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Mathematical modeling on obligate mutualism: Interactions between leaf-cutter ants and their fungus garden

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ABSTRACT

We propose a simple mathematical model by applying Michaelis–Menton equations of enzyme kinetics to study the mutualistic interaction between the leaf cutter ant and its fungus garden at the early stage of colony expansion. We derive sufficient conditions on the extinction and coexistence of these two species. In addition, we give a region of initial condition that leads to the extinction of two species when the model has an interior attractor. Our global analysis indicates that the division of labor by worker ants and initial conditions are two important factors that determine whether leaf cutter ants' colonies and their fungus garden can survive and grow or not. We validate the model by comparing model simulations and data on fungal and ant colony growth rates under laboratory conditions. We perform sensitive analysis of the model based on the experimental data to gain more biological insights on ecological interactions between leaf-cutter ants and their fungus garden. Finally, we give conclusions and discuss potential future work.

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1. Introduction

Mutualistic interactions, although ubiquitous in nature, are not well understood theoretically (Boucher, 1985; Hoeksema and Bruna, 2000; Hoeksema and Schwartz, 2002; Holland et al., 2002; Neuhauser and Fargione, 2004). Mathematical models of mutualism that correspond well with natural observed population dynamics have historically been difficult to formulate (Heithaus et al., 1980), and there is little work in this area despite the frequency of mutualism in nature (Holland and DeAngelis, 2010). The standard approach to date for modeling two-species mutualism derives from modified Lotka–Volterra equations, which have been applied to a variety of community ecological interactions, including plant–pollinator interactions (Soberon and Martinez del Rio, 1981; Wells, 1983; Holland et al., 2002) and legume rhizobium interactions (Vandermeer and Boucher, 1978; Simms and Taylor, 2002; West et al., 2002). However, these models generally predict unstable population dynamics that do not match the dynamics actually observed in nature, particularly for obligate mutualism which tend towards stability (Holland and DeAngelis, 2010). Recently, Holland and DeAngelis (2010) proposed a general framework using a consumer–resource approach to model the density-dependent population dynamics of mutualism. Here we apply Holland and

DeAngelis' approach to derive a new and simple mathematical model of the population dynamics within colonies of the obligate mutualism between leaf cutter ants (*Acromyrmex versicolor*) and their fungus garden that can be validated by experimental data.

The mutualism between leafcutters and their fungus is particularly interesting as an example of obligate mutualism. The colony functions simultaneously at two trophic levels, as a population of ants and a particular species of fungus that is dependent upon the ants for survival and growth while serving as their primary nutrient source. Growth of the fungus and the worker ant population is mutually interdependent. The social organization of the colony is an important contributor to this mutualism, because the colony faces a trade-off in allocation of worker effort towards growth and maintenance of the fungus and other necessary behaviors, including production and care of new offspring. This model is unique because it considers this trade-off by using the division of labor within the colony as a key explanatory variable. It also focuses on a critical colony life stage, early colony growth, which has empirically been demonstrated to be the most vulnerable life stage for the colony (Clark and Jennifer, in preparation). Finally, it applies a population dynamics framework to the problem by treating individual colonies as distinct populations. Viewing the colony as a population is relevant for models of growth in social insect colonies, because (similar to other populations), offspring production is limited by resource availability, including food and offspring care, which are a function of population size. Growth is not limited by queen egg production rate which is an order of magnitude higher than actual

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offspring production (Clark and Jennifer, in preparation). Incorporation of these behavioral, life-history, and organizational components make the model generally relevant to contexts where starting populations are small, and where there are trade-offs in how partners participating in the mutualism should allocate effort towards maintaining the mutualism versus other activities.

In this paper, we adapted a model for incipient colony growth based on simple density-dependent ant growth and death rates coupled with a fungus growth model which can be described by a generalized Michaelis–Menton equation of enzyme kinetics. The main purposes of this paper are three-fold:

1. Model functional response/numerical response based on ecological properties of leaf cutter ants and their fungus garden.
2. Explore how the division of labor and initial conditions can be key factors that determine the successful colony expansion at its early stage.
3. Validate the model with experimental data and perform sensitivity analysis to understand the influences of parameters and initial condition on the model outcomes.

The rest of this paper is organized as follows: In Section 2, we introduce the biological background of leaf-cutter ants and their fungus garden, and we formulate a simple mathematical model based on ecological assumptions that are supported by data and literature. In Section 3, we perform mathematical analyses of the proposed model: We derive the sufficient conditions for the extinction and coexistence of the two species and give a region of initial conditions that leads to the extinction of ants and fungus with a model also containing an interior attractor (Theorems 3.1 and 3.2). These global analyses indicate that the division of labor by ants and initial conditions are two important factors in determining whether leaf cutter ants and their fungus garden can coexist or not (Theorem 3.3 and Corollary 3.1). In Section 4, we compare simulations to data on growth rates for laboratory leaf cutter ant colonies, perform sensitivity analysis for all the parameters and initial conditions around the nominal value. The study suggests not only that the fit of our model to data is significantly accurate, but also that the model can provide parameter values that are difficult to measure in the experiments. In Section 5, we summarize our results and discuss future work. All proofs of mathematical results presented in Section 3 are provided in the last section.

2. Biological background and model formulation

This paper focuses on early colony growth in a species of leafcutter ants. Leafcutters are fungus farmers that harvest leaves and use them to cultivate a specific fungus that serves as the colonies primary nutritional source. The fungus feeds the larvae, and adult workers also feed off of it for maintenance. Thus, leaf-cutter ants and their fungus garden form an obligate mutualistic relationship, in which the increasing population of ants in the colony is due to consumption of the fungus while the increasing population of fungus is due to the agricultural services provided by the ants. The interaction between ants and fungus can be categorized as a consumer–resource mutualism according to Holland and DeAngelis (2010).

Although *Acromyrmex* colonies grow to several thousand workers, we focus on the early growth stage from first worker production to about 29 weeks of age, with worker populations of approximately 200. The relationship between fungal growth and worker production at this stage is less stable than at larger colony sizes (Clark and Jennifer, in preparation), and colony deaths are common even in conditions of ad libitum food availability. As a

practical consideration for model validation, colonies at this stage can be meticulously tracked for changes in fungal and worker mass or number as well as for brood production and division of labor, measured as task performance by each worker. New leafcutter colonies are started when mature colonies produce new winged females, queens, which mate with males and then excavate new nests in the soil. Leafcutter ant queens then expel a fungus pellet carried from their natal nest and lay eggs on the fungus. They forage for leaves to expand the fungus garden, which they feed to their brood. The first workers emerge 6–9 weeks after eggs are laid (Hölldobler and Wilson, 1994). These workers enlarge the nest, feed the queen and larvae, tend to the fungus garden and hunt for leaves for the fungus substrate. Once workers emerge, the queen's only task is egg production. This transition begins the colony's ergonomic growth phase, which may last for years until the colony matures (Oster and Wilson, 1979).

Division of labor, the allocation of workers to different tasks, is a key feature of social groups, and addresses how individual worker behavior integrates into colony-level task organization (Hölldobler and Wilson, 1990; Beshers and Fewell, 2001). Although the importance of division of labor in colony functioning is widely recognized, models of division of labor have not previously been integrated with effects on colony growth. More broadly, the role of division of labor in the leafcutter–fungus mutualism reflects the trade-offs that species must make in ecological mutualism between behaviors promoting the mutualism versus other maintenance behaviors.

Leafcutter colonies face a trade-off in allocation to different tasks affecting fungal growth, brood production and colony maintenance. This trade-off is particularly important at early growth stages, when the number of workers is low relative to the number of tasks and/or total work needed to be performed (Brown et al., 2006; Jeanson et al., 2007). The workers of established leafcutter colonies may perform up to 20–30 tasks (Wilson, 1983). Incipient colonies perform considerably fewer; of these, feeding fungus to larvae, tending the fungus garden, and collecting leaves for the fungus substrate require the bulk of worker activity budgets, which translate into energy expenditure of workers (Julian and Fewell, 2004). These tasks can be generalized into two categories: energy or time spent outside the colony for collecting and processing leaves and energy or time spent inside the colony for tending and cleaning the fungus garden and taking care of queens and larvae. We quantify time or energy expenditure for these two category tasks as the biomass of ant as our modeling approach.

Our study in this section aims to model the obligate mutualism interaction between leaf cutter ants and fungus at the incipient stage of the colony: From the time of first brood production to about 29 weeks of age. Let $A(t)$ be the total biomass of ants including workers, larvae, pupae and eggs at time t , where pA ($0 < p < 1$) is the biomass of workers and $(1-p)A$ is the biomass of the remaining ants. Let $F(t)$ be the total biomass of the fungus at time t . Ecological assumptions of the interaction between workers and fungus at the early stage of colony are as follows:

- A1: Assume that each worker has a fixed ratio of the energy spent outside the colony to the energy spent inside the colony which is $q/(1-q)$. This assumption is equivalent to a situation where workers with a population of qpA ($0 < q < 1$) collect leaves, and the rest of workers $(1-q)pA$ tend the fungus garden and take care of queen ants as well as larvae.
- A2: The ants' population increases as the queen, larvae and adult ants feed on fungus. Thus we can assume that the numerical response function for ants is the Holling Type I function, i.e., fungus biomass F multiplied by a constant number r_a . In addition, we assume that ants suffer from density-dependent

Table 1
Biological meanings of parameters in the system (1)–(2).

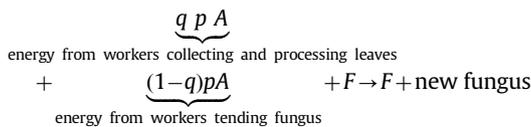
Parameters	Biological meaning
r_a	Maximum growth rate of ants
r_f	Maximum growth rate of fungus
c	Conversion rate between fungus and ants
d_a	Death rate of ants
d_f	Death rate of fungus
p	Proportion of ants that are workers
q	Proportion of workers that take care of fungus
b	Half-saturation constant

mortality due to energy consumed by foraging for leaves and taking care of the larvae and fungus garden as well as its multiple life stages, which will modify population growth through density-dependent self-limitation (Holland and DeAngelis, 2010). Therefore, the population dynamics of ants can be described as follows:

$$\frac{dA}{dt} = (r_a F - d_a A)A \tag{1}$$

where r_a is a parameter that measures the maximum growth rate of ants and d_a is the mortality rate of ants.

A3: The leaf-cutter ant mutualism is unique because the workers perform specific tasks to maintain the life of the fungus. The population of fungus can increase only if: there are qpA workers bringing back and processing leaves for the fungus; there are $(1-q)pA$ workers taking care of the fungus garden and there is healthy fungus F in the garden. Thus, fungus growth is a product of two different sets of tasks performed by workers which can be represented by the following diagram:



Therefore, by applying the concept of the kinetics of functional response (Real, 1977) we can assume that the numerical response of fungus to ants is a Holling Type III function

$$\frac{p^2 q(1-q)A^2}{b + p^2 q(1-q)A^2}$$

where b is the half-saturation constant. The population of fungus decreases due to the consumption by ants and its mortality. Here, we assume that the fungus suffers from density-dependent mortality due to self-limiting (Holland and DeAngelis, 2010). Thus, the population dynamics of fungus can be described as follows:

$$\frac{dF}{dt} = \left(\frac{r_f p^2 q(1-q)A^2}{b + p^2 q(1-q)A^2} - d_f F - r_c A \right) F \tag{2}$$

where r_f is the maximum growth rate of the fungus; c is the conversion rate between fungus and ants and d_f is the mortality rate of the fungus. See Table 1 for the biological meanings of the completed list of parameters.

Note: Holling (1959) and Murdoch (1969) have discussed the application of a type III functional response associated with learning processes of predators being able to adjust their feeding rate actively based on the quantity and density of available prey. An ant colony similarly changes allocation of workers to different tasks as needs change with changing colony size (Hölldobler and Wilson, 1990). In this way the ants actively modify their time expenditure and task allocation just as predators actively modify their feeding behaviors.

3. Mathematical analysis

Let $a = p^2 q(1-q)$ and $r_c = cr_a$, then based on the assumptions listed in Section 2, an interaction between ants and fungus at the early stage of colony may be modeled by the following differential equation:

$$\frac{dA}{dt} = (r_a F - d_a A)A \tag{3}$$

$$\frac{dF}{dt} = \left(\frac{r_f a A^2}{b + a A^2} - d_f F - r_c A \right) F \tag{4}$$

where a can be considered as a parameter measuring the division of labor in the colony of ants and other parameters are strictly positive. Since

$$a = p^2 q(1-q), \quad p \in [0, 1] \text{ and } q \in [0, 1],$$

therefore,

$$a \in [0, 0.25]$$

where a achieves its maximum 0.25 when $p = 1, q = 1/2$. In reality, p is always less than 1 since the biomass of queen and other stages of ants (i.e., larvae, pupae) other than adult ants is greater than 0. Thus, a is strictly less than 0.25 in the real biological system.

In this section, we focus on global dynamics of the proposed model (3)–(4) to explore the following ecological questions:

1. What is the pattern of population dynamics of leaf-cutter ants and fungus at the early stage of colony expansion?
2. What are the main factors determined the coexistence of ants and fungus?

To answer these questions, we first show the following lemma:

Lemma 3.1. *The system (3)–(4) is positively invariant and bounded in \mathbb{R}_+^2 . In particular, if both $A(0) > 0$ and $F(0) > 0$, then $A(t) > 0$ and $F(t) > 0$ for all $t > 0$.*

Note: Lemma 3.1 indicates that the population of leaf cutter ants and their fungus is bounded due to the limited resource in nature. Let $\mathbb{R}_+^2 = \{(A, F) \in \mathbb{R}_+^2 : A > 0, F > 0\}$, then from Lemma 3.1, we know that $(A(0), F(0)) \in \mathbb{R}_+^2$ implies that $(A(t), F(t)) \in \mathbb{R}_+^2$ for all $t > 0$.

Proposition 3.1. *For any initial condition taken in \mathbb{R}_+^2 , the trajectory of the system (3)–(4) is converging to an equilibrium point.*

Note: Proposition 3.1 implies that the population dynamics of leaf cutter ants and their fungus is simple in the sense that they do not have a limit cycle, i.e., if time t is large enough, then the population of leaf cutter ants and their fungus approach to some fixed point. Therefore, the short time dynamics of leaf cutter ants and their fungus garden is more important since it can give us more information on the dynamics of the interaction between ants and fungus. This answers our first question listed at the beginning of this section.

Proposition 3.2. *If $a < 4b((r_c r_a + d_f d_a)/r_a r_f)^2$, then the system (3)–(4) has only trivial equilibrium (0,0); while if $a = 4b((r_c r_a + d_f d_a)/r_a r_f)^2$, then the system (3)–(4) has the only positive equilibria*

$$(A^i, F^i) = \left(\frac{r_f r_a}{2(r_c r_a + d_f d_a)}, \frac{r_f r_a d_a}{2r_a(r_c r_a + d_f d_a)} \right)$$

in addition to (0,0); while if $a > 4b((r_c r_a + d_f d_a)/r_a r_f)^2$, then the system (3)–(4) has the following two positive equilibria in addition to (0,0):

$$(A^{i1}, F^{i1}) = \left(A^{i1}, \frac{d_a}{r_a} A^{i1} \right) \quad \text{and} \quad (A^{i2}, F^{i2}) = \left(A^{i2}, \frac{d_a}{r_a} A^{i2} \right) \tag{5}$$

where

$$A^{i1} = \frac{r_f r_a}{2(r_c r_a + d_f d_a)} - \sqrt{\left(\frac{r_f r_a}{2(r_c r_a + d_f d_a)}\right)^2 - \frac{b}{a}}$$

$$A^{i2} = \frac{r_f r_a}{2(r_c r_a + d_f d_a)} + \sqrt{\left(\frac{r_f r_a}{2(r_c r_a + d_f d_a)}\right)^2 - \frac{b}{a}}$$

Note: Recall that a is a parameter measuring the division of labor of workers. Proposition 3.2 implies that if a is too small, i.e., the ratio of adult ants that take care of fungus to adult ants that forage for leaves, $q/(1-q)$, is too small, then the system (3)–(4) has only trivial equilibrium point $(0,0)$. This leads to the following theorem:

Theorem 3.1 (Extinction of two species). *If $a < 4b((r_c r_a + d_f d_a)/r_a r_f)^2$, then the system (3)–(4) has global stability at $(0,0)$.*

Biological implications: Theorem 3.1 indicates that division of labor is an important factor determining whether the early colony stage of leaf cutter ants can survive or not. Recall that the proportion of ants performing a task is essentially equivalent to energy devoted to a given task. In the case that the population of adult ants is too small, i.e., q is too small, or the population of adult ants foraging for food is too small, i.e., $(1-q)$ is too small, then $a < q(1-q)$ will be too small such that $a < 4b((r_c r_a + d_f d_a)/r_a r_f)^2$. This leads to the extinction of both ants and fungus.

In order to investigate the biological conditions when leaf cutter ants and their fungus can coexist, we have the following theorem:

Theorem 3.2 (Coexistence of two species). *If $a > 4b((r_c r_a + d_f d_a)/r_a r_f)^2$, then the system (3)–(4) has two positive equilibria (A^{i1}, F^{i1}) and (A^{i2}, F^{i2}) where (A^{i1}, F^{i1}) is always unstable and (A^{i2}, F^{i2}) is always locally asymptotically stable.*

Biological implications: Theorem 3.2 implies that if allocation of workers to different tasks is in a good range, i.e., $a > 4b((r_c r_a + d_f d_a)/r_a r_f)^2$, then both leaf cutter ants and their fungus garden can coexist, because the system (3)–(4) has a locally asymptotically stable interior equilibrium (A^{i2}, F^{i2}) . On the other hand, for a fixed value of a , if d_a/r_c and d_a/r_a are small enough, then $a > 4b((r_c r_a + d_f d_a)/r_a r_f)^2$ holds, thus two species can coexist. Now the more interesting question is whether relative allocation among tasks is the only factor determining whether ants and fungus can coexist. The next theorem will answer this question.

Theorem 3.3 (Basin of attraction of $(0,0)$). *The trivial equilibrium $(0,0)$ is always locally asymptotically stable if $a \neq 4b((r_c r_a + d_f d_a)/r_a r_f)^2$. Moreover, if $a > 4b((r_c r_a + d_f d_a)/r_a r_f)^2$, then the basin of attraction of $(0,0)$ contains in the region $B_{(0,0)} \setminus S_{(A^{i1}, F^{i1})}$ where*

$$B_{(0,0)} = \left\{ (A, F) \in \mathbb{R}_+^2 : \frac{r_f a A^2}{d_f (b + a A^2)} - \frac{r_c A}{d_f} \leq F < F^{i1} \right\}$$

and

$$S_{(A^{i1}, F^{i1})} = \left\{ (A, F) \in \mathbb{R}_+^2 : \lim_{t \rightarrow \infty} (A(t), F(t)) = (A^{i1}, F^{i1}) \right\}.$$

A direct corollary of Proposition 3.1, Theorems 3.2 and 3.3 is as follows:

Corollary 3.1. *If $a > 4b((r_c r_a + d_f d_a)/r_a r_f)^2$, then the system (3)–(4) has two attractors*

$(0, 0)$ and (A^{i2}, F^{i2}) .

If the initial condition $(A(0), F(0))$ is too small such that it contained in $B_{(0,0)} \setminus S_{(A^{i1}, F^{i1})}$, then

$$\lim_{t \rightarrow \infty} (A(t), F(t)) = (0, 0);$$

while the initial condition $(A(0), F(0))$ is large enough, then

$$\lim_{t \rightarrow \infty} (A(t), F(t)) = (A^{i2}, F^{i2}).$$

Biological implications: Theorem 3.3 and Corollary 3.1 suggest that the initial population of leaf cutter ants and fungus is another important factor that determines whether ants and fungus can coexist or not. If initial population is contained in $B_{(0,0)} \setminus S_{(A^{i1}, F^{i1})}$, then both ants and fungus will go extinct even if the division of labor is in a good range, i.e., $a > 4b((r_c r_a + d_f d_a)/r_a r_f)^2$.

Our analysis performed in Proposition 3.1 indicates that the population pattern of ants and fungus are relatively simple, i.e., there are no fluctuations and converge to either the extinction state $(0, 0)$ or the coexistence state (A^{i2}, F^{i2}) . Theorem 3.1 suggests that both ants and fungus go to extinction if the division of labor by ants is too small, i.e., $a < 4b((r_c r_a + d_f d_a)/r_a r_f)^2$. Theorems 3.2, 3.3 and Corollary 3.1 suggest that the division of labor by ants and initial conditions are two important factors in determining whether leaf cutter ants and their fungus garden can coexist or not. This implies that the coexistence of two species needs two conditions: 1. the division of labor by ants is large enough, i.e., $a > 4b((r_c r_a + d_f d_a)/r_a r_f)^2$; 2. the initial population of ants and fungus are large enough, e.g., $A(0) > A^{i1}, F(0) > F^{i1}$. All the detailed proofs of these analytical results are presented in Section 6.

4. Numerical simulations, data and sensitive analysis

In this section, we validate our model (3)–(4) by performing numerical simulations, sensitivity analysis and parameter estimations based on the experiment data. The numerical simulations fit the data very well (see Figs. 1 and 2), which suggests that our model (3)–(4) is well defined. Sensitivity analysis around these chosen parameter values provides information on the governing factors for the ecological process modeled by (3)–(4).

4.1. Numerical simulations and experimental data

In this subsection, we compare the numerical simulations of the model (3)–(4) by using parameter values in certain intervals (see these values in Table 2). These intervals are obtained from the approximations according to data and literature (Brown et al., 2006; Clark and Fewell, in preparation). Measurements of leafcutter fungus and worker population growth, and of refuse production, were performed in the Fewell lab at Arizona State University from 2005 to 2007. Briefly, estimates of the maximum growth rates of the ants and fungus came from a six-month observational study of leafcutter colonies that were established and maintained in the laboratory ($n=11$), where the size (mass) of worker populations and fungus gardens were estimated once a week using noninvasive methods that correspond well with the actual worker population mass and fungus garden mass. Estimates of fungus death rates came from a two-month study of 21 colonies reared under the same conditions. Colony waste material (dead fungus and workers) was collected, sorted, dried and weighed to determine the rate at which dead fungus material was produced. Table 2 lists the range of parameters and the specific values (i.e., $r_a = 0.1, r_f = 0.7, d_a = 0.1, d_f = 0.2, b = 0.002, r_c = 0.0045, a = 0.2, A(6) = 0.05, F(6) = 0.3$) that generate dashed lines in Figs. 1 and 2 from weeks 6 to 29. Other values in the interval can generate similar dynamics as the chosen values, but the chosen values were selected because they match the empirical data well.

Figs. 1 and 2 provide the comparison between ecological data (solid lines with error bars) and simulations (dashed lines)

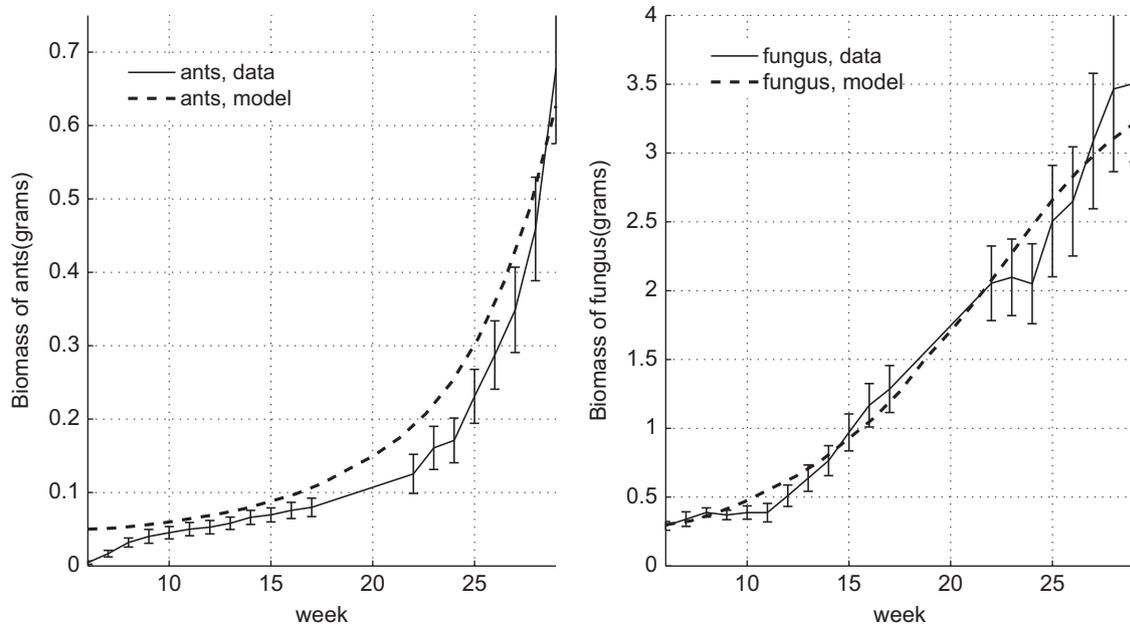


Fig. 1. The solid lines with error bars are from data and the dashed lines are simulations generated from the model (3)–(4) when $r_a = 0.1$, $r_f = 0.7$, $d_a = 0.1$, $d_f = 0.2$, $b = 0.002$, $r_c = 0.0045$, $a = 0.2$, $A(6) = 0.05$, $F(6) = 0.3$. The left figure is the biomass of ants vs. time in weeks and the right figure is the biomass of fungus vs. time in weeks.

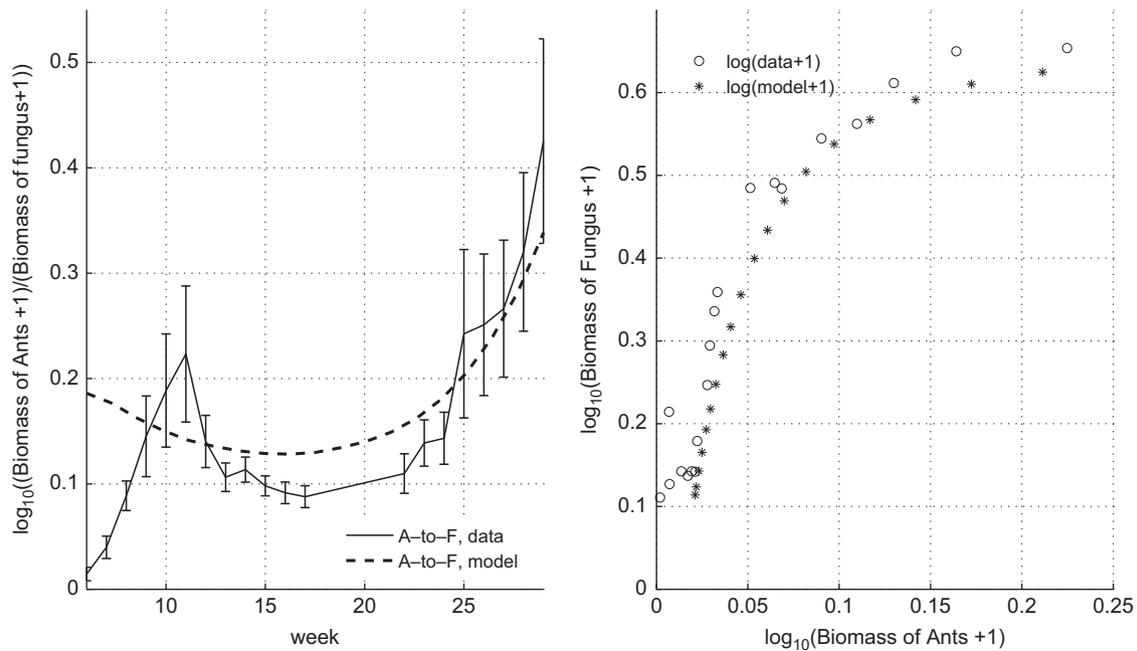


Fig. 2. The solid lines with error bars are from data and the dashed lines are simulations generated from the model (3)–(4) when $r_a = 0.1$, $r_f = 0.7$, $d_a = 0.1$, $d_f = 0.2$, $b = 0.002$, $r_c = 0.0045$, $a = 0.2$, $A(6) = 0.05$, $F(6) = 0.3$. In the left figure, the x-axis represents time in weeks and the y-axis represents the ratio of \log_{10} (the biomass of ants + 1) to \log_{10} (the biomass of fungus + 1); while in the right figure, the x-axis represents the value of \log_{10} (the biomass of ants + 1) and the y-axis represents the value of \log_{10} (the biomass of fungus + 1).

generated by the model (3)–(4) when $r_a = 0.1$, $r_f = 0.7$, $d_a = 0.1$, $d_f = 0.2$, $b = 0.002$, $r_c = 0.0045$, $a = 0.2$, $A(6) = 0.05$, $F(6) = 0.3$. The fitting of the model to the data for the ants and fungus population is evident from these two figures. The comparison between simulations generated by the model (3)–(4) and data suggests not only that the fit of the model to data is accurate but also that parameters match with expected values for growth, death, and division of labor. A recent experiment study by Clark and Fewell (in preparation) on leaf-cutter ants shows that the parameter values for r_a , r_f , d_f that generate Figs. 1 and 2 are very close to

actual data. The initial condition used for generating Figs. 1 and 2 is the mean value from the experimental data by Clark et al. (preprint). A study by Brown et al. (2006) on mortality rates of leaf cutter ants and division of labor suggests that the death rate matches with the parameter d_a . Notice that the values of parameters such as r_c , b are difficult to measure in the experiments. The good fit of the model to data (see Figs. 1 and 2) generated by the values listed in Table 2 provides an approximation of r_c and b . The parameter d_f is difficult to measure experimentally because of the efficiency of the mutualistic relationship between the ants

Table 2
Intervals and chosen values of parameters in the system (3)–(4).

Parameters	Intervals	Chosen values
r_a : Maximum growth rate of ants	(0.05, 0.3)	0.1
r_f : Maximum growth rate of fungus	(0.01, 1)	0.7
r_c : Conversion rate between fungus and ants	(0.001, 10)	0.0045
d_a : Death rate of ants	(0.001, 1)	0.1
d_f : Death rate of fungus	(0.001, 1)	0.2
b : Half-saturation constant	(0.001, 10)	0.002
a : Measurement of the division of labor	(0, 0.25)	0.2
$A(6)$: Biomass of ants at week 6	(0.001, 0.1)	0.05
$F(6)$: Biomass of ants at week 6	(0.001, 1)	0.3

and fungus. In the next subsection, we will examine the sensitivity of these parameter values and the initial condition.

The detailed information of Figs. 1 and 2 are presented as follows:

- In Fig. 1, the left figure is the biomass of ants vs. time in weeks and the right figure is the biomass of fungus vs. time in weeks. By comparison, we can see that the simulations fit the data very well, especially for biomass of fungus. Overall, the simulation of the biomass of ants is larger than the experimental data (the right figure). This is expected, because Eq. (3) models the biomass of all ants including the queen, eggs, larvae, pupae and workers, while the experiment only measures the biomass of workers. In addition, both data and simulations suggest that ants have exponential growth while the fungus has linear-like growth from weeks 6 to 29. Recall that our focus is the ergonomic growth stage of the ants which starts when the first workers appear. The exponential growth of ants at this growth stage confirms the study by Oster and Wilson (1979).
- The right figure of Fig. 2 represents $\log_{10}(\text{the biomass of ants} + 1)$ vs. $\log_{10}(\text{the biomass of fungus} + 1)$, which provides the information on the relationship between the growth rate of ants and the growth rate of fungus. Simulations fit data extremely well. Both suggest that the growth rate of ants and fungus increase over time, and the growth rate of ants increases faster than fungus, which may be caused by changes in the efficiency of the conversion between ants and fungus at the early colony stage.
The left figure of Fig. 2 is the ratio of $\log_{10}(\text{the biomass of ants} + 1)$ to $\log_{10}(\text{the biomass of fungus} + 1)$ vs. time in weeks, which provides information on the relative growth rate of ants to fungus: the simulation fits data very well from weeks 10 to 29 but shows some inconsistency between the data and the model fitting during weeks 6 to 9. In this case the model is a more accurate descriptor of population dynamics than the collected data because the biomass of the ants during this time consists almost entirely of immature workers or ants in the larvae/pupae stage. The data do not account for this ant biomass and thus, from weeks 6 to 9 the ant population may be largely underrepresented. This under-representation leads to an increase in the slope of data when the actual result should be closer to the model output during this time. Thus, the possible explanations for the inconsistency between the data and the model fitting during weeks 6–9 can be summarized as follows: 1. Eq. (3) models the biomass of all ants while the experiment only measures the biomass of workers; thus, the model (3)–(4) should generate the larger ratio of $\log_{10}(\text{the biomass of ants} + 1)$ to $\log_{10}(\text{the biomass of fungus} + 1)$; 2. For the first few weeks (weeks 6–9), the real population

dynamics of ants and fungus are highly unstable and may have very different ecological properties than our model assumptions. Stochasticity and multiple life stages of leaf cutter ants (e.g., eggs, larvae, pupae) may be considered in future models.

4.2. Implications from sensitivity analysis and parameter estimations

Input factors for our mathematical model (3)–(4) consist of seven parameters and two initial conditions for independent and dependent variables of the model. Because of natural variation, error in measurements, or simply a lack of current techniques to measure some parameters, it is necessary to perform sensitivity analysis to identify critical inputs (parameters and initial conditions) of our model and quantifying how input uncertainty impacts model outcomes (i.e., the dynamics of the ants and fungus biomass of ants $A(t)$, $F(t)$). In this subsection, sensitivity measure of the model (3)–(4) is computed numerically by performing multiple simulations varying input factors around the nominal value listed in Table 2.

The sensitivity of all parameters and initial conditions around $r_a = 0.1$, $r_f = 0.7$, $d_a = 0.1$, $d_f = 0.2$, $b = 0.002$, $r_c = 0.0045$, $a = 0.2$, $A(6) = 0.05$, $F(6) = 0.3$ from weeks 6 to 29 are shown in Figs. 3 and 4 (see Appendix). Sensitivity analysis for parameters and initial conditions were performed using an extension of the MATLAB function ODE23tb, a stiff solver for ordinary differential equations. The algorithms used in this instance are the internal numerical differentiation and iterative approximation based on directional derivative methods described by Bock (1981) and Maly and Petzold (1996) respectively. The output of the function is similar to that of ODE23tb with an additional array containing the derivatives (sensitivities) of the solution with respect to a given parameter vector. Brief summary on sensitivity results is as follows (the detailed information and figures on sensitivity results are presented in Appendix):

- The effects of all the parameters are strictly cumulative. The effects of each parameter at the beginning of the colony are relatively small. As time progresses the parameters have a much greater effect on the model, most of them having their largest effect on the biomass of fungus at around week 25.
- There is a time shift between the effect on the biomass of ants and the biomass of fungus: the largest effect on the biomass of fungus is always earlier than the largest effect on the biomass of ant.
- All the parameters have larger effects on the biomass of fungus than on the biomass of ants.
- The growth parameters r_a, r_f , the division of labor parameter a and the initial conditions $A(6), F(6)$ have positive effects on the biomass of ants and fungus while the death parameters d_a, d_f , the conversion rate r_c and the half-saturation constant b have negative effects on the biomass of ants and fungus.
- Among all these parameters and the initial conditions, the parameter b has the largest sensitivity and the conversion rate r_c , the death rate of ants d_a and the initial value of fungus $F(6)$ have relative small sensitivity to the output of the model.

In addition, we use nonlinear grey-box models (System Identification Toolbox provided in MATLAB) to perform parameter estimations based on experimental data by fixing the estimated interval of a to be (0, 0.25) and the estimated intervals of other parameters to be (0, ∞). The estimated values can be varied

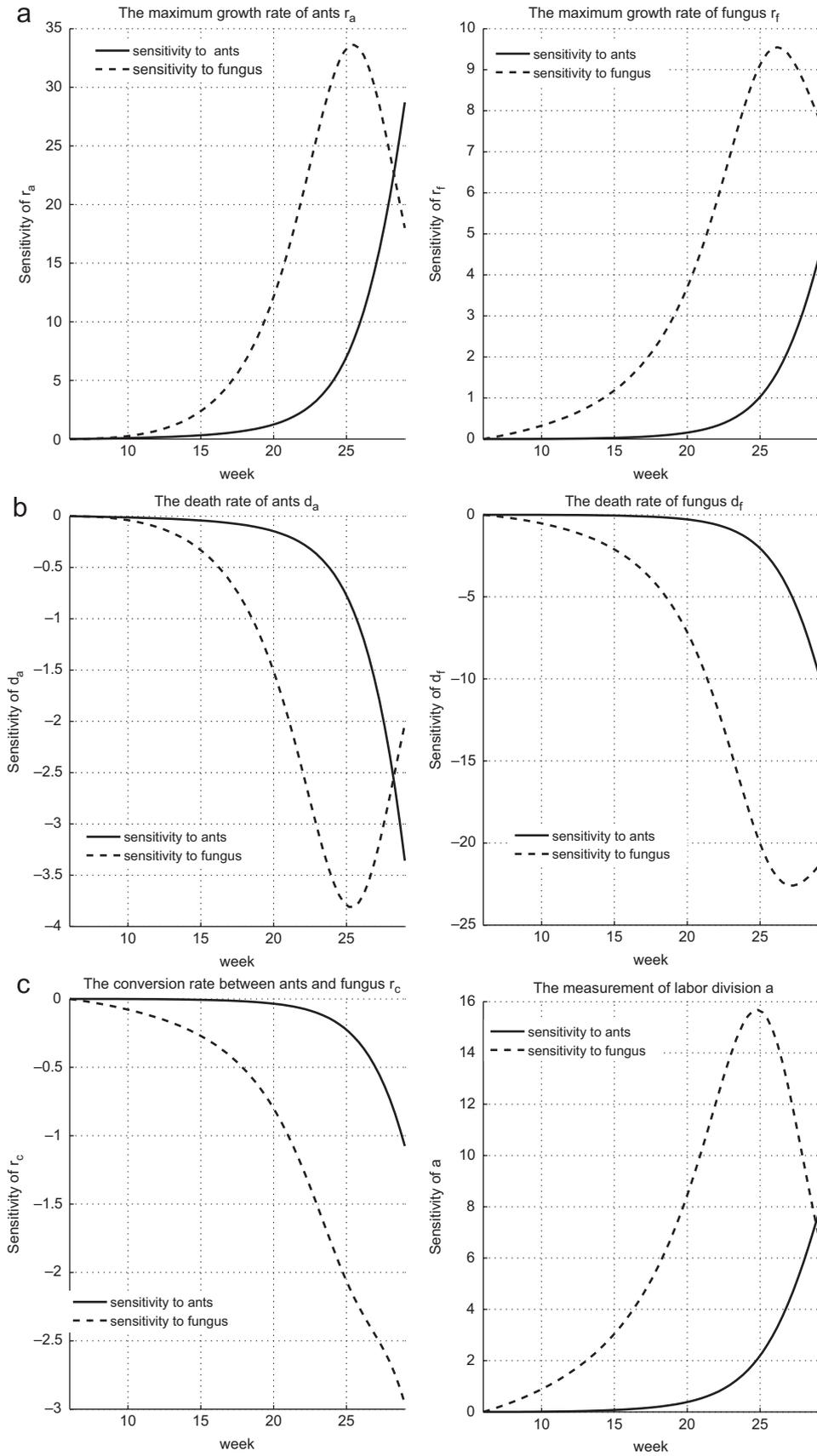


Fig. 3. Sensitivity of parameters r_a, r_f, d_a, d_f, r_c and a for model (3)–(4) around the chosen values where $r_a = 0.1, r_f = 0.7, d_a = 0.1, d_f = 0.2, b = 0.002, r_c = 0.0045, a = 0.2, A(6) = 0.05, F(6) = 0.3$. The x-axis is time with units in weeks and y-axis is the sensitivity. (a) The left figure is the sensitivity of the maximum growth rate of ants r_a and the right figure is the sensitivity of the maximum growth rate of fungus r_f . (b) The left figure is the sensitivity of the death rate of ants d_a and the right figure is the sensitivity of the death rate of fungus d_f . (c) The left figure is the sensitivity of the conversion rate between ants and fungus r_c and the right figure is the sensitivity of the measurement of the division of labor of ants a .

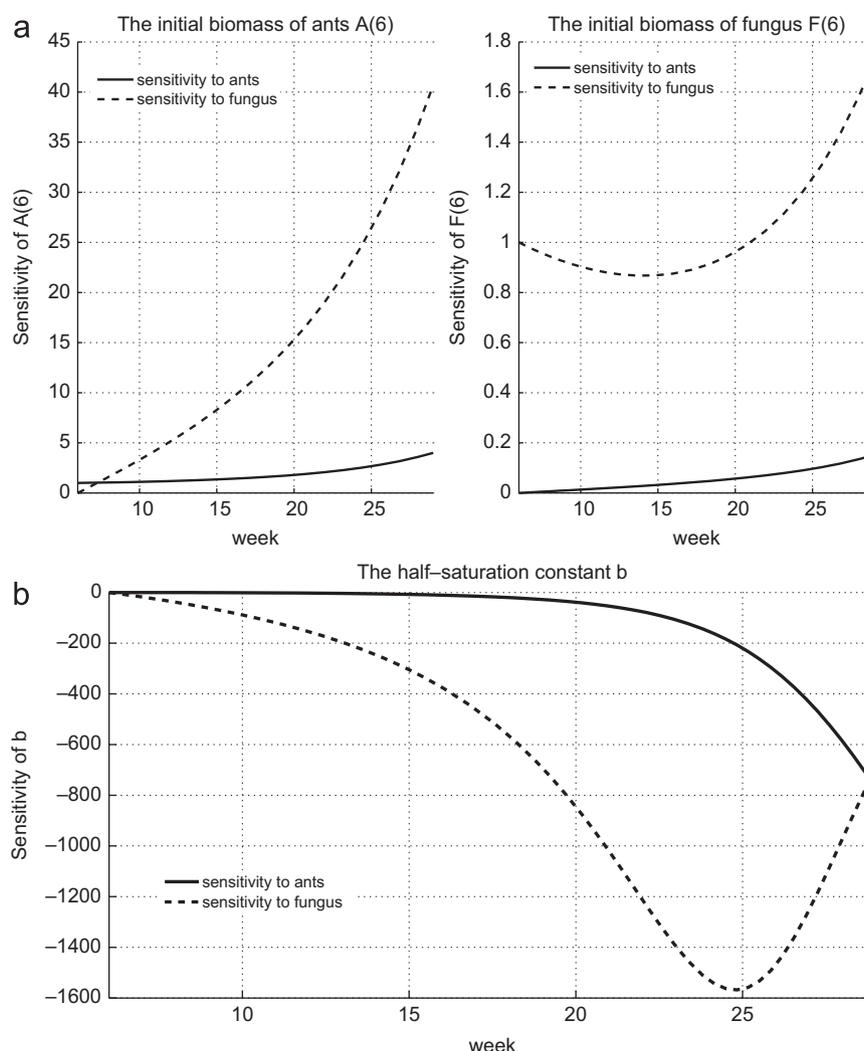


Fig. 4. Sensitivity of parameter b and initial conditions for model (3)–(4) around the chosen values where $r_a = 0.1$, $r_f = 0.7$, $d_a = 0.1$, $d_f = 0.2$, $b = 0.002$, $r_c = 0.0045$, $a = 0.2$, $A(6) = 0.05$, $F(6) = 0.3$. The x-axis is time with units in weeks and y-axis is the sensitivity. (a) The left figure is the sensitivity of the biomass of ants in week 6, $A(6)$ and the right figure is the sensitivity of the biomass of fungus in week 6, $F(6)$. (b) The sensitivity of the half-saturation constant b .

depending on the initial guesses and the estimated intervals. Parameter estimations suggest follows:

1. The different initial guess values will give different estimated values. This may be caused by the fact that the model (3)–(4) has multiple attractors.
2. The estimated values of r_c and d_a are both extremely small. In addition, the smaller the initial guesses of d_a and r_c , the smaller the estimated values of these parameters. This may suggest that r_c and d_a have little effect on the population dynamics of ants and fungus at the early colony stage, which has been confirmed by their small sensitivity (see Fig. 3(b) and (c)).
3. The standard deviations of estimated a, b, r_c, d_a are extremely large, which may be caused by two factors: 1. These parameters are not independent; 2. the extremely small value of d_a and r_c .

Thus the population dynamics of ants and fungus may be highly unstable at the early stage of colony development, as supported by the empirical data (Clark and Fewell, in preparation). Notice that collected data is from the successful colonies only. The extremely small estimated value of r_c and d_a may suggest that the conversion rate between ants and fungus and the death rate

of ants are not as important as other factors such as the growth rate parameters r_a, r_f and the death rate of fungus d_f . Possibly, a multiple-stage model that includes the stages of eggs, larvae, pupae or even a stochastic model should be introduced in order to get a better understanding of the detailed ecological processes.

5. Discussion

The traditional two-species mutualism models for plant-pollinator interactions (Soberon and Martinez del Rio, 1981; Wells, 1983; Holland et al., 2002) and legume rhizobium interactions (Vandermeer and Boucher, 1978; Simms and Taylor, 2002; West et al., 2002) are modified from Lotka-Volterra equations, which do not match the dynamics actually observed in nature (Holland and DeAngelis, 2010). In addition, these models make a general assumption that all individuals within the growing population are equivalent, which are less useful for predicting events at small scales like the population dynamics of leaf-cutter ants and their fungus garden at the early colony expansion, where internal organizational and demographic factors may play a larger role in determining the population growth. Thus, we need a new approach to study the mutualism interaction

between leaf-cutter ants and their fungus garden at the ergonomic growth stage starting from when the first brood of ant workers reaches the adult stage.

The interaction between ants and fungus can be categorized as a consumer–resource mutualism according to Holland and DeAngelis's (2010) study. In this paper, we apply Holland and DeAngelis' approach to derive a new and simple mathematical model (3)–(4) of the population dynamics of leaf cutter ants and their fungus garden at the early colony stage by applying Michaelis–Menton equations of enzyme kinetics. Our model not only includes the division of labor within the colony and the particular colony stage; incorporation of these behavioral and life-history components make it distinct from other obligate mutualism models such as plant–pollinator interactions but also can be validated by the experiment data. The unique features of model (3)–(4) can be summarized as follows:

1. The net benefit of the obligate fungus to leaf cutter ants is determined by the difference between the overall performance of collecting leaves and cultivating fungus by worker ants and the amount of fungus eaten by queen, larvae and workers; while the net benefit of obligate ants to fungus is determined by the difference between the amount of consumed fungus and the mortality rate due to the energy spent on collecting leaves and cultivating fungus.
2. The division of labor of leaf cutter ants: workers perform different tasks to maintain their fungus gardens. This feature allows us to apply the concept of the kinetics of functional response to model the numerical functional response of fungus.

The mathematical analysis (Theorems 3.1–3.3 and Corollary 3.1) of (3)–(4) gives a completed picture of the global dynamics of the interactions between leaf cutter ants and their fungus garden. These theoretical results suggests that:

1. The division of labor of ants can determine whether leaf cutter ants and their fungus garden are able to coexist.
2. When the division of labor is in a good range, the initial populations of leaf cutter ants and fungus are needed to be larger than some threshold in order to coexist.

We validated the model (3)–(4) by using empirical data. The comparison between model simulations and data supports the fact that (3)–(4) is well defined for modeling the population dynamics of the leaf cutter ants and fungus during the incipient colony stage (the early ergonomic growth stage). The good fit between the model and data also provides us an approximation of the values of difficult measured parameters such as the conversion rate between fungus and ants r_c and the half-saturation constant b . Sensitivity analysis implies b has the largest effect on the output of the model. Sensitivity analysis suggests that the growth rate parameters r_a, r_f and the death rate of fungus d_f are important factors for determining the population dynamics for the successful colony.

The inconsistency between the data and the model fitting during weeks 6–9 (Fig. 2) suggests that a more realistic and detailed model is needed during this period. Thus, consideration of multiple life cycle stages, including eggs, larvae and pupae, or even stochasticity should be included in further modeling work. In addition, for future experiments, the biomass of larvae and pupae should be measured as well. These different life cycle stages are likely to represent a larger proportion of the total ant biomass in early stages of colony growth and additionally likely have more variance. This could be our future work.

Overall, the present model provides methods for understanding and predicting mutualism growth dynamics in systems at small population sizes. Specifically, we show that a simple form of behavioral variation – classified here in terms of the division of labor – can play a key role in determining the system's outcome, whether stable growth or collapse of the system. Further, we found that the initial relative population sizes of the two mutualists are critical for successful establishment and growth.

In contrast, many studies of mutualistic interactions focus on population dynamics in large, or well-established populations. These sorts of populations are unlikely to reveal much about how such stable mutualisms arise or become established in new environments. The current model should be useful for predicting constraints on the evolution of mutualisms in certain systems, particularly those that experience population bottlenecks, followed by periods of rapid growth. These population characteristics are typical for digestive endosymbiotic bacteria that must be transferred from host mother to offspring (Hosokawa et al., 2007), and in situations where novel nutritional mutualisms arise and subsequently spread through a population (Hillesland and Stahl, 2010). It is much more challenging to empirically quantify establishment and exchanges between partners in such mutualisms; hopefully, the current work provides a framework for considering relevant factors for the initiation and growth of such systems.

6. Proof

Proof of Lemma 3.1. Notice that $A(0)=0$ then $A(t)=0$ for all $t \geq 0$; $F(0)=0$ then $F(t)=0$ for all $t \geq 0$. Therefore,

1. If $A(0)=0$ and $F(0)=0$, then $(A(t), F(t))=(0, 0)$ for all $t \geq 0$.
2. If $A(0)=0$ and $F(0)>0$, then

$$\frac{dF}{dt} = \left(\frac{r_f a A^2}{b + a A^2} - d_f F - r_c A \right) F = -d_f F^2 < 0.$$

$$\text{Thus, } \lim_{t \rightarrow \infty} F(t) = \lim_{t \rightarrow \infty} F(0)/(1 + d_f t) = 0.$$

3. If $F(0)=0$ and $A(0)>0$, then

$$\frac{dA}{dt} = (r_a F - d_a A) A = -d_a A^2 < 0.$$

$$\text{Thus, } \lim_{t \rightarrow \infty} A(t) = \lim_{t \rightarrow \infty} A(0)/(1 + d_a t) = 0.$$

If $A(0)>0$ and $F(0)>0$, then due to the continuity of the system, it is impossible for either $A(t)$ or $F(t)$ to drop below 0. Thus, for any $A(0) \geq 0, F(0) \geq 0$, we have $A(t) \geq 0$ and $F(t) \geq 0$ for all $t \geq 0$. Now assume $A(0) \geq 0, F(0) \geq 0$, then according to the expression of $dF(t)/dt$, we have

$$\frac{dF}{dt} = \left(\frac{r_f a A^2}{b + a A^2} - d_f F - r_c A \right) F \leq (r_f - d_f F) F.$$

Thus, $\limsup_{t \rightarrow \infty} F(t) \leq r_f/d_f$. This indicates that for any $\varepsilon > 0$, there exists T large enough, such that

$$F(t) < \frac{r_f}{d_f} + \varepsilon \quad \text{for all } t > T.$$

Therefore, we have

$$\frac{dA}{dt} = (r_a F - d_a A) A \leq \left(r_a \left(\frac{r_f}{d_f} + \varepsilon \right) - d_a A \right) A \quad \text{for all } t > T.$$

Since ε can be arbitrarily small, thus $\limsup_{t \rightarrow \infty} A(t) \leq r_a r_f / d_a d_f$. Therefore, we have shown that the system (3)–(4) is positively invariant and bounded in \mathbb{R}_+^2 . More specifically, the compact set $[0, r_a r_f / d_a d_f] \times [0, r_f / d_f]$ attracts all points in \mathbb{R}_+^2 .

Moreover, if both $A(0) > 0$ and $F(0) > 0$, then we have follows:

$$\frac{dA}{dt} = (r_a F - d_a A)A \geq -d_a A^2 \Rightarrow A(t) \geq \frac{A(0)}{1 + d_a t} > 0$$

$$\frac{dF}{dt} = \left(\frac{r_f a A^2}{b + a A^2} - d_f F - r_c A \right) F \geq -d_f F^2 \Rightarrow F(t) \geq \frac{F(0)}{1 + d_f t} > 0$$

Therefore, if both $A(0) > 0$ and $F(0) > 0$, then $A(t) > 0$ and $F(t) > 0$ for all $t > 0$. □

Proof of Proposition 3.1. By Poincaré–Bendixson Theorem (Guckenheimer and Holmes, 1983), the omega limit set of the system (3)–(4) is either a fixed point or a limit cycle. If there exists a function $B(A, F) : \mathbb{R}_+^2 \rightarrow \mathbb{R}_+$, such that

$$\frac{\partial}{\partial A} [B(A, F)(r_a F - d_a A)A] + \frac{\partial}{\partial F} \left[B(A, F) \left(\frac{r_f a A^2}{b + a A^2} - d_f F - r_c A \right) F \right] < 0,$$

then we can use Dulac’s criterion (Guckenheimer and Holmes, 1983) to exclude the existence of a limit cycle for the system (3)–(4). Let $B(A, F) = 1/AF$. Then,

$$\frac{\partial}{\partial A} [B(A, F)(r_a F - d_a A)A] + \frac{\partial}{\partial F} \left[B(A, F) \left(\frac{r_f a A^2}{b + a A^2} - d_f F - r_c A \right) F \right] = -\frac{d_a}{F} - \frac{d_f}{A} < 0$$

holds for any $(A(0), F(0)) \in \mathbb{R}_+^2$. Therefore, by Dulac’s criterion, the system (3)–(4) has no limit cycle, i.e., any trajectory of (3)–(4) starting with a non-negative initial condition converges to a fixed point. □

Proof of Proposition 3.2. It is easy to see that (0,0) is always an equilibrium of the system (3)–(4). The nullclines of (3)–(4) can be founded as

$$\frac{dA}{dt} = 0 \Rightarrow A = 0 \quad \text{or} \quad F = \frac{d_a}{r_a} A$$

$$\frac{dF}{dt} = 0 \Rightarrow F = 0 \quad \text{or} \quad F = \frac{r_f a A^2}{d_f (b + a A^2)} - \frac{r_c}{d_f} A$$

By solving $r_f a A^2 / d_f (b + a A^2) - (r_c / d_f) A = (d_a / r_a) A$ for A , we have the following two cases:

1. If $a > 4b((r_c r_a + d_f d_a) / r_a r_f)^2$, then by simple algebraic calculations, there are the following two positive solutions of $r_f a A^2 / d_f (b + a A^2) - (r_c / d_f) A = (d_a / r_a) A$:

$$A^{i1} = \frac{r_f r_a}{2(r_c r_a + d_f d_a)} - \sqrt{\left(\frac{r_f r_a}{2(r_c r_a + d_f d_a)} \right)^2 - \frac{b}{a}}$$

$$A^{i2} = \frac{r_f r_a}{2(r_c r_a + d_f d_a)} + \sqrt{\left(\frac{r_f r_a}{2(r_c r_a + d_f d_a)} \right)^2 - \frac{b}{a}}$$

Thus, the two interior equilibria are

$$(A^{i1}, F^{i1}) = \left(A^{i1}, \frac{d_a}{r_a} A^{i1} \right) \quad \text{and} \quad (A^{i2}, F^{i2}) = \left(A^{i2}, \frac{d_a}{r_a} A^{i2} \right).$$

2. If $a = 4b((r_c r_a + d_f d_a) / r_a r_f)^2$, then the system (3)–(4) has only one positive equilibria (A^i, F^i) where

$$(A^i, F^i) = \left(\frac{r_f r_a}{2(r_c r_a + d_f d_a)}, \frac{r_f r_a d_a}{2r_a(r_c r_a + d_f d_a)} \right)$$

3. If $a < 4b((r_c r_a + d_f d_a) / r_a r_f)^2$, then there is only one trivial equilibrium: $A = 0$ and $F = 0$.

Therefore, the statement of Proposition 3.2 holds. □

Proof of Theorem 3.1. From Proposition 3.2, we know that for any initial condition taken in \mathbb{R}_+^2 , the trajectory of the system (3)–(4) is converging to an equilibrium point. If $a < 4b$

$((r_c r_a + d_f d_a) / r_a r_f)^2$, then according to Proposition 3.2, the only equilibrium of the system (3)–(4) is the origin (0,0). Therefore, we can conclude that the system (3)–(4) has global stability at (0,0) when $a < 4b((r_c r_a + d_f d_a) / r_a r_f)^2$. □

Proof of Theorem 3.2. From Proposition 3.2, we know that the system (3)–(4) has two positive equilibria (A^{i1}, F^{i1}) and (A^{i2}, F^{i2}) when $a > 4b((r_c r_a + d_f d_a) / r_a r_f)^2$. The local stability can be determined from the eigenvalues of its Jacobian matrices evaluated at these equilibria.

Assume that (A^*, F^*) is an equilibrium point of (3)–(4), then its Jacobian matrices evaluated at this equilibrium can be written as follows:

$$J|_{(A^*, F^*)} = \begin{bmatrix} -d_a A^* & r_a A^* \\ \frac{d_a A^*}{r_a} \left(2 \left(r_c + \frac{d_a d_f}{r_a} \right) \left(1 - A^* \left(\frac{r_c}{r_f} + \frac{d_a d_f}{r_a r_f} \right) \right) - r_c \right) & -\frac{d_a d_f A^*}{r_a} \end{bmatrix} \quad (6)$$

Then we have

$$\text{trace}(J|_{(A^*, F^*)}) = -d_a A^* \frac{r_a + d_f}{r_a} < 0$$

$$\det(J|_{(A^*, F^*)}) = \frac{d_a (A^*)^2 (r_a r_c + d_a d_f) (2 r_a r_c A^* - r_a r_f + 2 d_a d_f A^*)}{r_a^2 d_f}$$

This implies that if $A^* > r_a r_f / 2(r_a r_c + d_a d_f)$, then (A^*, F^*) is locally asymptotically stable; while if $A^* < r_a r_f / 2(r_a r_c + d_a d_f)$, then (A^*, F^*) is a saddle node, i.e., unstable. Since

$$A^{i1} = \frac{r_f r_a}{2(r_c r_a + d_f d_a)} - \sqrt{\left(\frac{r_f r_a}{2(r_c r_a + d_f d_a)} \right)^2 - \frac{b}{a}} < \frac{r_a r_f}{2(r_a r_c + d_a d_f)}$$

$$A^{i2} = \frac{r_f r_a}{2(r_c r_a + d_f d_a)} + \sqrt{\left(\frac{r_f r_a}{2(r_c r_a + d_f d_a)} \right)^2 - \frac{b}{a}} > \frac{r_a r_f}{2(r_a r_c + d_a d_f)}$$

Therefore, (A^{i1}, F^{i1}) is always unstable and (A^{i2}, F^{i2}) is always locally asymptotically stable when $a > 4b((r_c r_a + d_f d_a) / r_a r_f)^2$. □

Proof of Theorem 3.3. If $a < 4b((r_c r_a + d_f d_a) / r_a r_f)^2$, then according to Theorem 3.1, (0,0) is global stable in \mathbb{R}_+^2 , thus it is locally asymptotically stable. Now we need to consider the case that $a > 4b((r_c r_a + d_f d_a) / r_a r_f)^2$.

First, we claim that the region defined by

$$\Omega_1 = \left\{ (A, F) \in \mathbb{R}_+^2 : \frac{r_f a A^2}{d_f (b + a A^2)} - \frac{r_c A}{d_f} \leq F \leq \frac{d_a A}{r_a} \right\}$$

is positively invariant. Assume that this is not true. Then there is some initial condition $(A(0), F(0))$ taken in Ω_1 such that for some future time T such that $(A(T), F(T))$ is leaving Ω_1 . From Proposition 3.2, we know that the system (3)–(4) has only one equilibrium point (A^{i1}, F^{i1}) in Ω_1 , thus due to the continuity of the system, there exists some time T such that we have one of the following two cases:

1. For all $0 < t < T$,

$$\frac{r_f a A^2(t)}{d_f (b + a A^2(t))} - \frac{r_c A(t)}{d_f} < F(t) < \frac{d_a A(T)}{r_a}$$

at $t = T$,

$$\frac{r_f a A^2(T)}{d_f (b + a A^2(T))} - \frac{r_c A(T)}{d_f} = F(T) \quad \text{and} \quad F(T) < \frac{d_a A(T)}{r_a}$$

and for some $\varepsilon > 0$ and $T < t < T + \varepsilon$, we have

$$\frac{r_f a A^2(t)}{d_f(b + aA^2(t))} - \frac{r_c A(t)}{d_f} > F(t) \quad \text{and} \quad F(t) < \frac{d_a A(T)}{r_a}$$

2. For all $0 < t < T$,

$$\frac{r_f a A^2(t)}{d_f(b + aA^2(t))} - \frac{r_c A(t)}{d_f} < F(t) < \frac{d_a A(T)}{r_a}$$

at $t = T$,

$$\frac{r_f a A^2(T)}{d_f(b + aA^2(T))} - \frac{r_c A(T)}{d_f} < F(T) \quad \text{and} \quad F(T) = \frac{d_a A(T)}{r_a}$$

and for some $\varepsilon > 0$ and $T < t < T + \varepsilon$, we have

$$F(t) > \frac{d_a A(t)}{r_a}$$

If the first case holds, then at time $t = T$ we have

$$\left. \frac{dA}{dt} \right|_{t=T} = (r_a F(T) - d_a A(T))A(T) = 0$$

$$\left. \frac{dF}{dt} \right|_{t=T} = \left(\frac{r_f a A^2(T)}{b + aA^2(T)} - d_f F(T) - r_c A(T) \right) F(T) < 0$$

This implies that there exists some small ε such that $A(t) \leq A(T)$, $F(t) < F(T)$ for all $T < t < T + \varepsilon$, which contradicts the conditions for the first case. Similarly, we can show it is impossible for the second case to be held. Therefore, Ω_1 is positively invariant.

Now we will show that $B_{(0,0)}$ is positively invariant. Define

$$\Omega_2 = B_{(0,0)} \setminus \Omega_1 \quad \text{and} \quad \Omega_3 = \Omega_1 \setminus \{(A^{i1}, F^{i1})\}.$$

Then, Ω_3 is also positively invariant since (A^{i1}, F^{i1}) is an equilibrium point and $B_{(0,0)} = \Omega_2 \cup \Omega_3$. For any initial condition $(A(0), F(0))$ taken in $B_{(0,0)}$, there are the following two cases:

1. If $(A(0), F(0)) \in \Omega_3$, then $(A(t), F(t)) \in \Omega_3$ for all $t > 0$ since Ω_3 is positively invariant.
2. If $(A(0), F(0)) \in \Omega_2$, then either $(A(t), F(t)) \in \Omega_2$ for all $t > 0$ or there exists some T such that

$$\left. \frac{dA}{dt} \right|_{t=T} = (r_a F(T) - d_a A(T))A(T) = 0$$

$$\left. \frac{dF}{dt} \right|_{t=T} = \left(\frac{r_f a A^2(T)}{b + aA^2(T)} - d_f F(T) - r_c A(T) \right) F(T) < 0$$

This implies that $(A(T), F(T)) \in \Omega_3$. Since Ω_3 is positively invariant, then $(A(t), F(t)) \in \Omega_3$ for all $t > T$.

Therefore, $B_{(0,0)}$ is positively invariant.

Define a Lyapunov function $V = A^\alpha F^\beta : B_{(0,0)} \rightarrow \mathbb{R}_+^2$ where both α and β are positive. Then we have

$$\begin{aligned} \frac{dV}{dt} &= \alpha A^{\alpha-1} F^\beta \frac{dA}{dt} + \beta A^\alpha F^{\beta-1} \frac{dF}{dt} = \alpha A^\alpha F^\beta (r_a F - d_a A) \\ &\quad + \beta A^\alpha F^\beta \left(\frac{r_f a A^2}{b + aA^2} - d_f F - r_c A \right) \\ &= V \left[\alpha \left(r_a - \frac{\beta d_f}{\alpha} \right) F + \beta \left(\frac{r_f a A^2}{b + aA^2} - \left(r_c + \frac{\alpha d_a}{\beta} \right) A \right) \right] \end{aligned}$$

Choose α, β such that $r_a - \beta d_f / \alpha = 0$, i.e., $\beta / \alpha = r_a / d_f$, then the expression of dV/dt can be simplified as

$$\begin{aligned} \frac{dV}{dt} &= V \left[\beta \left(\frac{r_f a A^2}{b + aA^2} - \left(r_c + \frac{d_f d_a}{r_a} \right) A \right) \right] \\ &= \beta V A \left[\frac{r_f a A - b \left(r_c + \frac{d_f d_a}{r_a} \right) - a \left(r_c + \frac{d_f d_a}{r_a} \right) A^2}{b + aA^2} \right] \end{aligned}$$

Define $f(A) = r_f a A - b(r_c + (d_f d_a / r_a)) - a(r_c + (d_f d_a / r_a))A^2$, then the sign of dV/dt depends on the sign of $f(A)$. Notice that $f(A) = (A - A^{i1})(A^{i2} - A)$ is negative if $0 < A < A^{i1}$ and any point $(A, F) \in B_{(0,0)}$ satisfying $0 < A < A^{i1}, 0 < F < F^{i1}$.

Since $B_{(0,0)}$ is positively invariant, for any initial condition taken in $B_{(0,0)}$, we have $dV/dt < 0$ for all future time. This indicates that $A(t)$ and $F(t)$ approach to some fixed point contained in $\overline{B_{(0,0)}}$ (the closure of $B_{(0,0)}$). Notice that $\overline{B_{(0,0)}}$ contains only $(0, 0)$ and (A^{i1}, F^{i1}) . According to Theorem 3.2, (A^{i1}, F^{i1}) is unstable when $a > 4b((r_c r_a + d_f d_a) / r_a r_f)^2$. Then based on Hartman–Grobman Theorem (Robinson, 1998), any point in $B_{(0,0)} \setminus S_{(A^{i1}, F^{i1})}$ will not approach to (A^{i1}, F^{i1}) , and therefore, it will approach to $(0, 0)$.

Therefore, the statement of Theorem 3.3 holds. \square

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Appendix

The detailed results on sensitivity analysis for each parameter and the initial condition can be summarized as follows:

1. Fig. 3(a) shows that the growth parameters r_a and r_f have a positive effect on the model as a function of time; as the model progresses, r_a and r_f have a larger effect on the increase of both biomass of ants and fungus. The largest effect of both r_a and r_f on biomass of fungus occurs at week 25, while the effect of both r_a and r_f on biomass of ants is an increasing function of time and achieves the largest effect at the end of experiment, i.e., week 29.
2. Fig. 3(b) shows that the death parameters d_a and d_f have a negative effect on the model as a function of time. As the model progresses forward in time the decrease in the biomass of ants and fungus respectively is increasingly affected by parameters d_a and d_f . The largest effect of r_a and r_f on biomass of fungus occurs at weeks 25 and 26 respectively while the effect of both d_a and d_f on biomass of ants is an increasing function of time and achieves the largest effect at the end of experiment, i.e., week 29.
3. Fig. 3(c) shows the conversion rate between ants and fungus r_c has a negative effect on the output of the model while the measurement of the division of labor of ants has a positive effect. The effect of r_c on the biomass of both ants and fungus is a decreasing function of time while the division of labor parameter a shows very similar behavior to the growth parameters r_a and r_f ; this suggests that by maximizing the

efficiency of division of labor, both the ants' and fungus's population growth will be maximized. This agrees with paper main purpose number 2.

4. Fig. 4(a) shows that both initial condition $A(6)$ and $F(6)$ have positive effects on the model as a function of time. The effect of $A(6)$ on biomass of both ants and fungus is an increasing function of time. The effect of $F(6)$ on the biomass of ants is an increasing function of time while the effect on the biomass of fungus is decreasing until week 15 and then increasing until week 29.
5. Fig. 4(b) shows that the half-saturation constant b has a negative effect on the model as a function of time. As the model progresses forward in time, the decrease in biomass of ants and fungus respectively is increasingly affected by b . The largest effect of b on fungal biomass occurs at week 25 while the effect of b on biomass of ants is an increasing function of time and achieves the largest effect at the end of experiment, i.e., week 29. Notice that b has the largest sensitivity among all the parameters and initial conditions.

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