Costs, benefits, and loss of vertically transmitted symbionts affect host population dynamics

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The costs and benefits of symbiotic interactions may vary with host and symbiont ontogeny. Effects of symbionts at different stages of host development or on different host demographic rates do not contribute equally to fitness. Although rarely applied, a population dynamics approach that integrates over the host life cycle is therefore necessary for capturing the net costs or benefits and, thus, the mutualistic or parasitic nature of symbioses. Using the native, disturbance-specialist grass *Agrostis hyemalis*, we asked how a symbiotic endophyte affected the population dynamics of its host and how imperfect vertical transmission influenced symbiont frequency in a late successional environment. A size-structured integral projection model (IPM) parameterized with experimental field data showed that greater rates of individual growth and reproduction for endophyte-symbiotic (E+) hosts outweighed their lower rates of survival, leading to a net positive effect of symbiosis on equilibrium plant population growth (slower rate of extinction). Given that populations undergoing successional transitions are unlikely to be at an equilibrium size structure, we also conducted transient analysis that showed an initial short-term cost to endophyte symbiosis. We used a megamatrix approach to link E− and E+ IPMs via imperfect vertical transmission and found that this parameter strongly influenced the frequency of symbiosis via complex interactions with host demographic rates. Overall, our population dynamics approach improves the ability to characterize the outcome of symbiotic interactions, and results suggest that particular attention should be paid to interactions between the rate of vertical transmission and host demography.

Symbioses are ubiquitous in nature (Douglas 1994) and have the potential to influence population and community dynamics by altering host ecology (White and Torres 2009). Yet, quantifying the net influence of symbionts on hosts can be challenging. Throughout host ontogeny, the effects on different components of fitness can differ in magnitude or even sign as the symbiont shifts along a continuum from mutualistic to neutral to parasitic (Bronstein 1994). Generally, the effect of a symbiotic interaction at any one time may not be a good predictor of the net impact over the host's lifetime (Palmer et al. 2010). Depending on the relative importance of the fitness costs and benefits at each host stage or size, a net positive (mutualistic) or negative (parasitic) outcome may result from the relationship. Thus, a demographic approach that quantifies the effects of symbionts on growth, survival, and reproductive rates across the entire host ontogeny is necessary to characterize the ecological dynamics of symbioses (Rudgers et al. 2010, 2012).

Vertical transmission is a widespread feature of symbiotic interactions (Bright and Bulgheresi 2010). As these symbionts are passed directly from parents to offspring, they have the potential to affect hosts during all developmental stages and can often be experimentally manipulated within host populations without contagious spread. One common and intriguing feature of vertically transmitted symbiosis is that rates of transmission can be variable and are often imperfect, with symbiotic hosts producing some offspring that lack the symbiont. For example, the fungal symbionts of plants may be lost via imperfect transmission to seeds (Afkhami and Rudgers 2008), and vertically transmitted bacteria may be lost from maternal lineages of aphids (Moran and Dunbar 2006). Theory suggests that imperfect transmission can lead to symbiont extirpation from the host population even when it is strongly beneficial (Ravel et al. 1997, Gundel et al. 2008); yet, this prediction has not been evaluated with empirical data. Thus, the potential for imperfect transmission suggests that a simple contrast of symbiotic and non-symbiotic individuals is not sufficient to understand the consequences of host–symbiont interactions. Instead, we must explicitly account for connections between symbiotic and non-symbiotic individuals via the process of imperfect transmission and for the potential interactions between the transmission rate and demographic rates of the host.

Demographic models provide useful tools for evaluating the consequences of interspecific interactions but have been

under-utilized for this purpose (Crone et al. 2011). Size-structured demographic models, such as matrix models or integral projection models, can identify which demographic rates contribute most to population growth, which are most affected by symbiotic interactions, or which merit greater empirical attention. Integral projection models (IPM) (Easterling et al. 2000, Ellner and Rees 2006) are appropriate when demographic rates and the impacts of interacting species vary continuously with size (Williams and Crone 2006, Miller et al. 2009). On the other hand, matrix models are useful for describing transitions among discrete states, such as the loss of symbiosis due to imperfect vertical transmission (Gundel et al. 2008). The natural history of many symbioses indicates a need for models that combine these two approaches, accommodating both the discrete nature of symbiont status (infected or not) as well as continuous variation in host size. We develop this approach here.

We examined the population dynamics of the symbiosis between a vertically-transmitted fungus and its grass host. The epichloeae fungal endophytes (Clavipitaceae) are common, intercellular symbionts found within the above-ground tissues of cool-season grasses (Poaceae, sub-family Pooideae) (Clay 1990, Schardl 2010). In agronomic grasses, endophytes are known for the production of protective defenses (anti-herbivore alkaloids; Schardl et al. 2007). Fungal endophytes can also ameliorate abiotic stressors, such as drought (reviewed by Malinowski and Belesky 2000). The majority of studies have focused on economically important host species that may have undergone artificial selection in association with agriculture (Cheplick and Faeth 2009). Fewer native grass–endophyte symbioses have been examined in detail, but costs are sometimes documented (Faeth et al. 2004). A meta-analysis has suggested that native grass-endophyte interactions have weaker herbivore deterrence than those in agronomic systems (Saikkonen et al. 2006). However, recent experiments showed that endophytes of several native grasses can have potent anti-herbivore effects (Crawford et al. 2010). Thus, the role of endophytes in the ecology of native grasses remains an open problem.

By altering the way their grass hosts respond to abiotic and biotic stressors, endophytes can play an important role in succession. In colonizing host species, endophytes can accelerate succession by enhancing host population growth rate or establishment (Rudgers et al. 2005, Uchitel et al. 2011). Alternatively, Rudgers et al. (2007) showed that endophyte symbiosis in an invasive, agronomic grass can retard succession by maintaining the dominance of the resident host species. To date, most studies of endophytes in a successional context have focused on only one life history stage (but see Uchitel et al. 2011). In order to predict how symbionts can affect succession by altering the establishment or persistence of their hosts, we need to combine effects across demographic stages to project rates of population growth. Due to imperfect vertical transmission, hosts may persist as mosaics of symbiotic and symbiont-free patches that can produce alternative stable states in the system through succession (Janos 1980). Therefore, it is necessary to explore symbiont transmission dynamics, in addition to host demographic stages, in order to understand the possible population-level effects of endophytes on the results of succession.

We employed a combination of field-based symbiont removal experiments and demographic models that incorporated continuous variation in host size and discrete loss of symbiosis due to imperfect transmission. Using a disturbance-specialized native grass host, we studied how fungal symbiosis influences the persistence or local extinction dynamics of an early-successional host in a late-successional context. Specifically, we investigated the following questions. 1) How does a vertically transmitted fungal symbiont affect individual-level demographic rates of its host? 2) What are the equilibrium population growth rates of symbiotic and non-symbiotic hosts, and which demographic processes account for any difference between them? In the context of succession, populations may be far from their stable size structure. We therefore additionally asked: 3) what are the transient dynamics of symbiotic and non-symbiotic hosts starting from a non-equilibrium size distribution? Finally, we asked: 4) how does imperfect vertical transmission interact with demographic rates to affect host population growth and the frequency of symbiosis? We address these questions using a novel modeling approach that accommodates dynamic transitions among both continuous (size) and discrete (symbiont status) state variables. While our models are grounded in the ecology of grass–epichloae symbioses, they are widely applicable to vertically transmitted symbioses in both plants and animals.

Material and methods

Study system

Agrostis hyemalis (winter bentgrass) is a C3 (cool season) short-lived cespitose grass native to the eastern United States and Canada, the Caribbean, Mexico, Ecuador, and Peru (Soreng and Peterson 2003, Barkworth et al. 2007). Able to disperse long distances when the culm breaks and tumbles in the wind, *A. hyemalis* is thought to specialize on patches of disturbed habitat through rapid colonization (Rabinowitz 1978). Throughout its range, populations are naturally infected with the vertically-transmitted endophyte *Epichloë amarillans* (mean infection frequency: 69%; range 0–100%; Rudgers et al. 2009). The endophyte of *A. hyemalis* synthesizes loline alkaloids, which can deter herbivores (Schardl et al. 2007, P. Nagabhyru and C. L. Schardl unpubl.). We have never observed horizontal (contagious) endophyte transmission in *A. hyemalis*, though some populations are capable of producing the fungal reproductive structures responsible for contagious transmission (stromata and ascospores, unpubl.).

Plant material

Seeds from 30 randomly chosen *A. hyemalis* plants were collected from Stephen F. Austin Experimental Forest in Nacogdoches, Texas (31°29′58″N, 94°46′01″W). Seedlings grown from these seeds were 97% endophyte-symbiotic. The seeds were pooled across the 30 parents and half of them were heat-treated for 6 min in a 62°C water bath to remove the endophyte (Davitt et al. 2011). All seeds were dried overnight and sown directly into to 115-ml pots with

potting soil during Jun–Aug 2007. Plants were grown in the greenhouse under relatively constant conditions (13–25°C, no supplemental light) and watered daily. Endophyte status was checked for every plant by staining thin sections of inner leaf sheath with aniline blue-lactic acid stain (Bacon and White 1994) and examining the tissue with a compound brightfield microscope at 200–400×.

Site selection

Agrostis hyemalis individuals were planted into the natural vegetation in a mixed hardwood understory habitat at Stephen F. Austin Experimental Forest in Nacogdoches, Texas (31°29′55″N, 94°46′14″W, elevation 61 m) on 10 Nov 2007. To ensure that this site selection would not bias our results, we compared our site to two nearby natural *A. hyemalis* populations with respect to a number of biotic and abiotic conditions and also the size distributions of *A. hyemalis* individuals. At least one of the two sites of natural populations was statistically indistinguishable from our experimental populations for all of the following variables: soil moisture content, soil pH, soil conductivity, macro and micro nutrient levels, percentage cover by vegetation. The main differences between natural and experimental populations were that experimental plots received lower photosynthetically active radiation and had lower species richness. Thus, we expect that the experimental plots are representative of a later successional stage, in which *A. hyemalis* populations would be in decline. Additionally, the size distribution of experimental plants was consistent with natural populations. See Supplementary material Appendix 1 for full descriptions and statistical analyses of conditions at the experimental and natural sites.

Field experimental design

Each 3×2.5 m plot consisted of either endophyte-symbiotic (E+) or endophyte-free (E−) plants, with 20 individually tagged plants placed 0.5 m apart in a 4×5 grid. Plots were separated by a minimum of 5 m to limit seed dispersal between plots. Five replicate plots were established for each endophyte treatment. One E− plot was excluded from our analyses because of a major tree-fall disturbance, which dramatically increased population growth rate, consistent with the importance of disturbance for this species. Within a single plot, each plant came from a different parent; therefore, all plots had similar levels of initial genetic diversity. We quantified survival, size (number of tillers), and seed production for all individuals from 2008 through 2010, including new individuals that recruited from within-plot seed production. Since our experimental populations were planted at microsites where *A. hyemalis* did not occur, we were able to confidently assign endophyte status to new individuals recruiting in years following plot establishment. Additionally, we determined rates of vertical transmission of the endophyte for each plot every year of the experiment. Further details regarding demographic response variables are provided in the Supplementary material Appendix 2.

Integral projection model

Field data were used to parameterize a size-structured integral projection model (Easterling et al. 2000, Ellner and Rees 2006). The IPM predicts change in population size structure, $n(y)$, over one year (Δt) as:

$$n(y)_{t+1} = \int_\Omega [p(y, x) + f(y, x)] n(x)_t \, dx \qquad (1)$$

Size-dependent survival and growth are represented by:

$$p(y, x) = s(x) \, g(y, x) \qquad (2)$$

Here, $s(x)$ is the probability that an x-sized individual survives the one year period, and g(y, x) is the probability of growth from size x to size y over the one year period. Reproduction, $f(y, x)$, represents the production of seedlings of size y from maternal plants of size x, and it can be decomposed into the number of seeds produced by a size x plant ($f_n(x)$), the probability of seedling establishment (p_E), and the distribution of seedling size ($d(y)$):

$$f(y, x) = f_n(x) \, p_E \, d(y) \qquad (3)$$

The survival, growth, and reproduction functions together form the IPM kernel, a surface that describes all possible transitions in a single year.

We first built separate models for E+ and E− populations of *A. hyemalis*, and then we integrated these into one model by linking the E+ and E− sub-populations by vertical transmission. Construction and analysis of the IPM were conducted in R ver. 2.12.2 (R Development Core Team).

Fitting demographic functions

Data were pooled across years (2008–2010) into a one-year (t to $t+1$) census interval. The size variable (x) was the natural logarithm of tiller number (range: 1–42). While tiller number is itself a discrete variable, we used it as a proxy for continuous variation in biomass (see also Williams and Crone 2006).

We fit the size-dependent demographic functions (survival, growth and reproduction) with data collected from our field experiments using generalized linear models (glm). For the survival and reproduction functions, we fit four candidate models (hypotheses): 1) no effect of endophyte status (null model), 2) effects of endophyte status on only the slope with respect to size, 3) effects of endophyte status only on the intercept, and 4) effects of endophyte status on both parameters. For growth, we constrained the function through the origin (log(one tiller), log(one tiller)), because plants cannot be measured as smaller than one tiller. Thus, we fit only two candidate models for the growth function: 1) and 2). We quantified model fit using Akaike's information criterion (AIC) and AIC weights, which give the proportional weight of evidence in favor of each candidate model (Burnham and Anderson 2002). If no model had an AIC weight > 0.9, we used model-averaging (Burnham and Anderson 2002). To assess contributions of spatial variation among plots to endophyte effects, we re-fit all functions with plot as a random effect in all models (lmer() function in R), and the results did not differ

qualitatively. We proceeded with the simpler analysis because it allowed us to bootstrap a confidence interval around the effect of endophytes on λ (below).

For the growth function $g(x)$, linear regressions were fit to sizes in years t and $t+1$ (n = 188), using a normal distribution of residuals. We also tested whether the residual variance of the growth function varied with plant size and it did not. For the survival function $s(x)$, a binomial glm was fit to the mortality data (0/1) in year t based on size in year t (n = 365). For the fecundity function, $f_n(x)$, a Poisson glm was fit to data for size-dependent seed production (n = 697). Seed production by two E− plants was excluded from the analysis as outliers.

The probability of establishment (p_E) was estimated as (recruits per plot in year $t+1$)/(total seeds produced per plot in year t). p_E did not differ significantly between endophyte treatments ($t = 1.017$, DF = 17, p = 0.324). Therefore, the overall average p_E (0.0023) was used in the model. For the distribution of seedling sizes, all but one

of 364 recruits across all plots consisted of one tiller at the time of the census (one plant had three tillers). We therefore used a common exponential size distribution ($d(y)$) for the E− and E+ models (mean = log(1.005 tillers)).

The IPM kernel for each endophyte treatment was discretized into a 150×150 matrix from which we calculated the population growth rate (λ) as the dominant eigenvalue (values for λ stabilized around this matrix size). The lower integration limit of the model was the minimum observed size, and the upper integration limit of the model was 1.1* the maximum observed size. However, because small plants were predicted to shrink (Results), these plants were being distributed below the lower integration limit, which artificially inflated mortality at the smallest sizes. To solve this problem, we used a truncated normal distribution to constrain the residual variance of the growth function at the integration limits (Williams et al. 2012).

Testing effects of endophyte symbiosis on population growth (λ)

We used a randomization approach to generate a null expectation for the difference in λ between E+ and E− populations (Manly 1991). In each of 10 000 iterations, endophyte status was randomly re-assigned to all individuals, and we re-calculated λ for E− and E+ populations. We estimated the confidence interval of the null difference ($\lambda_{E+} - \lambda_{E-}$) as the inner 95% quantile of the distribution of differences. We then asked whether the observed difference due to the endophyte treatment fell within this interval.

Transient dynamics

Our equilibrium results indicated rapidly declining population growth rates with stable size distributions dominated by seedlings and small plants. We asked whether the effects of fungal endophytes would differ during the initial stages

of local extinction, starting from a population size structure with more large plants (as expected in a population that was previously stable). We therefore simulated transient dynamics of E− and E+ populations based on vital rates from our experimental plots but starting from a size distribution that was estimated from one of the naturally occurring *A. hyemalis* populations that we censused. We numerically estimated the transient population growth rate (λ_t) for E− and E+ populations during the first ten years of the simulation.

Imperfect vertical transmission

To link the E− and E+ populations from the experimental plots by imperfect vertical transmission, we nested the IPM kernels within a larger 2×2 matrix that described transitions between E+ and E− plants. This approach is akin to the mega-matrix approach for populations with multiple, discrete state variables (Caswell 2001), only our sub-matrices were the approximating matrices for the continuous IPM kernels. Our mega-matrix model took the form:

$$\begin{pmatrix} E^-(y)_{t+1} \\ E^+(y)_{t+1} \end{pmatrix} = \begin{pmatrix} \int\left[p^-(y,x) + f^-(y,x) \right]dx & \int\left[(1-\tau)f^+(y,x) \right]dx \\ 0 & \int\left[p^+(y,x) + \tau f^+(y,x) \right]dx \end{pmatrix} \begin{pmatrix} E^-(x)_t \\ E^+(x)_t \end{pmatrix} \quad (4)$$

The p and f IPM components are super-scripted with endophyte status. The vertical transmission rate (proportion of E+ seeds produced by E+ plants) is represented by τ. Since no incidences of horizontal transition have been documented in these experimental plots, the lower-left quadrant of the mega-matrix was a matrix of zeroes. Because endophyte loss occurred during seed production, only reproduction ($f(x, y)$) contributed to the E+ to E− transition. We estimated the asymptotic rate of population growth (λ) for the entire *A. hyemalis* population using estimates of τ from seedlings checked in each year. We estimated the asymptotic frequency of endophyte symbiosis ($w+$) by calculating the stable distribution (right eigenvector) of the mega-matrix and dividing the sum of the E+ size distribution by the total population size distribution. Due to high variability in both τ and p_E, we explored their effects on λ and $w+$ by varying τ from 0 to 1 and p_E from 0 to 0.032 (approximately 1.5 times the maximum p_E in our field experiment). This allowed us to better understand how the vertical transmission rate of a symbiont and the probability that seedlings establish interactively alter plant population growth and the equilibrium endophyte frequency.

Results

1) How does a vertically transmitted fungal symbiont affect individual-level demographic rates of its host?

When field data is used to parameterize demographic functions, endophyte symbiosis was found to modify size-dependent growth, reproduction, and survival, but in contrasting ways. On average, all plants shrunk to smaller sizes over the census interval (Fig. 1). However, E+ plants
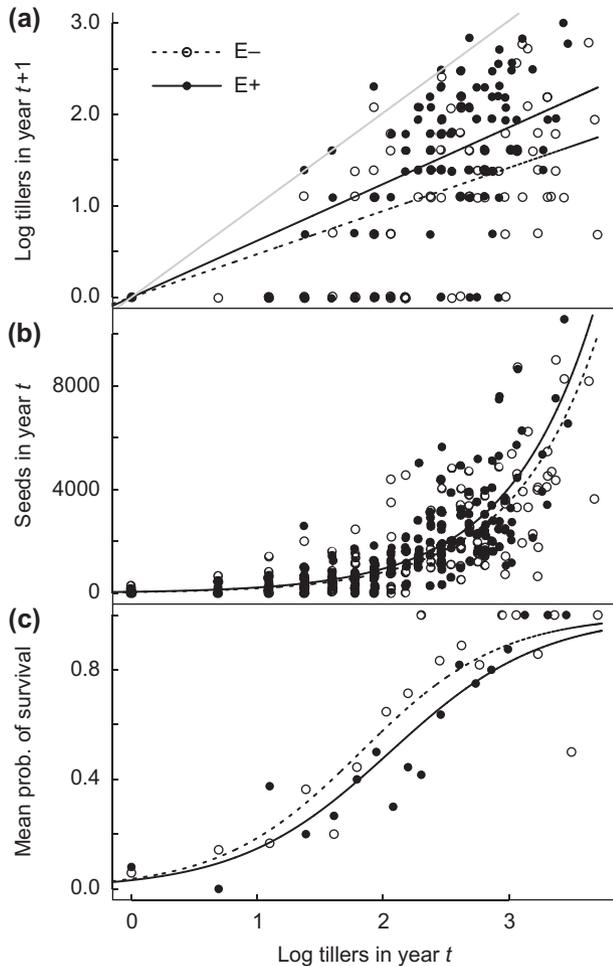
Figure 1. Growth (a), reproduction (b), and survival (c) of endophyte-symbiotic (E+, filled symbols) and endophyte-free (E−, open symbols) *Agrostis hyemalis* plants in relation to plant size (ln(number of tillers)). (a) Growth lines represent the best-fit linear models for the change in size from year t to year $t + 1$. (E− n = 86; E+ n = 101). The gray line shows the 1:1 relationship (size stasis). (b) Reproduction lines represent the best-fit Poisson models for the relationship between size in year t and the number of seeds in year t (E− n = 398, E+ n = 299). (c) Survival lines represent the binomial models for the relationship between size in year t and survival from year t to year $t + 1$, averaged across candidate models based on AIC weights (Table 1). Points represent mean probabilities of survival for binned plants within a discrete size class (E− n = 158, E+ n = 207).

Table 1. Candidate models of *Agrostis hyemalis* population dynamics (growth, reproduction and survival) and model fitting.

Model	ΔAIC	AIC weight
Growth		
$size_{t+1} = b \times size_t$	7.2	0.027
$size_{t+1} = b_e \times size_t$	**0**	**0.973**
Reproduction		
$\ln(seeds_t) = a + b \times size_t$	3070	0
$\ln(seeds_t) = a_e + b \times size_t$	7	0.022
$\ln(seeds_t) = a + b_e \times size_t$	287	0
$\ln(seeds_t) = a_e + b_e \times size_t$	**0**	**0.978**
Survival		
$logit(survival_{t+1}) = a + b \times size_t$	1.9	0.154
$logit(survival_{t+1}) = a_e + b \times size_t$	0.5	0.312
$logit(survival_{t+1}) = a + b_e \times size_t$	0	0.390
$logit(survival_{t+1}) = a_e + b_e \times size_t$	2.0	0.144

Best fit models are shown in bold. Subscript *e* indicates endophyte effect on the intercept (a) or slope (b), and subscript *t* indicates year. Data and best fit models are shown in Fig. 1.

2) What are the equilibrium population growth rates of symbiotic and non-symbiotic hosts, and which demographic processes account for any difference between them?

Both E+ and E− populations were predicted to decline ($\lambda \ll 1$) and the stable size distributions were dominated by small plants due to deterministic shrinkage. However, the estimated λ for the E+ population (0.233) was significantly and 22% greater than for the E− population (0.192), despite the survival cost of endophyte symbiosis depicted in Fig. 1 (observed difference [E− − E+ = −0.041] fell outside the bootstrapped 95% confidence interval of the null difference [E− − E+: (−0.000163, 0.000127)]). LTRE analysis (Supplementary material Appendix 3) indicated that the greater growth rate of the E+ population was driven by the positive effect of endophyte symbiosis on plant growth, that this positive effect over-whelmed the negative effect on survival, and that the positive effect on fecundity contributed negligibly to the observed difference in λ (Fig. 2). Individual plot-level estimates of λ were variable (E+ = 0.219 ± 0.038; E− = 0.196 ± 0.062). However, accounting for spatial variation with the random effect of plot in the demographic models did not qualitatively change the results for λ, which was 26% higher for E+ than E− (E+ = 0.172, E− = 0.136).

3) What are the transient dynamics of symbiotic and non-symbiotic hosts, starting from a non-equilibrium size distribution?

For both symbiotic and symbiont-free populations the transient population growth rates were two times larger than the equilibrium predictions for λ (~0.40). While the equilibrium results indicate that benefits of endophyte symbiosis outweighed the survival cost, results of our transient analysis revealed that this is not necessarily the case during the initial stages of local extinction. When populations were initiated at a size distribution estimated from a nearby natural population, the net effect of endophytes on λ was at first negative. In fact, the transient decrease in λ due to

exhibited greater growth (weaker shrinkage) than E− plants, and including endophyte status significantly improved the fit of the growth model (Table 1). On average, E+ plants also produced more seeds than E− plants of the same size, and this increase was greater for larger plants (Fig. 1). Thus, a seed production model with endophyte effects on both the intercept and slope provided the best fit (Table 1). For survival, larger plants were generally more likely to survive to the next year (Fig. 1). No single model provided the best fit to the survival data (Table 1). The model-averaged function indicates that E− plants tended toward higher survival than E+ of the same size, particularly in the middle and upper end of the size distribution (Fig. 1, Table 1).
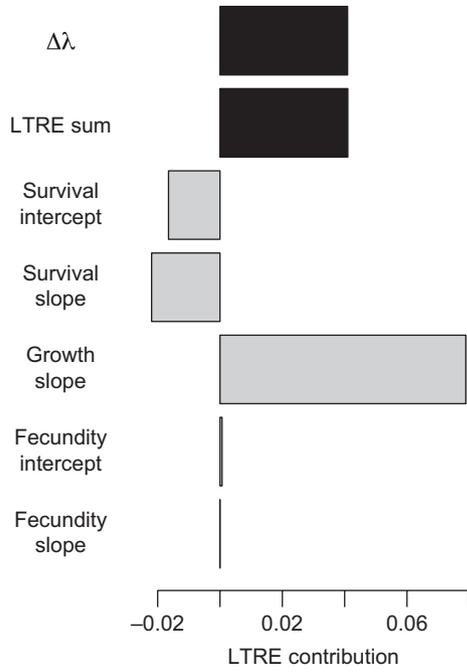
Figure 2. Contributions of demographic parameters to the total effect of endophytes on λ, estimated by a life table response experiment (LTRE). Bars represent the difference in parameter values (E+ minus E−) multiplied by parameter sensitivity evaluated at the midpoint value (Eq. A1). The sum of all such differences is represented as by the black bar labeled LTRE sum, which is equal to Δλ, or the total difference in growth rate between endophyte-symbiotic and endophyte-free populations (E+ minus E−).

endophyte symbiosis was equal in magnitude to the long-term positive endophyte effect (Fig. 3). Thus, when larger plants dominate populations, the survival cost of endophytes (greater in larger plants) can temporarily outweigh their positive effects on growth and fecundity. However, the net negative effect was short-lived (< 2 years of projection);
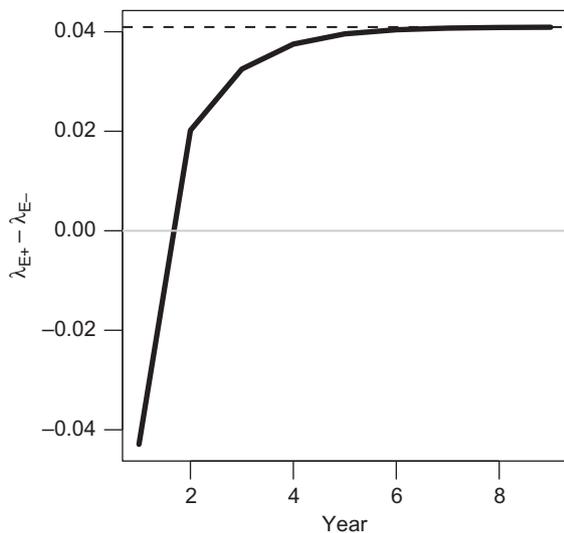

Figure 3. Difference in transient population growth rates (E+ minus E−) over time. Equal λ are represented by the solid gray line while the asymptotic difference in λ is indicated by the dashed line.
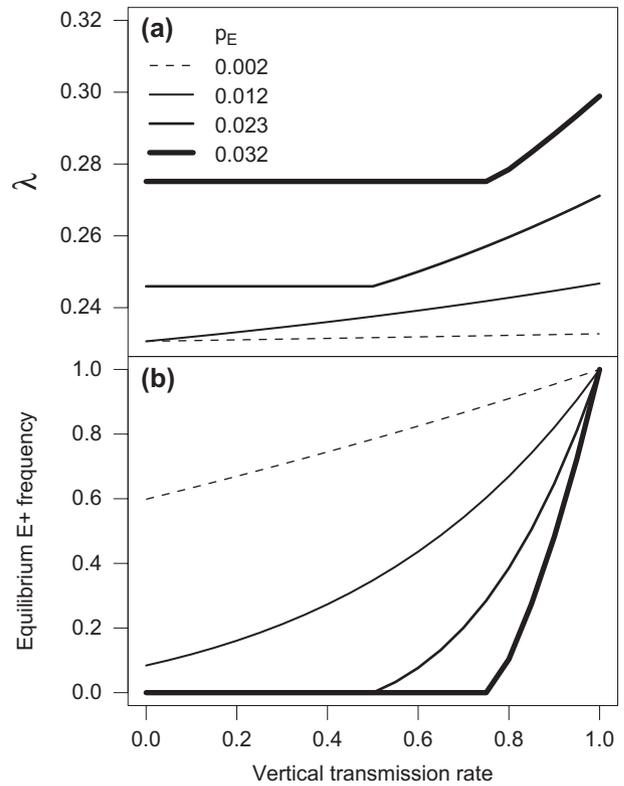

Figure 4. Variation in the population growth rate (λ) (a) and equilibrium frequency of endophyte symbiosis ($w+$) (b) with variation in endophyte vertical transmission rate. Line types and widths correspond to probabilities of establishment (dashed line gray lines shows results for the approximate observed mean value).

simulated populations converged on asymptotic predictions of net endophyte benefit after only six years of projection (Fig. 3).

4) How does imperfect vertical transmission interact with demographic rates to affect host population growth rate and the equilibrium frequency of symbiosis?

Integrating the E− and E+ sub-populations within the megamatrix framework allowed us to explore the effects of vertical transmission rate on the dynamics of the entire host population. In general, there was a positive relationship between τ and λ due to the net benefits of endophyte symbiosis (Fig. 4). In the hypothetical case of perfect transmission (τ = 1.0), λ was equal to that determined for the E+ population alone and E+ plants dominated the population ($w+$ = 1.0), as expected. Surprisingly, when τ = 0.0, λ was much greater than that determined for the E− population alone and $w+$ = 0.56, despite no source of new infected plants. This result occurred because the stable size distribution of the declining population included some large E+ plants. Through enhanced growth and fecundity, these plants retarded the rate of extinction despite producing no new E+ plants. Our simulation analyses of the megamatrix model (not shown) verified that when τ = 0.0 populations initially lacking E+ plants declined at the rate predicted by the E− model. Thus, the presence of

any endophyte symbiosis can boost population growth rates and retard the local extinction of plant hosts, even if vertical transmission rates are very low.

Variation in the establishment probability (p_E) modified the effects of vertical transmission (τ) on population growth (λ) and equilibrium symbiont frequency ($w+$) in interesting and complex ways for these shrinking populations (Fig. 4). While increasing p_E generally increased λ, it also decreased $w+$ for all but perfect vertical transmission rates. This surprising result can be understood by considering two things: 1) increasing p_E increases the relative abundance of seedlings (E− and E+ equally) in the host population, and 2) imperfect transmission provides two sources of E− seedlings (E− and E+ maternal plants), whereas there is only once source of E+ seedlings (E+ maternal plants). Thus, increasing the rate of recruitment disproportionately affected the non-symbiotic component of the host population because the stable size distribution was dominated by seedlings. At high p_E, there is a threshold value of τ below which E+ plants cannot persist in the host population (Fig. 4b) despite their enhanced demographic performance. Accordingly, τ has no effect on λ below these thresholds. Above the threshold, increasing τ has increasingly positive effects on λ with larger values of p_E due to the greater abundance, growth, and fecundity of E+ plants relative to E− plants.

Discussion

Integrating over the host life cycle revealed substantial population-level effects of symbiosis, some obvious and some surprising. With an IPM parameterized from experimental field populations of *Agrostis hyemalis*, symbiosis with the endophyte *Epichloë amarillans* was predicted to increase the equilibrium population growth rate by approximately 22%. As in our study, Faeth (2009) documented positive effects of fungal endophytes on host seed production and interpreted this as evidence for reproductive parasitism, leading to survival costs for the host. Indeed, we found evidence for reduced survival of E+ plants. However, our demographic models, which account for processes over the entire life cycle, demonstrate that positive effects of endophytes on seed production and, especially, on plant growth outweighed the survival cost. While the benefits of endophytes for growth and seed production are consistent with some previous studies (reviewed by Cheplick and Faeth 2009), prior work has not been able to predict the effects of endophyte symbiosis on host populations or the equilibrium frequency of symbiosis. Other studies have also shown net positive effects of symbionts despite negative effects on some demographic rates (Damiani 2005, Rudgers et al. 2012). Our new results and these previous studies highlight the value of a population dynamics approach for understanding host–symbiont interactions.

What ecological traits may underlie the net benefits of endophyte-symbiosis for host population dynamics? First, vertically transmitted endophyte-symbioses in agronomic grasses have generally been shown to decrease herbivory (Clay et al. 1993), but the effect of endophytes on herbivores, especially for native grasses, is often dependent upon the identity of both the herbivore and the host (Afkhami and Rudgers 2009). Despite these contingencies, the same experimental field populations studied here showed a significant reduction in leaf damage to E+ plants by insect herbivores, supporting the hypothesis of enhanced resistance (Crawford et al. 2010). The mechanism for this resistance is likely the production by *E. amarillans* of insect-deterring loline alkaloids through the biosynthetic pathway regulated by the *LOL*-cluster of genes (Schardl et al. 2007). Second, endophyte frequency is positively associated with drought occurrence in some natural populations of grasses (Lewis et al. 1997), and experiments have shown that reduced water availability can increase endophyte benefits to hosts (reviewed by Malinowski and Belesky 2000, Cheplick and Faeth 2009). Plants from our *A. hyemalis* population grown in the greenhouse received benefits of endophyte symbiosis (faster development time and greater number of inflorescences, seed mass, and reproductive effort) only under low water availability (Davitt et al. 2011). All growing seasons (Oct–May 2007–2010) included in the current study were relatively dry (average of 50.1 ± 13.3 cm total rainfall in comparison to the average precipitation of 87.8 cm expected during these months). Thus, enhanced tolerance to drought stress may be a second mechanism for the observed increase in growth and reproduction of endophyte-symbiotic plants over endophyte-free plants. Third, grass species restricted to low light environments, like the late-succession shaded pine and mixed hardwood understory of our experimental plots, are more likely to harbor endophytes, and endophyte-symbiosis increased the number of inflorescences produced under high shade levels for the closely related host grass *Agrostis perennans*, which also hosts the endophyte *E. amarillans* (Davitt et al. 2010). Thus, a variety of mechanisms of endophyte-mediated protection against biotic and abiotic stressors likely underlie the net boost to population growth rates observed here.

Despite positive effects of endophyte symbiosis, the estimated equilibrium population growth rates were strikingly low due to a predicted mean decrease in future size for all plant sizes and for both E+ and E− plants; as a result, the equilibrium size distributions were dominated by very small plants. However, natural populations of early-successional species, such as *A. hyemalis*, experiencing forest encroachment would not be expected to have a stable size distribution. Rather, they would begin this successional transition at a larger size distribution characteristic of their former growth rate. Therefore, analysis of the transient dynamics of the population was able to provide more realistic approximations of the experimental population that was started with adult plants. This analysis elucidated an initial net negative effect of endophyte symbiosis due to the enhanced survival cost on large sized plants (which are rare in stable, declining populations). As the size distribution changed, the net effect of endophyte symbiosis quickly became positive, and, overall, endophyte-symbiosis was predicted to lengthen the persistence of *A. hyemalis* populations after disturbance. Previous studies have shown that the mutualistic effects of endophyte symbiosis on their hosts can alter the rate of successional transitions (Rudgers et al. 2005, 2007, Uchitel et al. 2011), and our results

suggest this is a possibility for *A. hyemalis* populations subject to canopy closure by woody plants. However, more work is needed to characterize the effect of *A. hyemalis* on invasions by late-successional species in order to determine whether endophyte symbiosis in this host species has the potential to affect community-wide succession.

In addition to the effect of endophytes on host population dynamics, the results from our megamatrix analyses shed new light on the factors that determine the frequency of vertically transmitted symbionts within size-structured host populations. Theory predicts that even when symbionts enhance host population growth, transmission rates below a certain threshold can lead to symbiont extinction; this occurs because the magnitude of beneficial effect does not sufficiently compensate for the rate of symbiont loss (Ravel et al. 1997, Gundel et al. 2008). Ours is the first study, to our knowledge, to document the occurrence of such transmission thresholds in an empirical system. Interestingly, the seedling establishment parameter (p_E) critically determined the occurrence and location of the extinction threshold. This surprising result could not be predicted by current symbiosis theory, which does not account for realistic demographic complexity in the host population, and highlights the potentially complicated interactions that determine the balance of symbiont gain and loss. While increasing seedling establishment generally had positive effects on population growth, it actually exacerbated the effects of imperfect transmission to seeds, increasing the relative abundance of E− hosts and increasing the demographic burden on E+ hosts for maintenance of endophytes in the population. Such antagonism between early life-cycle establishment processes and symbiont frequency is likely to be a widespread feature of symbioses with imperfect vertical transmission. We expect that our approach of combining size-continuous and symbiont-discrete demographic models will be broadly applicable to other symbioses and will focus much-needed empirical attention on imperfect vertical transmission (see also Gundel et al. 2011).

Conclusion

This work provides evidence that the application of demographic models can better inform theories of and predictions for how symbioses affect host populations in nature. Such models can allow researchers to link positive and negative effects of these relationships throughout a participant's lifetime to equilibrium and transient population growth rates and extinction risks, which have implications for conservation and management. Here, our size- and symbiont-structured model predicts that endophyte symbiosis can boost a disturbance-specialized native grass species' population growth rates and persistence in late-successional contexts through benefits for growth and reproduction, despite a survival cost. Yet, population growth rates and the equilibrium frequency of symbiosis are sensitive to the complex interactions between vertical transmission rate and early demographic stages.

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