

Context-dependent variability in the population prevalence and individual fitness effects of plant–fungal symbiosis

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Abstract

1. Heritable symbionts are often observed at intermediate prevalence within host populations, despite expectations that positive fitness feedbacks should drive beneficial symbionts to fixation. Intermediate prevalence may reflect neutral dynamics of symbionts with weak fitness effects, transient dynamics of symbionts trending towards fixation (or elimination), or a stable intermediate outcome determined by the balance of fitness effects and failed symbiont transmission. Theory suggests that these outcomes should depend on symbiont-conferred demographic effects and vertical transmission efficiency, which may both depend on environmental context.
2. We established experimental populations of winter bent grass *Agrostis hyemalis* across a range of prevalence of the heritable fungal endophyte *Epichloë amarillans*. Using irrigation, we elevated the precipitation for half of the populations, which we hypothesized would weaken the benefits of symbiosis. Across two annual transitions, we assayed 5,485 individuals to determine prevalence and censused 954 individuals for demographic (survival, flowering, reproduction and recruitment) and vertical transmission data. We used hierarchical Bayesian models to infer long-run equilibria from short-term changes in symbiont prevalence and estimated demographic vital rates to link individual-level effects to population-level outcomes.
3. We found evidence for all three proposed mechanisms for intermediate symbiont prevalence, but the outcome differed qualitatively across years and precipitation treatments. In the first year, symbionts trended towards fixation under drought conditions but drifted neutrally under elevated precipitation. Fixation likely arose from symbiont-conferred recruitment benefits outweighing reproductive costs under the drought conditions, while elevated precipitation tempered these effects. In the second transition year, we inferred stable intermediate prevalence across both precipitation treatments, which indicated a balance between symbiont-conferred recruitment benefits that allowed low-prevalence populations to increase and imperfect transmission that caused high-prevalence populations to decrease.

4. *Synthesis*. We find support for neutral, transient and stable mechanisms underlying symbiont prevalence, indicating that symbiont prevalence is often pushed and pulled in different directions by the composite outcome of symbiont effects on demographic rates and transmission efficiency, and the way in which these processes respond to environmental context.

KEYWORDS

Agrostis hyemalis, plant demography, dynamic stability, *Epichloë amarillans*, hierarchical Bayesian model, long-term outcomes, mutualism, plant-microbe

1 | INTRODUCTION

Symbioses between micro- and macro-organisms are found across the tree of life (Gibson & Hunter, 2010; McFall-Ngai et al., 2013; Roossinck, 2015), and transmission can be a defining feature. Symbiont transmission can occur contagiously, as seen in bacteria and arthropods (Kim & Lee, 2017; Renoz et al., 2019), squids (McFall-Ngai et al., 2013) and legumes (Young & Johnston, 1989), or hereditarily, as observed with bacteria and viruses in arthropods (Hedges et al., 2008; Koga et al., 2012; Longdon et al., 2017), zooxanthellae in corals (Hirose et al., 2001), fungi in grasses and arthropods (Clay, 1990; Gibson & Hunter, 2009) and organelles in eukaryotes (Margulis, 1996). Symbionts may have diverse and ecologically important effects on their hosts, affecting nutrient uptake (Gündüz & Douglas, 2009), abiotic stress tolerance (Malinowski & Belesky, 2000) and pathogen and parasitoid resistance (Corbin et al., 2017; Heyworth & Ferrari, 2015; Oliver et al., 2005).

While some symbioses are so integrated that they are effectively obligate (e.g. eukaryotic organelles), many of them are more flexible (facultative for one or both partners). This means that populations may consist of a mix of symbiotic and non-symbiotic hosts. For heritable symbionts, these mixed host populations are puzzling. Heritability links the host and symbiont interaction through successive generations, thereby tightly coupling their fitness, and should lead to the evolution of host-symbiont mutualism and fixation within the host population, whereas a detrimental symbiont is expected to be eliminated due to its fitness costs. Despite these clear expectations, intermediate prevalence is commonly found in arthropod-bacterial (Chen & Purcell, 1997; Hilgenboecker et al., 2008; Jaenike et al., 2010; Renoz et al., 2019; White et al., 2013) and grass-fungal symbioses (Rudgers et al., 2009; Semmartin et al., 2015), and is likely pervasive across other heritable symbioses that have received less attention (e.g. Baker et al., 2018; Linneman et al., 2014).

Three hypotheses may explain the intermediate prevalence of heritable symbionts. First, in the absence of any fitness effects on hosts, prevalence may follow a process akin to genetic or ecological drift. In the long-term, neutral dynamics should lead to the random fixation or elimination of symbionts, but this process may proceed slowly in large host populations, resulting in long-term mixtures of symbiotic and non-symbiotic hosts. Second, under the expectation

that fitness feedbacks lead to fixation of heritable mutualists (or elimination of heritable parasites), intermediate symbiont prevalence may be a transient snapshot of a system trending towards symbiont fixation. As is the case for other types of ecological dynamics, extrinsic perturbations (Connell, 1978; Sousa, 1979) and long transients (Hastings et al., 2018) may prevent symbiont prevalence from reaching an equilibrium state. Third, intermediate prevalence may be a stable outcome even for symbionts with strong, positive fitness effects. Imperfect vertical transmission, where symbiotic hosts produce some symbiont-free offspring, is the stabilizing mechanism most commonly invoked (Bibian et al., 2016; Cavazos et al., 2018; Gundel et al., 2008), and has been documented from grasses (Afkhami & Rudgers, 2008; Cavazos et al., 2018), mosquitoes (Bosio et al., 1992) and budworms (van Frankenhuyzen et al., 2007). Theory for population dynamics of heritable symbionts is now well developed (Bibian et al., 2016; Gundel et al., 2008; Hancock et al., 2011; Turelli, 1994), but field-based experimental tests remain relatively rare (Cavazos et al., 2018; Clay et al., 2005; Santangelo et al., 2015). Consequently, the relative contributions of these processes to symbiont prevalence in natural populations remain poorly resolved.

While a single snapshot could reflect any one of these three mechanisms, they may be differentiated by considering the dynamics of symbiont prevalence in host populations through time (Figure 1a–c). Under the neutral scenario, symbiont prevalence observed in a given population (prevalence_t) should, on average, reflect the previous condition (prevalence_{t-1}) and there should be no systematic change in prevalence (Figure 1a). Under the hypothesis of intermediate prevalence as a transient state, prevalence should tend to increase (or decrease) for any initial condition (Figure 1b), given a positive (or negative) fitness effect. Lastly, if intermediate prevalence is dynamically stable, then it should exhibit a return tendency, with prevalence increasing below and decreasing above the equilibrium (Figure 1c).

These hypotheses are unified by two individual-level processes that give rise to population-level outcomes: the fitness effects of the symbiont on its host and the efficiency of vertical transmission. Most symbiosis studies focus on either individual- or population-level processes, but rarely both in parallel. Observational surveys in plants (Afkhami & Rudgers, 2008; Rudgers et al., 2009; Semmartin

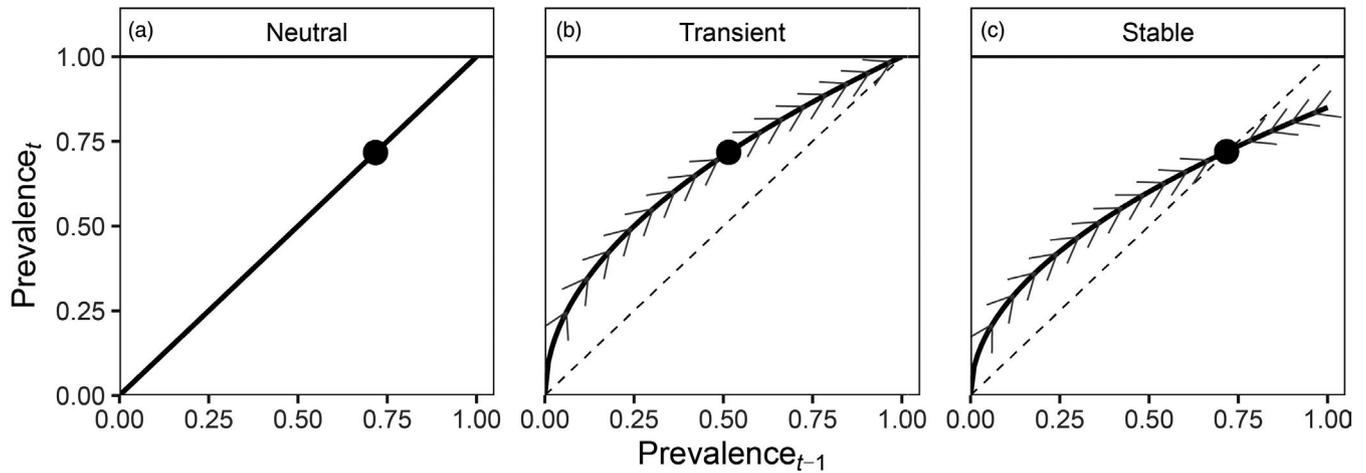


FIGURE 1 Conceptual representation of alternative hypotheses for intermediate prevalence of heritable symbionts. Circles represent a hypothetical snapshot of symbiont prevalence (*c.* 72% symbiotic) in a population at time *t*, which may arise from any one of these mechanisms. (a) Neutral dynamics: on average, prevalence tracks previous conditions with no deterministic forces that push it in any direction. (b) Transient dynamics: prevalence increases for any non-zero initial condition for beneficial symbionts and leads to eventual fixation of mutualists (dashed line shows prevalence_{*t*} = prevalence_{*t-1*}). (c) Stable intermediate: prevalence increases below and decreases above an intermediate equilibrium of mutualists

et al., 2015; Sneek et al., 2017) and animals (Chen & Purcell, 1997; Darby & Douglas, 2003; Fukatsu et al., 2001; Hilgenboecker et al., 2008; Jaenike et al., 2010) have documented variability in population prevalence of heritable microbes, typically with little or no knowledge of individual fitness effects. A different body of work has contrasted the fitness of symbiotic and non-symbiotic hosts (Oliver et al., 2007; Sakurai et al., 2005), often with limited insight into population-scale outcomes. Still fewer studies have used demographic models to infer population-level outcomes from individual-level effects (Chung et al., 2015; Gibert et al., 2015; Rudgers et al., 2012; Yule et al., 2013). However, direct observation and experimentation at both scales are rare (Cavazos et al., 2018; Himler et al., 2011), leaving open questions regarding how well the symbiont fitness affects its host and the efficiency of vertical transmission predict population dynamics of symbionts.

Fitness effects and efficiency of vertical transmission may vary across environmental contexts. Indeed, many heritable symbionts confer benefits to hosts in the face of stressors, such as natural enemies (Cockburn et al., 2013; Oliver et al., 2007; Reyes et al., 2019) and environmental harshness (Afkhami et al., 2014; Clay & Schardl, 2002; Giauque et al., 2019; Oliveira & Castro, 1998). In the absence of—or shift in—stressors, otherwise beneficial symbionts may become neutral (Oliver et al., 2007) or even parasitic (Baker et al., 2018; Reyes et al., 2019). Making matters more complicated, symbiont effects may vary across host life stages and vital rates (e.g. survival, growth, reproduction and recruitment; Bibian et al., 2016; Chung et al., 2015; Gibert et al., 2015; Palmer et al., 2010; Rudgers et al., 2012; Yule et al., 2013). Context-dependent variation in vertical transmission has received less attention, yet some studies have shown different transmission patterns dependent on abiotic (Sneek et al., 2017) or biotic (Rock et al., 2017) factors. Taken together, context-dependent variability

in fitness effects and transmission raises the possibility that the qualitative dynamics of host–symbiont interactions (Figure 1) may vary with environmental context.

We conducted a field experiment with winter bent grass (*Agrostis hyemalis* (Walter)) and its vertically transmitted fungal endophyte (*Epichloë amarillans*) to determine long-term outcomes and test the drivers of variation in symbiont prevalence. This facultative symbiosis (plants may be endophyte-positive [E^+] or endophyte-free [E^-]) is a well-suited model system to evaluate hypotheses for the drivers of symbiont prevalence (Figure 1). The short-lived *A. hyemalis* exhibits relatively fast population dynamics, allowing us to detect change in symbiont prevalence in a short time span. Additionally, the prevalence of *E. amarillans* varies across natural *A. hyemalis* populations in our Texas study region (mean: 74.6% E^+ , SE: 30.3%, range: 0%–100%, $N = 37$ populations; J. Fowler and T.E.X. Miller, unpubl. data, Rudgers et al., 2009). *Epichloë amarillans* can be experimentally removed from the host grass (Davitt et al., 2011), and can confer fitness benefits generally (Miller & Rudgers, 2014), under herbivory (Crawford et al., 2010), and during drought stress (Davitt et al., 2011). Our manipulation of environmental context focused on water availability, motivated by projected shifts in rainfall regimes (Trenberth, 2011), an unprecedented drought in the south-central United States in the years preceding our Texas field experiment (Nielsen-Gammon, 2012; Rippey, 2015), and evidence that water availability mediates the grass–endophyte mutualism (Davitt et al., 2011).

Using this model system, we addressed the following questions: (a) Does symbiont prevalence in host populations drift neutrally, trend towards fixation or elimination or persist at a stable intermediate? (b) Do the individual-level demographic fitness effects and transmission rates predict population-level outcomes? (c) Do the qualitative outcomes of symbiont prevalence and effects on host demography and transmission fluctuate in response

to environmental context in space (manipulation of water availability) or time (variation across years)? To determine long-term outcomes, we employed a space-for-time substitution to infer the temporal trajectory and dynamic stability of symbiont prevalence (Figure 1). We tracked shifts in symbiont prevalence from a continuous range of initial prevalence in replicated *A. hyemalis* populations, within which we collected data on individual demographic vital rates and symbiont transmission. As we tracked demographic performance of 954 plants and assayed symbiont status of 5,485 plants within 47 host-symbiont populations, our study is the largest scale experiment of host-symbiont population dynamics to date, to our knowledge.

2 | MATERIALS AND METHODS

2.1 | Study system and plant material

Agrostis hyemalis is a short-lived perennial, C_3 (cool-season), bunch grass native to eastern North America (Gould, 1975; Soreng & Peterson, 2003). *Agrostis hyemalis* germinates and grows during winter when temperatures are between 10 and 20°C (Mitchell, 1956; Thompson, 1989), then flowers in spring and panicles break off from the plant, with seeds dispersing by early summer. *Agrostis hyemalis* facultatively hosts the fungal endophyte *E. amarillans* (Craven et al., 2001; White, 1994). While *E. amarillans* is able to transmit both vertically and horizontally (Moon et al., 2004), vertical transmission appears to be the dominant transmission mode: in our experimental populations, formation of fungal stromata (whereby a fungal reproductive structure overtakes the host's reproductive structure) was exceedingly rare (0.37% or 9 out of 2,424 observed inflorescences across the 3-year study). However, this endophyte may also be transmitted horizontally via epiphyllous conidia (White et al., 1996), yet this has not been widely documented as a common form of endophyte transmission (Rodriguez et al., 2009).

Epichloë endophytes are readily detectable by microscopy and immunoblot assay. Specifically, fungal hyphae in seeds and leaf tissue stained with aniline blue lactic acid (Bacon & White, 1994) can be observed with a compound brightfield microscope at 200–400 \times (Yule et al., 2013). The immunoblot assay (Agrinostics Ltd. Co.) identifies E^+ and E^- plants through a monoclonal antibody that targets proteins of *Epichloë* endophytes and chromagen to visually indicate presence or absence (Koh et al., 2006). The efficacy of this method has been previously verified, and yields similar detection rates to that of visual microscopy endophyte detection (Hiatt et al., 1999).

Plant material for this experiment was derived from a natural population of *A. hyemalis* at the Stephen F. Austin Experimental Forest near Nacogdoches, Texas (31°29'N, 94.6°45'W). We generated E^- hosts from E^+ hosts using heat treatment of seeds, as described in our previous studies (Davitt et al., 2011; Yule et al., 2013). E^- seeds used for the present experiment were a minimum of three generations removed from heat treatment. From these seed stocks, 167

E^+ and 165 E^- plants were germinated on 10% agarose and grown to seedlings on ProMix potting soil (Quakertown, PA) in 3.8 cm \times 12.7 cm cylindrical containers. Due to the large number of plants required to start our experiment ($N = 960$), we relied on vegetative cloning of these greenhouse germinated plants to reach our target number. We confirmed symbiont status via microscopy for 59 plants from the E^- seed stock (all of which were negative) and 209 plants from the E^+ seed stock before we transplanted them into the field experiment.

2.2 | Field experimental design

We conducted the field experiment at the USDA-NRCS East Texas Plant Material Center near Nacogdoches, Texas, USA (31°31'N, 94.6°45'W). We established forty-eight 2 m \times 2 m experimental plots in a freshly tilled area at the field site in February 2013, with 3 m spacing between adjacent plots. Natural populations of *A. hyemalis* were not present at our field site but were present c. two km away. We enclosed plots with mesh netting to prevent browsing by vertebrate herbivores and dispersal of panicles (and thus seeds) between plots. We randomly assigned each population to ambient or elevated precipitation, with 24 populations per precipitation treatment, and to one of four levels of starting prevalence (5%, 35%, 65% or 95% E^+), which allowed us to estimate prevalence from across the full range of prevalence. In February 2013, we transplanted 20 founding individuals into each plot in a 4 \times 5 grid, drawing from greenhouse-raised E^+ and E^- plants to meet the target prevalence, ensuring that plants cloned from the same parent plant were distributed across populations. One population in the 5% initial endophyte prevalence category was planted incorrectly in the elevated precipitation treatment group; this population was dropped. We applied a broad-leaf herbicide one to two times per year to prevent our experimental grass populations from being overgrown with other non-grass vegetation.

2.3 | Precipitation treatment

Because our experiment was initiated towards the end of an extreme multi-year drought in Texas (which peaked in 2011, Nielsen-Gammon, 2012; Rippey, 2015), we designed the precipitation treatment to elevate rainfall above ambient levels. We hypothesized that elevated precipitation would alleviate drought stress, weaken the beneficial effects of endophytes on host individuals (Davitt et al., 2011) and alter population-level outcomes. We designed and implemented the treatment to approximate an extremely wet year in this study region as described in Cavazos et al. (2018). The elevated precipitation treatment ran for the duration of the experiment until its conclusion in May 2016. Ambient precipitation plots were unmanipulated. We monitored the effect of the precipitation treatment on soil moisture availability with Onset HOB0 soil moisture loggers (Onset Computer Corporation), which were installed in four

ambient and four elevated precipitation plots. Two sensors (one in an ambient and one in an elevated precipitation plot) malfunctioned and were not included in the analyses.

Additionally, as Mitchell (1956) and Thompson (1989) found that *Agrostis* spp. grow best at 10–20°C, we downloaded daily max temperature data from PRISM for the duration of our study period for Nacogdoches, Texas (PRISM Climate Group, 2016) to determine when during the calendar year these optimal temperatures occurred.

2.4 | Data collection

Data collection focused on the population-level symbiont prevalence and individual-level performance, symbiont status and transmission efficiency of plants that recruited into our experimental populations from 2014 through 2016, and not on the founders that we planted in 2013. Data were collected from three subplots within each population once at the beginning of each transition year, which we defined as May to April.

We quantified population-level symbiont prevalence by destructively harvesting all individuals within a 0.25 × 0.1 m strip immediately adjacent to each of the three subplots in each population. We harvested individuals from a different 'side' (corresponding to a cardinal direction) of the subplots in different years (2014: $N = 2,734$, 2015: $N = 1,299$, 2016: $N = 1,452$). These samples included new recruits and survivors from the previous year. The plants were placed in Falcon tubes, stored on dry ice during transfer from the field to the laboratory and stored at -20°C until processing for the immunoblot assay.

Plant performance was assessed by four demographic traits (survival and flowering status, reproduction and recruitment), which determine the relative fitness of E^+ and E^- plants, and therefore affect the change in prevalence. In the first year, we tagged four randomly selected individuals in each subplot for demographic census. In the following years, we replaced individuals that died by tagging a living individual in the subplot to maintain four census plants per subplot. We recorded survival status and size (in number of tillers) of each census plant. If a census plant was flowering, we counted all panicles and collected up to three of them. In the laboratory, we extracted and recorded mass of seeds from the panicles collected ($N = 384$ panicles). We recorded densities of recruited vegetative and flowering *A. hymenalis* within each of the subplots; recruit density ranged from 0 to 2,272 plants per m² (Figure S1). In total, we tracked 954 census individuals, with 442 and 512 plants in ambient and elevated precipitation populations respectively.

We determined symbiont status and vertical transmission efficiency from the seeds collected from the demographic census plants. To assign symbiont status, we scored four seeds from each individual. Seeds were soaked in 5% NaOH overnight, squashed and stained, then examined for fungal hyphae. For plants designated as E^+ (*Epichloë* hyphae observed in any seeds), we typically scored an additional 16 seeds to assess the vertical transmission rate (the fraction of seeds produced by E^+ plants that were E^+). In total, we

assessed 5,323 seeds. For some individuals, we were unable to collect seeds—either they did not flower, or their seeds dispersed prior to collection. For these individuals that were still alive in 2015 and 2016, we harvested a tiller to determine endophyte status via immunoblot assay. However, as many census plants died prior to ascertaining endophyte status, we did not know symbiont status of 72.4% and 50.4% of census individuals in 2014–2015 and 2015–2016. However, we knew the symbiont status for 154 and 314 plants in each of those transition years, and our statistical analysis allowed us to use plants of known and unknown symbiont status to estimate demographic rates.

2.5 | Statistical analysis

We estimated population-level symbiont prevalence and individual-level vital rates using a hierarchical Bayesian statistical framework, implemented in JAGS (Plummer, 2003) and R2JAGS (Su & Yajima, 2014) within R version 3.5.1 (R Core Team, 2020). Bayesian analysis was particularly useful in our estimation of symbiont-dependent vital rates, where data came from individuals of known and unknown symbiont status. In the latter case, for each vital rate we drew a probabilistic inference of individual symbiont status—a latent state—based on symbiont prevalence at the population level. In Supporting Information S1 (Figure S4) we show how parameter estimates changed when we used data only from plants of known symbiont status. We find that had we limited our analysis to plants of known symbiont status, estimates of reproductive rates would have been biased because we often only knew symbiont status if the plant flowered and produced seeds (and thus seeds were scored). Therefore, using both direct observations and latent-state inference in our analyses allowed us to reduce bias in our demographic estimates. All of the following sub-models were fit simultaneously as part of the larger hierarchical model. We ran three Markov Chain Monte Carlo chains with 10,000 iterations each and discarded the first 2,000 iterations as initialization. All parameters were given vague priors. We assessed model fit using posterior predictive checks.

We fit statistical models to characterize the change in symbiont prevalence between years (Figure 1). Using the symbiont scores from immunoblot screening of destructively harvested samples, we modelled the number of E^+ plants associated with subplot i within population j assigned to precipitation treatment l in year k (y_{ijk}^+) as binomial 'successes' given total samples N_{ijk} . In preliminary analyses, we found that a beta-binomial distribution, accounting for overdispersion, fit the data better than a binomial; this reflected the fact that even 50/50 populations may not be well-mixed because of localized seed dispersal, leading to aggregated clusters of E^+ and E^- individuals. This model was:

$$y_{ijk}^+ \sim \text{BetaBinomial} \left(q * p_{ijk}^+, q * (1 - p_{ijk}^+), N_{ijk} \right). \quad (1)$$

With this parameterization, the expected probability of success is p_{ijk}^+ and parameter q generated overdispersion in the binomial samples. For

the first year of samples ($k = 1$), we estimated p_{jk}^+ as a simple model-of-the-mean with no predictor variables, representing the starting conditions of each population. For the following 2 years ($k > 1$), we modelled symbiont prevalence of the current year as a linear function of prevalence in the previous year:

$$\text{logit}(p_{jk}^+) = \beta_{kl}^0 + \varepsilon_{jk}^0 + (\beta_{kl}^1 + \varepsilon_{jk}^1) * p_{jk(k-1)}^+ \quad (2)$$

Here we used the logit-link transformation with coefficients β_{kl}^0 and β_{kl}^1 specific to year and precipitation treatment. We incorporated population- and year- specific random effects on the intercept (ε_{jk}^0) and slope (ε_{jk}^1), which allowed populations to deviate from expected values; these were normally distributed with mean zero and variance σ^2 . For each transition year and precipitation treatment, we used Equation 2 and fitted parameters to estimate long-run equilibrium symbiont prevalence, which satisfies $p_{jk}^+ = p_{jk(k-1)}^+$. This is an inferred equilibrium that represents the long-run expectation if the conditions of that treatment and transition year remained constant. We used posterior distributions of the coefficients in Equation 2 to derive posterior probabilities for equilibrium prevalence, accounting for parameter uncertainty.

We then linked population-level outcomes to individual-level demography by estimating four vital rates of E^+ and E^- hosts (survival probability, flowering probability, fertility (seed production) of flowering plants and per-seed recruitment probability) and vertical transmission. We did not explicitly analyse host size because the census individuals had low size variation (~71% had <3 tillers), but host size is implicitly incorporated into reproductive output (larger plants produced more seeds). We also estimated the vertical transmission rate for E^+ hosts in each treatment and year. We then estimated recruitment probability of E^+ and E^- seeds based on the ratio of seedlings to seeds of each type produced in the preceding year. Estimation of all vital rates included fixed effects of precipitation treatment and year and population-specific random effects, and full details of these statistical methods are provided in Supporting Information S1. We present results in the form of posterior distributions of symbiont effects, accounting for parameter uncertainty ($\Delta = E^+$ rate - E^- rate) and absolute vital rate estimates are shown in Figure S3.

3 | RESULTS

3.1 | Precipitation treatment

Across both transition years, elevated precipitation populations had increased mean available soil moisture compared to ambient precipitation populations (Figure 2). Specifically, mean soil moisture was increased by approximately 27% in 2014–2015 and 26% in 2015–2016 for elevated precipitation populations (2014–2015:0.186 m^3/m^3 and 2015–2016:0.196 m^3/m^3) compared to that in ambient populations (2014–2015:0.146 m^3/m^3 and 2015–2016:0.155 m^3/m^3). While the total amount of ambient precipitation was similar between transition years (May to April; 156 cm in 2014–2015 and 161 cm in 2015–2016), the amount of ambient precipitation did not fall consistently throughout the year (Figure S2a). Because of these differences in the timing ambient precipitation, the precipitation deficit (and thus the amount of water added under the elevated precipitation treatment) also varied across years (Figure S2b). November to March appears to coincide with optimal temperature for recruitment and growth of *Agrostis* spp. (Figure 2 shaded grey area). During this period, ambient precipitation was 20.79% lower in 2014–2015 than in the same time period of 2015–2016 (2014–2015:62.5 cm and 2015–2016:78.6 cm).

3.2 | Population-level symbiont prevalence

We found evidence for all three of the proposed mechanisms underlying symbiont prevalence—neutral dynamics, transient dynamics, and stable intermediate—depending on the year and precipitation treatment (Figure 3). In the first transition year (2014–2015), symbiont prevalence under ambient precipitation increased in all 24 populations regardless of initial prevalence, indicating a temporal trajectory (Figure 3c) with a long-run outcome of certain symbiont fixation (Figure 3a, 100% CI: 98.5%–100%), such that populations at intermediate prevalence were in a transient state on a path to fixation. By contrast, populations that received elevated precipitation over the same transition year tracked their initial prevalence, thereby exhibiting neutral symbiont dynamics with no defined equilibrium, that is,

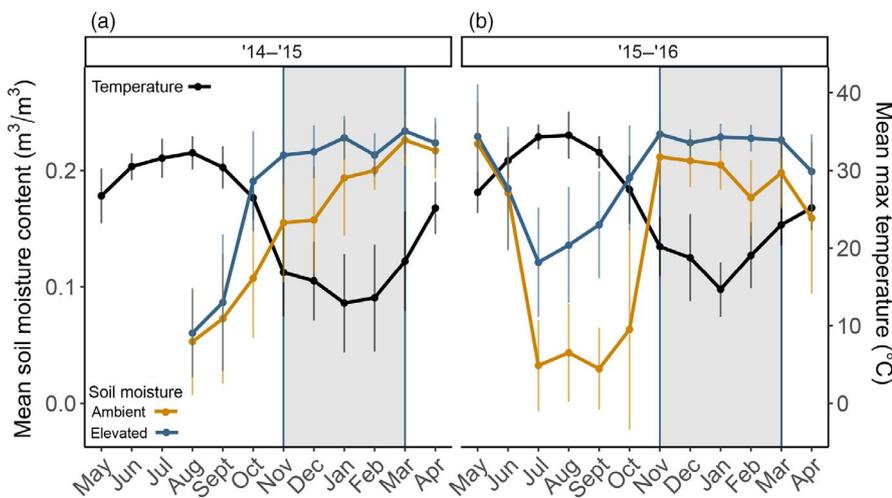


FIGURE 2 Monthly mean soil moisture (m^3/m^3) in ambient (orange) and elevated (blue) precipitation treatments and monthly mean maximum temperatures (black line) in (a) 2014–2015 and (b) 2015–2016. Points and error bars represent mean and standard deviation respectively. Grey shading from November to March identifies the time period coinciding with optimal growth and recruitment temperatures for *Agrostis* species (Mitchell, 1956; Thompson, 1989)

FIGURE 3 (a and b) Posterior distributions of equilibrium symbiont prevalence in host populations, visualized as density distributions (reflecting parameter uncertainty) and inferred from the temporal trajectories shown below, and (c and d) prevalence estimates for individual populations (points) across two transition years (2014–2015 and 2015–2016). Variation in final symbiont prevalence was plotted against prevalence in the preceding year. Thick lines show fitted relationships. Line colours indicate precipitation treatment, where orange and blue represent ambient and elevated precipitation populations respectively. Thin grey line is the identity line

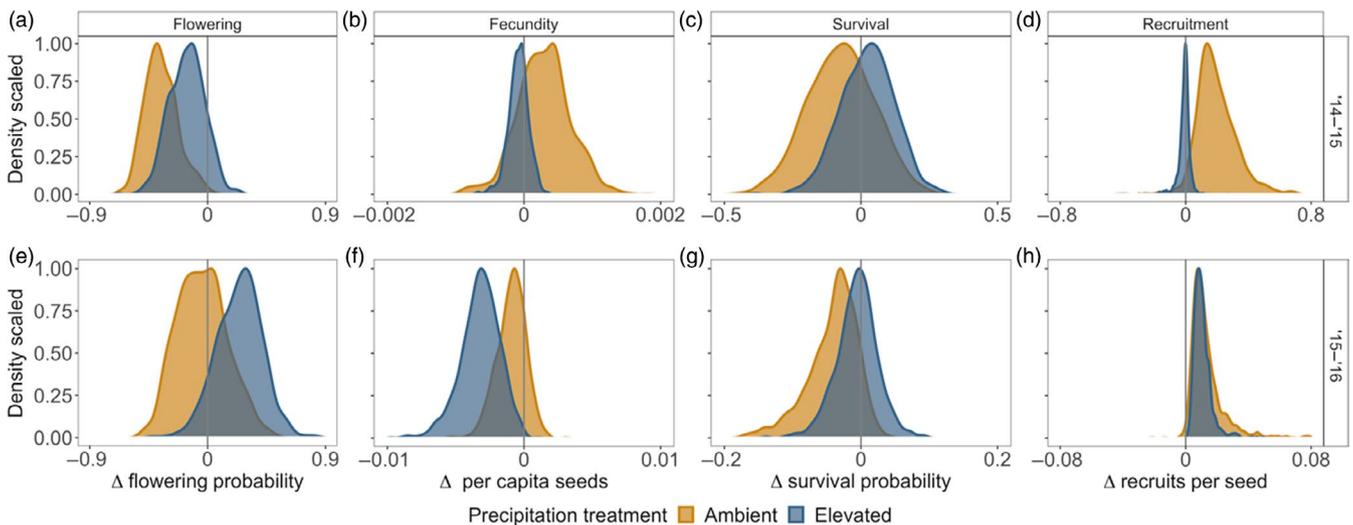
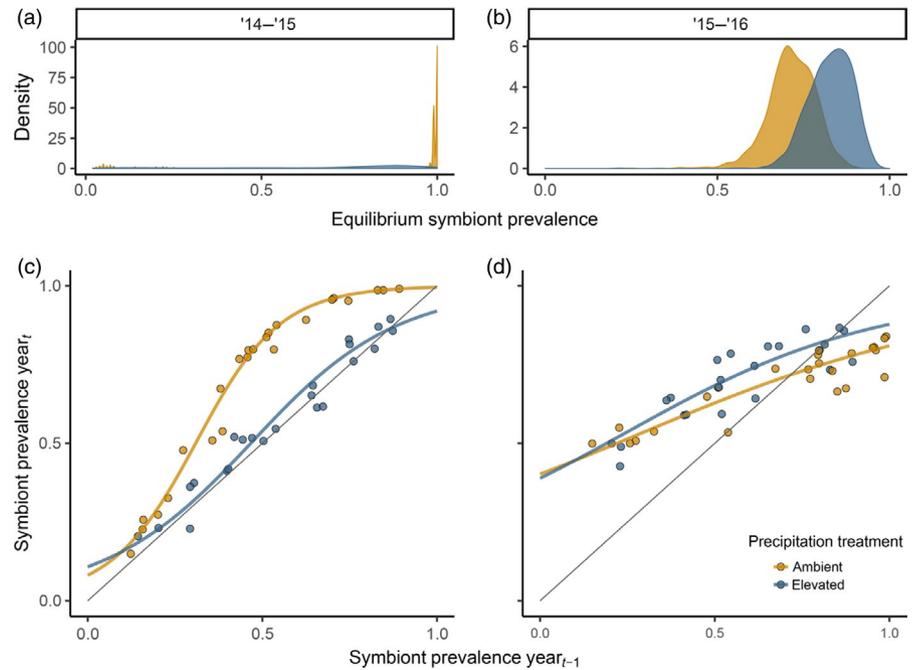


FIGURE 4 Posterior probability distributions of the effect of endophyte symbiosis $\Delta (E^+ - E^-)$ on four demographic vital rates: (a and e) the probability that a plant flowered, (b and f) the estimated amount of seeds a flowering plant produced in grams, (c and g) the probability that a plant survived and (d and h) the estimated number of new plants that recruited per seed. The vertical grey line corresponds to no difference between E^+ and E^- hosts. Positive values indicate greater vital rate estimates of E^+ plants compared to E^- plants, while negative values indicate the opposite. Orange and blue represent vital rate estimates from ambient and elevated precipitation treatments respectively. Rows represent the transition years, and columns represent individual vital rates. Note that the x axes vary in scale

all prevalence values were effectively equi-probable (Figure 3a). In the second transition year (2015–2016), low-prevalence populations increased, and high-prevalence populations decreased in prevalence, and these trajectories of change were similar between the ambient and elevated precipitation treatments (Figure 3b,d). Thus, the conditions of the second transition year favoured a stable intermediate prevalence, regardless of precipitation treatment. The long-run outcome of symbiont prevalence expected under these conditions was higher for elevated (84% CI: 75.5%–93.7%) than ambient (72% CI: 63.5%–79.5%) precipitation (Figure 3b).

3.3 | Individual-level host demography

The effect of symbiosis on host demography varied across the host life cycle and with environmental context related to precipitation treatment and year (Figure 4).

Host reproduction – Endophyte symbiosis depressed the probability of flowering, strongly so in the first transition year under ambient precipitation—the Δ posterior distribution was entirely negative—and moderately so under elevated precipitation (Figure 4a). In contrast in the second transition year, symbiosis did not strongly

affect the probability of flowering under ambient conditions but did promote flowering under elevated precipitation (Figure 4e). Symbiosis conferred a modest benefit on seed production (g) by flowering plants under ambient precipitation, and this effect was neutralized in the elevated precipitation treatment in the first transition year (Figure 4b). However, in the second transition year, endophyte symbiosis reduced seed production, particularly under elevated precipitation (Figure 4f).

Host survival – Endophyte symbiosis did not strongly affect the probability of host survival across transition years or precipitation treatments, as the all Δ posterior distributions were centred near zero, though symbiont effects on survival were somewhat more negative under ambient precipitation (Figure 4c,g).

Host recruitment – Endophyte symbiosis increased the per-seed probability of recruitment. Δ posterior distributions for this vital rate were almost entirely positive (Figure 4d,h), indicating high certainty in the beneficial effects of symbionts. This benefit was strongest under ambient precipitation in the first transition year. Elevated precipitation weakened the recruitment benefit of endophyte symbiosis but more so in the first transition year than the second. Specifically, the median endophyte effect on the recruitment rate was 5.25 times greater under ambient versus elevated precipitation in the first year, but only 1.8 times greater in the second.

Symbiont transmission – Vertical transmission of symbionts was consistently high across years and precipitation treatments (Figure S4a,b). The mean vertical transmission rate in 2014 was 84.8% (95% CI: 73.72%–93.2%) and 84.2% (95% CI: 70.4%–93.4%) under ambient and elevated precipitation treatments respectively. This was elevated slightly in 2015, with a mean vertical transmission rate of 88.3% (95% CI: 80.6%–93.8%) and 90.9% (95% CI: 84.2%–95.6%) in ambient and irrigated populations.

4 | DISCUSSION

Despite longstanding recognition of the varied and important roles heritable symbionts play in individual host fitness and the well-developed theory for population-level outcomes of host-symbiont interactions, identifying the drivers underpinning variation in symbiont prevalence in host populations remains an outstanding ecological goal. Our results of the observed temporal trajectories and inferred equilibria of symbiont prevalence supported that all three proposed mechanisms (Figure 1) can underlie symbiont prevalence, depending on environmental context corresponding to treatment and year (Figure 3). Furthermore, we connected these population-level outcomes to context-dependence in individual-level effects of symbiosis (Figure 4). Based on these findings, symbiont prevalence in nature may fluctuate across years, with certain years and conditions favouring different outcomes and likely giving rise to the variable patterns of symbiont prevalence observed in nature.

The inferred fixation of the symbiont under ambient precipitation and drift-like dynamics under elevated precipitation (Figure 3a,c) are consistent with the stress gradient hypothesis that fungal

endophytes, and symbionts in general, can function as defensive mutualists under stress, yet confer little to no benefit under benign conditions (Afkhani et al., 2014; Bertness & Callaway, 1994; Clay & Schardl, 2002; Giauque et al., 2019; Oliver et al., 2005; Russell & Moran, 2006). The corresponding results for individual-level vital rates add nuance to this interpretation, revealing costs and benefits of symbiosis that can manifest in different parts of the life cycle; the overall population-level outcome reflects the balance of these costs and benefits. Specifically, in the first transition year the stress-dependent benefit of symbiosis was limited to seedling recruitment, while endophyte effects on other vital rates were neutral to costly. We infer that the recruitment benefit outweighed the survival cost under ambient conditions, leading to consistent increases in symbiont prevalence, and that elevated precipitation weakened the recruitment advantage of E^+ hosts such that costs and benefits roughly balanced.

In the second transition year, the increase in symbiont prevalence when initially rare, declines in prevalence when initially common, and the resulting stable intermediate equilibria are consistent with a balance of symbiont-conferred fitness benefits and imperfect transmission, which theory predicts can lead to stable mixtures of symbiotic and symbiont-free hosts (Bibian et al., 2016; Gundel et al., 2008). While there was not a strong decline in vertical transmission in the second year to account for the prevalence decline in high-prevalence populations, the same vertical transmission rate can have different outcomes depending on the background demographic rates. The strong increases observed in low-prevalence populations clearly indicate an advantage of symbiosis but, because these outcomes were similar between ambient and elevated precipitation treatments, we conclude that benefits in this year were not related to drought protection. As symbionts can often confer multiple distinct benefits (reviewed in Oliver et al., 2014; Rodriguez et al., 2009), this increase may reflect enhanced nutrient acquisition (Malinowski & Belesky, 2000) or herbivore deterrence (Breen, 1994; Crawford et al., 2010). Future work could incorporate additional factors, such as nutrient acquisition and herbivore deterrence, in a combined experiment to parse out the symbiont-conferred contributions and resulting prevalence.

Interestingly, despite the very different population-level outcomes, symbiont effects on individual-level vital rates were generally not qualitatively different between years. Across the study period, we identified symbiont-associated costs for reproduction (with the effect on flowering more pronounced in the first year and fecundity more pronounced in the second year), no strong effect on survival and benefits for recruitment, though the recruitment benefit was much weaker in the second transition year (Figure 4a–h). The explanation for different outcomes in different years likely lies in the absolute values of the demographic vital rates (Figure S3), which provide additional information beyond the relative effects shown in Figure 4. In the second transition year, flowering probability ranged from approximately 1–3 times greater than the first year, while fecundity ranged from approximately 3–18 times greater per capita seed production (Figure S3,b,e,f). However, survival probability was

approximately 5–20 times lower depending on endophyte status and water treatment (Figure S3c,g) in the second year compared with the first year. These results indicate the change in symbiont prevalence in the second year was dominated by population turnover and influx of new recruits. We hypothesize that these changes in absolute survival and reproduction increased the importance of the modest recruitment benefit and imperfect transmission in the second transition year. As these were both similar between precipitation treatments, this likely led to the outcome of stable mixtures in both treatments. Our analyses did not explicitly track plant age, but it is possible that the increase in fertility and decline in survival between years reflects an ageing cohort instead of or in addition to changes in the environment.

Besides the difference in the long-run prevalence trajectories between years, the other striking inter-annual difference was in the effect of elevated precipitation. Our temporally well-resolved environmental data suggest that the two transition years of our study were not simply temporal replicates, but rather distinct periods that expanded coverage of environmental context. Across both transition years, the elevated precipitation treatment increased soil moisture to approximately the same average level (~27%) above ambient conditions (Figure 2). However, at a finer, monthly temporal resolution, ambient rainfall differed across years (Figure S2a). Rainfall was 20.79% lower and soil moisture was approximately 10.5% lower during November to March 2014–2015 compared to the same period in 2015–2016 (Figure 2); this is a critical period of recruitment and growth for *A. hyemalis* (Mitchell, 1956; Thompson, 1989). Specifically, rainfall during November 2015 greatly exceeded the amount we were scheduled to add (Figure S2), which resulted in similar mean soil moisture in the ambient and elevated precipitation treatments (Figure 2b). Considering these environmental conditions alongside the demographic rates suggest that drought-protective benefits of symbiosis were limited to seasonal events that occurred in a narrow temporal window in 2014–2015. Previous work has shown that individual vital rates can respond to short-term environmental variability, such as shifts in precipitation (Shriver, 2016). As changes in the amount and seasonal distribution of precipitation are forecasted under global climate change (Trenberth, 2011), these shifts may affect the strength of host-symbiont mutualism, the resulting individual-level demographic vital rates and the overall prevalence of symbionts within host populations.

Our results support previous work showing that symbionts of plant and animals can vary in sign and magnitude of their effects across time, context, and host life cycle (Chen et al., 2000; Chung et al., 2015; Goheen & Palmer, 2010; Thomas et al., 2016; Yule et al., 2013). These varied effects do not preclude host-symbiont mutualism and local persistence of the symbiont, so long as the outcome of the cost-benefit balance is a net positive fitness effect (Bibian et al., 2016). A common signature of heritable symbionts is increased host reproduction, which has been documented in flies (Himler et al., 2011), mites (Zhang et al., 2018) and grasses (Cavazos et al., 2018; Faeth et al., 2004; Rudgers et al., 2012). Our findings of detrimental effects of symbiosis on host reproduction were therefore

surprising, although similar results have been reported from studies on arthropods (Chen et al., 2000; Chong & Moran, 2016; Hoffmann et al., 1990) and plants (Chung et al., 2015; Pan & Clay, 2003). Our population-level results, where symbionts with reproductive costs still increased in prevalence, provide novel evidence that such reproductive costs do not preclude positive fitness feedbacks as long as they are balanced by benefits elsewhere in the life cycle. It was further surprising that elevated precipitation appeared to alleviate reproductive costs to flowering even as it weakened benefits in recruitment (Figure 4a,d,e,h). These results are consistent with recent hypotheses that effects of microbes on host responses to environmental stress fall along a continuum from mitigation (e.g. stronger recruitment benefits under drier conditions) to exacerbation (e.g. stronger reproduction costs under drier conditions; Chamberland et al., 2017; David et al., 2018). In our study, the benefits appear to have been greater than or equal to the costs; however, there may be conditions that would tip the outcome to a net cost. Alternating years or locations of net benefits and net costs would likely increase spatio-temporal variation in symbiont prevalence.

In addition to the possible roles of enhanced recruitment and imperfect seed transmission, the finding of stable intermediate symbiont prevalence may also be explained by other mechanisms. Our modelling approach estimated demographic rates that were averaged over plant density and endophyte frequency. However, the rising and falling of prevalence below and above a certain level is often considered a hallmark of frequency-dependent mechanisms (Omacini et al., 2006). Previous greenhouse work on *A. hyemalis* identified negative-frequency dependence (Miller & Rudgers, 2014), whereby the fitness advantage of E^+ over E^- hosts declined when E^+ were very common. It is possible that this process prevented symbiont fixation in the second transition year, as we detected negative-frequency dependence in the components of reproduction (flowering and fecundity) especially under ambient conditions in 2015–2016 (Figure S5b,h). However, we also detected positive-frequency dependence in recruitment and survival in this transition year (S5f,j). Future work should incorporate frequency and density dependence into vital rate estimates to parse out their influence in the resulting population-level symbiont prevalence.

Our finding that the long-run outcomes varied between years and across precipitation treatments indicates that symbiont prevalence is likely pushed and pulled in different directions. Thus, prevalence at a given time and place may be far from the equilibrium favoured at that time and place. In this sense, our results mirror expectations for structured populations in stochastic environments, where the relative abundance of different life stages or size classes constantly fluctuates due to variability in vital rates and may be far from asymptotic expectations (Ellis & Crone, 2013; Williams et al., 2011). While there is a large body of literature on short-term surveys from broad geographic ranges, we are aware of no long-term studies of symbiont dynamics from single locations, which would be valuable for testing whether the fluctuations in outcome that we induced experimentally could be observed under natural conditions. Combining geographic surveys with geographically distributed

space–time substitution experiments such as ours would be valuable for quantifying the distance between observed symbiont prevalence and environmentally determined equilibria.

Like any experimental study, our results should be interpreted in light of several caveats and limitations. First, we focused on inter-annual change in symbiont prevalence assuming there were no longer term legacies, as might arise from a persistent seed bank (Bibian et al., 2016). We know little about seed-banking in *A. hyemalis*, but this could be an important component underlying change in symbiont prevalence. Second, we focused on a single species, and one that we knew to have variable endophyte prevalence in natural populations. There are other species of endophyte-associated grasses with consistently higher endophyte prevalence, often at or near fixation, which may reflect consistent transmission and benefits of symbiosis. Comparative work with multiple species of each type could yield new insight into why symbiont associations are more consistent in some species than in others. Third, while this endophyte can transmit both horizontally and vertically, we focused solely on vertical transmission. As our low-prevalence populations generally remained at low prevalence, we posit that there was no large contribution of contagious transmission in our study. Finally, we intentionally averaged across *A. hyemalis* genotypes in our populations to reduce complexity and assumed they were all symbiotic with the same endophyte strain. It is possible that there was cryptic and functionally important symbiont diversity within the E^+ class, though previous work suggests that within-population endophyte genetic diversity is likely low, as expected for symbionts with predominantly asexual regeneration (Arroyo Garcia et al., 2002). Plant and fungal genotypes may interact, such that responses of different traits—including the demographic traits measured in our study—may be genotype-specific, and future work should address this by explicitly considering these interactions.

5 | CONCLUSIONS

In summary, our work identifies demographic and context-dependent drivers underlying population-level outcomes of symbiosis, and provides evidence for a balance of symbiotic costs and benefits on individual-level vital rates that serves to promote, depress or neutralize symbiont prevalence in response to spatio-temporal contexts. Overall, we find strong evidence for host–symbiont mutualism, especially during life cycle events that are sensitive to seasonal drought stress, yet we also document benefits that are not dependent on drought. Our work is among the first to link individual- and population-level processes in a field setting, detect dynamic stability in symbiont prevalence and reveal variability in the underlying temporal dynamics of symbiosis. Intermediate prevalence of heritable microbial symbionts, which is widely documented in plant and animal hosts, is likely to reflect some combination of neutral, transient and stable mechanisms, and the context-dependent fluctuations among them.

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AUTHORS' CONTRIBUTIONS

M.L.D., J.A.R. and T.E.X.M. designed the research; M.L.D., T.F.B., K.M.K., R.A.S. and T.E.X.M. performed the research; M.L.D. and T.E.X.M. analysed data and wrote the manuscript, with input from all co-authors. All authors gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.hmgqnk9f5> (Donald et al., 2020a) and code for statistical analyses is archived on Zenodo <https://zenodo.org/badge/latestdoi/215358398> (Donald et al., 2020b).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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