

Expansions on a multistability toy model

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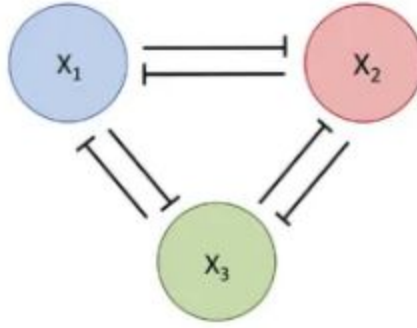
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Background

Multi-stability, or the characteristic of having multiple alternative stable states, is critical for understanding dynamics. Local stability analysis places primary focus on the destabilizing effects of small scale perturbations. However, in empirical observations, we often see the degree of perturbation on ecological systems to often be large. These perturbations can drastically change where communities exist on the stability landscape or the stability landscape itself (Beisner et al.).

A major component behind the multi-stability theory is hysteresis (Beisner et al.). Hysteresis occurs when a parameter change introduces a perturbation to the system and the community shifts in response to a new community state. If initial conditions are applied and the system is allowed to stabilize, the community does not return to the initial community state, but to another new community stable state. The alternative stable states produced in hysteresis exhibit a classification of multi-stability. In order to return the system back to the initial community state, the community must return from an alternative path direction.

Recent models for hysteresis have involved simple systems for the demonstration of hysteresis, particularly for microbial systems (Gonze et al. 2017). This toy system involves a three-member positive circuit of inhibition (Fig. 1) and the hysteresis demonstrated from it is recreated in Figure 2. While this system is still far from representing the complexities of an empirical system, the basic components necessary for hysteresis are present. Gonze et al. also extended the 3-member system to a 15-member but by subdividing that community into 3 modules of 5 weakly-interacting sub-communities to engender 3 alternative stable states. However, this model is relatively simplistic and is designed to be primarily a conceptual model, and is not parameterized to reflect empirical structures.



$$\begin{aligned}
 \frac{dX_1}{dt} &= X_1(b_1 f_1(X_2, X_3) - k_1 X_1) & f_1(X_2, X_3) &= \frac{K_{12}^n}{K_{12}^n + X_2^n} \frac{K_{13}^n}{K_{13}^n + X_3^n} \\
 \frac{dX_2}{dt} &= X_2(b_2 f_2(X_1, X_3) - k_2 X_2) & f_2(X_1, X_3) &= \frac{K_{21}^n}{K_{21}^n + X_1^n} \frac{K_{23}^n}{K_{23}^n + X_3^n} \\
 \frac{dX_3}{dt} &= X_3(b_3 f_3(X_1, X_2) - k_3 X_3) & f_3(X_1, X_2) &= \frac{K_{31}^n}{K_{31}^n + X_1^n} \frac{K_{32}^n}{K_{32}^n + X_2^n}
 \end{aligned}$$

Figure 1. (Taken from Gonze et al.) The three member system with positive circuit composed of mutual inhibitory interactions. Each has growth rates described with the ODEs on the bottom left, with X_i representing the individual population abundances of each species, b_i representing the growth rates of individual population abundances, and k_i representing the individual death rates. The inhibition functions f_i are describing species interactions with one other on the right by acting as a multiplier to lessen the growth rate. Inhibition coefficients (K_{ij} , meaning the inhibition of j on i) and Hill number (n) are defined according to Gonze et al. Large K values signify weaker inhibition.

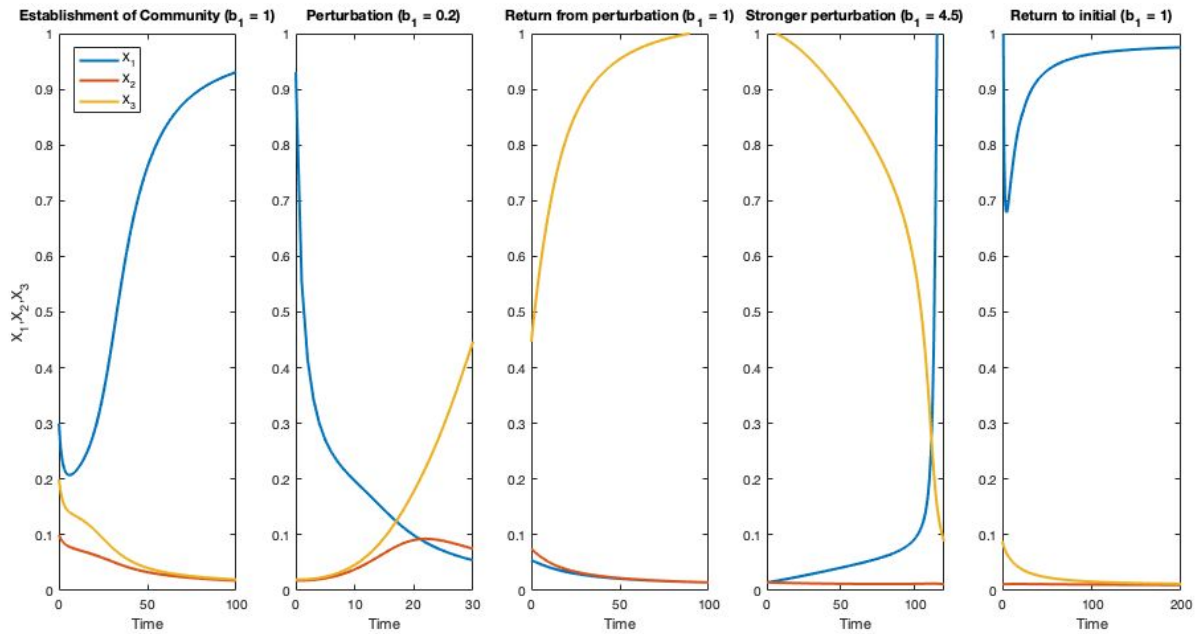


Figure 2. Replicating the hysteresis dynamics from Gonze et al. with our own code. In the first stage, the community is established, allowing X_1 in blue to dominate with its growth rate at $b_1 = 1$. After establishment, the system is perturbed by changing b_1 to 0.2, allowing X_3 to dominate the community. Increasing b_1 from 0.2 to 1 doesn't change the community back to the X_1 dominated community, but instead results in a X_3 dominated community. A stronger perturbation is required ($b_1 = 4.5$) to increase the

abundance of X_1 over X_3 , and ultimately allow X_1 to establish dominance when b_1 is brought back to the initial value of 1. Time on the y-axis is the time from the start of the simulation with the new b_1 value. Each new graph takes the community composition of the last, running as one continuous simulation.

The human vaginal microbiome has very strong suggestions of multi-stability. A longitudinal study of the vaginal microbiome of various healthy women has led to the discovery of five universal microbiome states: four of them are dominated by a distinct *Lactobacillus* species, and the last is referred to as the “diverse” type, composed of many species (Ravel et al.). *Lactobacillus* species are able to maintain their dominance by imposing very strong inhibitory interactions onto other species through lactic acid production and other antibacterial compounds (Ravel et al.), but throughout the course of a woman’s life, she can transition between these five states. This can be problematic because the diverse group, composed of many more trophic and mutualistic interactions (Aldunate et al.), tends to be more susceptible to bacterial vaginosis (Gajer et al.). There is debate over whether or not these five states are a result of typical changes in community composition due to a gradient along with environmental parameters or if it is due to true alternative stable states that can exhibit hysteresis (Gajer et al, Gonze et al.).

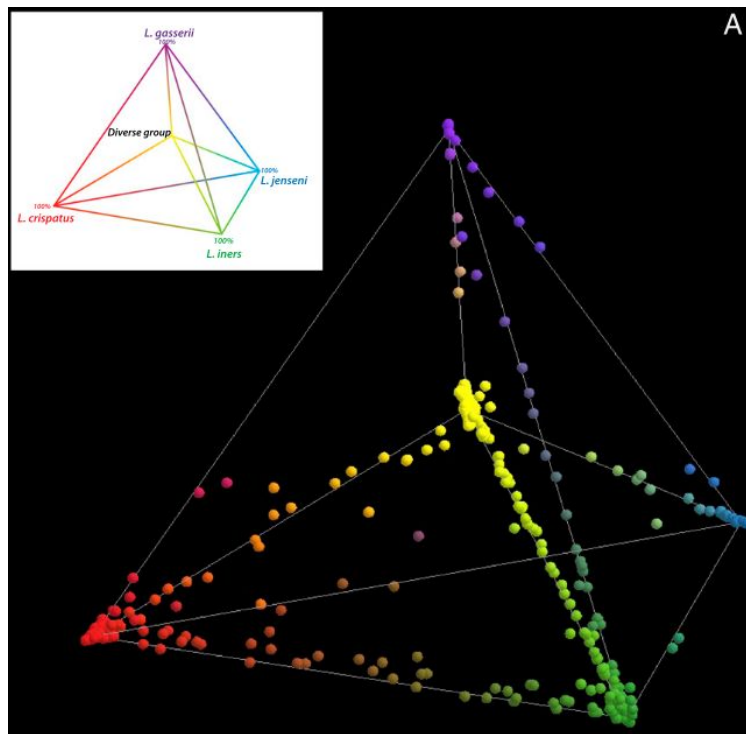


Figure 3. Taken from Ravel et al. (2011). The human vaginal microbiome falls within 5 clusters: four dominated by *Lactobacillus* species (*L. gasseri*, *L. jensenii*, *L. iners*, *L. crispatus*) and one *diverse* group composed of other anaerobic fermenter species.

Motivation

Multi-stability provides implications both to stability analysis in ecology as well as for a variety of existing ecological systems. With common local stability analysis, large perturbations are thought to be largely destabilizing. This stability analysis was utilized by May et al. (1972) to conclude that increased system complexity leads to a decrease in system stability. The existence of alternative stable states

changes the landscape of this debate, increasing the overall robustness of the system in regards to system perturbation and in higher levels of system complexity.

Multistability also holds great weight in the discussion of ecological restoration and conservation efforts. Available research on microbial systems already indicates alternative stable states potentially contributing to large degrees of human microbiome diversity. However, to expand to a larger scale ecological practice such as aquaculture, could multi-stability provide an explanation to marine species increased/decreased persistence given levels of human fishing pressure? An understanding of multistability being applicable across a variety of ecological networks and network interactions could improve models to reflect increased community system robustness and/or dependence on individual species.

Objectives

While the conclusions from Gonze et al. are promising in the application of multi-stability to 3 modal inhibition based systems, we expanded the premises of the toy model system (Gonze et al. 2017) to demonstrate the relevance of multistability on more varied ecological frameworks. Our first steps were to replicate the toy model architecture and test the theoretical results of alternative stable states with four members instead of three members. By comparing the conclusions of an increased member system, we test whether additional species types would demonstrate similar trends on multi-stability or highlight the specificity and optimization of the toy-model systems present in Gonze et al 2017.

Our second steps were to expand the toy model system to reflect observations of empirical microbial data/ By comparing observation of microbial community structure from the human vaginal microbiome to the output of the prediction by the toy-model system with similar input parameters, we can establish ecological relevance of the inhibition based multistability to varied microbial ecosystems.

The final steps are to scale away from microbial inhibition and test the existence of multistability with not only competitive interactions but with additional consumer-resource and mutualistic interactions as well. Utilizing Lotka-Volterra models for competition, Rosensweig - Macarthur models for consumer-resource interactions, and the modified model for mutualistic networks from Holland, Deangelis et al., we can modify the toy model to test the implication of multistability across trophic levels and vertical network arrangement.

Methods

Expansion to four members from three members

In our initial stability analysis of the four-species system (see Figure 4.), we keep ecosystem parameters constant and vary initial population parameters for each species. The system in Figure 2. was solved for these initial conditions and simulated in MATLAB for $t = 100$ time steps. The inhibitory coefficients in the Hill function were set as $K_{ij} = 0.1$ for all $i \neq j$.

$$\begin{aligned} \frac{dX_1}{dt} &= X_1(b_1 f_1(X_2, X_3, X_4) - k_1 X_1) \\ \frac{dX_2}{dt} &= X_2(b_2 f_2(X_1, X_3, X_4) - k_2 X_2) \\ \frac{dX_3}{dt} &= X_3(b_3 f_3(X_1, X_2, X_4) - k_3 X_3) \\ \frac{dX_4}{dt} &= X_4(b_4 f_4(X_1, X_2, X_3) - k_4 X_4) \end{aligned} \quad f_i(\{X_k\}) = \prod_{k=1, k \neq i}^4 \frac{K_{ik}^n}{K_{ik}^n + X_k^n}$$

Figure 4. The expanded toy model with four species based on the model from Gonze et. al. 2017. All mortality rates are kept at $k_i = 0.1$, and the Hill number was kept at $n = 2$. Birth rates and initial populations vary depending on the simulation.

We then conduct a stability analysis of the four-species system by varying an environmental parameter (in this case, b_1) in a bifurcation diagram. Initial conditions and all other birth and death rates were kept constant (initial conditions were all set to 0.2, since this was close to the numerically solved equilibrium point). The diagram will show all possible states of a system given the parameter values as well as its stability. If hysteresis is present, then we will observe multiple stable states for a given value of b_1 with instability between these states. All bifurcation analyses were done in the MATLAB package MATCONT.

Expansion to the community structure resembling the vaginal microbiome

The main difference from the toy model and the previous expansion to four members was the inclusion of a diverse sub-community, a five-member group with mutualistic interactions with each other. This community structure is shown in Figure 6. The main question of this objective is whether or not this system was capable of emerging with five states, with one being a group of diverse species, despite the fact that the diverse species was not holding a strong inhibitory effect against the other *Lactobacillus* species.

While the birth, inhibition, and death components are unchanged from the ordinary differential equations in the toy model, the only addition added was the addition of a mutualism term and an α matrix, which was composed of the α_{ik} , the mutualistic effect of species k on species i .

$$\frac{dX_i}{dt} = X_i(b_i f_i(X_k)) - k_i X_i + \sum_k \alpha_{ik} X_k$$

Because there was already a death term included in the model, there wasn't a need for self-inhibition. The *Lactobacillus* species maintain a strong inhibitory effect towards each other and members of the diverse group ($K = 0.15$ indicates strong inhibition), while the diverse group holds weak inhibition ($K = 0.9$) towards the *Lactobacillus* species and even weaker inhibition towards each other ($K = 1$). To add some variation reflecting some degree of heterogeneity, these K values were generated with a variation between 0 and 0.1 based on a random uniform distribution. For the mutualistic interactions, the only interactions added are between the diverse members, which were given values of 0.15 with additions between 0 and 0.03 based on a random uniform distribution.

Finally, to allow different stable states to emerge from the system, we varied the initial community composition of the nine-member community to vary between 0 and 0.1 based on a random uniform distribution. The birth rates also were the same, but were set at 0.9 with the variation, while the death rates were maintained at 1, similar to the Gonze et al. toy model.

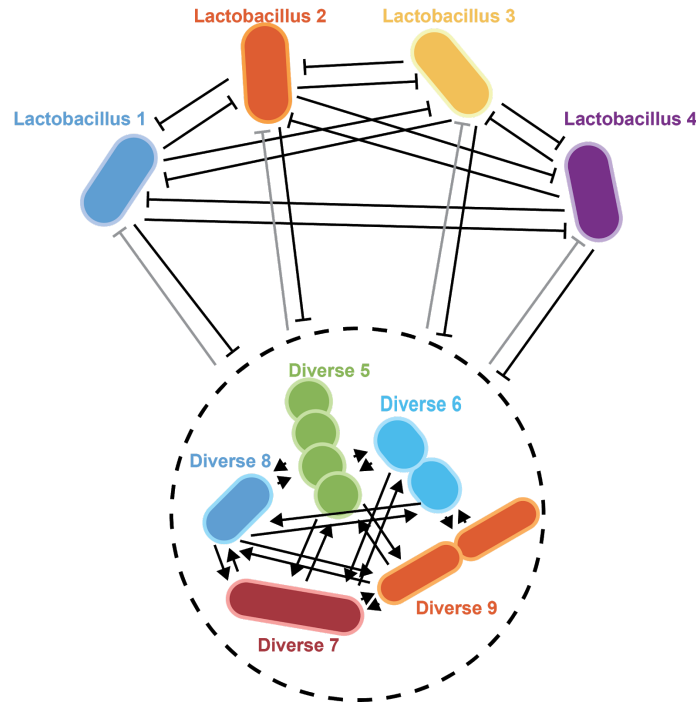


Figure 6. Simplified vaginal microbiome community structure. The *Lactobacillus* species resemble the chain on inhibition exhibited by the species from Gonze et al. However, there is the addition of the diverse subgroup within the community which has mutualistic interactions with each other, based on empirical evidence that mutualistic interactions are more abundant in the diverse system. The diverse subgroup still has a very weak inhibitory effect towards *Lactobacillus* species, but at a much smaller magnitude than what the *Lactobacillus* species exhibit towards each other and towards the diverse group.

Expansion of system interactions to involve varied ecological interactions

The toy model included competition as the interactions between each species within the system in the form of inhibition seen in Figure 1. The objective of this experiment is to test the presence of alternative stable states when we modify the interactions between each species of the model to reflect more ecologically generalized models of competition. consumer-resource theory and mutualistic theory. To identify which of these interactions apply to each species, we will be utilizing the concept of ecological web nodules (McCann et al. 2012) to select a subset of 3 species systems which are observed base units across trophic levels. Given the importance of competition between the three species as well as previous literature examining multispecies stability given consumer-resource interactions and 2+ trophic levels, there are ** combinations we consider of consumer-resource based interactions with an additional **combinations when a species acts as a mutualistic partner, as shown below Figure 6.1 .

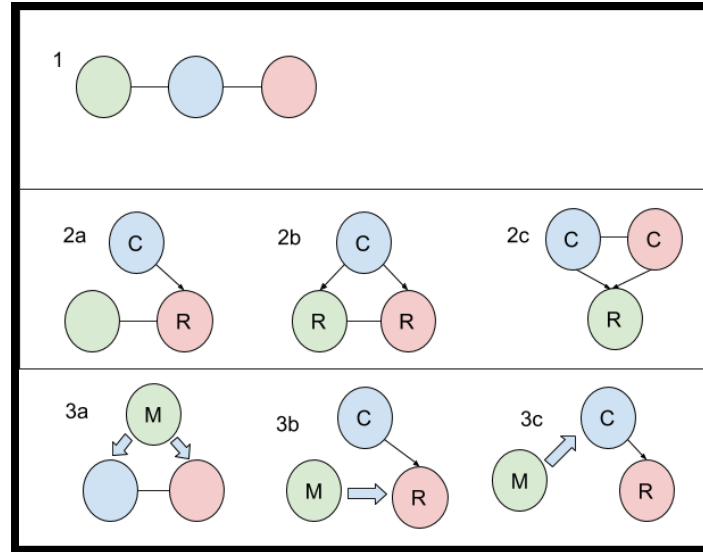


Figure 6.1: [1.] Represents the nodal pattern of the original toy model system represented by Gonze et al 2017. [2,3] Represents the three species nodal patterns from (Mcann et al. 2012). C denotes a Consumer and M denotes a Mutualistic partner.

To model the interactions between each 3 species system type, we utilized the Lotka-Volterra model for Intra and Interspecific competition, the Rosenzweig - Macarthur model for consumer-resource gain and loss coefficients, as the modified mutualistic consumption and cost coefficients from Holland, Deangelis et al 2010 . These models were combined in different combinations to produce population growth functions for each species depending if it acts primarily as a consumer (Eq 1.3), resource (Eq 1.1), or mutualistic partner (Eq 1.2).

Resource

$$\frac{dR}{dt} = R * \left[\underbrace{r_R \left(\frac{K_R - (Comp_{R_1 R_2} + R_2)}{K_R} \right)}_{\text{Growth given Interspecific and Intraspecific Resource Competition}} - \underbrace{\left(\frac{a_C C}{1 + a_C H_C R} \right)}_{\text{Loss due to Consumption}} + \underbrace{c_R \left(\frac{\alpha_{MR} M}{h_M + M} \right)}_{\text{Gain from Mutualism}} \right] \quad \text{Eqn. 1.1}$$

Mutualistic Partner

$$\frac{dM}{dt} = M \left[\underbrace{r_M \left(\frac{K_M - M}{K_M} \right)}_{\text{Growth Rate given Intraspecific Competition}} + \underbrace{c_M \left(\frac{\alpha_{RM} R}{h_R + R} \right)}_{\text{Gain from Mutualism}} - \underbrace{q_M \left(\frac{\beta_R R}{e_M + M} \right)}_{\text{Loss due to Mutualistic Consumption}} \right] \quad \text{Eqn. 1.2}$$

Consumer

$$\frac{dC}{dt} = C * \left[\underbrace{\left(\frac{B_C a_C R}{1 + a_P H_P R} \right) \left(\frac{K_C - (Comp_{C_1 C_2} + C_2)}{K_C} \right)}_{\text{Growth Rate obtained from Consumption and Growth given Interspecific and Intraspecific Competition}} + \underbrace{c_C \left(\frac{\alpha_{MC} C}{h_C + C} \right)}_{\text{Gain from Mutualism}} \right] \quad \text{Eqn. 1.3}$$

Given the interaction between species and results from the 3 modal inhibitive toy model, In order to provide the highest level of control, we chose to modulate only the competitive and interaction parameters between each species growth equation. This amounts to the variables of competition (*Comp*), mutualistic gain, and attack rate (*a*). We then set all the other parameters to constant values. The initial population parameters (*r*, *K*) were set as constants given the parameter range present in the initial 3-species toy model (Gonze et al 2017). The consumer resource parameters (*B*, *H*) were based on an allometric consumer: resource differential of 10:1 utilizing the Z Optimal value from (Schenieder et al 2016). The mutualistic parameters (*alpha*, *h*, *q*) were based on average values of strong partners found in Holland, DeAngelis et al 2010. Controlling for constants, we modify our equations to resemble Eq (2.1 – 2.3). These equations were then modified to reflect each species system through the removal or addition of consumption or mutualistic terms.

Constants

$$r_R, r_M = 0.1$$

$$K_R = 0.8$$

$$K_M, K_C = 0.3$$

$$B_C = 0.5$$

$$H_C = 4.5$$

$$\alpha_{MR}, \alpha_{RM}, \alpha_{MC} = 0.6$$

$$h_R, h_M, h_C = 0.6$$

$$q_M = 0.5$$

$$\beta_M = 0.2$$

$$e_M = 0.3$$

Resource

$$\frac{dR}{dt} = R * \left[\left(\frac{0.8 - (R + [Comp_{RR_2} R_2])}{8} \right) - \left(\frac{a_C C}{0.01 + 4.5 a_C R} \right) + c_R \left(\frac{6M}{0.6 + M} \right) \right] \quad \text{Eqn. 2.1}$$

Mutualistic Partner

$$\frac{dM}{dt} = M * \left[\left(\frac{0.3 - M}{3} \right) + c_M \left(\frac{0.6R}{0.6 + R} \right) - \left(\frac{0.1R}{0.3 + M} \right) \right] \quad \text{Eqn. 2.2}$$

Consumer

$$\frac{dC}{dt} = C * \left[\left(\frac{a_C R}{5 + 3 a_C R} \right) \left(\frac{0.3 - (C + [Comp_{CC_2} C_2])}{0.3} \right) + c_C \left(\frac{0.6M}{0.6 + M} \right) \right] \quad \text{Eqn. 2.3}$$

Finally, the population growth functions for the original toy model simulation were modified to reflect these new functions for each species. In addition, only the competitive and interaction parameters were modulated and tested for multistability. Alternative stable states were confirmed if the hysteresis present in the initial experiment, a series of two perturbations to a parameter of choice, resulted in alternative stable states when all parameters were returned to the original state and neither perturbation resulted in a species extinction.

Results and Analysis

Expansion to four members from three members

The simulation of populations over $t = 100$ time steps in the four-species model clearly shows that one species dominates the others depending on its initial conditions (see Figure 5.). It should be noted that although a species remains dominant by beginning with a higher population, the species sees a much more rapid increase in its population if its birth rate is higher.

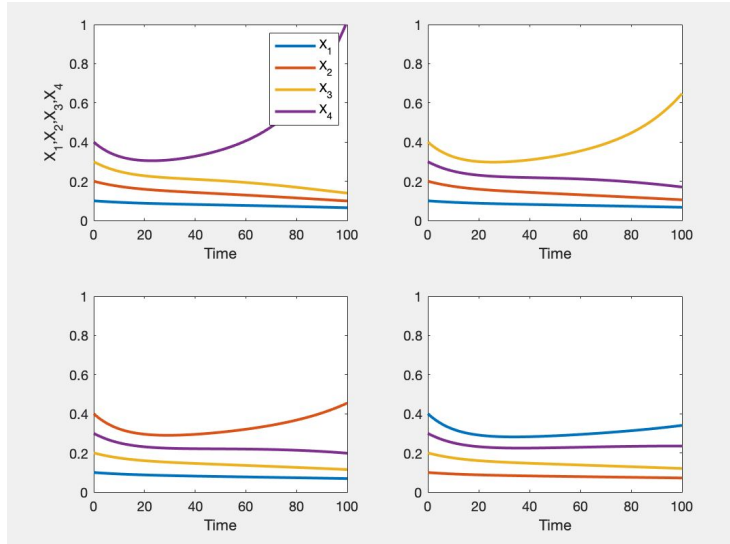


Figure 5. A simple demonstration of multistability. Birth rates for each species were kept constant ($b_1 = 0.95$, $b_2 = 1$, $b_3 = 1.05$, $b_4 = 1.1$) while initial conditions were varied for each species. Each species admits a new stable state depending on initial conditions.

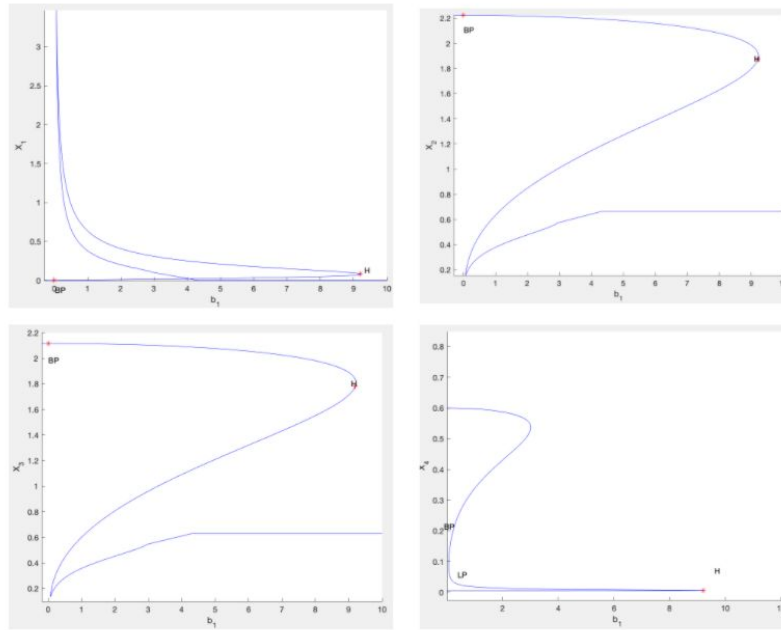


Figure 6. A one-parameter bifurcation diagram where the birth rate of species 1 is varied. Initial conditions were set at 0.2 for all species. The points on each curve denote: H = neutral saddle equilibrium, LP = limit point, BP = branch point. We assume that birth rates are positive.

When an environmental parameter was varied and all others were kept constant, we notice different stability behavior for each species. Figure 6. shows the community behavior for a four-species system while varying with the birth rate of species 1. Note that the stability for species 2 and 3 are exactly identical, and both display bistable behavior. We can conclude this behavior exists since there is a neutral saddle point at the cusp of the curve for each, which indicates a change in instability. The only other point

is the left tip of the curve, which is a critical point. For species 1, we also have the presence of a neutral saddle point at the end of our curve, indicating a change in the stability of the species once its birth rate exceeds 9.200. It is also apparent that multiple stable states occur simultaneously for this species since the branching point (BP), where the maximum eigenvalue changes signs, occurs near the origin. The two changes in stability are indicative of multiple stable states for the parameters between them. Likewise, for species 4, we have a saddle point and a limit point, which MATCONT denotes as changes in stability, indicating two simultaneously stable states.

Expansion to the community structure resembling the vaginal microbiome

Due to the chaotic nature of the model to the initial densities and various variations in the parameters which were based off of a random uniform distribution, in order to see the emergence of alternative community types after establishment, the model was run 100 times and each time was run until the system had reached equilibrium. From this, the following heatmap was generated in Figure 9. The result of which was a confirmation that from a clustering using the `clustergram()` function in MATLAB, the community abundances at equilibrium fell into five main groups. Four were dominated by high abundances of the four separate *Lactobacillus* species and the fifth was dominated by much lower abundances of those *Lactobacillus* species and virtually equal distribution of the diverse members. Although these five clusters emerged, it also seems there are multiple stable states beyond these five general clusters. Within the diverse group emerged a substantial number of communities in which the final equilibrium was an equal amount of diverse species and one *Lactobacillus* species. This probably represents a diversity of analytical stability points even within the diverse community group due to varying parameter values. This presents some degree of empirical realism, as for other studies that cluster microbiomes from vaginal microbiomes, even within these five groups, there is a great deal of variation in abundances and memberships, particularly for the diverse group (Ravel et al.). For simplicity sake, each cluster will be referred to as a different stability group.

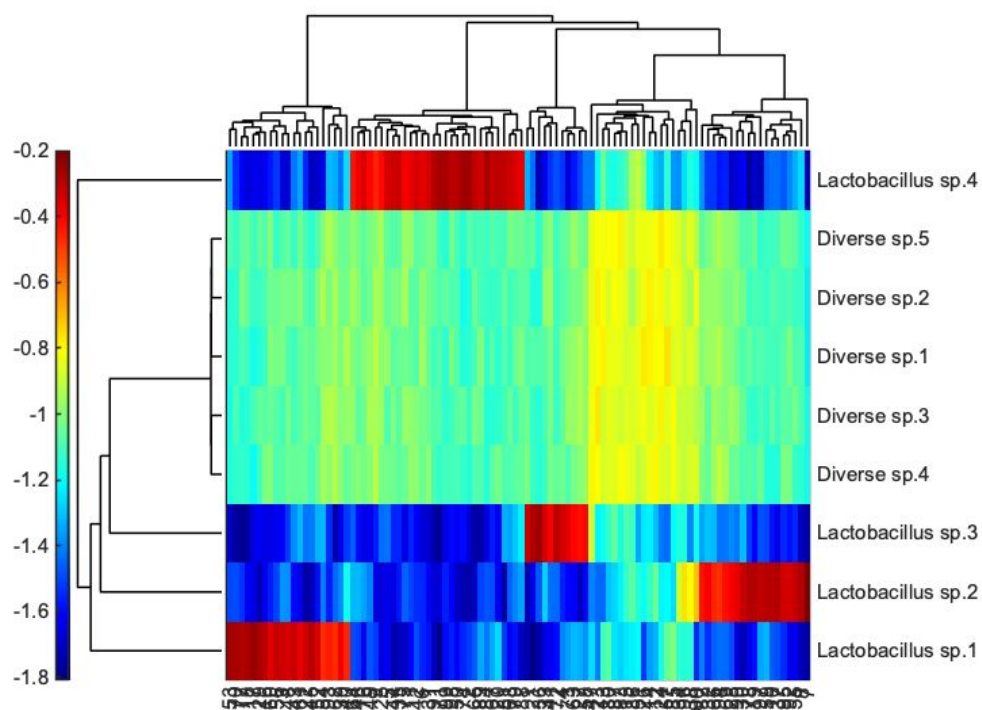


Figure 7. A heatmap of the equilibrium states from 100 runs of the vaginal community model. Each column is a different community resulting from a different vectors/matrices of initial abundances, inhibition and mutualism values, and birth rates. The colormap represents the log of the relative abundance (0-1) of each species to help better resolve the differences between the abundances. Clustering demonstrates that five stability groups emerge, each representing empirical structures seen in the vaginal microbiome.

Although these different stability groups emerged, are they actual alternative stable states? The best way to determine these outside of an analytical bifurcation diagram is to demonstrate hysteresis. To show this, a community was picked from the diverse stability group, its parameters and matrices extracted, and run similar to the hysteresis demonstrated in Figure 2. This is shown in Figure 10, which demonstrates the system shifting from the diverse state to the *Lactobacillus* dominated state when perturbed and failing to return back to the diverse state when perturbed back. This confirms the diverse stability groups as real alternative stable states.

What is interesting about this system compared to the toy model system is that the parameters do not need to shift dramatically to shift the community to alternative community states. In Fig. 2, b_1 needs to decrease to 0.2 or increase to 4.5 to alter community states. In this scenario, b_1 only needed to be shifted from 0.92 to 0.98 to 0.85 to shift the alternative stable state. In the toy model, the system is composed of very strong inhibitory species. In this system, due to the added number of competing species all attempting to impose inhibition and the strong overtaking effect of mutualism in the diverse group, it seems the alternative stable states may be more evenly matched. This results in more sensitivity towards perturbation, which may explain such high degrees of sensitivity in the vaginal microbiome as well (Gajer et al.).

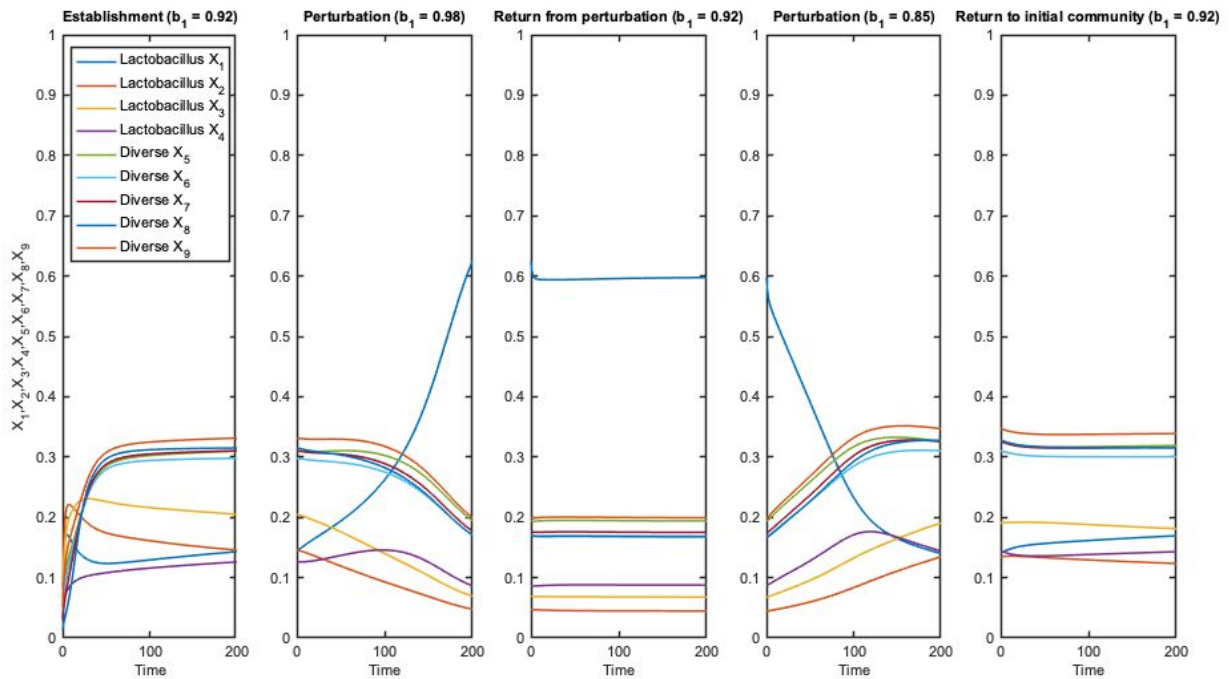


Figure 10. Demonstration of hysteresis dynamics with the vaginal microbiome. In the first stage, the diverse community is established, allowing X_{5-9} to dominate. After establishment, the system is perturbed by changing b_1 of the first *Lactobacillus* sp. 1 to 0.98 from 0.92, allowing it to dominate the community and shift the community towards the *Lactobacillus* 1 state. Decreasing b_1 back to the original value doesn't change the community back to the diverse state, demonstrating . A stronger perturbation is required ($b_1 = 0.85$) to decrease the abundance of X_1 , and ultimately allow the diverse group to establish dominance when parameters are reset back to the initial. Time on the y-axis is the time from the start of the simulation with the new b_1 value. Each new graph takes the community composition of the last, running as one continuous simulation.

Expansion of system interactions to involve varied ecological interactions

After each simulation was run with the three nodal networks, alternative stable states were observed in five of the six three-species systems. These three species system structures were [2a, 2b, 2c, 3a, 3b] from Figure 6.1. To conserve space on this paper, we will examine the system structure of [3a] which translates to a three species system with two competing species on the same trophic level who share a mutualistic partner. The modified population equations for this system are represented in Eq 3.1-3.3.

Mutualistic Partner

$$\frac{dM}{dt} = M * \left[\left(\frac{0.3 - M}{3} \right) + c_{M1} \left(\frac{0.6R_1}{0.6 + R_1} \right) + c_{M2} \left(\frac{0.6R_2}{0.6 + R_2} \right) - \left(\frac{0.1R_1 + 0.1R_2}{0.3 + M} \right) \right] \quad \text{Eqn. 3.1}$$

Resource 1

$$\frac{dR_1}{dt} = R_1 * \left[\left(\frac{0.8 - (R_1 + [Comp_{R1R2}R_2])}{8} \right) + c_{R1} \left(\frac{6M}{0.6 + M} \right) \right] \quad \text{Eqn. 3.2}$$

Resource 2

$$\frac{dR_2}{dt} = R_2 * \left[\left(\frac{0.8 - (R_2 + [Comp_{R2R1}R_1])}{8} \right) + c_{R2} \left(\frac{6M}{0.6 + M} \right) \right] \quad \text{Eqn. 3.3}$$

Alternative stable states occurred when this species system was placed with the input parameters of $[Comp_{R12} = 1.55, Comp_{R21} = 1, c_{R1} = 0.5, c_{R2} = 0.1, c_{M1} = 0.3, c_{M2} = 0.2]$ and initial population abundances of $[M = 0.2, R1 = 0.1, R2 = 0.7]$. The resulting population dynamics given a hysteresis change in c_{M1} are in Figure 10.1.

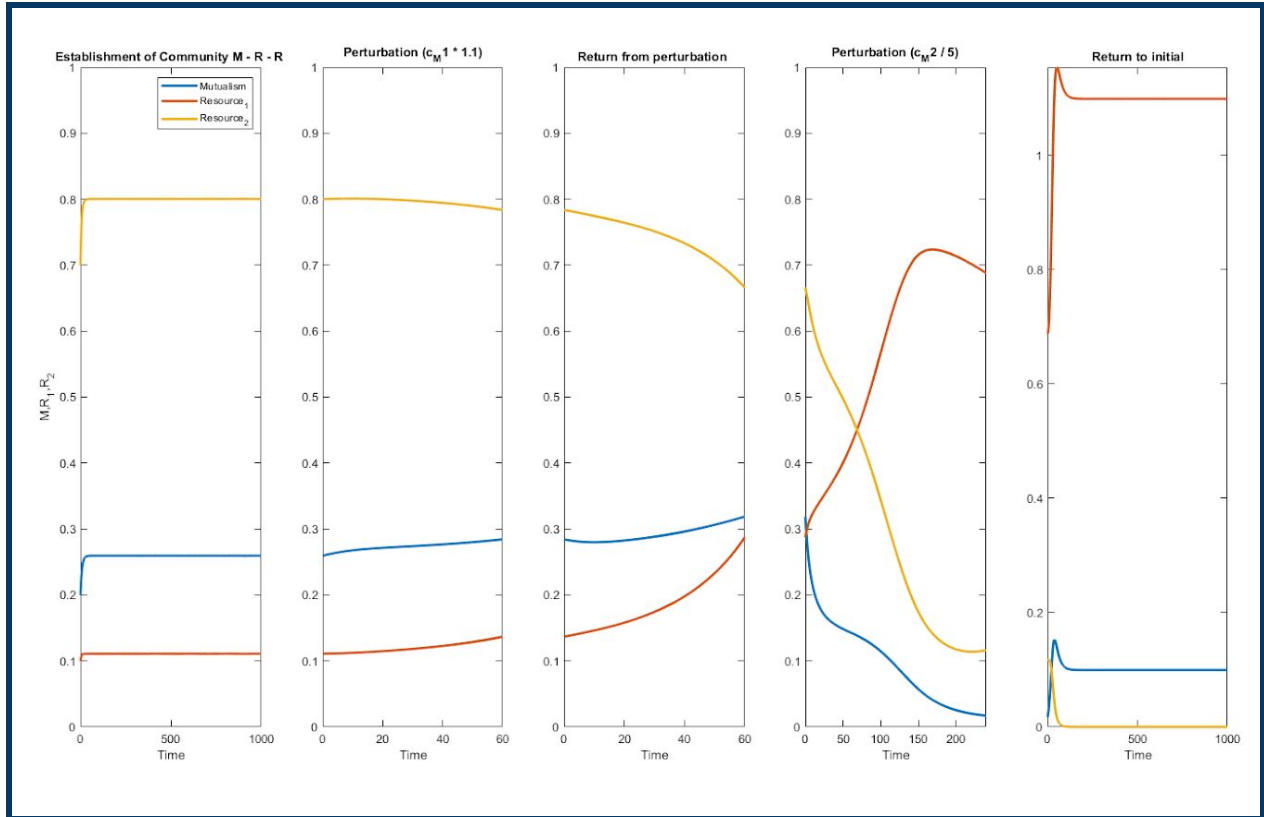


Figure 10.1: The output of the toy simulation from (Gonze et al. 2017) given the input Eq 3.1-3.3 and input parameters of $[Comp_R12 = 1.55, Comp_R21 = 1, c_R1 = 0.5, c_R2 = 0.1, c_M1 = 0.3, c_M2 = 0.2]$ and initial population abundances of $[M = 0.2, R1 = 0.1, R2 = 0.7]$. Mutualistic partner is denoted by Blue, Resource 1 with Red, Resource 2 with Yellow. Interpretation is similar to Figure 2.

The results of this simulation were interesting and are very dependent on competitive and mutualistic parameters. Given the initial parameters, Resource 1 had intrinsic growth parameters which were greater than Resource 2, However the high competitive effect of Resource 2 on Resource 1 stabilized Resource 1 at a lower level of population. The mutualistic benefit that the mutualistic partner gained from Resource 1 was not enough to grow and push the population of Resource 1 conversely. However when the mutualistic partner was perturbed and grew, it allowed Resource 1 to grow to a point where it's population growth outweighed the competitive effect and the final stable states after return to initial parameters projected an extinction of Resource 2.

Overall the other stable states that were observed followed similar interactions between competition and consumption/mutualism in which the hysteresis in the initial stable state resulted in a shift of equilibriums resulting in compounding effects of an inhibited species. It is noted that mathematically, the presence of two independent variables and interaction between all species in Eq 2.1-2.3 indicate the potential existence of stability points for all species combination in Figure 6.1, thus it is possible that I may not have discovered all the stable states in my modulation of parameters.

Discussion

In the extension of our model to four species, it is clear from Figure 5. that multiple stable states in the population exist as initial populations are varied. It is also clear that each species can enter one of four

stable states, depending on its initial population. This makes the interpretation of Figure 6. More complicated, since varying the birth rate of species 1 causes strong shifts in the stability of the population for that particular parameter value, but only a maximum of twice (when $b_1 > 0$). This indicates that the population can only enter at most two alternative stable states rather than the expected number of four. The analysis in Gonze et. al. 2017 showed three stable states for a competitive system of three species, but it seems that in systems of four species, if all parameters are constant, then we have only two stable states at positive population values. This result is further supported by the similarity in state values for species 2 and 3, as was shown in Figure 6. Regarding whether or not hysteresis is present, we clearly see in all bifurcation diagrams in the four-species system that populations pass through a change in stability which indicates that there is an intermediate unstable state between the high-abundance and low-abundance states. Thus, we require a change in the initial population to enter these alternative states as was shown in Figure 5. One limitation of our multistability analysis was the lack of analysis on other parameter variations, such as mortality. There also may have been an appearance of the two other expected alternative stable states if different parameter values were chosen in the bifurcation diagram (perhaps less inhibition from the Hill function).

In the vaginal microbiome extension, we were able to demonstrate the potential of hysteresis to explain the five empirically alternative stable states. Additionally, this required only minor modifications to the toy model. Mutualism is able to be incorporated to the diverse group, demonstrating that although the diverse group did not have one strong inhibition against the *Lactobacillus* competitors, with enough cooperation within the diverse group with “runaway” mutualism, the collective effort of five weakly inhibiting species was able to keep other strong inhibitors at bay. This community structure may help explain how groups of “weaker” species are able to cooperate to suppress very dangerous “strong” species, such as pathogens or invaders. While these factors have been implicated by previous studies of the vaginal system, none of these studies synthesize empirical observations into a model framework and show the presence of alternative stable states.

Synthesizing the results of the expansion of interaction types between species in the toy model species, we found that alternative stable states were indeed possible and prevalent in many varied ecologically realistic combinations of consumer-resource dynamics, mutualism and competition. However, given many of these stable states led to species extinction or provided compounding effects, further study is necessary to determine contribution to overall system stability. Overall the implication of multistability over trophic levels is possible and heavily reliant on competitive forces as well as combinations of mutualistic and consumption forces.

However, a large limitation of this extension is that the values for inhibition or mutualism were based on qualitative observations, not empirical quantitative parameters. The parameters were selected to allow for alternative stable states and hysteresis to emerge. In future work, a parameter sweep or sensitivity analysis could be applied to determine which range of values are necessary for multi-stability to emerge. Alternatively, experimental work can be done in pair-wise co-cultures to determine these inhibition, mutualistic, and growth rates more empirically. Including those values would solidify if multi-stability is expected to exist. However, the study demonstrates that multi-stability can exist in the vaginal microbiome. In regards to ecological expansion, the parameter values chosen in and modulated in this study could be a source of limitation and thus empirical network data would be critical in future expansion of the toy model with varying ecological interactions.

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