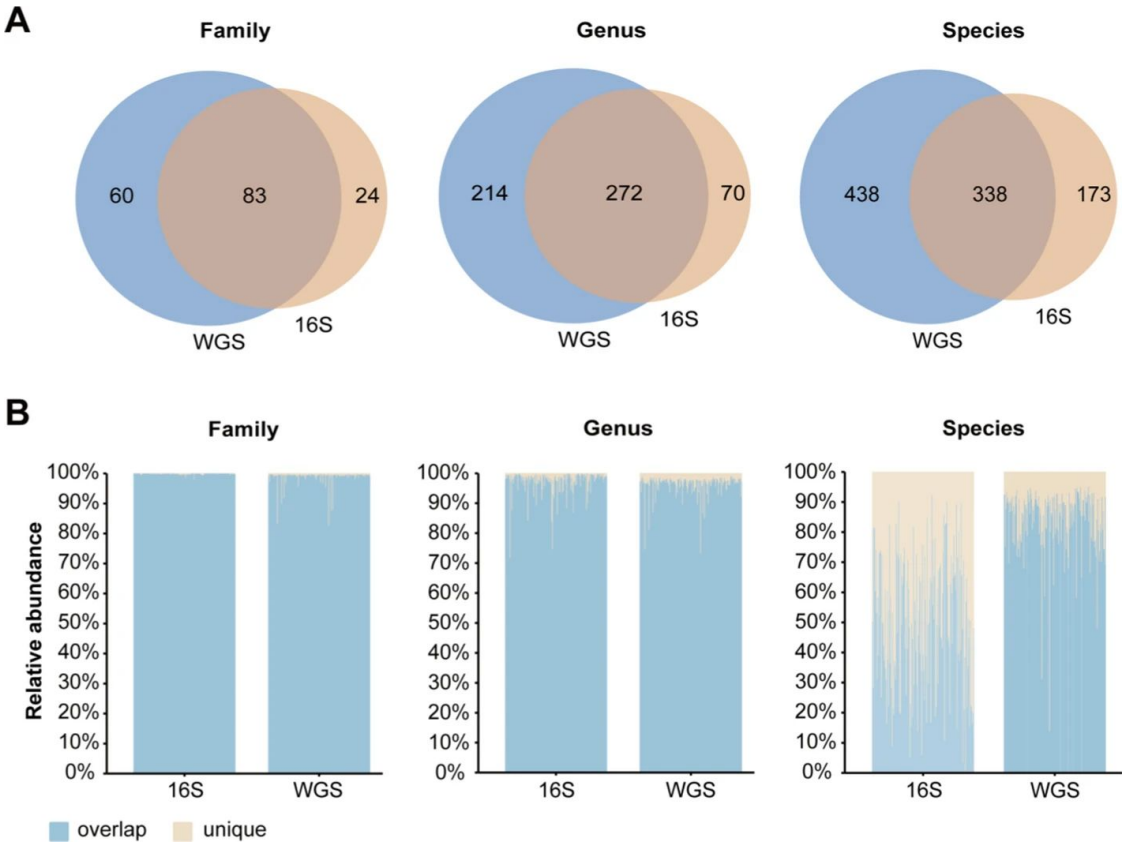
Pairing metagenomic and 16S data for enhanced representations: a proposal

Kevin Chen, November 2025

16S and metagenomic sampling

- Given a sample, there are two different ways to figure out the bacterial composition
- **16S (cheap but low resolution)** sequencing reads the number of 16S sequences once amplified
  - one bacteria can have multiple 16S reads. Usually this is dependent on bacterial species/strain
  - The 16S sequences of some bacteria are more sensitive to being amplified/read
  - Issue: no species resolution
- **Metagenomic (expensive)**
  - read and reconstruct the entire bacterial genome to determine whether a bacteria is present
- Currently, we have been learning with 16S sequence abundance counts



Differences between 16S and metagenomic WGS at multiple taxonomic levels of in-house dataset 1. A Number of overlapped and unique taxa between 16S and metagenomic samples. B Richness of overlapped and unique taxa between 16S and metagenomic samples. https://doi.org/10.1186/s12859-025-06156-7

Why new approach

- “[The 16S gene] alone is not a reliable indicator of the functional ability of a microbiome. [1]
- “16S data may underestimate functional ability of microbiomes, due to strain-level adaptations to local conditions, or overestimate functional ability, due to functional redundancy across taxa.” [1]
- The alternative is metagenomic Sequencing
- New masters thesis appears to show some success applying scGPT (the method we are using) to a smaller dataset (size 13,524) of metagenomic abundances.[2]
    - in contrast, we have been using a dataset of only 16S abundances (size 160,000)
    - They have more success with disease-prediction related tasks, which we are struggling with.

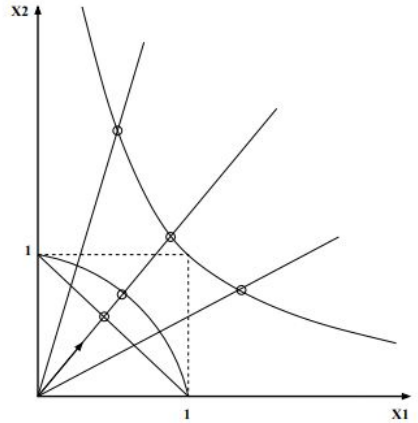
1. https://doi.org/10.3389/fmicb.2020.00101
2. https://dspace.mit.edu/bitstream/handle/1721.1/162973/medearis-medearis-meng-eecs-2025-thesis.pdf?sequence=1&isAllowed=y

Compositionality

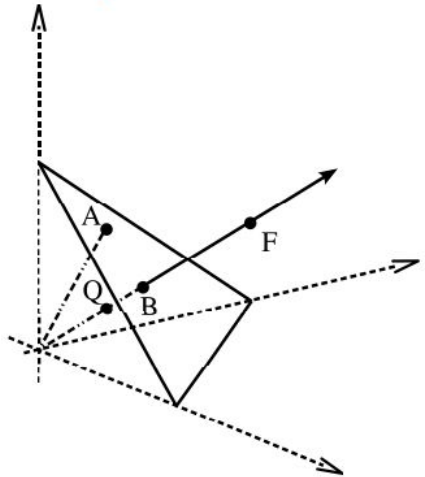
- Both are *compositional*, in this context meaning each datapoint represents ratios and therefore sum to 1
- Mathematically, **the sample space is a simplex**
- A simplex is a finite-dimensional inner-product space (called an Aitchison geometry)
- To convert to real number space, can transform with log operations
  - centre-log and isometric-log ratios are commonly chosen
- However, the data is sparse so this must be dealt with first (through imputation, zero-inflation, or other methods)

Compositionality

definition: parts of some whole which carry only **relative information** $\iff$ compositional data are **equivalence classes**



compositional data in $\mathbb{R}^2$



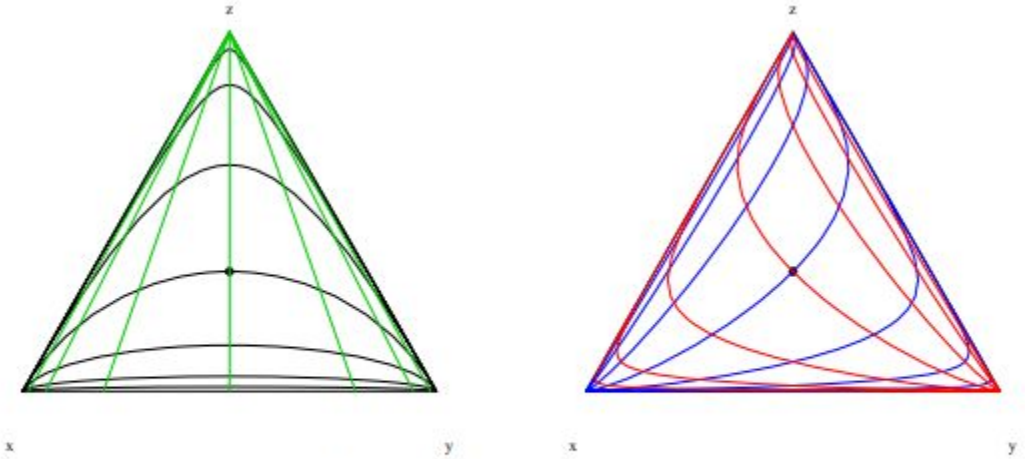
compositional data in $\mathbb{R}^3$

usual representation: subject to a **constant sum constraint**

V. Pawlowsky-Glahn and J. J. Egozcue

https://laboratoriomatematicas.uniandes.edu.co/cursocoda/04-Vera-geometry.pdf

Compositionality



orthogonal grids in $\mathcal{S}^3$, equally spaced, 1 unit in Aitchison distance; the right grid is rotated 45^o with respect to the left grid

https://laboratoriomatematicas.uniandes.edu.co/cursocoda/04-Vera-geometry.pdf

Centre log ratio

Center log ratio transform [edit]

The center log ratio (clr) transform is both an isomorphism and an isometry where $\operatorname{clr} : S^D \to U, \quad U \subset \mathbb{R}^D$

$$\operatorname{clr}(x) = \left[\log \frac{x_1}{g(x)}, \cdots, \log \frac{x_D}{g(x)}\right]$$

Where $g(x)$ is the geometric mean of x. The inverse of this function is also known as the softmax function.

Assumptions

- For a given genus, can we assume the given difference between 16S and metagenomic counts is a constant scaling (assuming sensitivity of 16S and metagenomic are constant but different for different bacteria)?
- In this case, in log space (after a centre-log-ratio or isometric-log-ratio transformation), the counts would be shifted by a constant vector.
- There may be cross-effects. Could be captured by linear model.

Potential Issues with these assumptions

- will have to do more literature search. Linearity may not be a good assumption
- what should we do if this is not a good assumption?

Models

- linear model
- bayesian hierarchical model
- training set: paired 16S and metagenomic representations from same sample

- An issue is that pairs of 16S and metagenomic is not commonly done.
  - dataset size probably only about 400.

Pipeline

- After converting existing 16S data to metagenomic data, rerun model
- Or, somehow integrate this transformation into the model