



Alternative statistical and data analyses

Network studies applied to microbial communities interaction

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Funding



Sponsorship



BIOPORTUGAL S.A.
Químico, Farmacéutica



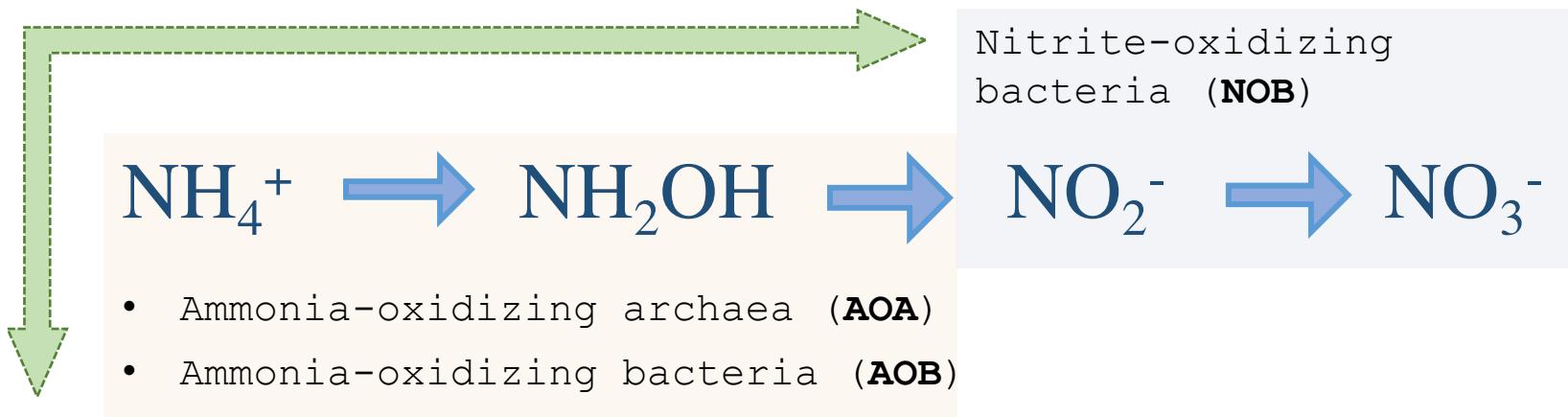
Support



Why to study interactions?

Modularity of metabolic pathways is prevalent in microbial communities

Nitrification (chemoautotrophs)



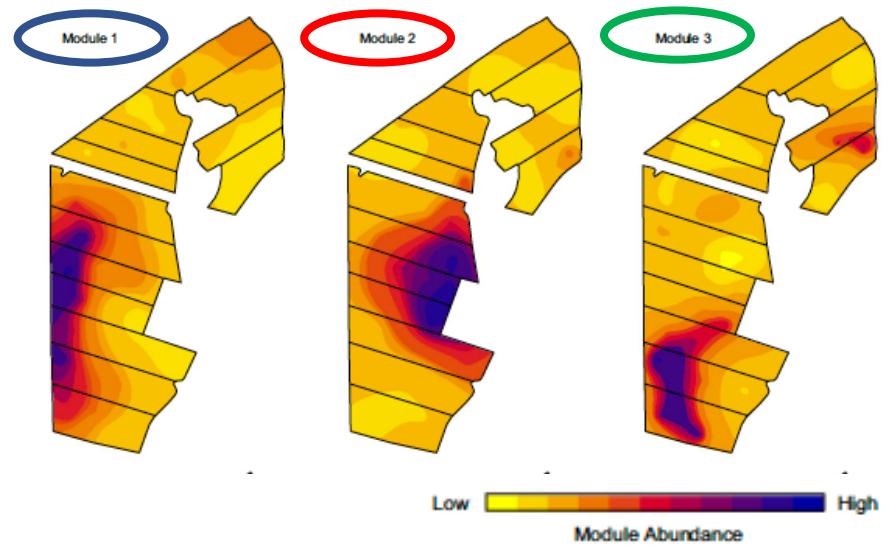
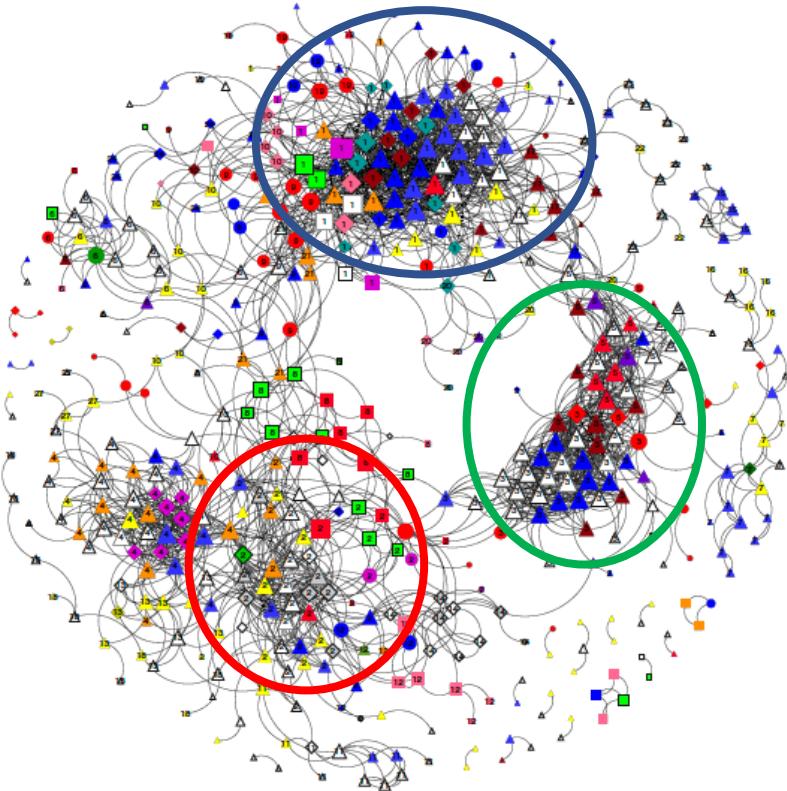
Mutualistic interaction

More complex in some cases (e.g. NOB urease, heterotrophs)

Why to study interactions?

Biological interactions within and between functional groups can play a vital role in microbial community structure.

Network of Pearson correlations



"(...) distinct modules of co occurring AO and NOB groups occupying disparate areas, with each module dominated by different lineages and associated with different edaphic factors."

Why to study interactions?

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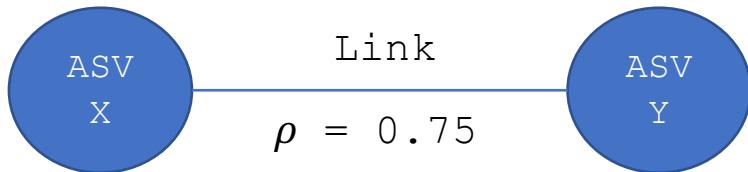
"Although network analysis **does not explain causal mechanisms** (...) these findings provide useful **starting points for future manipulation experiments** that can confirm, or refute, inferences on lineage-specific interactions and mechanisms underlying nonrandom associations within and between functional groups..."

In Jones and Hallin, 2018, Molecular Ecology.

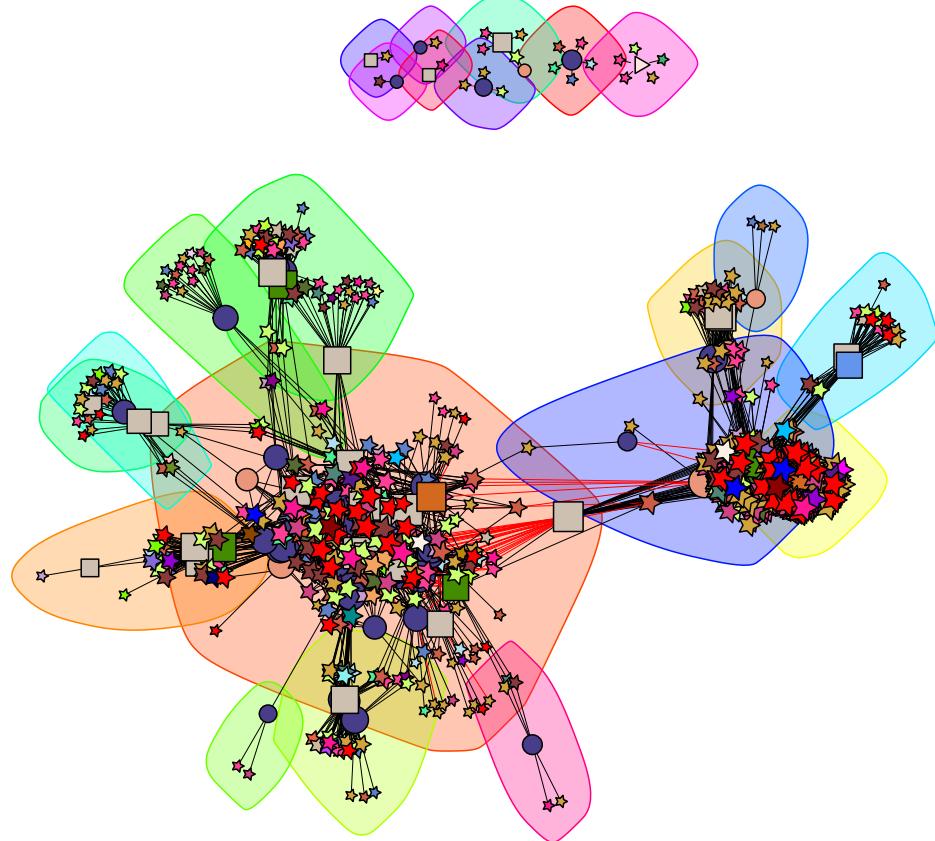
Interaction networks

- **Spatial arrangement of multiple interactions** (as opposed to isolated pairwise interactions). Tightly linked **groups of organisms** will cluster together.
- **Subcommunities**/modules/clusters

Co-occurrence/interaction networks are **usually based on some correlation unit** (e.g. Spearman or Pearson).



Correlations Network Between Nitrifying Genera and the Overall Community

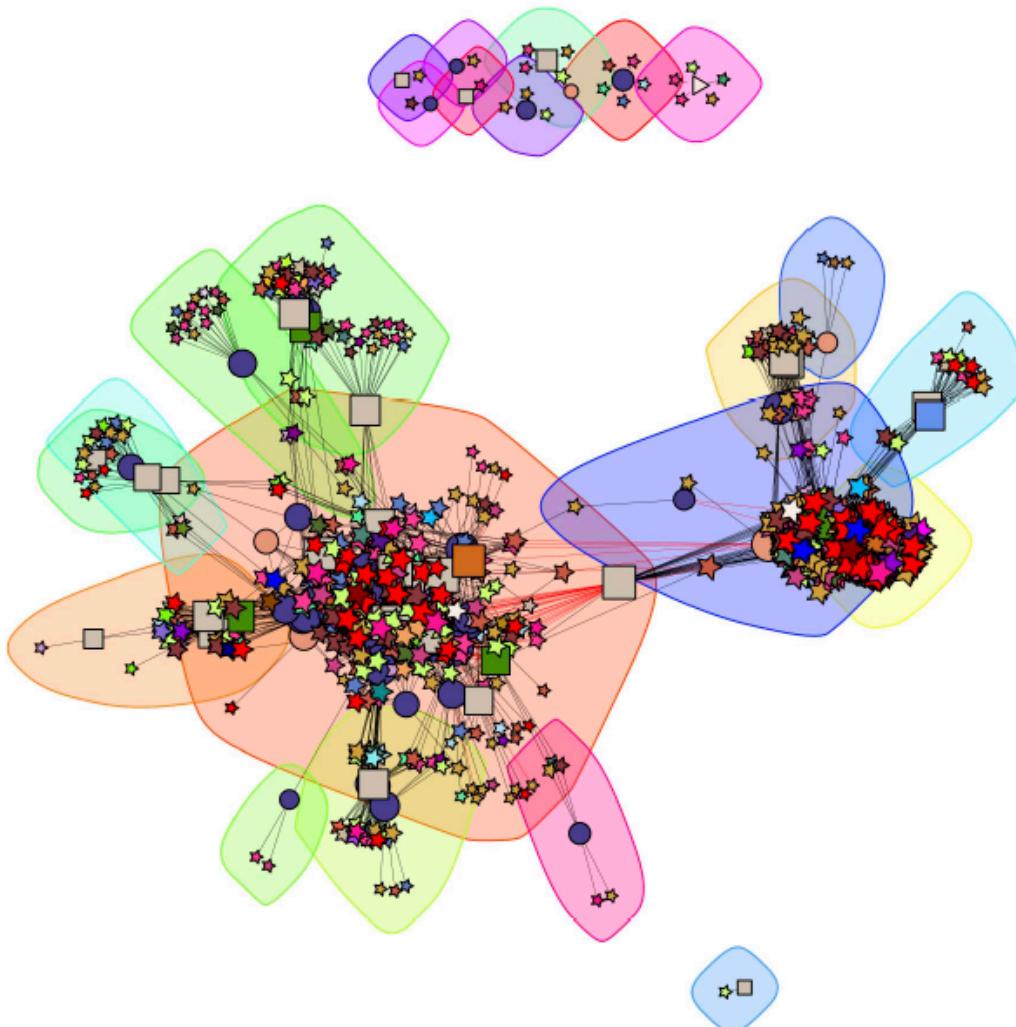


Semedo et al., 2021, Frontiers in Microbiology

Interaction networks

Correlations Network Between Nitrifying Genera and the Overall Community

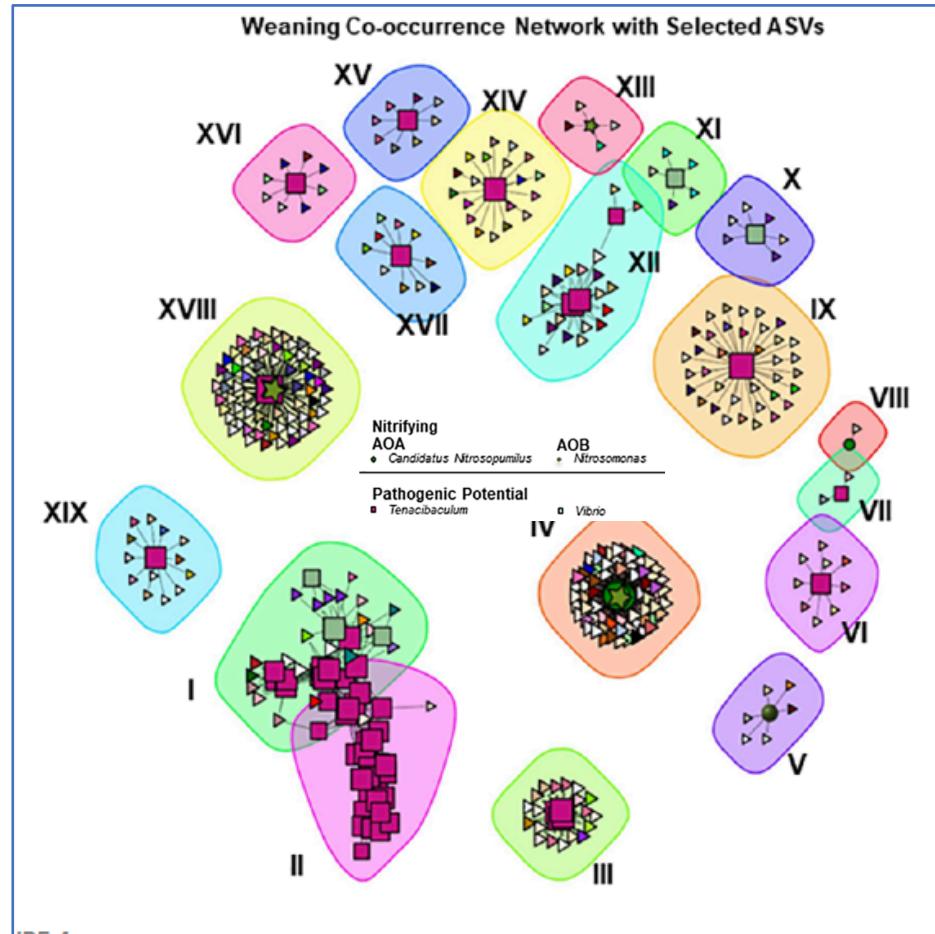
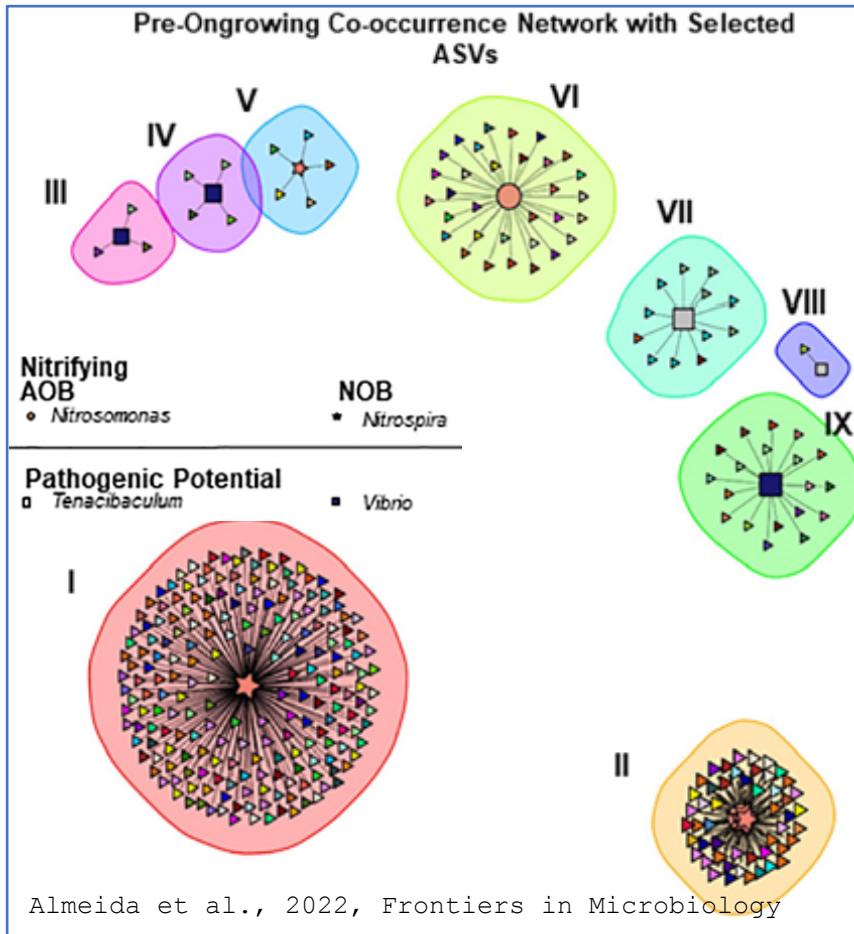
Are unclassified AOAs isolated from NOBs?



NITRIFYING GENERA		
AOA (Thaumarchaeota)		NOB
● Nitrosopelagicus	■ Nitrospira (Nitrospirae)	
● Nitrosopumilus	■ LS-NOB (Nitrospiniae)	
● Unclassified	■ Nitrospina (Nitrospiniae)	
AOB (Gammaproteobacteria)		■ Unclassified (Nitrospiniae)
△ FS142-36B-02		
OVERALL COMMUNITY		
Gammaproteobacteria	Planctomycetes	Other Unclassified
★ Endoeteinascidia	★ JL-ETNP-F28	★ Unclassified Euryarchaeota
★ Enhydrobacter	★ Pir4 lineage	★ Unclassified Gemmatimonadetes
★ Marinobacter	★ Rhodopirellula	★ Unclassified Hydrogenedentes
★ Methylophaga	★ Unclassified	★ Unclassified Kiritimatiellaeota
★ OM43 Clade	★ Urana-1B-19	★ Unclassified Margulisbacteria
★ Pseudohongiella		★ Unclassified Marinimicrobia
★ SUP05 Cluster		★ Unclassified PAUC34f
★ Woesia		★ Unclassified Schekmanbacteria
★ Unclassified		★ Unclassified AnkK6
Alphaproteobacteria		★ Unclassified Chlamydiae
★ AT-s3-44	★ Verruc-01	★ Unclassified Chloroflexi
★ Clade Ia	★ Cerasicoccus	★ Unclassified Crenarchaeota
★ Clade Ib	★ MB11C04	★ Unclassified Proteobacteria
★ Magnetospira	★ Unclassified	★ Unclassified Bacteria
★ Unclassified		
Deltaproteobacteria	Actinobacteria	
★ OM27 Clade	★ Actinomarina	
★ Unclassified	★ Mycobacterium	
	★ Unclassified	
Cyanobacteria	Bacteroidetes	
★ Prochlorococcus	★ Gramella	
★ Unclassified	★ Marinoscillum	
	★ Unclassified	
Deinococcus-Thermus	Acidobacteria	
★ Truepera	★ PAUC26f	
	★ Unclassified	

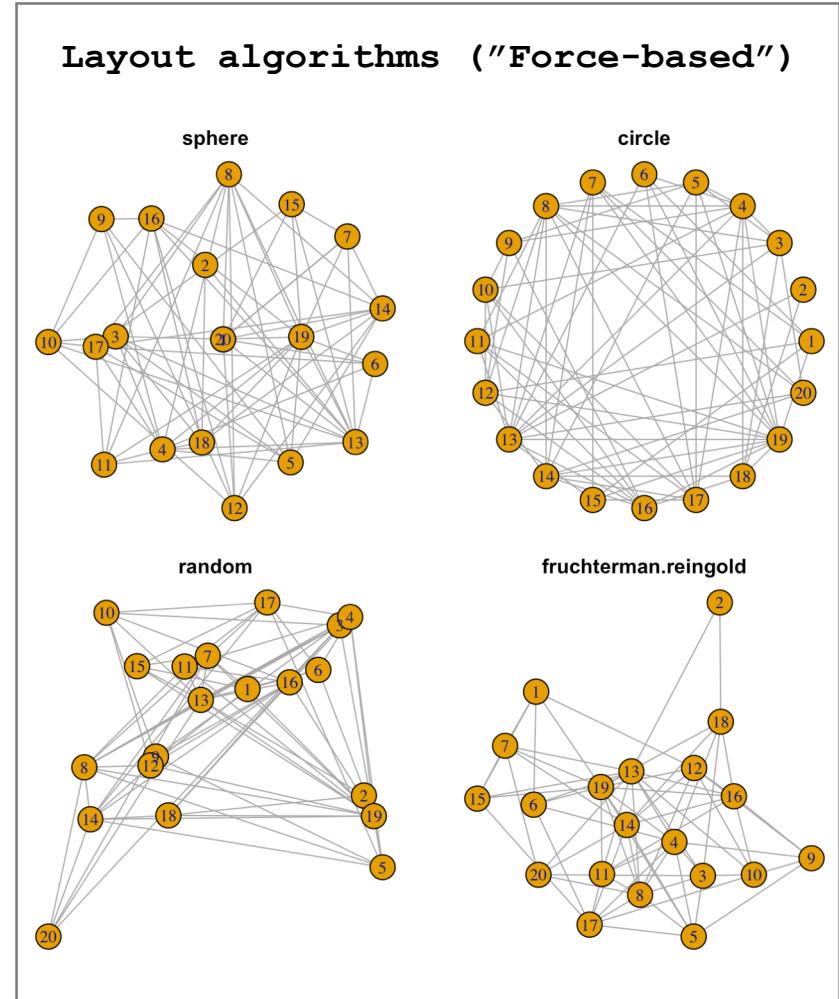
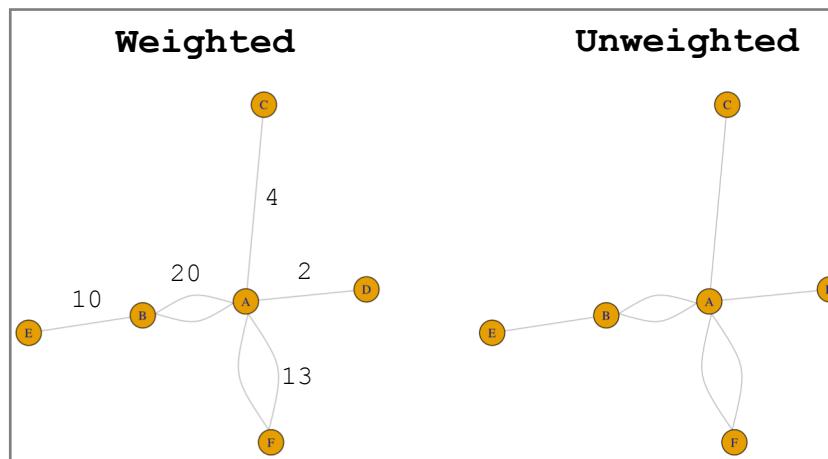
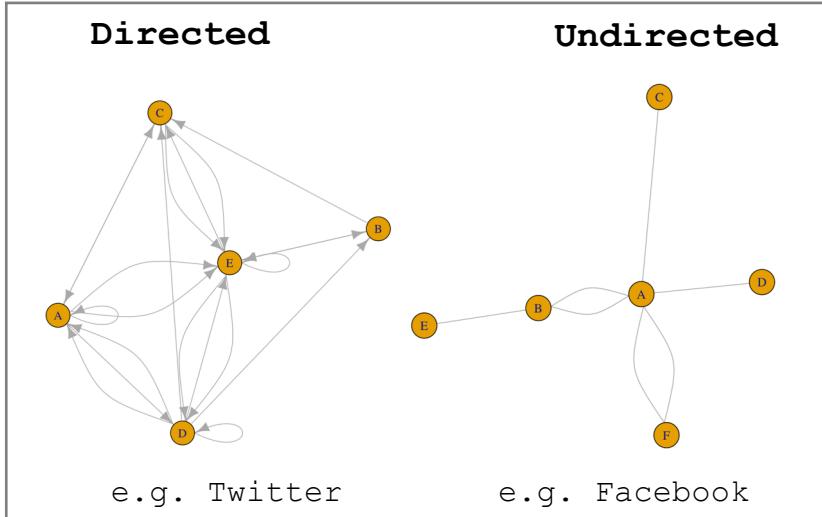
Interaction networks

Are two potentially pathogenic genera tightly linked or negatively correlated?



Not negatively correlated (no evidence of exclusion competition) and data suggests positive correlations in one of the systems (weaning)

Types of networks



Community detection: Louvain method, Leiden algorithm, etc.

Networks glossary

- **Node (or vertex)**: connecting points
- **Link (or edge)**: connecting lines
- **Module**: subcommunity/group/cluster of tightly linked nodes
- **Degree**: total number of links (per node, per network, per module)
- **Network layout**: coordinates algorithm used to spatially arrange nodes and links in order to minimize crossing and overlap.
- **Hub nodes or hub taxa**: highly connected nodes
- **Module-hub**: highly connected node within a module ("specialists")
- **Module connector**: node with multiple connections between modules ("generalists")

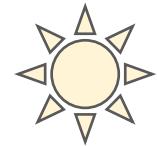
Concerns, limitations, and other approaches

- **Autocorrelation** (unless you want to test it):
Nodes interacting due to a third major force (e.g. habitat effect).
- Correlation coefficient and **threshold value**.
- Do you have a **specific hypothesis or research question?**
Avoid performing a network analysis "just to check".

Other approaches that can evaluate interactions:

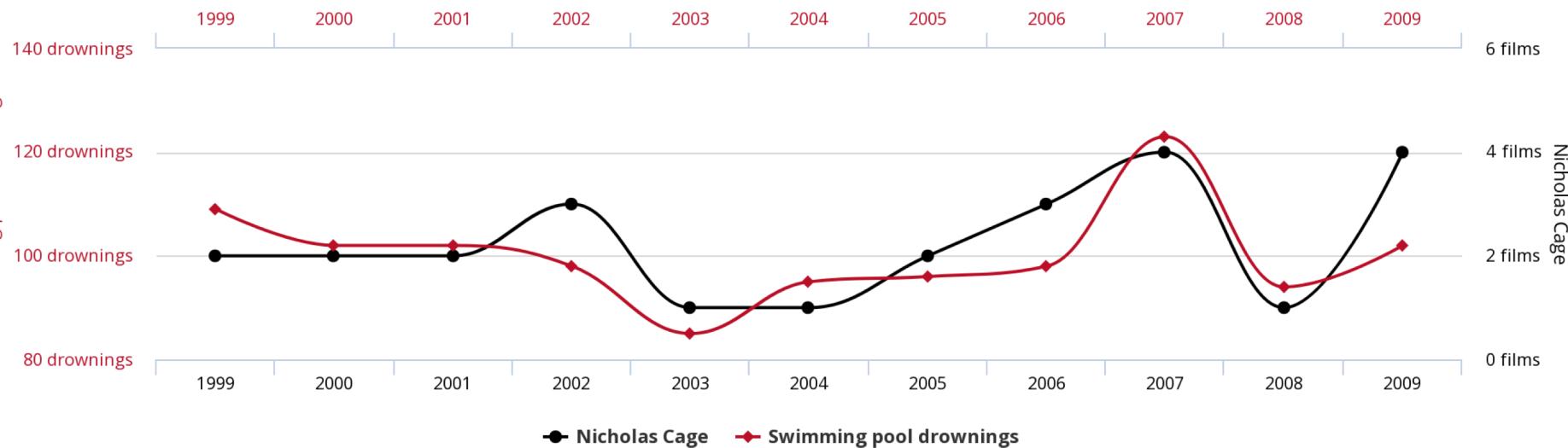
- Structural equation modelling
- Akaike-information criterion (AIC) analysis
- Association rules

Spurious correlations



- CORRELATION IS NOT CAUSATION !
- OCCASIONAL OBSERVATION DOES NOT REPLACE EXPERIMENTATION !

Number of people who drowned by falling into a pool
correlates with
Films Nicolas Cage appeared in



Data source: US CDC and IMDB

tylervigen.com

Questions?