Temporal dimensions of a microbial invader in a plant-microbiome feedback model

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Summary

- Plant-soil feedback (PSF) theory leverages the increasingly recognized ecological significance of microbiomes to provide insight into plant coexistence. However, PSF theory generally assumes that microbiomes have static composition and properties. In reality, plant communities are thought to experience high rates of colonization by new microbial strains.
- Here we use a PSF model to study the conditions under which soil-associated fungi invade a community of two plant species and their associated microbiomes at different stages of assembly. We manipulate fungal colonization timing, initial plant and microbiome evenness, and properties of all actors, then analyze the resulting community structure.
- Microbial invasion is most successful early in community assembly. However, invasion outcomes are highly variable across colonization timing. We derive an expression showing that the net support for the invader by the plants determines invasion success. When successful, the invader disrupts the negative feedback among the plants and their microbiomes that enables plant coexistence.
- Our results highlight the importance of plant-microbial interactions in microbial invasion success, as well as the susceptibility of plant coexistence to soil-associated microbial invaders during transient dynamics. Broadly, our approach integrating a host-microbiome framework with time-dependent interactions is promising for predicting biodiversity dynamics in a changing world.

Key words: Bever model, coexistence, fungal invasion, host microbiome, plant-soil feedback, priority effects, time-dependent interactions

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Introduction

Theoretical and empirical studies on feedback between host plants and their microbiomes, known as “plant-soil feedback,” highlight that microbes are important drivers of plant community structure (Mangan *et al.*, 2010; Bever *et al.*, 2015). Mutualists and pathogens can generate negative feedback (e.g., coexistence) or positive feedback (e.g., extinction) during plant-plant interactions (Jiang *et al.*, 2020). The ecological conditions that give rise to either positive or negative feedback in plant communities depend on the life-history traits of both microbes and plants (Holt & Lawton, 2003; Parker & Gilbert, 2004; Mordecai, 2013a,b; Spear *et al.*, 2015). However, microbes have high dispersal rates, meaning colonization by new microbes is frequent (Finlay & Clarke, 1999; Chaudhary *et al.*, 2022). Additionally, variation in microbial arrival time can be an important driver of plant community structure. For example, Peay (2018) showed that delayed arrival of an ectomycorrhizal fungal partner resulted in reduced growth of its conspecific host, while benefiting its heterospecific plant competitor. This suggests that variation in microbial properties and colonization can have important implications for plant coexistence. Yet, plant-soil feedback studies have assumed microbiomes to be static in their properties and composition, and generally have only implicitly considered the temporal context of microbial responses to plant hosts (i.e. conditioning phase) and plant responses to the microbes (i.e. feedback phase).

Over the last few decades, theoreticians have developed an influential body of work explaining the potential facilitation or constraints to plant coexistence induced by plant-soil feedbacks. Bever et *al.* (1997) developed a foundational model which departs from the Lotka-Volterra competition approach, instead relying on feedback between plant species and their associated microbiomes alone to determine plant coexistence outcomes. This two-plant model finds that if microbes inhibit the growth of their associated plant (i.e., negative feedback), plant coexistence is enhanced, while positive plant-soil feedback inhibits coexistence. More recently, Abbott et *al.* (2021) merged this model with classic community ecology theory by including interspecific and intraspecific competition among the plant species, allowing deeper understanding and predictability without losing the tractability or empirical applicability of the original model. Subsequent studies have incorporated priority effects – i.e., differential outcomes based on plant species arrival or germination order (Ke & Levine, 2021; Senthilnathan & D’Andrea, 2024), including for a third non-native plant species (Zou *et al.*, 2024). However, the effect of fungal

invasions and their timing on plant coexistence in this plant-soil feedback context have not yet been explored.

Host-associated microbiomes can prevent or facilitate invading microbes from establishing in ecosystems. In invasion ecology, the “biotic resistance hypothesis” posits that highly diverse ecosystems are more resistant to invaders as fewer niches become available, while regions with lower biodiversity are more prone to invasion (Elton, 2020). The biotic resistance hypothesis predicts an inverse correlation between native species diversity and invasive species (Jeschke *et al.*, 2014). While there is some empirical support for biotic resistance at smaller spatial scales (Maron & Vilà, 2001; Espinosa-García *et al.*, 2004), positive correlations at larger spatial scales have been more frequently documented (Stohlgren *et al.*, 2003, 2005; Fridley *et al.*, 2007). Similarly, in microbial communities both positive and negative associations have been found between microbial biodiversity and colonization resistance (Sorbara & Pamer, 2019; Madi *et al.*, 2020). While the mechanisms underlying colonization resistance remain unknown, the order and timing of colonization (i.e. priority effects) play an important role in the assembly of microbial communities and ecological success of invading microbes (Litvak & Bäumler, 2019; Debray *et al.*, 2022).

Here, we extend the canonical plant-soil feedback model to explore how the invasion of a soil-associated fungus or consortium can influence coexistence in a community of two host plants and their associated microbiomes. We analyze the model and conduct extensive simulations to identify the temporal conditions as well as invader and native community properties that result in successful invasions. Furthermore, we explore the relationship between invasion success and plant and microbiome diversity. Finally, we examine the resulting possible final community states and their relationship to plant-microbiome feedback. We find that fungal invader success is determined by the support of the host plants rather than microbiome or host plant diversity, and is most likely during the initial phase of community assembly. Establishment of the invader disrupts the feedback dynamics that maintain plant coexistence, highlighting the susceptibility of plant diversity to invasions of soil-associated microbes absent additional coexistence mechanisms.

Description

Model

We extend the host-microbiome model developed by Abbot et al. (2021) by incorporating a non-host-associated microbial invader into the community of two host-microbiome pairs. The model developed by Abbot et al. (2021) is based on the canonical plant-soil feedback model first proposed by Bever et al. (1997). The community consists of two plant hosts, N_1 and N_2, and their corresponding host-associated microbiomes M_1 and M_2, respectively. The density of each plant host positively affects the growth of their own microbiome. Parameter v represents the strength of support for M_2 by its host N_2 relative to the support for M_1 by N_1 which is assumed to have magnitude 1 (Fig. 1). The microbiome densities feed back on their corresponding host densities: M_1 influences N_1 via the parameter α_{11} and M_2 influences N_2 via α_{22}. Additionally, the microbiomes influence the growth of their non-host plant: M_1 impacts the density of plant host N_2 via the parameter α_{21}, and M_2 impacts N_1 via α_{12}. The two plants N_1 and N_2 affect each other's growth via interspecific competition represented by c_{21} and c_{12}, and through intraspecific density dependent regulation represented by c_{11} and c_{22} (Bever *et al.*, 1997; Bever, 2003; Abbott *et al.*, 2021). Departing from previous models, we include a microbial invader M_3. M_3 directly affects the densities of plant hosts N_1 and N_2 via the parameters α_{13} and α_{23}, respectively, and N_1 and N_2 affect the density of M_3 via the parameters e_1 and e_2, respectively (Fig. 1). Note that M_3 is not a plant-associated microbiome, but it still influences N_1 and N_2 and is affected by them.

The changes in host densities and microbiome frequencies are described using the Lotka-Volterra competition model. The per-capita growth rate of plant host N_i is represented by the parameter r_i. Interspecific and intraspecific coefficients, c_i, describe density-dependent resource competition between and within plant hosts. Plant hosts N_1 and N_2 promote the growth of their respective microbiomes, M_1 and M_2, at a rate proportional to their frequency in the community. Plant densities also cause changes in the frequency of the non-host associated microbe M_3. Microbiome growth is a function of the relative densities of plant hosts and the product of microbiome frequencies. The strength of plant support for the frequency of M_3 is quantified by the parameters e_i where positive values indicate that N_i supports M_3 and negative values indicate that N_i decreases M_3 relative frequency. Furthermore, M_3 modifies the growth rate of both hosts. The strength of growth rate modification by M_3 on N_i is quantified by the model parameter α_{i3}, which can be positive or negative. We define the frequency of M_3 implicitly in the model: $M_3 = 1 - (M_1 + M_2)$.

$$\frac{dN_1}{dt} = r_1 N_1 (1 + \alpha_{11} M_1 + \alpha_{12} M_2 + \alpha_{13} M_3) - c_{11} N_1^{\ 2} - c_{12} N_1 N_2$$

$$\frac{dN_2}{dt} = r_2 N_2 (1 + \alpha_{21} M_1 + \alpha_{22} M_2 + \alpha_{23} M_3) - c_{22} N_2^{\ 2} - c_{21} N_1 N_2$$

$$\frac{dM_1}{dt} = M_1 M_2 \frac{N_1}{N_1 + N_2} + M_1 M_3 \frac{N_1}{N_1 + N_2} - M_1 M_2 v \frac{N_2}{N_1 + N_2} - M_1 M_3 e_1 \frac{N_1}{N_1 + N_2} - M_1 M_3 e_2 \frac{N_2}{N_1 + N_2}$$

$$\frac{dM_2}{dt} = M_1 M_2 v \frac{N_2}{N_1 + N_2} + M_2 M_3 v \frac{N_1}{N_1 + N_2} - M_1 M_2 \frac{N_1}{N_1 + N_2} - M_2 M_3 e_1 \frac{N_1}{N_1 + N_2} - M_2 M_3 e_2 \frac{N_2}{N_1 + N_2}$$

Model Assumptions

Our model assumption that the growth rate modification of hosts as a result of the microbiome environment is proportional to the relative frequency of the active microbiome in the overall microbial community has been proposed to model 2-host 2-microbiome plant-soil feedback systems (Bever *et al.*, 1997). Other attempts to extend this model to describe systems with 3 or more distinct microbiomes have relied on quantifying the density of the microbiome in the environment (Abbott *et al.*, 2021). However, this approach abandons the benefits of the relative frequency assumption and increases the complexity of the model by at least one dimension.

In our extension of the plant-soil feedback model to include a non-host associated microbe or consortium, we retain the assumption that the effects of the microbiomes on host growth rates are proportional to the relative frequencies of these microbiomes in the environment. Practically, we attribute any increase in plant density when grown in a specific microbiome to the effects of this microbiome on its growth rate. Furthermore, if that plant were grown in a microbial community that is partially composed of that microbiome, the strength of this growth rate modification will be multiplied by the frequency of that microbiome in that microbial community. For example, if a plant were exposed to a microbial community that is 30% M_1, 40% M_2, and 30% M_3, its growth rate would be multiplied by a factor of $0.3\alpha_{i1} + 0.4\alpha_{i2} + 0.3\alpha_{i3}$, where α_{ij} is the multiplicative growth rate modification of M_j on N_i when grown in a microbial community that is 100% M_j. Since we are only concerned with the effect of each microbiome, we are free to model the effects of an individual strain or an ensemble of non-host associated microbes with the M_3 state variable.

Numerical simulations

The behavior of the system according to the model dynamics was simulated in R version 4.1.3 (R Core Team, 2022), with the *lhs* package for parameter sampling (Carnell, 2022), the *deSolve* package for numerical integration (Karline Soetaert *et al.*, 2010), and the *tidyverse* (Wickham *et al.*, 2019), *patchwork* (Pedersen, 2022), and *ggrepel* packages for visualization (Slowikowski, 2024). The *lsoda* solver was selected for its speed and dynamic switching between stiff and non-stiff methods as needed (Hindmarsh & Petzold, 2005). As a further protection against numerical instability, an extinction threshold of 10^{-4} was used for all elements of the system under which any host or microbiome concentration that drops below 10^{-4} is considered extinct and considered to have density 0 in future iterations of the simulation.

For the initial investigation of the effects of system parameters on system outcomes, 1,000,000 sets of parameters were sampled from reasonable parameter ranges (exact parameter value samples are given in Table S1 of the SI) using a Latin hypercube sampling method to efficiently and uniformly sample the 11-dimensional parameter space. For each of the sampled parameter sets, the equilibrium density of each plant species if it were alone was calculated using the one-plant equilibrium values for the system and the initial conditions for the model were set such that each plant was at its individual equilibrium density and the microbiome composition was proportional to the relative frequency of each plant. This initial value simulates the introduction of two plants that had been growing individually to the same environment.

From the initial conditions described, the simulation was run for 500 time-steps to allow the system to reach equilibrium and the resulting densities were recorded to determine the outcomes associated with each set of parameters. Those parameter outcomes that give rise to internal equilibria (all system members at nonzero density) were then used for invasion simulations. In initial invasion simulations, 1000 of the parameter combinations that gave rise to internal equilibria were simulated for an additional 100 time-steps from their achieved equilibrium point with 1% of the microbiome replaced with the invader. This process was repeated 1000 times per set of parameters with each invader having different characteristics sampled using the same Latin hypercube sampling method used to sample native system characteristics. The final state of the system following these simulations was recorded and analyzed to determine the probability of any plant extinction (defined as N_1 or N_2 density below

the 10^{-4} extinction threshold) and probability of invader persistence (M_3 frequency greater than 10^{-4} extinction threshold) as a function of pre-invasion microbiome diversity (% M_1).

Model analysis

The first step in analyzing the long-term behavior of the system was to identify the equilibria of the system. This was accomplished by using Mathematica version 13.1 to solve for the densities of the plant hosts and microbiomes for which there is no change to the state of the system (Wolfram Research Inc, 2022), i.e. the derivatives of all densities with respect to time are uniformly zero. Importantly, this process often returns equilibria in which densities are negative. We exclude these solutions as they are not biologically feasible.

While the non-linearity of the system brings about increasingly complex dynamics, it also complicates analytical investigation of the system. A common solution to this issue is to use the stability of the equilibria of a system to describe the long-term behavior of the system. The Routh-Hurwitz stability criterion is a common method to determine the stability of an equilibrium point of a nonlinear system. This method approximates the system-governing differential equations as a linear transformation around a given equilibrium point (the Jacobian matrix of the system evaluated at that equilibrium point), then uses the signs of the eigenvalues to determine if a small perturbation from equilibrium will grow or shrink. As small perturbations are commonplace in any biological system, the Routh-Hurwitz conditions give a good idea of the biological viability of a given equilibrium point.

To investigate the effects of invasion timing on invasion dynamics, 1000 parameter combinations that both reached an internal equilibrium and were locally stable were selected and simulated one time-step at a time until they reached equilibrium (defined as less than a 10^{-4} unit change in all parameters over 1 time-step), and this time to equilibrium was recorded. Then the invader was introduced to these systems at the beginning of the simulation and the system was simulated until it reached its new post-invasion equilibrium. This process was repeated with the invader instead being introduced at the time-point halfway to the time point at which the system had reached equilibrium without the invader, and again at the time-point at which the system reached equilibrium. Finally, all of these steps were repeated 100 times for each parameter combination with invader parameters sampled using a Latin hypercube sampling method.

To investigate the effects of invader characteristics on invasion outcomes, one set of native system parameters for which the internal equilibrium is stable was chosen to limit the computational complexity of the analysis. This set of parameters was obtained by using Mathematica's built-in *Solve* function to select a set of parameters for which the Routh-Hurwitz stability conditions for the two-host two-microbiome (pre-invasion) equilibrium was satisfied. Then the stability of each equilibrium point was computed for given values of α_{13}, α_{23}, e_1, and e_2 to determine the long term behavior of the system if an invader with those characteristics was introduced in a small dose. If the internal equilibrium remained stable, the system resisted invasion. If the internal equilibrium became unstable, the stability of other equilibria was used to determine the long-term behavior of the system. If the only stable equilibria for a given set of invader characteristics were those in which only N_1 remains, this region in parameter space was recorded and the same was done for N_2. For equilibria where N_1 and/or N_2 remained, we were unable to determine the long-term behavior of the system, so these regions were those in which priority effects dominate based on what stable equilibrium point was first reached by the system. These investigations were conducted for invader characteristics for every combination of α_{13}, $\alpha_{23} \in \{-1,1\}$ and e_1, $e_2 \in [-2,2]$. We chose e_1 and e_2 to be the continuous variables of the analysis because they more directly determine whether the invader is able to persist in the system.

Results

Inclusion of microbiome dynamics alters the qualitative outcomes of the model. In the example simulation shown in Figure 2a and b, plant densities begin to stabilize by time 50 with plant N_1 at a greater density than N_2. Upon introduction of the invading microbe, the system's microbiome composition is altered such that M_1 is nearly extinct by time 90 at which point the density of N_1 decreases, having lost the support of its native microbiome. Ultimately this allows N_2 to increase in density and the system re-stabilizes with the opposite dominant plant compared to pre-invasion, and N_1 is driven extinct. The invader throws the system off its trajectory to a stable equilibrium with both plants, towards a single-plant equilibrium (Fig. 2c and d).

The probability of invader persistence decreases as the timing of invasion approaches the time at which the system reaches its equilibrium in the absence of a fungal invader (Fig. 3a). Plants are also more likely to go extinct as the invader colonizes earlier, although the effect is weaker (Fig. 3b). The probability of presence of the microbial invader at post-invasion equilibrium is

independent of initial microbiome and plant evenness (Fig. 4a and b). Likewise, the probability of any plant extinction following invasion is independent of microbiome and plant evenness at pre-invasion equilibrium (Fig 4c and d). The data points at high probability values indicate the existence of parameter combinations that are particularly susceptible to plant extinction. The overall probability of invader persistence is greater than plant extinction.

An analysis of the Routh-Hurwitz stability criteria for all system equilibrium points proved useful for predicting invader characteristics and community outcomes for successful invasions at equilibrium (Fig. 5). Equilibrium stability analysis showed the existence of three unique equilibrium outcomes following fungal invasion: the fungal invader is resisted and the system returns to its pre-invasion equilibrium, or the fungal invader persists and either N_1 or N_2 goes extinct. In Figure 5, these outcomes are labeled as “Invasion resisted” when the system returns to pre-invasion equilibrium, “Only N1 remains” when N_2 goes extinct, “Only N2 remains” when N_1 goes extinct, and “Priority effects” when N_1 or N_2 may go extinct. In this latter case, the post-invasion equilibrium depends on which plant goes extinct first, although this information cannot be determined from equilibrium stability analysis alone because equilibria in which both plants go extinct are feasible.

For the parameter combination modeled, the invader successfully established itself if $e_1 + e_2v > 0$ independent of α_{13} and α_{23} (Fig. 5a, b). Since v is the ratio of N_2 density to N_1 density at equilibrium, the necessary and sufficient inequality for microbial invasion translates to the following statement: the sum of the support provided by N_1 and N_2 weighted by their equilibrium densities completely determines the success of a microbial invasion such that when the total support is positive the invasion is successful, and when the total support is negative, the invasion is unsuccessful. Although this inequality was algebraically derived by fixing all parameters except e_1, e_2, and v, the result holds when any other single system parameter is allowed to vary. However, we were not able to allow more than four variables to vary in our analyses, so it is unclear whether the inequality result holds for an arbitrary set of parameters giving rise to a stable two-host two-microbiome equilibrium. Among parameter combinations that gave successful invasions, the particular outcome was determined by α_{23}. In regions in which a particular extinction outcome is guaranteed, when $\alpha_{23} = 1$, N_1 goes extinct (Fig. 5a) and when $\alpha_{23} = -1$, N_2 goes extinct (Fig. 5b). Otherwise, priority effects dominate and long-term dynamics are not discernible through Routh-Hurwitz stability analysis (Fig. 5).

The dependence of the final community composition on α_{23} is likely due to the particular v parameter chosen for the analysis. Since this value was less than 1, the equilibrium density of N_1 was greater than N_2, so with the magnitude of the effects of M_3 on the plants being equal, N_2 would go extinct before N_1. Furthermore, for fixed e_1 and e_2 values facilitating fungal invasion, priority effects dominate for most α_{13} and α_{23} values (Fig 5c & d). However, when $e_1 = e_2 = 1$, there is a region of α_{13} and α_{23} values for which extinction of N_2 is guaranteed (Fig. 5c) while priority effects dominate for all α_{13} and α_{23} values when $e_1 = -1$ and $e_2 = 1$ (Fig. 5d). Extinction of one plant is guaranteed even when the invader has a small or zero effect on plant growth rates because the collapse of microbiome dynamics as a result of fungal invasion eliminates microbiome-mediated negative feedback mechanisms that enable plant coexistence.

Discussion

We extend the canonical plant-soil feedback model to examine the conditions allowing fungal invasions – including temporal dynamics – as well as the consequences of these invasions for plant-microbiome coexistence (Bever *et al.*, 1997; Abbott *et al.*, 2021). Our model is sufficiently general to represent the invasion of any soil-associated organism or group of organisms into a community of two plant species. For instance, the invader could be a species of fungus, bacterium, or even a consortium of soil microbes – but not a plant-associated microbiome. Our analyses suggest that early arrivals, i.e. invaders arriving well before the community approaches equilibrium while plant and microbiome densities are fluctuating, will be more likely to persist. Otherwise, the net support for the invader by the plants, and not plant or microbiome diversity, determines invader success in our model. Given that the invader establishes in the community, it interferes with the negative feedback among plant species and their microbiomes that allows plant coexistence, thereby causing local extinction of one of the plant species and its native microbiome.

While invasion success is highest during the initial phase of community assembly, community-level outcomes did not depend on initial microbiome evenness. This finding indicates that invasion in our model is primarily mediated by plant-invader interactions rather than native microbiome-invader interactions, a conclusion that is further supported by the results of our invader characteristic studies. This result is consistent with the basin of attraction from dynamical system theory: as a greater amount of time passes before the introduction of the invader, the system state travels farther into the basin of attraction of its equilibrium. As a

consequence, the perturbation to the system caused by the invader must be larger to send the system to a dysbiotic equilibrium. Therefore the dysbiosis of the system that is caused by the introduction of the fungal invader is a direct effect of the invader's ecological interactions in the community, rather than a perturbation of the initial state of the system. Our invasibility analyses of the model reveal four final qualitative community states: 1) community resistance to invasion, 2) fungal invader persistence and extinction of one of the plant hosts and their associated microbiome, 3) monocultures of one or the other plant species in a microbiome dominated by the fungal invader, and 4) alternative stable states of either plant species as a consequence of priority effects.

Studying the relationship between at-equilibrium invasion outcomes and microbial invader characteristics yielded insight into the relative importance of different kinds of plant-invader interactions. For the system analyzed, we found that the success or failure of invasion was determined by the inequality $e_1 + e_2v > 0$. If $e_1 + e_2v > 0$, then the invasion was successful, otherwise the invasion was unsuccessful. This inequality relates the amount of support N_1 and N_2 provide the invader, e_1 and e_2 respectively, with the relative density of N_2 to N_1 at equilibrium, v. Thus $e_1 + e_2v$ can be thought of as the net support for the invader by the hosts at equilibrium. So biologically, this inequality states that the invasion is successful if the invader is supported by the hosts at equilibrium and unsuccessful if the invader is resisted. While it is possible for the support for the invader to turn negative in a system where the inequality is satisfied, the inequality guarantees that the support for the invader will always be positive in some neighborhood around the native equilibrium, preventing the system from ever returning to the native equilibrium. While the success of an invasion is determined by the e_1, e_2 and v parameters, the α_{13} and α_{23} parameters determine which host goes extinct upon a successful invasion. In some cases, which host will go extinct upon invasion can be determined using only system parameters. However, in many other cases feasible equilibria exist that correspond to the extinction of either host. In these cases priority effects dominate, so determination of the long-term behavior of the system is best obtained through direct modeling of the system with the particular parameters and initial conditions relevant to the application rather than the more general equilibrium stability analysis used in this study.

The nonlinearity of the model introduces complex relationships between system parameter values and modeled outcomes that increase the representational capacity of the model but pose challenges to the analysis of the model. Overall, the large variation in invasion probabilities (the

vertical spread of plotted points in Figure 3) indicates that the probability of host extinction and invader fixation are largely determined by the particular native system characteristics. So, although there is a trend in overall invasion probability as the timing of fungal invasion varies, this trend must be considered in the context of the particular system parameters modeled to accurately predict the probability of invasion when modeling real systems. Likewise, in Figure 4, the tighter clustering of points around the mean in the panels describing the probability of fungal invader persistence (Fig. 4a, 4b) relative to the panels describing the probability of plant extinction (Fig. 4c, 4d) suggests that the variance in extinction outcomes is largely due to native system characteristics as opposed to invader characteristics. As such, the parameters of the native community must be carefully considered in addition to invader characteristics when modeling invasions of soil microbes into plant communities.

Our study demonstrates that the mechanism for plant coexistence in the classic plant-soil feedback model breaks down when a soil-associated fungus successfully invades the system. The model originally proposed by Bever et al. (1997) predicts that two competing plants can coexist when soil microbes provide a negative feedback on plant density. On the contrary, positive feedback on plant density results in the exclusion of one of the plant species. In the absence of microbial invasion, our model results in neutral oscillations, effectively reducing back to the Bever model. However, when the invader establishes itself in the community, we find that it interferes with the negative feedback among the plant species and their microbiomes, allowing one of the plants and its microbiome to go extinct. While the condition for species persistence in the Bever model requires a negative feedback ($I_s < 0$), this is not sufficient for long-term plant coexistence in the presence of an invader. Given that microbial invasions are likely common, additional mechanisms may be needed to account for plant coexistence in nature such as trait trade-offs and metacommunity processes (Thompson *et al.*, 2020).

Studies on the temporal dimension of plant-soil microbiome interactions have primarily focused on the demographic processes involved in soil microbial conditioning and plant response phases. For example, patch-occupancy models have been used to study the effects of soil microbial conditioning on plant establishment (Krishnadas & Stump, 2021; Ke & Levine, 2021; Miller & Allesina, 2021). Ke and Levine (2021) found that variable microbial decay rates impacted the persistence of plant communities, with faster decay rates resulting in variable species fecundity rates and higher plant diversity. Models have also considered the microbial effects on plant demographic processes. For example, Mordecai (2013) found that a shared

fungal pathogen between an invader annual and native perennial plant host differently impacted seed infection rates, germination rates, and adult host survival. Contrary to expectation, pathogen spillover in multi host communities had a broad range of outcomes including coexistence, exclusion, and priority effects (Mordecai, 2013b). Subsequent work in the same study system found that foliar fungal pathogens had minimal effects on perennial plant competitors and coexistence outcomes as compared to fungal seed pathogens (Uricchio *et al.*, 2019). Furthermore, foliar fungal pathogens did not promote negative frequency dependence and plant coexistence (Spear & Mordecai, 2018). While our model didn't consider the impacts of fungal invasion on the demographic rates of plants and their associated microbiomes, we do find qualitative agreement with previous models exploring long term outcomes of invasion on plant communities (Mordecai *et al.*, 2015). In particular, we find that the inclusion of the fungal invader in the community does not result in the long-term coexistence of plants and microbiomes.

Finally, we must note the limitations of the analysis presented. Namely, we varied e_1, e_2, α_{13}, and α_{23} for fixed values of all other system parameters. While repeat analyses with one other system parameter allowed to vary yield results in agreement with those presented, the computational complexity of conducting the same analyses with all system parameters left free is too great. Overall, this analysis indicates that action of the hosts on the invader determines invasion success whereas action of the invader on the hosts determines post-invasion system behavior.

There are many important sources of variation in real ecosystems which we were not able to explicitly investigate within the scope of our study. Firstly, our results regarding invasion timing are most applicable to relatively stable environments, where priority effects are more likely to be important. Under significant environmental variability, arrival time of species becomes less consequential as fluctuating conditions can facilitate invasion (Tucker & Fukami, 2014). Another source of variation is microbiome type. Theory suggests that microbiomes with a more facilitative effect on their host plant – e.g. dominated by mycorrhizae – encourage priority effects and lower plant diversity, while microbiomes with a more negative effect – e.g. dominated by pathogens – encourage plant coexistence (Bever *et al.*, 1997; Umbanhowar & McCann, 2005; Crawford *et al.*, 2019). While we did not assume neutral effects of microbiomes on their hosts, we randomized these effects and did not focus our study on the influence of their sign. Therefore, further research could more explicitly investigate different patterns expected when positive or negative microbiome effects dominate. Different fungal functional groups (e.g. AMF

vs. pathogens) can also exhibit different community assembly patterns. For example, some functional groups can be more strongly structured by dispersal while others are more strongly structured by environmental filtering, although studies disagree on the particular hierarchy (Wang *et al.*, 2022; Liang *et al.*, 2023; Gacura *et al.*, 2024). Assembly patterns are also likely to depend on spatial scale, where priority effects are more likely to be important at small scales and factors such as dispersal can be more important at larger scales. For example, using an approach focusing on spatial effects, Gacura and colleagues (2024) were able to explain the assembly of fungal functional groups across forest stands but not at small scales, citing priority effects as a likely explanation. Our results may therefore be most applicable at small spatial scales. Further research is needed to extend our approach to incorporate metacommunity processes and community coalescence (Castledine *et al.*, 2020; Thompson *et al.*, 2020).

Our study contributes to a new node of ecological theory at the intersection of multispecies coexistence, host-microbiome dynamics, and time-dependent interactions, all of which appear to be important for understanding and predicting ecosystem dynamics (Zou *et al.*, 2024). While many previous studies have either assumed all species are initially present or assume an invader enters a system at equilibrium, in reality communities rarely appear to be in a demonstrable equilibrium. Plant communities (including their microbiomes) are frequently undergoing long-term succession as well as phenological changes. Meanwhile, individual plants and microbes are constantly undergoing development and senescence. Therefore, microbial invasions may well have differential impacts on plant communities depending on their timing with respect to plant succession and development. It is increasingly important to understand how species interactions change over successional and phenological timelines as ecosystems are recovering more frequently from anthropogenic disturbances and interventions, and as climate change creates phenological mismatches among interacting species. In the years to come, research in this emerging sub-field of complex time-dependent interactions among multiple species and microbiomes has the potential to provide managers with the knowledge necessary to become stewards of biodiversity and ecosystem services in a new era of species migrations and global change.

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Competing interests

The authors have no competing interests to report.

Author contributions

WHP and SY planned and designed the research. WHP, SY, and CJR wrote the manuscript. WHP performed numerical simulations, analysed data, and produced the figures.

Data availability

The data that support the findings of this study are openly available in Zenodo at https://doi.org/10.5281,14783188.

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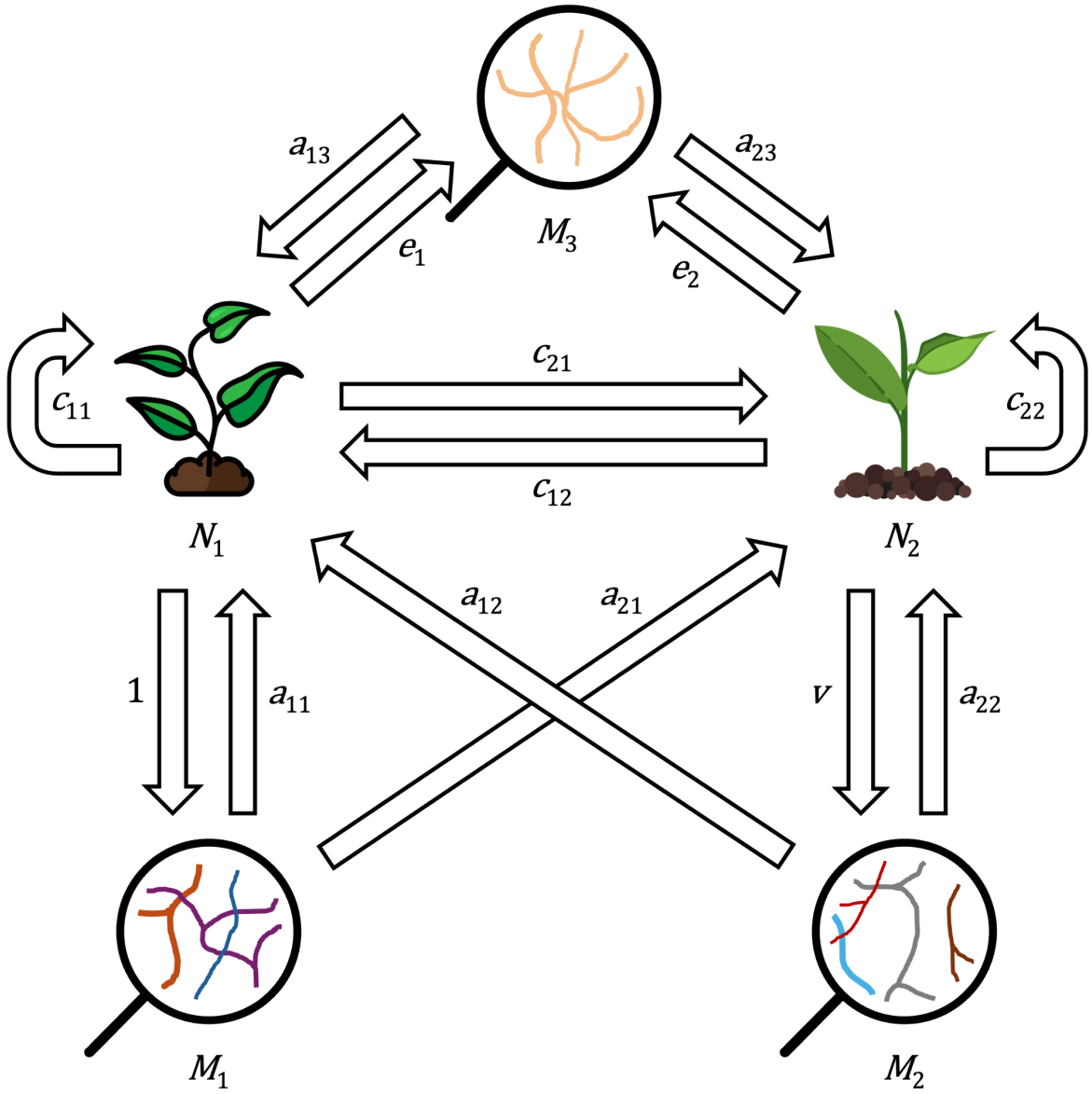

Figure 1: Diagram representing the state variables in the model as well as the direct interactions between them. Arrows are labeled with the key parameters that determine the strength and sign of the interaction. State variables, clockwise from bottom left: M_1, the microbiome associated with host plant N_1; N_1, host plant 1; M_3, the invading microbe; N_2, host plant 2; and M_2, the microbiome associated with host plant N_2. M_1, M_2, and M_3 each influence the density of both host plants, N_1 and N_2. Meanwhile, N_1 and N_2 both influence the density of only their associated microbiome and of M_3 (e.g. N_1 directly affects M_1 and M_3 but not M_2). Lastly, both N_1 and N_2 influence the density of each other and of themselves via interspecific and intraspecific competition, respectively.

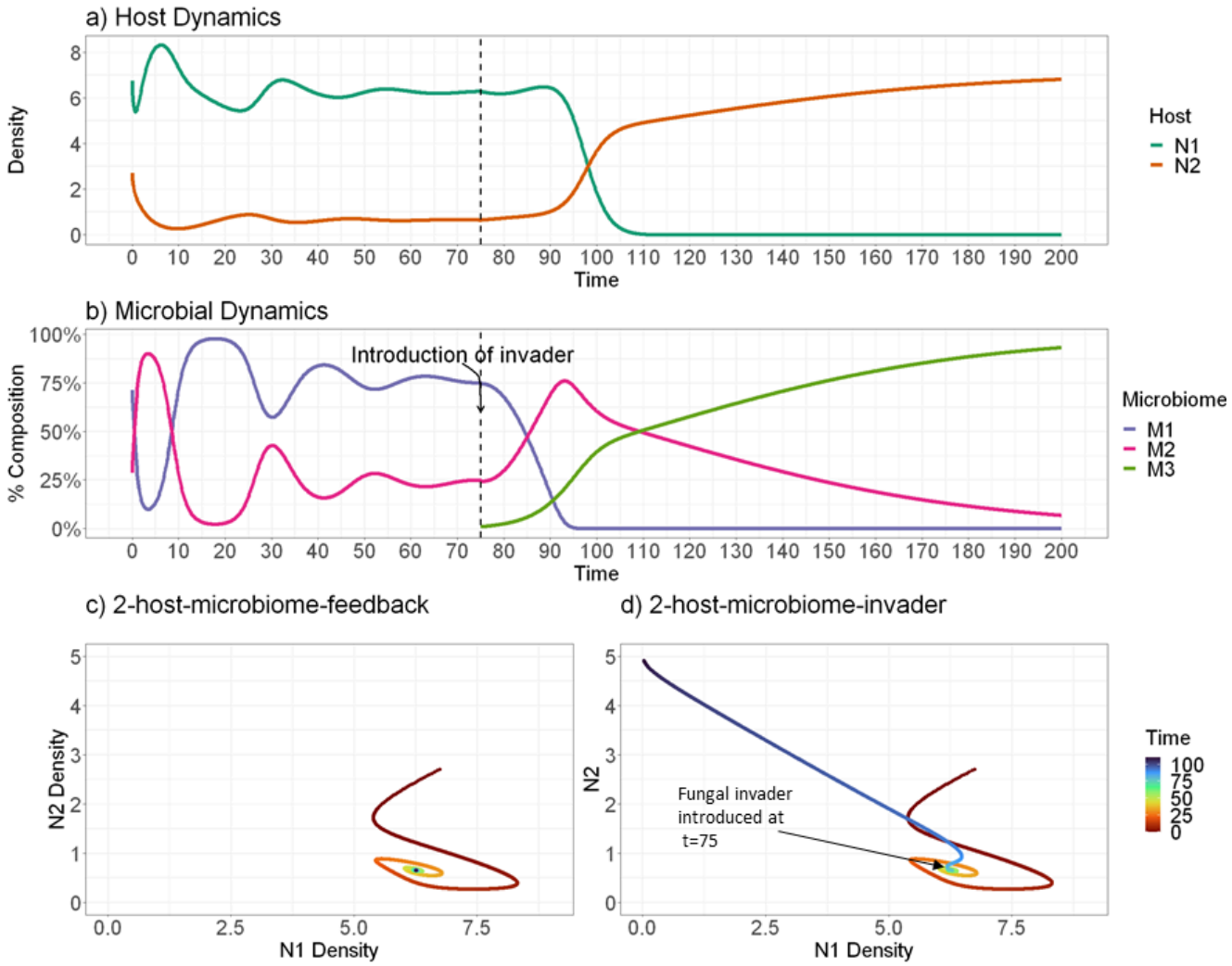

Figure 2: a) Example time series showing a change in host dynamics upon inoculation with a pathogen invader leading to extinction of host N_1. b) Time series of the changing microbial community associated with the host dynamics presented in panel a. Pre-invasion, microbiome diversity shifts to stabilize host dynamics through negative feedback. Post-invasion, the fungal invader dominates and the stabilizing effect of the microbiome dynamics is compromised. c-d) Plant species densities over time for two systems with the same parameters. c) With no invasion, the community approaches a stable equilibrium in which both plant hosts are present, as described by the Bever et al. (1997) model. d) A fungus invades after 75 time-steps, sending the system to a new equilibrium in which plant N_1 goes extinct.

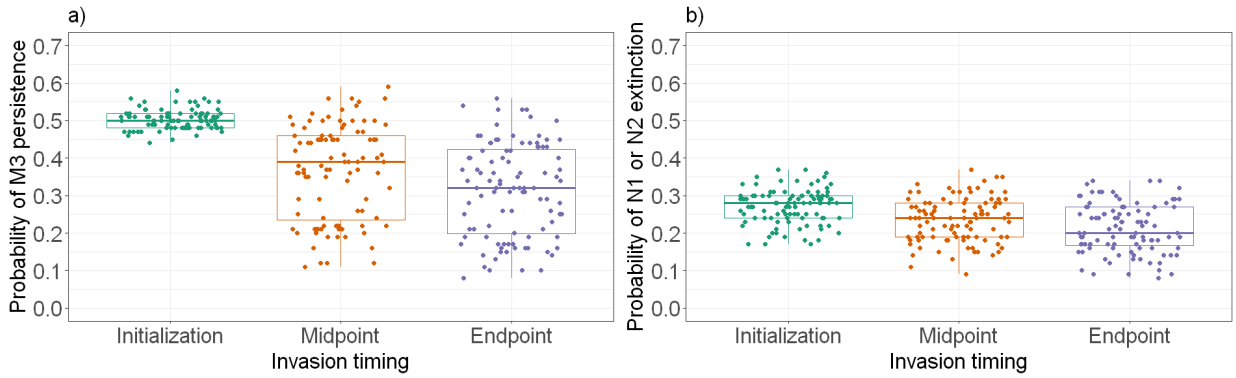

Figure 3: a) The probability of the persistence of the invading microbe M_3 as a function of invasion timing. b) The probability of any host extinction as a function of invasion timing. Across both plots, individual points represent a probability averaged over 1000 combinations of invader parameters while native system parameters are held constant.

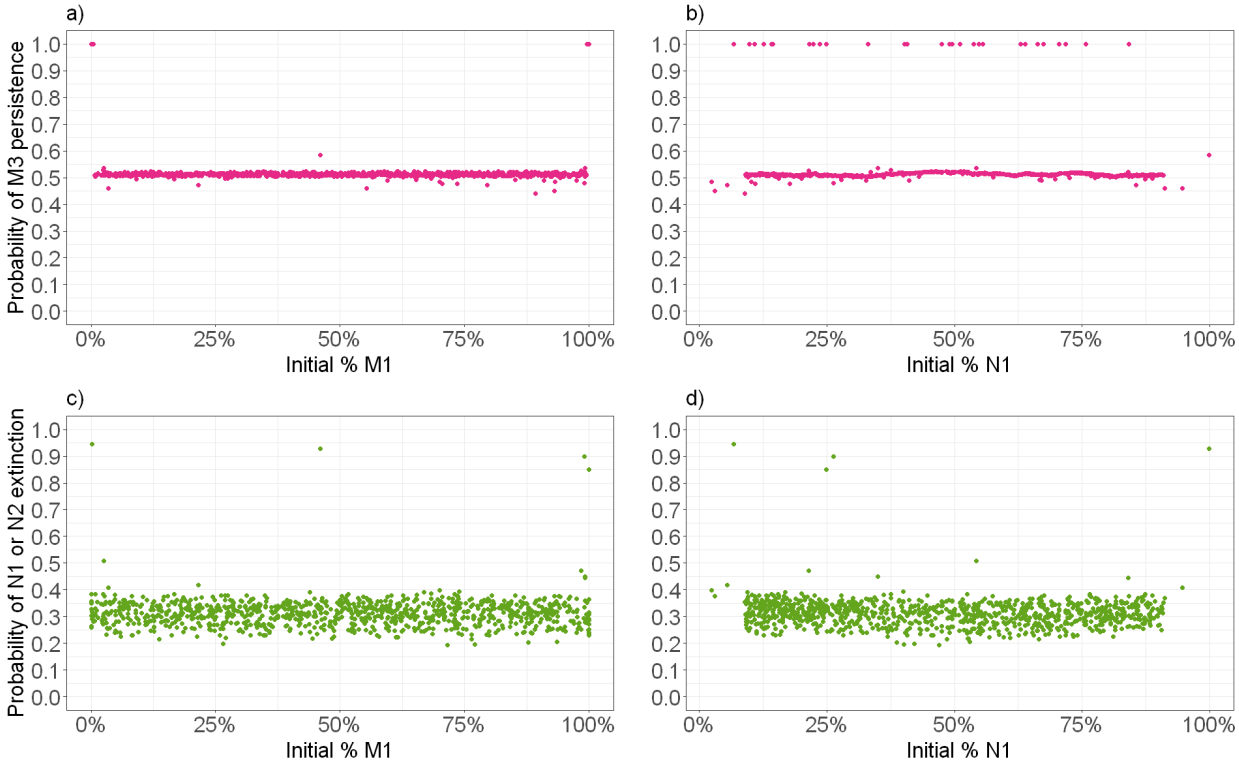

Figure 4: a-b) The probability of invader success as a function of a) microbiome diversity pre-inoculation and b) plant diversity pre-inoculation. c-d) The probability of any plant extinction as a function of c) microbiome diversity pre-inoculation and d) plant diversity pre-inoculation. Individual points represent a probability averaged over 1000 combinations of invader parameters while native system parameters are held constant.

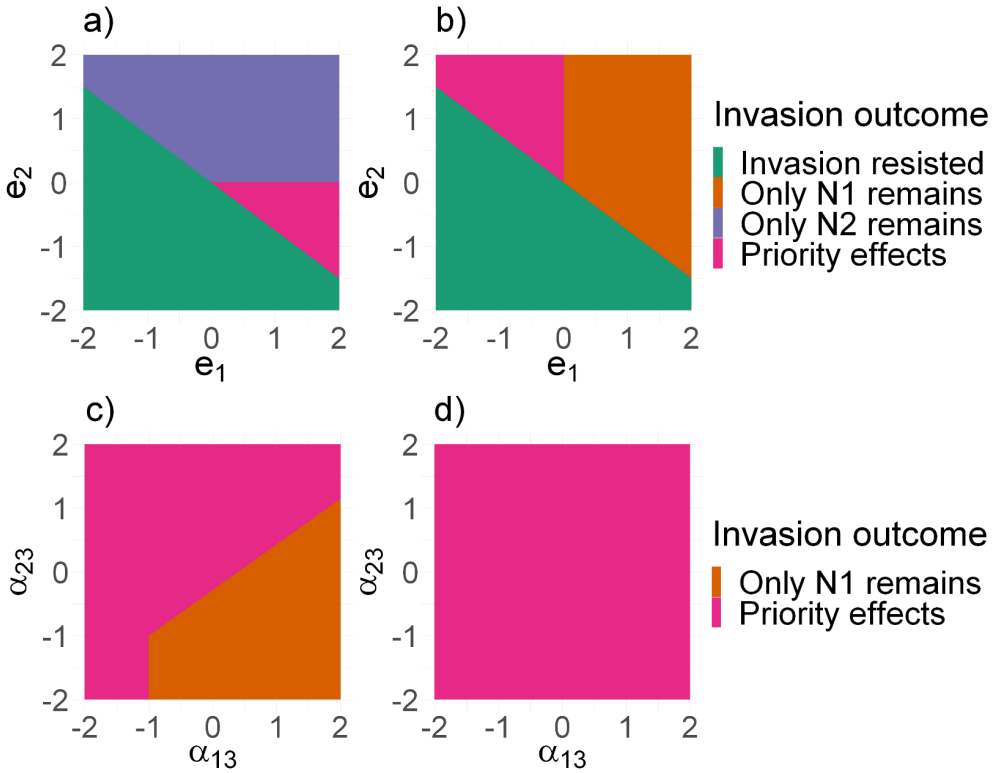

Figure 5: a-b) Plots showing the relationship between plant host support for the fungal invader and invasion outcomes. Results are displayed for a constant set of native community parameters when the invader has a strong effect on plant growth rate, a) for α_{13} = 1, -1 and α_{23} = 1 and b) when setting α_{13} = 1, -1 and α_{23} = -1. c-d) Plots showing the relationship between invasion outcome and the sign and magnitude of the fungal invader's modification of plant growth rates. Results are displayed for a constant set of native community parameters when the plant hosts support the fungal invader, and c) for e_1 = 1 and e_2 = 1 and d) for e_1 = -1 and e_2 = 1.

Supplemental Information

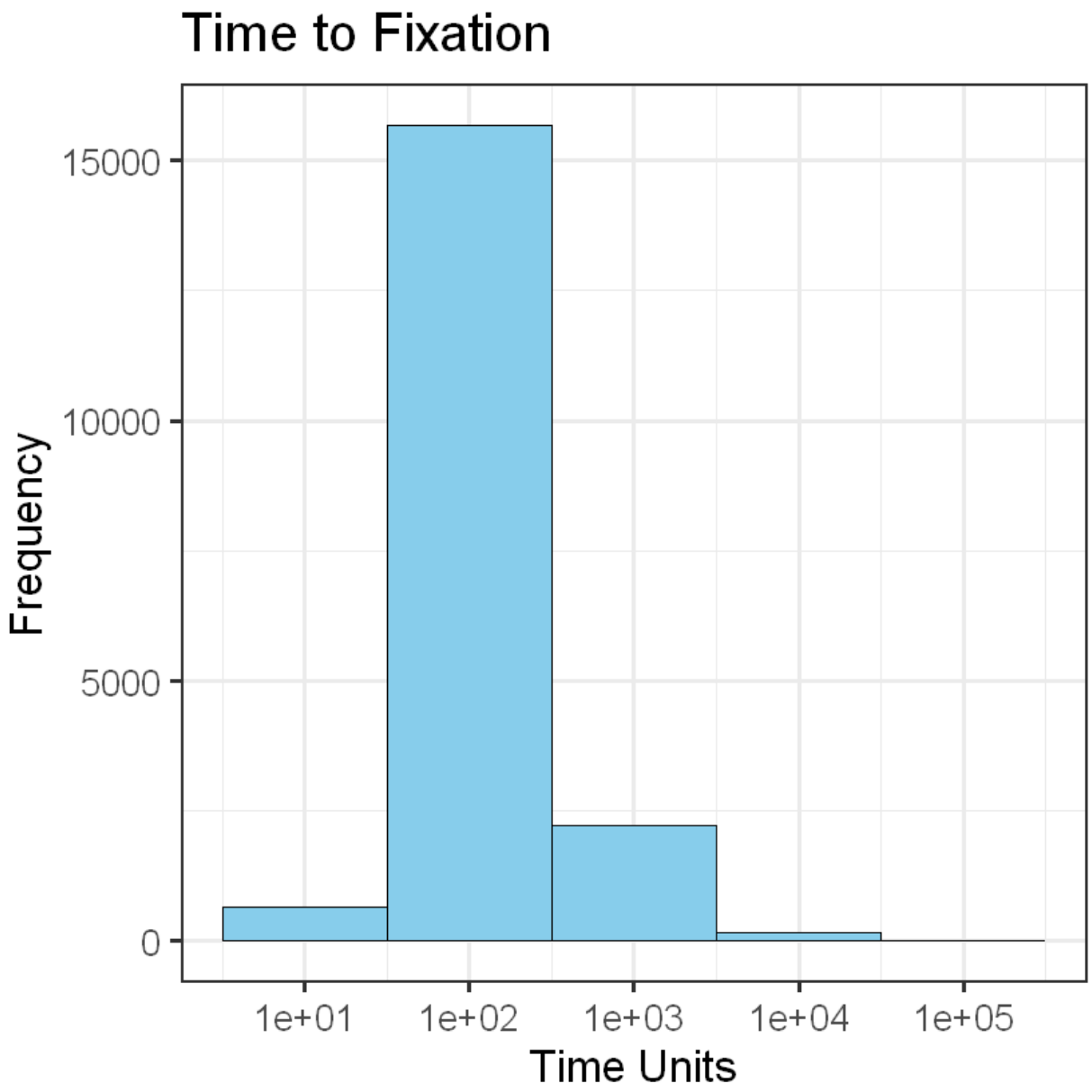

Figure S1: Histogram of time steps required to reach equilibrium for various combinations of native community parameters sampled in our simulations. The time to fixation depends on the system parameters, but a fixation time on the order of 100 time units was most frequently observed.

Table S1: Parameter value ranges sampled in the analyses.

Parameter	Interpretation	Minimum value sampled	Maximum value sampled
r_1	Growth rate of N_1	0.5	2
r_2	Growth rate of N_2	0.5	2
α_{11}	Frequency-dependent growth rate modification of N_1 by M_1	0	1
α_{12}	Frequency-dependent growth rate modification of N_1 by M_2	0	1
α_{13}	Frequency-dependent growth rate modification of N_1 by M_3	-2	2
α_{21}	Frequency-dependent growth rate modification of N_2 by M_1	0	1
α_{22}	Frequency-dependent growth rate modification of N_2 by M_2	0	1
α_{23}	Frequency-dependent growth rate modification of N_2 by M_3	-2	2
c_{11}	Density-dependent effect of N_1 on N_1	0.1	0.5
c_{12}	Density-dependent effect of N_2 on N_1	0.1	0.5
c_{21}	Density-dependent effect of N_1 on N_2	0.1	0.5
c_{22}	Density-dependent effect of N_2 on N_2	0.1	0.5
v	Strength of support for M_2 by N_2 as a factor of the support for M_1 by N_1	0.1	10
e_1	Strength of support for M_3 by N_1 as a factor of the support for M_1 by N_1	-2	2
e_2	Strength of support for M_3 by N_2 as a factor of the support for M_1 by N_1	-2	2