7/22/2024 10:24 AM	File folder	
env.txt	11/19/2017 4:32 PM	Text Document	
group_time-series_samples.txt	7/23/2018 5:45 PM	Text Document	
OTU_category.txt	4/10/2018 9:47 AM	Text Document	
OTUcategory.txt	8/25/2019 9:07 PM	Text Document	
	10:06 AM	Text Document	
	7 4:26 PM	Text Document	
	9:42 AM	Text Document	

Prepare environmental factor table according to the format requirements, then upload.

Module-EigenGene analyses (2)



Main -> Analysis

Module-EigenGene settings

Summary of your uploaded data:

Experiment name	warmingtest20241123a
# Gene/OTU	228
# Samples	14
Settings	Missing values were filled with 0.0100 by fill_paired; don't take logarithm; and then calculate Pearson Correlation.

Your environmental traits:

sample	pH	Moisture	TC
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Warming9	7.1	22	12
Warming10	9	20	14
Warming11	5.4	16	19
Warming12	4.6	12	17
Warming13	3.9	11	13
Warming14	8	10	18

Remove file

Or re-upload your file of environmental traits here

You already ran module-separation methods on t… Please choose one:

● greedy modularity optimization

Ignore the small modules with less than 5 **members**

Main -> Analysis

Module-EigenGene analysis

Your first-time to run Module-eigengene analysis on this network. Record this webpage and come back to view results later or refresh this page to check the status...

Wait until you can refresh the page

Main -> Analysis

Module-EigenGene analysis

The results of Module Eigengene analysis:

The Module-EigenGenes with module members in each module:

The Module Eigengene hierarchy structure:

The Module correlation with environmental factors:

The Module Membership table can be download here

The Module Separation table can be download here

The Module-Trait Relationship table can be download here

The Module EigenGene table can be download here

Back to Outline

End