

“Metabolic modeling of species interaction in the human microbiome elucidates community-level assembly rules”

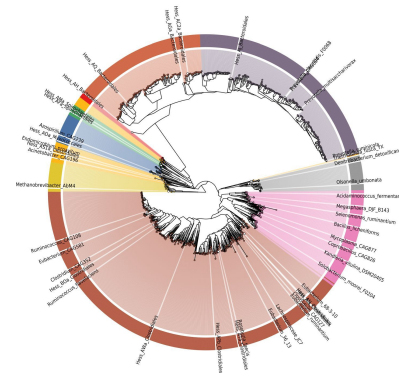
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Agenda

- The problem set definition
- A sketch of what data we need to evaluate and test the problem
- Prerequisites knowledge - Metabolic modeling
- The method
- Results and experiment
- Discussion



Which forces govern the microbiome assembly?

(And how we can distinguish between them?)

Possible forces

- Phylogeny
- Macroecological and environment
 - Sick/Healthy
 - pH level
 - Oxygen content
 - Nationality
- Habitat filtering process - The inability of a species to persist in all environments
- Species interactions
 - Metabolic
 - Signaling

What data we will need to answer

- Species data:
 - Metabolic/nutrition profile
 - Genome sequence
- Community data:
 - Abundance
 - Co-occurrence index
 - Growth rate
 - Phylogeny relatedness
- Macroecology (environment/habitat)
 - Nationality
 - BMI
 - Health - symptoms

The article main focus

Habitat filtering



Species Interaction

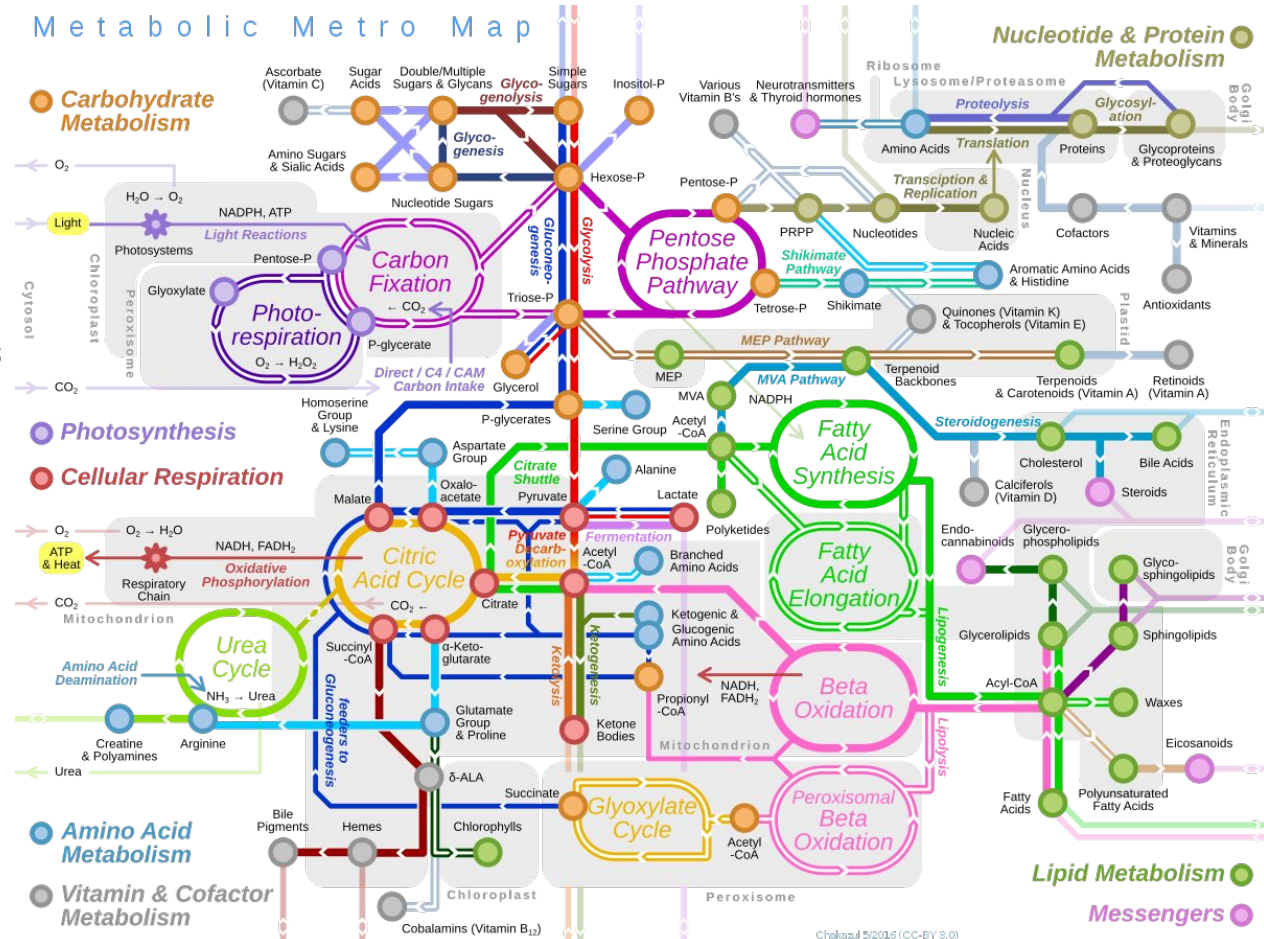
Metabolic relations
Between species

Metabolic modeling

- We will use the Reverse-Ecology Framework to predict species interaction
- This framework takes in Metabolic Networks and extracts information about ecology of microorganisms and their environments

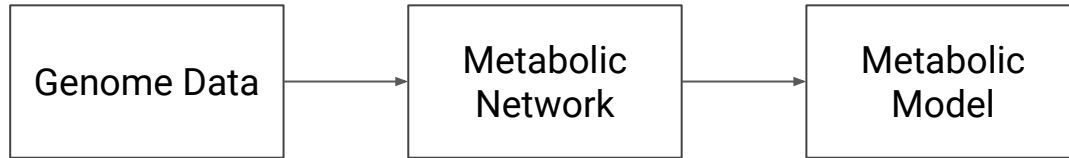
- Metabolic networks

Deriving the biochemical reactions of the organism from its genomes sequencing



Metabolic modeling

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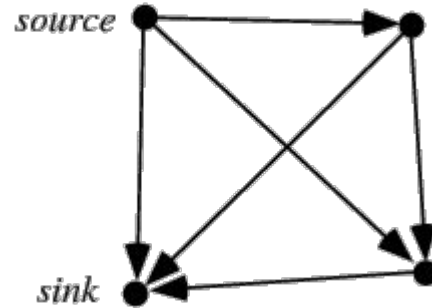


Metabolic modeling - selected model (prev. article)

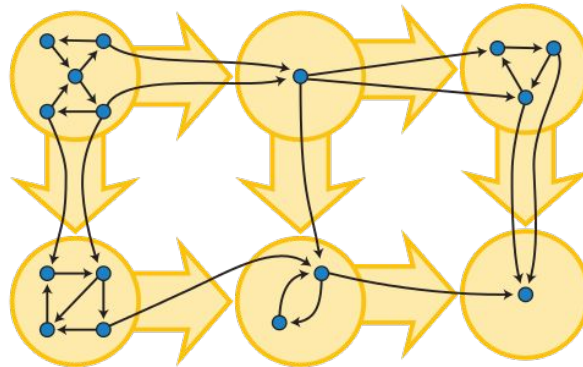
- “Seeds Detection” algorithm
 - Seed - A compound an organism exogenously acquire from its environment
- Graph theory based algorithm

Graph - DAG

- Directed Graph

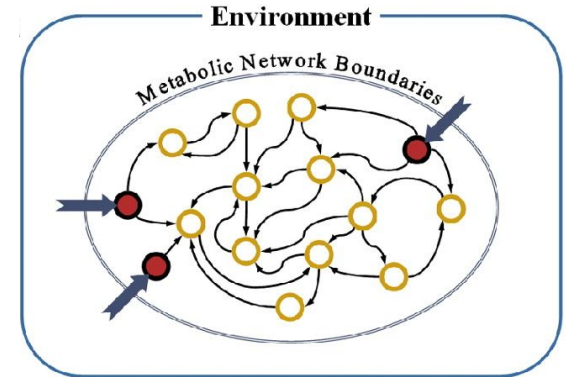
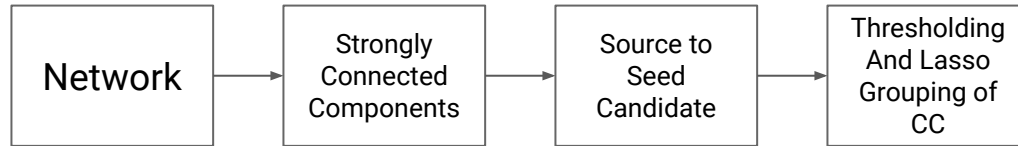


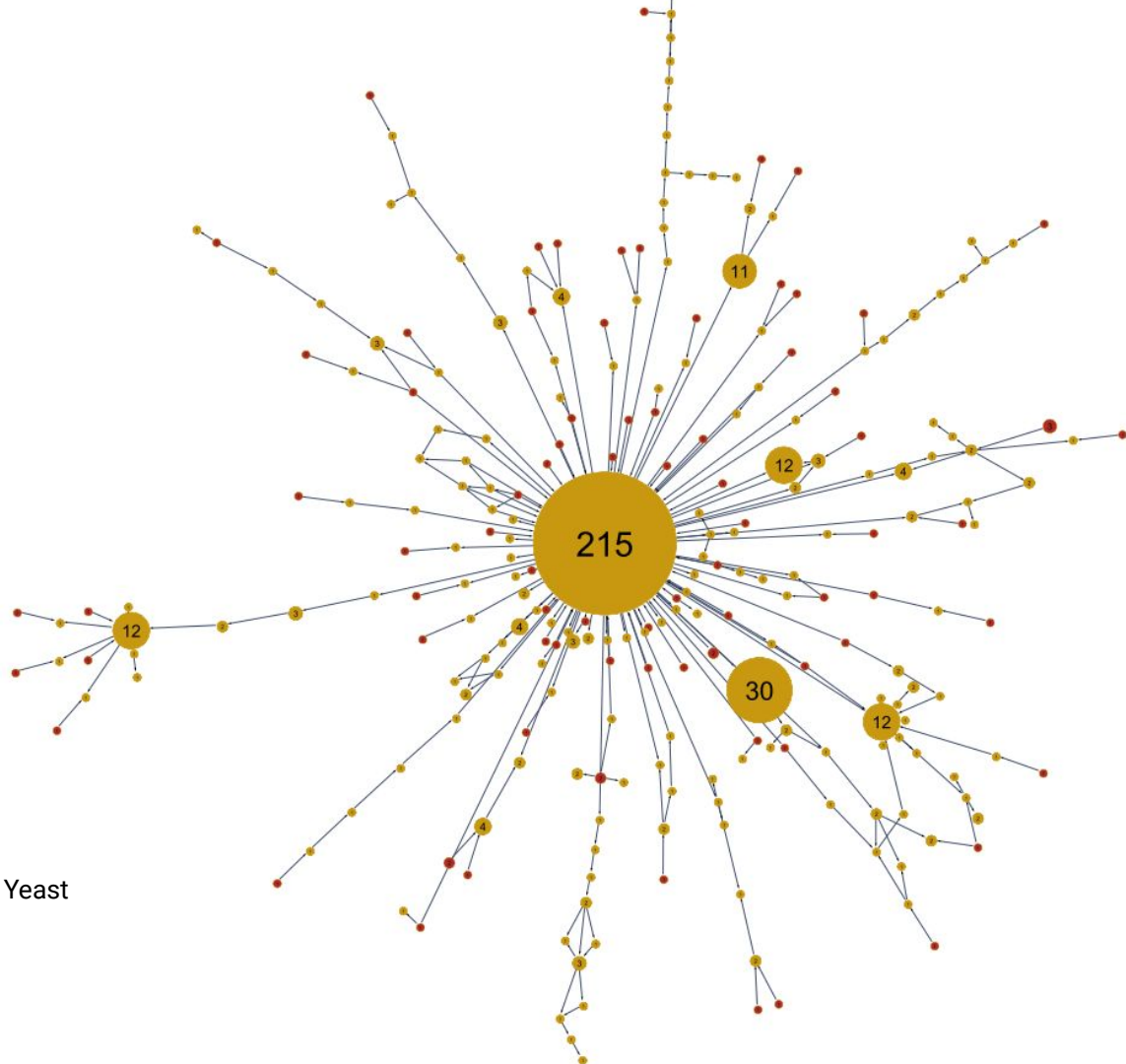
- Strongly Connected Components -> Directed Acyclic Graph



Metabolic modeling - selected model (prev. article)

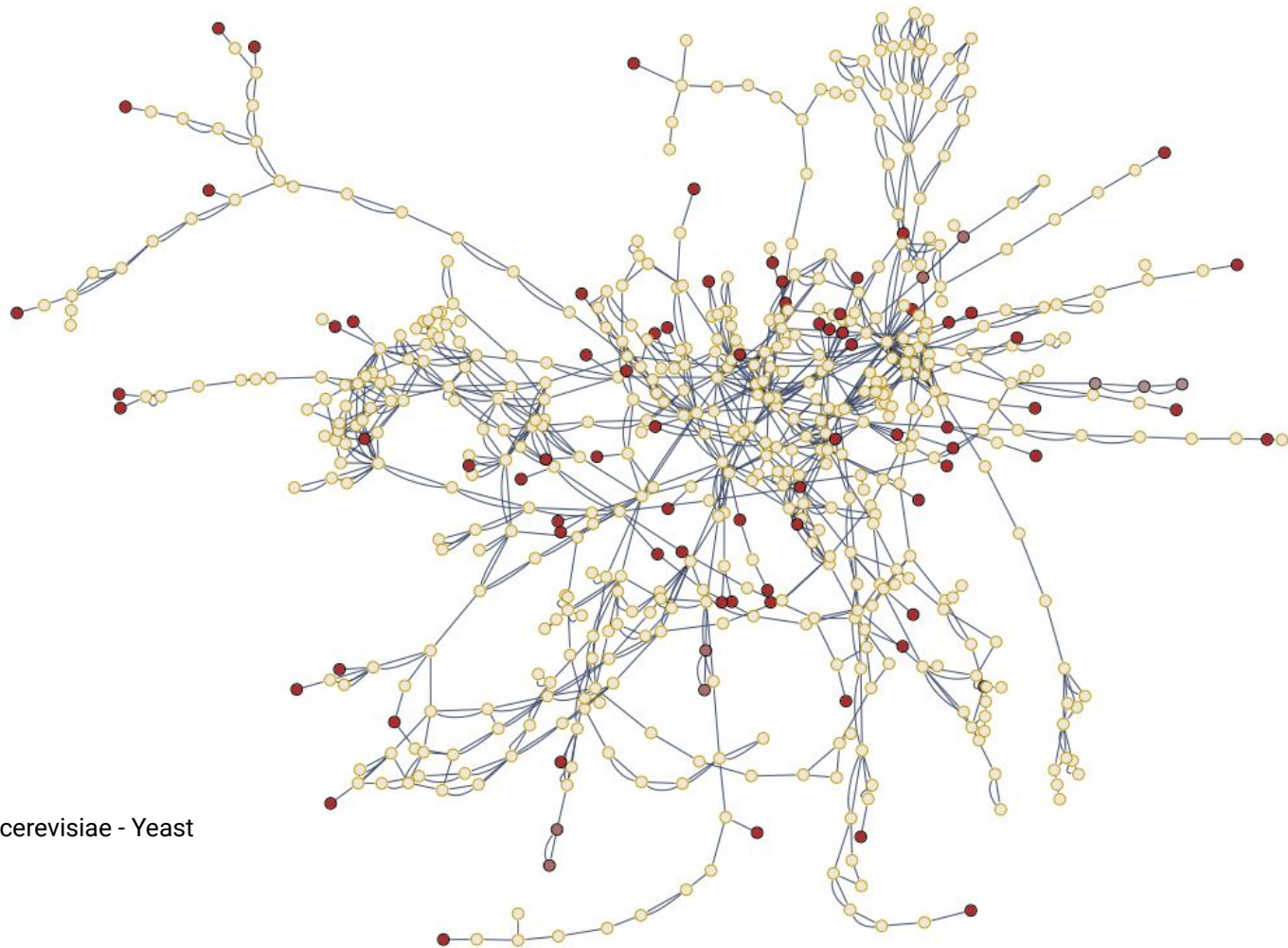
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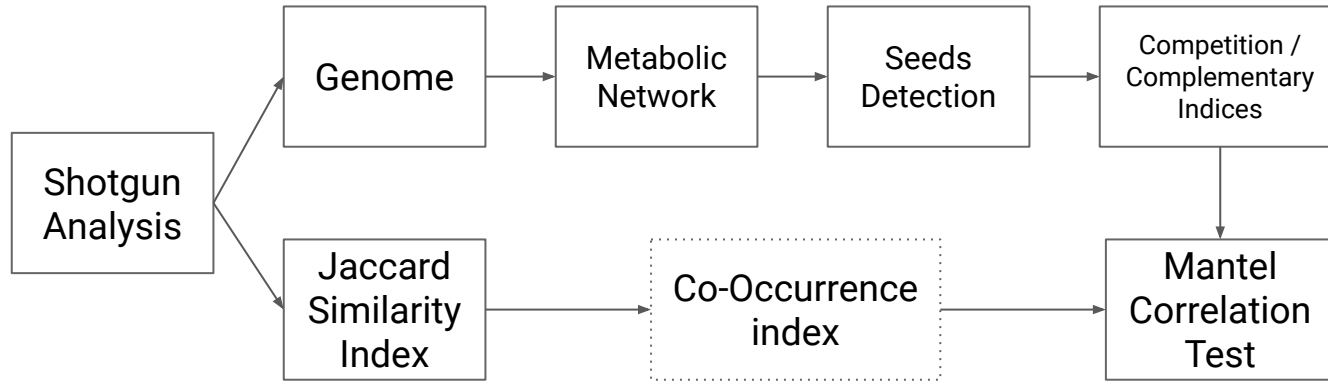
Saccharomyces cerevisiae - Yeast

Saccharomyces cerevisiae - Yeast

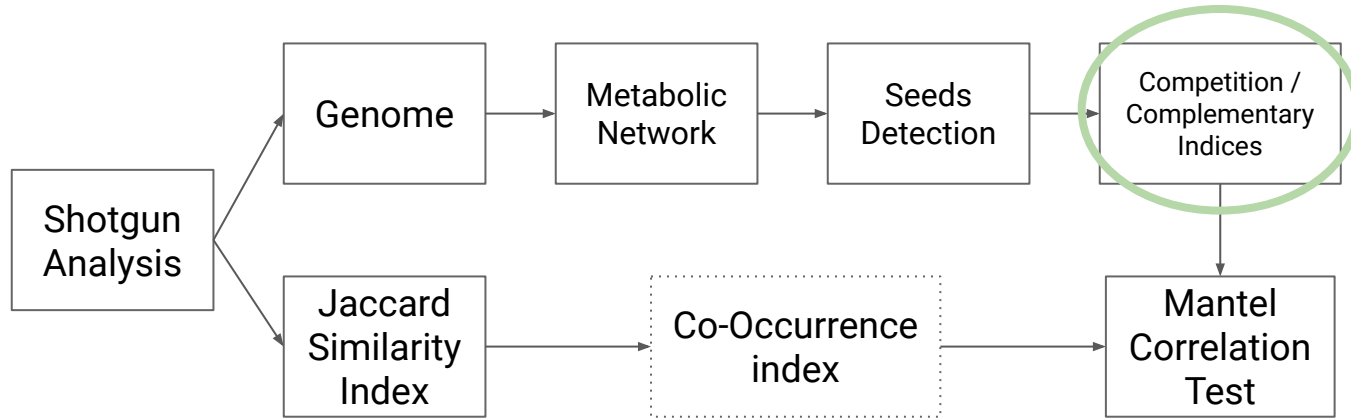


The Method

Reverse-Ecology framework for predicting species interaction



Reverse-Ecology framework for predicting species interaction



Reverse-Ecology framework for predicting species interaction

Competition & Complementary pairwise indices

- A proxy indicating species interacting

Competition Index:

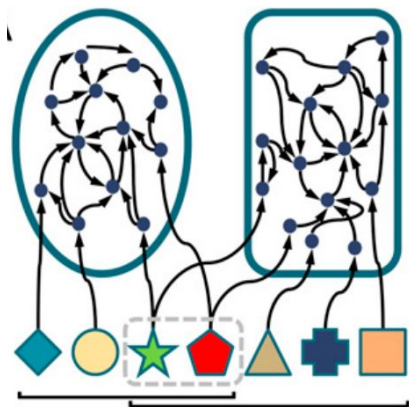
- The fraction of compounds (seeds) in a species' nutritional profile that are also included in its partner's nutritional profile

Complementary Index:

- The fraction of compounds (seeds) in one species' nutritional profile appearing as byproduct from its partner metabolic model

Reverse-Ecology framework for predicting species interaction

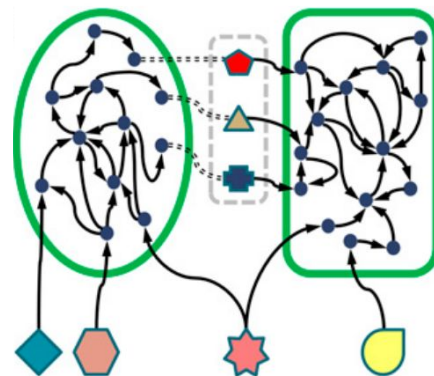
Competition



First relative to second: $2/4$

Second relative to first: $2/5$

Complementary



Second relative to first: $3/5$

Reverse-Ecology framework for predicting species interaction

Partners & Excluders pairwise definition

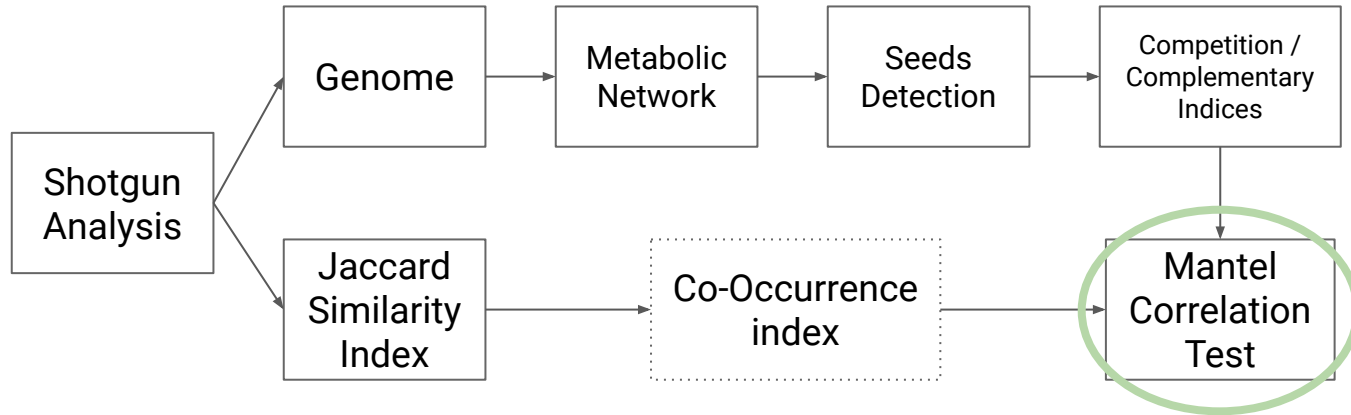
Species' Partners:

- Those 25% of species with which it has the highest co-occurrence index

Species' Excluders:

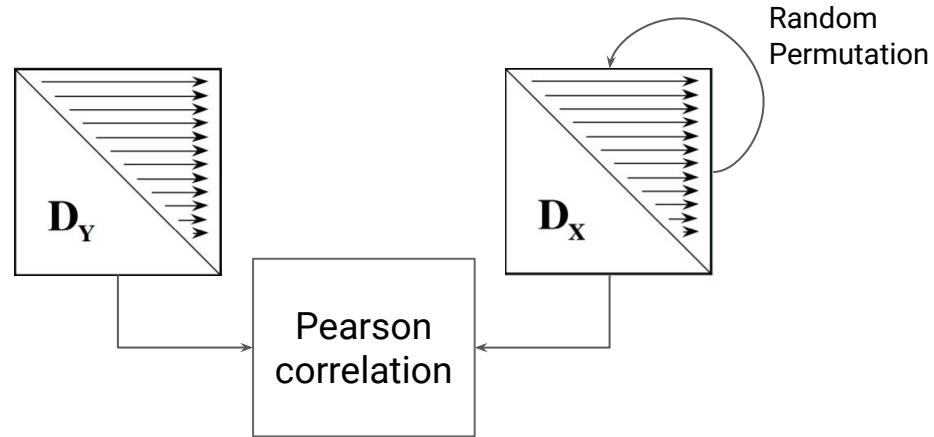
- Those 25% of species with which it has the lowest co-occurrence index

Reverse-Ecology framework for predicting species interaction



Reverse-Ecology framework for predicting species interaction

Mantel correlation test takes two distance matrices



Higher correlation after permutation indicate higher significant score

Experiments and Results



Experiments - Framework validation

Predicted interactions recapitulate species interaction between oral microorganisms

- Well characterized and cultured
- These species interact via signaling as well as metabolic mechanisms
- Colonization pattern - cycle of different organisms
 - For example: Dental Plaque formation
 - Pathogens typically arrive later in the cycle
- Purpose: Examine if complementary and competition indices correlate to the observed cycle in a **fixed environment**



Experiments - Framework validation

Predicted interactions recapitulate species interaction between oral microorganisms

Table S1A. Oral strains analyzed, with genome sequence and characteristic colonization time.

Species	Strain in growth assays	Genome used*	Colonization
Aa	<i>Aggregatibacter actinomycetemcomitans</i> JP2	<i>Aggregatibacter actinomycetemcomitans</i> D7S-1	Late
Ao	<i>Actinomyces oris</i> ATCC 43146	<i>Actinomyces oris</i> K20	Initial
Fn	<i>Fusobacterium nucleatum</i> ATCC 10953	<i>Fusobacterium nucleatum polymorphum</i> ATCC 10953	Middle
Pg	<i>Porphyromonas gingivalis</i> ATCC 33277	<i>Porphyromonas gingivalis</i> ATCC 33277	Early
Sg	<i>Streptococcus gordonii</i> DL1	<i>Streptococcus gordonii</i> str. Challis substr. CH1	Initial
So	<i>Streptococcus oralis</i> 34	<i>Streptococcus oralis</i> SK23, ATCC 35037	Initial
Va	<i>Veillonella</i> sp. PK1910	<i>Veillonella atypica</i> ACS-134-V-Col7a	Early

* The genome sequence used for metabolic network reconstruction. When annotations were not available for the strain used in the original growth assay, the alternative strain listed was used.

Experiments - Framework validation

Predicted interactions recapitulate species interaction between oral microorganisms

Table S1B. Metabolic competition index of human oral species

	Aa	Ao	Fn	Pg	Sg	So	Va
Aa		0.362	0.451	0.313	0.491	0.473	0.357
Ao	0.413		0.393	0.321	0.551	0.51	0.316
Fn	0.432	0.322		0.364	0.466	0.475	0.508
Pg	0.419	0.375	0.484		0.419	0.425	0.405
Sg	0.571	0.571	0.561	0.388		0.847	0.388
So	0.541	0.520	0.577	0.383	0.847		0.408
Va	0.435	0.335	0.652	0.359	0.413	0.435	

Table S1C. Metabolic complementarity index of human oral species

	Aa	Ao	Fn	Pg	Sg	So	Va
Aa		0.161	0.143	0.089	0.089	0.107	0.161
Ao	0.224		0.143	0.143	0.163	0.184	0.204
Fn	0.237	0.169		0.119	0.102	0.119	0.136
Pg	0.310	0.238	0.31		0.19	0.214	0.310
Sg	0.184	0.163	0.122	0.122		0.02	0.143
So	0.163	0.184	0.122	0.122	0.020		0.143
Va	0.196	0.152	0.174	0.152	0.174	0.174	

Experiments - Species assembly reasoning

Examining which forces are the main contributors to the microbiome composition

- Comparing predicted interactions and co-occurrence patterns suggests that Habitat-Filtering shapes the gut microbiome
- Metabolic Interactions of Species' Partners and Excluders
- Habitat Filtering in the Gut Microbiome Cannot Be Explained by the Co-occurrence of Phylogenetically Related Species
- Compositional Shifts Associated with Host Health and Body Mass index Do Not Fully Account for Observed Habitat-Filtering Patterns
- Analysis of Data from the Human Microbiome Project Validates a Habitat-Filtering Model

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Interactions vs Co-Occurrence in Gut Microbiome

- Focused on a set of 154 gut species
- Abundances across 124 individuals were obtained from shotgun metagenomic analysis
- Co-occurrence using Jaccard similarity index
- Predicting interaction by calculating competition and complementary indices
- Using Mantel correlation:
 - $p=0.211, P<10^{-4}$ for Metabolic competition index & Co-occurrence
 - $p=-0.193, P<10^{-4}$ for Metabolic complementary index & Co-occurrence
 - No null models produce higher result than $p=0.211$

Meaning habitat filtering, rather than species assortment, is the dominant structuring force in the intestinal microbiome

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Metabolic Interactions of Species' Partners and Excluders

- Quantitative test on the gut microbiome
- Compared the mean competition and complementarity indices of partners and excluders for each species

Competition index:

- In 82% of species higher with partners than with excluders

Complementary index:

- In 86% of species lower with partners than with excluders

Verified consistency with several ecological labels and species (different environments)

Experiments - Species assembly reasoning

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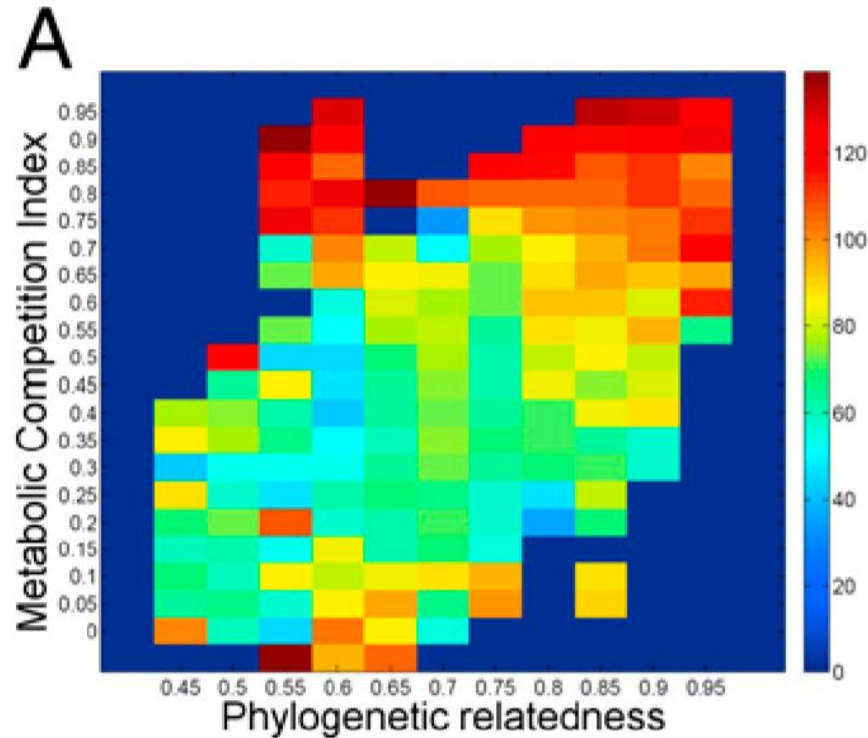
Rule out Phylogenetically Relations

- Studies found phylogenetically related species tend to co-occur in the gut
- Nutritional preferences is strongly linked to phylogeny

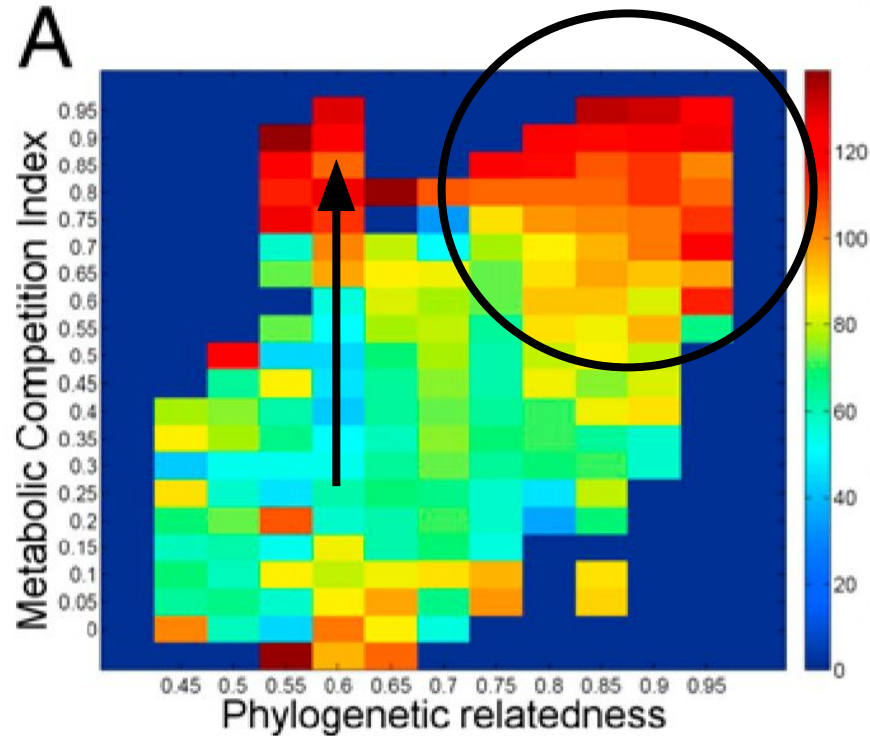
- Purpose: Confirm the association between co-occurrence and nutritional profile overlap is not a simple derivative of phylogenetic relatedness

Rule out Phylogenetically Relations

- Use 16s rRNA sequence similarity to estimate the phylogenetic distance
- Heat is higher co-occurrence



Rule out Phylogenetically Relations



Experiments - Species assembly reasoning

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Macroecological testing

- Previous studies found strong influence of environmental filters and microbiome composition
 - Like obesity and Inflammatory bowel disease (IBD)
- Purpose: Examine effect on habitat filtering pattern we observed
- Divided 124 samples into four groups:
 - Healthy/Lean
 - Healthy/Obese
 - IBD/Lean
 - IBD/Obese
- **Found the same correlations within all groups**

Conclusion and Discussion

Summary and Conclusion

- We established a framework from sequence to metabolic interaction and abundance relations
 - And correlated them
- We saw a positive correlation between competition and co-occurrence
- We saw a negative correlation between complementary and co-occurrence
- We concluded that habitat filtering is the dominant force in microbiome assembly
- We thoroughly examine this correlation by investigating the effect of other known factors to microbiome composition

Discussion points

- Does the scale of data and other missing macroecological labels will change the results?
 - Meaning do we have a bias towards environment that we can reproduce (or culture)
- We still don't know to what extent the species interaction play a role. We saw an example of that in the oral saliva experiment
 - Meaning only metabolic mechanism did not completely explained the colonization cycle
 - And, when looking only at low, or rare compounds, how is the interaction govern the composition
 - And, only metabolic mechanism was examined, and specifically for proving habitat-filtering