

Analysis of conditions necessary for mycorrhizal mutualism  
through a mechanistic model of nutrient exchange

Niko Darci-Maher

UID #504924547

UCLA Ecology and Evolutionary Biology 219B

Professor Lloyd-Smith

10 June 2022

**Abstract**

Trees and fungi interact belowground in relationships called mycorrhizae, in which nutrients are exchanged between tree roots and fungal hyphae. Biological differences between trees and fungi lead to a complementary imbalance in their specialties for metabolizing carbon (C) and nitrogen (N), which some theories suggest results in mutualism between the species. Here, I present a mechanistic model of the mycorrhizal interaction, and use it to discover the conditions required for mutualism. By simulating the system in time, then characterizing the resulting relationship based on steady-state outputs over a range of critical inputs, I discover that a relatively equal amount of environmental C and N is required for mutualism. I also show that an imbalance in nutrient availability leads to parasitism. These findings have the potential to improve the greater understanding of the nature of the mycorrhizal relationship, and thus inform more effective forest management.

## Introduction

A mycorrhiza is a belowground interaction between a tree and fungus<sup>1,2</sup>, which 80-90% of trees in boreal forests participate in<sup>3</sup>. This interaction can be ectomycorrhizal, in which the fungal hyphae, or filamentous tips, wrap around the surface of the tree roots, or arbuscular, in which the fungal hyphae penetrate the cell walls of the tree roots<sup>4</sup>. In both cases, the tree and fungus interact over a large amount of surface area, and nutrients are exchanged between the two across the mycorrhizal connection<sup>1,2</sup>.

Theories suggest that the most critical of these nutrients are carbon (C), which the tree photosynthesizes from the air, and nitrogen (N), which the fungus is highly efficient at metabolizing from the soil<sup>1</sup>. While both organisms are capable of surviving independently, there is a distinct imbalance in their ability to metabolize and make use of both C and N. Trees are effective at photosynthesis, but are diminished in comparison to the fungus at metabolizing soil nutrients<sup>1</sup>. The tree's root system and availability of metabolic compounds is greatly limited compared to the abilities of the fungus<sup>1</sup>. Conversely, fungi are efficient at extracting soil nutrients, but cannot photosynthesize<sup>1</sup>. Fungi are capable of spreading throughout a soil area far more rapidly than a system of roots, and can metabolize some carbon sources from the soil, but are lacking in their ability to sequester carbon compared to trees<sup>1</sup>.

Prevailing theories implicate that these complementary specialties and needs give rise to mutualism, however studies have characterized the mycorrhizal relationship as parasitism or commensalism, and a definitive conclusion is lacking<sup>3</sup>. Here, I present a mechanistic model of the mycorrhiza based on established methods<sup>5</sup>, and use the model to answer a central research question: what environmental conditions are required for a stable mutualistic exchange of C and N in the mycorrhizal relationship between a tree and fungus? Through computational simulations

of the model system, I discover that a relatively equal environmental availability of C and N is required for mutualism, and in an imbalanced environment, the mycorrhizal relationship shifts into parasitism. This result could contribute to an improved understanding of forest ecology and thus result in more informed management of healthy forests.

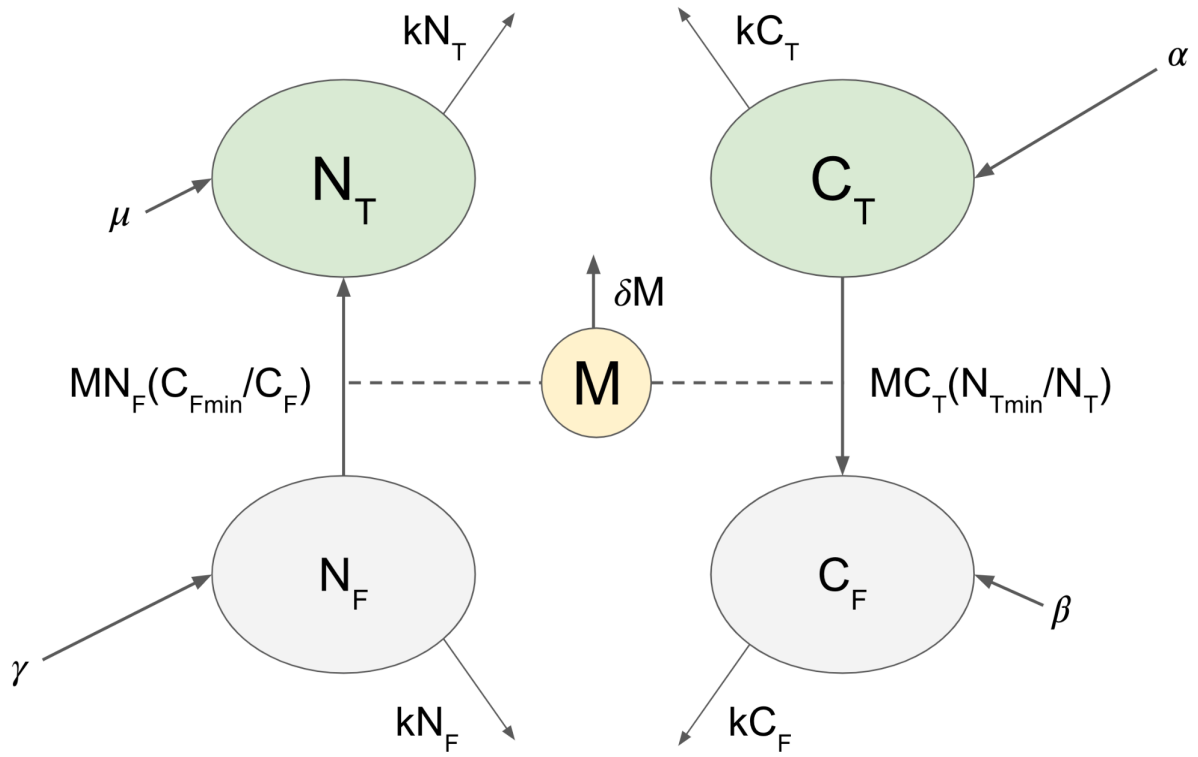
## **Results**

### *Study design*

To determine the environmental conditions required for mycorrhizal mutualism, I first conceived of a mechanistic model representing the exchange of C and N in the mycorrhizal relationship between a tree and fungus. The model consists of distinct state variables representing the free C and N available to the tree and fungus (4 nutrient state variables in total), and an additional state variable representing the degree of mycorrhizal colonization by the fungus on the tree's roots (Figure 1, Equations 1-5, see Methods). I simulated the dynamics of this model over time while varying the crucial input conditions, and determined the resulting ecological relationship for each combination of input parameters. From these results, I drew conclusions about the ecological balance required for mutualism in a mycorrhizal relationship.

### *In four extreme cases, abundance of both C and N results in mutualism*

Before simulating the system over a wide range of parameters, I first aimed to test the behavior of the model in four extreme cases: scarce C and N; scarce C and abundant N; abundant C and scarce N; and abundant C and N. For simplicity, I assumed that the only C coming into the system came in through the tree, and the only N coming into the system came in through the fungus. I then simulated the model in the above cases (Figure 2).



**Figure 1: Mechanistic model of mycorrhizal nutrient exchange.** I constructed a compartmental model to represent the exchange of carbon (C) and nitrogen (N) in the mycorrhizal relationship between a tree and fungus. Ovals and circles represent compartmental state variables, which are represented by ordinary differential equations (ODEs). Solid lines represent flow of nutrients (either C or N) into or out of state variable compartments. Dashed lines represent the dependency of both mycorrhizal flows on the M state variable. Abbreviations:  $C_T$  = free C available to tree;  $N_T$  = free N available to tree;  $C_F$  = free C available to fungus;  $N_F$  = free N available to fungus; M = proportion of tree roots colonized by fungus;  $\alpha$  = C input to tree per unit time;  $\mu$  = N input to tree per unit time;  $\beta$  = C input to fungus per unit time;  $\gamma$  = N input to fungus per unit time;  $\delta$  = scaling parameter of root colonization decay; k = scaling parameter of tree and fungal growth requirements;  $N_{Tmin}$  = minimum amount of free N required for a healthy tree;  $C_{Fmin}$  = minimum amount of free C required for a healthy fungus.

$$\frac{dC_T(t)}{dt} = \alpha - kC_T(t) - M(t)C_T(t) \frac{N_{Tmin}}{N_T(t)} \quad (\text{Eq. 1})$$

$$\frac{dN_F(t)}{dt} = \gamma - kN_F(t) - M(t)N_F(t) \frac{C_{Fmin}}{C_F(t)} \quad (\text{Eq. 2})$$

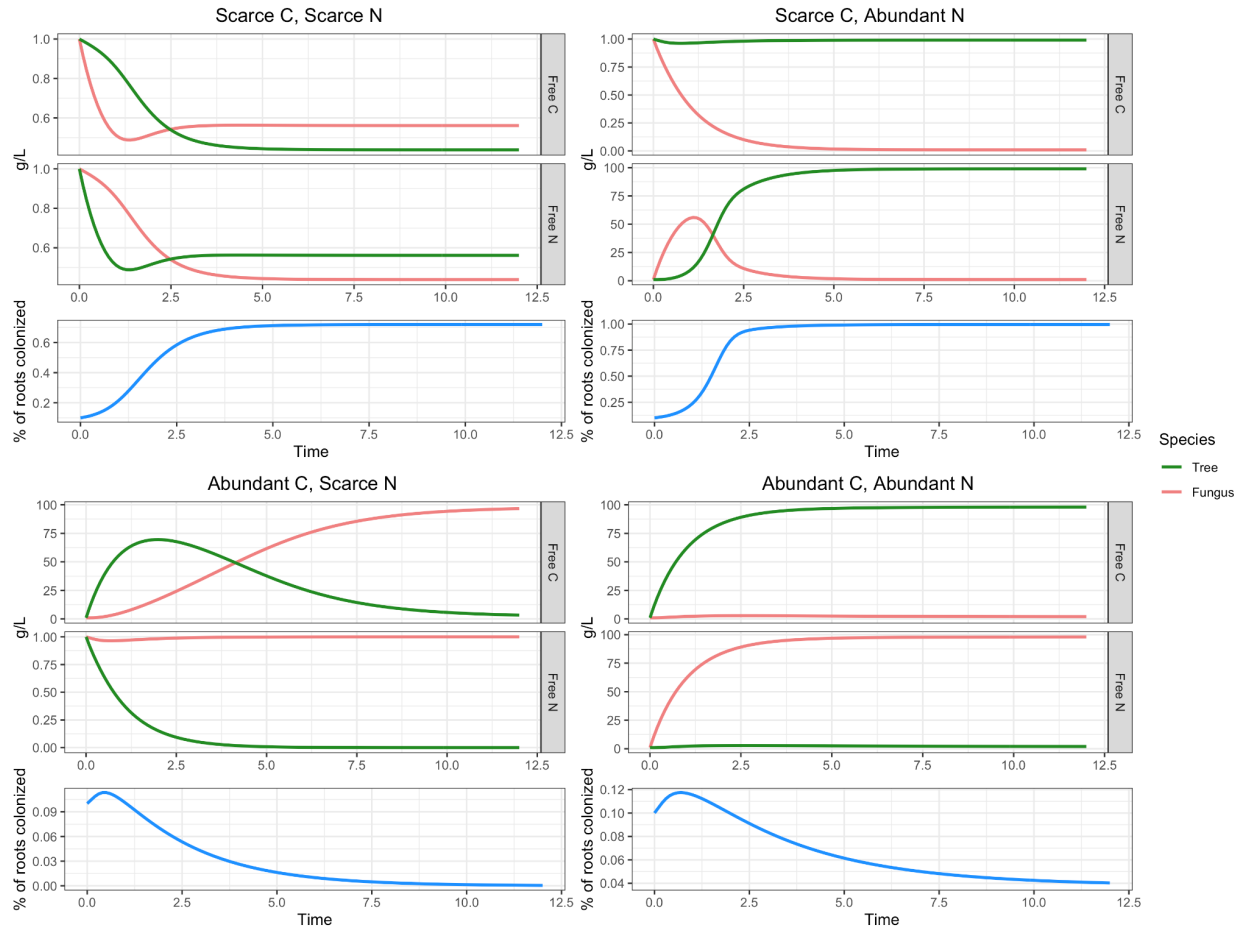
$$\frac{dN_T(t)}{dt} = \mu - kN_T(t) + M(t)N_F(t) \frac{C_{Fmin}}{C_F(t)} \quad (\text{Eq. 3})$$

$$\frac{dC_F(t)}{dt} = \beta - kC_F(t) + M(t)C_T(t) \frac{N_{Tmin}}{N_T(t)} \quad (\text{Eq. 4})$$

$$\frac{dM(t)}{dt} = \frac{C_{Fmin}}{C_F(t)} M(t)(1 - M(t)) - \delta M(t) \quad (\text{Eq. 5})$$

**Equations 1-5: Differential equations to represent the mechanistic model of mycorrhizal**

**nutrient exchange.** Abbreviations:  $C_T$  = free C available to tree;  $N_T$  = free N available to tree;  $C_F$  = free C available to fungus;  $N_F$  = free N available to fungus;  $M$  = proportion of tree roots colonized by fungus;  $\alpha$  = C input to tree per unit time;  $\mu$  = N input to tree per unit time;  $\beta$  = C input to fungus per unit time;  $\gamma$  = N input to fungus per unit time;  $\delta$  = scaling parameter of root colonization decay;  $k$  = scaling parameter of tree and fungal growth requirements;  $N_{Tmin}$  = minimum amount of free N required for a healthy tree;  $C_{Fmin}$  = minimum amount of free C required for a healthy fungus.



**Figure 2: Dynamics of state variables in time across four extreme cases of environmental conditions.** I simulated the dynamic system over time in four extreme cases: scarce carbon (C) and nitrogen (N) (top left), scarce C and abundant N (top right), abundant C and scarce N (bottom left), and abundant C and N (bottom right). For all nutrient plots (red and green), the X-axis represents time, and the Y-axis represents nutrient concentration in g/L. For root colonization plots (blue), the Y-axis represents the proportion of roots colonized by the fungus. I observed that the case of abundant C and N is the only case of these four that results in mutualism in the mycorrhizal relationship. With scarce C and scarce N, the system crashes, and when either C or N is scarce while the other nutrient is abundant, the organism that can directly metabolize the scarce resource dominates.

In the case of scarce C and N, I discovered that the nutrients of both organisms crash. Neither organism can sustain its own growth, and while they both need the mycorrhizal relationship desperately for the resource they are incapable of metabolizing alone, neither organism has any of their own resource remaining. Thus, the degree of root colonization grows significantly but cannot sustain the system.

In the case of scarce C and abundant N, I discovered that while the free C of both organisms dwindles, the tree dominates the free N available to the system. Because the fungus is severely lacking in free C, mycorrhizal colonization surges, but the tree has no surplus C to offer.

In the case of abundant C and scarce N, the opposite occurs, with the fungus dominating the free C in the system. However, here the process happens much slower, as the tree has no control over the degree of colonization.

Finally, in the case of abundant C and N, the mycorrhizal colonization spikes early on, as both organisms are incentivized to participate in the relationship and have plenty of surplus of the nutrient they can metabolize. In this case, as both organisms are satiated, the mycorrhizal colonization decreases over time. Of the four extreme cases tested, the case of abundant C and N is the lone case that gives rise to mutualism.

By plotting the nutrient state variables for each organism against each other with color indicating the degree of root colonization (Figure 3), I observed the behavior of increases in one state variable “rescuing” the other within one organism through an increase in mycorrhizal root colonization, especially in the case of scarce C and abundant N.

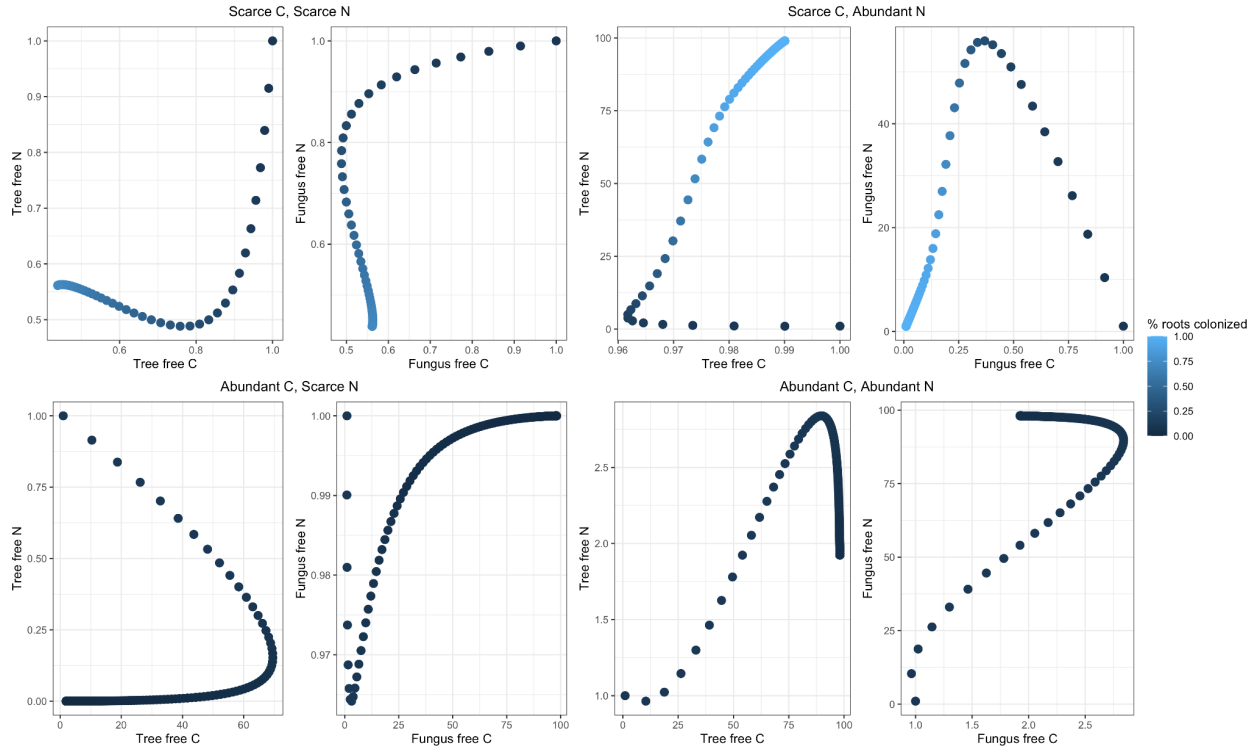
*Over a range of environmental inputs, relatively equal amounts of C and N result in mutualism*

To expand the four extreme simulations to a spectrum of input parameters, I assessed the steady-state relationship of the system across a range of combinations of input C and N. To

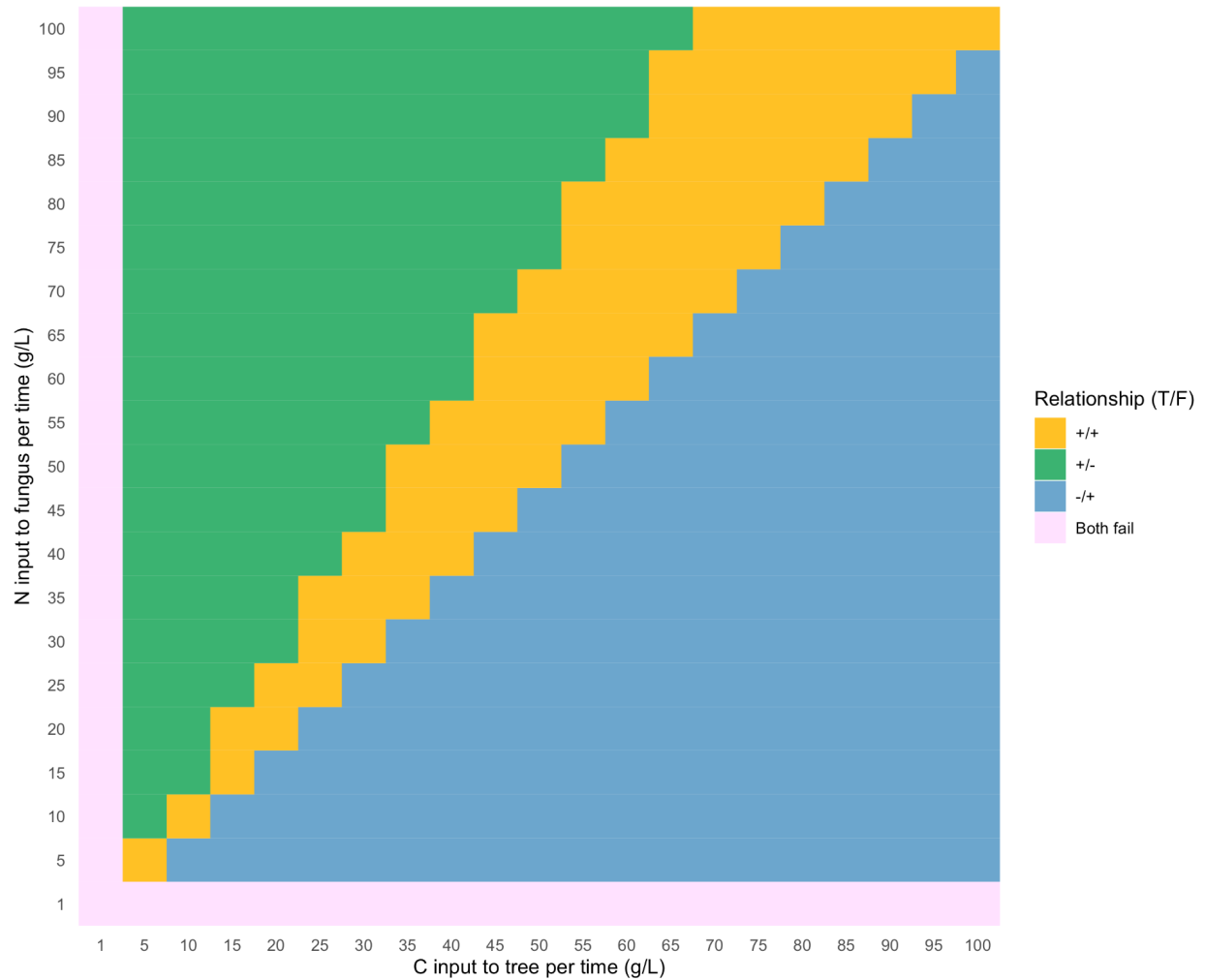
accomplish this, I defined the mycorrhizal relationship as either mutualism, parasitism, or mutual failure, based on whether the steady states of the nutrient state variables exceeded their (low) initial values (see Methods). I then simulated the system over a range of input parameters, characterizing the relationship arising from every combination of  $\alpha$  and  $\gamma$ , where each parameter took the values of 1 to 100, with a step size of 5 (Figure 4, see Methods).

I found that the trend observed in the extreme cases held across the expanded spectrum of parameters. When both resources were scarce, both organisms fail. When one resource is scarce and one is abundant, the organism capable of directly metabolizing the scarce nutrient dominates. When both resources are sufficiently abundant, both organisms survive through mutualism. The diagonal band of mutualism is asymmetrical because the fungus is in full control of the degree of root colonization—the system has a higher tolerance to decreasing input C, because the fungus increases the degree of root colonization proportionally to its need for C.





**Figure 3: Dynamics of state variables against each other in four extreme cases of environmental conditions.** I simulated the dynamic system over time in four extreme cases: scarce carbon (C) and nitrogen (N) (top left), scarce C and abundant N (top right), abundant C and scarce N (bottom left), and abundant C and N (bottom right). For all plots, the X-axis represents free C concentration in g/L, and the Y-axis represents the free N concentration in g/L. Plots in the first and third column correspond to tree nutrients, while the second and fourth column correspond to fungus nutrients. Color indicates proportion of tree roots colonized by the fungus. Each plot can be traced through time by starting at the point (1, 1), which are the initial conditions for all nutrient state variables.



**Figure 4: Relatively equal inputs of C and N into the mycorrhizal system give rise to mutualism between tree and fungus.** I defined mutualism and parasitism based on the steady-state dynamics of the system (see Methods), and simulated the model over a wide range of input conditions to assess the resulting relationship classifications. X-axis represents the C input to the tree per unit time in g/L ( $\alpha$ ), and Y-axis represents the N input to the fungus per unit time ( $\gamma$ ). Colors correspond to the characterization of the steady-state mycorrhizal relationship resulting from each combination of input conditions.

## Discussion

In this project, I developed a mechanistic model of the mycorrhizal relationship between a tree and fungus, and used it to determine the environmental conditions required for mutualism between the two species. The structure of the model represents the biological differences between trees and fungi, namely the ability of the tree to photosynthesize and the effectiveness of the fungus at metabolizing soil nutrients. By simulating the system under a range of input conditions, I discovered that a relatively equal inflow of environmental C and N is required for the tree and fungus to exchange nutrients mutualistically. Additionally, I found that when one nutrient is significantly more abundant than the other, the species that is more efficient at metabolizing the scarce nutrient dominates the interaction, which I characterized as parasitism.

Previous work has been conducted on interactions between organisms that depend on each others' metabolic products for survival<sup>5</sup>, however the model I present here expands on this work because it involves two resources being available to both organisms, with an imbalance in the metabolic abilities of each species. Additionally, my model represents the degree of root colonization, an important factor influencing nutrient transfer, as its own dynamic state variable, which is a structural feature of the biology of mycorrhizae that could be applied to other multi-species interactions.

While my model is not yet validated with field data, its behavior under the relative parameters employed in this project is in agreement with established ecological principles. The  $R^*$  theory proposed by Tilman et al.<sup>6</sup> states that, when two species are in competition for a common resource, the species with the smaller  $R^*$  value will dominate as resource availability decreases. The  $R^*$  value, which is species-specific, is defined as the amount of environmental resource availability at which the species is at equilibrium (i.e. when birth rate = death rate)<sup>6</sup>. By

definition, the species with the smaller  $R^*$  value is capable of surviving on a lower level of available resources, so when the resource availability dips below the higher  $R^*$  value, the higher- $R^*$  species will begin to decline as the lower- $R^*$  species remains in a growth state. I observed this same trend in the output of my model when the mycorrhizal relationship shifted to parasitism, validating the applicability of my model to the true biological system. The structure of my model results in this  $R^*$  difference, because both organisms depend on the surplus nutrients of the other organism for either C or N. The fungus has a lower  $R^*$  than the tree for N, because the fungus takes in N directly, but the tree depends on the fungus sharing N through the mycorrhiza, which only occurs when N is abundant. This imbalance is reversed for C.

The accordance of the model with  $R^*$  theory indicates that some biology is being effectively represented, however further validation is needed. A crucial next step for this work would be to collect the relevant field or greenhouse data from a plot of trees colonized by mycorrhizal fungi, including circulating nutrient concentrations in both the tree and fungus as well as the degree of root colonization by the fungus. This data could be used to fit the model parameters, using a maximum likelihood or expectation maximization approach, which would allow the model outputs to be more directly related to real-world biological values. Additionally, I made a significant assumption in this work that the tree was incapable of metabolizing N from the soil, and that the fungus was incapable of obtaining C without the tree ( $\mu = \beta = 0$ ). To more accurately represent the true biological system, this assumption would need to be relaxed, as these parameters would likely be nonzero after fitting the model to field data.

In conclusion, while the model presented here is not without limitations, it is a promising step towards understanding the interleaved ecological strategies of a tree and fungus as they interact through their mycorrhizal connection. By simulating the model across a wide range of

input conditions and characterizing the resulting relationship, I succeeded in determining the relative environmental conditions required for mutualism. These results contribute to the existing body of work on mycorrhiza, and may assist in the educated management of healthy forests in the future.

## Methods

### *Conception of a mechanistic model for mycorrhizal nutrient exchange*

I designed a compartmental model representing the free nutrients available to the tree and fungus. The model is made up of five state variables, which are represented by a set of coupled ordinary differential equations (ODEs), and eight constant parameters.

To determine the necessary compartments to represent nutrient exchange in the mycorrhiza, I began by selecting carbon (C) as the primary air nutrient, and nitrogen (N) as the primary soil nutrient. It was essential to model distinct nutrients separately for this problem, because the precedent for mycorrhizal interaction relies on the fact that these nutrients are non-interchangeable. This initial choice resulted in four state variables, representing the free C and N stored in the tree, and the free C and N stored in the fungus ( $C_T$ ,  $N_T$ ,  $C_F$ , and  $N_F$ , respectively). For simplicity, I incorporated constant inflows of nutrients into each of these compartments ( $\alpha$ ,  $\mu$ ,  $\beta$ , and  $\gamma$  for  $C_T$ ,  $N_T$ ,  $C_F$ , and  $N_F$ , respectively), to represent the influx of nutrients via the natural processes of each organism. I also added outflows to each of these four compartments to represent the amount of free C and N each organism contributed to their own growth and fixed into tissue, all scaled by a universal parameter  $k$ .

Additionally, I created a fifth state variable  $M$ , which represents the proportion of tree roots colonized by the fungus.  $M$  grows logistically, with a rate scaled by the amount that the fungus is lacking in C ( $C_{Fmin}/C_F$ ). The logistic growth equation,  $M(1-M)$ , represents the fact that

it gets harder to spread onto more roots when there are fewer uncolonized roots to spread to.  $M$  also decays at a rate proportional to itself and a constant  $\delta$ , representing the fact that the fungus has less incentive to maintain extensive colonization of the tree's roots if the mycorrhizal relationship is not being heavily used. Additionally, because the mycorrhizal nutrient exchange depends on the surface area available between the tree roots and fungus body, the amount of nutrients sent across the mycorrhizal exchange is scaled by  $M$ . I modeled this quantity dynamically in time in order to capture the changing ecological strategies of the fungus over the course of the simulation.

To capture the nature of the mycorrhizal relationship, the model includes a flow from  $C_T$  into  $C_F$ , and a flow from  $N_F$  into  $N_T$ . Each of these flows are proportional to  $M$ , the amount of free nutrient available from the sending organism ( $C_T$  for tree,  $N_F$  for fungus), and the amount the sending organism is lacking in the *other* nutrient ( $N_{Tmin}/N_T$  for tree,  $C_{Fmin}/C_F$  for fungus).

### *Implementation of the mechanistic model*

To implement the mechanistic model, I simulated each of the ODEs using the R<sup>7</sup> package `deSolve`<sup>8</sup>, using timepoints from 0 to 100 with a step size of 0.1. I used the following constants as parameter values and initial conditions:  $C_T(0) = N_T(0) = C_F(0) = N_F(0) = 1$ ;  $M(0) = 0.1$ ;  $N_{Tmin} = 1$ ;  $C_{Fmin} = 1$ ;  $\mu = 0$ ;  $\beta = 0$ ;  $k = 1$ ;  $\delta = 0.5$ . I simulated the dynamics of the system for every combination of  $\alpha$  and  $\gamma$  between 1 and 100 with a step size of 5 (i.e. 1, 5, 10, ..., 100). For each combination of  $\alpha$  and  $\gamma$ , this produced a dataframe of each state variable at a series of discrete timepoints, which I then manipulated using the R package `tidyverse`<sup>9</sup>, and visualized using the R packages `ggplot2`<sup>10</sup>, `grid`, `gridExtra`<sup>11</sup>, and `cowplot`<sup>12</sup>.

### *Application of the mechanistic model to determine conditions required for mutualism*

To determine the environmental conditions required for mutualism between tree and fungus in the mycorrhiza, I queried the model for aggregated summary outputs based on vital inputs. Because the raw output of the model consists of the values of the state variables over time, I needed to compute a single summary output representing the overall state of the mycorrhizal relationship under the input conditions. To do this, I first computed the steady state of each state variable, which was the value of the state variable at time = 100, provided that the same state variable at time = 99.9 was within 0.001 of the final value. Next, I created a binary organism health variable, which was computed by testing whether the steady state of both an organism's free C and free N variables increased from its (very low) initial value of 1. From these health variables, I defined a single output to represent the nature of the mycorrhizal relationship between tree and fungus for each set of input conditions: mutualism (+/+) = both tree and fungus are healthy; parasitism (+/-) = one organism is healthy and one is unhealthy; Both fail = both organisms are unhealthy. I then used this characterization of the mycorrhizal relationship based on model outputs to generate a heatmap showing summary-level output of the model across every tested combination of input conditions.

### **Research plan**

For me, the scope of this project ends with this class in mid-June. However, I plan to refer to this project in my application to the Scientists in Parks (SIP) program during the same week that the school year ends. I aim to use this project to demonstrate my ability to apply my computational skills to ecological questions, and will hopefully get the opportunity to continue in ecological research starting next fall.

## References

1. Wang, W. *et al.* Nutrient Exchange and Regulation in Arbuscular Mycorrhizal Symbiosis. *Mol. Plant* **10**, 1147–1158 (2017).
2. Simard, S. W. *et al.* Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* **388**, 579–582 (1997).
3. Stuart, E. K. & Plett, K. L. Digging Deeper: In Search of the Mechanisms of Carbon and Nitrogen Exchange in Ectomycorrhizal Symbioses. *Front. Plant Sci.* **10**, 1658 (2019).
4. Averill, C., Bhatnagar, J. M., Dietze, M. C., Pearse, W. D. & Kivlin, S. N. Global imprint of mycorrhizal fungi on whole-plant nutrient economics. *Proceedings of the National Academy of Sciences* vol. 116 23163–23168 (2019).
5. Fischer, M. M. A mechanistic model of metabolic symbioses in microbes recapitulates experimental data and identifies a continuum of symbiotic interactions. *Theory Biosci.* **139**, 265–278 (2020).
6. Tilman, D. Resource competition between plankton algae: An experimental and theoretical approach. *Ecology* **58**, 338–348 (1977).
7. Team, R. C. & Others. R: A language and environment for statistical computing. (2013).
8. Soetaert, K., Petzoldt, T. & Woodrow Setzer, R. Solving Differential Equations in R: Package deSolve. *J. Stat. Softw.* **33**, 1–25 (2010).
9. Wickham, H. *et al.* Welcome to the tidyverse. *J. Open Source Softw.* **4**, 1686 (2019).
10. Wickham, H. *ggplot2: Elegant Graphics for Data Analysis*. (Springer, 2016).
11. Auguie & Antonov. gridExtra: miscellaneous functions for ‘grid’ graphics. *R package version*.
12. Wilke. cowplot: streamlined plot theme and plot annotations for ‘ggplot2’. *R package version 0.9*.