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Effects of Warming on Growth and Leaf Colonization by Litter Mat-Forming Fungi in a Wet Tropical Forest in Puerto Rico

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ABSTRACT

Wet tropical forests are experiencing rising temperatures and increased frequency and intensity of extreme climatic events, such as cyclones, which can increase rates of soil erosion and surface runoff. Fungal litter mats, formed by agaric decomposer fungi, play a crucial role in stabilizing slopes, preventing erosion, and aiding nutrient cycling; however, little is known about how warming affects litter mat growth and function. We investigated two litter mat-forming fungi, *Gymnopus johnstonii* and *Marasmius* aff. *crinis-equi*, in warmed (+4°C above ambient) and control plots in the Luquillo Experimental Forest, Puerto Rico. Growth and time-to-leaf colonization were monitored over 6 weeks in spring (both species) and summer (*G. johnstonii* only). We hypothesized that warming would inhibit fungal mat growth and slow leaf colonization, particularly for *G. johnstonii* since it is drought sensitive. As expected, warming significantly reduced relative growth rates (RGR) in spring, though *M.* aff. *crinis-equi* showed slightly higher RGR than *G. johnstonii*. Leaf colonization was also delayed by 22% in warmed plots, with *M.* aff. *crinis-equi* colonizing leaves 4.3 times faster than *G. johnstonii*. There were significant seasonal differences in response to warming for *G. johnstonii*, with warming increasing RGR during the consistently wetter summer sampling period. Overall, warming led to significant inhibition of leaf colonization when conditions were dry, whereas there was a trend toward increased colonization in warm and wet conditions. Our findings suggest that warming, combined with drier conditions, is likely to suppress drought-sensitive fungal mat growth, reducing their ability to prevent nutrient and soil loss via erosion.

RESUMEN

Los bosques húmedos tropicales están experimentando un aumento en temperatura acompañado de un aumento en la frecuencia e intensidad de eventos climatológicos extremos, tal como los ciclones, los cuales pueden aumentar las tasas de erosión del suelo y escorrentías de agua superficiales. Hongos formadores de tapetes miceliares también llamados esteras, formadas por hongos descomponedores del orden Agaricales, juegan un rol importante en la estabilización de laderas y el reciclaje de nutrientes. Sin embargo, se conoce poco sobre los efectos del calentamiento en el funcionamiento y crecimiento de las esteras de hongos en la

hojarasca. Se investigaron dos hongos formadores de esteras, *Gymnopus johnstonii* y *Marasmius* aff. *crinis-equi*, en parcelas calentadas (+4°C sobre la temperatura ambiental) y controles en el Luquillo Experimental Forest, Puerto Rico. El crecimiento y el tiempo de colonización sobre las hojas fueron monitoreados durante seis semanas en la primavera (ambas especies) y verano (solamente *G. johnstonii*). Hipotetizamos que el calentamiento inhibiría el crecimiento de las esteras de hongos y reduciría la colonización de las hojas, particularmente para *G. johnstonii*. Acorde a los resultados esperados, el calentamiento redujo significativamente las tasas de crecimiento relativas (RGR por sus siglas en inglés) en la primavera, pero *M.* aff. *crinis-equi* demostró un RGR levemente más alto que *G. johnstonii*. Además, la colonización de las hojas se retrasó 22% en las parcelas calentadas, donde *M.* aff. *crinis-equi* colonizó las hojas 4.3 veces más rápido que *G. johnstonii*. Hubo diferencias significativas entre las temporadas para *G. johnstonii*, ya que calentamiento tuvo un efecto positivo durante el periodo de muestreo más húmedo del verano. En general, el calentamiento inhibió significativamente la colonización de las hojas bajo condiciones secas, mientras que se observaron patrones de aumento en la colonización bajo condiciones cálidas y húmedas. Nuestros hallazgos sugieren que el calentamiento, combinado con condiciones más secas, es probable que suprima el crecimiento de hongos sensibles a la sequía, lo cual reduce su capacidad de prevenir la pérdida de nutrientes y suelo mediante la erosión.

1 | Introduction

Fungal litter mats reduce the loss of nutrients via erosion in wet tropical forests (Lodge et al. 2008, Lodge et al. 1994; Lodge and Asbury 1988), which is important because the water vapor holding capacity of the atmosphere increases with temperature and is expected to increase high-intensity rainfall and flooding globally (Whitfield 2012), which could increase erosional forces. In the North Atlantic, total tropical cyclone rain has trended upward at a rate of 24% per decade between 1988 and 2007 (Lau and Zhou 2012). At the same time, there is a drying trend with increased frequency of droughts in the Caribbean region, which is present in the Greater Antilles but especially strong in the Lesser Antilles (Moraes et al. 2022). Decreases in annual precipitation of 10%–20% are projected for the Caribbean region, Mesoamerica, and central Brazil, with summer precipitation reductions exceeding 40%–50% for Mesoamerica and the Caribbean region (Reyer et al. 2017). Together, these contrasting extreme climatic events will result in an intensification of wetting and drying cycles in these systems that could have significant effects on both biological (i.e., decomposer fungi) and physical (i.e., soil erosion) processes important for biogeochemical cycling in wet tropical forests (Lodge et al. 1994).

In wet tropical forests, the forest floor litter layer, which contributes a substantial flux of nutrients to soils (Vincent et al. 2009), may be particularly sensitive to changes in moisture regime (Lodge 1993; Lodge and Cantrell 1995; Lodge et al. 1994, Lodge et al. 2008, Lodge et al. 2022). Decomposition experiments throughout the tropics and in Puerto Rico demonstrate that litter moisture content is important in controlling rates of litter decomposition and that litter organisms, including litter decomposing macrofungi, are affected by changes in moisture (Lodge 1993; Lodge and Cantrell 1995; Lodge et al. 1994, Lodge et al. 2008, Lodge et al. 2022). Fungal litter mats are formed when leaf litter is bound together by agaric fungal mycelium and rootlike structures (hyphal strands, cords, and rhizomorphs). These agaric decomposer fungi that physically bind leaves together are called non-unit-restricted, and unlike unit-restricted decomposer microfungi, they play an important physical role in slowing the loss of litter and soil from steep forest slopes (Bibbo and Lodge 2022; Lodge and Asbury 1988; Lodge et al. 2008, 2014; Osono 2007). Non-unit-restricted agaric fungi maintain dominance in litter containing low concentrations of mineral nutrients by translocating nutrients that limit fungal growth and leaf decomposition, via their root-like structures, from partly decomposed leaves below to colonize fresh leaf litter above, fulfilling an important role in nutrient cycling (Lodge et al. 2008, 2014; Cantrell et al. 2014; Osono 2007). Some species such as those in the *Marasmius crinis-equi* F. Muell. ex Kalchbr (Marasmiaceae) species complex, produce powerful antibiotics that inhibit growth of all other fungi and bacteria (R.H. Petersen cited in Lodge and Cantrell 2023).

Despite their ecological importance, we have little understanding of how agaric fungi will respond to novel temperature and moisture regimes that are expected over the coming century. While there have been studies showing resilience in soil-dwelling microfungal communities and their response to drought in dry temperate grasslands, Austrian alpine soil, and Amazonian rain forests (Narayanan et al. 2021; Canarini et al. 2024; Buscardo et al. 2021), there have been none, to our knowledge, that have looked at the effects of warming on litter mat-forming agaric fungi. There is increasing evidence that suggests that agaric fungi are sensitive to warm and dry conditions; however, teasing apart key drivers is challenging given the prevalence of multiple covarying factors. Open canopies or gaps in canopy cover increase exposure to light and solar radiation, which can in turn drive warmer temperatures that reduce relative humidity and litter moisture (Lodge et al. 2008, 2014, 2022). Further, responses to changes in environmental conditions differ among basidiomycete litter mat species (Lodge and Cantrell 1995; Lodge et al. 2008, 2014, 2022). For example, many but not all agaric fungi that form litter mats in humid tropical forests are sensitive to disturbances that open the forest canopy (Lodge and Cantrell 1995, 2023; Lodge et al. 2008, 2014, 2022).

Studies in disturbed forest sites within the Luquillo Experimental Forest in Puerto Rico found a negative correlation between temperature and humidity (i.e., when temperatures are high, humidity is low) with percent litter mat cover for some seasons, but not others (Lodge et al. 2022). A drought in the spring of 2015 in the Luquillo Mountains (Sierra de Luquillo) significantly decreased fungal litter mat cover in plots where the canopy had been experimentally trimmed as well as in the controls, suggesting sensitivity of fungal mats to drying in the litter layer (Lodge et al. 2022). However, this decline in fungal mat cover did not

hold for one control plot that was dominated by a drought-tolerant fungus, *M*. aff. *crinis-equi*. Other studies at Luquillo that span pre- and post-hurricanes Hugo and Maria have found that *Gymnopus johnstonii* (Murrill) A.W. Wilson, Desjardin & E. Horak (Omphalotaceae), which has superficial mycelial fans on leaf surfaces, is sensitive to drought, canopy opening, and exposure to wind and direct sunlight (Lodge and Cantrell 1995; Lodge et al. 2022). In contrast, *M*. aff. *crinis-equi*, which creates litter mats suspended in the subcanopy as well as on the ground, was more resilient to canopy opening from a canopy trimming treatment, a large treefall gap, and disturbance from major hurricanes (Lodge et al. 2022). *Marasmius* aff. *crinis-equi* was also found to have the same high rates of attachment to freshly fallen leaves on a steep road embankment regardless of whether the litter mats were placed in shade with low evaporative potential versus partial shade with high evaporative potential (Lodge et al. 2008). Taken together, there is ample evidence that agaric fungi are responding to environmental changes, but determining the primary factor that is driving these responses remains challenging.

Within the tropics, temperatures are expected to increase between 1°C and 6°C over the next century (IPCC 2014), which would shift these systems into a novel climatic regime that is warmer than anything that has been experienced historically, particularly for lowland tropical systems (Mean Annual Temperature [MAT] > 20°C; Wood et al. 2012; Khalyani et al. 2015). Rising atmospheric temperatures are likely to influence fungal litter mat activity in the tropics, yet we have very little evidence to prove this. We utilized an ongoing field warming experiment in the Luquillo Experimental Forest in Puerto Rico, called the Tropical Responses to Altered Climate Experiment (TRACE; Kimball et al. 2018), to explore how increased temperatures influence litter mat-forming species *M*. aff. *crinis-equi* and *G. johnstonii* under warmed (+4°C) and ambient conditions. Our goals were to determine whether these species react differently to warming, and how warming affects litter mat growth and ability to colonize freshly senesced leaves. We looked at interspecies differences as well as seasonal differences that spanned both wet and dry conditions. We hypothesized that warming would negatively affect growth and leaf colonization times due to indirect effects of warming on litter moisture, but that *M*. aff. *crinis-equi* would be less hindered by heat than *G. johnstonii* given inter-specific differences in drought tolerance. Given projected changes in climate for these regions, research exploring the mechanisms behind and ecological components of natural mitigations, such as litter mats, may provide opportunities for conserving key ecosystem services in a changing world, as well as improving our ability to better predict effects on biogeochemical cycling.

2 | Methods

2.1 | Study Site

The study was conducted in an evergreen wet tropical forest in the Luquillo Experimental Forest in Luquillo, Puerto Rico (18°19′ N, 65°44′ W). The mean annual temperature is 24°C (Kimball et al. 2018), mean annual rainfall is 2879 mm (García-Martinó et al. 1996), and the Holdridge Life Zone is Wet Subtropical Forest (Holdridge 1967). While the climate is relatively aseasonal, there is a drier period from January to April, which experiences lower monthly rainfall than that of May to December (Heartsill-Scalley et al. 2007). Additionally, the two sampling periods, spring and summer, had different mean temperatures of 24°C and 27°C, respectively (Barreto-Vélez, T., Ortiz-Iglesias, D. A., Rubio-Lebrón, L. C., Wood, T. E., Cavaleri, M. A., Reed, S. C.). The site is 100 m asl with acidic and clay-rich soils characterized as Oxisols in the Cristal-Zarzal series (Beinroth et al. 2003; Heartsill-Scalley et al. 2007). The forest floor has a thin layer of leaf litter and partly decomposed litter (called the fermentation layer) overlying mineral soil (Muñoz et al. 2018), which is typical of mull-type litter in many wet tropical forests. A humus layer is absent, unlike moder type soils of many temperate and boreal forests. Total rainfall and average temperature during the 2024 study year were 2984 mm and 25°C, respectively (González et al. 2025; Barreto-Vélez, T., Ortiz-Iglesias, D. A., Rubio-Lebrón L. C., Wood, T. E., Cavaleri, M.A., Reed, S. C.). The experimental area contains six hexagonal 12 m^2 plots, three of which are experimentally heated +4°C above ambient temperatures using an array of infrared heaters (warmed), and three controls (ambient) which have the same infrastructure but receive no warming, as part of the Tropical Responses to Altered Climate Experiment (TRACE), a long-term in situ warming experiment (Kimball et al. 2018). Using a temperature of +4°C above ambient temperatures fits in with IPCC projected warming scenarios and this temperature is the mean seasonal variation from month to month in this forest (IPCC 2014; Khalyani et al. 2015; Kimball et al. 2018). The plots have a similar plant community composition, and the dominant species include *Palicourea brachiata* (Sw.) Borhidi, *Piper glabrescens* (Miq.) C.DC., *Miconia prasina* (Sw.) DC., and *Swietenia macrophylla* King.

2.2 | Experimental Design

We placed fungal litter mats (leaf litter bound together by hyphal strands, cords, or rhizomorphs) that were collected from the forest floor into warmed (+4°C) and control plots and monitored the growth of the litter mats by marking the perimeter of the colonized leaves biweekly and taking photographs. We also placed a recently senesced *Swietenia mahagoni* (L.) Jacq. leaf on top of the litter mats and documented the time it took for the mat-forming fungi to attach the fresh leaf to the litter mat. *Gymnopus johnstonii* and *M*. aff. *crinis-equi* were selected as the litter mat species because their vegetative colonies in leaf litter could be readily identified in the field and previous research in the Luquillo Mountains of Puerto Rico indicated they differed in drought tolerance (Lodge and Cantrell 1995; Lodge et al. 2022). The first round of sampling was conducted over a 6-week period from March 18 to April 29, 2024, which we refer to as the spring sampling period (mean weekly rainfall = 49.2 mm; total rainfall = 360 mm; mean number of dry days 4.5 $week^{-1}$; Figure 1). In each of the TRACE plots, three subplots (30 cm × 25 cm) per species were established on the forest floor of the northern side of the plots, where long-term chambers and sensors are located. Fungal litter mats were collected from the surrounding forest a minimum of 10 m distance from the TRACE plots to ensure there was no prior interaction between the plots and fungal mats. The litter mats were collected from the forest floor and put into paper bags, then examined under stronger lighting in

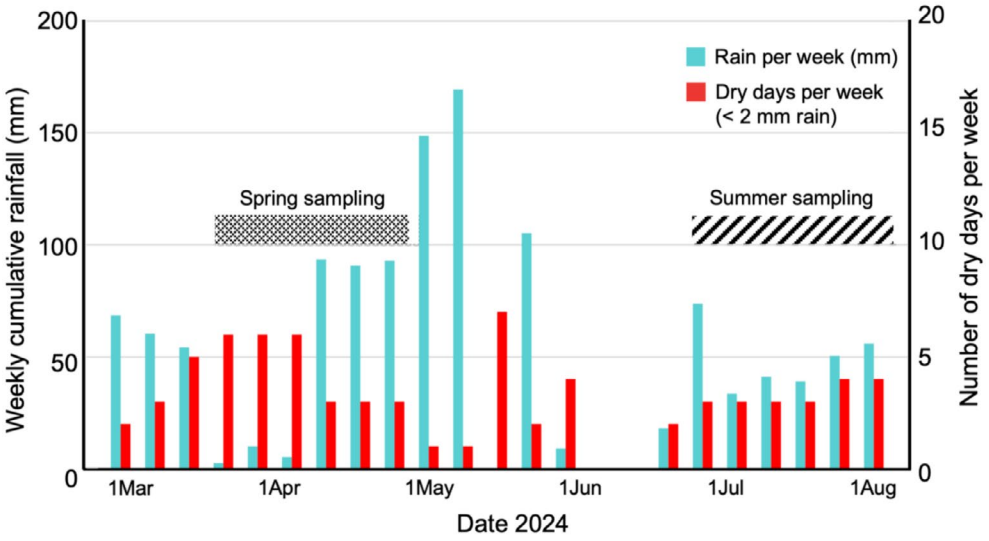

FIGURE 1 | Weekly cumulative rainfall and number of dry days week^{-1} (days < 2 mm rain) during the spring (Mar 18–Apr 29) and summer (Jun 15–Aug 2) sampling periods in 2024 at Luquillo in Puerto Rico.

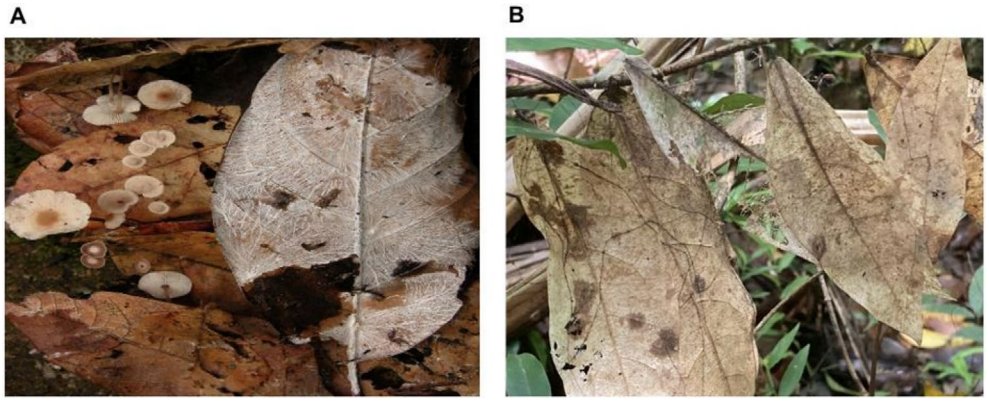

FIGURE 2 | (A) *Gymnopus johnstonii* fruiting on litter mat, showing the characteristic white mycelial fans on leaf surface, and (B) *Marasmius* aff. *crinis-equi*, showing black shiny rhizomorphs on leaf surfaces. Surface mycelium is not produced by *M.* aff. *crinis-equi*. The bleaching of leaves being decomposed by both species results from degradation of lignin-like phenolic compounds. Photographs by D. Jean Lodge.

the laboratory for subsequent identification confirmation based on presence of superficial white mycelial fans, hyphal strands, and cords in *G. johnstonii* and smooth thin-diameter black rhizomorphs in *M.* aff. *crinis-equi* (Figure 2). For more information on identification of fungal mats see Supporting Information. Initial size of the litter mats depended on how many leaves were connected and what species of leaves the fungi had colonized but averaged 31.9 cm^2 for *M.* aff. *crinis-equi* and 28.1 cm^2 for *G. johnstonii* in spring, and 55.3 cm^2 for *G. johnstonii* in summer. There was no significant difference in sizes between the initial mat size of the two species in spring (Welch's two-sample *t*-test, $p = 0.553$), but there was a significant difference between the initial mat size of *G. johnstonii* among seasons (Welch's two-sample *t*-test, $p = 0.003$).

Two complementary measurements were used to assess fungal responses to the warming treatment, that is, vertical colonization of a fresh leaf (time-to-leaf colonization) and relative growth rate (RGR) to measure colony expansion. Leaf colonization measured how quickly the fungus extended cords vertically to colonize a fresh leaf whereas RGR measured horizontal expansion through the surrounding leaf litter. Agaric litter mat fungi may use different growth strategies depending on the microclimatic conditions and the nutritional value of the leaf litter surrounding them versus the fresh leaves above. Bibbo and Lodge (2022) showed that preconditioning of leaf litter by microfungi facilitates subsequent colonization by cords of *G. johnstonii* if preconditioning lasts < 3 months. A previous experiment to measure the effect of *G. johnstonii* on rate of leaf litter mass loss at this same field site had "failed" because some of the mycelia migrated into surrounding litter instead of colonizing the fresh leaf litter placed in mesh bags on top of them whereas others migrated into bagged leaf litter colonized by microfungi in the non-white-rot treatment (Lodge et al. 2008). A subsequent experiment "succeeded" by using *G. johnstonii* mycelia that were caged inside or outside the treatments, showing 15% greater mass loss over 5 weeks in litter bags colonized by *G. johnstonii* (Lodge et al. 2008).

Fungal mats were installed in the established subplots and a recently senesced *Swietenia mahagoni* leaf, also collected a

minimum of 10 m distance from the plots, was placed on top of the fungal mats. The perimeter of the fungal mat was marked with different colored toothpicks indicating each sampling period, and the mats were photographed. Toothpicks were used so that the hyphal movement into the surrounding leaf litter could be visualized. The fungal mats were evaluated weekly and time-to colonization of the recently senesced *S. mahagoni* leaf was recorded. Time to colonization was determined by gently lifting the leaf and checking for hyphal cord or rhizomorph connections. Litter mats were monitored biweekly. Photographs of the mats were taken from above with a ruler grid for scale. At the end of the sampling period, fungal mats were removed from the subplots and all mat components were separated and laid out onto a smooth surface with a ruler for scale, then photographed. Initial and final surface area of the fungal mats were calculated by analyzing images on ImageJ and these calculations were used to determine growth. We repeated this experiment for a 7-week period over the summer (June 15 to August 2, 2024) with only *G. johnstonii*. The summer period was overall 11% drier than the spring sampling period (mean weekly rainfall = 42.6 mm; total rainfall = 320 mm), though dry days were less frequent than in spring (3.0 vs. 4.5 mean dry days week^{-1}; Figure 1).

2.3 | Statistical Analyses

Within TRACE, warmed and control plots are paired based on topographic position at the site (i.e., upper-slope, mid-slope, lower-slope). As is common in tropical climates, it is difficult to keep electronic equipment running 100% of the time, and for each of the seasonal field campaigns one heater malfunctioned. We therefore analyzed two pairs of warmed and control plots for each sampling period. All statistical analyses were conducted in R version 4.4.2 (R Core Team 2024). To look at the effects of warming on growth, the data were first tested for normality using the Shapiro–Wilk normality test and Q–Q plots for visualization. Growth data were not normally distributed, but converting the growth data to relative growth rate (RGR) [(ln(final surface area) − ln(initial surface area))/(time)] met the assumptions of normality. Relative growth rate (RGR) produces a unitless rate relative to the initial size of the fungal mat and how much it grew per unit of time (week). Since RGR is relative to the initial size of the fungal mats, it accounts for the differences in initial mat size and thus comparisons of RGR across treatments, species, and seasons are not confounded by initial size variation. RGR was used for all analyses.

A linear mixed model analysis (LMM) was used to test for species and treatment effects on RGR and account for random variation between plots. Relative growth rate was the dependent variable used in the interspecies LMM, and the fixed effects were treatment, species, and treatment × species interaction; plots were included as a random effect. For the seasonal LMM (only *G. johnstonii*), the RGR was the dependent variable, and the fixed effects were treatment, season, and the interaction between treatment and season; plots were included as a random effect. The residuals of the models were checked for normality to ensure the LMM was a suitable test; all residuals met the assumptions of normality. To further evaluate the statistical significance of the effect of warming on RGR, Welch's two sample *t*-tests were performed to evaluate differences between warmed and control treatments for each species in the spring, and the differences between seasons for *G. johnstonii* in the summer.

We analyzed the effects of warming on time to leaf colonization using time-to-event analysis, more commonly known as survival analysis. This test was the best suited for these data because not all leaves were colonized by the end of the study period, and this test is able to account for these censored data. First, Kaplan–Meier survival curves were fitted to visualize effects of warming on time to leaf colonization for each species and season separately. We tested the effects of treatment, species, and season on time to leaf colonization using Cox proportional hazard tests with and without Firth's penalized likelihood correction to better account for the small sample sizes. For the interspecies comparison, we used the Cox mixed effects model with fixed effects of treatment, species, and treatment × species interaction, using plot as a random effect. For the seasonal comparison of *G. johnstonii*, the fixed effects were treatment, and season with plot as a random effect. Additional Cox tests were performed using temperature, number of dry days (< 2 mm rain), and temperature × dry days as fixed variables to evaluate the effects of moisture on time to leaf colonization.

3 | Results

3.1 | Effect of Warming on Relative Growth Rates

The warmed treatment reduced fungal litter mat relative growth rate (RGR) by an average of 0.188 per week compared to the control during the spring sampling period (Figure 3A), which had more frequent dry days (4.5 days week^{-1}; Figure 1), although this difference in RGR between treatments in the spring was not significant ($p = 0.244$; Table 1). Growth of litter mats (final–initial surface area) ranged from 7.5 to 627 cm^2 for *G. johnstonii* and −0.5 to 1139 cm^2 for *M.* aff. *crinis-equi*. The RGR of *M.* aff. *crinis-equi* did not differ significantly between warmed and control treatments in the spring campaign, but the RGR of *G. johnstonii* did (Figure 3A). During the summer sampling period, which had less frequent dry days (3 days week^{-1}; Figure 1), there was a significant effect of warming on the RGR of *G. johnstonii* ($p = 0.024$), which was on average 0.163 per week greater in the warmed plots compared to the controls (Figure 3B). Further, there was a significant interaction of warming and season on the RGR of *G. johnstonii* ($p < 0.004$; Table 1), such that the response of RGR to warming during the consistently wetter summer sampling campaign encouraged growth rather than hindering it, in contrast to what was seen during the spring. The main effect of treatment was also significant ($p = 0.045$), while the effect of season alone was marginal ($p = 0.087$; Table 1). Growth of *G. johnstonii* litter mats ranged from 15 to 2770 cm^2 during the summer campaign.

3.2 | Effect of Warming on Time to Leaf Colonization

During the spring sampling period, warming significantly slowed leaf colonization by an average of 22% ($p = 0.021$).

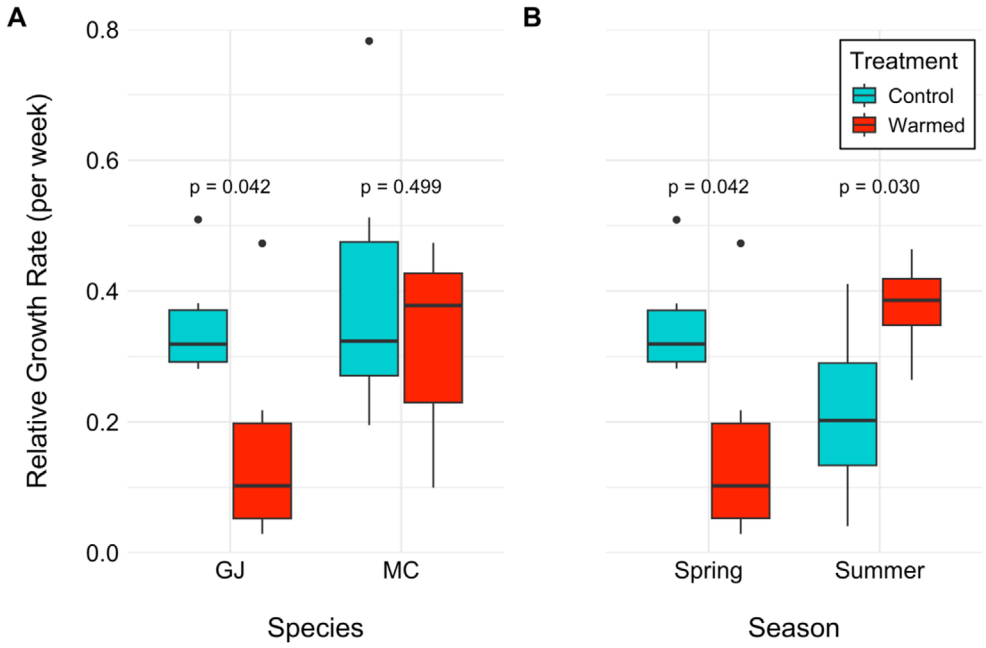

FIGURE 3 | Box and whisker plots showing the distribution of the relative growth rate (RGR) of fungal litter mats formed by (A) *Gymnopus johnstonii* (GJ) and *Marasmius* aff. *crinis-equi* (MC) in heated versus control treatments in Puerto Rico in spring and (B) *Gymnopus johnstonii* (GJ) in warmed and control plots in Puerto Rico during the spring and summer sampling periods. *p*-values indicate the results from Welch's two-sample *t*-test ($\alpha = 0.05$) which evaluated differences between warmed and control treatments.

TABLE 1 | Results from the linear mixed effects model analyses for the spring and summer sampling campaigns examining experimental warming on fungal mat growth in Puerto Rico.

Season	Fixed effects	*B* (CI)	df	*t*	*p*
Spring	(Intercept)	0.350 (0.15 to 0.55)	3.31	3.726	**0.028**
	Treatment (Warmed)	−0.188 (−0.47 to 0.09)	3.31	−1.414	0.244
	Species (MC)	0.051 (−0.14 to 0.24)	18.00	0.567	0.577
	Treatment (Warmed) × Species (MC)	0.054 (−0.21 to 0.32)	18.00	0.431	0.671
Summer	(Intercept)	0.348 (0.24 to 0.46)	6.21	6.513	**< 0.001**
	Treatment (Warmed)	−0.190 (−0.35 to −0.03)	6.21	−2.507	**0.045**
	Season (Summer)	−0.130 (−0.28 to 0.02)	15.79	−1.823	0.087
	Treatment (Warmed) × Season (Summer)	0.341 (0.13 to 0.55)	15.79	3.368	**0.004**

Note: For both seasons, relative growth rate is the dependent variable, and the fixed effects are as listed. During spring, the two species *Marasmius* aff. *crinis-equi* (MC) and *Gymnopus johnstonii* (GJ) were compared, while the summer analysis only looks at the seasonal differences for GJ. Plot was included as a random variable but explained very little variance across either season (variance = 0.0096 in spring and variance = 0.0012 in summer). *B* represents the fixed effects estimates ± the confidence interval. Bold values indicate statistical significance at $p < 0.05$.

Marasmius aff. *crinis-equi* colonized leaves 4.3 times faster than *G. johnstonii* ($p = 0.032$) (Figure 4A,B). The odds ratio of colonization was 0.300, meaning the odds of colonization for the heated treatment were about 70% lower than for controls based on the 95% confidence interval which did not span 0. Increased temperatures and the number of dry days prior to sampling did not decrease the time to colonization when evaluated independently ($p = 0.005$, $p = 0.002$ respectively); however, there was a significant interaction of dry days and temperature such that the time to leaf colonization increased as ambient temperatures and the frequency of dry days increased ($p = 0.002$).

During the summer sampling period, the warmed treatment significantly increased time to colonization and reduced colonization odds for *G. johnstonii* when assessed by the likelihood ratio test ($p = 0.008$, Figure 4C). The Wald test only yielded a marginally significant result ($p = 0.086$) but may not capture statistical significance for smaller sample sizes. Independently, the increased number of dry days significantly increased the time to leaf colonization ($p = 0.024$), while increased temperatures only marginally increased the time to leaf colonization ($p = 0.074$). The interaction between temperature and dry days (combined effect of increasing temperature and number of dry days on RGR) was not significant ($p = 0.789$). The seasonal comparison

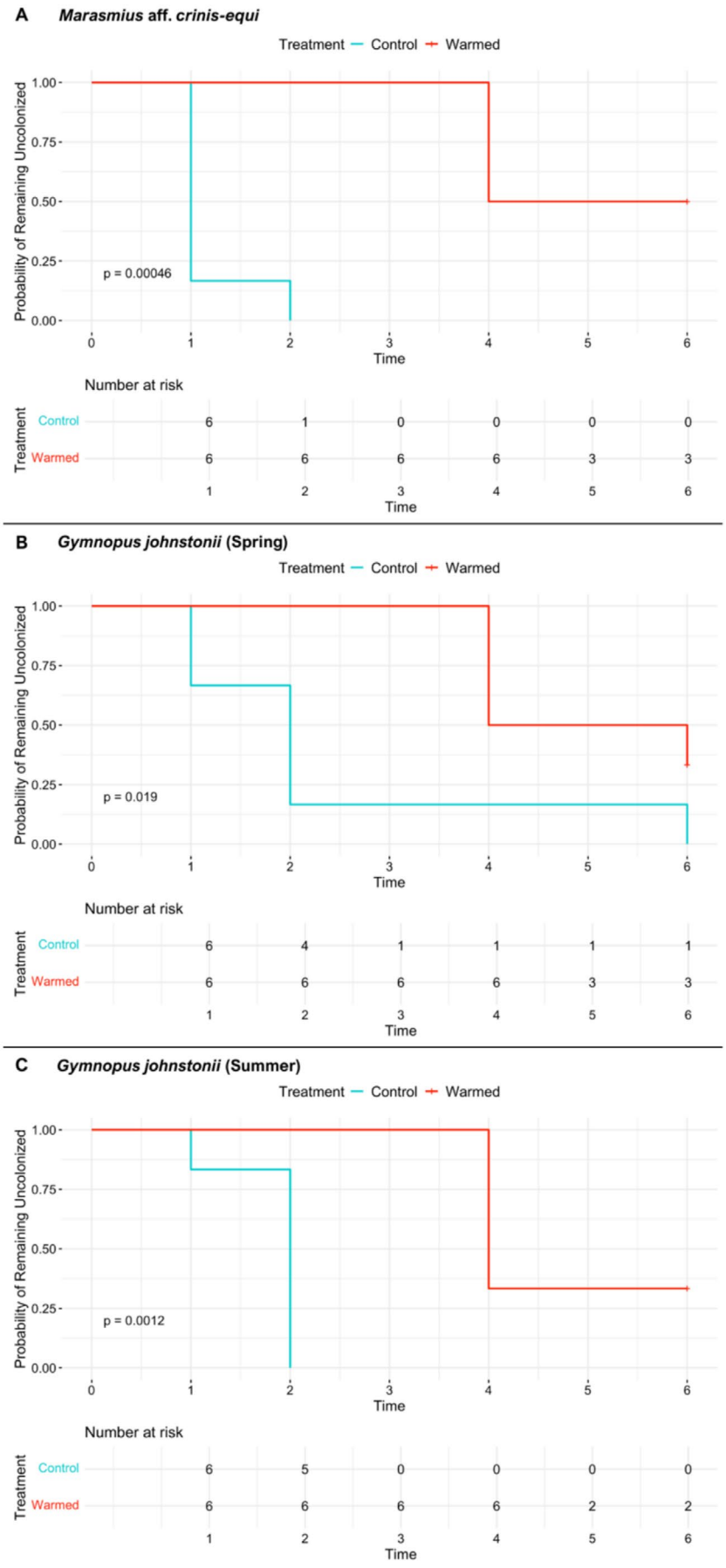

FIGURE 4 | Legend on next page.

FIGURE 4 | Survival curves showing time to leaf colonization for species (A) *Marasmius* aff. *crinis-equi* and (B) *Gymnopus johnstonii* during the spring sampling season, and (C) *Gymnopus johnstonii* during the summer sampling season. Time indicates the weeks it will take for the fungus to colonize a leaf. A steeper curve indicates faster colonization rates, while a flatter curve indicates slower ones. The number at risk table indicates how many individuals had not yet colonized the leaves and were therefore at risk of doing so at different time points ($n = 12$).

showed no significant main effect of season on leaf colonization ($p = 0.559$), but there was a trend toward increased odds in the summer.

4 | Discussion

4.1 | Effect of Warming on Relative Growth Rates

Experimental warming significantly affected fungal relative growth rate (RGR); however, the directions and magnitude of the response differed across the field sampling seasons (spring vs. summer). While more sampling would be needed to disentangle the controls, these seasonal differences were likely driven by differing moisture regimes during these periods, emphasizing the complex interplay between temperature and moisture on fungal growth dynamics. During the spring campaign, which maintained drier conditions in the first 2 weeks and overall based on the frequency of dry days, warming suppressed fungal growth by an average of 0.188 cm^2 $week^{-1}$ compared to the controls, potentially due to physiological stress.

Prior work has demonstrated that elevated temperatures can directly affect fungal metabolism by disrupting enzymatic activity and nutrient acquisition by leaf decomposer fungi (Carreiro et al. 2000). Warming may also indirectly affect fungal growth via drying of the litter layer, which could be exacerbated during dry periods or under more open canopies. For example, research in a neighboring watershed within the Luquillo Mountains (El Verde), found that three consecutive days of no throughfall reaching the forest floor led to significant drying of the litter layer and a 50% reduction in fungal hyphal volume (Lodge 1993; Lodge et al. 1994). Furthermore, direct solar irradiation on the forest floor at El Verde increased drying of the leaf litter via increased air temperature which reduced relative humidity, causing greater water loss via a significant increase in the evaporation potential (Lodge et al. 2008). It follows that warmer temperatures combined with more frequent days without throughfall during the spring sampling period (12 dry days during the first 2 weeks; mean 4.5 days $week^{-1}$ over the spring sampling period) also coincided with a drier litter layer, which negatively affected fungal growth.

Interestingly, the summer sampling period, which had fewer dry days and more consistent rainfall than the spring (mean of 3.0 vs. 4.5 dry days $week^{-1}$), revealed a reversal of this trend with warming significantly increasing RGR for *G. johnstonii* by an average of 0.163 per week. The significant interaction between warming treatment and season further highlights the potential role of seasonal moisture availability in modulating fungal responses to temperature. Increased growth rates during the summer sampling season may reflect the synergistic effects of greater humidity and warmth, which could enhance enzymatic activity and substrate utilization efficiency (Paulus et al. 2006).

A previous study using the warmed plots at our site found that warming by +4°C decreased moisture in litter by 36% and significantly slowed decomposition rates by 3% (data not shown). In the Luquillo Experimental Forest, moisture availability is a key driver of fungal activity, but it is the consistent availability of moisture rather than the amount of rainfall that is of primary importance to fungal leaf litter decomposer abundance and activity (Lodge 1993; Lodge et al. 2008). The litter layer in wet tropical forests is often thin, making it susceptible to drying if not regularly moistened by throughfall. Lodge (1993) found that the litter layer at El Verde near our study site in Puerto Rico dried completely if there were three or more consecutive days lacking throughfall inputs, and fungal hyphal abundance in litter was related to the extent of contiguous dry days and not to the amount of rain in the preceding week.

4.2 | Effect of Warming on Time to Leaf Colonization

Leaf colonization was significantly delayed in the warmed plots during both spring and summer sampling periods, but with notable differences between fungal species. In the wetter spring season, warming reduced *G. johnstonii* colonization rates by 22%, with *M.* aff. *crinis-equi* colonizing leaves 4.3 times faster than *G. johnstonii*. Our results are consistent with previous observations of the susceptibility of *G. johnstonii* to drought and exposure of the litter layer to solar irradiance, which dries it (Lodge and Cantrell 1995, 2023). The reduced colonization rate under warmed conditions is consistent with findings that elevated temperatures can inhibit fungal hyphal extension (Hawkes et al. 2011). The relative resilience of *M.* aff. *crinis-equi* to drier conditions is also consistent with observations from previous studies that suggest this species is more drought-resistant (Hedger et al. 1993; Lodge et al. 2008, 2022; Lodge and Cantrell 2023). These results underscore the idea that different fungal species could respond differently to the drivers of global change, and that over longer timescales, the composition of the fungal community could also be altered. The fact that temperature was negatively correlated with the rate of colonization during both sampling periods suggests that a 4°C temperature increase can disrupt fungal establishment and ecosystem functioning. This effect may be especially detrimental to *G. johnstonii* since it likely relies on rapid colonization times to outcompete other litter inhabiting fungi (Lodge and Asbury 1988).

4.3 | Effect of Climate Variability

The observed seasonal differences in fungal mat responses may be influenced by broader climatic patterns, such as those associated with El Niño events. El Niño years are characterized by reduced rainfall and altered moisture availability in many tropical regions, including Puerto Rico (Taylor et al. 2002). During the

dry season in Puerto Rico (December–April), El Niño years usually experience greater than average precipitation, while during the wet season (May–November) rainfall is normally lower than average (NOAA's National Weather Service, n.d.). We experienced these patterns during our study period in 2024, which could explain the observed pronounced seasonal interactions. During the spring campaign, which coincided with the typical "dry season" in Puerto Rico, there were more intense rainfall events and greater total rainfall than in the summer period, but there were also more total dry days. This sampling period experienced heavy rainfall events and drought-like conditions consecutively, which likely contributed to the significant negative interaction of warming on litter mat growth. These climatic patterns of intense rainfall and consecutive drought are projected to continue as atmospheric temperatures rise (Whitfield 2012; Lau and Zhou 2012; Moraes et al. 2022, Reyer et al. 2017). Future studies incorporating long-term climate data could help disentangle these controls and better predict fungal responses under variable climatic conditions.

4.4 | Implications and Future Directions

These findings highlight the nuanced effects of temperature and moisture on fungal growth and colonization processes, which have broad ecological implications. Since fungal mats are important for rapid decomposition of low-quality leaf litter by recycling limiting nutrients and reducing loss of mineral nutrients by controlling leaf and surface soil erosion, such disruptions could have cascading effects on decomposition and nutrient cycling (Lodge and Asbury 1988; Lodge et al. 2004, 2008, 2014, 2022; van der Wal et al. 2013). The observed seasonal variations emphasize the value of considering temporal context and regional climate patterns, such as those driven by El Niño, when assessing fungal responses to climatic changes. Our observed responses of litter mat-forming fungal species indicate some capacity for resilience in response to warming from drought-tolerant species such as *M.* aff. *crinis-equi*, as well as showing that warming effects could not only vary in magnitude but also in their sign, even across the seasons of a single year.

Our results also indicate that drought-sensitive species such as *G. johnstonii* may be significantly hindered in a much warmer and drier climate, meaning there could likely be a change in community composition of these litter mat forming fungi under intense climatic conditions. The loss of superficial mycelial fans of *G. johnstonii* could affect nutrient cycling via the food web by decreasing fungivorous invertebrates. This change in community composition could also decrease the effectiveness of fungal mats at reducing erosion and stabilizing slopes, especially since the drought-tolerant *M.* aff. *crinis-equi* also inhabits a sub-canopy niche, meaning it is not restricted to the forest floor like many other litter mat-forming species. Furthermore, species in the *M. crinis-equi* complex are necrotrophic plant pathogens that can switch to pathogenic mode when resources are scarce, thereby diminishing the shrub layer in wet tropical forests (Hedger et al. 1993; Lodge and Cantrell 2023). Results from Lodge et al. (2008) showed that placing litter mats formed by drought-tolerant agaric fungi can be used in remediation to reduce litter and soil erosion on steep slopes of road banks that only have limited shade, but caution is needed in species selection as some are facultative plant pathogens as noted above. Future research exploring the mechanistic underpinnings of these responses, including the role of enzyme activity, substrate availability, and fungal community composition could greatly benefit our mechanistic understanding of the consequences of global change effects on litter mat-forming fungi. Long-term experiments that incorporate multiple environmental stressors, such as drought or altered nutrient inputs, could provide deeper insights into the resilience of fungal litter mat ecosystems. Additionally, investigating the thermal thresholds of different fungal species could inform predictive models of ecosystem responses to global warming.

5 | Conclusion

Our study reveals that warming exerts complex and context-dependent effects on tropical litter mat forming fungal growth and leaf colonization rates, with implications for ecosystem functioning in a warming world. These results underscore the importance of integrating seasonal dynamics, species-specific traits, and regional climatic variability into models of fungal responses to climate change, thereby enhancing our ability to predict and mitigate ecosystem impacts.

Author Contributions

A.E.P., D.J.L., and T.E.W. conceived the idea and designed the methodology. A.E.P., H.C., L.C.R.-L., and D.A.O.-I. collected data. A.E.P. and D.J.L. worked on data analysis and visualization. A.E.P., D.J.L., T.E.W., D.A.I., T.B.-V., and C.S.O. wrote the manuscript. S.C.R. provided resources used for data collection. T.E.W. provided project funding.

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Disclosure

The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in ESS-DIVE at https://doi.org/10.15485/3020155.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** btp70201-sup-0001-Supinfo.docx.