Modelling the effect of *Lactobacillus* on tryptophan metabolism in the gut

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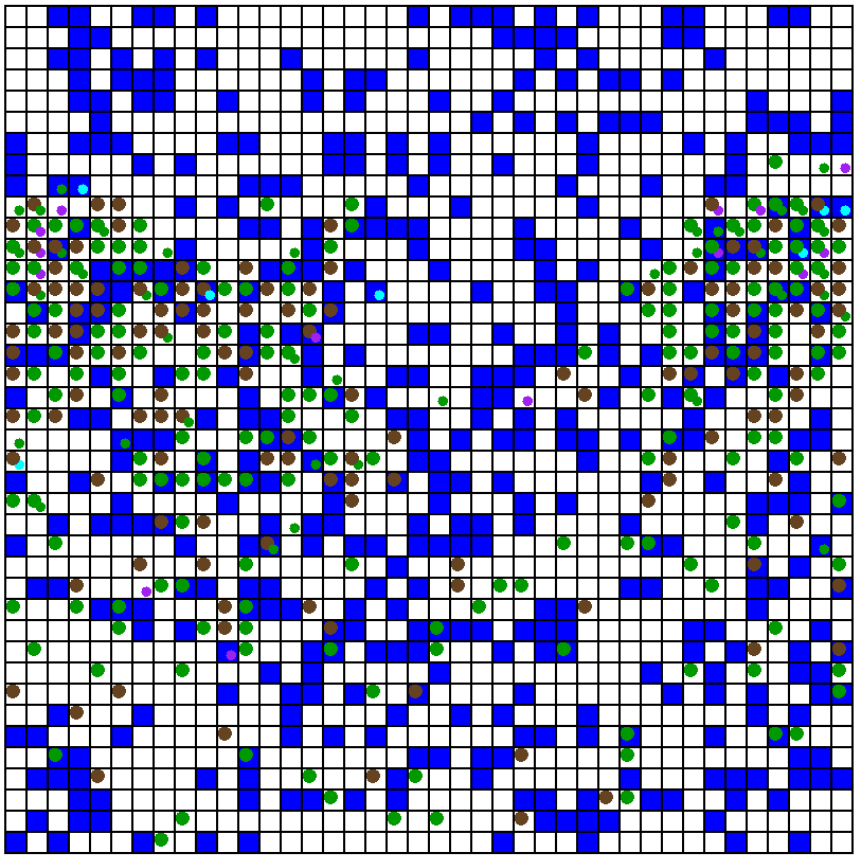

Background

Investigating the microbiome's effect on overall health is a burgeoning new field of study. It has been shown that the microbiome is important for healthy mental function; studies have shown that germ-free mice (mice with no gut bacteria) having higher anxiety than mice with normal gut bacteria (O'Mahony et al, 2015). Several studies have focused on the effects of *Lactobacillus* on the metabolism of tryptophan, which is a precursor to serotonin, and important neurotransmitter involved in depression, to link the microbiome to the brain. Low levels of serotonin contribute to a lower mood state (Jenkins et al., 2016). Tryptophan is metabolized in two major pathways, the kynurenine pathway, which accounts for 90% of tryptophan metabolism, and the pathway leading to serotonin synthesis, which accounts for 10% of tryptophan metabolism (Jenkins et al, 2016). The presence of different bacteria can influence the pathway that tryptophan goes down. Recent studies have shown that *Lactobacillus* inhibit the kynurenine pathway, increasing the production of serotonin (O'Mahony et al, 2015). Studies indicate that this occurs due to the production of a reactive oxygen species by *Lactobacillus* which then inhibits enzymes in the kynurenine pathway (Marin et al, 2017). We decided to use agent-based modeling to model the effects of probiotic *Lactobacillus* on the production of serotonin in the gut.

This is a model of a cylindrical section of the gut, with each grid cell representing one gut, or enterochromaffin, cell. Each cell can be populated by bacteria, which will be on the surface of the entire cell. To be clear, when a gut cell has bacteria on it, biologically speaking it is many, many bacteria, since bacterial cells are much smaller than human cells. In our program, we have simplified it to be one "bacterium" per gut cell. Nutrients in the form of tryptophan and carbohydrates start at the top of the grid and move downwards, simulating the movement of a meal down through the gut. The tryptophan is metabolized by the gut cells, while the carbohydrates are metabolized by both the gut cells and the bacteria. Tryptophan gets converted to either kynurenine or serotonin with certain probabilities depending on the presence of the *Lactobacillus.*

The Gut Cells

The gut cells are instances of the Cell class. When they are created in the SimulationManager, they are assigned a position and whether or not a bacterium is present. The presence of the bacterium can change if the bacteria die and the nutrient molecules can move through the gut cell on their way from the top of the grid to the bottom. The user specifies how large the square grid of gut cells is.

The Bacteria

All bacteria are instances of the Bacterium class. When they are created in the SimulationManager, they are assigned a position (row, col), a death time, energy points, and an id. All of the bacteria of one bacterial species in the system are identical to each other. In our current model, bacterium die at their assigned death time or when their energy points fall below a certain threshold. The assigned death simulates death by competition with the underlying native microbiome of the gut. Bacteria can metabolize carbohydrates, which add to

their store of energy points. They lose energy points at a constant rate of -1.5 energy points per time unit. In this model, all bacteria are *Lactobacillus*.

The Nutrient Molecules (Agents)

The nutrient molecules are the tryptophan and carbohydrates. During the simulation, a row full of nutrients is generated one at a time between every event until the total number of nutrients the user has specified have been added to the system. The nutrient molecules travel downwards and can move one cell diagonally down and to the left, directly down, or diagonally down and to the right, with equal probabilities of each direction. When they are created, each agent is randomly assigned a convert time and end convert time, which is when they will be used by the cells and disappear off of the grid (into the body). It is important to note that this is not the way this happens biologically. In the body, cells have ways of transporting nutrients in when they need them, the nutrients themselves don't determine that. For the sake of simplification, we decided to make it the agent's trait.

The Events

MOVE: This event changes the position of a nutrient molecule. In a move event, either an tryptophan or a carbohydrate moves down the grid by one cell. They can move one cell diagonally down and to the left, directly down, or diagonally down and to the right, with equal probabilities of each direction. Since the grid is a cylinder, when a nutrient molecule moves off of the left or right side, it appears on the opposite side. When a nutrient molecule moves past the last row of the grid, it is removed from the system.

CONVERT and ENDCONVERT: These events change the number of tryptophan, carbohydrates, kynurenine, and serotonin in the system. When the convert time starts, tryptophan becomes either kynurenine or serotonin and is still shown on the grid as a different color and in the bottom right of the cell. Carbohydrates remain the same color but also move to the bottom right of the cell. Carbohydrates are metabolized by either the gut cell or the bacterial cell, with a 0.5 chance for each. When an end convert event occurs, the carbohydrate, kynurenine, or serotonin is removed from the system. When a gut cell is in the process of converting a nutrient, other nutrient molecules can still move through that cell. The end convert event also checks if the bacteria in the cell is still alive according to the number of energy points it has and triggers a bacterial death event if needed.

BACTERIUM DEATH: This event changes the number of bacteria in the system. It can be triggered during an end convert event if the bacterium's energy level is below a certain threshold. One limitation of this method of checking for deaths is that we don't check the energy status of the bacteria once all of the nutrients have passed them. This doesn't have consequences in the conversion of the nutrients, but it does affect the visualization and the final counts of bacteria. A death event also happens at a designated time that is set when the bacterium is created. This is simulating the interaction with the other bacteria in the gut that might compete with the *Lactobacillus.* A death event removes the bacteria from the grid.

All of the events are kept track of in a PriorityQueue List, with easy access to the next event.

Experimentation

To experiment with our model, we altered the probability of bacteria from 0 to 1 and looked at the resulting kynurenine (purple) and serotonin (cyan) production. As the presence of *Lactobacillus* increases, the more serotonin is produced relative to kynurenine (Figure 1). As the lactobacillus influence, or how much the lactobacillus shifted tryptophan's metabolism towards serotonin, increased, even more serotonin was produced with increased bacterial presence.

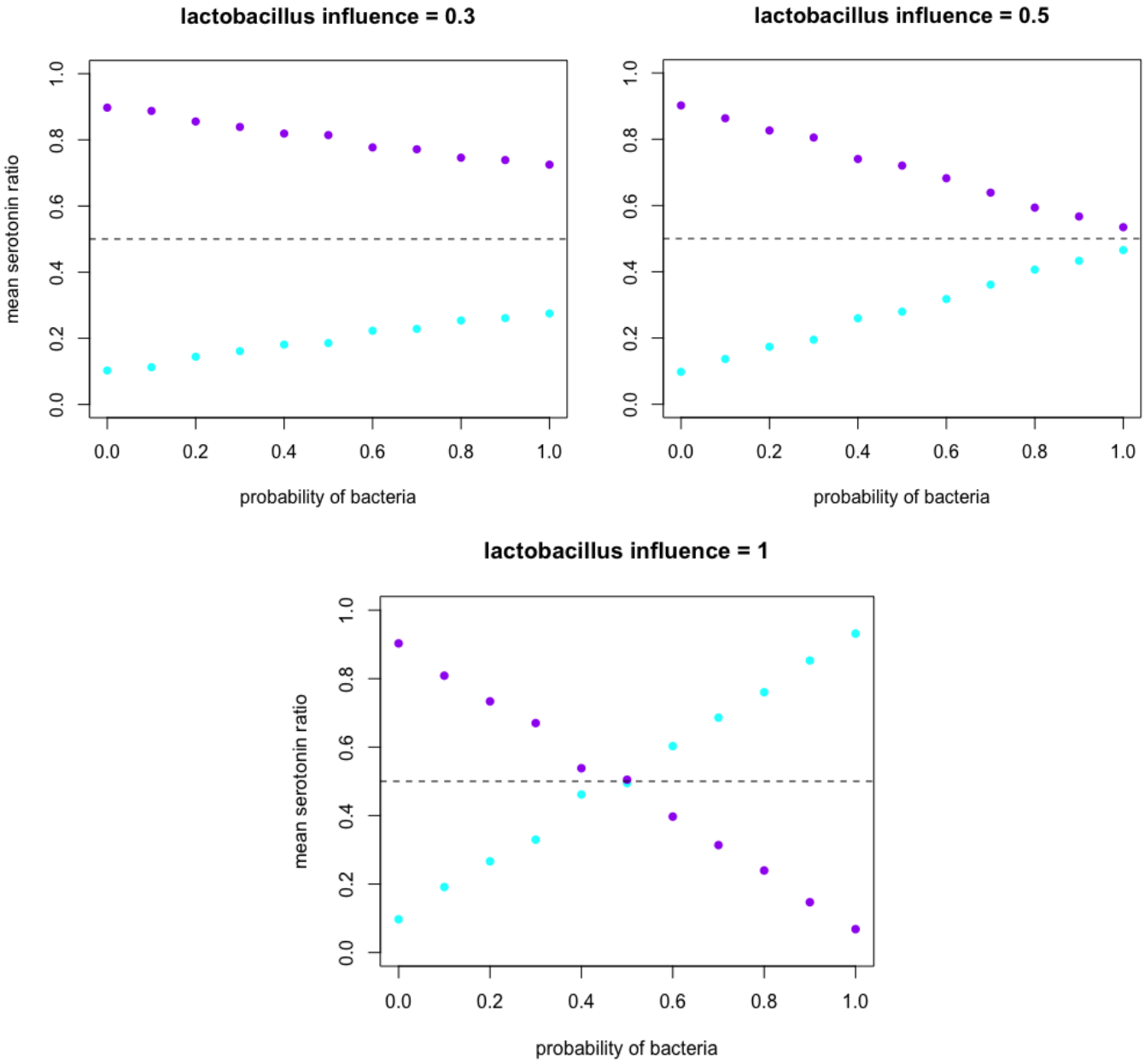

Figure 1. Increasing the number of bacteria increases the production of serotonin. gridSize = 40, num_agents = 1000, initial_seed = 8675309, tryptophan_ratio = 0.5, carbohydrate_ratio = 0.5. Each point is the mean of 15 samples. The purple represents kynurenine and the cyan represents serotonin.

To further investigate the effect of lactobacillus influence, we plotted lactobacillus influence from 0 to 1 with one probability of bacteria (number of bacteria). Increasing the influence increases the serotonin production (Figure 2), as one would expect.

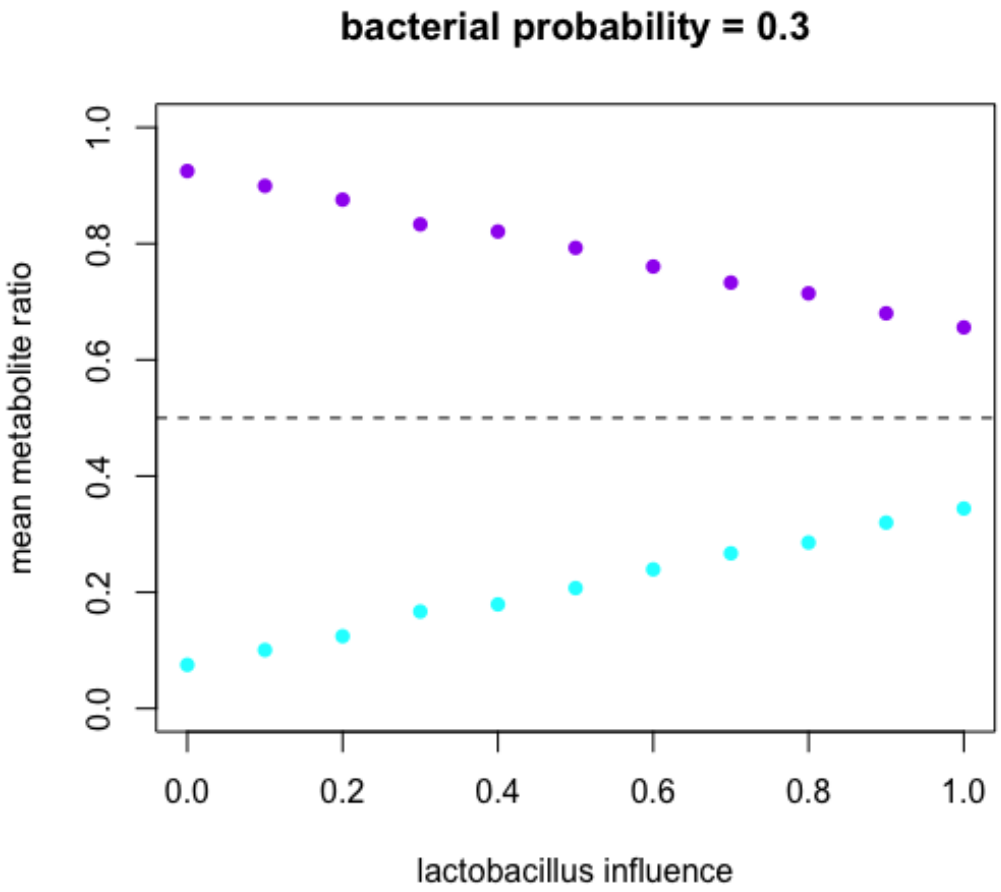

Figure 2. Increasing lactobacillus influence increases serotonin production.
grid_size = 40, num_agents = 1000, bacteria_prob = 0.3, initial_seed = 8675309, tryptophan_ratio = 0.5, carbohydrate_ratio = 0.5. Each point is the mean of 15 samples. The purple represents kynurenine and the cyan represents serotonin.

To look at the effect of the nutrient ratios, we looked at tryptophan/carbohydrate levels. For the increasing tryptophan ratio, we decreased the carbohydrate ratio so that they sum to 1. The serotonin and kynurenine production is stable across the different tryptophan ratios (Figure 3). We expected that the serotonin production at high levels of tryptophan would be much lower because of bacterial deaths due to lack of carbohydrates. With these conditions, we did not see this, perhaps because the death parameters are not strict enough.

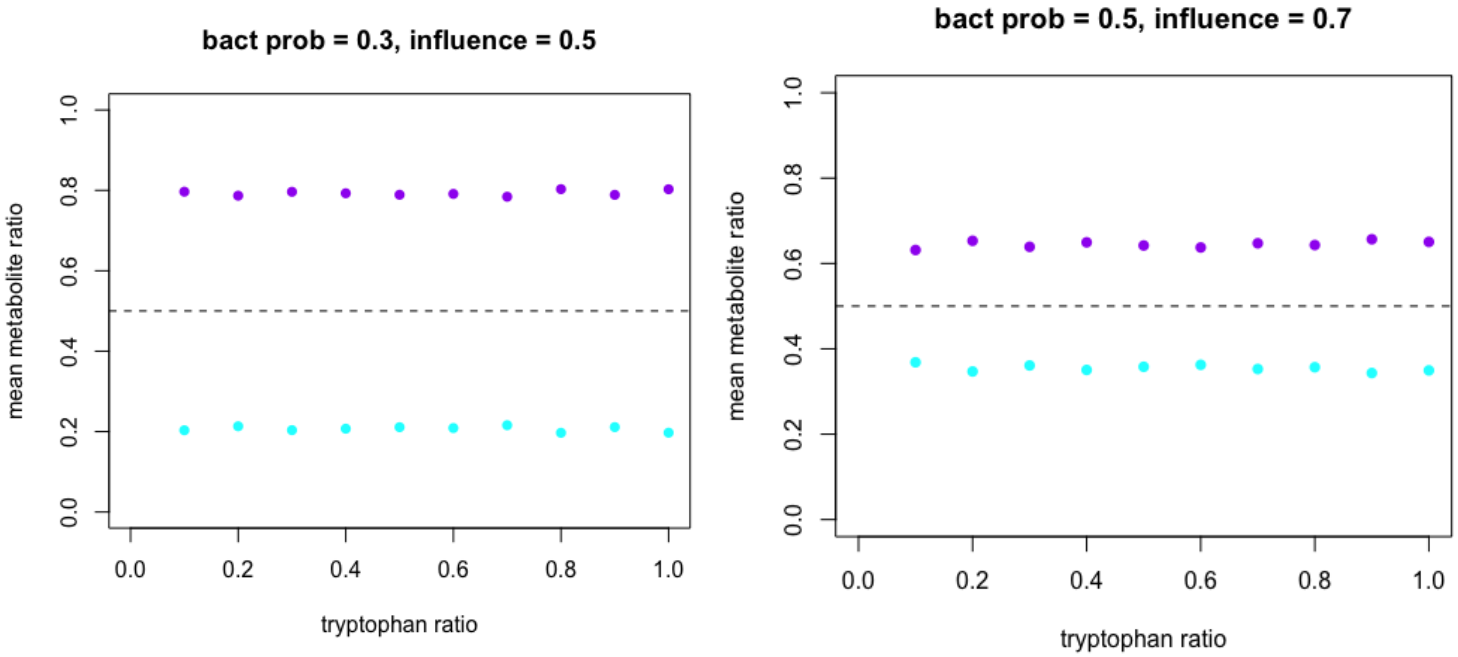

Figure 3. Investigating the effect of the nutrient ratios on serotonin production. grid_size = 40, num_agents = 1000, initial_seed = 8675309. Each point is the mean of 15 samples. The purple represents kynurenine and the cyan represents serotonin.

To investigate the effect of bacterial deaths, I modified the code directly to change the maximum energy points of each bacterium to 30, keeping the same death threshold of 20 energy points. This resulted in the slight decrease of production of serotonin at high levels of tryptophan (low levels of carbohydrates). This effect was more obvious when there were more bacteria on the grid (bact prob = 1) and their influence was higher (Figure 4). These results were more similar to what I was expecting to happen because of the reduced presence of bacteria because of starvation.

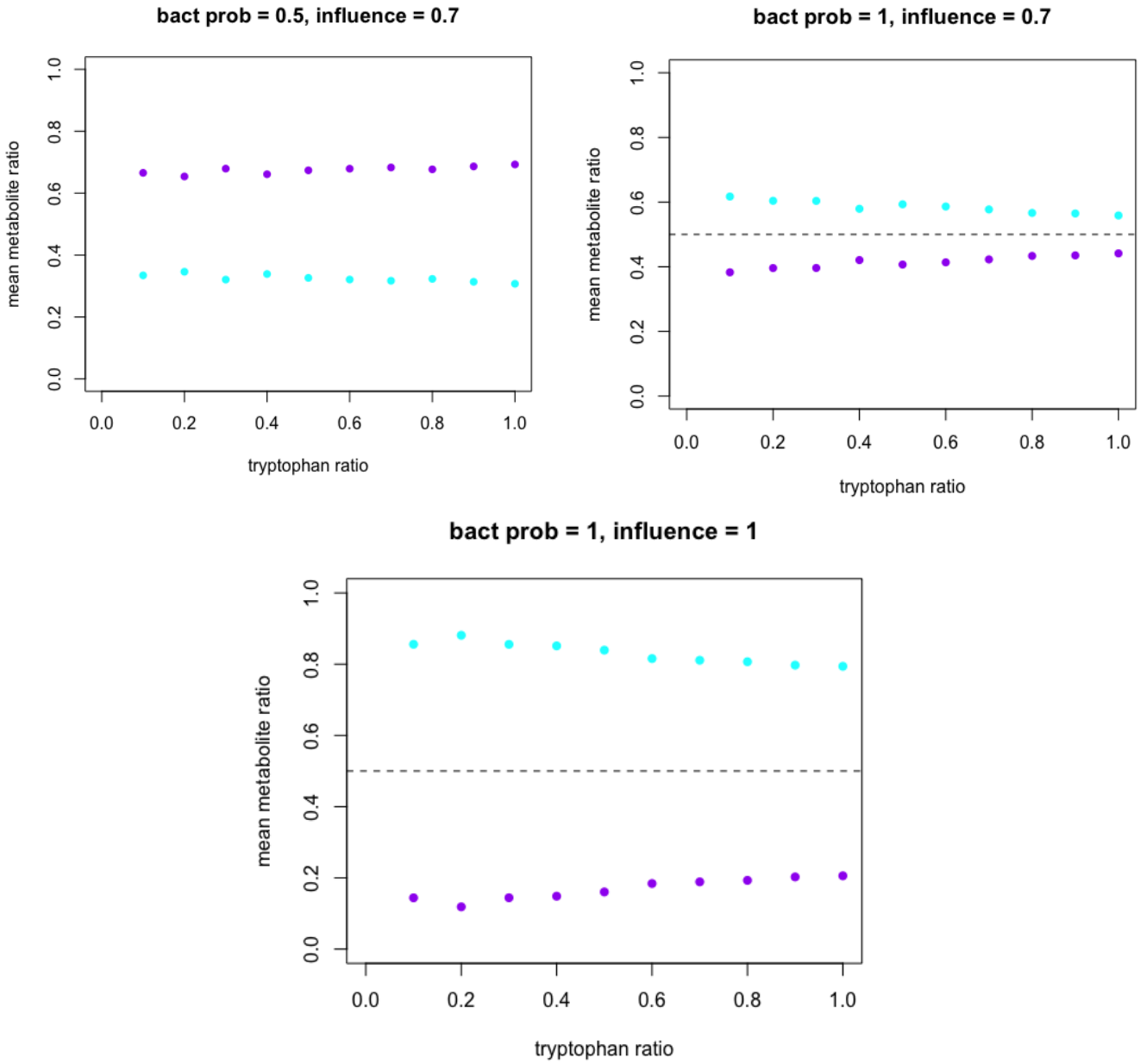

Figure 4. Effect of stricter death parameters on production of serotonin. The energy points were restricted to 30, with the threshold for death set at 20. grid_size = 40, num_agents = 1000, initial_seed = 8675309. Each point is the mean of 15 samples. The purple represents kynurenine and the cyan represents serotonin.

We didn't have enough time to really pour through the literature to find numbers for the model that are supported by experimental evidence. The values we chose for probabilities are somewhat arbitrary. An extensive literature search would improve the predictive abilities of the model.

This model would also be improved by factoring in competition with other bacteria that have been observed to be beneficial and bacteria that are harmful. Modeling those interactions could lead to predictive hypotheses that could be experimentally tested. We could also model the actual production of the reactive oxygen species and other products that the bacteria make, short chain fatty acids (SFCAs). It has recently been shown that these SCFAs can promote serotonin synthesis (Yano et al, 2015), so that is another way that bacteria influence the production of serotonin. Bacterial growth can also be modelled, but it is important to note that many studies have seen that probiotic supplementation with bacteria like *Lactobacillus* does not lead to a permanent *Lactobacillus* presence, indicating that they do not thrive in the gut. The reason is unknown.

User Specifications

We have created an input file for easy changing of the parameters. This input file should be provided as the first command line argument. The name of an output file should be indicated as the second command line argument. The program will output some statistics from the simulation.

The input file should contain the following parameters:

grid_size = 40 *(size of the grid)*
num_agents = 1000 *(this is the total number of nutrients)*
bacteria_prob = 0.3 *(the probability of one gut cell being occupied by a bacterium)*
initial_seed = 8675309 *(initial seed for random generator)*
lactobacillus_influence = 0.5 *(the probability of tryptophan becoming serotonin when lactobacillus is present)*
tryptophan_ratio = 0.5 *(tryptophan and carbohydrate should equal zero)*
carbohydrate_ratio = 0.5

There are parameters within the Bacterium class that could also be changed, like the energy points and death threshold.

We have also included the R script that we used to make the figures. To generate the data for the scripts, we used some for loop in the main method of SimulationManager. They are still there, just commented out.

Literature Cited

Jenkins, T., Nguyen, J., Polglaze, K., & Bertrand, P. (2016). Influence of Tryptophan and Serotonin on Mood and Cognition with a Possible Role of the Gut-Brain Axis. *Nutrients,8*(1), 56. doi:10.3390/nu8010056

Marin, I. A., Goertz, J. E., Ren, T., Rich, S. S., Onengut-Gumuscu, S., Farber, E., . . . Gaultier, A. (2017). Microbiota alteration is associated with the development of stress-induced despair behavior. *Scientific Reports,7*, 43859. doi:10.1038/srep43859

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