

## RESEARCH ARTICLE

# Temporal host–symbiont dynamics in community contexts: Impacts of host fitness and vertical transmission efficiency on symbiosis prevalence

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## Abstract

1. Symbiotic associations play a role in plant ecology and evolution, but the outcome of the interaction depends on the life-history traits of the partners and the environmental context. Although symbiosis with vertically transmitted microorganisms should result in mutualism, it is not clear how the transmission process aligns with the outcome of the context-dependent symbiosis.
2. For 3 years, we sampled individuals of an annual plant species that forms symbiosis with a vertically transmitted fungal endophyte, in paired stands of two contrasting vegetation communities (humid mesophytic meadows [HMM]: productive/low stress, and humid prairies [HP]: less productive/high stress). We estimated the prevalence of symbiosis at the population level, and the fitness of the plant, the symbiotic status and vertical transmission efficiency at the individual level.
3. Over 3 years, the prevalence of symbiosis was  $\approx 100\%$  in HMM and  $\approx 75\%$  in HP. Plant fitness was very low and high in years with precipitation below and above the yearly mean, respectively. The higher fitness of endophyte-symbiotic plants was evident in the HMM and high precipitation years. Vertical transmission of endophytes was higher in HMM ( $\approx 96\%$ ) compared to HP ( $\approx 93\%$ ) and was not related to plant fitness. Despite transmission inefficiencies in HP, changes in prevalence within the growing season (from seeds to the final plant stand) suggest a fitness advantage for symbiotic plants.
4. Vertical transmission is expected to promote mutualism as it aligns partners' fitness. Although symbiotic plants showed higher fitness and the probability of transmission failures was higher among low-fitness plants, the variation

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in transmission efficiency between plants and vegetation communities was not related to the fitness of the individual host. Our study provides evidence that context-dependent vertical transmission efficiency and endophyte-mediated fitness advantages interact complexly to determine the prevalence of symbiosis in populations that occur in contrasting vegetation communities.

#### KEYWORDS

annual plants, *Epichloë occultans*, fungal endophytes, incidence of symbiosis, *Lolium multiflorum*, mutualism, plant population and community dynamics, plant–microorganism interaction

## 1 | INTRODUCTION

Microorganisms are commonly associated with plants in tightly interconnected interactions known as symbiosis. These interactions have the potential to influence evolution and ecological functions in host species, as well as indirectly impact other species within the community (Thrall et al., 2007). Symbioses can display varying interaction outcomes, depending on whether microorganisms exert detrimental or beneficial effects on host fitness. Categorizing symbiosis as parasitic or mutualistic can be challenging due to the context-dependent nature of interaction outcomes, and even mutualistic microorganisms can exert variable fitness effects on host plants (Bronstein, 1994; Thompson, 2005). The probability of evolutionary integration and fixation of a symbiotic microorganism into the host phenotype, as well as its prevalence in host plant populations, can be significantly influenced by the dependence of the microorganism on the host (e.g. free-living or obligate symbiont), mode of transmission (horizontal and/or vertical) and transmission efficiency (high or low; Ewald, 1987; Gundel, Rudgers, & Ghersa, 2011; Newman et al., 2022). In this article, we investigated the controls on the prevalence of a symbiosis between an annual plant species and its vertically transmitted fungal endophytes in two distinct vegetation communities over three consecutive years. Our study aims to unravel the relative contributions of host plant fitness and vertical transmission efficiency to variation in symbiont prevalence, considering spatial heterogeneity and temporal variability.

Asexual *Epichloë* fungal endophytes (formerly *Neotyphodium*) (Ascomycota, Clavicipitaceae) are obligate symbionts of grasses in the subfamily Pooideae, inherited maternally (i.e. vertical transmission; Leuchtman et al., 2014; Schardl, 2010). These endophytes grow asymptotically and synchronized with host plants (Christensen et al., 2008). A fungus inhabits aerial plant parts, colonizing tillers, inflorescences and developing seeds (Gundel, Rudgers, et al., 2011; Liu et al., 2017). Considered an evolutionary transition that led to fungal symbionts with increased ability to produce bioactive alkaloids with antiherbivore properties, most endophytes are diploid or triploid interspecific hybrids derived from haploid pathogenic ancestors (Selosse & Schardl, 2007; Schardl et al., 2023). Based on the production of alkaloids, *Epichloë* fungal endophytes are considered defensive mutualists (Bastías et al., 2017; Clay, 1988). Fungal

endophytes can also promote plant growth and reproduction, and tolerance to abiotic stress factors (Bastías et al., 2021; Decunta et al., 2021). However, under conditions of low resources or high stress, symbiotic plants may exhibit reduced performance due to the maintenance costs of the endophyte (Cheplick, 2007). These symbioses have been framed in the mutualism–parasitism continuum (Müller & Krauss, 2005; Newman et al., 2022). While the fitness alignment of both partners is apparent, the endophyte fitness also depends on its transmission to the seeds (Gundel et al., 2008; Gundel, Garibaldi, et al., 2011). Although vertical transmission efficiency was initially thought to be high and invariant, accumulating evidence shows that the process can be imperfect (Afkhami & Rudgers, 2008; Clay & Schardl, 2002; García Parisi et al., 2012; Gibert et al., 2015; Gundel, Rudgers, et al., 2011). Although positive correlations between endophyte transmission efficiency and host fitness have been observed under experimental conditions (Gundel, Rudgers, et al., 2011; Gundel et al., 2020), the role of transmission efficiency in determining or contributing to determine the endophyte prevalence in plant populations has been less investigated.

Factors influencing endophyte prevalence in grass populations have recently become a subject of research interest. Regional and global field surveys have revealed substantial variability in the prevalence of endophyte symbiosis within natural populations of various species (Casas et al., 2022; Dirihan et al., 2016; Iannone et al., 2015; Semmartin et al., 2015; Sneek et al., 2017). Efforts to understand this variation focus on investigating bioclimatic variables that influence differential fitness between endophyte-symbiotic and non-symbiotic plants (see e.g. Afkhami, 2012; Clay et al., 2005; Żurek et al., 2013). For instance, endophyte-conferred drought tolerance was crucial in expanding *Bromus laevipes* distribution into arid regions (Afkhami et al., 2014). Similarly, a higher prevalence of endophytes in *Lolium perenne* populations in drier sites was correlated with greater survival of endophyte-symbiotic plants during drought (Gibert et al., 2012). However, *Hordeum comosum* in Patagonia exhibited a low prevalence of endophytes in extreme aridity, suggesting impaired maintenance of symbiosis due to water scarcity (Casas et al., 2022). Furthermore, modelling exercises have indicated that vertical transmission is crucial in determining endophyte prevalence in populations (Gundel et al., 2008; Newman et al., 2022). Consistent with this, field surveys in *Lolium multiflorum*, *Elymus virginicus* and

*E. canadensis* found positive correlations between the prevalence of endophytes in populations and the mean vertical transmission efficiency of plants (Gundel et al., 2009; Sneek et al., 2017). A study of *Festuca eskia* plants in a common garden found that the genetically determined vertical transmission efficiency likely explained variations in the prevalence of population endophytes (Gibert & Hazard, 2013). Both theoretical and experimental research suggests that imperfect transmission can influence intermediate population-level endophyte prevalence, preventing symbiosis fixation (Cavazos et al., 2018; Gundel et al., 2008; Newman et al., 2022), yet there is no natural evidence.

The prevalence of endophytes in populations can be influenced by local environmental characteristics and population genetic structure, affecting symbiont effects on plant fitness and transmission efficiency. Local selection forces can favour specific plant genotypes, plant-by-endophyte combinations or non-symbiotic plants when the symbiosis does not enhance fitness, thereby affecting population genetic structure and endophyte prevalence (Thompson, 2005). Locally adapted, compatible plant-endophyte combinations can be temporarily disrupted by seed- and/or pollen-mediated gene flow (Saikkonen et al., 2004; Thompson, 2005). The relevance of these processes is expected to depend on landscape configuration, patch size variations in physical and biological characteristics and life-history traits of interacting species (Thompson, 2005). Some endophyte host species are obligate out-crossing, wind-pollinated and exhibit high intrinsic genetic variability (Gundel, Omacini, et al., 2010). For example, no spatial organization of genetic diversity was observed in the annual, outcrossing species *Lolium rigidum* (Balfourier et al., 1998). Still, genetically structured populations can emerge if strong, spatially variable environmental filters differentially impact plant populations (Thompson, 2005). Annual plant species, being yearly re-established, may evolutionarily respond to selection pressures (Gundel, Omacini, et al., 2010). For example, an alkali-tolerant ecotype of *L. multiflorum* was discovered despite the short distance between inundated alkali sinks and surrounding areas (Dawson et al., 2007). Pollen-mediated gene flow may disrupt locally selected plant-by-endophyte combinations, since ovaries from flowers carrying hyphae are pollinated by not necessarily locally adapted congeners during anthesis (Saikkonen et al., 2004). While this process can disrupt plant-endophyte compatibility (Saikkonen et al., 2010), it can also enhance fitness and evolutionary potential by increasing or maintaining heterozygosity and hybrid vigour in the host (Gundel, Omacini, et al., 2010). Experimental evidence shows that *L. multiflorum* can experience inbreeding depression, but also benefit from hybrid vigour and endophyte symbiosis, resulting in increased fitness (Firestone & Jasieniuk, 2012; Gundel et al., 2012).

This study aimed to assess the temporal dynamics of endophyte prevalence in *L. multiflorum* populations in different vegetation communities. We focused on the effects of symbiosis on plant fitness and vertical transmission efficiency as determinants of the prevalence of endophytes in populations. The sampling was carried out in paired stands of humid mesophytic meadows (HMM) and humid prairies (HP) in the Flooding Pampa subregion of Argentina.

We predicted that endophyte-based fitness benefits are higher in productive vegetation communities and years, and positively aligned with transmission efficiency. Genetic markers were used to identify potential differences between populations due to genetic variations that influence endophyte prevalence and transmission (Gibert & Hazard, 2013). Since *L. multiflorum* is allogamous and wind-pollinated, we expected no significant population genetic structure unless there are strong local selection pressures that vary between vegetation communities (Dawson et al., 2007). The proportion of endophyte-symbiotic and non-symbiotic individuals at the beginning of the growth cycle was estimated by integrating the contributions of fitness and vertical transmission efficiency on the prevalence of endophytes in the populations (Gundel et al., 2008). This approach, suitable for annual species such as *L. multiflorum*, allowed us to compare the initial proportion of seeds with endophyte to the proportion of plants at the end of the growing season, providing insights into whether selection favoured or not symbiosis.

## 2 | MATERIALS AND METHODS

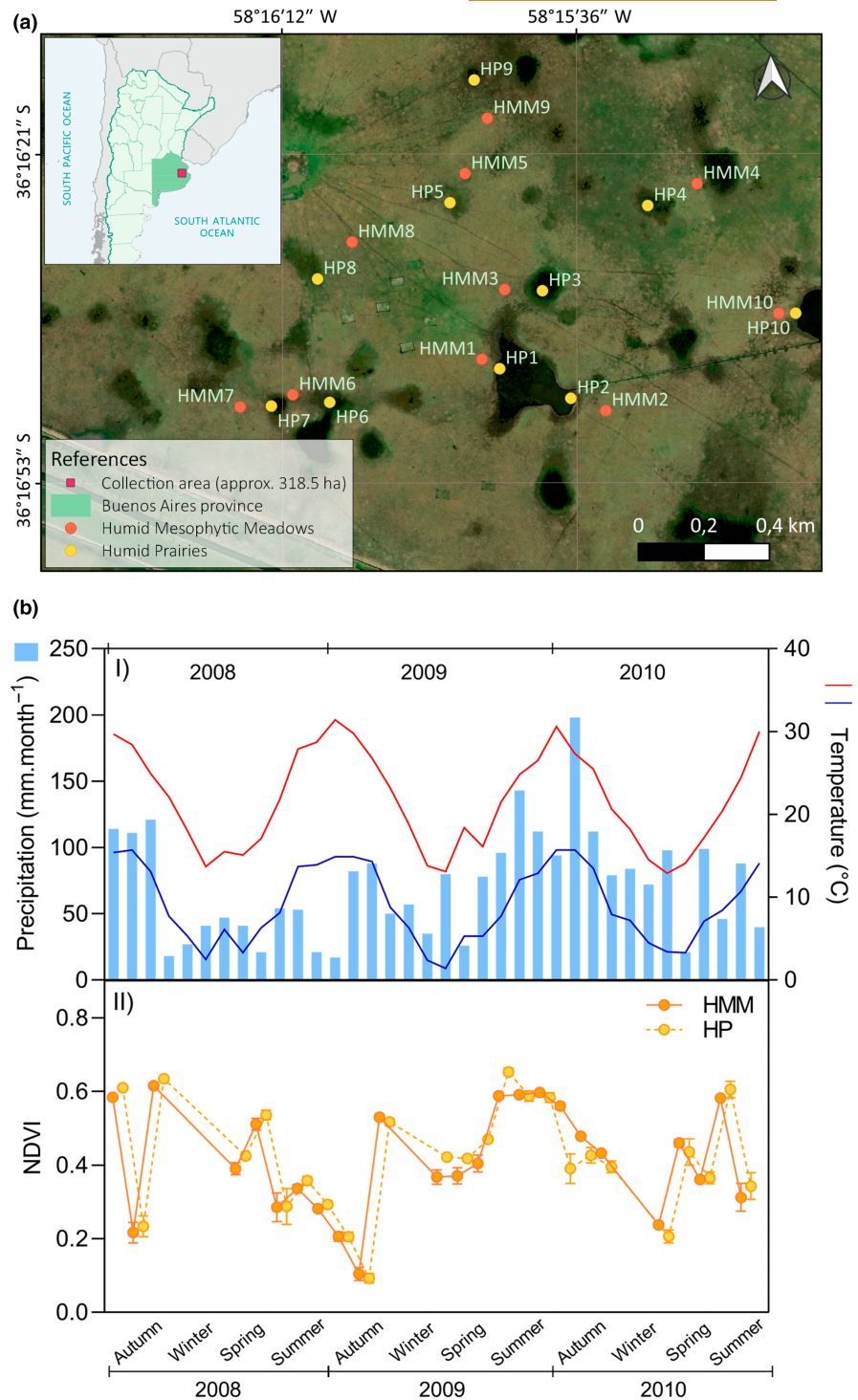
### 2.1 | Sampling design and plant collection

We conducted paired sampling of *Lolium multiflorum* populations in contiguous stands of two vegetation communities, namely 'humid mesophytic meadows' (HMM) and 'humid prairies' (HP), in Flooding Pampa, Argentina (see below *Landscape Characteristics and Vegetation Communities*; Figure 1; Burkart et al., 1990; Perelman et al., 2001). *Lolium multiflorum*, originally from the Mediterranean, is now naturalized and widespread worldwide, including in the Pampean grassland communities (Chaneton et al., 2002; Soriano et al., 1991). In previous research, we observed that *L. multiflorum* plants are typically associated with the common endophyte *E. occulta*, but endophyte prevalence and transmission vary between populations in different vegetation communities (Bastías et al., 2017; Gundel et al., 2009; Moon et al., 2000).

From 2008 to 2009, we identified 10 sites, each with paired stands of 'humid mesophytic meadows' (HMM) and 'humid prairies' (HP) (Figure 1a). In 2010, only 6 of these 10 were sampled (see Table S1). The mean distance between the paired stands was 128.41 m, ranging from 61.75 to 181.57 m. Each year, we collected at least 10, but no more than 20, individual plants of *L. multiflorum* in each stand. Due to the higher abundance of *L. multiflorum* in HMM (see Section 2.4), the sampling effort there was typically lower than in HP. The plant collection took place at the beginning of summer, when the *L. multiflorum* plants were at the end of their life cycle (Figure 2). All above-ground parts (stem, leaves, spikes and seeds) of each plant were collected and placed in a paper bag.

In the laboratory, harvested plants were subjected to an estimation of biomass and seed production. After threshing, all seeds per plant were weighed precisely ( $\pm 0.001$  g). The dry weight of the shoot was determined by weighing the entire plant (excluding seeds) after 48 h in a 65°C drying oven. Each plant's endophyte status (E+

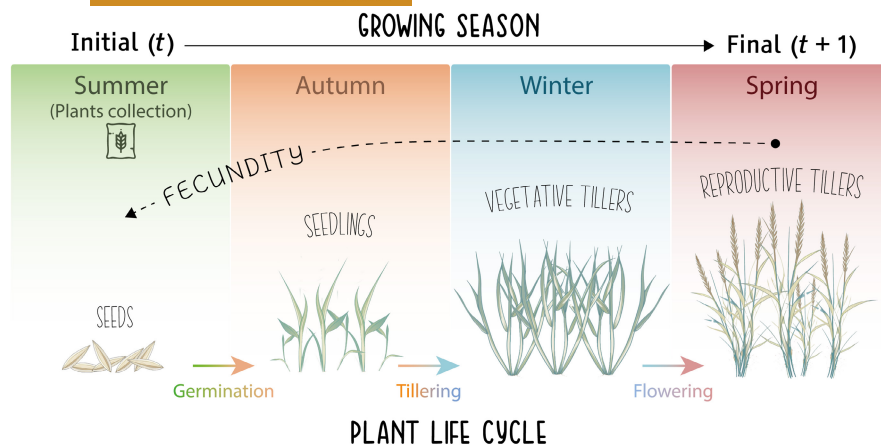
**FIGURE 1** (a) Geographic positions of the paired stands of the two plant communities (humid mesophytic meadows [HMM] and humid prairies [HP]) where the *Lolium multiflorum* plants were collected at the end of the growing cycle, in three consecutive years (2008, 2009 and 2010) in the Flooding Pampa, Argentina. The number of paired stands that were surveyed was 10 in 2008, 2009 and 6 in 2010. Source of satellite image: Esri, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo and the GIS User Community. (b) Monthly values of (I) climatic conditions (precipitation and minimum and maximum temperatures) and (II) Normalized Difference Vegetation Index (NDVI) that describes the productivity of vegetation for both vegetation communities (HMM and HP).



or E-) and vertical transmission efficiency were evaluated, with 10 seeds per plant examined. The seeds were soaked in 5% NaOH for 8+ hours, squashed and stained with Bengal rose. Endophyte positivity was confirmed under a microscope ( $\times 100$ ) by observing typical hyphae of *Epichloë occultans* (Gundel et al., 2018; Moon et al., 2000). If the initial 10 seeds were endophyte-free, an additional 10 were examined to reduce false negatives. A plant was considered non-symbiotic if all 20 inspected seeds lacked endophyte hyphae; otherwise, it was considered symbiotic.

## 2.2 | Landscape characteristics and vegetation communities

The study took place in the Flooding Pampa grasslands of the Pampa region, Argentina ( $36^{\circ}40' S$ ,  $58^{\circ}16' W$ ; Soriano et al., 1991). The climate is subhumid temperate, with a mean temperature of  $15^{\circ}C$  and an annual precipitation of 1023 mm (Chaneton et al., 1996; Perelman et al., 2001; Pérez et al., 2015). The flat landscape, while showing minimal topographic variation, creates diverse habitats and specific



**FIGURE 2** Representative schematic illustrating the stages of the *Lolium multiflorum* life cycle and their associations with the seasons of the year. The schematic depicts the autumn–winter–spring stages for germination, vegetation and reproduction, with seed stage occurring in summer.  $t$  and  $t + 1$  indicate the times when endophyte symbiosis prevalence was estimated in seeds and plants, respectively, during autumn and early summer.

vegetation communities, well characterized structurally (Burkart et al., 1990; Perelman et al., 2001) and functionally (Aragón & Oesterheld, 2008). Among the five major vegetation communities, we focused on ‘Humid Mesophytic Meadows’ (HMM) in intermediate positions and ‘Humid Prairies’ (HP) in lowlands. Despite differing in size, these communities were selected due to their frequent association, allowing paired stands in our sampling design. Both HMM and HP are prone to flooding and occasional droughts due to flat landscape and poor soil draining capacity (Posse et al., 2005). The area, predominantly used for cattle breeding with natural grasslands as the primary forage resource, encompassed a paddock of approximately 350 ha with a low stocking rate typical for the region (0.7 cow/ha; Cid et al., 2011).

### 2.3 | Climatic variables and functional characterization of vegetation communities

We obtained monthly precipitation and temperature data from TerraClimate, sourced at a resolution of 4638.3 m ( $1/24^\circ$ ,  $\approx 4$ -km), using the Google Earth Engine platform (<https://code.earthengine.google.com/>, Accessed: October 2022) for the years 2008–2010. The normalized difference vegetation index (NDVI) was employed to characterize vegetation community dynamics (Aragón & Oesterheld, 2008; Gundel et al., 2009). NDVI for each community (HMM and HP) was derived from Landsat5 TM false colour images with a spatial resolution of  $30\text{m} \times 30\text{m}$ , composited every 32 days (Image collection LANDSAT/LT05/C01/T1\_32DAY\_NDVI). The corresponding plot illustrates NDVI values for each vegetation community throughout the study duration (Figure 1b).

The climatic conditions varied slightly during the 3 years of the field survey. Although maximum and minimum temperatures exhibited consistent dynamics, mean annual precipitation differed notably. In 2008, accumulated precipitation was significantly low (669 mm, representing 34.61% below the regional mean), gradually recovering in 2009 and 2010 (864 and 1031 mm/year, respectively). Precipitation strongly influenced NDVI levels, with the lowest values recorded at the end of 2008 and the beginning of 2009 (Figure 1b,

I and II). The dynamics of NDVI between stands of the two communities were subtle. For instance, during the 2008 drought, NDVI values in HP stands were higher than those in HMM stands. On the contrary, in the summer of 2009, when precipitation exceeded the average, HMM stands exhibited higher NDVI values than HP stands (Figure 1b, I and II).

### 2.4 | Estimation of the changes in endophyte symbiosis prevalence within a growing season

*Lolium multiflorum*, an annual species without a persistent soil seed bank (Gundel et al., 2012; Gundel, Martínez-Ghersa, et al., 2010), relies on seeds produced in a given year for the upcoming growing season. The frequency of E+ seeds available at the beginning of the growing season ( $\gamma_t$ ) may not align with the frequency of E+ plants at the cycle's end ( $\gamma_{t+1}$ ) (Figure 2). Changes in prevalence could reflect the relative fitness of E+ and E- plants and/or the variation in vertical transmission efficiency of E+ plants. Our calculations are based on two key assumptions: (i) local plant dynamics are unaffected by migration among stands (closed populations) and (ii) the abundance of *L. multiflorum* plants remains constant within each vegetation community during the sampled years. The abundance of *L. multiflorum* has been characterized in different years for these vegetation communities. Overall, previous studies have shown that its winter–spring cover ranges from 10% to 20% in HMM stands, while it ranges from 3% to 10% in HP stands (Casas et al., 2016; Gundel et al., 2009; Longo et al., 2013). We denote  $t$  and  $t + 1$  as the initial (autumn) and final (early summer) of the growing season, respectively (Figure 2). With plant collection over three consecutive years (2008, 2009 and 2010), we have data for two complete growing seasons (initial and final of 2009 and 2010). This framework allows us to identify contributors to the initial input of seeds at the beginning of a growing cycle (fitness of E+ and E- plants, and endophyte vertical transmission) and its outcome (population endophyte prevalence) at the end of the cycle.

We started by estimating the frequency of endophyte-symbiotic and non-symbiotic extant plants in each population (i.e.

associated with each stand) and the mean fitness of individuals either E+ or E-. Thus, if the population endophyte prevalence at the beginning of the growing season ( $t$ ) is 70%, there are 7 E+ plants and 3 E- ones. We know the mean fitness of E+ and E- plants in terms of seed number ( $\omega$ ), the endophyte vertical transmission in E+ plants only ( $\tau$ ) and its complement ( $1 - \tau$ ) that contributes with E- seeds. Therefore, we estimated the initial seed input ( $t$ ) of E+ and E- seeds as follows:

$$E_{+t} = \omega_{E+} \times \tau \times \#plants_{E+} \quad (1)$$

$$E_{-t} = (\omega_{E-} \times \#plants_{E-}) + [\omega_{E+} \times (1 - \tau) \times \#plants_{E+}] \quad (2)$$

The initial input in relative frequency ( $\gamma$ ) of E+ individuals was estimated as:

$$\gamma_t = \frac{E_{+t}}{[E_{+t} + E_{-t}]} \quad (3)$$

Thus, the population endophyte prevalence at the beginning of a growing season ( $\gamma_t$ ) is compared with the directly estimated population endophyte prevalence at the end of a growing season ( $\gamma_{t+1}$ ) (Figure 2). As examples, possible outcomes are as follows:

$\gamma_t = \gamma_{t+1}$ : there is an equilibrium in population endophyte prevalence ( $\gamma$ ).

$\gamma_t > \gamma_{t+1}$ : there is a selection against E+ individuals.

$\gamma_t < \gamma_{t+1}$ : selection favours E+ individuals over E- ones.

## 2.5 | Characterization of the genetic structure of community-associated plant populations

We investigated population genetic structure influenced by biophysical differences in vegetation communities using nuclear microsatellites (SSR, single sequence repeat). Due to their high polymorphism, codominant inheritance and abundance throughout the genome, these markers are widely used in plant genetic ecology in general and have been successfully applied in *Lolium* species (Guan et al., 2017; Zane et al., 2002). Our study involved 45 and 35 plants from five paired stands (1, 4, 6, 9 and 10) of HMM and HP, respectively, which represents on average nine plants per HMM stand and seven plants per HP stand (Table S2). These plants originated from seeds collected in 2008, with three seeds from a sampled plant sown in a 1.5-L pot filled with commercial potting mix. After emergence, each pot was thinned to one seedling. Thus, each plant used in the molecular analysis represented a plant originally in 2008. Molecular analysis was conducted when these plants reached sufficient biomass (3–4 tillers and 4–5 leaves/tiller). We employed a set of 34 microsatellites (SSR; Jones et al., 2002; Momotaz et al., 2004) to identify polymorphic variability among populations. SSR has proven effective in genetically characterizing species in the *Lolium* genus and populations within *L. multiflorum* (Gundel et al., 2012). The molecular procedures followed the protocols described in Cuyeu et al. (2013) and Cuyeu et al. (2015, see *Analysis of genetic variability* in Supporting Information).

## 2.6 | Replication statement

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Landscape or community	Stands of vegetation communities	10 vegetation community stand and each year (except in 2010 that it was 6)
Population	Individual plant	10–20 individual plants per vegetation community stand

## 2.7 | Statistical analysis

All analyses were performed in R (version 4.2.1) unless specified otherwise (R Development Core Team, 2022). Population endophyte prevalence, estimated as the proportion of plants hosting endophytes per population (Table S1), was analysed using generalized linear mixed models (glmer function in lme4 package; Bates et al. (2015)) with a Poisson error distribution and a log-link function. The response variable was expressed as an integer value between 0 and 100. The model included year (2008, 2009 and 2010), vegetation community (HMM and HP) and their interaction as predictors, with year/sites/stands/plants as random effects. Dispersion, estimated by the dispersion\_glmer function in R (blmeco package; Korner-Nievergelt et al., 2015), was close to 1. Inference was performed with Anova function (car package; Fox & Weisberg, 2019). When predictors or the interaction were significant, least squares means adjusted by 'sidak' and  $p$ -value  $< 0.05$  were used for mean comparisons between factor levels (emmeans function in emmeans package; Lenth, 2022). The strength of fixed effects was validated by calculating the difference between the AICc of the null model and the minimum model. The conditional coefficient of determination ( $R^2$ ), which integrates fixed and random effects, was calculated using the MuMIn package (Barton, 2024). Similar analyses were performed for endophyte vertical transmission, expressed as the percentage of E+ seeds relative to tested seeds per plant, except that plant performance, including seed production (fitness) or above-ground biomass, was included as an explanatory variable.

We used linear mixed models to assess the impacts of year, symbiotic status (E+ and E-), vegetation community and their interactions on plant fitness, with stands nested within sites as random effects. Data were assessed for normality and homogeneity of variances, and when assumptions were not met, log-transformations were applied (Zuur et al., 2009). The conditional coefficient of determination ( $R^2$ ), which integrates fixed and random effects, was calculated using the MuMIn package (Barton, 2024). Significance of fixed effects was tested using the ANOVA function of the car package for R (Fox & Weisberg, 2019) followed by post hoc Tukey's HSD tests ( $p < 0.05$ ) for all variables to examine differences between groups. In 2010, data for estimating the fitness of non-symbiotic

plants were absent because all sampled plants turned out to be endophyte-symbiotic.

We analysed plant population structure using 34 nuclear microsatellites (single sequence repeat) with 82 individuals of *L. multiflorum* from HMM and HP vegetation communities. Genetic population structure was further explored through analyses of molecular variance (AMOVA) using the Arlequin program v3.5.2.2 (Excoffier et al., 2007) with 10,000 permutations to determine significance. Fixation indexes (FCT, FSC and FST) were computed and tested by permutations to assess genetic variability within and between populations. To visualize similarity patterns in genetic population structure among vegetation communities, principal coordinate analyses (PCoA) were performed using a dissimilarity index matrix (Euclidean) constructed after data standardization (vegan package; Oksanen et al. (2016)).

To assess changes in population endophyte prevalence within the growing seasons of 2009 and 2010, we employed generalized linear mixed effect models with a Poisson error distribution and a log-link function (Bates et al. (2015)). Initially, we considered a complete model with the year (growing season) as a fixed factor, along with vegetation community and life stage (seed or adult plant) within the growing season (corresponding to the 'initial' and 'final' periods of the life cycle of *L. multiflorum*). After conducting model selection procedures based on the Akaike information criterion, the year did not significantly contribute to explaining our variables of interest. Therefore, the final model included the explanatory variables (vegetation community, life stage within the growing season and their interactions) with year as

random effects incorporated as year/site/stand. Differences between vegetation communities in each season were analysed using two-way ANOVAs, followed by Tukey's HSD test (significance level:  $p < 0.05$ ).

### 3 | RESULTS

#### 3.1 | Contrasting prevalence of endophyte symbiosis between vegetation communities

Prevalence of *Epichloë* fungal endophytes differed between *L. multiflorum* populations of the two vegetation communities, but did not vary over time (Table 1). Averaged over 3 years, the prevalence of *Epichloë* fungal endophytes was ~22% lower in humid prairies than in humid mesophytic meadows (76% and 98% of prevalence, respectively; Figure 3a).

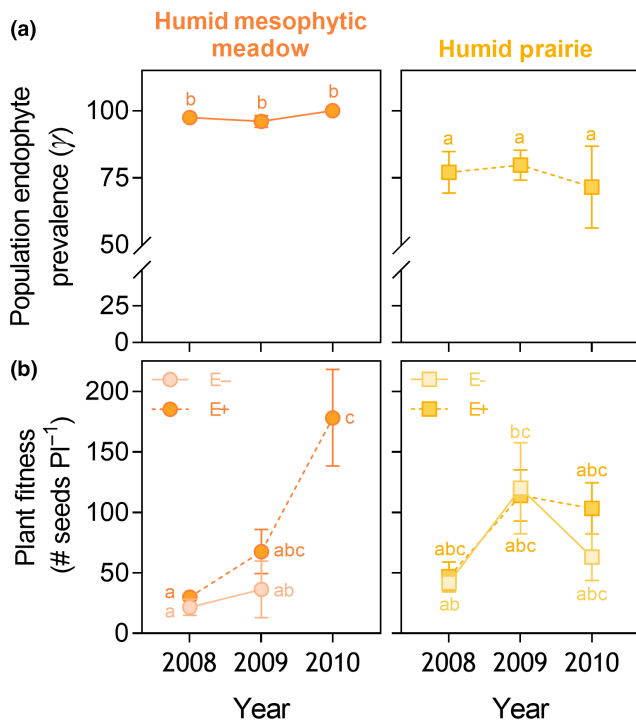
#### 3.2 | With a positive effect from endophyte symbiosis, plant fitness varied across years and communities

The fitness of plants (i.e. seed production) differed according to year and vegetation community (Table 1). Overall, the number of seeds per plant increased by 2.4 from 2008 to 2010 (Figure 3b). *Lolium multiflorum* plants in stands of humid prairies produced on average

Response variable	Source	df	$\chi^2$	<i>p</i> -value	<i>R</i> <sup>2</sup>
Population endophyte prevalence ( $\gamma$ )	Plant community (PC)	1	12.44	<b>&lt;0.001</b>	0.89
	Year	2	0.59	0.747	
	PC × Year	2	1.47	0.471	
Plant fitness (# seeds $PI^{-1}$ )	Plant community (PC)	1	7.25	<b>&lt;0.01</b>	0.41
	Year	2	24.80	<b>&lt;0.001</b>	
	Symbiosis	1	4.21	<b>0.040</b>	
	PC × Year	2	5.07	0.079	
	Symbiosis × Year	2	1.11	0.573	
	PC × Symbiosis	1	1.51	0.218	
	PC × Symbiosis × Year	1	0.00	0.955	
Endophyte vertical transmission ( $\tau$ )	Plant community (PC)	1	4.59	<b>0.031</b>	0.85
	Year	2	1.14	0.564	
	Plant fitness	1	0.66	0.413	
	PC × Year	2	3.05	0.216	
	PC × Plant fitness	1	0.29	0.588	
	Year × Plant fitness	2	2.33	0.311	
	PC × Year × Plant fitness	2	2.85	0.239	

Note: Additionally, for endophyte vertical transmission (the proportion of endophyte symbiotic seeds produced only symbiotic plants), the model included the same fixed factors, along with plant fitness. For the response variable plant fitness (seed number per plant), the model included symbiosis with endophytes and all interactions. Conditional *R*<sup>2</sup> are the proportion of variance explained by both fixed and random effects. Statistically significant effects ( $p < 0.05$ ) are highlighted in bold.

TABLE 1 Results of linear mixed model testing for the effect of vegetation community (humid mesophytic meadows and humid prairies), year (2008, 2009 and 2010) and the interaction on the endophyte population prevalence (proportion of endophyte-symbiotic plants per population) in accordance with Wald chi-square tests.



**FIGURE 3** Prevalence of *Epichloë* fungal endophytes (a) and plant fitness (b) in *Lolium multiflorum* plant populations occurring in stands of two different vegetation communities, humid mesophytic meadows (HMM) and humid prairies (HP), of the Flooding Pampa grasslands (Argentina) during three consecutive years (2008, 2009 and 2010). After determining the symbiotic status of each plant, plant fitness is presented separately for endophyte-symbiotic (E+) and non-symbiotic (E-) plants. Values are mean  $\pm$  SE. Different letters mean significant differences among mean values within and between vegetation communities (post hoc comparison,  $p < 0.05$ ). The missing data for E- plant fitness in the 2010 HMM were due to the absence of plants in this symbiotic category.

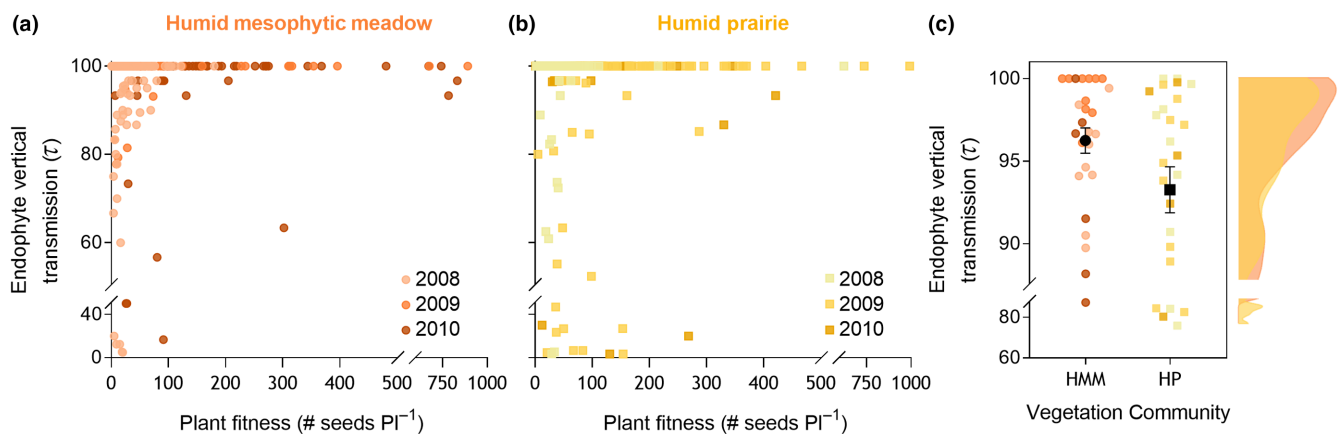
22% more seeds than plants in humid mesophytic meadows. Over the 3 years and in the stands of both vegetation communities, endophyte-symbiotic plants displayed a higher fitness ( $\approx 90$  seeds per plant) producing, on average, 59% more seeds than endophyte-free plants ( $\approx 56$  seeds per plant; Figure 3b, Table 1).

### 3.3 | Endophyte vertical transmission associated with vegetation communities, not plant performance

The endophyte transmission efficiency of endophyte symbiotic plants was different among populations of the two vegetation communities and did not vary significantly over time (Table 1). The efficiency of endophyte vertical transmission was lower in plants from humid prairies ( $\approx 93\%$ ) compared to plants from humid mesophytic meadows ( $\approx 96\%$ ) (Figure 4c, Table 1). Although the probability of inefficiency was higher among plants with low fitness, the difference in transmission efficiency between vegetation communities was not related to plant fitness (Figure 4a,b, Table 1) or plant biomass (Figure S3, Table S3).

### 3.4 | No evidence of genetically structured populations associated with vegetation communities

Genetic variability was mainly attributed to differences among populations but not to vegetation communities (Table 2). With axes 1 and 2 explaining 18.47% and 9.97%, respectively, the principal coordinate analysis explained 27.2% of the total variability among *L. multiflorum* plants from the two vegetation communities (Figure S1). Sampled plants did not cluster in ordination space according to vegetation communities (Figure S1).



**FIGURE 4** Relationship between endophyte vertical transmission efficiency and plant fitness of *Lolium multiflorum* from (a) humid mesophytic meadows (HMM) and (b) humid prairies (HP) of the Flooding Pampa grasslands (Argentina), during three consecutive years (2008, 2009 and 2010). Data points represent individual plants from HMM (2008: 174 plants, 2009: 100 plants, 2010: 62 plants) and HP (2008: 108 plants, 2009: 183 plants, 2010: 82 plants). (c) Mean endophyte vertical transmission efficiency of *L. multiflorum* plant populations in the same two vegetation communities. Coloured points represent stands from HMM and HP (2008: 10 stands, 2009: 10 stands, 2010: 6 stands), with density plots showing the distribution of stands for each plant community. Black points and error bars denote means  $\pm$  SE.



**TABLE 2** Fixation indexes obtained from the analysis of molecular variance (AMOVA) with nuclear microsatellite DNA (SSR, simple sequence repeat) tested on *Lolium multiflorum* plants from different stands of the two vegetation communities, humid mesophytic meadows (HMM) and humid prairies (HP) of the Flooding Pampa, Argentina.

Fixation index		
$F_{SC}$	0.1571	***
$F_{CT}$	0	ns
$F_{ST}$	0.1341	***

Note: Significance levels: ns:  $p < 0.1$ ; \*\*\* $p < 0.001$ .

**TABLE 3** Results of a generalized mixed model testing the effect of vegetation community (humid mesophytic meadows and humid prairies), plant life stage with growing season (initial: seed, and final: adult plant, respectively) and the interaction on the prevalence of *Epichloë* fungal endophytes ( $\gamma$ ) in populations of the annual host species *Lolium multiflorum*, according to the Wald chi-square tests.

Response variable	Source	df	$\chi^2$	$p$ -value	$R^2$
Endophyte population prevalence ( $\gamma$ )	Vegetation community (PC)	1	18.09	<b>&lt;0.001</b>	<b>0.91</b>
	Plant life stage (PLS)	1	11.24	<b>&lt;0.001</b>	
	PC $\times$ PLS	1	10.20	<b>&lt;0.01</b>	

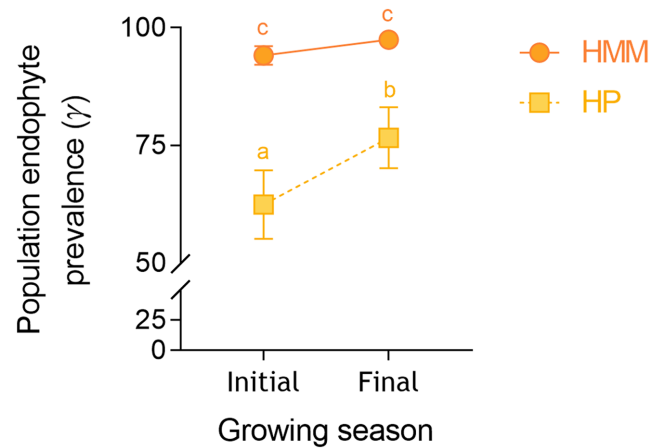
Note: Statistically significant effects ( $p < 0.05$ ) are highlighted in bold.

### 3.5 | Variation in endophyte symbiosis prevalence within growing seasons depended on the vegetation community

The change in endophyte prevalence within the growing season varied between vegetation communities (Table 3). Although there was no difference in endophyte prevalence between the seed and adult plant stages in the humid mesophytic meadow, there was a significant increase in endophyte prevalence in the humid prairies (Figure 5). In the humid mesophytic meadows, endophyte prevalence was high both at the seed stage (94%) and at the adult plant stage (98%). In the humid prairies, endophyte prevalence was lower at the seed stage (62%) and higher at the adult stage (78%), representing an increase of 23% from autumn to early summer.

## 4 | DISCUSSION

Our 3-year field survey consistently demonstrated the impact of vegetation communities on the prevalence of vertically transmitted endophyte fungi in populations of the studied annual plant species. Despite significant variation observed in the fitness of symbiotic and non-symbiotic plants across different vegetation communities and years, symbiotic plants exhibited overall superior fitness compared to the non-symbiotic plants. Regardless of plant fitness, the mean



**FIGURE 5** Population endophyte prevalence ( $\gamma$ ) at the initial (Autumn) and final (early Summer) of the life cycle of the annual host species *Lolium multiflorum* (seed and adult plant, respectively) in two vegetation communities (humid mesophytic meadow [HMM] and humid prairies [HP]) within the growing season. Note that  $\gamma$  values for the initial stage seed are estimated based on the subsection 'Estimation of the changes in endophyte symbiosis prevalence within a growing season' while for the final stage,  $\gamma$  values are measured on extant adult plants in the field. Values are mean  $\pm$  SE. Different letters mean significant differences among mean values (post hoc comparison,  $p < 0.05$ ).

vertical transmission of endophytes was consistently lower in Humid Prairies than in Humid Mesophytic Meadows. Analysis of changes in endophyte prevalence within the growing season indicated that selection favours endophyte symbiosis from seed to adult plant stage in humid prairies. Molecular marker analysis did not reveal genetic differences among plant populations, suggesting that endophyte prevalence and plant transmission efficiency are not linked to host plant genetics. Our study suggests that transmission efficiency is context-dependent and, together with symbiosis improving host fitness, determines symbiosis prevalence in host plant populations.

The plant-*Epichloë* symbiosis, proposed to span the parasitism-mutualism continuum (Müller & Krauss, 2005), receives support from experiments indicating lower performance of endophyte-symbiotic plants under certain treatments (Ahlholm et al., 2002; Cheplick, 2007). Potential costs associated with maintaining symbiotic microorganisms, especially under resource-limiting conditions, may explain this phenomenon (Bastías & Gundel, 2022; Cheplick, 2007). Models suggest that vertically transmitted symbionts must positively affect host fitness and exhibit high transmission efficiency to persist and achieve high local prevalence (Ewald, 1987; Gundel et al., 2008; Newman et al., 2022). In this study, *L. multiflorum* plants exhibited significant variation in fitness (i.e. seed production) across years and vegetation communities. Despite this variation, symbiotic plants generally showed higher fitness than endophyte-free plants. The incorporation of the vertical transmission process has improved our understanding of symbiosis prevalence (Afkhami & Rudgers, 2008; Donald et al., 2021; Gibert et al., 2015; Gundel et al., 2008; Gundel, Garibaldi, et al., 2011; Gundel, Rudgers, et al., 2011; Sneek et al., 2017). Although graphical observations of

the variation in transmission efficiency and plant fitness indicated a higher likelihood of transmission failures among low-fitness plants, we found that the mean endophyte transmission efficiency was primarily associated with the vegetation community and not with plant fitness. This may contrast with previous studies conducted under controlled conditions, in which the transmission efficiency of *L. multiflorum* individuals was positively correlated with plant fitness (García Parisi et al., 2012; Gundel, Garibaldi, et al., 2011). Unlike experimental plants, field populations in our study were mostly composed of individuals with low fitness levels. Plant growth and fitness under natural conditions are likely limited by resources affected by natural dynamics of precipitation, extreme temperature, plant–plant competition and/or herbivore damage. Within this range of fitness, vertical transmission of endophytes appears to be more influenced by the physical and/or biological characteristics of vegetation communities than by individual plant performance.

Symbiosis outcomes at the individual level, including the fitness of endophyte symbiotic and non-symbiotic plants and vertical transmission efficiency, offer insights into their roles in determining endophyte prevalence in populations (Cavazos et al., 2018; Donald et al., 2021; Gibert et al., 2015; Gundel et al., 2009; Sneek et al., 2017). To scale up to the population level, it is crucial to integrate individual-level processes with vertical transmission efficiency, as symbiotic plants can contribute non-symbiotic seeds (Afkhami & Rudgers, 2008; Gundel et al., 2008; Gundel, Rudgers, et al., 2011). Therefore, understanding whether symbiosis is selected or disfavoured requires deciphering the contribution of both endophyte-symbiotic and non-symbiotic plant fitness plus endophyte transmission efficiency (Gundel et al., 2008; Gundel, Rudgers, et al., 2011). For 2 years, we estimated the initial frequency of endophyte-symbiotic seeds at the beginning of the growing season and contrasted it with the frequency of extant symbiotic plants at the end of the same season. Our study system, involving an annual plant species without a persistent soil seed bank, avoids the impact of long lifespans that could slow down selection processes or seed banks acting as genotypic diversity reservoirs (Friedman, 2020; Honnay et al., 2008). We found that changes in symbiosis prevalence within the growing season from seed to adult plant depended on the vegetation community. Except for humid mesophytic meadows, where prevalence and transmission were consistently high (almost 100%), we observed positive selection for endophyte-symbiotic individuals from seed stage (62%) to adult plants (78%). This change in prevalence suggests that slightly impaired vertical transmission in humid prairies is compensated by endophyte-mediated fitness advantages in vital rates and fitness-related traits (other than seed production) of the host (e.g. seed and seedling survival, differential growth rates and resistance to herbivory).

The observed stability in endophyte population prevalence and transmission efficiency over the years in both vegetation communities suggests the involvement of environmental and genetic factors. Subtle yet significant differences between communities are reflected in NDVI satellite estimates, a widely accepted proxy

for primary productivity and environmental quality (Aragón & Oesterheld, 2008; Gundel et al., 2009). Mean individual fitness, a key indicator, responded significantly to communities in interaction with mean annual precipitation. For instance, E+ plant fitness in HMM varied four times from the driest year (2008) to the most productive one (2010). This pattern of plant fitness associated with annual precipitation was not as evident in the humid prairies. Notably, in the driest year, despite equally low fitness of E+ plants in both communities, variation in vertical transmission efficiency was solely attributed to the vegetation community. This is in line with results from an experiment in which endophyte vertical transmission was artificially manipulated, revealing its ability to influence symbiosis prevalence and drive intermediate but stable equilibria in populations (Cavazos et al., 2018). Therefore, our field study supports the idea that context-dependent imperfect vertical transmission influences population symbiosis prevalence in nature.

The genetic structure of plant populations, assessed through microsatellite markers, did not reveal spatial patterns. Despite potential phenological variations resulting from differences in vegetation communities (Aragón & Oesterheld, 2008; Chanton et al., 2002; Perelman et al., 2001), polymorphism was equally high within and between communities. This aligns with the annual life cycle, self-compatibility, and wind-mediated pollination traits of the host species (Balfourier et al., 1998; Gundel et al., 2012; Gundel, Omacini, et al., 2010). However, potential differences in specific genes for local adaptation or environmentally driven post-transcriptional modifications controlling transmission efficiency cannot be ruled out. Conducting a cross-transplant experiment with transcriptomic analysis could provide insights into these mechanisms. Symbiotic microorganisms, especially vertically transmitted ones influencing host fitness, can drive genetic population structure (Saikkonen et al., 2004; Thompson, 2005). Although few studies have explored how vertically transmitted *Epichloë* endophytes affect genetic population structure (Sullivan et al., 2023), existing evidence points towards structured endophyte populations (Arroyo García et al., 2002; Wäli et al., 2007). Further research is needed to assess genetic diversity in *E. occulta*s (Bastías et al., 2017; Moon et al., 2000), which could explain variations in endophyte prevalence and transmission efficiency among different vegetation communities.

In summary, the study highlights the significant impact of precipitation on plant fitness and the thriving of endophytes in more productive conditions. However, plant fitness alone does not fully explain variations in endophyte prevalence. Surprisingly, the lack of correlation between vertical transmission efficiency and plant fitness challenges conventional understanding of symbiosis dynamics, indicating a need for a deeper exploration into the factors influencing endophyte population prevalence.

#### AUTHOR CONTRIBUTIONS

Pedro E. Gundel and Marina Omacini conceived the idea and conceptualized the hypothesis. Pedro E. Gundel, Andrea C. Ueno, Cecilia Casas, Luis I. Pérez and Marina Omacini collected data. Andrea C.

Ueno, Cecilia Casas and Romina Cuyeu analysed the data. Pedro E. Gundel, Andrea C. Ueno, Cecilia Casas and Tom E. X. Miller wrote the manuscript. All authors contributed significantly to the last version of the manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

## DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.95x69p8v5> (Gundel et al., 2024).

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Table S1.** Number of plants (#) collected in stands (#) of the vegetation communities humid mesophytic meadows (HMM) and

humid prairies (HP) in the Flooding Pampa region, Argentina, during three consecutive years 2008, 2009, and 2010. The number of plants used for estimating "Population endophyte prevalence", "Endophyte vertical transmission" and "Plants fitness" (for both symbiotic and non-symbiotic plants) is shown.

**Table S2.** Number of *Lolium multiflorum* plants from various stands of the two vegetation communities (humid mesophytic meadows [HMM] and humid prairies [HP]) of the Flooding Pampa region, Argentina, used for the molecular analysis with nuclear microsatellite DNA (SSR, simple sequence repeat). The plants were cultivated in a greenhouse and sourced from seeds collected in 2008. We ensured that each seed originated from a different field sampled plant to avoid using half-siblings.

**Table S3.** Results from linear mixed model testing for the effect of vegetation community (humid mesophytic meadows and humid prairies), year (2008, 2009 and 2010), plant biomass and the interactions on the endophyte vertical transmission (proportion of endophyte-symbiotic seeds produced by symbiotic plants) in accordance with Wald chi-squared tests. Contidional  $R^2$  are the proportion of variance explained by both fixed and random effects. Statistically significant effects ( $P < 0.05$ ) are highlighted in bold.

**Figure S1.** Principal Coordinates Analysis (PCoA) based on 34 nuclear microsatellite DNA (SSR, simple sequence repeat) assessed on 45 and 37 *Lolium multiflorum* plants from paired stands of the humid mesophytic meadows (HMM, dark grey symbols) and humid prairies (HP, white symbols), respectively, of the Flooding Pampa region, Argentina. Values in parentheses on the axes 1 and 2, indicate the amount of variability explained by each axis.

**Figure S2.** Boxplots showing the distribution of fitness (# of seeds per plant) for *Lolium multiflorum* plants collected from the vegetation communities humid mesophytic meadows (HMM, orange symbols) and humid prairies (HP, yellow symbols), symbiotic (E+) and non-symbiotic (E-) with *Epichloë* fungal endophyte, during three consecutive years (2008, 2009, and 2010). Each data points represent an individual plant.

**Figure S3.** Relationship between endophyte vertical transmission efficiency and aboveground biomass for *Lolium multiflorum* individuals collected in stands of (A) Humid Mesophytic Meadows (HMM) and (B) Humid Prairies (HP) during three consecutive years (2008, 2009, and 2010). Colored point represent individual plants form HMM (2008: 174 plants, 2009: 100 plants, 2010: 62 plants) and HP (2008: 108 plants, 2009: 183 plants, 2010: 82 plants).

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