

Demographic consequences of partner diversity and turnover in a multi-species ant-plant mutualism

Keywords: Integral Projection Model, *Cylindropuntia imbricata*, population fitness, multi-species mutualism, complementarity, sampling effect, portfolio effect

Manuscript type: Article.

Prepared using the suggested L^AT_EX template for *Am. Nat.*

Abstract

Mutualisms commonly involve multiple partner species but the ecological consequences of partner diversity remain poorly understood. Partner diversity may benefit a focal mutualist through mechanisms that mirror positive effects of species diversity on ecosystem function, such as sampling effect, complementarity or portfolio effect. In sequential mutualisms, partner identity can change over time due to environmental variability or competitive displacement. Here we asked how partner diversity and turnover influence host demography and long-term fitness, and which mechanisms explain these effects. We studied the sequential multi-species food-for-protection mutualism involving an EFN-bearing plant (tree cholla cactus *Cylindropuntia imbricata*) and multiple species of nectar-feeding ants (*Crematogaster opuntiae*, *Liometopum apiculatum*, and other less frequent ants) that provide defense from herbivores. We used a 20-year dataset on plant vital rates and partner identity to quantify the demographic consequences of association with different ant partners and the frequency and direction of turnover between mutually exclusive partner associations across the plant life cycle. We then constructed a stochastic, multi-state Integral Projection Model (IPM) to investigate how different patterns of partner richness, composition, and sequential turnover affect population fitness under fluctuating environmental conditions. We found that, while ant partners had different impacts on plant vital rates (*C. opuntiae*-tended plants had advantages in growth and survival when small, and *L. apiculatum*-tended plants had floral viability advantages), plant fitness was similarly elevated by ant visitation for any level of partner identity and richness, because effects on the highest-sensitivity vital rates were consistent across partner species. Furthermore, there was no evidence that benefits of partner diversity manifest in the context of fluctuating environments (i.e., the portfolio effect). In contrast to much of the previous literature, this study highlights that demographic benefits of mutualism can be surprisingly robust to diversity and turnover of partner species.

Introduction

Mutualisms, species interactions where all participants receive net benefits, are key components of ecological communities and can enhance the fitness and population growth rates of the partners involved. While traditionally framed as pairwise (+/+) interactions, mutualisms are often embedded within multispecies communities in which a focal mutualist interacts with multiple partner species. Growing evidence suggests that pairwise interactions may be poor predictors of the net effects of multi-species mutualism (Afkhami et al., 2014; Bascompte, 2009; Dattilo et al., 2014; Palmer et al., 2010).

A focal mutualist may interact with multiple guilds of partner types (e.g., plants that interact with pollinators, seed dispersers, soil microbes, and ant defenders) or with multiple partner species within the same guild (e.g., plants visited by multiple pollinator species). Within a mutualist guild, partner species often differ in the amount or type of goods or services they provide, making partner identity an important source of contingency in mutualism (Stanton, 2003). Partner diversity can arise either from simultaneous interactions or sequential ones, where individual hosts experience turnover in partner identity over time (Djiéto-Lordon et al., 2005; Ness et al., 2006). Understanding how partner diversity and turnover affect the overall fitness benefits of mutualism is an open question, especially in the context of environmental variability, where different partners may confer benefits unevenly across space or time (Frederickson, 2005; Palmer et al., 2010; Rogers et al., 2014; Thibaut et al., 2012).

There are multiple mechanisms by which partner diversity can influence the net benefits accrued by a focal mutualist, mirroring the mechanisms by which, at a larger scale of organization, biodiversity can influence ecosystem function (Barrett et al., 2015; Ushio, 2020; Winfree, 2020). First, when there is a consistent ranking of best to worst mutualists, a more diverse sample of the partner community may be more likely to include the best partner (Frederickson, 2013). This can lead to an apparent benefit of diversity driven by a sampling effect (Batstone et al., 2018). However, if partner associations are mutually exclusive (a focal mutualist may engage with only one partner at

51 a time), then partner diversity may impose opportunity costs, leading to negative effects of a diverse
mutualist assemblage relative to exclusive association with the single best partner (Miller, 2007).
Second, even within a mutualist guild, the benefits conferred by alternative partner species can vary
54 by method, efficiency, or timing (Bronstein et al., 2006; Stachowicz and Whitlatch, 2005; Stanton,
2003). This can lead to a positive effect of partner diversity through complementarity, whereby
diversity increases the net benefits received by focal mutualist beyond what a single partner species
57 could offer (Batstone et al., 2018). Positive diversity effects through complementarity need not be
additive: interference or synergies between partners can make their combined effect different than
expected from the sum of complementary functions (Afkhami et al., 2014). Third, partner species
60 can have species-specific responses to environmental variation, either spatially (Ollerton et al.,
2006) or temporally (Alarcón et al., 2008). Multiple partners can therefore act as a ‘portfolio’
that stabilizes fitness benefits across spatial or temporal heterogeneity, leading to positive effects of
63 partner diversity through the portfolio effect (Batstone et al., 2018; Horvitz and Schemske, 1990;
Lázaro et al., 2022). In the context of temporally fluctuating environments, the portfolio effect
implies that inter-annual variation in partner identity could stabilize overall plant fitness across
66 years. For such a portfolio effect to operate, two conditions must be met: (i) partner species must
differ in the timing or magnitude of their responses to environmental variation (i.e., their benefits
fluctuate asynchronously), and (ii) the combined effect of these fluctuations must result in increased
69 net benefit to the host across years relative to the net benefit expected if all partners responded in
parallel (Hautier et al., 2014; Loreau, 1999; Tilman et al., 1994). While portfolio effects have been
well studied in plant and animal communities, their role in mutualisms—particularly where partner
72 diversity is expressed sequentially rather than simultaneously—remains less explored (Ushio, 2020).

For exclusive, sequential associations, the timing, frequency, and directionality of partner turnover
can influence the net fitness benefits to a focal mutualist, particularly if turnover allows accumula-
75 tion of complementary benefits or buffers environmental variability via portfolio effects (Fiala et al.,
1994; Sachs et al., 2004; Ushio, 2020). Turnover can happen at different timescales, from minutes
to years (Horvitz and Schemske, 1986; Nakazawa, 2020; Oliveira et al., 1999; Ventre-Lespiaucq

78 et al., 2021). If partner identity changes systematically across ontogeny, such as through succession
of partners aligned with host life stages, this may further affect fitness outcomes associated with
partner diversity (Dejean et al., 2008; Fonseca and Benson, 2003; Noe and Hammerstein, 1994). For
81 example, plant susceptibility to enemies can change across life stages (Barton and Koricheva, 2010;
Boege and Marquis, 2005), so the benefits of a diverse guild of defensive mutualists are greatest
when more defensive partner species align with more vulnerable life stages (Dejean et al., 2008;
84 Djitéo-Lordon et al., 2005). Thus, the duration and order of interactions may be just as important
as the identities of the interacting partners.

Defensive ant-plant mutualisms – where plants provide food and/or housing to ants that in turn
87 defend them from enemies – are widespread interactions that offer valuable model systems for the
ecology and evolution of mutualism (Bronstein, 1998; Bronstein et al., 2006). Extrafloral nectar
(EFN) -bearing plants can serve as dietary resources that promote ant abundance and colony size
90 (Byk and Del-Claro, 2011; Donald and Miller, 2022; Ness et al., 2006, 2009). In turn, presence
of defensive ant partners is often linked to reductions in herbivory (Rudgers, 2004; Trager et al.,
2010) and demographic advantages for the plant partner (Báez et al., 2016). Defensive ant-plant
93 mutualisms are commonly multi-species, where a guild of ant partner species share, and often
compete for, a plant mutualist (Agrawal and Rutter, 1998; Beattie, 1985; Bronstein, 1998; Trager
et al., 2010). Ant partners can vary in their ability to deter herbivores (Bruna et al., 2014) and
96 visitation by low quality ant partners can prevent visitation by higher quality partners, causing
a reduction in relative fitness through opportunity costs (Fraser et al., 2001; Frederickson, 2005).
Susceptibility to herbivory can also vary significantly throughout the life stages of the plant (Boege
99 and Marquis, 2005), suggesting that the order and timing of successive partners is important to the
fitness impacts of the combined partner guild (Barton and Koricheva, 2010; Boege and Marquis,
2005; Fonseca and Benson, 2003). Finally, herbivore identity and pressure can vary inter-annually
102 (Wetzel et al., 2023), much like mutualist identity and presence, meaning the threat plants face can
vary just as much as the protection they receive due to temporal stochasticity. Previous studies
have investigated how ant partner diversity affects plant fitness (Dattilo et al., 2014; Fiala et al.,

105 1994; Gaume et al., 1998; Ludka et al., 2015; Palmer et al., 2010), however little is known about
the combined effects of partner identity, directional partner turnover, and temporal stochasticity,
particularly because long-term demographic studies on ant-plant interactions are exceedingly rare
108 Donald and Miller (2022); Moraes and Vasconcelos (2009).

Long-term demographic data are essential to understanding the fitness consequences of multi-species mutualisms for several reasons. First, fitness effects of sequential partnering play out over
111 long time scales due to the accumulation of partners across time rather than the effects of multiple partners at once. While turnover of mutualist partners is known to occur (Djiéto-Lordon et al., 2005; Fonseca and Benson, 2003; Ness et al., 2006), long-term data documenting patterns
114 and consequences of partner switching are uncommon, especially in field settings (Palmer et al., 2010). Second, certain effects of partner diversity are fluctuation-dependent (i.e., portfolio effect) and would only be detectable in the context of temporally stochastic environments in which alter-
117 native partners may fluctuate asynchronously. Quantifying contributions from the portfolio effect therefore requires long-term data on partner (a)synchrony across inter-annual fluctuations. Finally, alternative partners may have different demographic effects (e.g., one that enhances survival and
120 another that enhances reproduction), and these effects may contribute differently to overall fitness of a focal mutualist. Most studies evaluate short-term fitness proxies, such as seed set or herbivory, often focusing on a single life stage (Palmer et al., 2010; Trager et al., 2010), while fewer trace
123 demographic effects across multiple stages (Bruna et al., 2014). Integrating long-term data from across the life cycle within a demographic modeling framework therefore provides a powerful op-
portunity to diagnose the fitness consequences of partner diversity and turnover in the context of
126 environmental stochasticity.

This study examined the consequences of partner diversity in a food-for-protection mutualism between the tree cholla cactus (*Cylindropuntia imbricata*), a long-lived EFN-bearing plant, and
129 multiple species of ant partners. Tree cholla are tended by two common ant partners and several additional rarer species, all of which collect EFN in exchange for defense against herbivores. These ant species locally co-occur, but individual plants are typically tended by only one species that

132 patrols the plant around-the-clock and maintains control of the plant's nectar resources, usually
133 for an entire growing season (Donald and Miller, 2022; Ohm and Miller, 2014). Switches between
partner species, or between vacancy and ant occupancy, commonly occur from one growing season
134 to the next (Miller, 2007). Prior experiments suggested a hierarchy of mutualist quality, with *Li-*
ometopum apiculatum providing strong anti-herbivore defense and *Crematogaster opuntiae* having
net negative effects because herbivore deterrence is outweighed by deterrence of pollinators (Miller,
135 2007; Ohm and Miller, 2014). However, previous studies in this system focused on single life stages
(adult plants) or vital rates (seed production), did not integrate the demographic effects of ant
defense across the life cycle, and did not incorporate temporal stochasticity – gaps we aimed to fill
136 in the present study.

137 Here we used a unique long-term data set that allows us to explore mutualistic associations
with multiple partner species, longitudinal dynamics of partner turnover, and demographic effects
138 of alternative partners that vary across plant size structure and across nearly 20 years of inter-
annual fluctuations. The long-term nature of this observational dataset gives us the power to
investigate whether and through which mechanism(s) partner diversity affects the fitness benefits
139 of ant visitation. Specifically, we asked:

140 1. What are the vital rate effects of association with alternative partners and how do these
effects fluctuate across years?

141 2. What are the frequency and direction of partner turnover across the plant life cycle?

142 3. What is the net effect of partner diversity on plant fitness, and what mechanism(s) explain(s)
this effect?

143 To answer these questions we used a hierarchical Bayesian statistical approach to estimate demo-
graphic vital rates for hosts in different states of ant occupancy and to quantify state-dependent
partner turnover from the long-term data. We then built a stochastic, multi-state integral projec-
144 tion model (IPM) which combines diverse effects on vital rates and pathways of partner turnover.

Finally, we used the model to quantify plant fitness under different scenarios of partner richness, composition, and synchrony.

159

Methods

Study System

This study was conducted at the Sevilleta Long-term Ecological Research site (SEV-LTER), located
162 within the Sevilleta National Wildlife Refuge in central New Mexico, USA. Our study area in the Los Piños mountains ($34^{\circ}20'5.3''N$, $106^{\circ}37'53.2''W$) is characterized by steep, rocky slopes and
perennial vegetation including grasses (*Bouteloua eriopoda* and *B. gracilis*), yuccas, cacti, and
165 junipers. Tree cholla cacti are common in such high Chihuahuan desert habitats (Benson, 1982).

These arborescent plants produce cylindrical segments with large spines. Early each growing season
(May to August in New Mexico), they initiate new vegetative segments and flowerbuds at the ends
168 of existing segments. While most plants produce new segments every season, only those which are
reproductively mature produce flowerbuds. Like other EFN-bearing cacti, tree cholla secrete nectar
from specialized glands on young vegetative segments and flowerbuds (Ness et al., 2006; Oliveira
171 et al., 1999). Flowerbuds produce more and higher-quality EFN than vegetative segments, making
reproductive cholla valuable mutualist partners (Miller, 2014). Small, non-flowering cholla produce
little to no EFN and are commonly vacant (no ant visitation) (Miller, 2014).

174 Tree cholla EFN is collected by several ground-nesting ant species. At SEV-LTER, cholla are vis-
ited primarily by two species, *Crematogaster opuntiae* and *Liometopum apiculatum*, as well as other
rarer species, including *Forelius pruinosus* and unidentified species in the genera *Aphaenogaster* and
177 *Camponotus*. *L. apiculatum* are the most frequent visitors with 25% – 60% of tree cholla tended by
these ants in a given year, followed by *C. opuntiae* (5% – 20%) (Donald and Miller, 2022). Between
30% – 80% of cacti are vacant in any given year. Workers of different species rarely co-occur on
180 individual plants, likely due to interspecific competition. For example, staged introductions of *C.*
opuntiae to *L. apiculatum*-tended plants, and vice versa, provoke aggressive responses by residents

(personal observation).

183 Several insect herbivores and seed predators specialize on tree cholla (Mann, 1969), and defense
against these enemies is the main pathway by which ant visitation affects plant demography. The
Cerambycid beetle *Moneilema appressum* and an unidentified weevil (Coleoptera: Curculionidae)
186 of the genus *Gerstaekeria* feed on vegetative and reproductive structures as adults and their larvae
feed internally. Two species of cactus bugs, *Narnia pallidicornis* and *Chelinidea vittiger* (Hemiptera:
Coreidae), feed on all cholla parts with a preference for flower buds; their damage can induce floral
189 abortion (Miller et al., 2006). A seed predator, *Cahela ponderosella* (Lepidoptera: Pyralidae),
oviposits in open flowers and larvae eat seeds in developing fruits. These consumers can have
significant negative impacts on plant fitness and depress population growth (Miller et al., 2009).
192 Prior experiments showed that ant-tended tree cholla experience less herbivory and seed predation
than plants from which ants were excluded (Miller, 2007; Ohm and Miller, 2014).

Data Collection

195 This study is based on long-term data spanning 2004 to 2023 that documented plant demographic
rates in relation to their ant partner status. From 2004 to 2008, we censused 134 plants distributed
across three spatial blocks. This initial census group was discontinued in 2009, when we established
198 six 30 × 30-meter plots and tagged all tree cholla within those plots. Two additional 30 × 30-meter
plots were added in 2011, and this group of eight plots (mean ± SD tree cholla per plot: 87.30
± 18.28) was censused annually through 2023 (with the exception of 2020 due to the pandemic
201 shutdown). For all plants, in May or early June of each year we recorded plant survival since the
last survey (if the plant had any green flesh it was considered alive) and, for survivors, we recorded
height (cm), maximum crown width (cm), and crown width perpendicular to the maximum (cm).
204 Size measurements were used to calculate plant volume (cm^3) based on the volume of an elliptical
cone. We measured reproduction by counting flowerbuds, and in most years we distinguished
between flowerbuds that were viable versus aborted (aborted flowerbuds are distinguishable by
207 dry and withered true leaves and petals). We recorded the ant species presence and identity (or

vacancy if no ants present). Occurrences of more than one ant species on one plant were rare (less than 5% of observations), and for the purpose of this analysis we classified the plant as being
210 occupied by the more abundant species. We also quantified the abundances and identities of any cactus herbivores observed, and recorded the occurrence of herbivore damage (described further in Appendix B). Each year, the field team searched exhaustively for new recruits by walking slowly in
213 a standard formation across the plot area, and these were added to the census (cactus seedlings are small (< 2cm in height) and cryptic so it is likely that some are missed even with a standardized search protocol). These data allowed us to link each plant's demographic fate (survival, growth,
216 and reproduction) to its state of ant visitation. In total, the data set includes a total of 9,787 observations of 1,141 individuals across 15 complete transition years (spanning May/June of year t to May/June of year $t + 1$) (Miller, 2020). In addition to missing the year 2020, there are
219 gaps in the time series where we switched plots or plants (and thus broke up transition years for growth/survival and partner turnover) or where we did not distinguish between viable and aborted flower buds (and therefore are missing some vital rate information for those years).

222 To complete the tree cholla life cycle, we used additional, smaller data sets from previously published studies to estimate seed and seed bank parameters. First, Ohm et al. 2014 provide data on the number of seeds per fruit for plants tended by *L. apiculatum*, *C. opuntiae*, or no ants
225 (experimental exclusion), accounting for their effects on pollinator visitation. Second, Elderd and Miller 2016 provide data on seed entry to the seed bank and seedling germination and survival rates based on surveys of fruit predation and seed addition experiments. Because these seed and
228 seed bank data were not collected as part of our long-term study, these demographic parameters are based on data from a single year and are assumed to be temporally constant.

Multi-state Integral Projection Model

231 Model overview

The demographic data were used to parameterize a multi-state Integral Projection Model (IPM). IPMs describe population dynamics in discrete time with functions that relate vital rates to continuous state variables, typically size (Ellner et al., 2016). While IPMs are a natural choice for populations with continuous size structure, they can also be modified to accommodate a combination of continuous and discrete state variables, as we do here. We constructed a stochastic, multi-state IPM that stitches together population structure associated with plant size and ant state, allowing us to determine the individual fitness effects of each ant species in isolation and the composite effects of multiple partners, with ant transition dynamics and inter-annual variability modeled explicitly.

Given the low frequency of ant occupancy states other than *L. apiculatum* and *C. opuntiae* (<8% of observations) we combined all other ants into an “Other” category, such that our multi-state IPM included four possible ant states: vacant, *L. apiculatum*, *C. opuntiae*, and Other. The “Other” category was made up of *Forelius pruinosus* (3.5% of observations), unidentified species belonging to the genera *Camponotus* (0.9%), *Aphaenogaster* (0.4%), *Myrmecocystus* (0.08%), *Tetramorium* (0.02%), *Brachymyrmex* (0.02%), and additional ants not identified to genus or species (2.8%). Given our objective of quantifying demographic effects across the plant size distribution, there was not sufficient size representation of these low-frequency partner species in the long-term data to treat these as separate partner states.

Ant state is included as a predictor variable in IPM sub-models where there are biologically realistic pathways through which ants could have an impact. For example, prior experimental work indicates that ant tending can reduce vegetative tissue loss and floral abortion, while also potentially reducing pollinator visitation (Miller et al., 2009; Ohm and Miller, 2014). Therefore, ant state was included in sub-models for survival, growth, flowerbud viability, and seed number per flowerbud. In contrast, we have no reason to expect that ant tending can directly influence

the probability of flowering or flowerbud production independently of its influence on plant size, so these sub-models do not include ant state as a predictor variable.

The biological “story” of the model is as follows: The tree cholla population is comprised of plants characterized by their size, which can take any continuous value between lower and upper limits, and ant visitation status, which can take one of the four discrete levels described above. An individual’s size and ant status jointly predict its demographic fate such as survival, growth, and reproductive contributions to the seed bank. Plants may be lost to mortality and new individuals may be gained through recruitment from the seed bank. Size and ant status are updated annually.

The environment fluctuates from year to year such that demographic rates exhibit stochastic deviations from their long-term average. Plants in different ant states can fluctuate independently; for example, a year that is better than average for *L. apiculatum*-tended plants may be worse than average for *C. opuntiae*, and vice versa, which could occur if there are year-to-year fluctuations in the natural enemies against which each defender species is most effective. Our long-term demographic data define the “rules” of tree cholla demography and ant partner transition. In the analyses that follow, we tinker with those rules to create novel scenarios of partner diversity and inter-annual synchrony.

Mathematical formulation

Mathematically, we modeled the tree cholla life cycle using continuously size-structured plants where number of plants of size x and ant state a in year t ($n(x, a)_t$) predicts the number of plants of size x' and ant state a' in year $t + 1$ ($n(x', a')_{t+1}$) based on a size- and ant-specific vital rates.

The model also includes two discrete seed banks (B_t^1 and B_t^2) corresponding to 1 and 2-year old seeds. Seed bank dynamics are given by:

$$B_{t+1}^1 = \delta \sum_{a=1}^A \int_L^U \kappa(a) P(x; \boldsymbol{\tau}^P) F(x; \boldsymbol{\tau}^F) V(a; \boldsymbol{\tau}_a^V) n(x, a)_t dx \quad (1)$$

$$B_{t+1}^2 = (1 - \gamma_1) B_t^1 \quad (2)$$

Functions $P(x; \boldsymbol{\tau}^P)$ and $F(x; \boldsymbol{\tau}^F)$ give the probability of flowering in year t and the number of flowerbuds produced in year t , respectively, by plants of size x in year t . The proportion of flowerbuds that remain viable through fruit set ($V(a; \boldsymbol{\tau}_a^V)$) and the number of seeds per fruit ($\kappa(a)$) are dependent on ant state a . The vectors $\boldsymbol{\tau}$ give year-specific deviates (mean zero) and appear in functions for which we can estimate temporal stochasticity from the long-term data; superscripts indicate the corresponding vital rate and, when present, the a subscript indicates that deviates are specific to plants in ant state a . For example, temporal deviates $\boldsymbol{\tau}_a^V$ describe better- and worse-than-average years for flowerbud viability, and plants in different ant states can fluctuate independently.

Seed production is integrated over the size distribution, from the lower L to upper U size limits, and summed over all possible ant states ($A = 4$) giving total seed production. Seeds are multiplied by the probability of escaping post-dispersal seed predation (δ) to give the number of seeds that enter the one-year old seed bank. Plants can recruit out of the year-one seed bank with probability γ_1 or transition to the two-year seed bank with a probability of $1 - \gamma_1$. **Seeds in the two-year seed bank are assumed to either germinate with probability γ_2 or die.**

For the above-ground part of the life cycle, the number of plants of size x' and ant state a' in year $t + 1$ is given by survival/growth transitions from size x and ant state a in year t , plus germination out of the seed banks:

$$n(x', a')_{t+1} = (\gamma_1 B_t^1 + \gamma_2 B_t^2) \eta(x') \omega \rho_0(a') + \sum_{a=1}^A \int_L^U S(x, a; \boldsymbol{\tau}_a^S) G(x', x, a; \boldsymbol{\tau}_a^G) \rho(x, a, a') n(x, a)_t dx \quad (3)$$

The first term in Eq. (3) estimates the number of individuals recruiting from a one- or two-year seed bank to a plant of size x' and ant state a' based on the recruit size distribution $\eta(x')$ and the probability of over-winter seedling survival (ω) from germination (late summer) to the census (May). This term is multiplied by $\rho_0(a')$, which gives the probability that a new recruit has ant state a' upon its first appearance in the census ($\sum \rho_0(a') = 1$). The second term represents all possible transitions from size x and ant a to size x' and ant a' , conditioned on survival. Survival ($S(x, a; \boldsymbol{\tau}_a^S)$) and growth from size x to x' ($G(x', x, a; \boldsymbol{\tau}_a^G)$) are both dependent on initial size and

ant state. As above, these functions include inter-annual variability through year-specific deviates
 306 that can vary by ant state (τ_a). Finally, ant transition function $\rho(a', a, x)$ gives the probability that
 an individual transitions from ant state a to a' in the next census, conditional on initial size x .

Statistical modeling and parameter estimation

309 We parameterized the IPM using a series of generalized linear mixed models in a hierarchical
 Bayesian framework. Vital rate models included spatial and temporal random effects associated
 with plot and year variation, respectively (only year variation is used in the IPM), and included
 312 plant size (the natural logarithm of volume, $\log(cm^3)$; x, x'), ant partner state (a, a'), or both as
 fixed-effect predictor variables. As in the IPM, our statistical modeling assumed that demographic
 effects of ant occupancy are limited to survival, growth, and flowerbud viability.

315 *Growth.* Following best practices for modeling size transitions (Miller and Ellner, 2025), we fit
 the growth sub-model ($G(x', x, a; \tau_a^G)$) to data on size in year $t + 1$ (y^G) using the skewed normal
 distribution to account for left-skewed size transitions (at some initial sizes, transitions below the
 318 expected future size were more common than transitions above it). The skew-normal has three
 parameters corresponding to location (\hat{G}), shape (σ), and scale (α):

$$y_i^G \sim SkewedNormal(\hat{G}_i, \sigma_i, \alpha_i) \quad (4)$$

$$\hat{G}_i = \beta_{a[i]}^0 + \beta_{a[i]}^1 x_i + \beta_{a[i]}^2 x_i^2 + u_{year[i], a[i]} + w_{plot[i]} \quad (5)$$

$$\log(\sigma_i) = \beta^3 + \beta^4 x_i \quad (6)$$

$$\alpha_i = \beta^5 + \beta^6 x_i \quad (7)$$

324 Here, the location parameter for the i th observation \hat{G}_i is defined as a second-order polynomial
 with ant-size interactions because preliminary analysis found this was an improvement over a linear
 relationship. The location parameter of the skew-normal is not the mean, but the mean can be
 327 derived as $\hat{G} + \frac{\sigma\alpha}{\sqrt{1+\alpha^2}}\sqrt{\frac{2}{\pi}}$. The year- and ant-specific random effect u (which parameterizes the τ_a^G
 vectors) and plot-specific random effect w are normally distributed with variances σ_{year}^2 and σ_{plot}^2 ,

respectively. Parameters σ_i and α_i control residual variance and skewness, respectively, and were
330 defined as linear functions of initial size x_i (σ_i is strictly positive and was modeled with a log link
function). Due to data constraints, we modeled growth variance and skewness as size-dependent
but not dependent on ant occupancy state.

333 *Survival.* The survival sub-model ($S(a, x; \tau_a^S)$) estimates the probability of survival from year t to
year $t + 1$, with fixed effects of size x and ant partner a in year t . We fit this model to the survival
data (alive or dead) using a Bernoulli distribution with a similar linear predictor for the probability
336 of survival as in the growth model but with a logit link function and without the second-order
influence of size.

339 *Reproduction.* The flowering sub-model ($P(x; \tau^P)$) estimates the probability of reproducing in
year t , with fixed effects of size x in year t and random effects of plot and year. We fit this model
to the reproductive status data (vegetative or flowering) using a Bernoulli distribution and a logit
link function, similar to the survival model above but with no ant effects. The flowerbud function
342 $F(x; \tau^F)$ estimates the total flowers produced by a reproducing plant in year t , with fixed effects of
size x in year t . We fit this model to flowerbud count data (sum of viable and aborted buds) using
a zero-truncated negative binomial distribution with a log link and normally distributed year and
345 plot random effects.

348 The flowerbud viability sub-model ($V(a; \tau_a^V)$) estimates the probability that flowerbuds pro-
duced in year t remain viable, with fixed effects of ant partner a in year t . We fit this model to
floral viability data using a binomial distribution where trials and successes are given by the total
number of flowerbuds and the number that are viable, respectively. This model used a logit link
function and included random effects for plot and year following the same structure as the survival
351 model, with ant-specific year random effects.

Estimates for the number of seeds per fruit were obtained from a field experiment which excluded
ants from flowering plants (Ohm and Miller, 2014). This experiment only included *L. apiculatum*
354 and *C. opuntiae*-tended plants. We therefore assumed that plants tended by Other ants had

the same number of seeds per fruit as vacant plants (our results showed very little sensitivity to this assumption). Additional reproductive parameters for probability of entry to the seed bank,
357 germination rates, and recruit size were estimated following methods described in Appendix A. [All](#)
[of these parameters are assumed to be temporally constant.](#)

Ant Transitions. The ant transition model ($\rho(x, a, a'; \boldsymbol{\tau}^e)$) estimates the probability of a cactus
360 being occupied by ant partner a' in year $t + 1$ given that it was occupied by initial ant partner a in
year t , with fixed effect of initial size x . We fit this model to ant partner data using a multinomial
distribution with a logit link function.

363 *Model-fitting.* We fit all models using Stan run through version 4.0.2 of R (R Core Team, 2023;
Stan Development Team, 2023). We used vague priors for all parameters. For each model, we
obtained three chains of 10,000 iterations, discarding the first 1,500 iterations. We visually assessed
366 parameter convergence between and within chains and assessed overall model fit with posterior
predictive checks to examine how well the fitted model can generate simulated data similar to the
real data.

369 *IPM Analysis*

Analyzing the IPM required that we discretize the continuous IPM kernel into an approximating
matrix. Size variable x was discretized into b bins, resulting in a $b \times b$ matrix. In our model there is
372 additional complexity in the form of transitions between A ant states and two additional discrete
states (year one and year two seed banks), leading to a matrix size of $A(b + 2) \times A(b + 2)$. We
used $b = 500$ bins, which we found to be sufficient for numerically stable outputs, and extended the
375 integration limits beyond the minimum (L) and maximum (U) observed sizes to avoid unintentional
eviction using the “floor-and-ceiling” method (Williams et al., 2012).

For stochastic analyses, we estimated the approximating matrix corresponding to each t to $t + 1$
transition year. To estimate population mean fitness in a stochastic environment (λ_S) we simulated

population dynamics for 500 years by randomly sampling among the 15 annual transition matrices, discarding the first 100 years of the simulation to minimize the influence of initial conditions. Sampling observed transition matrices (rather than independently sampling regression coefficients) produces demographic time series that realistically capture inter-annual variation by preserving correlations between vital rates (Metcalf et al., 2015). We tallied the total population size at each time step as $N_t = B_t^1 + B_t^2 + \sum_{a=1}^A \int n(x, a)_t dx$ and calculated the stochastic growth rate as

$$\log(\lambda_S) = E[\log(\frac{N_{t+1}}{N_t})]$$

(Rees and Ellner, 2009). We propagated uncertainty from the vital rate models using 100 draws
 378 from the joint posterior distribution of model parameters, resulting in a posterior distribution of λ_S and the derived quantities described next.

Partner diversity scenarios

381 Using the fully parameterized multi-state IPM, we conducted simulations to quantify how diversity
 and identity of ant partners influenced plant fitness. From the full version of the model (described
 above) corresponding to the observed assemblage of partners and observed frequencies of part-
 384 ner transition, we created [alternative model versions](#) corresponding to all eight “counter-factual”
 scenarios of diversity and composition: no ant partners (complete vacancy); one ant partner (*C.*
opuntiae only, *L. apiculatum* only, Other only); two partners (all pairwise combinations of *C. op-*
 387 *untiae*, *L. apiculatum*, and Other); and three partners (observed scenario of all ant states). These
 simulations were made possible by extrapolating ant-specific demographic performance across the
 size distribution, even for combinations of size and ant occupancy that were rarely observed. For
 390 example, the no-partner scenario modeled a hypothetically ant-free cactus population, even though
 no such population exists to our knowledge, by applying the statistical knowledge gleaned from
 vacant plants across the size distribution. We refer to stochastic fitness associated with partner
 393 number or identity using superscripts, e.g. λ_S^0 for vacant plants (zero partners), λ_S^{1+} for any state
 of ant tending, λ_S^C for tending by only *C. opuntiae*, λ_S^{CO} for tending by *C. opuntiae* and Other

ants, etc.

396 In all scenarios that included any ant partners, we preserved the observed pattern of size-dependent vacancy/occupancy (estimated through the ant transition sub-model) and manipulated partner identity conditional on occupancy by any ant. This means, for example, that the *C.* 399 *opuntiae*-only scenario included two possible states, vacancy and occupied by *C. opuntiae*. Our statistical models allow us to extrapolate the demographic performance of ant-tended plants to small sizes that are typically vacant, but the natural history of this system tells us that this is 402 not biologically sensible. Small, non-reproductive plants are typically vacant because they do not produce extrafloral nectar, and once plants begin producing nectar they are nearly always ant-tended (Miller, 2014). Our simulations preserved this basic biology, avoiding tiny ant-occupied 405 plants that do not and could not occur in nature. While technically such plants must exist in the model (because all ant states share the same L and U size limits), they are so infrequent, as guided by our data, as to have virtually no bearing on the results.

408 The partner diversity scenarios required additional assumptions about the mechanisms that give rise to observed occupancy patterns. Based on evidence that EFN-bearing cacti are nearly 411 always ant-occupied (Miller, 2014), we assume that ant partners competitively exclude one another from EFN-bearing cacti and that competition for plant partners is zero-sum. This means that, in scenarios that remove species from the partner community, remaining species gain access to 414 plants that the removed species would have tended. In Appendix C, we present results under an alternative assumption, that ant visitation is limited by factors other than availability of cactus EFN (e.g., nesting sites or off-plant dietary resources), such that when a species is removed from the partner community, the plants it would have occupied remain vacant.

417 *Partner synchrony scenarios*

Under the portfolio effect hypothesis, partner diversity may confer a fitness advantage when the 420 benefits of alternative partners are not perfectly synchronized across temporal environmental variation, yielding an advantage of a diverse “portfolio” of partners when the environment fluctuates.

We constructed two versions of the stochastic, multi-state IPMs that allowed us to test this hypothesis. The baseline, “non-synchronous” model described above included ants effects that could
 423 vary uniquely across time, according to the parameter estimates for the random effects (τ_a). We quantified from the fitted random effects how tightly inter-annual variation was correlated between ant states for each vital rate. The “synchronous” version included temporal fluctuations that were
 426 forced to be the same across ant states. To synchronize ant states, we averaged the ant-specific year random effects, thus ensuring that plants in all ant states fluctuated synchronously in response to temporal environmental variation. This second, synchronous version of the model effectively
 429 turns off any portfolio effect, holding all else equal. Both scenarios of temporal stochasticity, non-synchronized and synchronized, were run for all eight ant partner scenarios described above. In
 Appendix D, we explore additional scenarios that vary the correlation of ant-specific fluctuations,
 432 to determine if contributions from the portfolio effect are constrained by high correlations seen in the long-term data (Results).

Statistical inference on fitness consequences of partner identity and diversity

The range of models we created could generate many outputs; we focus our inference on the following specific contrasts. First, to determine whether ant occupancy and partner diversity are beneficial, we calculated a posterior distribution of λ_S for each of four partner richness levels (λ_S^0 , λ_S^1 , λ_S^2 ,
 438 λ_S^3), averaging over composition scenarios within each level. If cactus fitness increases with partner richness, this would be interpreted as evidence for benefits of partner diversity. Second, to determine whether each partner, in isolation, confers a fitness advantage and to rank alternative partners, we
 441 contrasted the fitness of each single partner scenario (λ_S^C , λ_S^L , λ_S^O) against vacancy (λ_S^0). Third, to determine whether any benefits of diversity are due to the sampling effect or complementarity, we contrasted the fitness of multi-partner scenarios against the single best partner scenario. If
 444 the best multi-partner scenario exceeds the fitness associated with the best single partner, this would be interpreted as evidence of complementarity. Alternatively, the sampling effect hypothesis predicts that no multi-partner scenario yields higher plant fitness than the best single partner. It

447 is also possible that multi-partner scenarios yield lower fitness than the single best partner, which
would be consistent with an opportunity cost of diversity. Fourth, to quantify any contribution of
the portfolio effect, we contrasted λ_S of the full (four-state) scenario to vacancy for synchronized
450 and non-synchronized responses to temporal stochasticity. If the portfolio effect confers a benefit
of diversity, the fitness advantage of having all vs. no partners should be greater when temporal
fluctuations are not synchronized across ant states.

453 We base our statistical inferences on the posterior probability distributions of the contrasts
described above. For example, the contrast of *C. opuntiae* (λ_S^C) with vacancy (λ_S^0) yields a posterior
distribution of the difference $\Delta\lambda_S^{C-0}$. We can quantify from this distribution our certainty in the
456 mutualistic effect of *C. opuntiae*, given the data, as $Pr(\Delta\lambda_S^{C-0} > 0)$. We apply similar logic
to other contrasts described above. We interpret contrast probabilities ≥ 0.95 as statistically
significant differences.

459 Results

*What are the effects of association with alternative partners on vital rates
and how do these effects fluctuate across years?*

462 Over the 20-year data set, we found that ant partners influenced cactus demographic performance,
and different ant partners had contrasting effects across host vital rates. Ant-tended plants had a
growth advantage over vacant plants, especially at smaller sizes (Figure 1). For the smallest sizes
465 that were likely to be ant-tended (minimum observed size of ant-tended plants was $0.8 \log(cm^3)$;
solid lines in Figure 1) there were modest differences between partner species, with the greatest
growth advantage associated with *C. opuntiae* followed by *L. apiculatum* and then Other ants. At
468 the largest sizes, growth trajectories of ant-tended and vacant plants were nearly indistinguishable.
For all ant states, growth was left-skewed, with transitions to sizes below the mean being more
frequent than sizes above the mean.

⁴⁷¹ Similarly, for plants that were large enough to have ant visitors, visitation enhanced cactus survival (Figure 2). Mean survival rates ranged from 7.7% to 99.9%, with the smallest plants being the most vulnerable to mortality. *C. opuntiae*-occupied plants had a survival advantage over ⁴⁷⁴ other ant-tended plants, particularly at smaller sizes, consistent with positive effects on growth. At larger sizes, plants in any state of ant occupancy had a survival advantage over vacant plants. Plants smaller than $-2 \log(cm^3)$ were predicted to experience negative effects of ant visitation, but ⁴⁷⁷ this was based entirely on extrapolated survival estimates of ant-tended plants. Plants in this size range were never observed to be ant-tended and, because the IPM preserves the size-dependence of vacancy, benefits of vacancy at small sizes are never realized in the IPM.

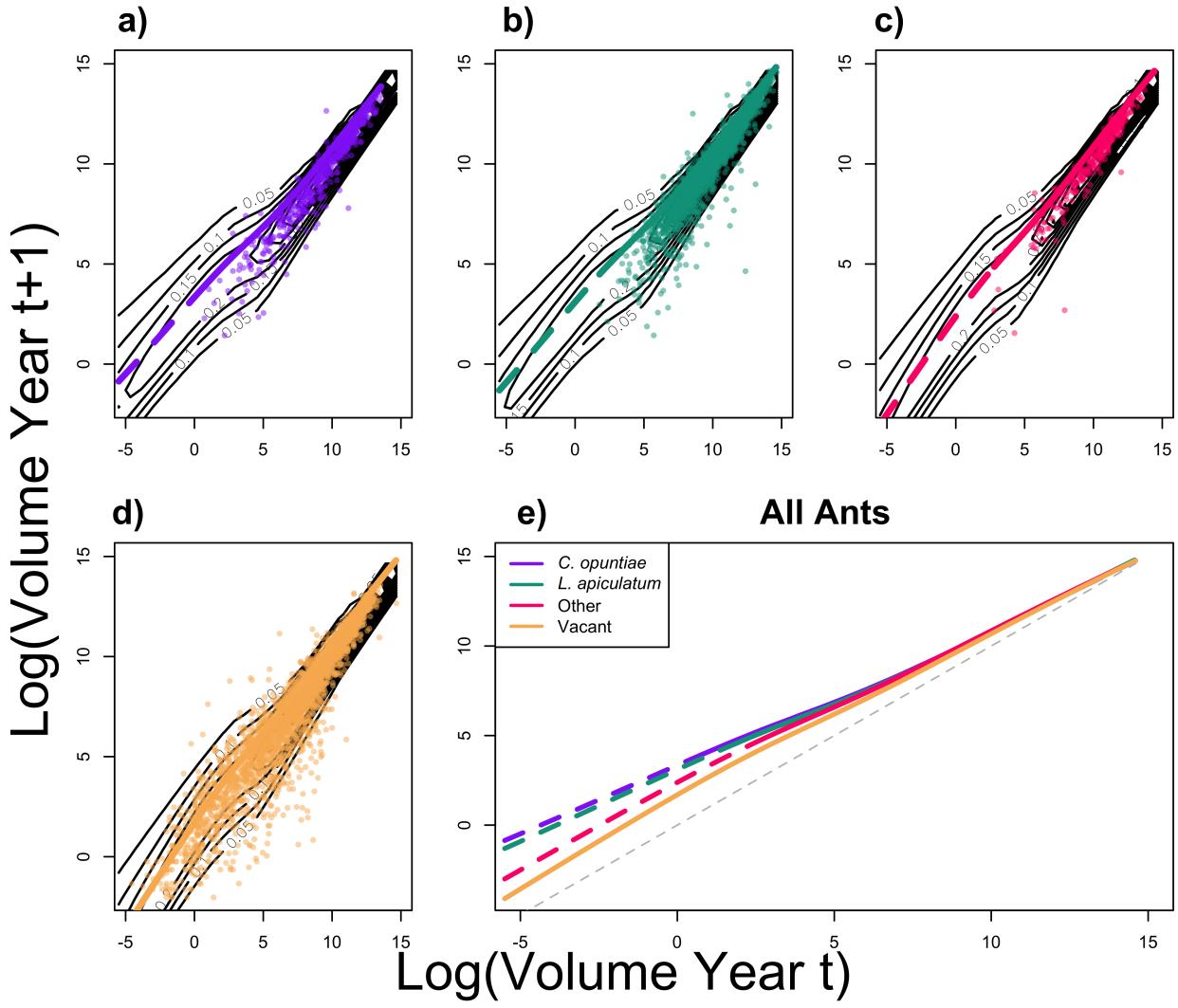


Figure 1: The next predicted size of tree cholla based on previous size in relation to ant partner (a, *C. opuntiae*; b, *L. apiculatum*; c, *Other*; d, *Vacant*, e, all states combined). Points show observed size transitions, colored lines are the mean next size, and contours show probability density of the skewed normal growth model. Dashed lines indicate extrapolation while solid lines indicate the range of observed data. The gray dashed line indicates no change in size (areas above this line show growth while areas under it show shrinkage).

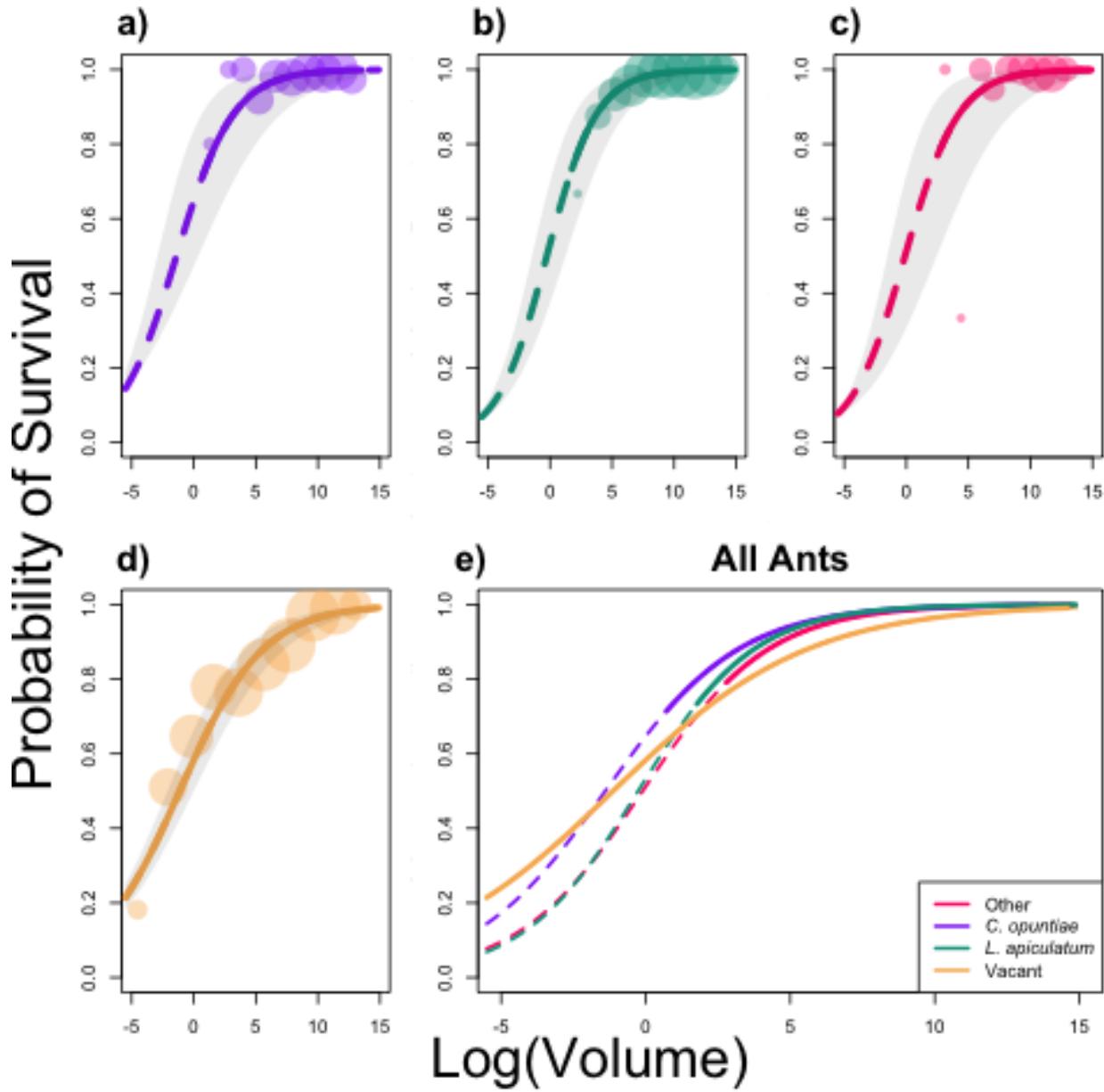


Figure 2: Probability of survival in relation to size and ant partner. Layout as in Fig. 1, except here points show data as binned means, where point size is proportional to number of observations. Grey areas show the 90% confidence interval for the mean.

Ant visitation was associated with increased floral viability and ant identity determined the

483 strength of viability benefits. Mean viability rates spanned 55–81% (Figure 3). *L. apiculatum*-
 tended plants had the highest mean viability rate (86.1% [95% credible interval: 77.6–92.4%]),
 while there were similar viability rates for vacant (60.0% [44.3–75.0%]), Other-tended (60.6% [43.7–
 486 75.5%]), and *C. opuntiae*-tended plants (57.1% [40.6–72%]). Furthermore, *C. opuntiae*-tended
 plants had lower mean seeds per fruit (115.0[79.5–165.5] seeds) than vacant (147.2[114.1–189.9]
 seeds) or *L. apiculatum*-tended plants (142.4[100.7–200.2] seeds), likely due to pollinator interfer-
 ence (Ohm and Miller, 2014).

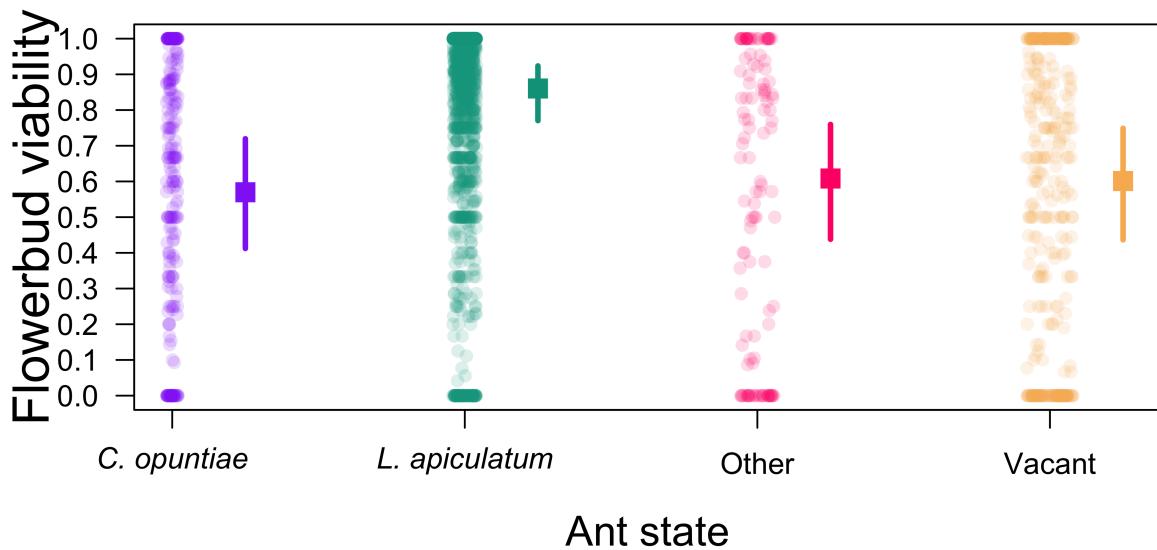


Figure 3: Flowerbud viability in relation to ant partner status. Points show observed data (fraction of initiated buds that were viable). Points and bars show the means and 95% credible intervals for each group.

Inter-annual fluctuations in demographic rates (estimated as statistical random effects) were
 generally positively correlated between ant states, limiting the potential for benefits of diversity
 492 through the portfolio effect (Figure 4). However, the degree of correlation depended on the vital
 rate and pair of ant partners. Across vital rates, random effects for cactus growth were the most

strongly correlated between ant states (mean pairwise correlation: 0.63) and random effects for
495 survival were the least correlated (mean pairwise correlation: 0.36). Certain partner pair / vital
rate combinations were effectively independent in their yearly fluctuations (such as survival of *C.*
opuntiae and Other-tended plants; mean pairwise correlation: -0.0016) while others were almost
498 completely synchronous (such as growth of Other and *L. apiculatum*-tended plants; mean pairwise
correlation: 0.93. The ant partner with the lowest overall variance between years across all vital
rates was the category other. This is likely due to different ant species which are grouped together
501 responding contrastingly to interannual fluctuations, resulting in a lower fluctuation variance. On
the other hand, the vacant cacti experienced the highest variance of performance across vital rates
and interannual fluctuations, indicating that ant presence may be an important buffer.

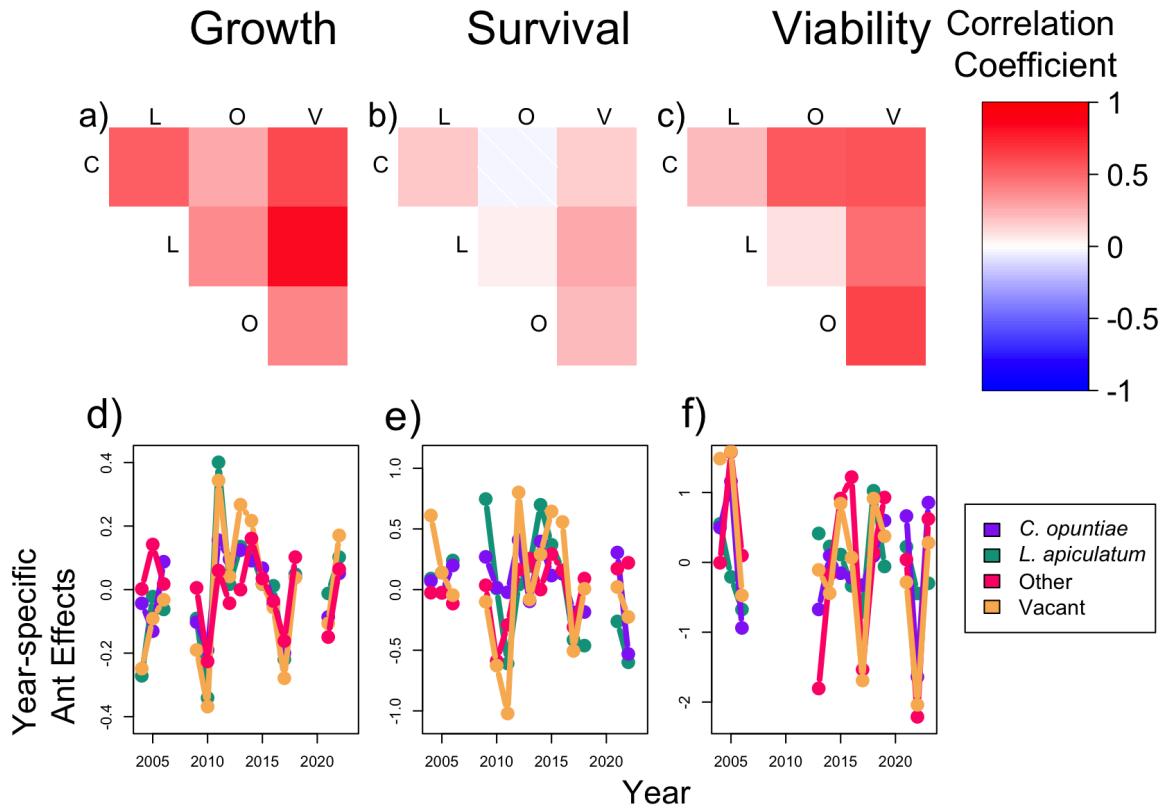


Figure 4: The correlation coefficients (Pearson’s r) of ant-specific year-random effects from statistical models for growth (a), survival (b), and floral viability (c). Dark red indicates negative correlation, dark blue indicates positive correlation, white indicates no correlation. The year-specific random effects for each ant state from the statistical models are shown in a time series for growth (d), survival (e), and floral viability (f). All random effects have mean zero such that positive values indicate better-than-average years and negative values indicate worse-than-average years. Values are missing where there were no data available for that year.

*What are the frequency and direction of partner turnover across the plant
life cycle?*

507 A majority (55%) of plants surveyed in the long-term data experienced at least one ant state
transition, with distinct size-dependent and directional patterns (Figure 5). Vacancy was the most
likely ant state of small plants ($\leq 10\log(cm^3)$). Even when small plants were ant-tended at the
510 start of the transition year, they were most likely to transition back to vacancy (Figure 5 b-d).
The probability of becoming ant-tended increased with size, though it was not equally likely to be
tended by any partner. For large plants that were initially vacant or tended by *L. apiculatum* or
513 Other ants, *L. apiculatum* was the most likely next partner, suggesting that this species is able to
colonize plants that were previously vacant or occupied by Other ants, and effectively retain plants
that it previously occupied. *C. opuntiae* were also able to retain plants they previously occupied,
516 but not as well as *L. apiculatum*: for plants that began the transition year with *C. opuntiae*, the
probability that those plants remain occupied by *C. opuntiae* at the end of the transition year
was only slightly greater than the probability of take-over by *L. apiculatum*, while take-over in the
519 other direction was extremely rare. It is also notable that transitions away from the initial state
of *L. apiculatum* were almost always transitions to vacancy (Figure 5 d), while transitions away
from the initial states of *C. opuntiae* and Other were often transitions to other ants. This suggests
522 a competitive hierarchy whereby *L. apiculatum* may abandon low-value plants with little nectar
production but is almost never displaced from high-value plants.

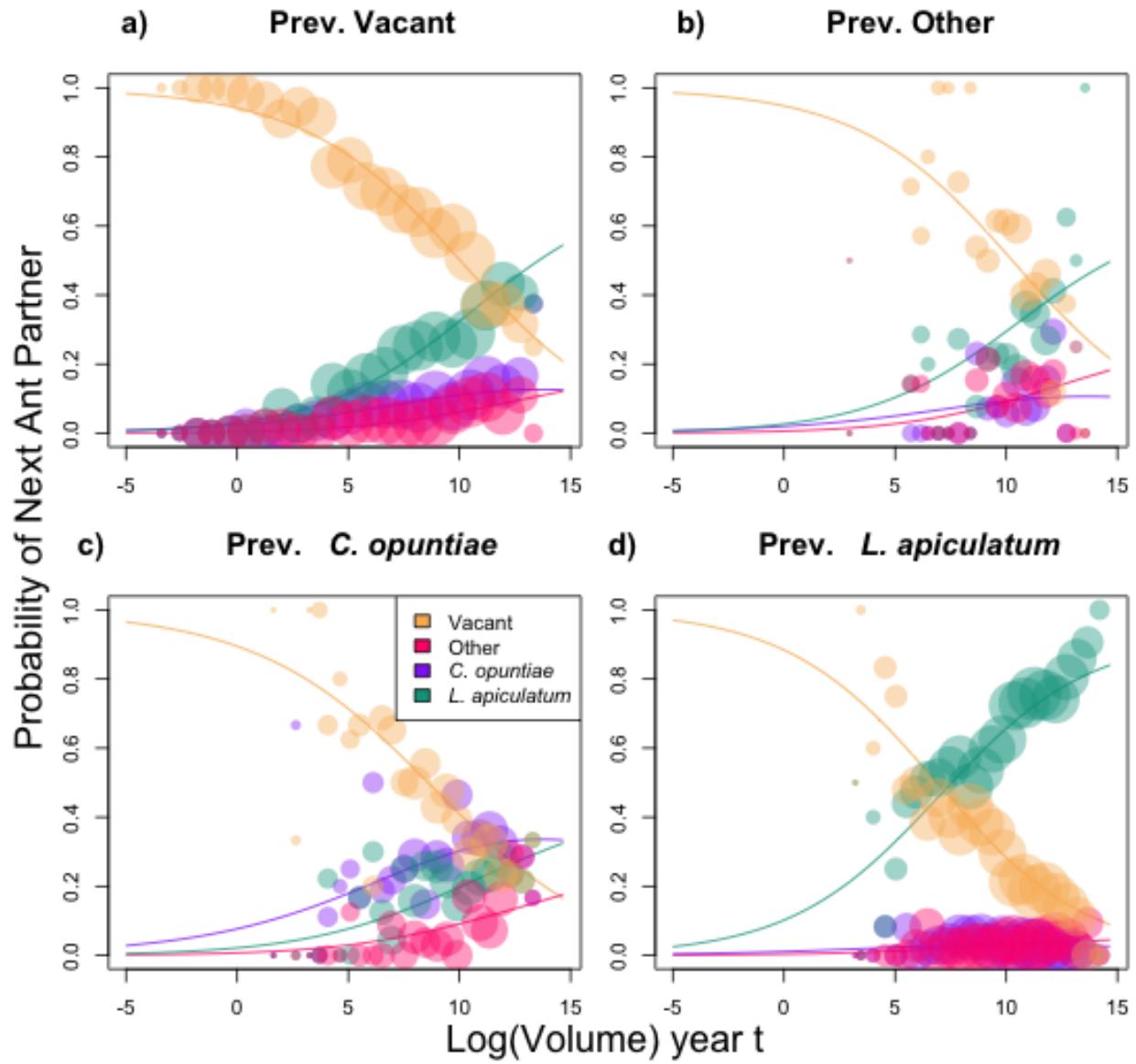


Figure 5: The probability of ant state based on plant size and previous ant state. Each panel shows size-dependent probabilities of the next ant state conditional on the previous ant state and size. Solid lines represent predictions of the multinomial statistical model and points show data binned over size intervals, where point size is proportional to the number of observations.

525 What is the net effect of partner diversity on plant fitness, and what
mechanism(s) explain(s) this effect?

By integrating vital rates, temporal fluctuations, and ant transition dynamics into the stochastic,
528 multi-state IPM we can evaluate the fitness implications of different scenarios of partner identity
and diversity (Figure 6). First, there was strong evidence that ant visitation had mutualistic fitness
effects on plant partners. The lowest mean stochastic fitness was λ_S^0 , the fitness of the cholla with no
531 partners (Figure 6 a). Across all 1+ partner scenarios, we found that the λ_S^{1+} posterior distributions
were greater than λ_S^0 with nearly 100% certainty. This indicates that ant visitation elevates fitness
no matter the number of partners. Furthermore, we found no benefit of partner diversity, with the
534 fitness associated with one, two, or three partners roughly equivalent (Figure 6 a). The one- and
two-partner scenarios were statistically consistent but there was a modest reduction in fitness in the
three-partner scenario compared to two partners ($Pr(\Delta\lambda_S^{2-3} > 0) = 0.95$), consistent with a weak
537 cost of diversity at the highest level. Patterns of λ_S in relation to partner richness were generally
consistent between scenarios of non-synchronous and synchronous inter-annual fluctuations.

Partner identity and composition were not strongly consequential for plant fitness (Figure 6 b-c).
540 Among the one-partner scenarios, there was no strong evidence for any single best partner species
(Figure 6 b). While a hypothetical *L. apiculatum*-only population had the highest mean fitness, it
was not significantly higher than *C. opuntiae-* ($Pr(\Delta\lambda_S^{L-C} > 0) = 0.7$) or Other- ($Pr(\Delta\lambda_S^{L-O} >$
543 0) = 0.69) only populations. Furthermore, the fitness of *L. apiculatum*-only was consistent with
all 2-partner scenarios ($Pr(\Delta\lambda_S > 0)$ ranged from 0.37 to 0.83). However, as above, there was
evidence for an opportunity cost of diversity wherein fitness of the three-partner scenario was lower
546 than any of the 2-partner scenarios ($Pr(\Delta\lambda_S > 0) > 0.95$). The lack of diversity benefits are not
driven by the high overall frequency of *L. apiculatum*.

Using simulations in which all ant species were artificially forced to be equally frequent (further
549 explained in Appendix C), we found the same fitness patterns as described above. We found no
evidence that the portfolio effect generated positive effects of partner diversity, as effects of partner

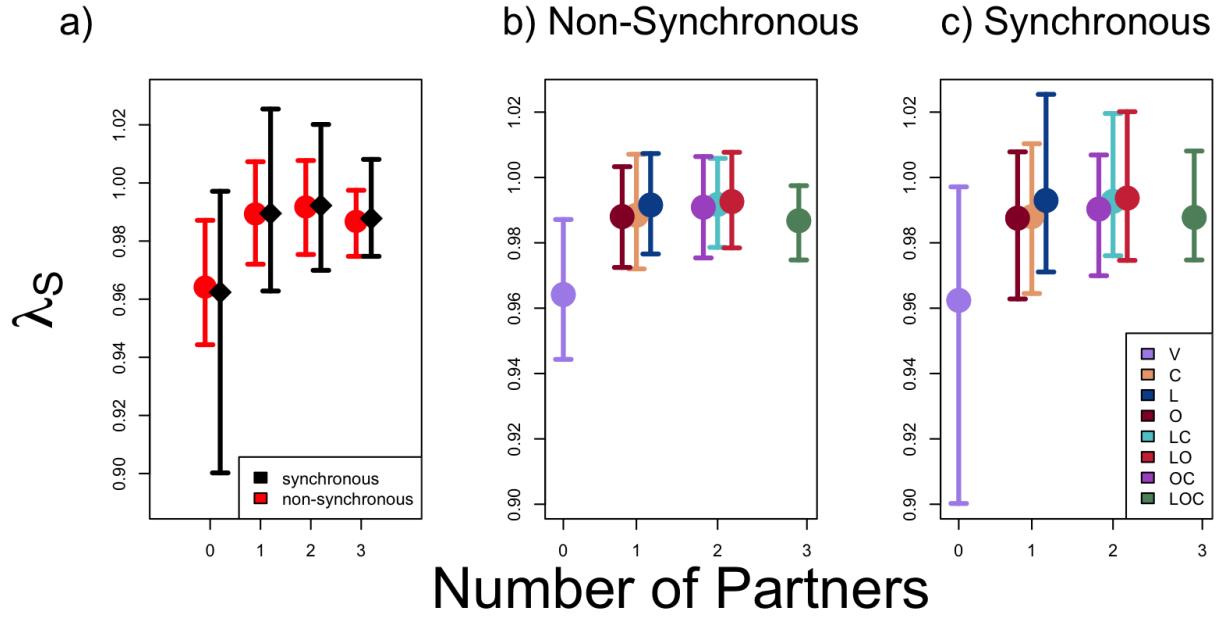


Figure 6: Tree cholla stochastic growth rate (λ_S) corresponding to number (a) or number and identity (b-c) of ant partners for synchronous and non-synchronous inter-annual fluctuations. Points and intervals show mean and 95% credible intervals from the posterior distributions of λ_S . In b-c letters in the legend correspond to ant partners as follows (V = Vacant, C = *C. opuntiae*, L = *L. apiculatum*, O = other).

richness and composition were highly consistent between the baseline, non-synchronous model and
552 the model version that synchronized all ant states (Figure 6). The effect of all ant partners can be measured as $\lambda_S^3 - \lambda_S^0$. We have high confidence that this contrast is positive and equally so for the synchronous and non-synchronous scenarios (Figure SD7), indicating that the full suite of partners
555 is similarly beneficial with and without the portfolio effect operating. In Appendix D, we show that contributions from the portfolio effect were constrained by positively correlated fluctuations between ant states (Figure 4). In scenarios in which partner fluctuations were uncorrelated or, especially,
558 negatively correlated, the non-synchronous model exhibited a stronger benefit of diversity than the synchronous model (Figure D8), as expected under the portfolio effect hypothesis.

This absence of a sampling effect in our primary model likely stems from the strong competitive
561 dominance and high frequency of *L. apiculatum*, which restricts the realized niche of alternative partners. To explore the conditions under which a sampling effect might emerge, we conducted an alternative simulation in which ant species did not competitively exclude one another (Appendix
564 D). In this counterfactual scenario, we found that a sampling effect can indeed emerge when partner transitions are governed by random colonization rather than competitive hierarchies. This suggests that the benefits of partner diversity are highly sensitive to the rules of partner turnover; in systems
567 like the cholla-ant mutualism, where a single high-quality partner is numerically and competitively dominant, the potential for diversity to increase fitness via sampling is ecologically constrained.

Discussion

570 Mutualisms commonly involve multiple partners but the ecological consequences of partner diversity remain poorly understood. Here we show that while alternative partners may be ecologically different, their fitness effects on a shared mutualist can be effectively interchangeable and redundant.
573 The results of our hierarchical models revealed that different ant partners exhibit different effects on vital rates, with *C. opuntiae*-tended plants experiencing advantages in growth and survival when small, and *L. apiculatum*-tended plants experiencing floral viability advantages. These results,

576 alone, would suggest some potential benefits of partner diversity through complementarity. Yet,
our stochastic, multi-state IPM revealed that all scenarios which included any partners resulted in
similarly high cactus fitness, with a statistically significant but quantitatively weak cost of diversity
579 at the highest level. Furthermore, evidence from nearly two decades of demographic data failed to
support the “portfolio effect” hypothesis, whereby benefits of diversity manifest in the context of
environmental fluctuations, **because fluctuations in the demographic effects of alternative partners**
582 **are too synchronized**. Overall, our results indicate that while ant visitation is strongly consequential
for plant demography, partner composition is much less so, suggesting that the fitness benefits of
mutualism from the plant’s perspective may be surprisingly resilient to changes in partner diversity.

585 We attribute weak effects of partner identity and diversity to the vital rate sensitivity struc-
ture of this population. Like other long-lived, iteroparous species (Franco and Silvertown, 2004),
tree cholla fitness is most sensitive to the growth and survival of established individuals (Elderd
588 and Miller, 2016; Miller et al., 2009), which are virtually guaranteed many years of reproductive
opportunities once they reach a size that is protected from mortality. Differences between alterna-
tive partners were most pronounced either in reproductive rates (floral viability and seed number),
591 which contribute relatively weakly to fitness because a long reproductive lifespan overrides individ-
ual reproductive bouts, or in growth and survival at small sizes, where mortality risk is relatively
high. At intermediate sizes, ant-tended plants had consistent growth and survival advantages over
594 vacant plants regardless of partner identity, and that result dominates our integrative measures of
fitness; differences between partner species in other vital rates and at other sizes do not register
nearly as strongly. The weak reduction in fitness at the highest partner diversity level is harder to
597 explain, but suggests some form of **opportunity cost** in a more crowded partner environment.

Our results broaden the literature on multi-species mutualisms, a majority of which has demon-
strated positive effects of partner diversity through complementarity (Afkhami et al., 2021; Afkhami
600 and Stinchcombe, 2016; Fehling, 2022; Gustafson and Casper, 2005; Hernandez et al., 2019; Larimer
et al., 2014; McKeon et al., 2012; Palmer et al., 2010; Stachowicz and Whitlatch, 2005; Yang et al.,
2024) or portfolio effect (Lázaro et al., 2022; Rogers et al., 2014; Stevens et al., 2024; Thibaut

603 et al., 2012). For example, working in another sequentially-partnered ant protective mutualism of
long-lived plants (*Acacia drepanolobium*), Palmer et al. 2010 showed that synergies between alter-
native partners elevate plant fitness above what could be expected from the single best partner.
606 However, the *Acacia* partner guild includes a “castrating” parasite that is uniquely able to boost
plant growth by suppressing reproduction. We speculate that partners that differ in degree but
not type, such as those in our system, may be less likely to synergize. Furthermore, our simu-
609 lations exploring the absence of competitive dominance (Appendix C) suggest that the dynamics
of the partner guild dictate which diversity mechanisms can manifest. Specifically, a sampling
effect emerged only when we removed the competitive hierarchy that otherwise allows *L. apicula-
612 tum* to dominate the host population. This provides evidence that when a high-quality partner is
also high-frequency due to competitive exclusion, the benefit of adding more species is essentially
nonexistent, as the host is already likely to be occupied by the best available partner. Fewer studies
615 have shown costs of mutualist diversity. Bruna et al. 2014 showed that ant diversity can depress
fitness of the sequentially-partnered ant-plant *Maieta guianensis* due to reduced interactions with
the better defender. Costs of diversity have also been documented in systems in which multiple
618 partners interact simultaneously with a shared mutualist, likely due to interference via the host
resource budget (Keller et al., 2018) or negative higher-order interactions (Barrett et al., 2015).
One previous study (in a different ant-cactus system) showed neutral effects of partner diversity,
621 but this study also showed that ant visitation was itself neutral, with no detectable effects on plant
fitness (Ford et al., 2015). To our knowledge, ours is the first study documenting a strong, positive
624 fitness effect of mutualism, regardless of mutualist identity or guild composition. As this field
grows, particularly with long-term studies that integrate multiple vital rates across the life cycle,
we will be better positioned to understand whether positive, neutral, or negative effects of partner
diversity predominate, and the conditions under which each might be more likely.

627 When partners exhibit different reactions to varying environments, interacting with a diverse
portfolio of partners can lead to more consistent benefits across time (Batstone et al., 2018). Our
work explicitly incorporated temporal environmental stochasticity, yet we find no evidence for

630 the portfolio effect as a mechanism of diversity benefits. In ant-plant defensive mutualisms, any
portfolio effect would emerge from fluctuations in the community of herbivores, and ant-specific
responses to each herbivore species. Instead, we found that inter-annual fluctuations in vital rates
633 were generally positively correlated across plants in different ant states, suggesting other drivers
of inter-annual variation that are shared across the population (notably, weather) override any
fluctuations in herbivore composition and ant-herbivore interactions. To further explore the poten-
636 tial for portfolio effects, we conducted simulations in which inter-annual fluctuations in ant effects
were manipulated to be independent or negatively correlated; we found evidence of the portfolio
effect in both scenarios, but especially when ant effects were negatively correlated (Appendix D).
639 These results indicate that while the portfolio effect can emerge under hypothetical conditions of
anti-synchronous partner responses, shared drivers of inter-annual variability in our study system
dampen this potential benefit of partner diversity. Dallas et al. 2022 found that while the portfolio
642 effect was easy to show in theoretical models, it is infrequently detected in empirical data across
many systems. This indicates that the portfolio effect may be difficult to detect, disguised by other
mechanisms, or uncommon in nature.

645 This study highlights the value of long-term data in investigating species interactions. Previ-
ously studies have analyzed how partner identity and partner turnover impact focal mutualist fitness
(Barrett et al., 2015; Bruna et al., 2014; Dejean et al., 2008; Fonseca and Benson, 2003; Noe and
648 Hammerstein, 1994; Trojelsgaard et al., 2015). Separate studies have analyzed how inter-annual
variability impacts focal mutualists (Alarcón et al., 2008; Alonso, 1998; Horvitz and Schemske,
1990; Lázaro et al., 2022; Ollerton et al., 2006). The long term data set we used gave us the abil-
651 ity to consider, for the first time, the combined effects of partner identity, partner turnover, and
temporal stochasticity. By piecing together complete life cycle information from long-term data,
we gain a more nuanced understanding of the fitness consequences of ant visitation. For example,
654 our previous study that focused only on reproductive vital rates suggested that *C. opuntiae* has
overall parasitic fitness effects because activity of this species within tree cholla flowers can deter
pollinators and reduce seed set (Figure A1) (Ohm and Miller, 2014). Yet, the more complete anal-

657 ysis presented here, which accounts for reduced seed set alongside other demographic advantages
in higher-sensitivity vital rates, indicates that this species is clearly a mutualist.

As with any study, there are limitations to consider when interpreting our results. First, we
660 used observational data to infer ant effects on plant demography. However, we have previously
conducted experimental manipulations which revealed that ant presence has causal effects on plant
demographic rates through anti-herbivore defense (Miller, 2007; Ohm and Miller, 2014). The combi-
663 nation of long-term observational data backed up by experimental results gives us greater confidence
in our causal interpretations. Second, we have not explicitly incorporated ant-herbivore interac-
tions, even though these are the primary pathway through which ants influence plant demography.
666 Surveys of herbivore damage from our long-term data (Figure B4) support the protective benefits
of ant visitation. Specifically, herbivore pressure was highest on plants in which no ant was present
(vacancy), intermediate under Other and *C. opuntiae* tending, and lowest under *L. apiculatum*
669 tending. This gradient aligns with the expected defensive hierarchy of ant species, providing indi-
rect evidence that differences among ant partners influence herbivory rates and, by extension, plant
demographic outcomes (Miller, 2007; Ohm and Miller, 2014). Future work explicitly incorporating
672 ant-herbivore-plant interactions in the demographic models could more directly quantify how vari-
ation in ant species composition mediates herbivore pressure and plant fitness. Third, the rarity of
several ant partner species imposed a practical constraint on assessing effects of partner diversity,
675 since we needed to combine these into an Other category that might mask important differences.
As more long-term data accumulate, we may gain additional information to further unpack this
group of species.

678 *Conclusion.* This study highlights that while partners within a mutualistic guild can be ecologi-
cally different, they may still be interchangeable in their net effects on fitness of a shared mutualist
based on where their effects occur in the life cycle – **highlighting the importance of a demographic**
681 **perspective in understanding the fitness consequences of mutualisms.** Contrary to much of the
literature on multi-species mutualisms, we find that while ant partners are beneficial, partner di-

versity has no measurable benefit to their shared mutualist and may even be somewhat costly. As
684 studies accumulate across different types of multi-partner mutualisms, particularly those spanning
simultaneous/sequential partnerships and leveraging demographic data from the complete life cycle,
our field will be better poised to understand whether, how, and under what conditions mutualist
687 diversity is neutral, costly, or beneficial.

Acknowledgments

Financial support for this work came from the Sevilleta Long-Term Ecological Research Program
690 (NSF DEB awards 1655499 and 1748133). We acknowledge the many students and technicians
who have helped collect annual census data for this project, including M. Donald, J. Fowler, M.
Saucedo, K. Dickens, T. Jordan-Millet, J. Moutouama, C. Oxley, K. Schraeder, B. Scherick, A.
693 Sears, M. Tucker, and J. Xiong. J. Moutouama, A. Bradley, B. Scherick, A. Gough, J. Martin, and
V. Rudolf provided helpful feedback on the manuscript. We also thank two reviewers for taking
the time to provide thoughtful comments and suggestions on earlier versions of this manuscript.

696 Data and Code Availability

This section will be filled out once double blind reviews are complete.

Literature Cited

- 699 Afkhami, M. E., Friesen, M. L., and Stinchcombe, J. R. (2021). Multiple mutualism effects generate
synergistic selection and strengthen fitness alignment in the interaction between legumes, rhizobia
and mycorrhizal fungi. *Ecology Letters*, 24(9):1824–1834.
- 702 Afkhami, M. E., Rudgers, J. A., and Stachowicz, J. J. (2014). Multiple mutualist effects: conflict
and synergy in multispecies mutualisms. *Ecology*, 95(4):833–844.

- Afkhami, M. E. and Stinchcombe, J. R. (2016). Multiple mutualist effects on genomewide expression in the tripartite association between *Medicago truncatula*, nitrogen-fixing bacteria and mycorrhizal fungi. *Molecular Ecology*, 25:4946–4962.
- Agrawal, A. A. and Rutter, M. T. (1998). Dynamic anti-herbivore defense in ant-plants: the role of induced responses. *Oikos*, 83(2):227–236.
- Alarcón, R., Waser, N. M., and Ollerton, J. (2008). Year-to-year variation in the topology of a plant-pollinator interaction network. *Oikos*, 117(11):1796–1807.
- Alonso, L. E. (1998). Spatial and temporal variation in the ant occupants of a facultative ant-plant. *Biotropica*, 30:201–213.
- Barrett, L. G., Bever, J. D., Bissett, A., and Thrall, P. H. (2015). Partner diversity and identity impacts on plant productivity in *Acacia*-rhizobial interactions. *Journal of Ecology*, 103(1):130–142.
- Barton, K. E. and Koricheva, J. (2010). The ontogeny of plant defense and herbivory: characterizing general patterns using meta-analysis. *The American Naturalist*, 175(4):481–493.
- Bascompte, J. (2009). Disentangling the web of life. *Science*, 325(5939):414–416.
- Batstone, R. T., Carscadden, K. A., Afkhami, M. E., and Frederickson, M. E. (2018). Using niche breadth theory to explain generalization in mutualisms. *Ecology*, 99(5):1039–1050.
- Beattie, A. (1985). *The Evolutionary Ecology of Ant-Plant Mutualisms*. Cambridge University Press, Cambridge, UK.
- Benson, L. (1982). *Cacti of the United States and Canada*. Stanford University Press, Stanford, CA.
- Boege, K. and Marquis, R. J. (2005). Facing herbivory as you grow up: The ontogeny of resistance in plants. *Trends in Ecology and Evolution*, 20(7):441–448.

- Bronstein, J. L. (1998). The contribution of ant-plant protection studies to our understanding of mutualism. *BioTropica*, 30(2):150–161.
- 729 Bronstein, J. L., Alarcón, R., and Geber, M. (2006). The evolution of plant–insect mutualisms. *New Phytologist*, 172(1):412–428.
- Bruna, E. M., Izzo, T. J., Inouye, B. D., and Vasconcelos, H. L. (2014). Effect of mutualist partner
732 identity on plant demography. *Ecology*, 95(12):3237–3243.
- Byk, J. and Del-Claro, K. (2011). Ant-plant interaction in the Neotropical savanna: direct beneficial effects of extrafloral nectar on ant colony fitness. *Population Ecology*, 53(3):327–332.
- 735 Báez, S., Donoso, D. A., Queenborough, S. A., Jaramillo, L., Valencia, R., and Dangles, O. (2016). Ant mutualism increases long-term growth and survival of a common Amazonian tree. *The American Naturalist*, 188(5):1–9.
- 738 Dallas, T. A. and Kramer, A. M. (2022). Temporal variability in population and community dynamics. *Ecology*, 103(2):e03577.
- Dattilo, W., Marquitti, F. M. D., Guimaraes, P. R., and Izzo, T. J. (2014). The structure of
741 ant-plant ecological networks: Is abundance enough? *Ecology*, 95(2):475–485.
- Dejean, A., Djieto-Lordon, C., Cereghino, R., and Leponce, M. (2008). Ontogenetic succession and the ant mosaic: an empirical approach using pioneer trees. *Basic and Applied Ecology*,
744 9(3):316–323.
- Djitéo-Lordon, C., Dejean, A., Ring, R. A., Nkongmeneck, B. A., Lauga, J., and McKey, D. (2005). Ecology of an improbable association: The pseudomyrmecine plant-ant *Tetraponera tessmanni* and the myrmecophytic liana *Vitex thyrsiflora* (lamiaceae) in Cameroon. *Biotropica*, 37(3):421–430.
- Donald, M. L. and Miller, T. E. X. (2022). Does ant–plant mutualism have spillover effects on the
750 non-partner ant community? *Ecology and Evolution*, 12(1):1–12.

- Elderd, B. D. and Miller, T. E. X. (2016). Quantifying demographic uncertainty: Bayesian methods for integral projection models. *Ecological Monographs*, 86(1):125–144.
- 753 Ellner, S. P., Childs, D. Z., and Rees, M. (2016). *Data-driven Modeling of Structured Populations: A Practical Guide to the Integral Projection Model*. Springer, Ithaca, NY.
- Fehling, L. S. (2022). Reward complementarity and context dependency in multispecies mutualist
756 interactions in partridge pea (*Chamaecrista fasciculata*). Master's thesis, Miami University,
Miami, FL.
- Fiala, B., Grunsky, H., Maschwitz, U., and Linsenmair, K. E. (1994). Diversity of ant-plant
759 interactions: protective efficacy in macaranga species with different degrees of ant association.
Oecologia, 97(2):186–192.
- Fonseca, C. R. and Benson, W. W. (2003). Ontogenetic succession in Amazonian ant trees. *Oikos*,
762 102(2):407–412.
- Ford, K. R., Ness, J. H., Bronstein, J. L., and Morris, W. F. (2015). The demographic consequences
of mutualism: ants increase host-plant fruit production but not population growth. *Oecologia*,
765 179:435–446.
- Franco, M. and Silvertown, J. (2004). A comparative demography of plants based upon elasticities
of vital rates. *Ecology*, 85(2):531–538.
- 768 Fraser, A. M., Axen, A. H., and Pierce, N. E. (2001). Assessing the quality of different ant species
as partners of a myrmecophilous butterfly. *Oecologia*, 129(4):452–460.
- Frederickson, M. E. (2005). Ant species confer different partner benefits on two Neotropical myrme-
771 cophytes. *Oecologia*, 143(3):387–395.
- Frederickson, M. E. (2013). Rethinking mutualism stability: Cheaters and the evolution of sanc-
tions. *Quarterly Review of Biology*, 88(4):269–295.

- ⁷⁷⁴ Gaume, L., McKey, D., and Terrin, S. (1998). Ant-plant-homopteran mutualism: how the third partner affects the interaction between a plant-specialist ant and its myrmecophyte host. *Ecological Entomology*, 26:569–575.
- ⁷⁷⁷ Gustafson, D. and Casper, B. (2005). Differential host plant performance as a function of soil arbuscular mycorrhizal fungal communities: experimentally manipulating co-occurring glomus species. *Plant Ecology*, 183:257–263.
- ⁷⁸⁰ Hautier, Y., Isbell, F., Borer, E. T., Seabloom, E. W., Harpole, W. S., Lind, E. M., MacDougall, A. S., Stevens, C. J., and Tilman, D. (2014). Eutrophication weakens stabilizing effects of diversity in natural grasslands. *Nature*, 508:521–525.
- ⁷⁸³ Hernandez, D., Kiesewetter, K., Palakurty, S., Stinchcombe, J., and Afkhami, M. (2019). Synergism and symbioses: unpacking complex mutualistic species interactions using transcriptomic approaches. In de Bruijn, F. J., editor, *The Model Legume Medicago truncatula*, pages 1045–1054. ⁷⁸⁶ John Wiley & Sons, Hoboken, New Jersey, USA.
- Horvitz, C. C. and Schemske, D. W. (1986). Seed dispersal of a Neotropical myrmecochore: variation in removal rates and dispersal distance. *bioTropica*, 18(4):319–323.
- ⁷⁸⁹ Horvitz, C. C. and Schemske, D. W. (1990). Spatiotemporal variation in insect mutualists of a Neotropical herb. *Ecology*, 71:1085–1097.
- ⁷⁹² Keller, K. R., Carabajal, S., Navarro, F., and Lau, J. A. (2018). Effects of multiple mutualists on plants and their associated arthropod communities. *Oecologia*, 186:185–194.
- Larimer, A. L., Clay, K., and Bever, J. D. (2014). Synergism and context dependency of interactions between arbuscular mycorrhizal fungi and rhizobia with a prairie legume. *Ecology*, 95:1045–1054.
- ⁷⁹⁵ Loreau, M. (1999). Biodiversity and ecosystem functioning: Effects of species diversity on productivity and stability in plant communities. *Proceedings of the Royal Society B: Biological Sciences*, 264(1381):127–130.

- 798 Ludka, J., Levan, K. E., and Holway, D. A. (2015). Infiltration of a facultative ant–plant mutualism by the introduced Argentine ant: effects on mutualist diversity and mutualism benefits. *Ecological Entomology*, 40(4):437–443.
- 801 Lázaro, A., Gómez-Martínez, C., González-Estévez, M. A., and Hidalgo, M. (2022). Portfolio effect and asynchrony as drivers of stability in plant–pollinator communities along a gradient of landscape heterogeneity. *Ecography*, 45(3):1–14.
- 804 Mann, J. (1969). *Cactus-feeding insects and mites*. United States National Museum Bulletin. Smithsonian Institution Press, Washington, D.C.
- McKeon, C. S., Stier, A. C., McIlroy, S. E., and Bolker, B. M. (2012). Multiple defender effects: synergistic coral defense by mutualist crustaceans. *Oecologia*, 169(4):1095–1103.
- 807 Metcalf, C. J. E., Ellner, S. P., Childs, D. Z., Salguero-Gómez, R., Merow, C., McMahon, S. M., Jongejans, E., and Rees, M. (2015). Statistical modelling of annual variation for inference on stochastic population dynamics using integral projection models. *Methods in Ecology and Evolution*, 6(9):1007–1017.
- Miller, T. (2020). Long-term study of tree cholla demography in the Los Piños mountains, Sevilleta
813 National Wildlife Refuge.
- Miller, T. E. and Ellner, S. P. (2025). My, how you've grown: A practical guide to modeling size transitions for integral projection model (ipm) applications. *Ecology*, 106(5):e70088.
- 816 Miller, T. E., Louda, S. M., Rose, K. A., and Eckberg, J. O. (2009). Impacts of insect herbivory on cactus population dynamics: experimental demography across an environmental gradient. *Ecological Monographs*, 79(1):155–172.
- 819 Miller, T. E. X. (2007). Does having multiple partners weaken the benefits of facultative mutualism? a test with cacti and cactus-tending ants. *Oikos*, 116(3):500–512.

- Miller, T. E. X. (2014). Plant size and reproductive state affect the quantity and quality of rewards
822 to animal mutualists. *Journal of Ecology*, 102(4):496–507.
- Miller, T. E. X., Tyre, A. J., and Louda, S. M. (2006). Plant reproductive allocation predicts
herbivore dynamics across spatial and temporal scales. *The American Naturalist*, 168(5):608–
825 616.
- Moraes, S. C. and Vasconcelos, H. L. (2009). Long-term persistence of a neotropical ant-plant
population in the absence of obligate plant-ants. *Ecology*, 90(8):2375–2383.
- Nakazawa, T. (2020). Ontogenetic shifts in mutualistic interactions: stage-structured models of
host-symbiont dynamics. *Oikos*, 129(6):857–869.
- Ness, J. H., Morris, W. F., and Bronstein, J. L. (2006). Integrating quality and quantity of
831 mutualistic service to contrast ant species protecting *Ferocactus wislizeni*. *Ecology*, 87(4):912–
921.
- Ness, J. H., Morris, W. F., and Bronstein, J. L. (2009). For ant-protected plants, the best defense
834 is a hungry offense. *Ecology*, 90(10):2823–2831.
- Noe, R. and Hammerstein, P. (1994). Biological markets: supply and demand determine the effect
of partner choice in cooperation, mutualism and mating. *Behavioral Ecology and Sociobiology*,
837 35(1):1–11.
- Ohm, J. R. and Miller, T. E. X. (2014). Balancing anti-herbivore benefits and anti-pollinator costs
of defensive mutualists. *Ecology*, 95(10):2924–2935.
- Oliveira, P. S., Rico-Gray, V., Díaz-Castelazo, C., and Castillo-Guevara, C. (1999). Interaction
between ants, extrafloral nectaries and insect herbivores in neotropical coastal sand dunes: her-
bivore deterrence by visiting ants increases fruit set in *Opuntia stricta* (cactaceae). *Functional
840 Ecology*, 13(5):623–631.
843

- Ollerton, J., Johnson, S. D., and Hingston, A. B. (2006). Geographical variation in diversity and specificity of pollination systems. In Waser, N. M. and Ollerton, J., editors, *Plant-Pollinator Interactions: From Specialization to Generalization*, pages 283–308. University of Chicago Press, Chicago, IL.
- Palmer, T. M., Doak, D. F., Stanton, M. L., Bronstein, J. L., Kiers, T. E., Young, T. P., Goheen, J. R., and Pringle, R. M. (2010). Synergy of multiple partners, including freeloaders, increases host fitness in a multispecies mutualism. *Proceedings of the National Academy of Sciences*, 107(40):17234–17239.
- R Core Team (2023). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Version 4.3.3.
- Rees, M. and Ellner, S. P. (2009). Integral projection models for populations in temporally varying environments. *Ecological Monographs*, 79(4):575–594.
- Rogers, S. R., Tarpy, D. R., and Burrack, H. J. (2014). Bee species diversity enhances productivity and stability in a perennial crop. *PloS One*, 9(5):e97307.
- Rudgers, J. A. (2004). Enemies of herbivores can shape plant traits: selection in a facultative ant-plant mutualism. *Ecology*, 85(1):192–205.
- Sachs, J. L., Mueller, U. G., Wilcox, T. P., and Bull, J. J. (2004). The evolution of cooperation. *Quarterly Review of Biology*, 79(2):135–160.
- Stachowicz, J. J. and Whitlatch, R. B. (2005). Multiple mutualists provide complementary benefits to their seaweed host. *Ecology*, 86(9):2418–2427.
- Stan Development Team (2023). RStan: the R interface to Stan. R package version 2.32.3.
- Stanton, M. L. (2003). Interacting guilds: moving beyond the pairwise perspective on mutualisms. *The American Naturalist*, 162:S10–S23.

- 867 Stevens, B. L. F., Howard, K., Bogar, L. M., et al. (2024). Environmental fluctuations promote
host reward strategies that maintain partner diversity in multispecies mutualisms. *The American
Naturalist*, 202(6):E000.
- 870 Thibaut, L. M., Connolly, S. R., and Sweatman, H. P. (2012). Diversity and stability of herbivorous
fishes on coral reefs. *Ecology*, 93(4):891–901.
- Tilman, D., Wedin, D., and Knops, J. (1994). Biodiversity and stability in grasslands. *Nature*,
873 367:363–365.
- Trager, M. D., Bhotika, S., Hostetler, J. A., Andrade, G. V., Rodriguez-Cabal, M. A., McKeon,
876 C. S., Osenberg, C. W., and Bolker, B. M. (2010). Benefits for plants in ant-plant protective
mutualisms: a meta-analysis. *PLoS ONE*, 5(12):e14308.
- Trojelsgaard, K., Jordano, P., Carstensen, D. W., and Olesen, J. M. (2015). Geographical variation
in mutualistic networks: Similarity, turnover and partner fidelity. *Proceedings of the Royal Society
879 B: Biological Sciences*, 282(18-2):1–9.
- Ushio, M. (2020). Interaction capacity as a potential driver of community diversity. *Proceedings of
the Royal Society B*, 289(1969):20212690.
- 882 Ventre-Lespiaucq, A., Jacquemyn, H., Ramirez, S., and Bidartondo, M. I. (2021). Temporal dy-
namics of orchid–mycorrhizal interactions: turnover and persistence of fungal symbionts across
life stages. *New Phytologist*, 230(2):697–708.
- 885 Wetzel, W. C., Inouye, B. D., Hahn, P. G., Whitehead, S. R., and Underwood, N. (2023). Variability
in plant-herbivore interactions. *Annual Review of Ecology, Evolution, and Systematics*, 54:451–
474.
- 888 Williams, J. L., Miller, T. E. X., and Ellner, S. P. (2012). Avoiding unintentional eviction from
integral projection models. *Ecology*, 93(9):2008–2014.

Winfree, R. (2020). How does biodiversity relate to ecosystem functioning in natural ecosystems? In
891 Dobson, A., Holt, R. D., and Tilman, D., editors, *Unsolved Problems in Ecology*, pages 302–318.
Princeton University Press, Princeton, NJ.

Yang, N., Røder, H. L., Wicaksono, W. A., Wassermann, B., Russel, J., Li, X., Nesme, J., Berg, G.,
894 Sørensen, S. J., and Burmølle, M. (2024). Interspecific interactions facilitate keystone species in
a multispecies biofilm that promotes plant growth. *International Society for Microbial Ecology
Journal*, 18(1):wrae012.

A Additional Methods and Parameters

In addition to the models described in the body of the paper, we fit several other models using
900 data from previous studies. These models are described below.

Seeds Per Fruit. With data from Miller (2007), we fit a model for the number of seeds produced by every fruit on a cholla ($\kappa(a')$) in year $t + 1$ based on the ant partner a' in year $t + 1$. We fit this model to seed data y^κ using a Negative Binomial distribution and the log link function:

$$y^\kappa \sim \text{NegativeBinomial}(\hat{\kappa}, \hat{\phi})$$

$$\hat{\phi} = \beta_0^\phi$$

The data used for this model did not include data on ants in the “other” category, so we used the data from vacant plants to parameterize seeds per flower for plants with “other” ants in the IPM.

903 We found that vacant plants produced the most mean seeds (147.2 per fruit), followed by *L. apiculatum* tended plants (142.4 per fruit), and finally, *C. opuntiae* tended plants (115.0 per fruit).

Recruit Size Distribution. We fit this model to recruit size data y^η using a Normal distribution with the identity link function:

$$y^\eta \sim N(\hat{\eta}, \hat{\sigma})$$

where $\hat{\sigma}$ is estimated with a non-informative prior.

906 We found that the mean size of recruits is $\log(-2.097)m^3$ with an interquartile range from $\log(-2.173)m^3$ to $\log(-1.712)m^3$.

Germination. With germination data Miller et al. (2009), we fit two models for the probability of germinating from the first year seedbank (γ_1) or the second year seedbank (γ_2) in year $t + 1$, with

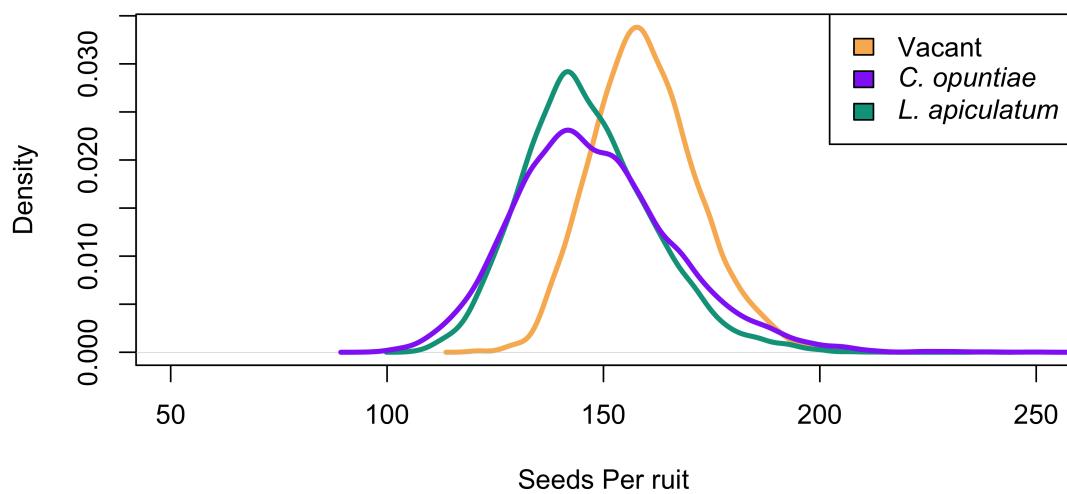


Figure A1: The posterior distributions of seeds produced per fruit on reproducing plants. These estimates are ant specific with the yellow representing vacant plant seed production, purple representing *C. opuntiae* tended plant seed production, and teal representing *L. apiculatum* tended plant seed production.

no fixed or random effects. These models were fit to germination data $y^{\gamma_1}, y^{\gamma_2}$ using the binomial distribution with logit link functions:

$$y^{\gamma_1} \sim \text{Binomial}(\hat{\gamma}_1)$$

$$y^{\gamma_2} \sim \text{Binomial}(\hat{\gamma}_2)$$

We found that the mean germination rates for seeds in the seedbank for one year is 0%, with

909 an interquartile range of 0% and 1%. We found that the mean germination rates for seeds in the seedbank for a second year is 0%, with an interquartile range of 0% to 0.4%. Seeds are more likely to germinate in their first year in the seedbank, but most seeds will never germinate.

Pre-Census Survival. With recruit census data Miller et al. (2006), we fit a model for the probability of a seedling (which germinates in early Fall) surviving to when we census in May (δ) of year $t + 1$ (accounting for missed mortality events), with fixed effects of the previous size x and random effects of the transect m . We fit this model to pre-census survival data y^δ using a Bernoulli distribution with a logit link function:

$$y^\delta \sim \text{Bern}(\hat{\delta})$$

912 where $m \sim N(0, \sigma_{\text{transect}}^2)$ is the random effect of transect where the recruited individual was analyzed for survival.

We found that plants have a 16.2% probability of surviving from germination to the next census.

915 Our model estimated this very well, expecting a 16.3% probability.

Probability of Flowering. We fit a model for the probability of flowering (P) in year $t + 1$ based on the size (x) in year $t + 1$ with random effects of year τ_a using a Bernoulli distribution with a logit link function:

$$y^P \sim \text{Bern}(\hat{P})$$

$$\hat{P}_i = \beta^0 x_i + \tau_{a[i]}$$

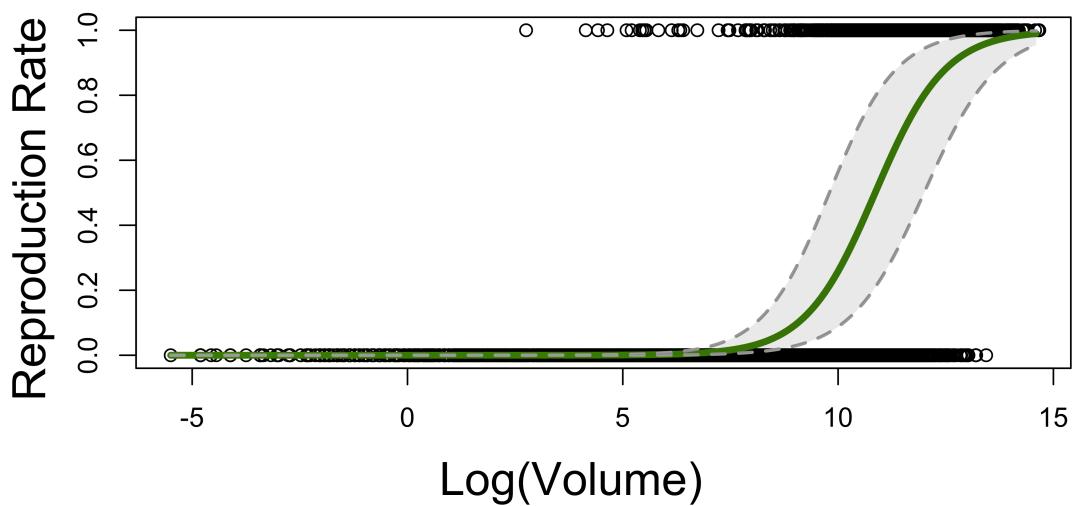


Figure A2: The probability of a cholla plant reproducing based on its size. The green line shows the average probability, the gray surrounding area shows the 90% confidence interval, and the black points show the actual data.

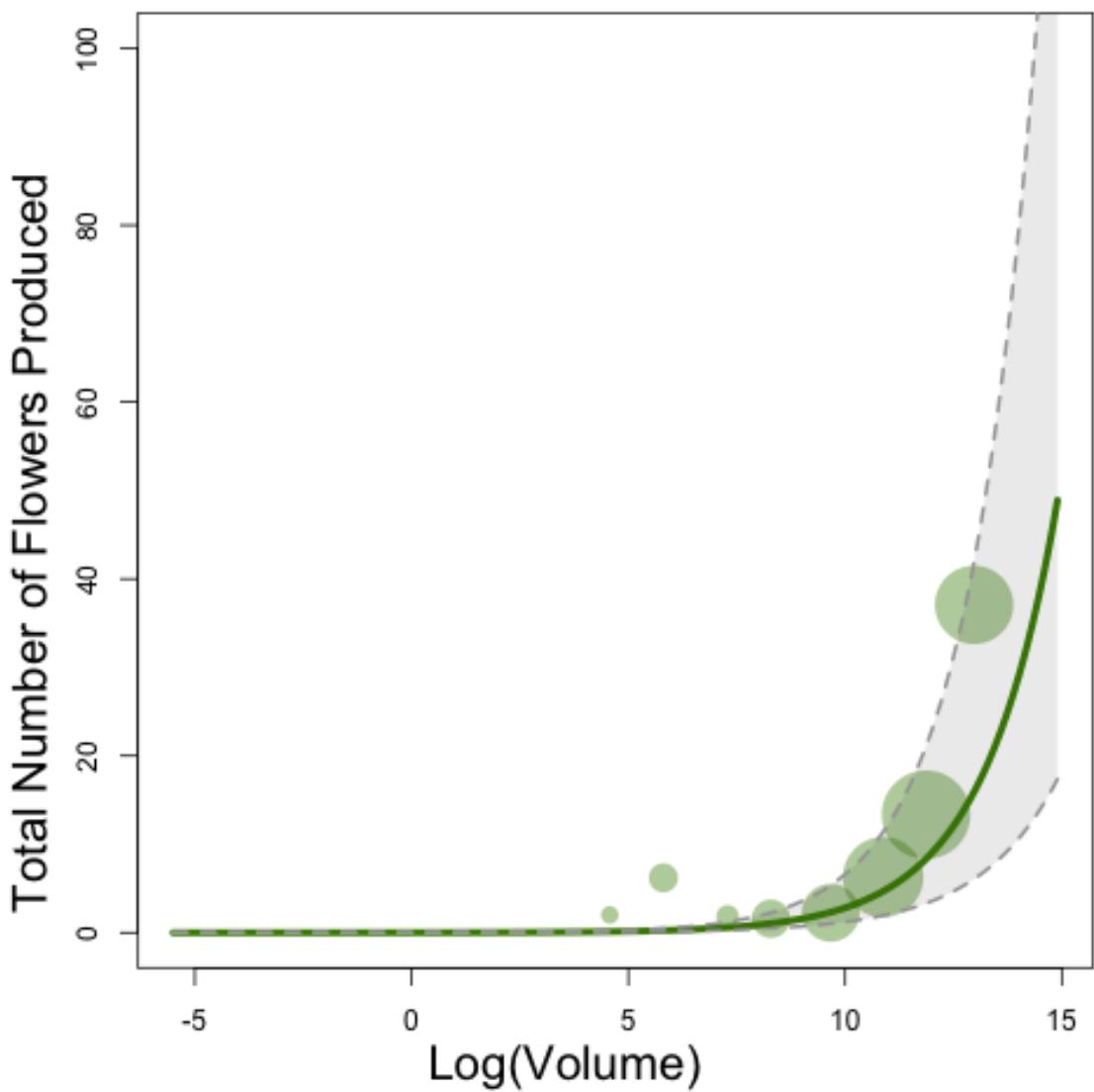


Figure A3: The number of flowers produced by a cholla plant based on its size. The green line shows the average number of flowers produced, the gray surrounding area shows the 90% confidence interval, and the green points show the binned data.

The probability of a plant reproducing in a given year is highly size dependent. The mean probability of reproducing remains at about 0% until the plant reaches a medium size, after which
918 the mean probability of reproducing increases steadily before reaching about 100% at large sizes (Figure A2).

Number of Flowers Produced. We fit a model for the flowers produced (F) in year $t + 1$ based on the size (x) in year $t + 1$ with random effects of year τ_a using a zero truncated negative binomial distribution:

$$y^F \text{Bern}(\hat{F})$$

$$\hat{F}_i = \beta^0 x_i + \tau_{a[i]}$$

The number of flowers produced by a reproducing plant is also highly size dependent. The
921 mean number of flowers produced are 0 until the plant reaches a medium size, after which the mean number of flowers produced increases to about 45 at the largest sizes (Figure A3).

B Observed Herbivory Data

924 Herbivory is an important driver in this population and shapes the range and demography of cholla. Herbivore presence has been shown to negatively impact growth and fecundity of cholla populations (Miller et al., 2009). Ant visitors are believed to offer defensive benefits to the plants
927 they tend in this system, leading to the hypothesis that ant presence would be correlated with reduced herbivory. Therefore, herbivore protection is the likely driver behind the increased survival, growth, and reproductive rates of tended plants. Fluctuations in herbivore communities across time
930 would also lead to potentially important fluctuations in ant visitor responses and therefore cholla demographic responses.

Here we conduct an analyses of herbivory levels on plants with different ant visitors and contrast
933 them to herbivory levels on plants with no visitors. Evidence that herbivory is decreased for tended plants further strengthens the results presented in the main text that ant presence is beneficial.

Herbivory data was collected during annual demographic censuses. This involved noting the
936 type and quantity of herbivores observed. Presence of herbivores is considered to be herbivore
pressure as it is assumed they will damage the plant if they are present. This data has been
taken consistently since 2014, so the analysis below considers 9 years of data. We considered only
939 plants which were reproducing, since prior work shows that flowering significantly elevates herbivore
pressure (Miller et al., 2006). The proportion of reproducing plants that experienced herbivory was
calculated for each ant state separately. Analysis showed that ant presence is correlated with lower
942 herbivore visitation. We observed herbivores on 40% of vacant cacti. Plants tended by Other
ants experienced similar, though lower, frequency of observed herbivores on reproducing plants
(herbivores detected on 37.5% of plants). Herbivores were detected on 25% of plants tended by
945 *C. opuntiae* ants and on 11% of plants tended by *L. apiculatum* ants. These results indicate that
ant presence is correlated with lower levels of herbivory and that partner identity has an impact
on the level of herbivory. They also indicate that the partner correlated with the lowest levels of
948 herbivory is *L. apiculatum* ants. These findings are consistent with literature findings which show
that *L. apiculatum* ants are the most aggressive (therefore the most effective against herbivores)
(Miller, 2007).

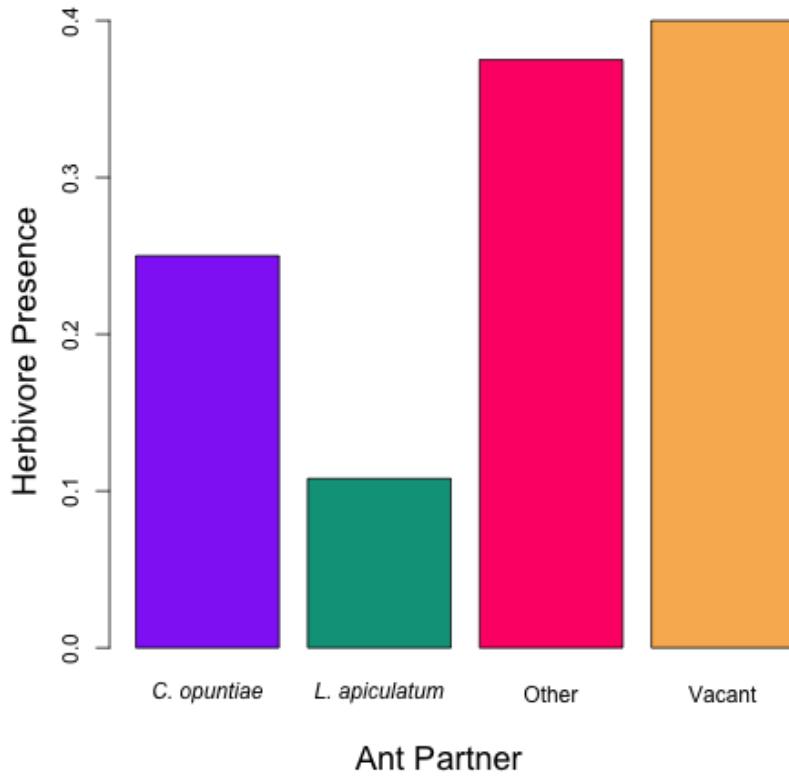


Figure B4: The proportion of reproducing plants which are visited by herbivores. Each bar represents the observed proportion of cacti in each ant state with observed herbivores.

951

C Alternative Ant Transition Simulations

In addition to the competitive exclusion model defined and analyzed in the body of the paper, we simulated results from several other potential models. We chose to include competitive exclusion as
 954 our primary results in the paper because we believe it to be the most biologically realistic. However, in building and testing of alternative models we found that the method of ants occupying plants significantly impacts the fitness of the population. We tested two alternative transition models,
 957 one called the frequency based model and one called the equal likelihood model.

Frequency Based Model. The first alternative hypothesis we tested was what we called the frequency based model. In this model rather than the proportion of vacant cacti being maintained, the proportion of cacti occupied by each species is maintained and when one is removed it is replaced with vacancy. This version of the model assumes that the frequency of each ant we see is reflective of the real frequency of populations rather than some other mechanism. With this model we found very clear evidence of Sampling Effect in the system. When only *C. opuntiae*, Other ants, or both ants are present, there is very little difference in the fitness of the cacti from when no partners are present. Only when *L. apiculatum* ants are present do we see an increase in the fitness of the focal mutualist (Figure C5a). In this simulation, the more partners that are present the higher the fitness of the focal mutualist is, confirming that partner diversity would be beneficial through sampling effect if this transition model were correct. (Figure C5b).

Equal Likelihood Model. The second alternative hypothesis we tested was what we called the equal likelihood model. In this model we preserved the observed pattern of size-dependent vacancy/occupancy, but occupancy was manipulated to be equally likely for all partner identities. This was designed to remove the effect overwhelming numbers of *L. apiculatum* ants may have. Despite very different proportions, we found very similar outcomes to the competitive exclusion model analyzed in the paper. All ants are beneficial, but having more than one is not necessarily any better than having an individual species as a partner (Figure C6b). Partner presence is beneficial, but neither identity nor number of partners appears to be important (Figure C6a).

D The Portfolio Effect

This appendix includes our evaluation of evidence for the portfolio effect under realistic conditions (those observed in the field) as well as simulated conditions.

Portfolio Effect Evidence. We compared the effects of partner diversity (estimated as $\lambda_S^4 - \lambda_S^0$) for the synchronized and non-synchronized models. We determined that non-synchronous effects

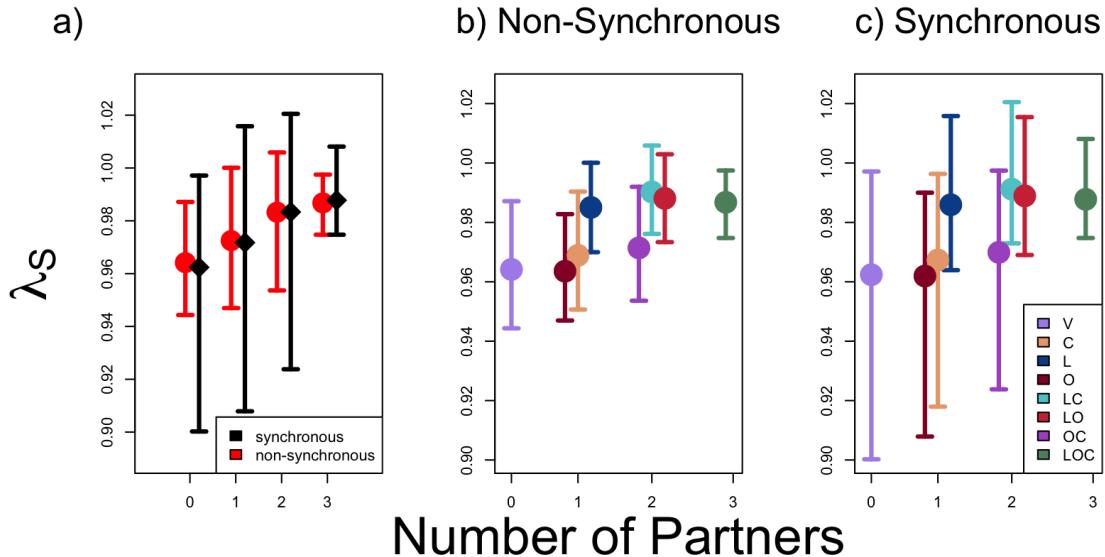


Figure C5: Panel a) the mean estimated λ_{NS} and λ_S for different numbers of partners (0-3) for the synchronous IPM (black circle) and the non-synchronous IPM (red diamond). The lines show the posterior distribution spread of the estimated λ values. Panels b-c) the mean estimated λ_{NS} and λ_S respectively, for each simulated combination of ant partner as the filled in circles. The lines show the posterior distribution spread of estimated λ values. The letters in the legend correspond to what ant partners are present (V = Vacant, C = *C. opuntiae*, L = *L. apiculatum*, O = other).

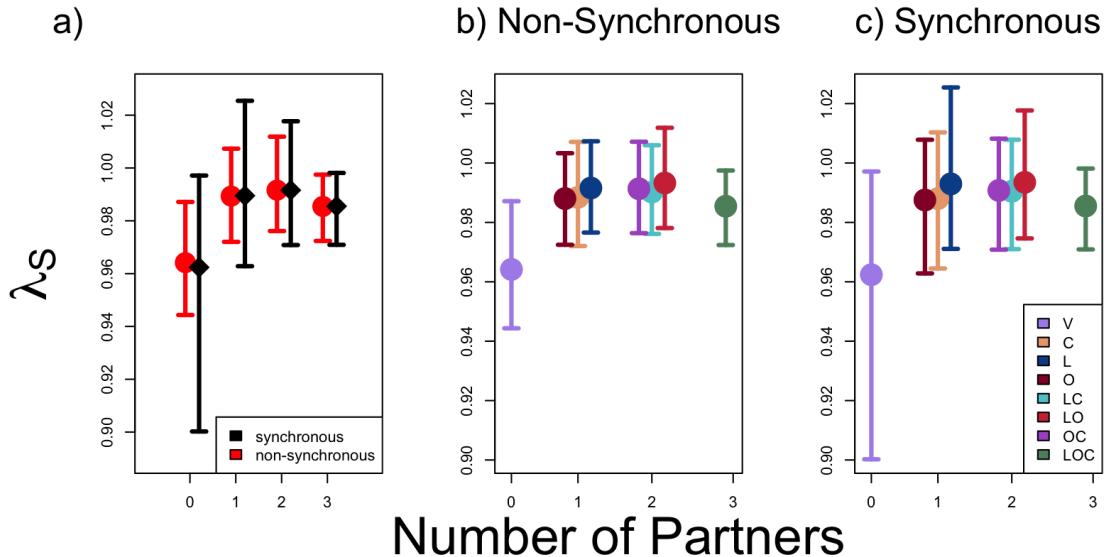


Figure C6: Panel a) the mean estimated λ_{NS} and λ_S for different numbers of partners (0-3) for the synchronous IPM (black circle) and the non-synchronous IPM (red diamond). The lines show the posterior distribution spread of the estimated λ values. Panels b-c) the mean estimated λ_{NS} and λ_S respectively, for each simulated combination of ant partner as the filled in circles. The lines show the posterior distribution spread of estimated λ values. The letters in the legend correspond to what ant partners are present (V = Vacant, C = *C. opuntiae*, L = *L. apiculatum*, O = other).

of ant partners are greater than synchronous effects of ant partners only 50% of the time. This means there is no evidence of portfolio effect in this system.

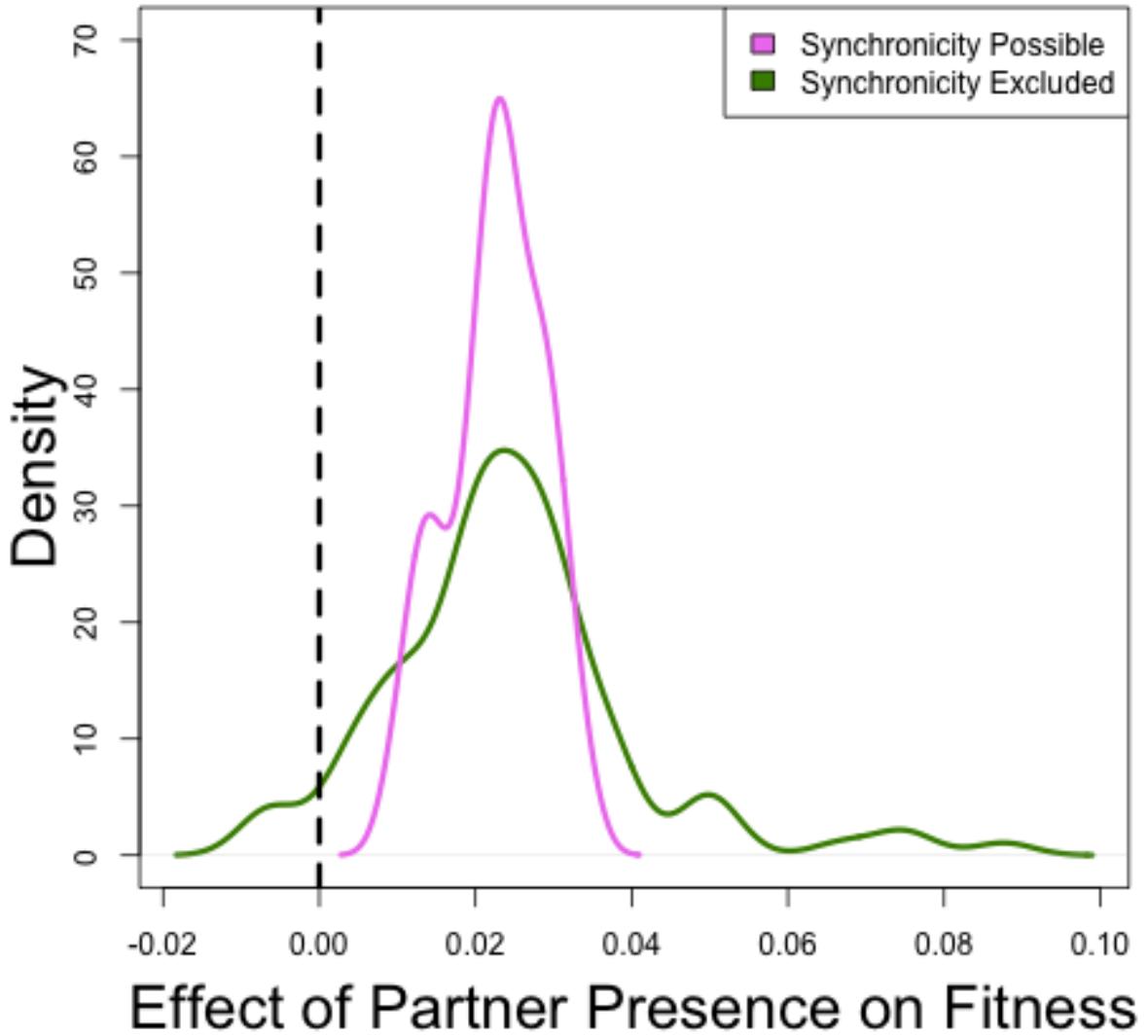


Figure D7: The distributions of $\lambda_S^4 - \lambda_S^0$ for the non-synchronous and synchronous models are shown in pink and green respectively. The vertical dashed line shows where the effect of partners on the fitness of the cholla is 0 (to the left the partners have a negative effect, to the right the partners are beneficial).

984 To further evaluate the potential for a portfolio effect, we conducted an additional analysis in

which we increased the degree of difference among ant partners. Specifically, we manipulated the correlation structure of ant-specific year random effects in the stochastic IPM. In the first scenario,
987 we forced the correlation among ant species to be zero, representing complete independence of partner responses across years. In the second scenario, we forced the correlation as close to -1 as possible, representing maximally anti-correlated partner responses. To reach a zero correlation
990 and a maximally negative one, we reordered the vector of mean ant-specific year random effects in each year so that high values for one ant corresponded to low values for another, and vice versa, while preserving the original distribution of effects. We continued reordering each vector until the
993 correlations reached the desired values. The resulting manipulated vectors allowed us to evaluate the fitness of the cacti under different partner diversity scenarios when the ants experienced different asynchronous responses to inter-annual fluctuations, allowing us to determine if portfolio effect was
996 possible under different ant conditions.

For each of these scenarios, we quantified the effect of partner diversity as the difference between projected population growth with no partners and with the full suite of partner diversity ($\lambda_S^4 - \lambda_S^0$),
999 and compared results under synchronous versus non-synchronous environmental variation. Both scenarios resulted in greater benefits of partner diversity under non-synchronous conditions, though the evidence was much stronger when the ants were anti-correlated, as expected under the portfolio
1002 effect hypothesis. This shows that portfolio effect is possible in this system if the ant partners diverged more greatly in their responses to annual variation.

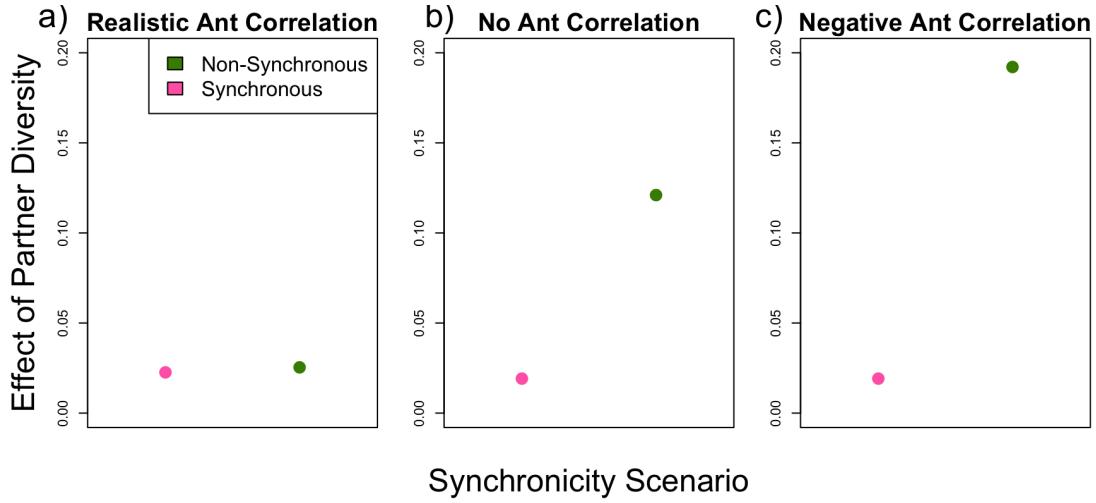


Figure D8: A comparison of synchronous and non-synchronous cactus responses to partner diversity across inter-annual fluctuations. If the non-synchronous effects of partner diversity are greater than the synchronous ones under fluctuation environments, evidence of the portfolio effect is found. Panel a) shows the mean effects of partner diversity ($\lambda_S^4 - \lambda_S^0$) under realistic levels of non-synchronicity (what was estimated from the field data), while panels b) and c) shows the mean effects of partner diversity under no synchronicity (where we artificially forced a correlation of 0 for the ant-specific year random effects) and negative synchronicity (where we artificially forced a correlation close to -1 for the ant-specific year random effects). The mean estimations of $\lambda_S^4 - \lambda_S^0$ for the non-synchronous and synchronous models are shown in green and pink respectively.