
MS Thesis - Mid Year Report

Understanding the Evolutionary Trajectories of Microbial Communities

Sabdhayini K B

20201014

Indian Institute of Science Education and Research, Tirupati

Supervisor: Dr. Karthik Raman

Professor, Department of Data Science and AI,

Wadhvani School of Data Science and AI,

Indian Institute of Technology Madras

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Abstract

Microbes are ubiquitously present across all environments and play a critical role in maintaining ecosystem stability and performing essential functions. They often work together as communities, engaging in dynamic interactions with other members present within the community. This complex network of interactions gives rise to the emergent properties that are essential for the normal functioning of the community. Understanding the basic framework and principles by which these microbial communities operate can unlock immense potential in applications across diverse fields, including human health, industrial biotechnology, environmental sciences, agriculture and bio-processing. Such insights can enable us to manipulate microbial communities for desired outcomes like improving health, bioremediation, optimizing industrial production of desired metabolites, etc. This work focuses on understanding the patterns exhibited by microbial communities through in-silico approaches, specifically through community modelling using genome-scale metabolic models. We initially investigate the interaction dynamics between two microbes and how the dynamics change when an additional species is present in the community. Initial analyses suggest that the proportion of negative interactions increases as the community size increases. Future work will extend this exploration to validate the formulated hypotheses and study the effects of other perturbations (removal of metabolites/reactions) on the interaction dynamics, stability and stress responses such as the resistance and resilience of the community.

Keywords: Microbial communities, genome-scale metabolic models, flux balance analysis, interaction dynamics

Report's total word count: 3560

GitHub link to the codes used: github.com/Sabdha07/metabolic_modelling

1 Introduction

1.1 Background

Microorganisms are an inherent part of all ecosystems, co-existing with us on Earth and even in outer space. Microbes rarely work alone (1); instead, they are team players, functioning together as communities. These communities are often highly specific to their environmental niches, making the study of microbial communities across diverse environments essential. Microbial communities exhibit dynamic interactions that drive the community's emergent properties, such as stability, productivity, adaptability, resistance, and resilience to perturbations.(2; 3; 4). A systems-level perspective is hence crucial for understanding the roles of these communities in their environments and the principles underlying their dynamics. By understanding such principles, we can gain insights into their natural functioning and manipulate them to achieve desired outcomes. For instance, microbial communities can be manipulated to transition systems from diseased to healthy states, polluted environments to clean environments, or infertile to fertile soils(5).

While experimental approaches to studying microbial communities are indispensable, they are often time-intensive. Computational and mathematical methods are a time-efficient alternative for studying microbial systems across different conditions and environments, allowing the abstraction of the various aspects of the biological system under study. These methods can enable the focus on specific components in detail, which is not possible in the case of experimental approaches.

One of the widely used computational approaches to study the metabolic aspect of microbial systems is the use of genome-scale metabolic models(GEMs)(6). This approach offers a metabolic perspective of the system, enabling researchers to derive meaningful insights into community properties and the patterns that they exhibit.

This work employs in-silico simulations to study the dynamics and stability of microbial communities, with a focus on gut microbes. Using genome-scale metabolic models, we initially simulate the

growth of two-membered microbial communities and analyze their interaction dynamics to identify common patterns across different communities. This study seeks to understand the fundamental properties of microbial communities, highlighting how the interaction dynamics evolve under perturbations. Additionally, checking whether these patterns are specific to the gut microbes or reflect universal trends observed in microbial communities across diverse environments would be an exciting direction to study.

1.2 Research Questions

This study's primary focus is to explore the evolutionary trajectories of microbial communities. Specifically, our goal is to:

1. Investigate how the interactions between species in the community change upon perturbations over time.
2. Examine how the community properties, such as stability, stress tolerance - resilience, and resistance, change in response to perturbations.
3. Analyze how these properties and dynamics vary across microbial communities of different sizes and compositions.

1.3 Review of Literature

1.3.1 Microbial Communities

Microbial communities are specific and sometimes unique to the environments in which they exist and play a vital role in the essential functioning of the ecosystem. Studying these communities has become necessary to understand their stability, dynamics, and their role in maintaining human health, agriculture, and other areas(5; 7). Gut microbes, in particular, have been a major area of study due to their direct effects on human health(7). The initial part of this work focuses on understanding the patterns observed in gut microbial communities.

1.3.2 Genome-scale Metabolic Models and Flux Balance Analysis

Genome-scale metabolic models(GEMs) are curated mathematical representations of the metabolic networks of individual species (microbes) constructed over genomic annotations from the literature. The models represent an organism's metabolic network using a stoichiometric matrix (S) and a flux vector(v)(6). Each column of the matrix corresponds to the reactions in the network, while the rows correspond to the metabolites. An entry in the matrix S_{ij} denotes the stoichiometric coefficient for the metabolite i in reaction j.

Flux Balance Analysis(FBA)(8) is a constraint-based computational approach widely used to analyze the metabolic networks in microbial systems. FBA optimizes a biological objective function, which is typically the microbial growth(biomass function - v_{gro}) while calculating the flow of metabolites - the fluxes of the reactions under the given constraints. FBA assumes a steady state of the system considered, and this assumption pertains to the idea that the concentrations of the metabolites in the system, on average, do not change over time. This steady-state assumption implies linear constraints on the fluxes of the reactions in the system while optimizing(maximizing (maximizing v_{gro}) for the objective such that

$$Sv = 0 \quad (1.1)$$

Performing FBA translates to solving a set of linear equations while optimizing for a particular objective function(s). However, in contrast to the steady-state assumption, biological systems are rarely in steady-state. In order to overcome this limitation, an extended version of FBA called dynamic FBA was developed, which incorporates time-dependent changes. dFBA has two implementation approaches - the dynamic optimization approach(DOA) and the static optimization approach(SOA)(9). Given the time duration of the study considered, DOA integrates over the whole time interval for the flux values and the metabolite concentration, becoming a computationally intensive task as the linear problem of FBA now becomes a non-linear problem to be solved.

Meanwhile, the SOA approach divides the simulation time frame into small intervals, solves ODEs for the nutrient concentration and microbial growth and performs FBA at the beginning of each interval. This approach maintains the optimization of the biomass and other fluxes as a linear problem. Since we want to explore the evolutionary trajectories of microbial communities, we use the static optimization

approach(SOA) of dFBA for the simulations, which is computationally less expensive than DOA.

1.3.3 Metabolic Modelling of Microbial Communities

The development of GEMs has significantly enhanced the metabolic modelling studies of microbial communities, as they offer an in-silico approach to understanding these interesting communities and patterns which can be missed out on in experimental setups. Though GEMs fail to capture the regulatory and other inherent aspects of microbial systems, they still have been proven to be instrumental in understanding community dynamics, stability, and interspecies interactions, with experimental validation reinforcing their utility in different environmental settings and scales.(10; 11; 12)

Several tools and databases facilitate the metabolic modelling and analysis of microbial communities. Some of them are the COBRA Toolbox(MMT)(13), COBRApy(14), MICOM(15), BacArena(16), COMETS(17), etc. Publicly available repositories like BiGG(18), AGORA(19; 20), ModelSEED(21), etc., contain curated GEMs of microbial species. This work uses models from the AGORA database, which houses GEMs for gut microbes.

Metabolic modelling can be used to simulate the growth and behaviour of entire microbial communities, moving beyond the simulations of individual organisms. These approaches enable the exploration of the metabolism of the species, in addition to growth(22) and other dynamics(23; 24; 25; 26). Metabolic modelling can help predict unique interactions in microbial communities existing in extreme environments(26), understand the stress tolerance properties of communities(27) and much more.

1.3.4 Interaction Dynamics in Microbial Communities

The structure, stability, and function of microbial communities are determined mainly by the interaction dynamics between the species in the system. A lot of internal (nutrient requirement - competition for resources) and external (environmental conditions - biotic and abiotic) factors affect these dynamics, making the interactions between members plastic, which helps the system as a whole to adapt to changes(28). Most interactions in bacterial communities have been observed to be polarized towards the two extremes - competition for the limited available resources and space and cooperation through crossfeeding(27; 29). So, a basic understanding of the underlying principles that govern these dynamics and the patterns exhibited can be translated into practical applications(30). Some interesting directions

we focus on in this work are understanding whether the microbes primarily cooperate or compete, how interactions change over time, and what influences the changes in interaction types.

Most studies on interaction dynamics focus on the interactions between a pair of species and do not account for higher-order interactions. However, analyzing higher-order interactions can provide new insights and enhance our theoretical understanding of how communities operate(3). While this work currently focuses on pairwise interactions in microbial communities, the future directions of work will involve looking at higher-order interactions to assess whether the patterns observed in pairwise interactions can be extrapolated to higher-order complex interactions.

2 Methodology

2.1 Interaction Dynamics of Two-membered Communities

2.1.1 Selection of Genome-Scale Metabolic Models and FBA

Initially, to familiarize myself with the tools, I followed a previous work(31) that focused on studying the evolution of interactions in two-membered communities where the notion of evolution was through successful swapping of reactions. However, I focused on studying the evolution of interactions as the community size increases from two to three by adding a new species to the two-membered communities.

Deriving from the previous work, the same 20 models of gut microbes from the AGORA 1 database(19) were used to perform community modelling. All possible two-membered communities($\binom{20}{2} = 190$) and three-membered communities($\binom{20}{3} = 1140$) of the 20 microbes were simulated in MATLAB using the *cFBA(createMultipleSpeciesModel)* function in the COBRA Toolbox(13). The communities were simulated to grow on a Western diet(19) in anaerobic conditions. The monoculture growth was also simulated for all species to serve as a baseline for further calculations and analyses.

2.1.2 Prediction of Interaction Type

The interaction type between two species can be predicted from their biomass values in the community and the monoculture biomass values. We calculate the relative change in biomass value for each species using the formula below:

$$\alpha_i = \frac{X_{i(\text{community})} - X_{i(\text{monoculture})}}{X_{i(\text{monoculture})}} \quad (2.1)$$

Based on the relative change in biomass values - alpha value of the two species in the pair, we classify the pairwise interaction into one of six types: amensalism(-/0), commensalism(+/0), neutralism(0/0),

competition(-/-), parasitism(+/-) and mutualism(+/+) as described in Table 2.1.

Condition on Alpha1	Condition on Alpha2	Interaction Type
$-10 \leq \alpha_1 \leq 10$	$\alpha_2 \leq -10$	Amensalism
$\alpha_1 \leq -10$	$-10 \leq \alpha_2 \leq 10$	
$-10 \leq \alpha_1 \leq 10$	$\alpha_2 \geq 10$	Commensalism
$\alpha_1 \geq 10$	$-10 \leq \alpha_2 \leq 10$	
$\alpha_1 \leq -10$	$\alpha_2 \geq 10$	Parasitism
$\alpha_1 \geq 10$	$\alpha_2 \leq -10$	
$\alpha_1 \leq -10$	$\alpha_2 \leq -10$	Competition
$\alpha_1 \geq 10$	$\alpha_2 \geq 10$	Mutualism
$-10 \leq \alpha_1 \leq 10$	$-10 \leq \alpha_2 \leq 10$	Neutralism

Table 2.1: Classification of pairwise interaction type based on alpha values

2.2 Interaction Dynamics between Pairs of Different Jaccard Distances

2.2.1 Selection of Genome-Scale Metabolic Models

One hundred models of gut microbes were selected from the AGORA 2 database(20). The Jaccard distance(32) between two given species is calculated using the reactions of the models based on the following formula:

$$\begin{aligned}
 \text{Jaccard Distance}(M_i, M_j) &= 1 - \text{Jaccard Similarity}(M_i, M_j) \\
 &= 1 - \frac{|\text{rxns}(M_i) \cap \text{rxns}(M_j)|}{|\text{rxns}(M_i) \cup \text{rxns}(M_j)|}
 \end{aligned} \tag{2.2}$$

After calculating the Jaccard distance between every two species in the pool of the hundred models, the pairs were binned into three categories(high distant pairs, mid-distant pairs and low distant pairs) based on their Jaccard distance as described in Table 2.2. The top 20 in each category were considered for further analysis. In addition to the three categories, 20 more communities were randomly sampled from all possible two-membered communities.

Category	Description
High Distant Pairs	Top 20 pairs with the highest Jaccard Distance ($JD > 0.8$).
Low Distant Pairs	Top 20 pairs with the least Jaccard Distance ($JD < 0.2$).
Mid Distant Pairs	20 pairs in the middle of the Jaccard Distance distribution ($JD \approx 0.6$).
Random Pairs	20 pairs sampled randomly from all possible pairs.

Table 2.2: Binning pairs based on Jaccard distance.

2.2.2 Dynamic FBA and Prediction of Interaction Type

The selected pairs were simulated using COMETS(Computation Of Microbial Ecosystems in Time and Space)(17), which implements the Static Optimization Approach(SOA) of dynamic FBA. The communities were simulated for 24 hours on a Western diet(19) under anaerobic conditions. In addition to the two-membered communities, monoculture simulations were also run with an initial biomass of 0.01 gDW of each species. Then the interaction type for each pair was predicted using the same alpha value-based approach described earlier.

2.2.3 Selection of the Third Member:

After the pairwise simulations, a third member was introduced to each community to explore how the interaction type between two species changes when a third species is present. The third species for each pair was selected based on specific constraints(Table 2.3), ensuring that the communities in each category still exhibited a distinct difference from the other categories.

Category	Average JD between all species
High Distant Pairs	$JD > 0.73$
Low Distant Pairs	$JD < 0.23$
Mid Distant Pairs	$0.55 < JD < 0.65$
Random Pairs	random

Table 2.3: Addition of third species to the existing pairs based on Jaccard Distance.

Then, the three-membered communities were simulated for 24 using the same parameters as the two-membered community simulations.

3 Results and Discussion

3.1 Interaction Dynamics of Two-membered Communities

3.1.1 Interaction Types

In this study, 20 models spanning four major phyla that are commonly observed in the gut microbiome, namely Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (Table 3.1), were used.

Phylum	Count
Firmicutes	9
Proteobacteria	5
Actinobacteria	3
Bacteroidetes	3
Grand Total	20

Table 3.1: Phylum count

All possible two-membered and three-membered communities were simulated and analyzed to understand the interaction dynamics between all two species in a given community and the impact of community size on the interaction dynamics. Parasitism was the most frequently observed interaction type across two-membered and three-membered communities (>50%) as can be seen from figure 3.1.

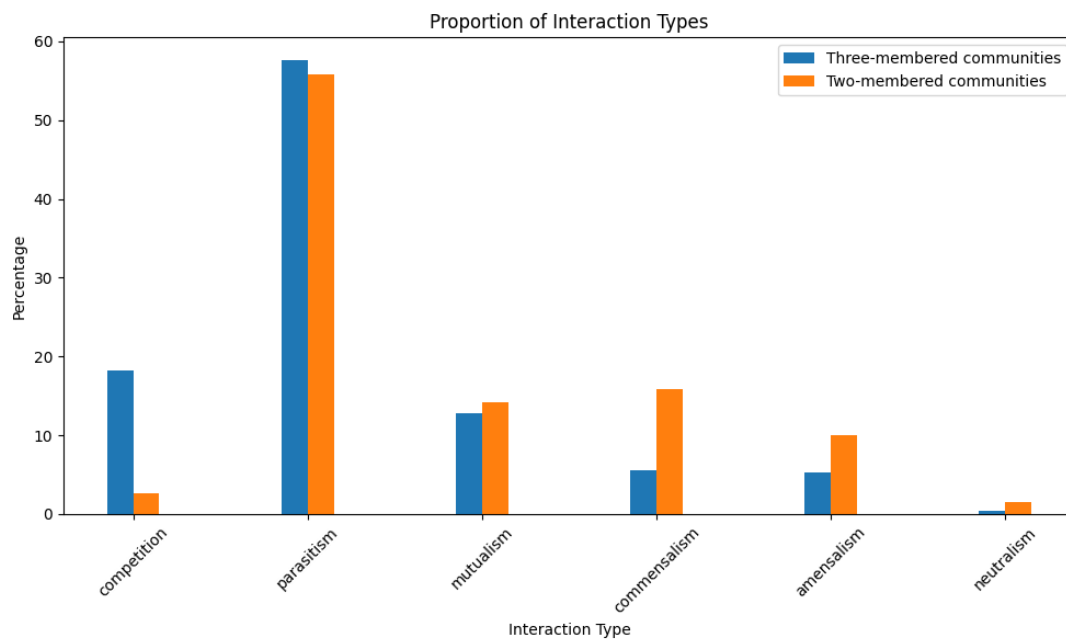


Figure 3.1: Interaction type in two and three-membered communities

This observation aligns with the general pattern observed in most microbial pairs: negative interactions dominate pairwise interactions(1), (33). Notably, the addition of a third member did not alter this pattern.

3.1.2 Interaction Switch in Response to the Addition of a New Species

To study the effect of the presence of a third member in the community on the existing pairwise interaction, we analyzed the change in interaction types between two and three-membered communities. The heatmap is shown in figure 3.2.

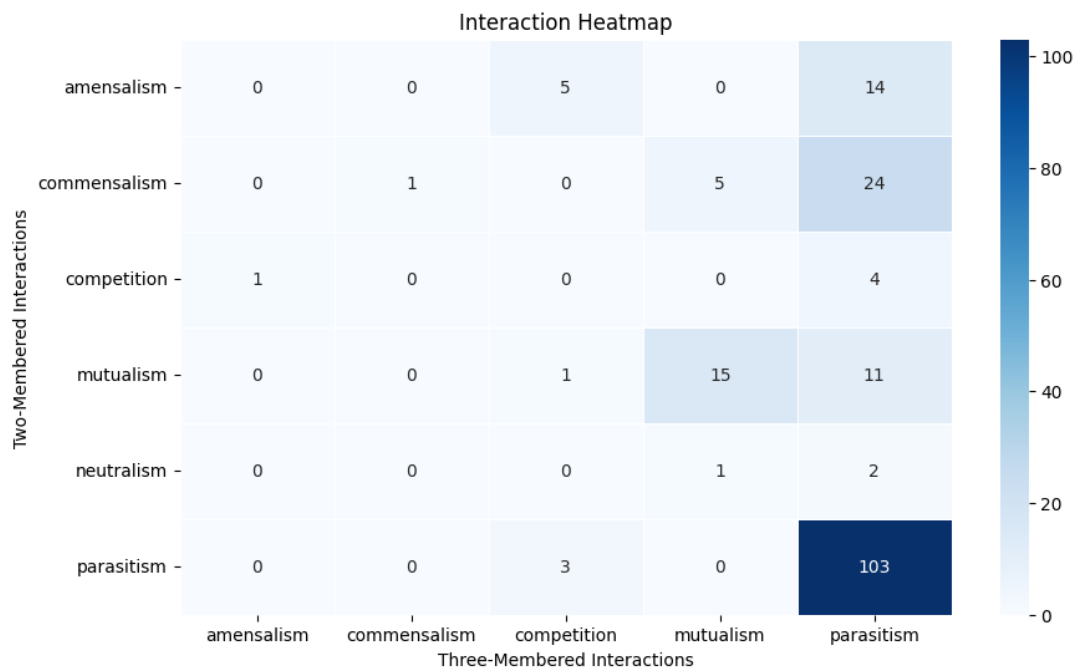


Figure 3.2: Interaction type switch - combined

Parasitism, followed by mutualism, remained the most consistent interaction between pairs, even after adding a new species. Interestingly, a major chunk of pairs exhibiting amensalism and commensalism switched to parasitism with the introduction of the third species. This suggests that adding a new species to a microbial community tends to increase negative interactions, as the existing nutrients must be shared with many more companions.

3.1.3 Discussion

Based on the observations, two hypotheses can be put forward:

1. As the community size increases, the proportion of negative interactions will increase.
2. Adding a new species to the community does not majorly alter the interaction dynamics but does change some of the neutral or positive interactions into negative ones.

However, it is essential to note that a steady-state FBA approach was used for these simulations. This assumption of a steady state might not hold true in real-world scenarios. A problem with the extension of these simulations to larger community sizes was that as the species number increased, FBA tried to maximize the objective function of all the members, eventually leading to some species having zero growth, which might not always be the case in microbial communities. One possible way to overcome

this hurdle is to use dFBA to simulate larger community sizes. This idea will be considered and taken forward in the future work.

3.2 Interaction Dynamics between Pairs of Different Jaccard Distances

3.2.1 Average Community Biomass over Time

A hundred models from AGORA 2 spanning Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, Fusobacteria and Tenericutes (table 3.2) were taken, and the Jaccard distance between every two species was calculated.

Phylum	Count
Firmicutes	48
Proteobacteria	18
Actinobacteria	18
Bacteroidetes	10
Fusobacteria	4
Tenericutes	2
Grand Total	100

Table 3.2: Phylum count

Based on the Jaccard distance, the pairs were binned into three categories (see methods). Additionally, 20 two-membered communities were randomly sampled from all possible pairwise communities. All 80 communities were simulated using dFBA for 24 hours on Western diet using COMETS. The average community biomass for all pairs in each category was calculated and plotted (figure 3.3). Similarly, the average community biomass for three-membered communities were also plotted (figure 3.4).

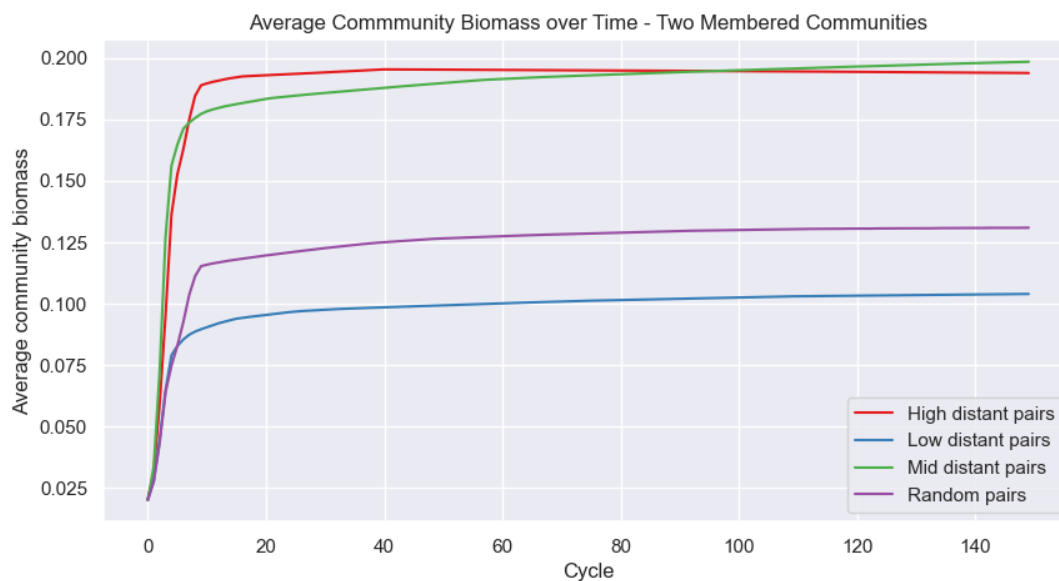


Figure 3.3: Average community biomass over time - two-membered communities

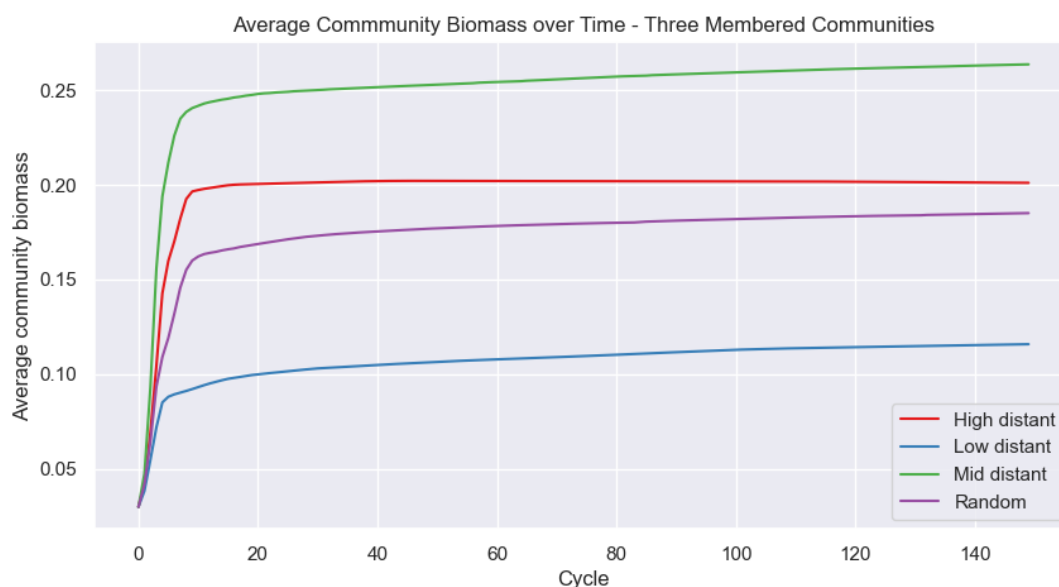


Figure 3.4: Average community biomass over time - three-membered communities

A common observation in both two-membered and three-membered communities was that the community biomass saturated after approximately ten cycles, indicating a steady-state condition or saturated growth rate. This observation can be utilized to design future experiments to study the invasion resistance of communities. The saturation time point can be considered optimal for introducing a new species into an existing community.

3.2.2 Interaction Type in Communities of Different Jaccard Distances

The interaction type between species was predicted at the 20th cycle(at 2 hours) of the simulations when the communities stabilized in terms of community growth. The count of the interaction types across different distances are shown in the plots below(figure 3.5, figure 3.6).

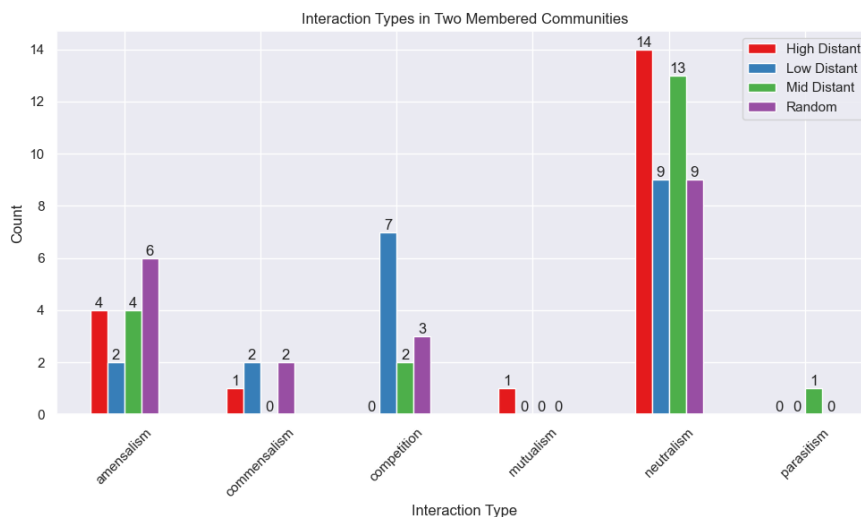


Figure 3.5: Interaction type in two-membered communities across different Jaccard distances

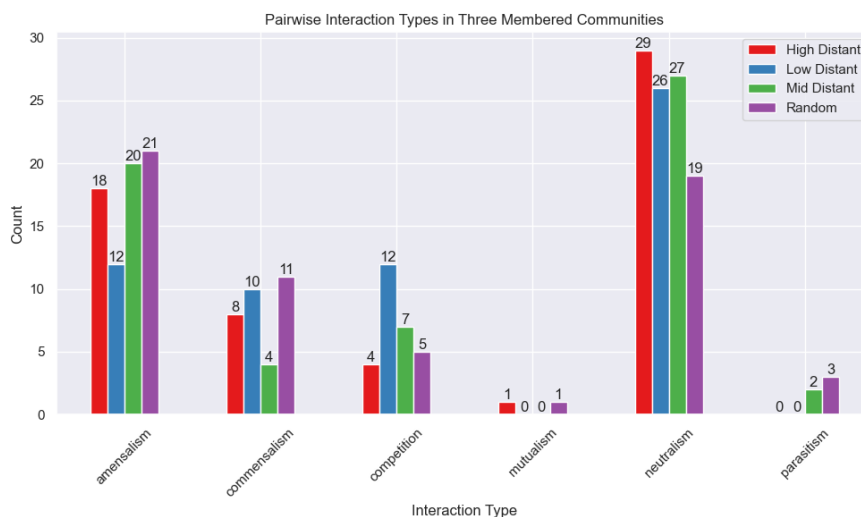


Figure 3.6: Interaction type in three-membered communities across different Jaccard distances

On analyzing the results, we saw that communities that had species with lower metabolic similarity (i.e., those with high and mid distances) tended to grow faster compared to those with higher metabolic similarity(or low distances). This observation can be explained by the idea that species with lesser shared metabolic pathways have access to a broader range of resources, thereby reducing potential competition

between them and enabling faster growth. On the other hand, metabolically similar species compete for the same resources, leading to slower growth as the chances for competition increase in such cases.

Another interesting observation was that the high and mid-distance pairs often exhibited neutralism (i.e., minimal or no interaction). In contrast, the distribution in the case of low-distance pairs was majorly shared between neutralism and competition.

3.2.3 Interaction Switch in Response to the Addition of a New Species

Furthermore, to study the effect of the presence of a new species on the existing interaction between pairs, we compared the interaction type shift from two-membered to three-membered communities (figure 3.7). In communities with higher and mid-distance pairs, introducing a third species tended to shift some neutral interactions to amensalism, although most interactions remained neutral. No other interesting patterns were observed in the other cases.



Figure 3.7: Interaction type switch in high distant pairs

Overall, on combining all four categories, we see that the interaction remains the same and is not affected by introducing a new species. However, there are very few cases where the switch happens. A plausible reason why neutralism is being observed the most in all cases could be that the resources are adequately available for all species to grow comfortably without having to interfere or compete with the

other species in the community.

3.2.4 Discussion

Based on the observations from this part of the work, a hypothesis can be formulated that dissimilar species are more likely to cooperate and grow faster, while similar species tend to engage in competitive or negative interactions. This hypothesis, along with the previous ones, can be statistically tested and checked for in communities of different environments and scales to gain a deeper understanding of microbial communities.

3.3 Dynamic FBA vs. Steady-State FBA

Among the two studies done so far, one was done using steady-state FBA and another was done using dynamic FBA (dFBA). Though the models were sourced from different versions of the AGORA database and the total number of communities differed in both cases, they are still microbes from the gut simulated under the same anaerobic conditions on Western diet. Moreover, the interaction type was predicted at a time point when the communities had stabilized - in some sense, moved into a steady state in the dFBA simulations. So it is expected that we get similar trends in the results, but the results differed significantly.

The steady-state FBA simulations consistently pointed toward parasitism as the dominant interaction type, whereas the dynamic FBA simulations leaned towards neutralism mostly. This contrast between these results could be due to several factors, such as the quality(completeness) of the models and the choice of parameters - dFBA had many factors to be considered for the simulations, like the initial biomass, K_m values, V_{max} , the duration of simulation, dilution rate, etc., which were not present in the steady-state case and the inherent assumptions of both methods.

This difference raises an important question: Does dynamic FBA offer a more accurate reflection of microbial community dynamics, as it accounts for the changes that happen over time, or does steady-state FBA better capture the long-term equilibrium? While dynamic FBA might better model real-world conditions by considering time-varying fluxes and changes in resource availability, further investigation into the metabolic fluxes from both sets of simulations can help us better understand this difference.

4 Conclusions and Future Work

4.1 Conclusions

This study is an initial effort to gain insights into microbial community dynamics, mainly focusing on the interaction patterns between pairs of species. A consistent pattern observed from the two sets of simulations and analysis done is that negative interactions become increasingly prevalent as the community expands. This observation supports the idea that resource competition majorly drives community dynamics.

This work also discusses the difference in results from the steady-state and dynamic FBA simulations. While results from the steady-state FBA simulations pointed to parasitism being the dominant interaction type, dynamic FBA simulations suggested neutralism as the most prevalent interaction type. This contrast in the results emphasizes that further analysis is required to assess which approach aligns with the results observed in the experimental setups.

Additionally, through this study, we have identified exciting areas and directions for future exploration. Understanding the relationship between community size, metabolic similarity, stability, resistance and resilience can enhance the existing theoretical understanding of microbial communities and point towards practical applications.

4.2 Future Work

As observed and formulated based on the current analysis, the immediate plan is to statistically test for the following hypothesis in the context of gut microbes and further extend to microbial communities in other environments like water, soil, built environments, etc.

1. The proportion of negative interactions increases as community size increases.
2. Microbial pairs with low metabolic similarity have fewer negative and more cooperative interac-

tions and grow faster than pairs with higher metabolic similarity.

3. The addition of a new species majorly does not alter the existing interaction dynamics (in smaller communities).

Along with that, we want to investigate the fluxes of metabolic exchanges in these simulated communities and see if the results can be correlated with the interaction patterns observed.

Later, we want to assess whether the results of the same communities correlate with each other if simulated through steady-state FBA and dFBA and previously conducted experimental results, which will be taken from the literature.

For the second phase of the work, we want to study the stress tolerance - resistance and resilience of microbial communities when faced with a disturbance. The goal is to understand how community stability changes as the system evolves through perturbations.

To summarize, the overall results from these proposed plans would not only build on the current observations but also would contribute to a better understanding of the dynamics of microbial communities, and can possibly point towards potential practical applications.

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