


Third-party mutualists have contrasting effects on host invasion under the enemy-release and biotic-resistance hypotheses

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Abstract Plants engage in complex multipartite interactions with mutualists and antagonists, but these interactions are rarely included in studies that explore plant invasiveness. When considered in isolation, we know that beneficial microbes can enhance an exotic plant's invasive ability and that herbivorous insects often decrease an exotic plant's likeliness of success. However, the effect of these partners on plant fitness has not been well characterized when all three species coevolve. We use computational evolutionary modeling of a trait-based system to test how microbes and herbivores simultaneously coevolving with an invading plant affect the invaders' probability of becoming established. Specifically, we designed a model that explores how a beneficial microbe would influence the outcome of an interaction between a plant and herbivore. To model novel interactions, we included a phenotypic trait shared by each species. Making this trait continuous and selectable allows us to explore how trait similarities between coevolving plants, herbivores and microbes affect fitness. Using this model, we answer the following questions: (1) Can a beneficial plant-microbe interaction influence the evolutionary outcome of antagonistic interactions between plants and herbivores? (2) How does the initial trait similarity between interacting organisms affect the likelihood of plant survival in novel locations? (3) Does the effect of tripartite interactions on the invasion success of a plant depend on whether organisms interact through trait similarity [Enemy Release Hypothesis (ERH)] or dissimilarity (Biotic Resistance Hypothesis)? We found that it was much more difficult for

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plants to invade under the ERH but that beneficial microbes increase the probability of plant survival in a novel range under both hypotheses. To our knowledge, this model is the first to use tripartite interactions to explore novel species introductions. It represents a step towards gaining a better understanding of the factors influencing establishment of exotic species to prevent future invasions.

Keywords Tripartite interactions · Evolutionary dynamics · Computational modeling · Plant invasion · Plant microbiome · BioSquare · Plant-herbivore

Introduction

The number of exotic plants has increased steadily during contemporary times and these plants are a pervasive feature of most communities (Maron and Vilà 2001; Orians and Ward 2010), with profound and often devastating effects on native species and ecosystem functions (Vitousek et al. 1987; Lovich 1996; Bezemer et al. 2014). Multiple theories have been proposed to explain what makes some plants successful invaders while others fail to establish, including the Enemy Release Hypothesis (ERH) and the Biotic Resistance Hypothesis (BRH). According to the ERH, exotic plants have greater success in their new environment because they have left behind coevolved natural enemies, and native generalist herbivores have a greater impact on native plants than on these exotics (Keane and Crawley 2002; Maron and Vilà 2001). Conversely, the BRH predicts the opposite—it posits that native herbivores preferentially feed on exotic plants because the herbivores are not adversely affected by defensive compounds produced by the plants. Some empirical studies have found support for each of these hypotheses while others have been inconclusive, leading to a lack of generality and clarity (Colautti et al. 2004; Hill and Kotanen 2009; Carrillo-Gavilán et al. 2012; Fan et al. 2013). For example, Morrison and Hay (2011) found that apple snails and crayfish prefer non-native plants to their more familiar native confamilials as predicted by the BRH while Tallamy and colleagues (2010) showed that exotic plants were detrimental to the growth of native generalist herbivores as predicted by the ERH. A meta-analysis by Chun et al. (2010) found that exotic plants did not always experience enemy release and relatedness to native host species did not predict the behavior of native generalists. Clearly, there is a missing piece to this puzzle. Adding microbes as a third interactor might be the key to making more general predictions about invasion success.

Research using plant-microbe-insect (PMI) interactions to explain invasiveness is in the early stages, since the field has focused upon visible above-ground antagonistic interactions (Van der Putten et al. 2007; Bever et al. 2010). However, beneficial microbes may be key players in plant invasions by impacting plant growth and reproduction, increasing plant tolerance to biotic and abiotic stress, and mediating volatile production (Pineda et al. 2010; Friesen et al. 2011; Turner et al. 2013; Schlaeppi and Bulgarelli 2015). Microbes could be instrumental in the success of invasive plants against the background of antagonistic herbivores that would normally prevent plant establishment (Dean et al. 2014; Simonsen and Stinchcombe 2014). When cucumbers were treated with plant growth-promoting bacteria, it resulted in higher growth but there was an antagonistic affect on the herbivore population (Zehnder et al. 1997). However, the effect of symbiotic relationships on herbivores is not straightforward. For example, arbuscular mycorrhizal fungi (AMF) have

been found to both induce and suppress herbivore defense in plants; the outcome appears to depend on the identity of all participants (Bennett et al. 2009; Kempel et al. 2013). White clover that was inoculated with rhizobia showed increased growth for both the plant and herbivore (Kempel et al. 2009). A recent extension of the tripartite interaction found that a combination of microbes with different functions- in this case arbuscular mycorrhizal fungi and diazotrophic bacteria- had additive effects on plant growth, which led to increased preference for spider mites (Khaitov et al. 2015). Thus, integrating tripartite interactions into our understanding of species invasions is critical.

Computational modeling has given insights into the role of antagonistic interactions in species' invasion (Hyder et al. 2008; Gilman et al. 2012; Venkatramanan et al. 2016) but has yet to be applied to multipartite systems. Modeling overcomes the difficulty faced when studying natural systems in terms of isolating specific relationships between multiple species and disentangling their complex interactions (Servedio et al. 2014). Species interactions can be modeled using a trait-based system where the degree of matching affects the outcome of the interaction (Nuismer et al. 2005, 2007). Trait matching is assumed in the enemy release hypothesis, while trait dissimilarity is assumed in the biotic resistance hypothesis. Trait matching between wild parsnip (*Pastinaca sativa*), an introduced weed, and the parsnip webworm (*Depressaria pastinacella*) is a prominent example of this system. Wild parsnip produce furanocoumarins, toxins that are under genetic control and are responsive to selection by the parsnip webworm, whose ability to metabolize and tolerate furanocoumarins is also heritable and subject to selection. The ability of the parsnip webworm to metabolize certain furanocoumarins mirrors the frequency of those compounds in wild parsnip populations (Berenbaum and Zangerl 1998). If wild parsnips were to invade a habitat lacking the parsnip webworm, they would experience enemy release. Similar trait-matching systems are also found in feather lice and doves (Clayton et al. 2003), red crossbills and lodgepole pine (Benkman et al. 2003), the Japanese camellia and the camellia weevil (Toju and Sota 2005), and the gene-for-gene matching system between flax and flax rust (Dodds et al. 2006). Trait matching can also underlie beneficial interactions. For example, compatibility between legumes and rhizobial symbionts can result from polymorphism at a single locus (Lie 1978; Spoerke et al. 1996; Parker 1999). Trait dissimilarity such as in the elicitor-receptor systems that underlie disease resistance between plants and pathogenic microbes can suppress invasions as this allows the enemies to go undetected by the host plants (Jones and Dangl 2006; Verhoeven et al. 2009).

We developed a model to bridge research in invasion biology and tripartite interactions involving plants, microbes, and herbivorous insects. Our goal is to unite theories from these disciplines to gain a better understanding of what may allow exotic plants to become successful invaders. To our knowledge, this is the first computational model to explore the idea that both ecological and evolutionary processes simultaneously affect novel interactions between species interacting in tripartite systems during species invasions. We use computational evolutionary modeling of a trait-based system to test how a third interaction partner (a symbiotic microbe) in the plant-herbivore interaction affects the invaders' ecological stability. In particular, the ratio between extinction and establishment should depend on the degree of trait similarity between the invader and the species in the invaded ecosystem and the manner in which trait similarity is translated into interaction strength.

Our agent-based coevolutionary computational model enables us to answer the following research questions:

1. Can a beneficial interaction between two parties influence the evolutionary outcome of an antagonistic interaction between one of the parties and a third?
2. How does the initial trait similarity between interacting organisms affect the likelihood of plant establishment in novel locations?
3. Does the effect of tripartite interactions on the invasion success of a plant depend on whether the organisms interact through trait similarity (ERH) or dissimilarity (BRH)?

Materials and methods

BioSquare

To study how plants, herbivores and microbes might interact, we created a computational model called BioSquare (<https://github.com/LSheneman/BioSquare>), to explore the stability and evolution of such a system. Our environment is designed to model the interactions of an invasive plant, a beneficial microbe, and herbivorous insect. Specifically, we use the model to test how a beneficial microbe influences the outcome of an interaction between a plant and herbivore. All interactions are mediated by a single trait in each species of the three species. To compare differences in outcomes between the ERH and the BRH, we set up our model so that the herbivore trait value should evolve towards the plant's in the former and evolve away in the latter.

In our model, the organisms have a genome that consists of two genes. We assume that these genotype values translate directly into phenotypic traits. Individuals receive negative or positive payoffs based on the distance between their phenotypic traits and the traits of the individuals around them. The probability of an individual being selected for reproduction is based on the cumulative payoff (organism fitness) where organisms with higher fitness are more likely to reproduce. In this model plants interact directly with herbivores and microbes, while herbivores and microbes do not interact with each other but are influenced by the outcomes of the plants interacting with the other.

We set each organism's genome as a 2-dimensional vector, $G = (X, Y)$ where the genotypic space is $0 \leq X, Y \leq 1$, that can be represented as a point on a 2-D surface. The two-dimensional vector allows coevolution to proceed in a manner more likely to allow the plant to survive exploitation (Gilman et al. 2012). The vector acts as a single trait, which for the plant determines interactions with both the mutualist and antagonist. Future extensions could model trade-offs or constraints between separate plant traits underlying interactions with different community members, but for simplicity in the present model the same trait is involved in both types of interactions.

We limited our biotic environment to a 128×128 toroidal surface. Organisms traverse this space through mutations to the genome, which is translated into species interactions. We assume that the genome encodes a trait that is important in the interaction and that the similarity between trait values determines the compatibility and hence the strength of the species interaction. The interaction strength (Δ) is determined by the phenotypic distance (d) between two organisms (i and j), which is defined using Hamming distance (Hamming 1950):

$$d_{i,j} = |X_i - X_j| + |Y_i - Y_j| \quad (1)$$

At every update of the model, each organism interacts with eight other random individuals. Payoffs produced by an individual interaction are based on what organisms are

involved and which theory is being tested (see Tables 1, 2). The fitness of each organism is the summation of all payoffs it experiences during the current generation. Each generation, random death is represented by eliminating 1% of the population (16,384 organisms) which are then replaced with an organism that is selected from the population using a Moran process (Moran 1958). Each newly created organism inherits the exact genome from its ancestor. In addition, each site in the genome has a 1% chance to mutate. The way that a site mutates matters only under very rare circumstances (Iliopoulos et al. 2010), so we setup our model so that the gene is being replaced by a random number drawn from a uniform distribution between $\in [0, 1]$. We don't expect a smaller mutational effect to change our results significantly, though the boundary conditions may, i.e., how values behave that are close to 0.0 or 1.0 (Adami et al. 2016).

Starting trait distances

In 2-D space, the trait distance between two individuals ranges from 0 to 2, where 0 represents traits that are completely matched and 2 would be traits that are completely dissimilar. We chose 6 starting distances between the plant and herbivore that spanned that range: 0.13, 0.29, 0.6, 1.0, 1.22 and 1.96. We only modeled trait similarity between the plant and the microbe as positive because we wished to focus more on the continuum of trait distances between the plant and the herbivore with the microbe's influence as more of a presence/absence metric. We could not remove the microbe from our environment because that would completely change the computational dynamics of the system, so we only compared an environment where the plant trait was very similar ($d = 0.18$) to our microbe to one where the plant was not ($d = 1.78$). This simplified our model but we do not believe that it was hugely disadvantageous because, while we do not have reciprocal effects, we have contrasting and matching trait systems.

Table 1 Biotic resistance hypothesis (BRH) payoff matrix: we set our model so that the plant and microbe would always have a positive payoff from interacting with each other, no matter the distance between their traits

	Plant	Herbivore	Microbes
Plant	0	$-\Delta$	$(2 - \Delta)$
Herbivore	$+\Delta$	0	0
Microbes	$(2 - \Delta)$	0	0

The herbivore has the greatest payoff (and plant the lowest) when the distance between its trait and the plant's are as distant as possible

Table 2 Enemy release hypothesis (ERH) payoff matrix: as with the BRH, the plant and microbe always have a positive payoff from interacting with each other, no matter the distance between their traits

	Plant	Herbivore	Microbes
Plant	0	$-(2 - \Delta)$	$(2 - \Delta)$
Herbivore	$(2 - \Delta)$	0	0
Microbes	$(2 - \Delta)$	0	0

However, the herbivore has the greatest payoff (and plant the lowest) when its trait and the plant's are as similar as possible

Model analysis

Under each hypothesis (BRH and ERH), we ran 50 replications of the model across 6 starting phenotypic (trait) distances for 500,000 generations. We recorded the frequency of each species in the community and the distribution of genetic values in 2-D space for each replicate run.

To determine the probability of survival of each species, we counted the number of rows where the frequency of each organism was zero, indicating that it went extinct. This was converted to either a 0 (extinct) or 1 (alive) and averaged over the 50 runs for each starting distance.

The phenotypic distance was computed by calculating the mean of the Hamming distance between every individual within a species pairing (Plant–Microbe, Plant–Herbivore, Microbe–Herbivore) within a run. All 50 runs were then averaged to get a final value per initial environmental condition.

To determine if the traits in our system are under selection, we calculated the variance of the value for a randomly selected gene for all organisms within the population. Using variance is a proxy for looking at the distribution of the gene values across the population. Because the mutation process is random, if a gene is not under selection it can take any value between 0.0 and 1.0 and those values will be uniformly distributed across the gene space. The standard deviation of a uniform distribution is:

$$\frac{1}{12}(a - b)^2 \quad (2)$$

here $a = 0.0$ is the lower bound, and $b = 1.0$ is the upper bound of that distribution. Therefore, a drifting gene can be identified as having a variance of $\frac{1}{12}$ and will allow us to determine which organisms in our system are under selection and which are not.

The frequency, phenotypic distance, and variance were also averaged over the 50 replicates per environmental condition.

Statistical analyses and figures

BioSquare was built using C++11. We used R version 3.3.0 (Core Team 2016) with the following packages: `data.table` (Dowle and Srinivasan 2016), `plyr` (Wickham 2011), `reshape2` (Wickham 2007), `Rmisc` (Hope 2013) to run the model and statistical analyses. The R packages `ggplot2` (Wickham 2009), `scales` (Wickham 2016), and `cowplot` (Wilke 2016) were used to create the figures.

To compare the relative importance of the variables for each hypothesis we used the `varImp` function from the `caret` package (Kuhn et al. 2016). We fit a generalized linear model with a binomial family. The function then uses the absolute value of the t-statistic for each parameter. We also used this same `glm` to test the significance of the parameters of the model.

Results

The initial trait distance between the plant and the microbe has a greater influence on plant invasion success under the ERH than the BRH

In our model, the two variables of interest were the initial trait distance between the plant and the microbe and the initial trait distance between the plant and the herbivore. Using the

probability of plant survival as our measure of invasion, we quantified the importance of each variable. The initial distance between the plant and the herbivore was the most important variable in both models with a score of 100. Interestingly, the initial distance between the plant and microbe was more important in the ERH than it was in the BRH (importance score = 37.91 vs. 0.0192).

The probability of plant invasion success depends on the initial trait distance between the plant and the herbivore

The presence of a beneficial microbe can increase plant survival in a novel habitat (Fig. 1) even if the initial trait similarity is low. Under the Biotic Resistance Hypothesis plant survival does not depend on the trait distance between the microbe and the plant until the trait distance between the plant and herbivore was 1, the midpoint of possible values that trait similarity can take (Fig. 1a). However, plant survival differed between the microbe treatments until the herbivore's traits were highly diverged from the plant when we modeled the Enemy Release Hypothesis (Fig. 1b). We tested this using a generalized linear model for the probability of survival for each hypothesis. For the BRH, probability of survival for the plant depended on the trait distance between the plant and the herbivore and whether or not the microbe was phenotypically similar to the plant but the interaction term was not significant (Table 3). However, under the ERH, the interaction term was significant along with the trait distance between the plant and herbivore and the trait distance between the microbe and plant (Table 3).

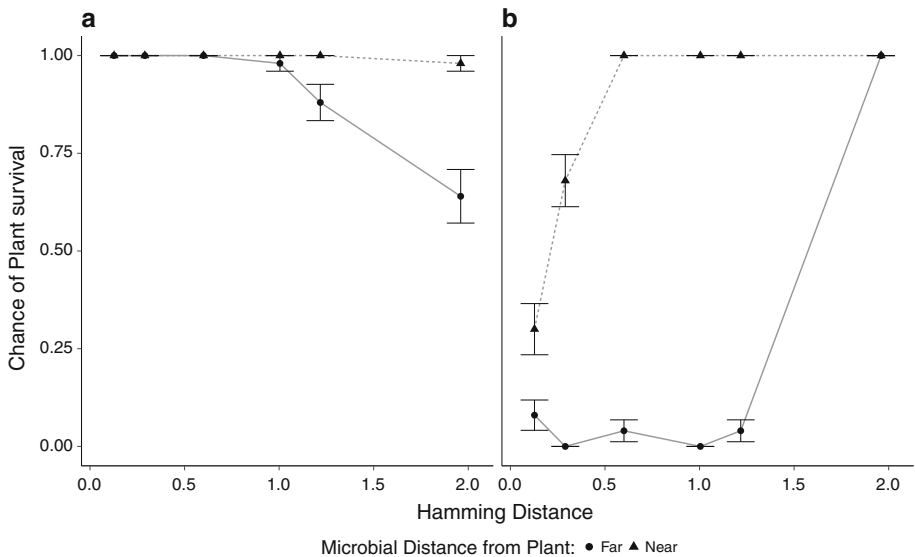


Fig. 1 **a** Under the biotic resistance hypothesis (BRH), plant survival gradually decreases as the trait distance between the invasive plant and the herbivore increases. **b** Under the enemy release hypothesis (ERH), plant survival increases. In both theories the presence of a closely matching microbe increases plant survival, but it is more dramatic under the ERH

Table 3 Results for GLM of trait similarity effects on probability of plant survival for each hypothesis

	BRH		ERH	
	df	χ^2	df	χ^2
$d_{plant,herbivore}$	5	60.238**	5	190.43**
$d_{plant,microbe}$	1	31.709**	1	412.91*
$d_{plant,herbivore} \times d_{plant,microbe}$	5	0.9926	5	42.87**

** p value < 0.0001; * p value < 0.001, () p value n.s

Population dynamics: the frequency and final outcome of all organisms in the community are influenced by their initial trait similarity to each other

The frequency of all species in the community is determined by the initial trait distances between the plant, microbe, and herbivore. Figure 2 shows the outcomes of all 1200 runs arranged by the hypothesis tested. Only in 2 runs were all three organisms able to coexist. Under both hypotheses, the herbivore went extinct most often but under the ERH, there was a greater range of the subset of species that went extinct. This includes 50 runs where all three species went extinct. The microbe only went extinct when the plant also went extinct. When an organism goes extinct, it tends to happen quickly (Fig. 3). Only when the initial trait distance between the plant and the herbivore is farthest away does the herbivore survive, although its frequency is tempered by how similar the plant and microbe are initially (Fig. 3a). Under the ERH, the organism that goes extinct is also dependent on the

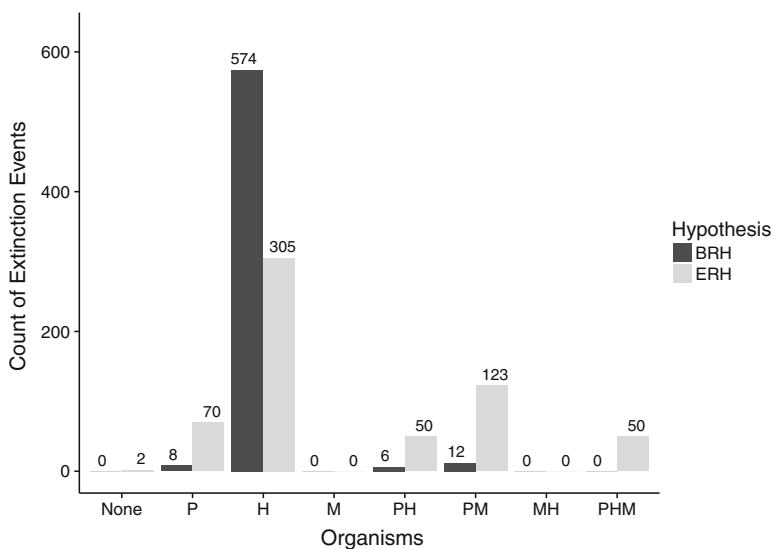


Fig. 2 Total count of organism extinctions for each run by hypothesis. This graph shows the outcomes of all 1200 runs grouped by which set of organisms went extinct and under which hypothesis. Under each hypothesis, herbivores went extinct the greatest number of times. The second highest grouping was when plants and microbes went extinct in the same run. This was the same for both groups but was several times higher in the ERH

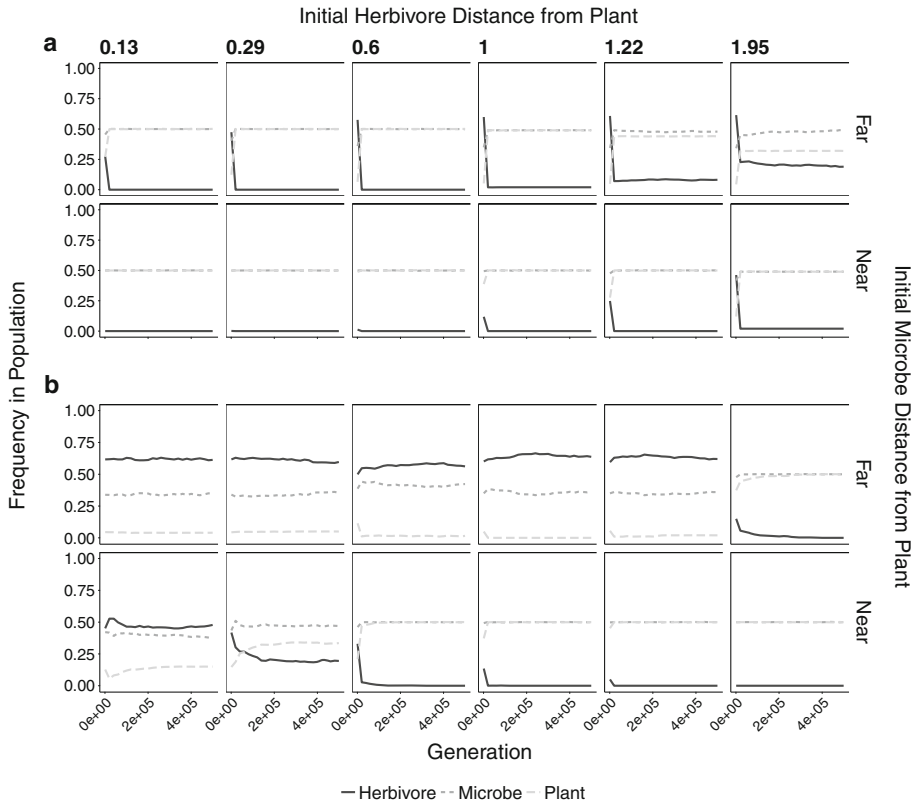


Fig. 3 Frequency of all species in the population depends on the initial trait distance between the plant, microbe, and herbivore. In some scenarios it is possible for all organisms to coexist but when an organism goes extinct, it tends to happen quickly. **a** Biotic resistance hypothesis, **b** enemy release hypothesis

initial trait distance between the microbe and the plant (Fig. 3b). Extinction of the herbivore and, to a lesser extent, the microbe may be an artifact of our system because there are not interactions with native plants included. However, invasive plants can lead to the extinction of native species over time if native plants are outcompeted.

The plant and microbe genomes evolve to yield matching traits

In all circumstances, the distance between the microbe and the plant decreases indicating that they are evolving towards each other in phenotype space (Fig. 4). In the BRH, the plant and the herbivore do not coevolve consistently enough (usually one or the other goes extinct) to find a pattern in trait coevolution (Fig. 4a). In the ERH, especially when the the microbe is phenotypically distant from the plant, the trait distance between the plant and the herbivore appears to oscillate (Fig. 4b).

Selection pressure changes trait values

The average variance of each organism in the model was compared to $\frac{1}{12}$, the variance for the uniform distribution to determine if the trait is under selection or if changes are a result

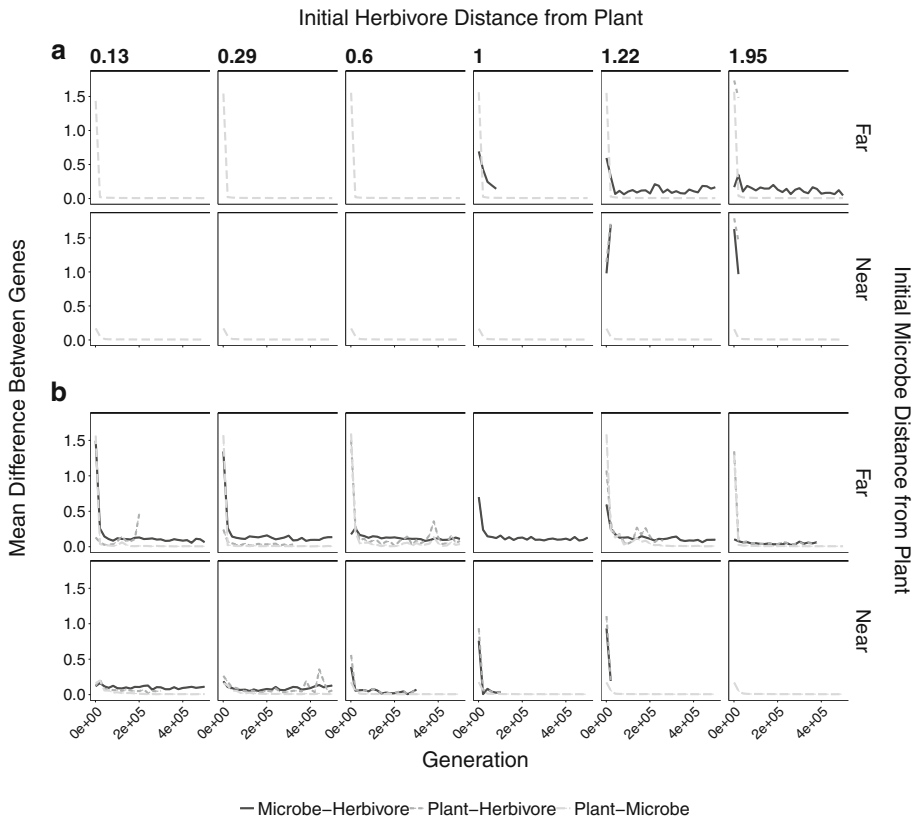


Fig. 4 Trait similarity between organisms over generations. Trait similarity between the plant and the microbe always increases as indicated by the decrease in mean difference between the genes. The similarity between the plant and the herbivore fluctuates over time. **a** Biotic resistance hypothesis, **b** enemy release hypothesis

of genetic drift (Fig. 5). In the BRH, the herbivore tends to go extinct under most conditions, so the plant does not feel antagonistic pressure to escape so there are only benefits to be gained from being similar to the microbe. The variance of the herbivore hovers around $\frac{1}{12}$ because when it is present in the simulation runs, the plant goes extinct so there is no selective pressure to move towards any particular trait value (Fig. 5a).

In the ERH, when the microbe is not close to the plant in trait space, the probability of the plant surviving is low (Fig. 1) and when it persists it tends to be at a low frequency in the community (Fig. 3b). This results in the herbivore not facing strong selective pressure and the herbivore trait variance hovers around $\frac{1}{12}$ because the microbe and the herbivore do not directly affect each other. For a similar reason, the microbe's variance is only slightly higher. When the plant is absent, there is no selective pressure on the microbe trait but when the plant is present, the plant and microbe traits converge. The plant trait appears to always be under selection, likely because it is always facing pressure to either escape the herbivore or move towards the microbe (Fig. 5b).

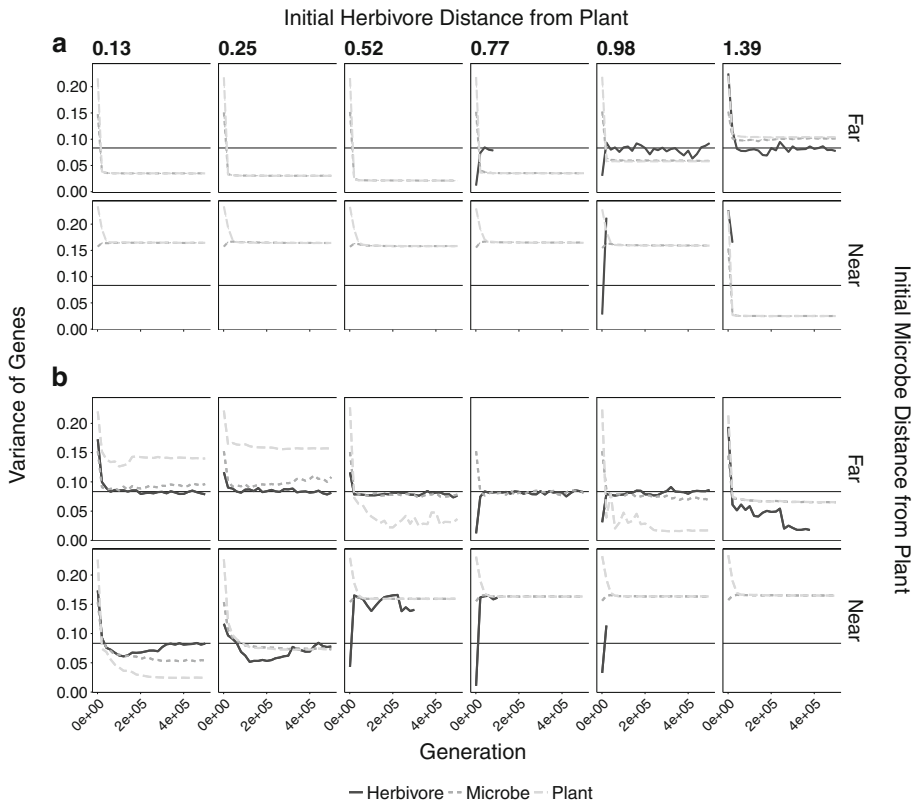


Fig. 5 Trait variance as an indicator of selection or genetic drift as the mechanism of evolution in our system. The variance of the genetically-determined trait in our system can determine whether the organisms are under selection or if changes in the traits are due to genetic drift. When the variance is at the red line (variance = $\frac{1}{12}$), then changes in trait values are due to genetic drift. Otherwise, the organisms are under selection. **a** Biotic resistance hypothesis, **b** enemy release hypothesis

Discussion

During invasion of novel habitats, plants encounter both antagonistic herbivores and beneficial microbes; coevolutionary dynamics with these partners may play a key role in determining invasion success. In our model we varied the initial similarity between the herbivore and the plant from very similar to highly dissimilar. This is biologically similar to environments where the plant may be invading a novel habitat that already contain plants that are phylogenetically similar and thus contain herbivores with trait values that match the invading plant. Many studies have found correlations between phylogenetic distance and antagonistic pressures on plants (Pearse and Hipp 2009; Ness et al. 2011; Parker et al. 2015). The phylogenetic composition of a community may have as great an effect on the outcome of invading plants as the ecological composition. Under both the Enemy Release and Biotic Resistance hypotheses, the presence of a microbe that had a phenotypically similar trait greatly increased the likelihood of the plant surviving. The acquisition of a beneficial symbiont, along with other traits that make exotic plants more competitive, such as higher growth rates or novel chemical defenses, may allow them to

outcompete native plants. Or, it may allow them to colonize uninhabited locations, similar to how *Leucaena leucocephala*, a nitrogen-fixing species, has invaded N-limited volcanic soils in Hawaii because there are no native species occupying that niche (Funk 2013).

Our model demonstrates that microbes can impact plant invasions even if plants encounter and form relationships with novel symbionts where trait similarity is low. We find that selective pressure will rapidly lead to an increase in similarity between the plant and its newly acquired mutualist. This may be relevant to several biological scenarios. For example, arbuscular mycorrhizal fungi (AMF) associate with over 80% of plant families and are thought to be globally distributed with low host-specificity (Pringle et al. 2009). A study by Ness and colleagues (2011) found that AMF from the native range of the palm *Trachycarpus fortunei* were replaced by AMF in the invasive range. However, recent work has found that some taxa do show host-specific growth responses (Helgason et al. 2002) and that they may positively influence invasive plant ability (Marler et al. 1999). We lack work examining whether the presence of invasive plants results in microevolutionary change in native microbes. However, there is evidence of native herbivores evolving in response to invasive plants. For example, native soapberry bugs have evolved changes in behavior and morphology in approximately 100 generations of coevolving with the exotic weeds *Koeleria* and *Cardiospermum* to where the insects have lowered performance on native plants (Strauss et al. 2006). If invasive plants can invoke evolutionary changes in native herbivores, there are likely to be evolutionary changes in microbes due to their much shorter generation times. Future experimental work in this area can help answer many questions about eco-evolutionary feedback between not only plants and microbe, but also more complex community interactions.

Interestingly, the microbe had a much greater influence on plant survival under the ERH as compared to the BRH. For the ERH, not only were initial similarity of the herbivore and the microbe to the plant significant factors in determining plant invasion success, but the interaction between microbe and herbivore was as well. This mirrors empirical studies that have found non-additive effects when investigating PMI interactions (Morris et al. 2007; Bennett 2013). This is most likely related to the plant having a harder time invading because benefits gained from being similar to the microbe are canceled by the increased similarity to the herbivore under the BRH.

Our model is based upon the assumption that a single trait mediates both the beneficial and antagonistic interactions, which while simplistic is likely a biologically realistic approximation since many interactions have single traits with strong effects. In the most extreme cases, gene-for-gene interactions can determine whether or not two partners are able to interact (Dodds et al. 2006; Yang et al. 2010). By interacting with multiple partners via the same trait, plants serve as a link between above- and below-ground organisms. Trait-mediated indirect interactions (TMII) have been used to explain plant-insect interactions (Werner and Peacor 2003; Utsumi et al. 2010). Feeding by an herbivorous insect induces changes in plant traits, which may positively or negatively affect the growth or fitness of another species. Herbivory by *Spodoptera exigua* on *Medicago truncatula* increased the fitness of the rhizobium *Sinorhizobium melliloti* (Heath and Lau 2011). Mechanical wounding of *M. truncatula* to simulate herbivory also increased AMF colonization (Landgraf et al. 2012). We envision that similar effects are found in plant-microbe interactions (Khaïtov et al. 2015). Leaf chemistry is a key component of many plant herbivore interactions and studies have found that soil microbes alter the leaf chemistry of their host plants (Meiners et al. 2017). *Pseudomonas fluorescens* colonization induced systemic resistance in *Arabidopsis*, which significantly reduced the growth of the herbivore *S. exigua*, but it also changed the composition of volatiles induced by the generalist aphid,

Myzus persicae, making the plant less attractive to the aphids' natural enemies (Van Oosten et al. 2008; Pineda et al. 2013; Sugio et al. 2015).

We know that the identity of the participants in both plant-microbe and plant-insect interactions are important factors in the outcomes of those interactions and their evolution (Bezemer et al. 2014; Simon et al. 2015; Bennett et al. 2016). In tripartite interactions, the identity of all three interacting species is important and may influence the results of interactions (Bennett et al. 2009; Kempel et al. 2013; Zytynska et al. 2014; Parker et al. 2017). A recent study by Bonte et al. (2010) found that spider mites adapted to the physiological change in snap beans induced by AMF in just fifteen generations. Schausberger et al. (2012) found that *Phytoseiulus persimilis*, a predatory mite that is the natural enemy of the spider mite that attacks bean plants, was more strongly attracted by the volatiles of plants that were induced by the fungus *Glomus mosseae*. These studies show that evolutionary change in one species can be influenced by interactions between two other interacting organisms even though there is not a direct link between all three. Our model captures this key element in a simplified manner.

We focus our paper on the association of plants with beneficial microbes and antagonistic herbivores but future work could use our model to explore other types of interactions. Our analyses are motivated by experimental studies that show how microbial symbionts increase plant fitness when interacting with herbivores by increasing plant resistance to herbivory through the production of compounds that either deter herbivory or decrease insect fitness. The plant *Nicotiana tabacum* produces nicotine as a defensive compound to ward against herbivory. When colonized with AMF, nicotine levels are much higher compared to non-AMF colonized plants (Subhashini 2012). Van Oosten et al. (2008) found that *P. fluorescens* WCS417r initiates an induced systemic response (ISR) in *Arabidopsis* which activates plant defenses prior to herbivore attack and results in reduced weight gain in *S. exigua*. Microbes may also allow plants to have compensatory growth after herbivory, a defensive strategy known as tolerance. Tallgrass prairie plants were found to regrow to their preherbivory levels when in conjunction with AMF, while non-associated plants had much lower levels of biomass (Kula et al. 2005). However, the power of our model lies in both its simplicity and flexibility. BioSquare is open source and available to anyone who wishes to use it. By making slight changes in the BioSquare code, many other scenarios can be modeled. We could change our input code to explore the effect on plant fitness if a plant simultaneously encounters a novel pathogen while interacting with an herbivore. If we were interested in exploring the evolution of the interaction between the Colorado potato beetle *Leptinotarsa decemlineata* and its microbial symbionts, we can model herbivore-associated microbes and how these decrease plant fitness (Chung et al. 2013). It can be used with other systems to explore different trophic levels as well.

Our model shows that plant invasion success depends on interactions with mutualists and enemies in complex ways that hinge on the mechanism underlying these interactions. To our knowledge, this study is the first computational model that explores coevolutionary tripartite interactions in the context of invasion by exotic plants. Even this simplistic model provides insight into the role that PMI interactions play in the establishment of invasive plant species. All plants engage in complex multipartite biotic interactions within their communities, so it is critical moving forward that both theoretical and empirical studies expand beyond pairwise interactions in order to predict and prevent future species' invasions.

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