## Network Science Basics

Savvas Paragkamian, post doc



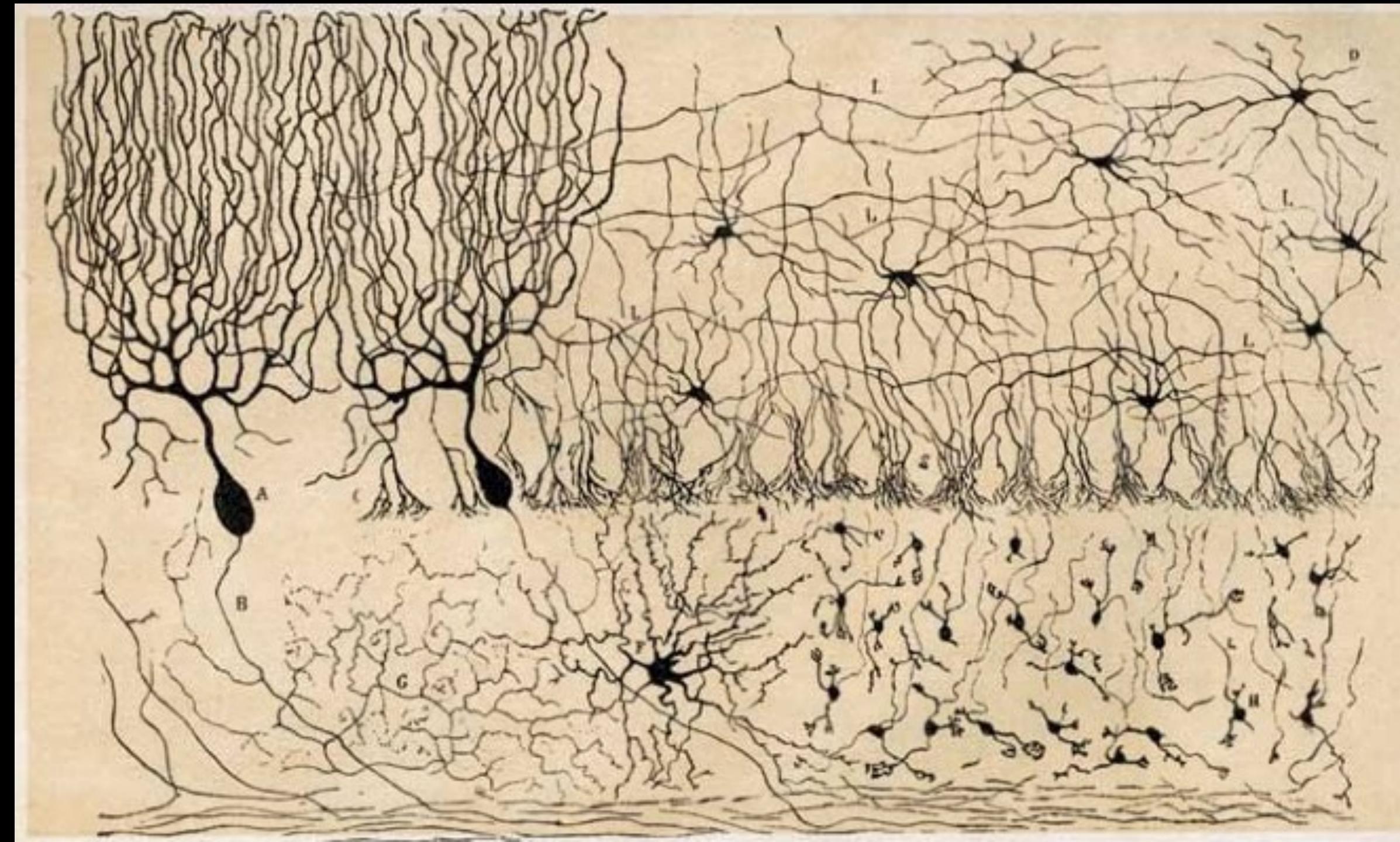


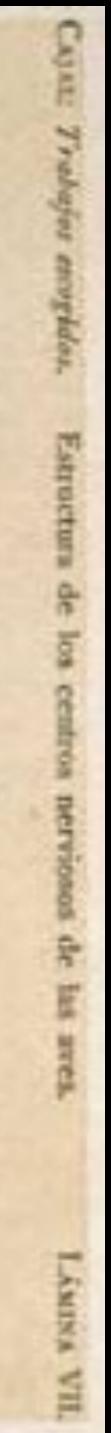
# What is a network?

# From omics to networks?



Examples





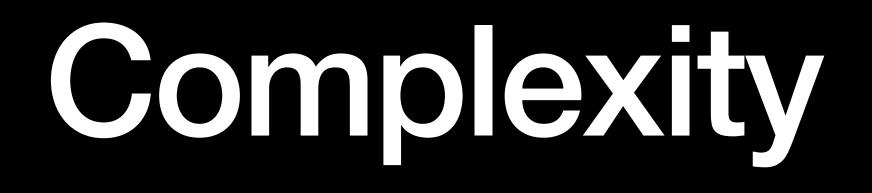
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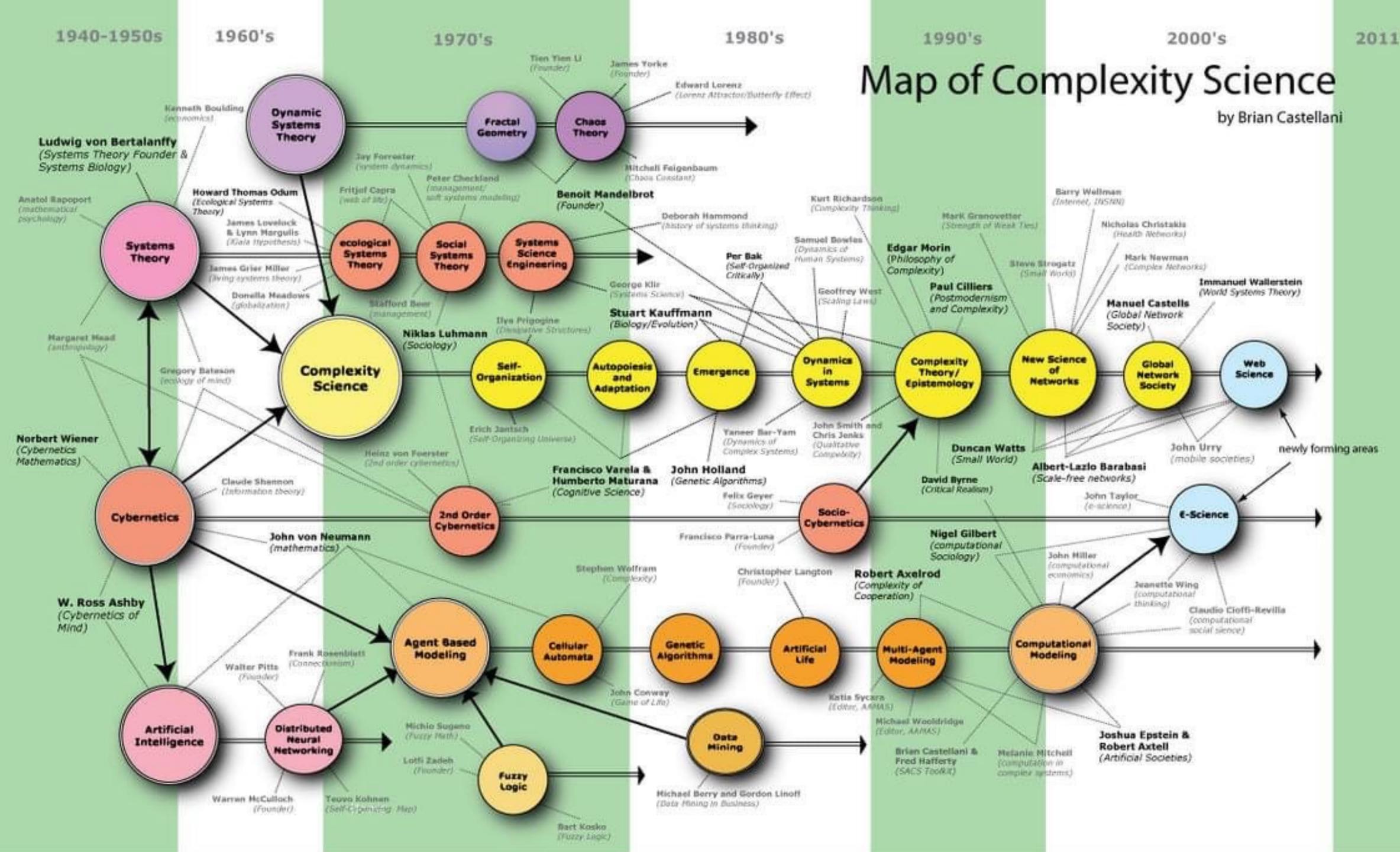




## From Prof I. Antoniou









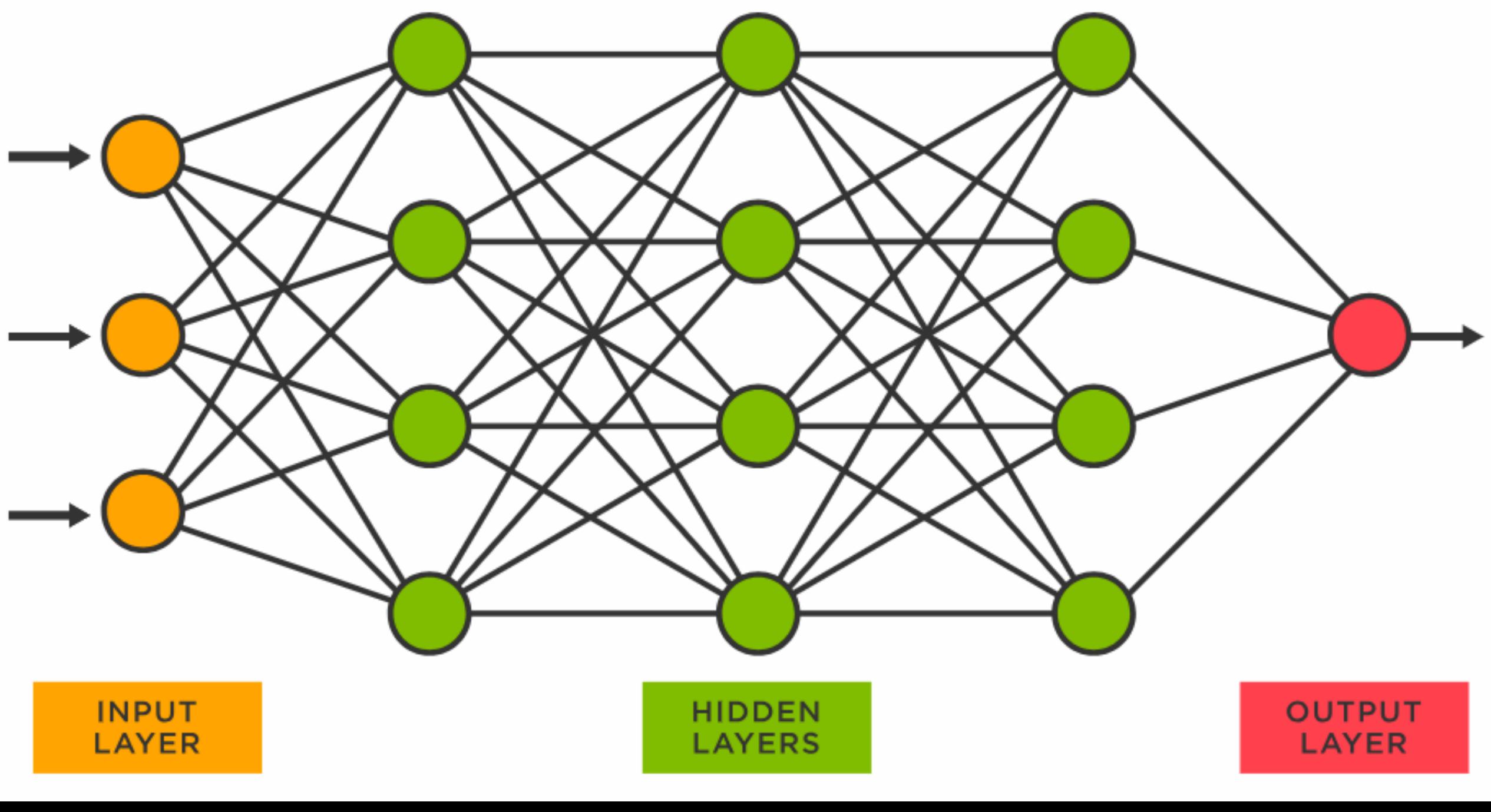


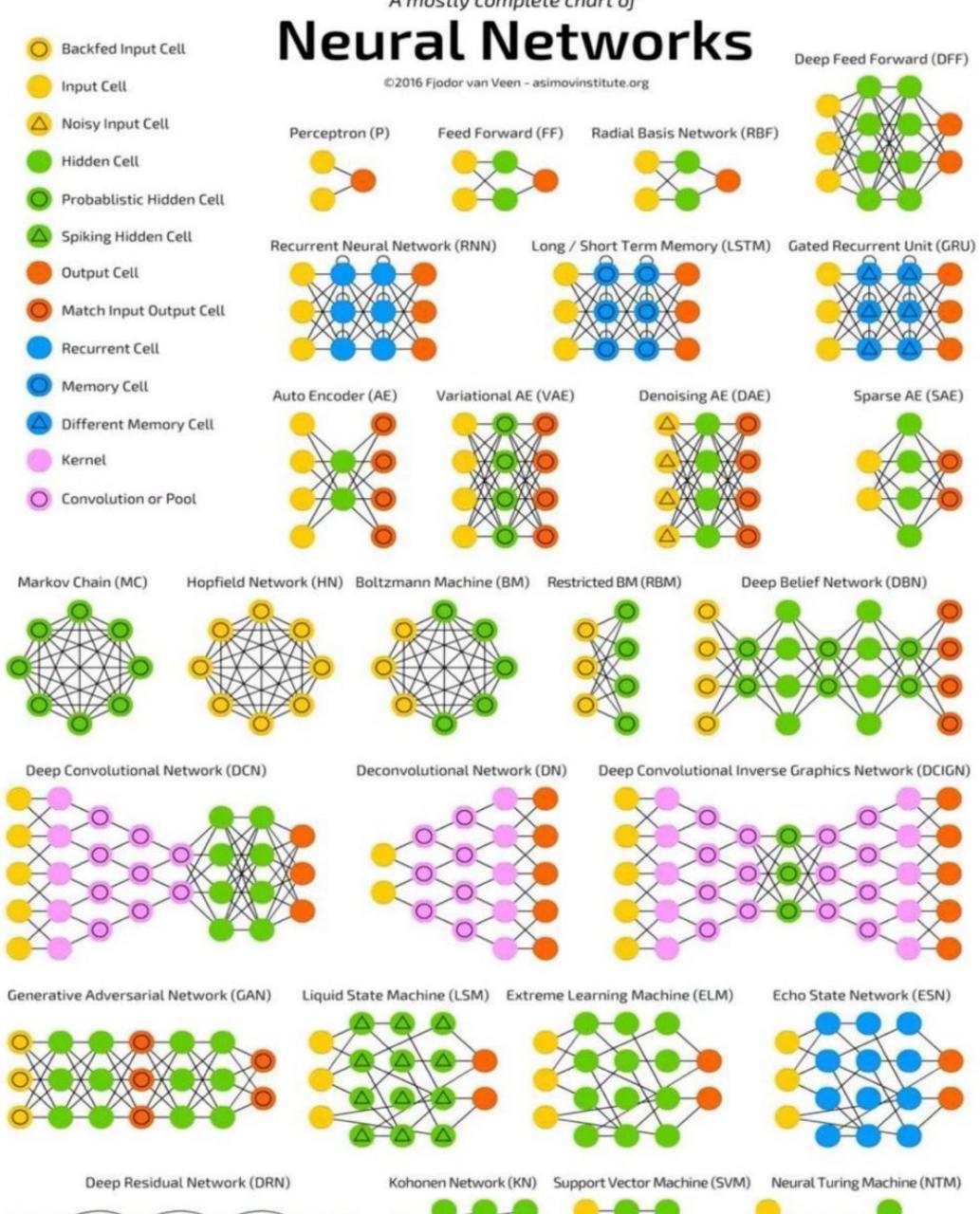
Less is more

# **More Is Different**

# Large Language Models Diffusion models Deep Neural Networks

# ChatGPT AlphaFold EVO

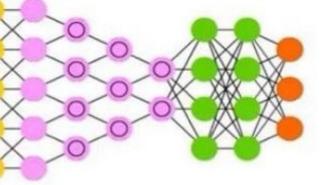


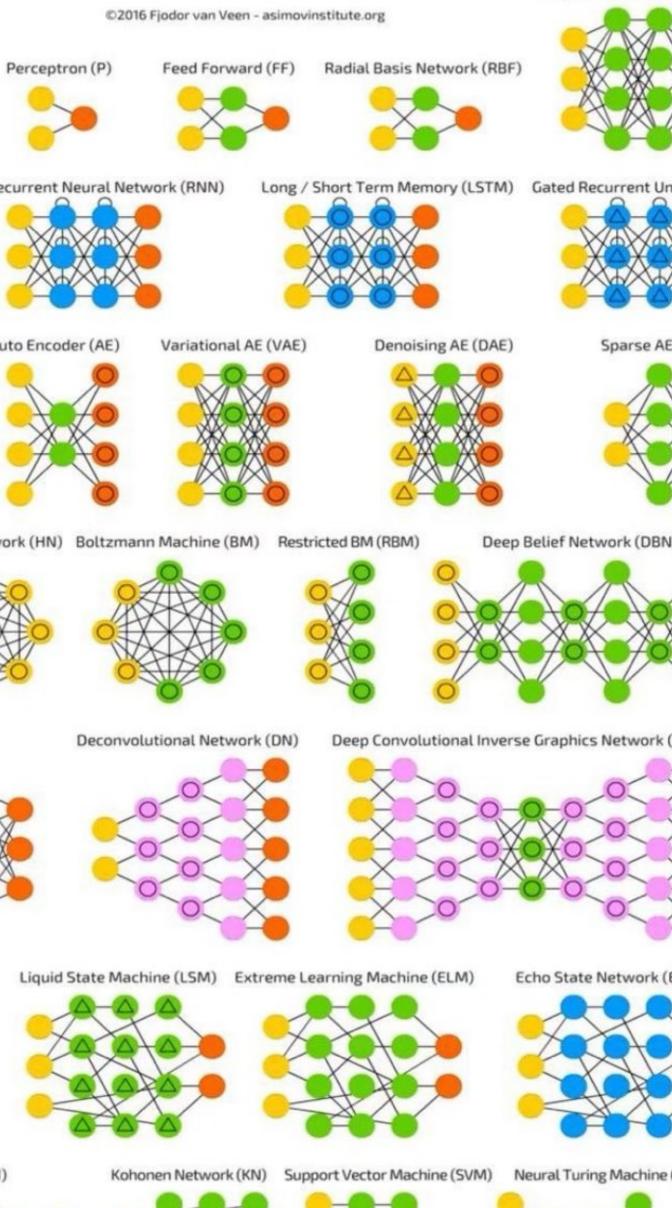




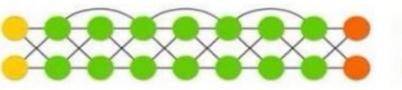


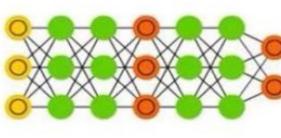






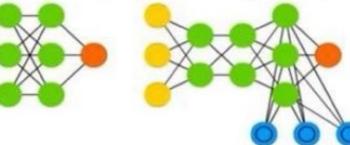














# Types of Networks



# Bipartite

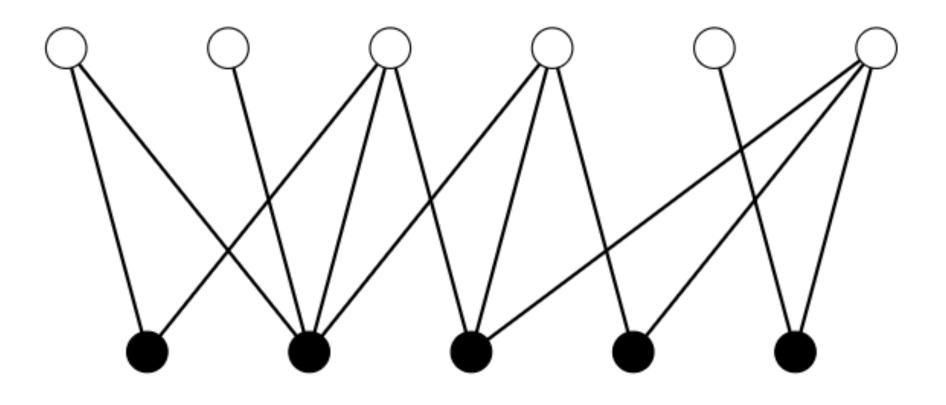


Figure 6.5: A small bipartite network. The open and closed circles represent two types of nodes and edges run only between nodes of different types. It is common to draw bipartite networks with the two sets of nodes arranged in lines, as here, to make the bipartite structure clearer. See Fig. 4.2 on page 50 for another example.

### Newman 2018





# Fypergraph

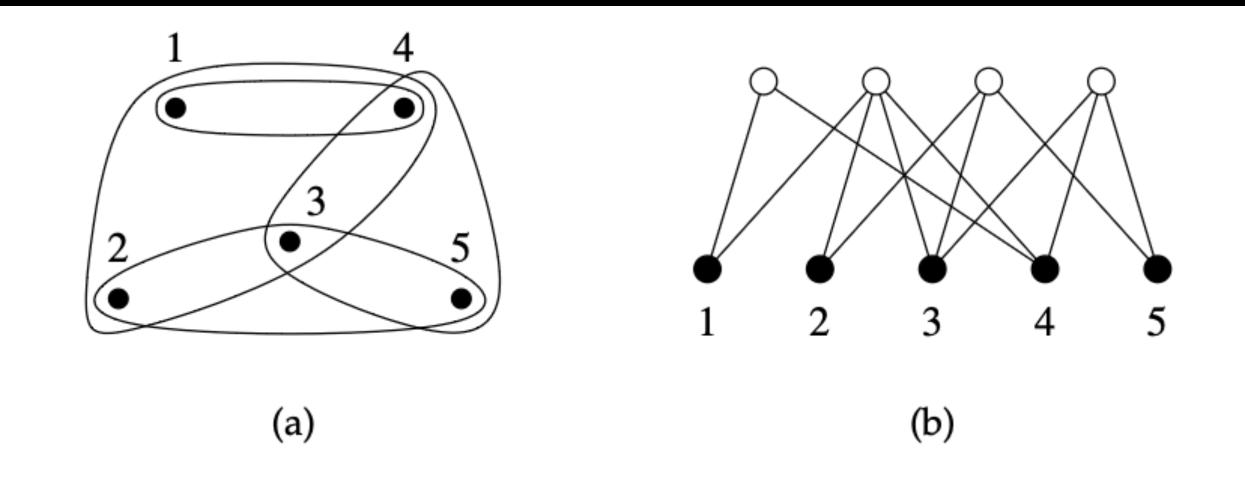
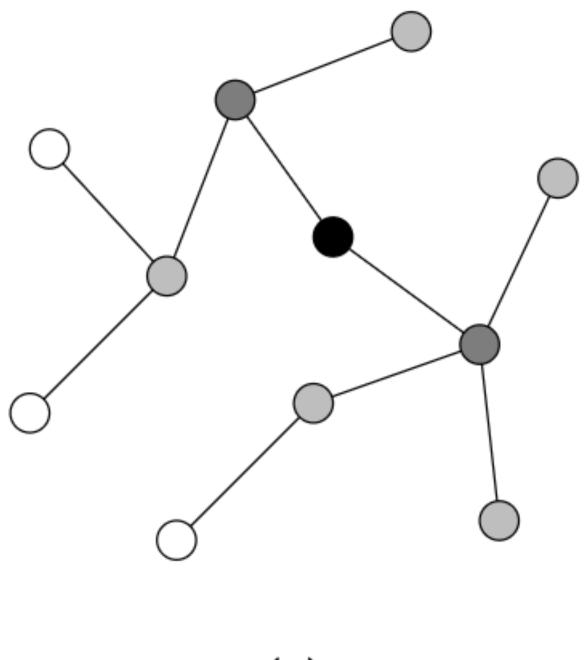


Figure 6.4: A hypergraph and corresponding bipartite graph. These two networks convey the same information—the membership of five nodes in four different groups. (a) The hypergraph representation in which the groups are represented as hyperedges, denoted by the loops circling sets of nodes. (b) The bipartite representation in which we introduce four new nodes (open circles at the top) representing the four groups, with edges connecting each of the original five nodes (bottom) to the groups to which it belongs.



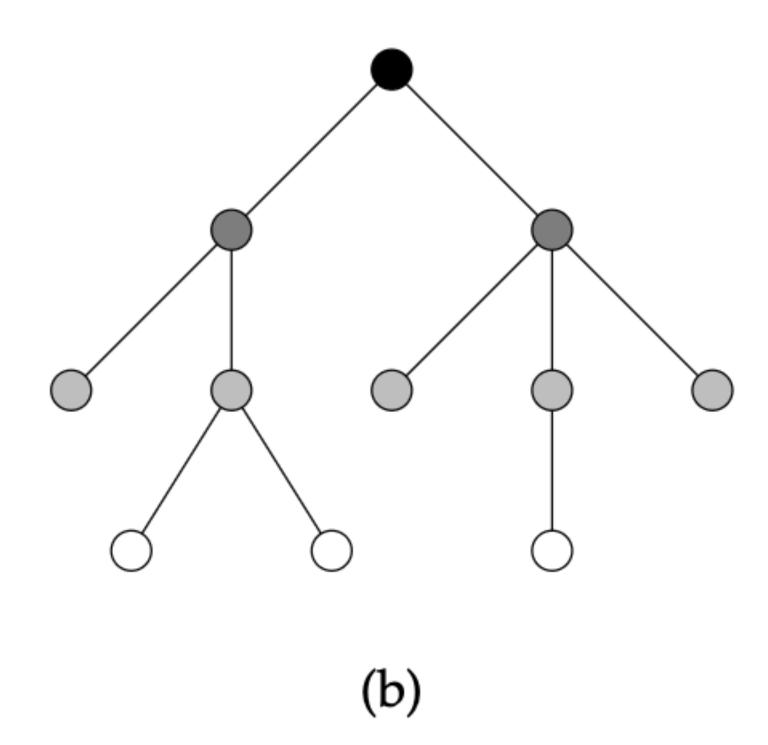






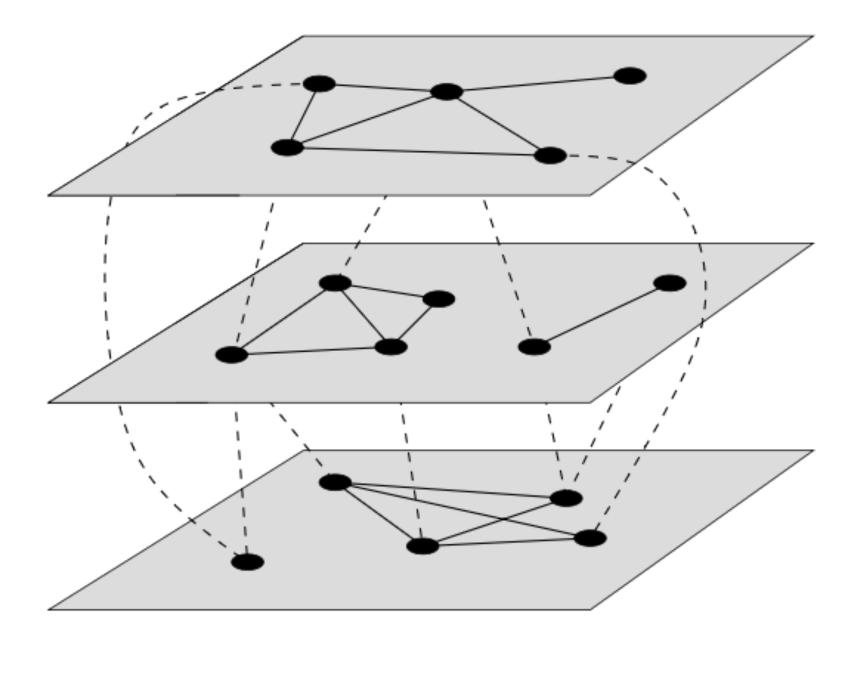
(a)

**Figure 6.8: Two sketches of the same tree.** The two panels here show two different depictions of a tree, a network with no closed loops. In (a) the nodes are positioned on the page in any convenient position. In (b) the tree is a laid out in a "rooted" fashion, with a root node at the top and branches leading down to "leaves" at the bottom.



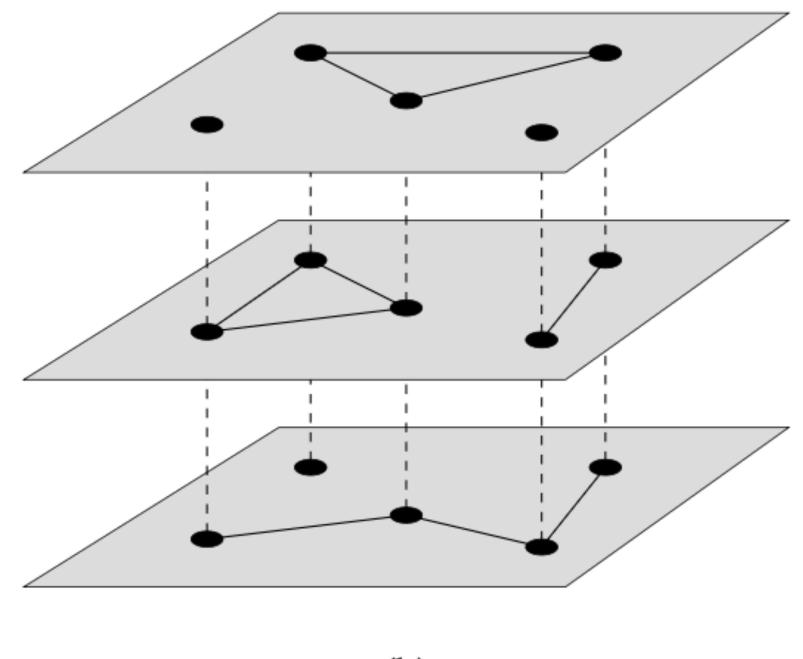
### Newman 2018





(a)

Figure 6.7: Multilayer and multiplex networks. (a) A multilayer network consists of a set of layers, each containing its own network, plus interlayer edges connecting nodes in different layers (dashed lines). An example is a transportation network with layers corresponding to airlines, trains, buses, and so forth. (b) A multiplex network is a special case of a multilayer network in which the nodes represent the same set of objects or people in each layer. For instance, a social network with several different types of connections could be represented as a multiplex network with one layer for each type. Dynamic or temporal networks are another example, where the layers represent snapshots over time of the structure of a single, time-varying network. In principle one can include interlayer edges in a multiplex network, as here, to represent the equivalence of nodes in different layers, although in practice these are often omitted.



(b)

### Newman 2018



## Summary of 21/05/2025

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## **Rmarkdown - Network Basics**

## **15 minutes Break**

Soil Metagenome - From sequences to microbe co-occurrences

## Take a look at Metagenomics course by Dr Lagkouvardos



field days

sampling sites

countries



The success of this complex international project is a testament to the dedication of all teams involved. Credit: Savvas Parakamian/HCMR, Gerals Pfister, Joanna Zukowska, and Kinga Lubowiecka, Creative Team/EMBL





Times of a sampling video shoots: Latest 02:10

carried on a video shooting day



For further info please contact AML team



## An expedition where land meets sea



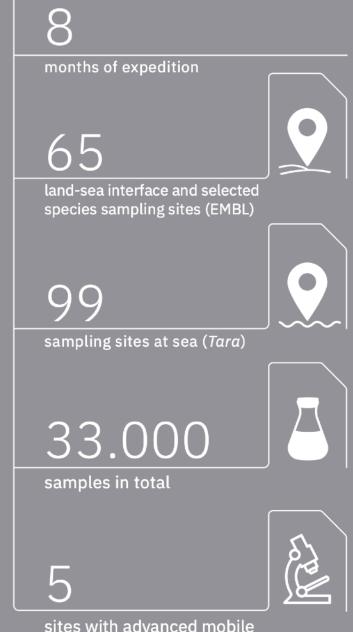
### **TREC expedition stops 2024**

Malaga, Spain	Late February
Mallorca, Spain	Early March
Barcelona, Spain	Mid-March
Banyuls, France	Late March
Villefranche-sur-Mer, France	Early April
Pisa, Italy	Mid-April
Naples, Italy	Late April
Calabria, Italy	Early May
Lesina, Italy	Mid-May
Chioggia, Italy	Late May
Split, Croatia	Early June
Kotor, Montenegro	Mid-June
Athens, Greece	Early-July
Thrace, Greece	Mid-July

Follow the expedition via our interactive map https://trec.embl.de/itinerary.cgi



### TREC 2023 IN NUMBERS



sites with advanced mobile lab support



### LEGEND

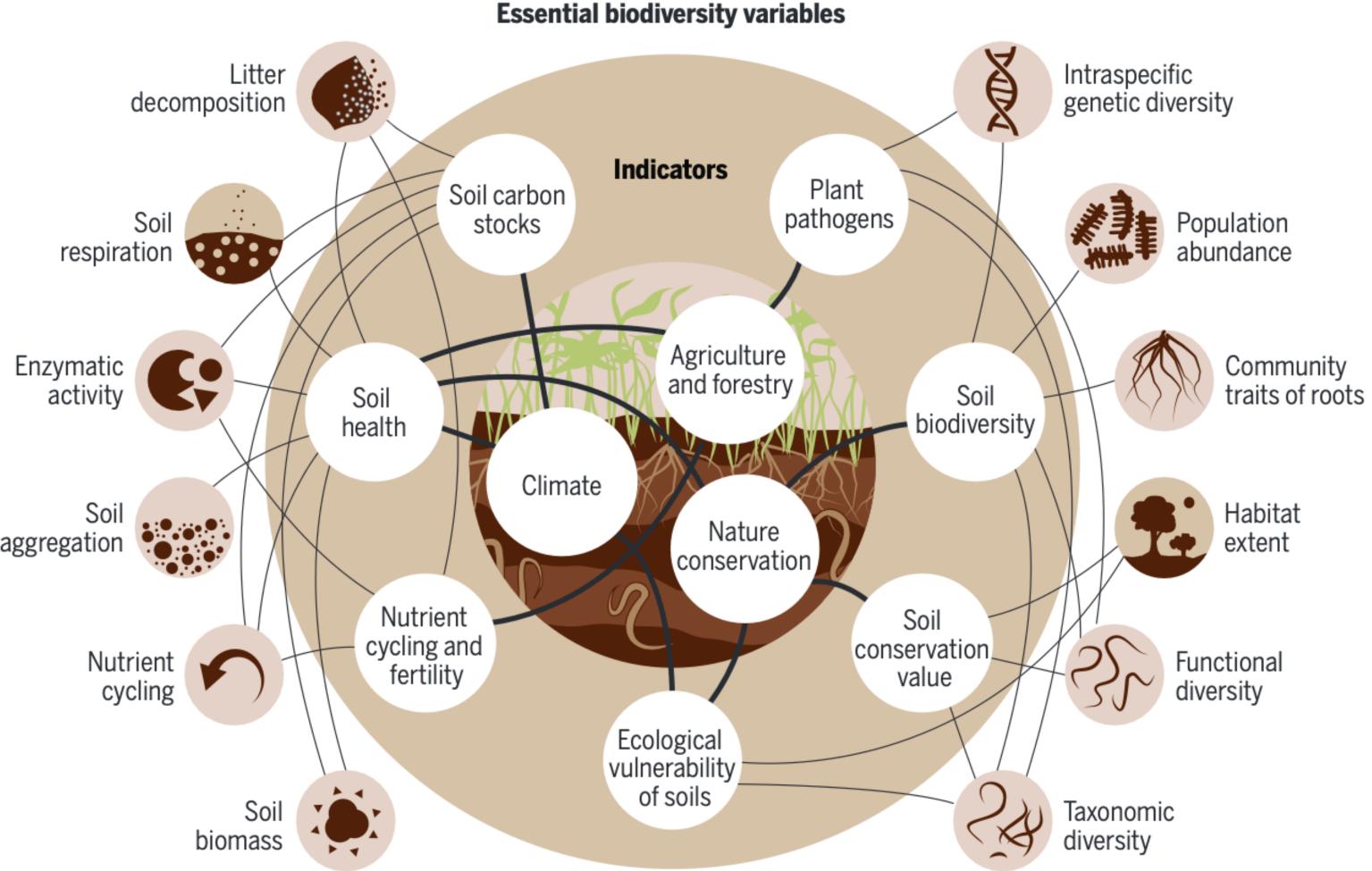
- Common stopovers TREC / Tara EUROPA
- Port calls Tara EUROPA
- Sampling Coastal Sites
- --- 2023
- \_\_\_\_\_ 2024

The TREC expedition began in Roscoff, France, in spring 2023 and will conclude in Thessaloniki, Greece, in July 2024. During this period, researchers from EMBL, the Tara OceanS consortium, together with the Tara Ocean Foundation, and numerous European collaborating institutes and organisations will be working at 120 sampling sites across 21 European countries.

- TREC https://www.embl.org/about/info/trec/
- Salamina <u>https://www.youtube.com/watch?v=FcYOZWyTmms</u>
- Psatha https://www.youtube.com/watch?v=BQ2hJBeSBwQ

### Linking soil biodiversity to policy

Links between global soil essential biodiversity variables (EBVs) (outer ring) are prioritized by the Soil Biodiversity Observation Network (SoilBON) and policy sectors (center) through the use of soil ecological indicators (inner ring; table S3). Thin lines correspond to links between EBVs and soil indicators; thicker lines refer to links between each soil indicator and specific policy sectors. The EBVs for soil systems are proposed as a holistic system approach (table S2), where soil organisms are intertwined with relevant soil chemical, physical, and functional properties, contributing to overall societal well-being. See table S1 for further information on links to specific policy targets and policies. See table S2 for details of the EBVs.



### VOGEL ET AL.

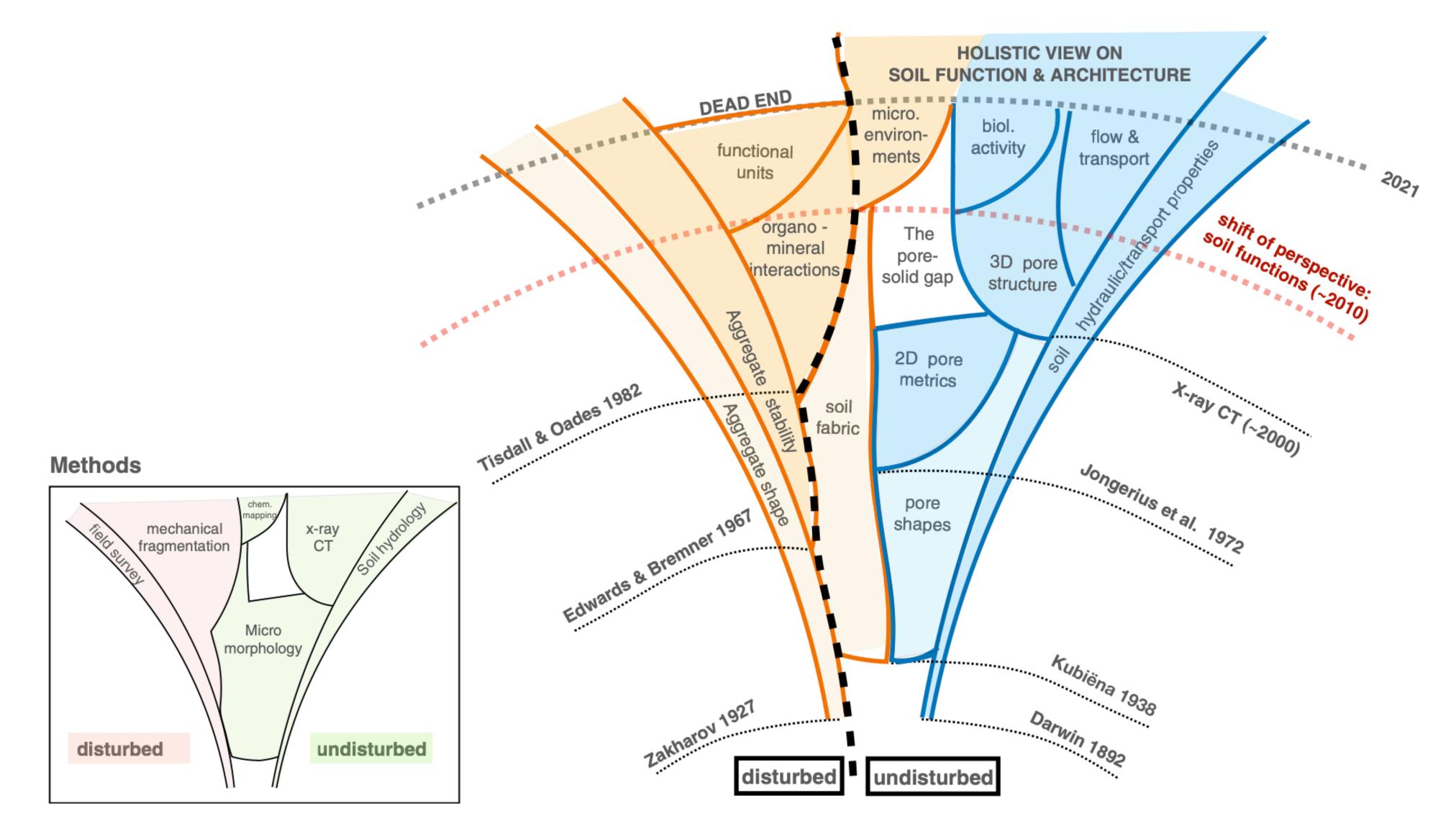
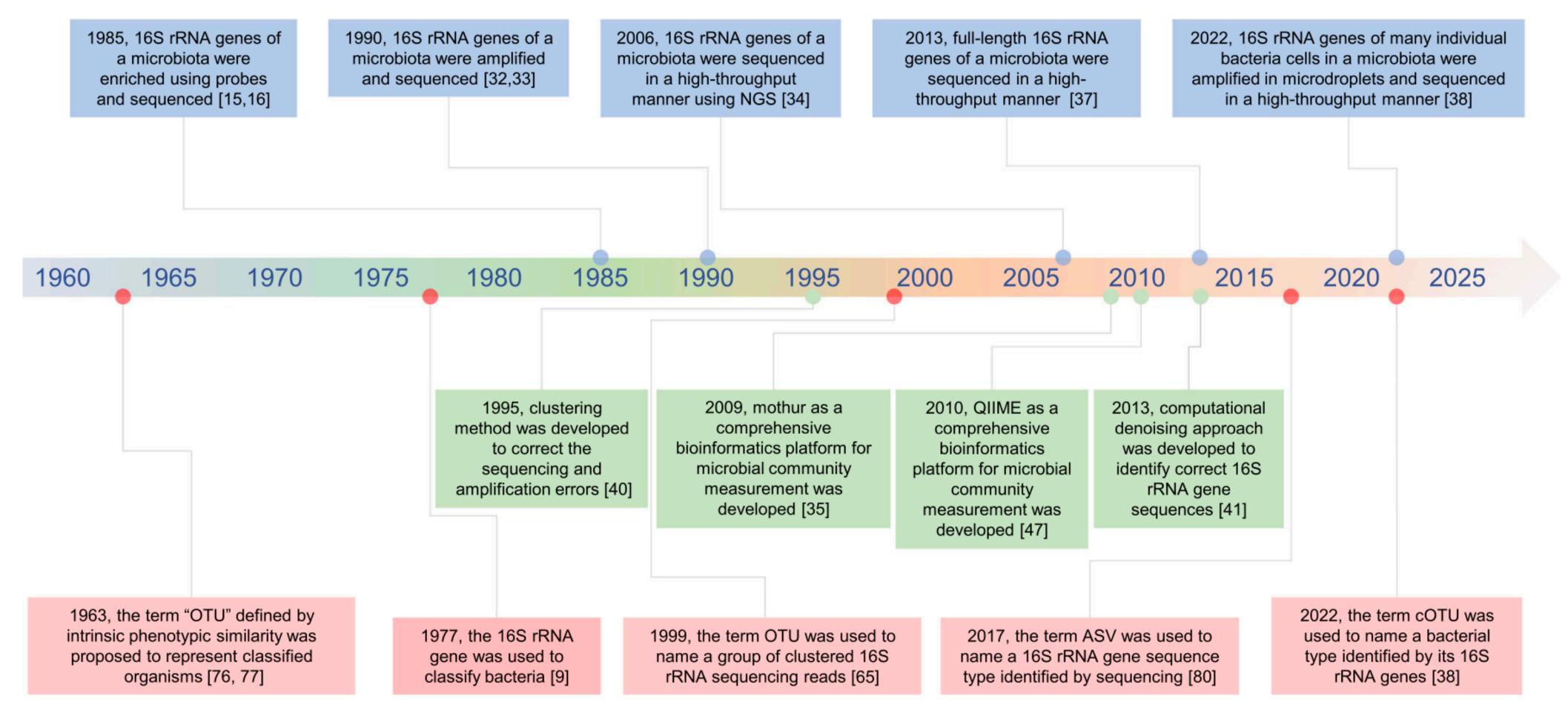


FIGURE 1 foci are marked by milestone publications or new technical developments

### European Journal of Soil Science –WILEY– 3 of 14

Historical development of exploring soil structure. The pore perspective is in blue, the solid perspective in ochre. Dark colours and light colours indicate quantitative and qualitative analysis, respectively. New branches of developments with respect to research

### Milestones of 16S rRNA gene-amplicon sequencing

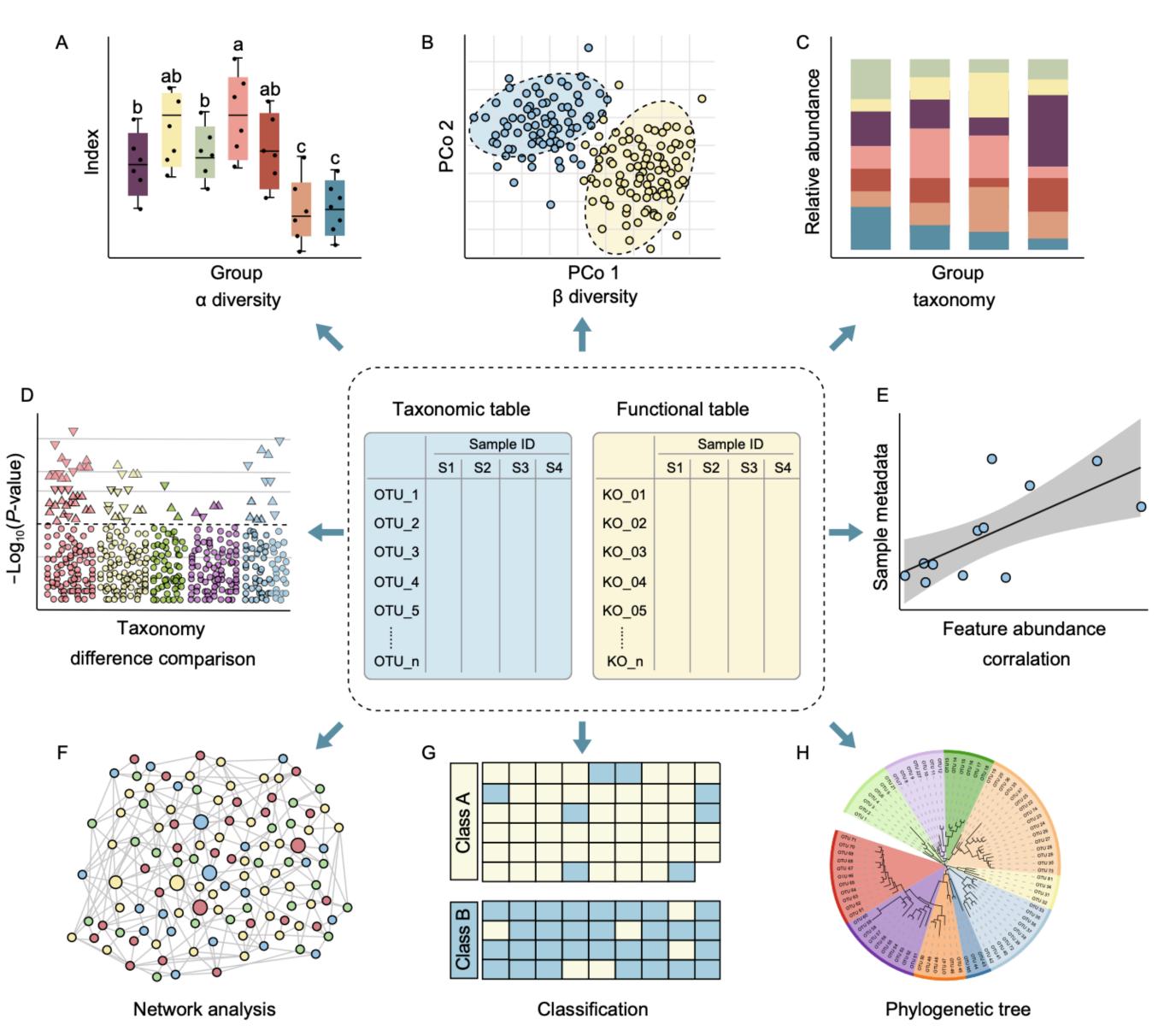


### Main units toward defining the microbiota and key analysis methods

**FIGURE 1** History of 16S rRNA gene-amplicon sequencing, key analysis methods, and related classification units. ASV, amplicon sequence variants; cOTU, cell-based operational taxonomic unit; NGS, next-generation sequencing; OTU, operational taxonomic unit; QIIME, quantitative insights into microbial ecology; rRNA, ribosomal RNA.



A practical guide to amplicon and metagenomic analysis of microbiome data В А

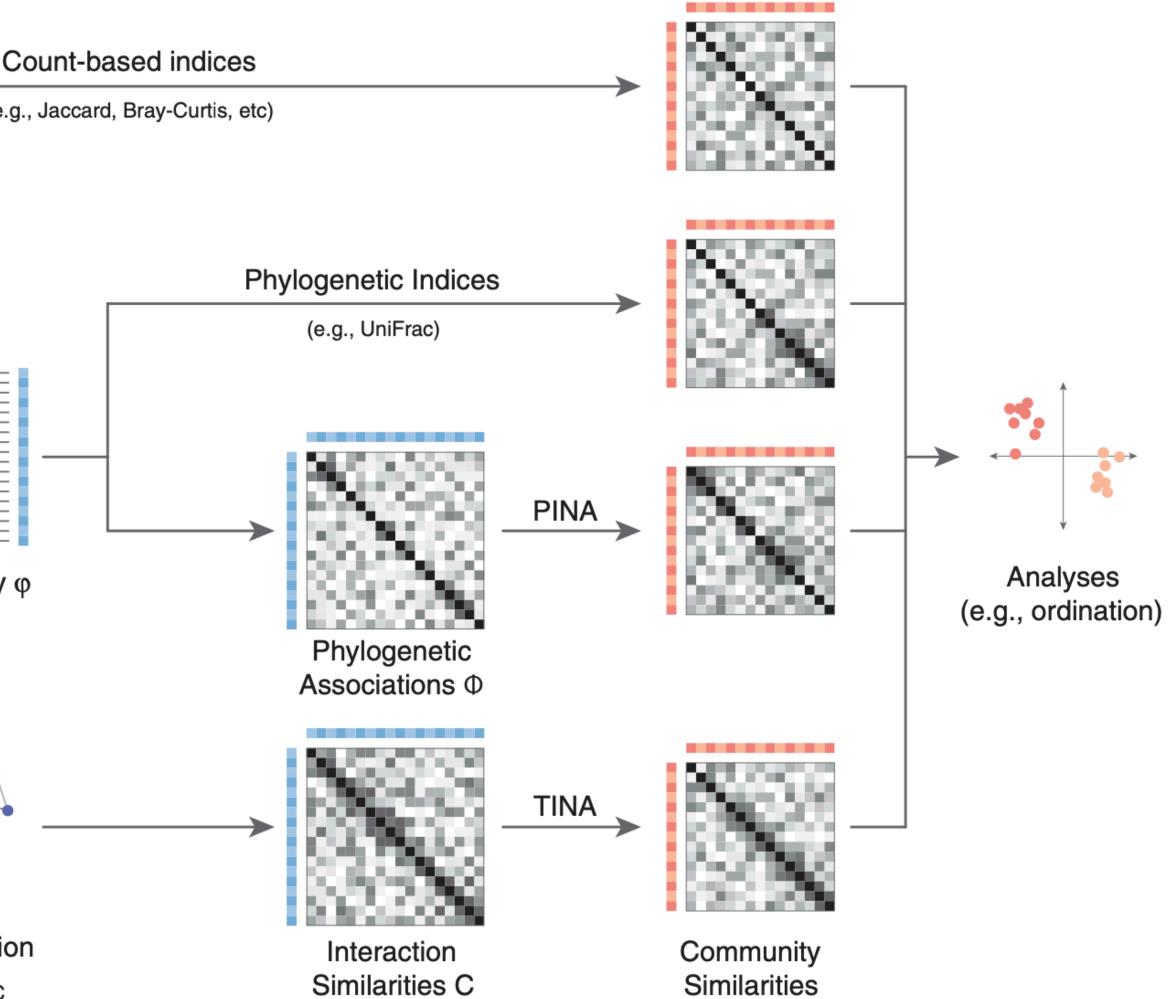


analysis (F), classification of machine learning (G), and phylogenetic tree (H). Please see Table 2 for more details.

**REVIEW** 

Figure 3. Overview of statistical and visualization methods for feature tables. Downstream analysis of microbiome feature tables, including alpha/beta-diversity (A/B), taxonomic composition (C), difference comparison (D), correlation analysis (E), network

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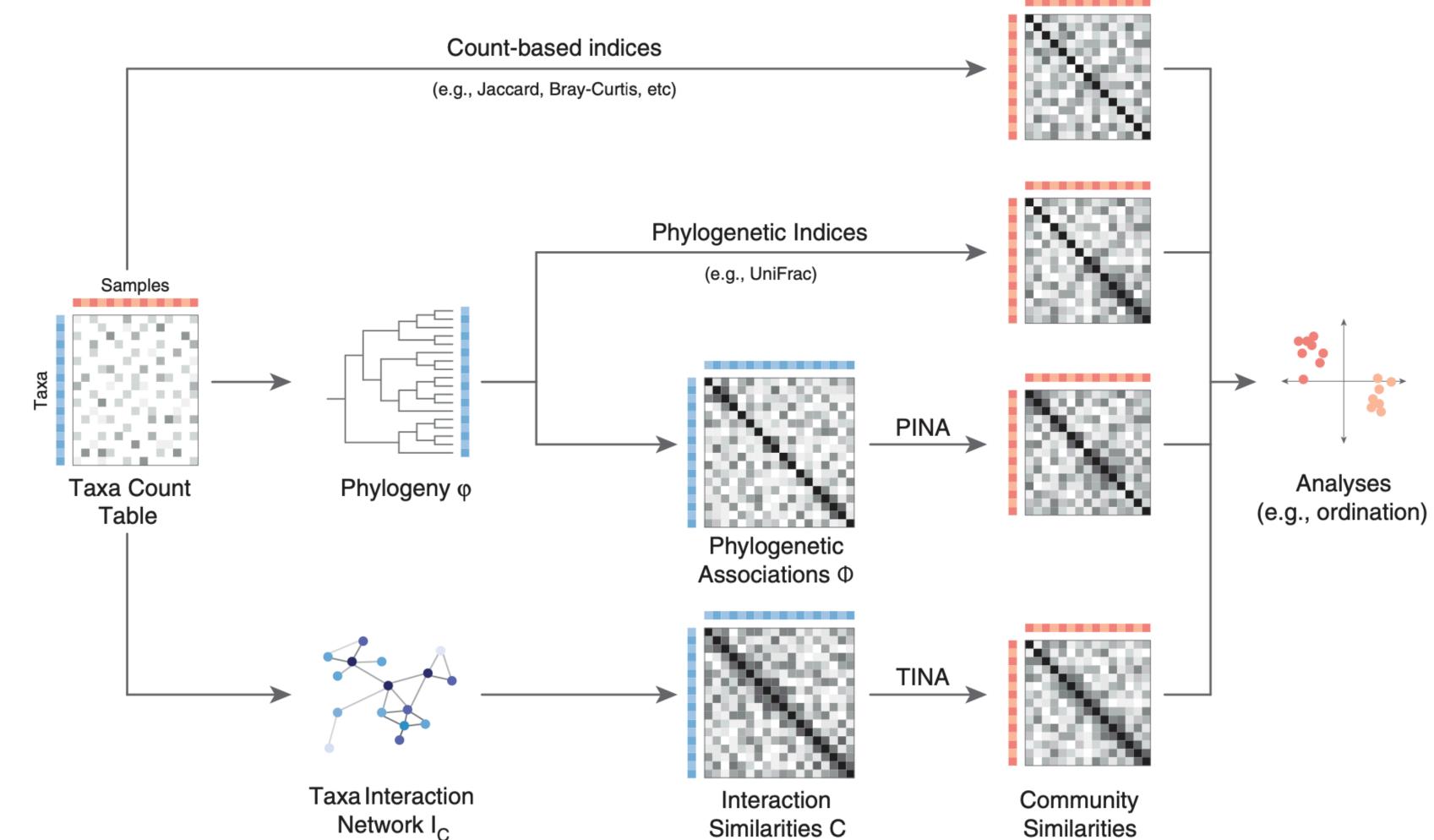
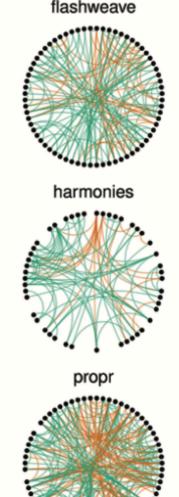


Figure 1 Overview of different approaches to quantifying community similarity. Based on a taxa-sample count table, traditional countbased indices such as Jaccard and Bray-Curtis quantify community similarity from the overlap in taxa composition (upper branch). In contrast, phylogenetic indices such as UniFrac take into account taxa relationships, quantifying community similarity as shared evolutionary history, based on taxa phylogeny (middle branch). Our proposed Taxa INteraction-Adjusted (TINA) and Phylogenetic INteraction-Adjusted (PINA) indices, in contrast, take into account similarities on a taxa co-occurrence network, codified in an interaction similarity matrix C, or in terms of cophenetic phylogenetic distances, represented in a phylogenetic association matrix  $\Phi$ .

А



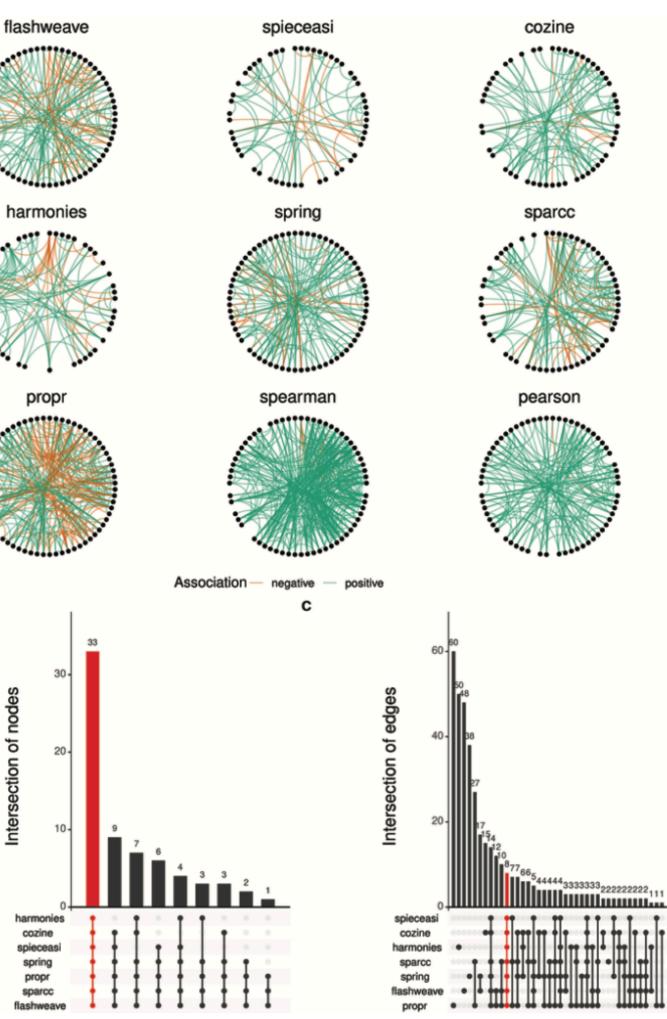


FIG 4 Networks generated using different network inference methods show notable differences in terms of edge-density and connectivity. (A) The nine different networks generated by the different network inference methods (excluding mLDM). The nodes for each network (representing taxa) are arranged in the same positions in a circular layout, and the differences in the connections can be directly visualized and compared. The green links are positive associations, and the orange links represent negative associations. The networks look dissimilar and vary widely in terms of connectivity, and it is notable that the correlation-based methods generally produce networks with higher edge-densities. A threshold of 0.3 was set for the correlation-based methods (sparcc, propr, spearman, and pearson), and a threshold of 0.01 was set for the direct association methods (flashweave, spieceasi, cozine, harmonies, and spring). (B) The node overlap Upset plot indicates that all the networks have a large proportion of common nodes involved in connections (33 out of 68). Conversely (C), the edge overlap Upset plot shows that a very small fraction of these connections are actually shared (8 out of 202). The data used in this analysis were the healthy stool samples from the FMT data set. mLDM is not shown in the comparisons because the algorithm failed to converge for the particular network combination used here (default setting of the MiCoNE pipeline).





8 Computational Biology | Research Article

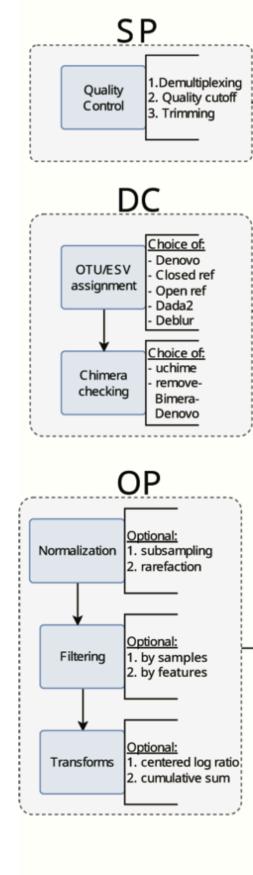
## Inferring microbial co-occurrence networks from amplicon data: a systematic evaluation

Dileep Kishore,<sup>1,2,3</sup> Gabriel Birzu,<sup>4,5</sup> Zhenjun Hu,<sup>1</sup> Charles DeLisi,<sup>1,4,6</sup> Kirill S. Korolev,<sup>1,2,4</sup> Daniel Segrè<sup>1,2,4,6,7</sup>

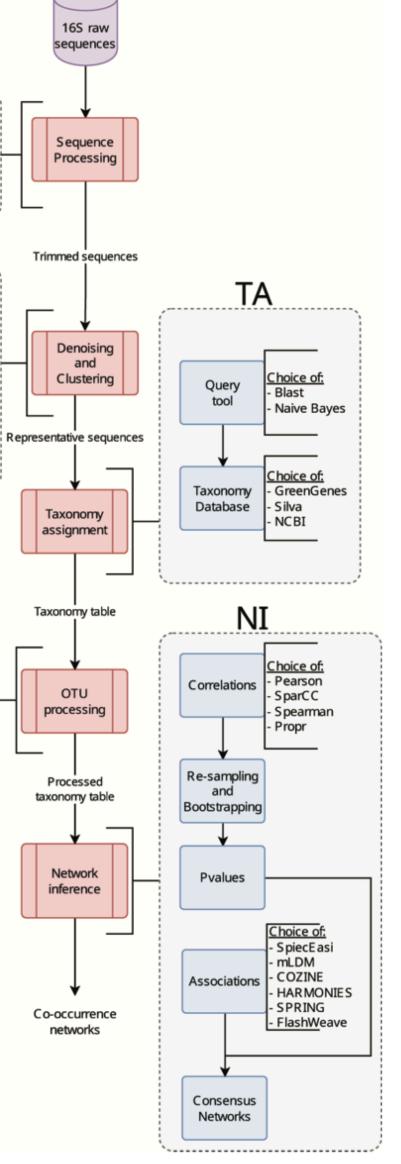
**AUTHOR AFFILIATIONS** See affiliation list on p. 25.







**FIG 1** The workflow of the MiCoNE pipeline. The steps of the workflow can be broken down into five major groups: (SP) sequence processing, (DC) denoising and clustering, (TA) taxonomy assignment, (OP) OTU and ESV processing, and (NI) network inference. Each step incorporates several processes (blue boxes), each of which, in turn, has several alternative algorithms for the same task (indicated by the text to the right of the blue boxes). Each arrow describes the data that is being passed from one step to another. The inputs to the pipeline are 16S rRNA sequencing reads, and the final output is the consensus network generated from the inferred co-occurrence networks. For details on each process and the different outputs, see Methods.



# **Microbiome Datasets Are Compositional: And This Is Not** Optional

## Gregory B. Gloor<sup>1\*</sup>, Jean M. Macklaim<sup>1</sup>, Vera Pawlowsky-Glahn<sup>2</sup> and Juan J. Egozcue<sup>3</sup>

<sup>1</sup> Department of Biochemistry, University of Western Ontario, London, ON, Canada, <sup>2</sup> Departments of Computer Science, Applied Mathematics, and Statistics, Universitat de Girona, Girona, Spain, <sup>3</sup> Department of Applied Mathematics, Universitat Politècnica de Catalunya, Barcelona, Spain



### 1. Current Challenges and Pitfalls in Soil Metagenomics

- rRNA-Based Studies
- Pyrosequencing
- 10. Reducing the Effects of PCR Amplification and Sequencing Artifacts on 16S rRNA-Based Studies
- 11. Batch effects removal for microbiome data via conditional quantile regression
- 12. Parsing ecological signal from noise in next generation amplicon sequencing
- 13. Comparison of Oxford Nanopore Technologies and Illumina MiSeq sequencing with mock communities and agricultural soil
- 14. Insight into biases and sequencing errors for amplicon sequencing with the Illumina MiSeq platform

- ecology
- 19. Analysis of microbial compositions/ a review of normalization and differential abundance analysis
- 20. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data
- 21. Applications and Comparison of Dimensionality Reduction Methods for Microbiome Data
- 22. Broadscale Ecological Patterns Are Robust to Use of Exact Sequence Variants versus Operational Taxonomic Units

- 33. Analysis of compositions of microbiomes with bias correction

## **16s amplicon workflow**

## Or How I Learned to Stop Worrying and Love the Errors

2. Pitfalls in the statistical analysis of microbiome amplicon sequencing data

### 3. Insight into biases and sequencing errors for amplicon sequencing with the Illumina MiSeq platform

4. The choice of the DNA extraction method may influence the outcome of the soil microbial community structure analysis

5. Biases in Prokaryotic Community Amplicon Sequencing Affected by DNA Extraction Methods in Both Saline and Non-saline Soil

6. Examining Sources of Error in PCR by Single-Molecule Sequencing, Reducing the Effects of PCR Amplification and Sequencing Artifacts on 16S

7. Reducing the Effects of PCR Amplification and Sequencing Artifacts on 16S rRNA-Based Studies

## 8. Groundtruthing Next-Gen Sequencing for Microbial Ecology–Biases and Errors in Community Structure Estimates from PCR Amplicon

9. Examining Sources of Error in PCR by Single- Molecule Sequencing

### **15. Ten quick tips for effective dimensionality reduction**

### 16. Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform

17. Handling of targeted amplicon sequencing data focusing on index hopping and demultiplexing using a nested metabarcoding approach in

18. Normalization and microbial differential abundance strategies depend upon data characteristics

## 23. Ranking the biases: The choice of OTUs vs. ASVs in 16S rRNA amplicon data analysis has stronger effects on diversity measures than rarefaction and OTU identity threshold

24. Uniform Manifold Approximation and Projection (UMAP) Reveals Composite Patterns and Resolves Visualization Artifacts in Microbiome Data

## 25. Blocking Factors and Hypothesis Tests in Ecology: Is Your Statistics Text Wrong?

## 26. Contamination in Low Microbial Biomass Microbiome Studies: Issues and Recommendations

## 27. Parsing ecological signal from noise in next generation amplicon sequencing

## 28. Batch effects removal for microbiome data via conditional quantile regression

29. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis

30. Amplicon Sequence Variants Artificially Split Bacterial Genomes into Separate Clusters

## **31.A field guide for the compositional analysis of any-omics data**

32.A family of interaction-adjusted indices of community similarity

34. Rapid Inference of Direct Interactions in Large-Scale Ecological Networks from Heterogeneous Microbial Sequencing Data

## 35. Microbiome Datasets Are Compositional: And This Is Not Optional

36. Microbiome differential abundance methods produce different results across 38 datasets

37. Normalization and microbial differential abundance strategies depend upon data characteristics

38. Improved normalization of species count data in ecology by scaling with ranked subsampling (SRS)/ application to microbial communities 39. From hairballs to hypotheses-biological insights from microbial networks

## 40. Inferring microbial co-occurrence networks from amplicon data: a systematic evaluation

41. Rapid Inference of Direct Interactions in Large-Scale Ecological Networks from Heterogeneous Microbial Sequencing Data

## 42. Phylogenies of the 16S rRNA gene and its hypervariable regions lack concordance with core genome phylogenies



Σφαλματα (	(Errors) που οδηγουν	ν <mark>σε Απατες (</mark> Ι			
Syntactic Processing Errors	Observation Errors	Τυχαια, Ακριβε			
		Συστηματικα,			
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Errors είναι Εσφαλμενες					

ΣΧΟΛΙΟ 1: Λαθος είναι το Σφαλμα που Λανθανει της Προσοχης και για το οποιο δεν εχουμε Επιγνωση

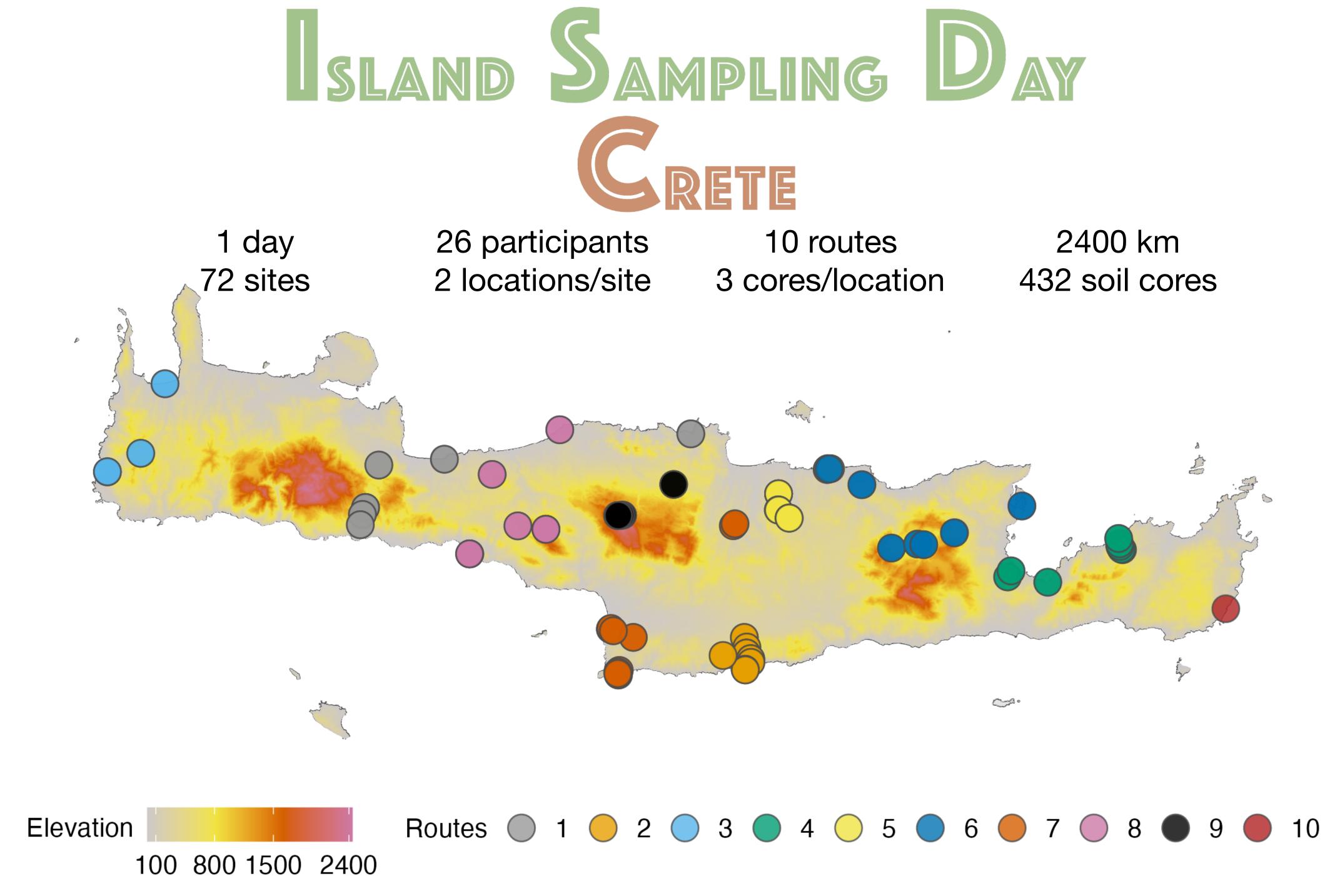
ΣΧΟΛΙΟ 2: Τα Σφαλματα μπορει να ωφειλονται και σε Υλικες Δυσλειτουργιες (Hardware Malfunctions), όπως Brain Deficits (Ανοια, Μωρια, Χημικες Παρεμβασεις-Αλλοιωσεις).

## Delusions) Ατομικες-Συλλογικες

- ειας (Accuracy) Τυπικη Αποκλιση Σαφηνειας (Precision) Εντροπια
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Slide from Prof I. Antoniou







# Island Sampling Day 2016

## One Day, One Island - 15th June 2016, Crete - GSC18





**16s rRNA amplicon study** 26 participants, 10 teams/routes 72 predefined locations across the island of Crete Second time point in 2022, same locations





# Sampling

Topsoil cores 72 sites (2 sub-sites in each) Each sub-site 3 replicates DNA - Chemistry - HCMR Designed for Citizen science by experts Single day event to avoid seasonality Capture as much ecosystem diversity as possible



- **Open data with immediate release**
- Available at ENA project **PRJEB21776**
- 140 samples available (Illumina HiSeq 2500) using Amplicon 16s rRNA V3V4 regions
- 3 Samples NOT sequenced
- Dr Lynn Schriml, University of Maryland

## Sequences

<b>XC</b>		
	ł	
	X	7

Reads	60.6 x 10 <sup>6</sup>	
Ns	76.000	
Filtered	50.1 x 10 <sup>6</sup>	
denoisedF	45.9 x 10 <sup>6</sup>	
denoisedR	48.5 x 10 <sup>6</sup>	
merged	35.0 x 10 <sup>6</sup>	
nonchim	33.3 x 10 <sup>6</sup>	

# Metadata

Onsite measurements GPS coordinates nearest plant Etc

total nitrogen water content total organic carbon pH

Dr Stephanie A Yarwood, University of Meryland

FAIR Data by Design - Findable - Accessible - Interoperable - Reusable



## Biosample: SAMEA104726343

metagenome from Crete soil

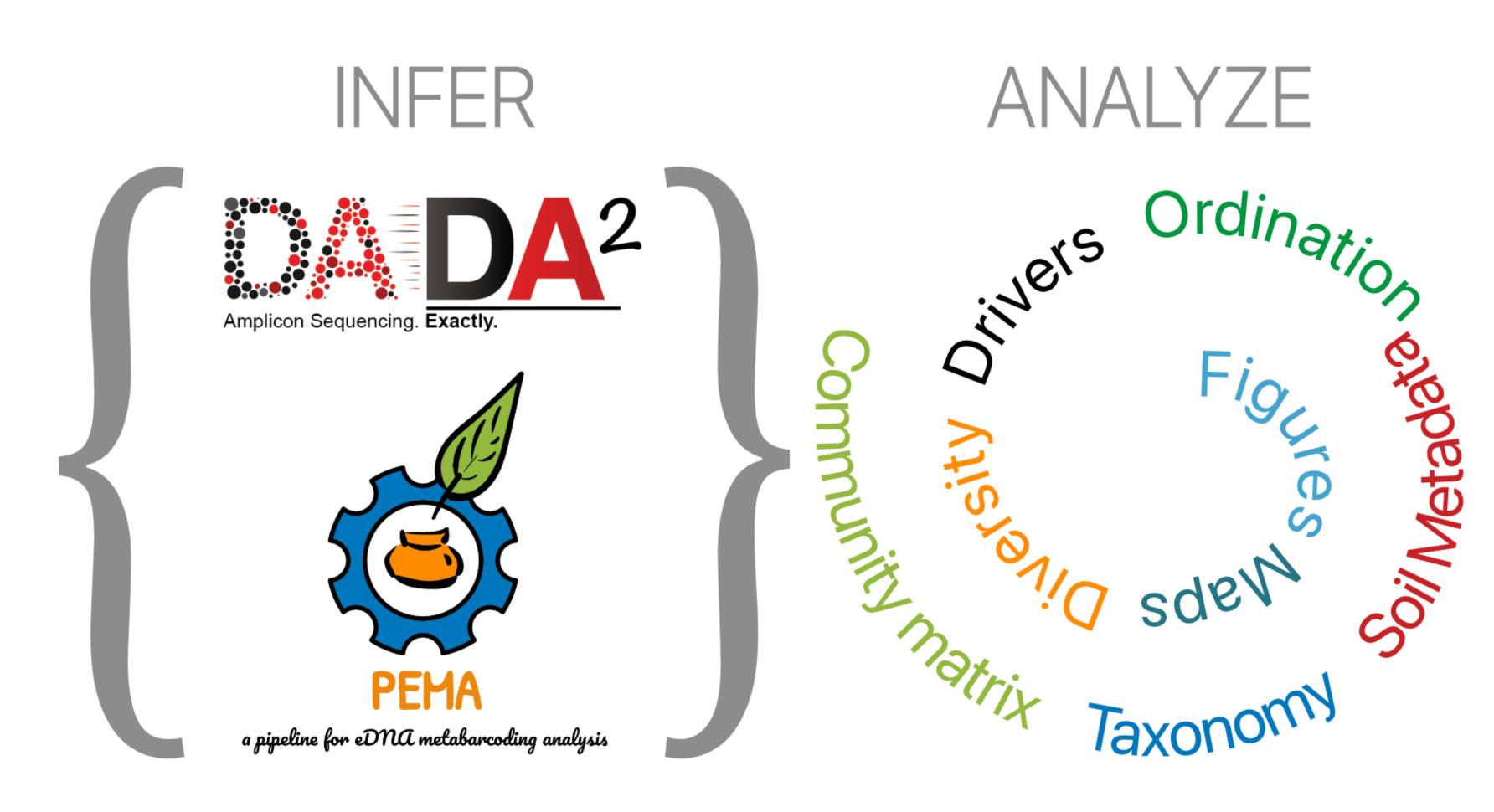
Organism:	soil metagenome	
Sample Accession:	SAMEA104726343	
Sample Title:	Crete soil metagenome	
Location:	35.3518526 N 24.3609229 E	
Center Name:	INSTITUTE FOR GENOME SCIENCES,	
Sample Alias:	3	
Checklist:	ERC000022	
Tag:	terrestrial terrestrial_medium_confidence metagenome datahub	
Geographic Location (Depth):	5 cm	
DNA Concentration:	7.81	
Environment (Material):	soil	
Sample Volume Or Weight For DNA Extraction:	0.2596	

# **ISD Crete - Reproducible analysis**



## **API** sequences (fastq) metadata (xml)





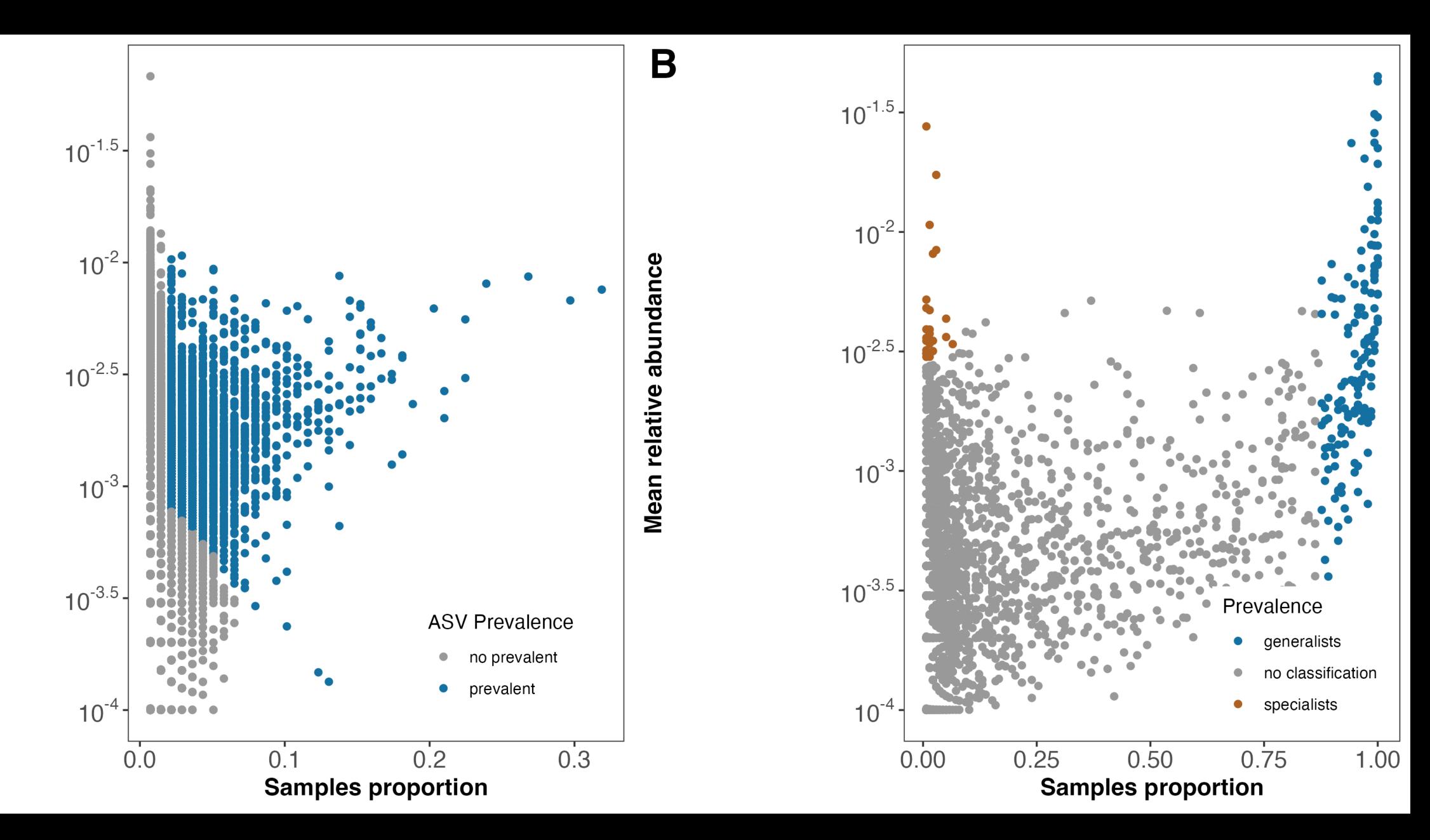
# Taxonomic assignment

## • DADA2

- Taxonomy Silva 138
- ASVs = 239000
- Taxa = 3102
- PEMA (VSEARCH)
  - Taxonomy Silva 132
  - OTUS 6890
  - Taxa = 1057

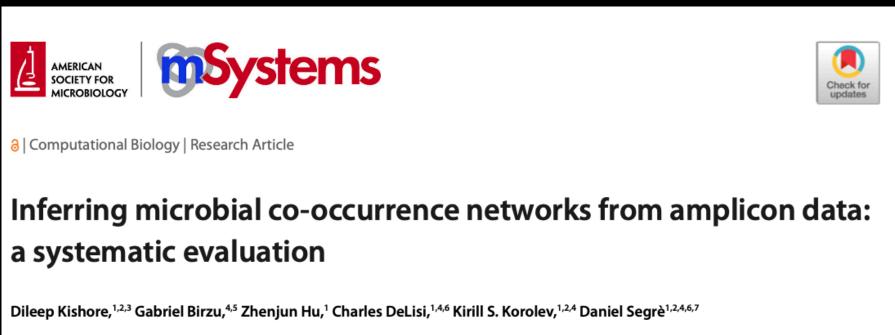
Classificatio depth Kingdom Phylum Order Class Family Genus Species Total

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	9120	1338	44	43
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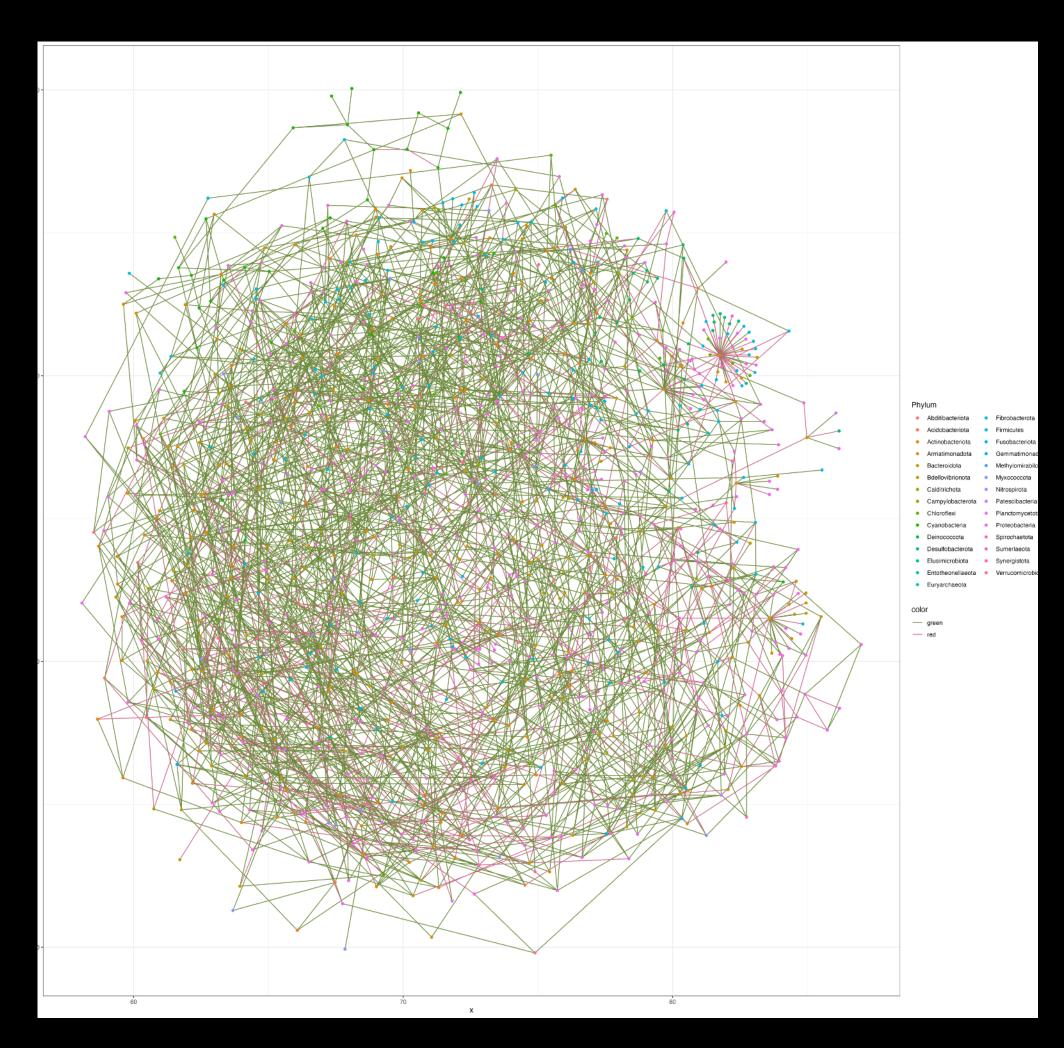


- Network analysis (FlashWeave v0.19.2)
- Focus on Richtis gorge



AUTHOR AFFILIATIONS See affiliation list on p. 25.

## Networks



Bioinformatics. Kacuropies Epjachiov Edgelist long format Wide 62 Metagenomics TTOIS Eiver 20 Episcufa Bapos kotlo2. Kot 603 1 Marablyzi Sample B & S him presabbri pisto Opnic > TL Elso a E180> 0p8. giant romp. tt ready Fiali Q 2 2  $|w\rangle$ 0 a 3 g(x)=F(x) a 0 0 goulga your gle roios a 25 cores - 2 Sample m 0 P=0,04 3 Evandondia apprisés ou Dires Xaos Q A a S