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ARTICLE

Disease Ecology

Invasive grass litter suppresses a native grass species and promotes disease

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Abstract

Plant litter can alter ecosystems and promote plant invasions by altering resource availability, depositing phytotoxins, and transmitting microorganisms to living plants. Transmission of microorganisms from invasive plant litter to live plants may gain importance as invasive plants, which often escape pathogens upon introduction to a new range, acquire new pathogens over time. It is unclear, however, if invasive plant litter affects native plant communities by promoting disease. *Microstegium vimineum* is an invasive grass that suppresses native populations, in part through litter production, and has acquired new fungal leaf spot diseases since its introduction to the United States. In a greenhouse experiment, we evaluated how *M. vimineum* litter and its pathogens mediated competition with the native grass *Elymus virginicus*. *M. vimineum* litter promoted disease on *E. virginicus* and suppressed establishment and biomass of both species. Litter had stronger negative effects on *E. virginicus* than *M. vimineum*, increasing the relative biomass of *M. vimineum*. Live plant competition reduced biomass of both species and live *M. vimineum* increased disease incidence on *E. virginicus*. Altogether, invasive grass litter suppressed both species, ultimately favoring the invasive species in competition, and increased disease incidence on the native species.

KEYWORDS
Bipolaris, *Elymus virginicus*, foliar fungal pathogen, interference competition, invasive species, *Microstegium vimineum*, thatch

INTRODUCTION

Dead organisms and tissues can influence populations, communities, and ecosystems (Facelli & Pickett, 1991; Renwick et al., 2007; Subalusky et al., 2017). Plant litter, for example, can release nutrients and allelopathic chemicals (Facelli & Pickett, 1991), block light penetration to the soil (Molinari & D'Antonio, 2020), mediate fire intensity (Flory et al., 2015), and host microorganisms that alter nutrient availability or cause disease (U'Ren & Arnold, 2016), which can modify plant competition. For example, litter can alter the outcome of resource competition through species-specific effects on nutrient cycling and light availability (Daufresne & Hedin, 2005; Eppinga et al., 2011). Indeed, litter impacts on resource availability (Aerts et al., 2017; Farrer & Goldberg, 2009;

Molinari & D'Antonio, 2020) and fire regimes (D'Antonio & Vitousek, 1992) have facilitated the dominance of invasive plant species. However, research on invasive litter-mediated competition rarely considers pathogens that may reside in litter and infect living plants (Beckstead et al., 2012). Because invasive plants can acquire new pathogens over time (Goss et al., 2020), apparent competition with native species (i.e., indirect competition through a shared enemy; Holt & Bonsall, 2017) becomes more likely the longer an invasive species has been established and may be mediated by its litter.

Invasive plants often dominate communities, produce abundant biomass (Vilà et al., 2011), and can disproportionately contribute to litter (D'Antonio & Vitousek, 1992; Farrer & Goldberg, 2009; Flory et al., 2015; Wolkovich, 2010). Litter can cause interference competition (i.e., block access to resources or directly induce mortality, Figure 1a; Amarasekare, 2002) and suppress establishment, growth, and survival of native (Flory et al., 2015; Molinari & D'Antonio, 2020; Walker & Vitousek, 1991) and invasive species (Chau et al., 2013; Warren et al., 2013). Conversely, litter can facilitate plant growth through increased soil moisture and nutrient availability (Chau et al., 2013; Wolkovich, 2010). Therefore, impacts of invasive plant litter on native species through newly acquired pathogens may compound other negative effects or counteract positive effects of litter.

Litter can mediate wild plant diseases (Facelli et al., 1999; Whitaker et al., 2017) and has long been recognized as a source of primary inoculum for crop diseases (Kerdraon et al., 2019). Plant pathogens associated with litter (Chand et al., 2002) can transmit following plant germination (Figure 1a) and throughout the plant life cycle (Figure 1b,c; Beckstead et al., 2012). Litter may also indirectly promote infection by altering microenvironments to favor pathogen growth, potentially through increased moisture, moderating temperature extremes, and enhancing nutrient availability (Beckstead et al., 2012; Bonanomi et al., 2011). Invasive litter impacts on disease are understudied, likely because invasive plants often escape pathogens upon introduction to a new range, and acquisition of new pathogens takes time (Goss et al., 2020). However, after invasive plants have acquired pathogens in their new range, litter may take on the additional role of promoting disease. It is unclear whether litter's impacts on disease, like its impacts on fire regimes and resource availability (Facelli & Pickett, 1991), can mediate competition between native and invasive species.

Here, we experimentally investigated the potential for invasive plant litter containing foliar fungal pathogens to mediate competition with a native species. *Microstegium vimineum* (Trin.) A. Camus (stiltgrass) is an invasive grass that was introduced to the United States from Asia in the early 1900s (Fairbrothers & Gray, 1972). Many
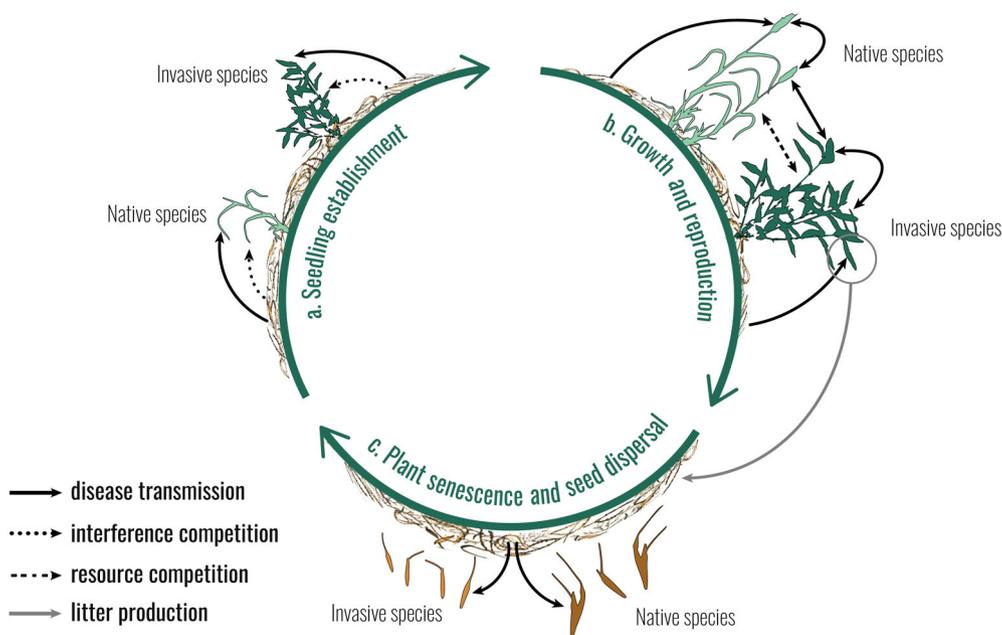

FIGURE 1 Litter is a potential source of pathogen propagules throughout the plant life cycle (a–c), which may be transmitted among live plants (b). Litter also has physical, chemical, and other biological effects on plant establishment and growth that can result in interference competition (a). The net effects of litter can alter establishment (a), growth, reproduction (b), and seed survival (c). Litter may mediate the negative effects of invasive plants (here, *Microstegium vimineum*) on native species (here, *Elymus virginicus*), such as resource competition, allelopathy, or plant–soil feedbacks

M. vimineum populations in the United States are infected with foliar fungal pathogens (Stricker et al., 2016). *M. vimineum* suppresses germination, establishment, and growth of other plant species through resource competition and in some cases, allelopathy or plant–soil feedbacks (Bauer & Flory, 2011; Corbett & Morrison, 2012). *M. vimineum* also affects native species through litter-mediated competition, blocking seedling establishment (Flory & Clay, 2010) and increasing the intensity of fires (Flory et al., 2015). *M. vimineum* litter may suppress native seedling establishment through light limitation (Flory & Clay, 2010), allelopathic chemicals (Cipollini & Greenawalt Bohrer, 2016; Corbett & Morrison, 2012; Pisula & Meiners, 2010), or harboring pathogens (i.e., apparent competition; Chand et al., 2002). In a greenhouse experiment, we manipulated *M. vimineum* litter and competition between *M. vimineum* and the co-occurring native grass *Elymus virginicus* (Virginia wild rye; Flory et al., 2011). We hypothesized that litter would promote disease on and interfere with both species (Figure 1). We expected that litter would increase the relative biomass of *M. vimineum* if litter suppressed *E. virginicus* more than *M. vimineum* and vice versa.

METHODS

Study system

Microstegium vimineum is a C-4 annual grass that is invasive in the eastern United States (USDA, 2020). It grows rapidly, produces large quantities of litter, and reduces surrounding plant abundances (Flory et al., 2017; Flory & Clay, 2010). Over the past 10 years, leaf spot diseases caused by fungal pathogens of the genus *Bipolaris* have been identified in US *M. vimineum* populations, where the diseases can reduce *M. vimineum* biomass and seed production (Flory et al., 2011; Stricker et al., 2016). *E. virginicus* is a perennial bunchgrass that is native to North America (USDA, 2020) and co-occurs in forest understory habitats with *M. vimineum* (Cole & Weltzin, 2004; Wisenhunt, 2008). The competitive effects of *M. vimineum* on *E. virginicus* in the field are unknown, but field experiments in which *E. virginicus* was a member of the native community demonstrate negative impacts of *M. vimineum* invasion on overall native plant biomass (Flory et al., 2017; Stricker et al., 2016). However, in one greenhouse study, the strength of *M. vimineum* interspecific competition was not significantly different than the strength of *E. virginicus* intraspecific competition (Jones, 2009). *Bipolaris* pathogens infect *E. virginicus* (Flory et al., 2011; Lane et al., 2020) and can reduce its biomass production (Kendig et al., 2021).

Experimental design

To evaluate the potential for invasive plant litter to mediate competition with a native species, we manipulated invasive *M. vimineum* litter amount and plant species composition in a greenhouse experiment and measured plant establishment, biomass, and disease incidence. The experiment consisted of 12 treatments: four levels of *M. vimineum* litter crossed with three planting treatments (*M. vimineum* alone, *E. virginicus* alone, and *M. vimineum* and *E. virginicus* together). We made three assumptions about the experimental treatments: (1) a significant effect of litter on establishment or biomass represented either interference competition or, if disease was involved, apparent competition; (2) a significant effect of planting treatment on establishment or biomass indicated interspecific competition (e.g., resource competition, allelopathy, plant–soil feedbacks or, if disease was involved, apparent competition); and (3) a significant effect of litter or planting treatment on disease incidence provided evidence for apparent competition mediated by *Bipolaris* fungi.

We obtained *M. vimineum* litter for the greenhouse experiment in May 2018 from a forested site that was heavily invaded by *M. vimineum* in Big Oaks National Wildlife Refuge (BONWR, Madison, IN, 38.9656, -85.3645). Dead *M. vimineum* leaves at the site showed symptoms of *Bipolaris* infection, including leaf spots with a dark brown border and light interior (Lane et al., 2020). Live *M. vimineum* and *E. virginicus* at the site had symptoms previously shown to be caused by *B. gigantea* and other *Bipolaris* spp. (Lane et al., 2020; Stricker et al., 2016). We transported *M. vimineum* litter to the University of Florida (Gainesville, FL).

Microstegium vimineum has a mixed mating system of cleistogamous (obligately selfed, hidden within leaf sheaths) and chasmogamous (potentially outcrossed, open) seeds (Baker & Dyer, 2011). The litter collected from BONWR contained cleistogamous seeds. With the goal of tracking intentionally planted seeds (described in next paragraph) and isolating the impacts of litter, we minimized the effects of cleistogamous seeds by hand-picking and discarding them along with any non-*M. vimineum* material from the litter. We created three litter treatments within the range of litter observed at BONWR (Appendix S1): 0.91 g/pot (50 g/m², "low"), 1.82 g/pot (100 g/m², "medium"), 3.64 g/pot (200 g/m², "high"), and a control treatment without litter ("none"). While the treatment without litter is unrealistic for *M. vimineum*-invaded communities, it allowed us to evaluate the impacts of live plant competition in isolation of litter effects. To help simulate conditions in the field and enhance sporulation of pathogenic fungi in the litter,

we incubated litter for 56 h prior to the experiment at room temperature in 3.78-L plastic bags with a paper towel saturated with deionized water.

We collected and combined *M. vimineum* seeds from multiple plants at multiple sites within BONWR in fall 2015 and purchased *E. virginicus* seeds from Prairie Moon Nursery, which were harvested in 2017 from a production field in Winona, MN. On 15 June 2018, we planted either 50 seeds of *E. virginicus*, 50 seeds of *M. vimineum* (both "alone"; i.e., intraspecific competition), or 50 seeds of each ("in competition"; i.e., inter- and intraspecific competition), into 1-L (15.2 cm diameter) plastic pots filled with Metromix 930 growing medium (Sungro Horticulture, Agawam, MA) saturated with tap water. We used this additive competition experimental design to evaluate the effects of interspecific competition between live plants, but we acknowledge that we are unable to evaluate the effects of intraspecific competition, the relative strength of inter- to intraspecific competition, or the sensitivity of our results to the densities we chose (Inouye, 2001). In addition, we refer to the planting treatments as a manipulation of interspecific competition but acknowledge that litter effects are also a potential form of interspecific competition between *M. vimineum* and *E. virginicus*. We added the litter treatments to pots, creating 12 treatments with six replicates each. The following day, we topped each pot with a clear plastic sheet (50.8 cm width, 17.8 cm height; 0.005 Grafix Dura-Lar film, Maple Heights, OH) formed into a cone to mimic humidity that seedlings may encounter in the field and potentially enhance fungal sporulation. Cones were opened to cylinders as plants outgrew them. We placed pots under shade tents in the greenhouse to reduce heat stress (photosynthetically active radiation: 224 ± 18 μmol $m^{-2} s^{-1}$), haphazardly rearranged them across two greenhouse benches weekly, and watered them daily.

Data collection

To measure establishment, we counted the number of plants per pot and divided this number by the number of seeds in the pot. To measure disease incidence, we counted the proportion of plants per pot with at least two foliar lesions. We measured establishment and disease incidence weekly for 6 weeks (Figure S1). Some *M. vimineum* seedlings grew in *E. virginicus*-only pots, likely from cleistogamous seeds left in the litter despite our attempts to remove them. We counted these seedlings in every census and removed them every 7–13 days beginning 18 days postplanting (Appendix S2: Figure S1). After 10 weeks, we cut all plant stems at the soil surface and stored each species from each pot in a separate paper

bag at 4°C. To obtain postharvest estimates of disease incidence, we counted the proportion of *M. vimineum* plants and *E. virginicus* tillers (individuals were not identifiable) with two or more foliar lesions. Because *M. vimineum* had few lesions, we searched for a maximum of 10 min per pot. To measure biomass, plants were oven-dried at 60°C to a constant mass and weighed.

To evaluate the presence of *Bipolaris* spores in the litter used for the experiment, we extracted *M. vimineum*-dominated litter that was collected from the same location and at the same time as the litter used in the experiment. We incubated 65 g of litter with 50 ml sterile deionized water at 27°C for 48 h. We then washed the litter in 120 ml sterile deionized water with 0.1% Tween 20 (Sigma-Aldrich, St. Louis, MO) and filtered rinsate through cheese cloth, resulting in a crude suspension of conidia. Conidia were quantified using a Bright-Line hemacytometer, where elongated multicelled conidia with longitudinal cell walls characteristic of *Bipolaris* species were counted (USDA-APHIS-PPQ permit PP526P-18-01688; Lane et al., 2020).

To confirm that pathogens found on leaves with lesions corresponded to the fungi isolated from the litter used for treatments (i.e., *Bipolaris* species), we selected five *M. vimineum* leaves and six *E. virginicus* leaves from the experiment with relatively high numbers of lesions or large lesions. The leaves came from the treatments: *M. vimineum* planted with medium and high litter; *E. virginicus* planted with no, low, and high litter; and both species planted with low and high litter. We placed each leaf in a Petri dish with 7-cm filter paper wetted with deionized water and incubated them at 26°C under 12-h light/dark cycle for 24 h. Under a dissecting microscope, we searched leaves for conidiophores and conidia, which we transferred to a V8 media agar plate (4:1 distilled water : V8 juice, 15 g/L agar, and 2 g/L $CaCO_3$) using a sterile dissecting needle. We incubated plates at 26°C under 12-h light/dark cycle for 5–7 days and identified the fungal genus or species based on conidia size and morphology (Lane et al., 2020).

Data analysis

To evaluate the effects of litter and interspecific competition on establishment and disease incidence, we fit generalized linear regressions with binomial error distributions (logit link) to the proportion of seeds that became plants for each species (establishment) and the proportion of *E. virginicus* tillers with lesions (disease incidence). Too few lesions were observed on *M. vimineum* to analyze disease incidence. Because unintentionally added cleistogamous seeds could have increased *M. vimineum*

establishment values, we estimated the number of cleistogamous seeds that were likely in pots planted with *M. vimineum* (based on *E. virginicus*-only pots with the same litter treatments) and added this value to the number of *M. vimineum* seeds planted. In two cases, the number of *M. vimineum* plants exceeded the sum of the number planted and estimated cleistogamous seeds so we increased the estimate of seeds planted to match the number of plants. Litter mass, planting treatment, and their interaction were the independent variables in the models. The intercept was the treatment in which species were alone and without litter, therefore experiencing only intraspecific competition. The main effect of planting treatment therefore estimated the effect of adding interspecific competition. We evaluated establishment at the time points when the greatest number of treatments reached their maximum establishment values (26 and 39 days postplanting for *M. vimineum* and *E. virginicus*, respectively; Appendix S2: Figure S1), and we evaluated disease incidence using data collected postharvest. To evaluate whether the effects of litter on *M. vimineum* establishment were detectable given the unintentional addition of cleistogamous seeds from the litter, we fit a generalized linear regression with a Poisson error distribution (logit link) to the number of *M. vimineum* plants per pot counted postharvest with the independent variables described earlier.

To evaluate the effects of litter and interspecific competition on plant growth, we fit normal linear regressions to two response variables per species: log-transformed biomass per pot and log-transformed biomass divided by number of plants (for *M. vimineum*) or tillers (for *E. virginicus*) per pot counted postharvest. The models again contained litter mass, planting treatment, and their interaction as the independent variables. To evaluate the effect of disease on establishment and plant biomass, we fit normal linear regressions to the residuals of the simplified establishment and biomass regressions (only containing significant independent variables) with disease incidence as the independent variable. To evaluate the effect of litter on relative biomass, we fit a normal linear regression to log-transformed *M. vimineum* biomass from "in competition" pots with litter as an independent variable and an offset variable of log-transformed total biomass, effectively creating a dependent variable of the log ratio of *M. vimineum* biomass to total biomass (Zuur et al., 2009).

Multiple response variables did not change monotonically with the continuous litter variable "litter mass," so we refit all models with litter as a categorical variable (i.e., none, low, medium, and high) and compared them to models with the continuous litter variable using Akaike's information criterion (AIC). We present results from models with lower AIC values (see Appendix S2 for ΔAIC). We used backward stepwise selection to test the statistical significance of interactions and, if applicable, main effects (Crawley, 2007), comparing nested models with chi-squared tests (binomial/Poisson) or F tests (normal). For models in which the categorical litter variable was statistically significant, we compared the effects of the litter levels on the response variable with Tukey's honestly significant difference test. We performed analyses in R version 4.0.1 (R Core Team, 2020). We report model-estimated average establishment, disease incidence, and biomass in the "Results" section.

RESULTS

The average proportion of seedlings that established from *M. vimineum* seeds without litter or *E. virginicus* present was 0.86 and high litter reduced *M. vimineum* establishment to 0.71 (Figure 2a, continuous litter effect). With *E. virginicus* present, *M. vimineum* establishment was 0.81 without litter and 0.73 with high litter (litter–competition interaction: $p = 0.04$, Appendix S2: Table S1). The negative effect of litter on *M. vimineum* establishment did not, however, translate to significantly lower plant numbers (Figure 2b; $p = 0.45$, Appendix S2: Table S2), likely because there were higher total numbers of seeds (unintentional cleistogamous seeds) in pots with more litter (Appendix S2: Figure S1). The average proportion of seedlings that established from *E. virginicus* seeds without litter or live *M. vimineum* present was 0.83 (Figure 2c). Litter reduced *E. virginicus* establishment to 0.73 in the low ($p < 0.01$) and high ($p < 0.01$) litter treatments and to 0.76 in the medium ($p = 0.01$) litter treatment (categorical litter effect), although there were no significant differences among the low, medium, and high litter treatments (Figure 2c, Appendix S2: Table S3). There was no significant effect of live *M. vimineum* on *E. virginicus* establishment ($p = 0.29$, Appendix S2: Table S3). Live *M. vimineum* were temporarily in some *E. virginicus*-only pots due to cleistogamous seed establishment, but their numbers were significantly lower than *M. vimineum* seedlings in the interspecific competition pots (difference of means = −37, $t = -23.15$, $p < 0.01$ for Welch two-sample t test of seedling numbers on the fourth observation day, Appendix S2: Figure S1).

Microstegium vimineum developed few lesions, with disease incidence of only 0.02 across all treatments (data not shown). *E. virginicus* had disease incidence of 0.05 without litter or live *M. vimineum* present (Figure 2). Low litter increased disease incidence to 0.23 ($p < 0.01$), medium litter increased disease incidence to 0.08
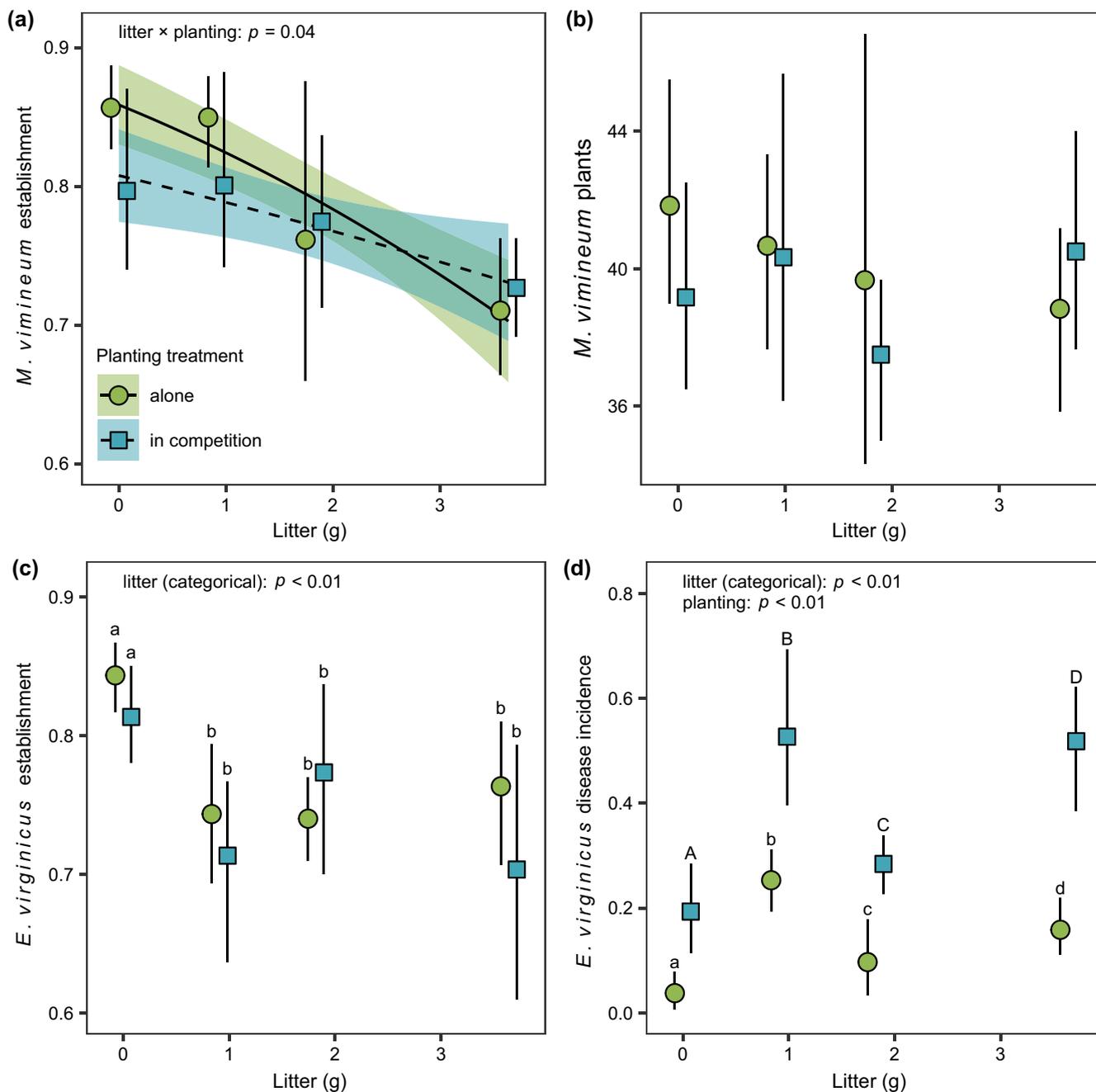
FIGURE 2 *Microstegium vimineum* litter and the presence of *Elymus virginicus* (i.e., "in competition") reduced *M. vimineum* establishment (i.e., the proportion of seeds that became seedlings; a) but did not significantly affect the number of *M. vimineum* plants postharvest (b). *Microstegium vimineum* litter reduced *E. virginicus* establishment (c). Live plants (i.e., "in competition") and litter of *M. vimineum* increased disease incidence on *E. virginicus* (i.e., the proportion of tillers with at least two lesions; d). Points and error bars represent raw data (mean ± 95% CI) and are nudged horizontally to reduce overlap. Lines and shading represent model estimates (mean ± 95% CI). Letters represent significantly different treatments (Tukey's honest significant difference: $p < 0.05$), where differences in capitalization represent significant differences between planting treatments while differences in letters represent significant differences among litter treatments

($p < 0.01$), and high litter increased disease incidence to 0.16 ($p < 0.01$), with significant differences among all litter treatments (categorical litter effect, Figure 2d, Appendix S2: Table S4). In addition, live *M. vimineum* increased *E. virginicus* disease incidence to 0.20 in the absence of litter ($p < 0.01$). The interaction between litter and live

M. vimineum was not statistically significant ($p = 0.08$, Appendix S2: Table S4).

An extraction of litter collected from the same location and at the same time as the experimental litter yielded 5500 *Bipolaris*-like conidia/g. To evaluate whether foliar lesions were also associated with *Bipolaris*

fungi, we assessed five *M. vimineum* and six *E. virginicus* leaves with lesions. We identified *Bipolaris gigantea* on four *M. vimineum* leaves and another *Bipolaris* species on the fifth *M. vimineum* leaf and two *E. virginicus* leaves. Other pathogens were also associated with *Bipolaris* species on the leaves. We identified a *Curvularia* species, a common saprophyte, either alone or in combination with

Bipolaris on one *M. vimineum* leaf and two *E. virginicus* leaves. We also identified a *Cladosporium* species, a common endophyte, on a *M. vimineum* leaf infected with *Bipolaris*.

Microstegium vimineum weighed an average of 37.4 g per pot and 0.8 g per plant without litter or *E. virginicus* present (Figure 3a,b). High litter reduced *M. vimineum*
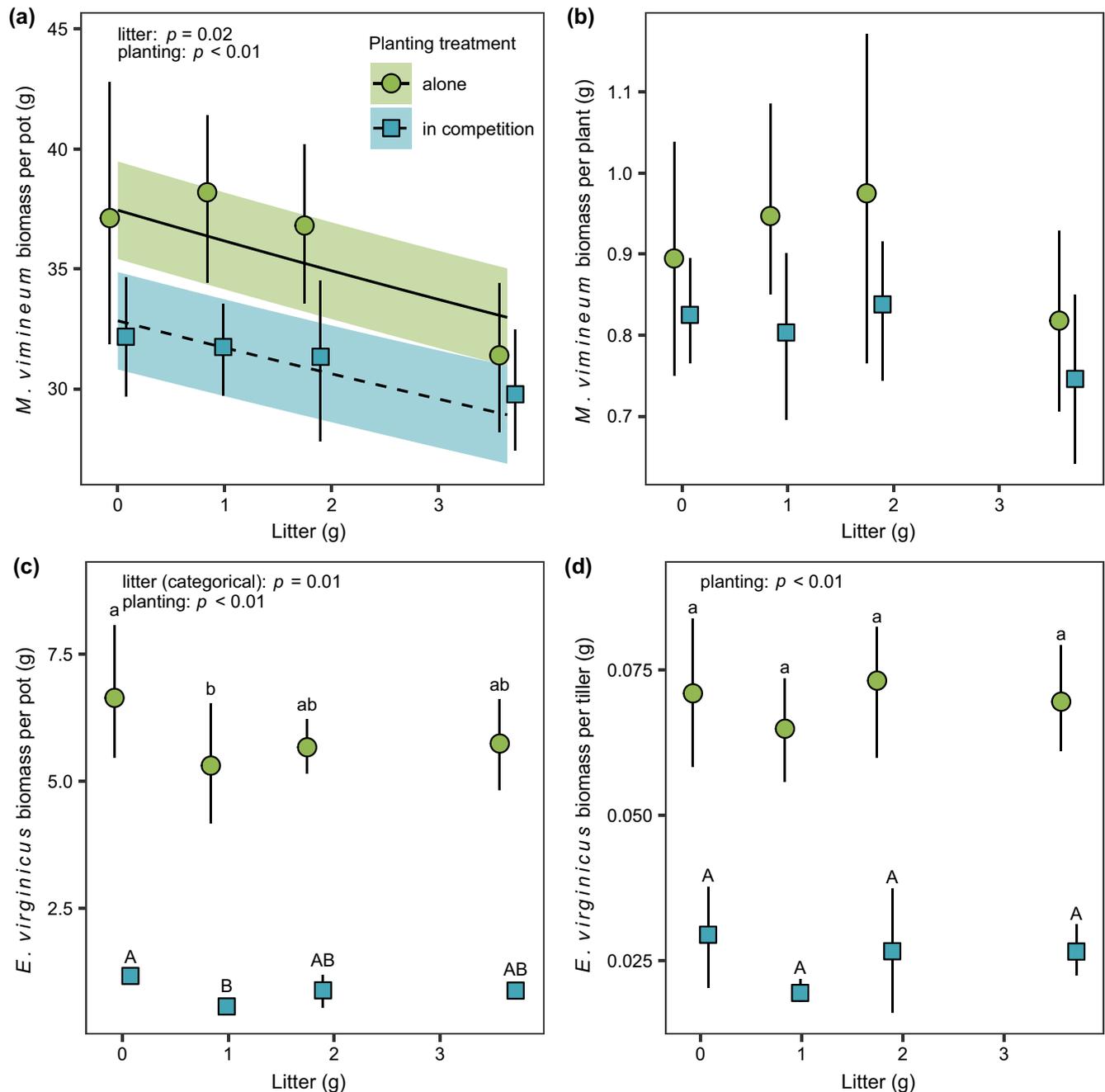

FIGURE 3 *Microstegium vimineum* litter and the presence of *Elymus virginicus* (i.e., "in competition") reduced *M. vimineum* biomass per pot (a) but did not significantly affect *M. vimineum* biomass per plant (b). Live *M. vimineum* (i.e., "in competition") reduced *E. virginicus* biomass per pot (c) and per plant (d) and *M. vimineum* litter reduced *E. virginicus* biomass per pot (c). Points and error bars represent raw data (mean ± 95% CI) and are nudged horizontally to reduce overlap. Lines and shading represent model estimates (mean ± 95% CI). Letters represent significantly different treatments (Tukey's honestly significant difference: $p < 0.05$), where differences in capitalization represent significant differences between planting treatments while differences in letters represent significant differences among litter treatments

biomass per pot by 11.9% ($p = 0.02$, continuous litter effect, Appendix S2: Table S5), but there was no significant effect of litter on *M. vimineum* biomass per plant ($p = 0.18$, Appendix S2: Table S6). *E. virginicus* reduced *M. vimineum* biomass per pot by 12.3% ($p < 0.01$) and had a marginally significant effect on *M. vimineum* biomass per plant (-10.5%, $p = 0.05$). Litter and *E. virginicus* did not interact to significantly affect *M. vimineum* biomass per pot ($p = 0.38$, Appendix S2: Table S5) or per plant ($p = 0.99$, Appendix S2: Table S6). *E. virginicus* weighed an average of 7.3 g per pot and 0.07 g per tiller without litter or live *M. vimineum* present (Figure 3c,d). Low litter reduced *E. virginicus* biomass per pot by 38.7% ($p = 0.01$, categorical litter effect), but there were no significant effects of the other litter treatments (Appendix S2: Table S7). In addition, litter did not significantly affect *E. virginicus* biomass per tiller ($p = 0.48$, Appendix S2: Table S8). Live *M. vimineum* reduced *E. virginicus* biomass per pot by 85.8% ($p < 0.01$) and biomass per tiller by 65.6% ($p < 0.01$). Litter and live *M. vimineum* did not interact to significantly affect *E. virginicus* biomass per pot ($p = 0.31$, Appendix S2: Table S7) or per tiller ($p = 0.75$, Appendix S2: Table S8). Disease incidence did not significantly affect *E. virginicus* establishment, biomass per pot, or biomass per tiller after accounting for treatment effects (Appendix S2: Tables S9–S11). *Microstegium vimineum* comprised 96.4% of the biomass in pots with both species and no litter. The low litter treatment increased the relative biomass of *M. vimineum* to 98.2% ($p = 0.01$, categorical litter effect), but there were no significant differences between any of the other litter treatments (Appendix S2: Table S12).

DISCUSSION

Invasive plant litter can play an important role in community dynamics (D'Antonio & Vitousek, 1992; Eppinga et al., 2011; Facelli & Pickett, 1991), but the degree to which it influences communities through disease is unclear (Beckstead et al., 2012). We evaluated the impacts of litter from the invasive grass *M. vimineum* on disease incidence and competition with the native grass *E. virginicus*. Although litter negatively impacted both species, it ultimately increased the relative biomass of *M. vimineum*. Because disease incidence on *E. virginicus* did not significantly affect its establishment or growth, it is likely that *M. vimineum* litter increased the relative biomass of *M. vimineum* through stronger suppression of *E. virginicus* establishment than *M. vimineum* and not through disease. Therefore, invasive plant litter can drive interference competition and promote disease on native species, which may ultimately benefit the invasive species in the field.

We expected pathogen transmission from litter to live plants to alter competition between the native and invasive species because pathogen exposure can reduce growth of both species (Kendig et al., 2021; Stricker et al., 2016). However, litter only promoted disease on *E. virginicus*, and there were no significant effects of disease incidence on *E. virginicus* establishment or biomass after accounting for litter and planting treatment effects. Further, there were no significant interactive effects of litter and competition on biomass. Due to the visually striking levels of disease at BONWR where the litter for this experiment was collected, we were surprised by the low levels of disease in the greenhouse. Dry conditions, despite our use of humidity chambers, may have limited lesion formation on *M. vimineum* (Appendix S3) and disease incidence on *E. virginicus*. Additionally, *M. vimineum* may have shed infected leaves between assessments (Vloutoglou & Kalogerakis, 2000). A similar experiment conducted in the field could yield greater disease incidence and lesion formation through season-long secondary infection and disease development at the discrete foci where pathogens perennate on litter. However, even with these limitations on disease, live *M. vimineum* promoted disease incidence on *E. virginicus*. Because *M. vimineum* had few foliar fungal lesions and therefore was likely not a prominent source of pathogen transmission, we hypothesize that *M. vimineum* biomass altered the microclimate to favor fungal sporulation. Therefore, *M. vimineum* may promote disease on co-occurring native species through both litter and live biomass, which could lead to apparent competition with native species that are susceptible to *Bipolaris* infection. Lesions on *E. virginicus* plants without litter suggest an additional pathogen source, such as unsterilized soil or seeds. While we cannot conclude that infection came from litter, we isolated *Bipolaris* species from both *M. vimineum* litter and leaf spot lesions on both plant species.

Litter reduced *M. vimineum* establishment and biomass. This negative feedback between *M. vimineum* growth and litter production may regulate or reduce *M. vimineum* density over time (Flory et al., 2017; Tilman & Wedin, 1991). While increasing litter amounts decreased *M. vimineum* establishment and biomass, *E. virginicus* biomass was most sensitive to low amounts of litter. Because litter can have both inhibitory and facilitative effects (Facelli & Pickett, 1991; Molinari & D'Antonio, 2020), we hypothesize that low amounts of litter had stronger net negative impacts than higher amounts of litter, where facilitative effects may be more prominent. Because litter had a stronger effect on biomass per pot than biomass per plant/tiller for both species, it is likely that litter reduced biomass through its impacts on establishment in addition to its impacts on

individual plant growth. Stronger litter-mediated interference competition on *E. virginicus* than *M. vimineum* likely drove increased *M. vimineum* relative biomass, which was greatest with low litter. Because *M. vimineum* better tolerated low amounts of litter than *E. virginicus*, litter may reinforce the negative impacts of *M. vimineum* on *E. virginicus* through live plant competition (Eppinga et al., 2011). We did not isolate the mechanism by which *M. vimineum* litter suppressed plant establishment and biomass, but *M. vimineum* litter reduces light penetration to the soil surface (Flory & Clay, 2010) and the leaves contain phytotoxins (Pisula & Meiners, 2010).

Despite our best efforts to remove cleistogamous seeds from litter, there were some unintended *M. vimineum* seeds in *E. virginicus*-only pots. While it is possible that seedlings that emerged from cleistogamous seeds competed with *E. virginicus* seedlings, we minimized these effects by removing *M. vimineum* seedlings when they were small. Additionally, to not overestimate *M. vimineum* establishment, we adjusted the planted number of seeds to account for probable germination of cleistogamous seeds. Cleistogamous seeds could have affected *M. vimineum* biomass per pot in two ways: increasing the number of plants per pot and decreasing the biomass per plant. We found evidence for the first effect because *M. vimineum* plant numbers were effectively constant across litter treatments despite reduced establishment with increasing litter (i.e., more litter deposited more cleistogamous seeds, offsetting establishment losses due to litter). We did not find evidence for the second effect because litter treatments did not significantly affect *M. vimineum* biomass per plant. Therefore, the negative effect of litter on *M. vimineum* biomass per pot is likely because litter reduced the carrying capacity of pots, rather than because of cleistogamous seeds. Because litter reduced *M. vimineum* establishment, but deposited cleistogamous seeds, it may increase the proportion of *M. vimineum* germinating from cleistogamous seeds (obligate selfing) relative to chasmogamous seeds (potentially outcrossing) in the field, which could reduce genetic diversity of *M. vimineum* populations (Baker & Dyer, 2011). To our knowledge, the role of litter in ecology and evolution of plant mating systems has not been studied and may be a fruitful direction of inquiry.

While the importance of invasive plant litter for resource availability (Aerts et al., 2017; Farrer & Goldberg, 2009) and fire regimes (D'Antonio & Vitousek, 1992) has been recognized for multiple systems, its role in mediating species interactions through disease has received little attention (Beckstead et al., 2012). We found that litter suppressed plant establishment and growth and promoted disease on the native species. While disease did not affect relative biomass, greater disease severity (e.g., due to more favorable environmental conditions) could affect *M. vimineum* competition with native species (Stricker et al., 2016). These processes can co-occur with interactions among live plants (Figure 1), and here, live invasive *M. vimineum* reduced biomass and promoted disease on the native species. Thus, consideration of invasive plant impacts on native communities and ecosystems should comprise both live plants and litter, including their potential roles in promoting disease.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
Data and code (Benitez et al., 2021) are available from the Environment Data Initiative (EDI) Data Portal: https://doi.org/10.6073/pasta/3688be04a98697c95efa5747eb3c9513.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Appendix S1 for "Invasive grass litter suppresses a native grass species and promotes disease" by Liliana Benitez, Amy E. Kendig, Ashish Adhikari, Keith Clay, Philip F. Harmon, Robert D. Holt, Erica M. Goss, and S. Luke Flory
Ecosphere

Assessment of litter density at BONWR

On April 4, 2018, we collected litter from six haphazardly selected 0.25 m^2 plots at the BONWR site described in the main text. We cut the litter around the inside edges of the PVC used to delineate the plots and put it in plastic bags. The following week, it was weighed at the University of Indiana (Bloomington, IN, USA). We did not separate *Microstegium vimineum* litter from other types of litter, but *M. vimineum* appeared to be the dominant species.

Table S1. Weights of litter harvested from BONWR.

Plot ID	Litter density (g/m^2)
A	197.36
B	291.00
C	369.76
D	364.76
E	458.88
F	204.72
Average	314.41
Standard error	41.93

Appendix S2 for "Invasive grass litter suppresses a native grass species and promotes disease "
by Liliana Benitez, Amy E. Kendig, Ashish Adhikari, Keith Clay, Philip F. Harmon, Robert D.
Holt, Erica M. Goss, and S. Luke Flory
Ecosphere

Table S1. Model summary of *Microstegium vimineum* establishment (proportion of seeds that
became plants, binomial distribution, logit link). Estimates, standard errors (SE), z statistic, and
associated *P*-value from maximal model (n = 48). Deviance and *P*-value from Chi-squared tests
of nested models. Model comparison with categorical litter model: ΔAIC = 4.7.

Predictor	Estimate	SE	z	Pr(>|z|)	Deviance	Pr(>Chi)
Intercept	1.80	0.12	14.98	$1.02e^{-50}$		
Litter (g)	-0.26	0.05	-5.22	$1.83e^{-7}$		
Elymus virginicus present	-0.37	0.16	-2.26	0.02		
Litter:*E. virginicus* present	0.14	0.07	2.01	0.04	-4.06	0.04

Table S2. Model summary of *Microstegium vimineum* plants per pot (Poisson distribution, logit link). Estimates, standard errors (SE), z statistic, and associated *P*-value from maximal model (*n* = 48). Deviances and *P*-values from Chi-squared tests of nested models. Model comparison with categorical litter model: ΔAIC = 7.1.

Predictor	Estimate	SE	z	Pr(>|z|)	Deviance	Pr(>Chi)
Intercept	3.73	0.05	75.50	0.00		
Litter (g)	-0.02	0.02	-0.84	0.40	-0.56	0.45
Elymus virginicus present	-0.06	0.07	-0.88	0.38	-0.23	0.63
Litter:*E. virginicus* present	0.03	0.03	0.75	0.45	-0.19	0.66

Table S3. Model summary of *Elymus virginicus* establishment (proportion of seeds that became plants, binomial distribution, logit link). Estimates, standard errors (SE), z statistic, and associated *P*-value from maximal model (*n* = 48). Deviances and *P*-values from Chi-squared tests of nested models. Model comparison with continuous litter model: ΔAIC = 8.4.

Predictor	Estimate	SE	z	Pr(>|z|)	Deviance	Pr(>Chi)
(Intercept)	1.68	0.16	10.60	$3.07e^{-26}$		
Low litter	-0.62	0.21	-3.00	$2.70e^{-3}$		
Medium litter	-0.64	0.21	-3.09	$2.01e^{-3}$	-22.04	$6.39e^{-5}$
High litter	-0.51	0.21	-2.45	0.01		
Microstegium vimineum present	-0.21	0.22	-0.97	0.33	-1.57	0.21
Low litter:*M. vimineum* present	0.06	0.28	0.21	0.83		
Med. litter:*M. vimineum* present	0.39	0.29	1.36	0.17	-3.74	0.29
High litter:*M. vimineum* present	-0.10	0.29	-0.34	0.74		
Litter comparison	Diff.	Lwr.	Upr.	*P*		
Low - None	-0.59	-0.95	-0.22	$1.68e^{-4}$		
Med - None	-0.44	-0.81	-0.07	0.01		
High - None	-0.56	-0.93	-0.20	$4.09e^{-4}$		
Med - Low	0.15	-0.19	0.49	0.68		
High - Low	0.03	-0.31	0.36	1.00		
High - Med	-0.12	-0.46	0.22	0.79		

Notes: Diff. = difference in observed means, Lwr. = lower 95% confidence interval, Upr. = upper 95% confidence interval, *P* = *P*-value adjusted after multiple comparisons

Table S4. Model summary of *Elymus virginicus* disease incidence (proportion of tillers with lesions, binomial distribution, logit link). Estimates, standard errors (SE), z statistic, and associated *P*-value from maximal model (*n* = 47). Deviances and *P*-values from Chi-squared tests of nested models. Comparison between categorial litter levels with Tukey's honest significant differences test. Model comparison with continuous litter model: ΔAIC = 143.2.

Predictor	Estimate	SE	z	Pr(>|z|)	Deviance	Pr(>Chi)
Intercept	-3.38	0.23	-14.48	$1.61e^{-47}$		
Low litter	2.28	0.26	8.69	$3.53e^{-18}$		
Medium litter	1.02	0.28	3.61	$3.05e^{-4}$	-180.64	$<2.2e^{-16}$
High litter	1.67	0.26	6.31	$2.89e^{-10}$		
Microstegium vimineum present	2.14	0.27	7.82	$5.12e^{-15}$	-247.28	$<2.2e^{-16}$
Low litter:*M. vimineum* present	-0.79	0.33	-2.37	0.02		
Med. litter:*M. vimineum* present	-0.69	0.35	-1.94	0.05	-6.62	0.08
High litter:*M. vimineum* present	-0.44	0.33	-1.32	0.19		
Litter comparison	Diff.	Lwr.	Upr.	*P*		
Low - None	1.82	1.42	2.22	0.00		
Med - None	0.58	0.16	1.00	$2.50e^{-3}$		
High - None	1.35	0.95	1.74	0.00		
Med - Low	-1.24	-1.62	-0.86	0.00		
High - Low	-0.47	-0.81	-0.13	$2.14e^{-3}$		
High - Med	0.76	0.39	1.14	$6.24e^{-7}$		

Notes: Diff. = difference in observed means, Lwr. = lower 95% confidence interval, Upr. = upper 95% confidence interval, *P* = *P*-value adjusted after multiple comparisons

Table S5. Model summary of log-transformed *Microstegium vimineum* biomass per pot. Estimates, standard errors (SE), t statistic, and associated *P*-value from maximal model ($n = 48$). F statistics and *P*-values from comparisons of nested models. Model comparison with categorical litter model: ΔAIC = 5.7.

Predictor	Estimate	SE	t	Pr(>|t|)	F	Pr(>F)
Intercept	3.64	0.04	85.37	$1.59e^{-50}$		
Litter (g)	-0.05	0.02	-2.34	0.02	5.84	0.02
Elymus virginicus present	-0.17	0.06	-2.86	0.01	11.41	$1.51e^{-3}$
Litter (g):*E. virginicus* present	0.03	0.03	0.89	0.38	0.80	0.38

Table S6. Model summary of log-transformed *Microstegium vimineum* biomass per plant. Estimates, standard errors (SE), t statistic, and associated *P*-value from maximal model ($n = 48$). F statistics and *P*-values from comparisons of nested models. Model comparison with categorical litter model: ΔAIC = 5.6.

Predictor	Estimate	SE	t	Pr(>|t|)	F	Pr(>F)
Intercept	-0.08	0.06	-1.25	0.22		
Litter (g)	-0.03	0.03	-0.94	0.35	1.82	0.18
Elymus virginicus present	-0.11	0.09	-1.27	0.21	3.96	0.05
Litter (g):*E. virginicus* present	$5.04e^{-4}$	0.04	-0.01	0.99	$1.00e^{-4}$	0.99

Table S7. Model summary of log-transformed *Elymus virginicus* biomass per pot. Estimates, standard errors (SE), t statistic, and associated *P*-value from maximal model (*n* = 48). F statistics and *P*-values from comparisons of nested models. Comparison between categorial litter levels with Tukey's honest significant differences test. Model comparison with continuous litter model: ΔAIC = 8.1.

Predictor	Estimate	SE	t	Pr(>|t|)	F	Pr(>F)
Intercept	1.86	0.14	13.39	$2.27e^{-16}$		
Low litter	-0.23	0.20	-1.18	0.25		
Medium litter	-0.13	0.20	-0.68	0.50	4.14	0.01
High litter	-0.14	0.20	-0.69	0.50		
Microstegium vimineum present	-1.71	0.20	-8.68	$9.75e^{-11}$	388.14	$<2.2e^{-16}$
Low litter:*M. vimineum* present	-0.52	0.28	-1.85	0.07		
Medium litter:*M. vimineum* present	-0.31	0.28	-1.10	0.28	1.24	0.31
High litter:*M. vimineum* present	-0.16	0.28	-0.59	0.56		
Litter comparison	Diff.	Lwr.	Upr.	*P*		
Low-None	-0.49	-0.86	-0.11	0.01		
Med-None	-0.29	-0.66	0.09	0.19		
High-None	-0.22	-0.59	0.16	0.42		
Med-Low	0.20	-0.17	0.58	0.48		
High-Low	0.27	-0.10	0.65	0.23		
High-Med	0.07	-0.31	0.44	0.96		

Notes: Diff. = difference in observed means, Lwr. = lower 95% confidence interval, Upr. = upper 95% confidence interval, *P* = *P*-value adjusted after multiple comparisons

Table S8. Model summary of log-transformed *Elymus virginicus* biomass per tiller. Estimates, standard errors (SE), t statistic, and associated *P*-value from maximal model ($n = 47$). F statistics and *P*-values from comparisons of nested models. Model comparison with continuous litter model: ΔAIC = 3.9.

Predictor	Estimate	SE	t	Pr(>|t|)	F	Pr(>F)
Intercept	-2.67	0.15	-18.28	$9.96e^{-21}$		
Low litter	-0.07	0.22	-0.34	0.74		
Medium litter	0.03	0.21	0.16	0.87	0.84	0.48
High litter	$-3.54e^{-3}$	0.21	-0.02	0.99		
Microstegium vimineum present	-0.93	0.21	-4.52	$5.70e^{-5}$	110.24	$1.11e^{-13}$
Low litter:*M. vimineum* present	-0.26	0.30	-0.88	0.38		
Medium litter:*M. vimineum* present	-0.23	0.29	-0.78	0.44	0.40	0.75
High litter:*M. vimineum* present	-0.03	0.29	-0.12	0.91		

Table S9. Model summary of the residuals of *Elymus virginicus* establishment regressed against litter treatments (Table S3). Estimates, standard errors (SE), t statistic, and associated *P*-value are shown (*n* = 47).

Predictor	Estimate	SE	t	Pr(>|t|)
Intercept	0.02	0.29	0.08	0.94
Disease incidence	-0.08	0.87	-0.09	0.93

Table S10. Model summary of the residuals of log-transformed *Elymus virginicus* biomass per pot regressed against treatments (Table S7). Estimates, standard errors (SE), t statistic, and associated *P*-value are shown ($n = 47$).

Predictor	Estimate	SE	t	Pr(>|t|)
Intercept	0.02	0.08	0.25	0.80
Disease incidence	-0.12	0.23	-0.50	0.62

Table S11. Model summary of the residuals of log-transformed *Elymus virginicus* biomass per tiller regressed against treatments (Table S8). Estimates, standard errors (SE), t statistic, and associated *P*-value are shown (*n* = 47).

Predictor	Estimate	SE	t	Pr(>|t|)
Intercept	0.08	0.08	1.02	0.32
Disease incidence	-0.32	0.24	-1.30	0.20

Table S12. Model summary of the relative abundance of *Microstegium vimineum* (ln[*M. vimineum* total biomass/(*M. vimineum* + *E. virginicus* total biomass)]). Estimates, standard errors (SE), t statistic, and associated *P*-value from maximal model ($n = 24$). F statistic and *P*-value from comparison of nested models. Comparison between categorial litter levels with Tukey's honest significant differences test. Model comparison with continuous litter model: $\Delta AIC = 7.8$.

Predictor	Estimate	SE	t	Pr(>|t|)	F	Pr(>F)
Intercept	-0.04	$3.64e^{-3}$	-9.99	$3.21e^{-9}$		
Low litter	0.02	0.01	3.55	$2.01e^{-3}$		
Medium litter	0.01	0.01	1.57	0.13	4.28	0.02
High litter	0.01	0.01	1.35	0.19		
Litter Comparison	Diff.	Lwr.	Upr.	*P*		
Low-None	0.02	$3.86e^{-3}$	0.03	0.01		
Med-None	0.01	-0.01	0.02	0.42		
High-None	0.01	-0.01	0.02	0.54		
Med-Low	-0.01	-0.02	$4.19e^{-3}$	0.23		
High-Low	-0.01	-0.03	$3.08e^{-3}$	0.16		
High-Med	$-1.11e^{-3}$	-0.02	0.01	1.00		

Notes: Diff. = difference in observed means, Lwr. = lower 95% confidence interval, Upr. = upper 95% confidence interval, *P* = *P*-value adjusted after multiple comparisons
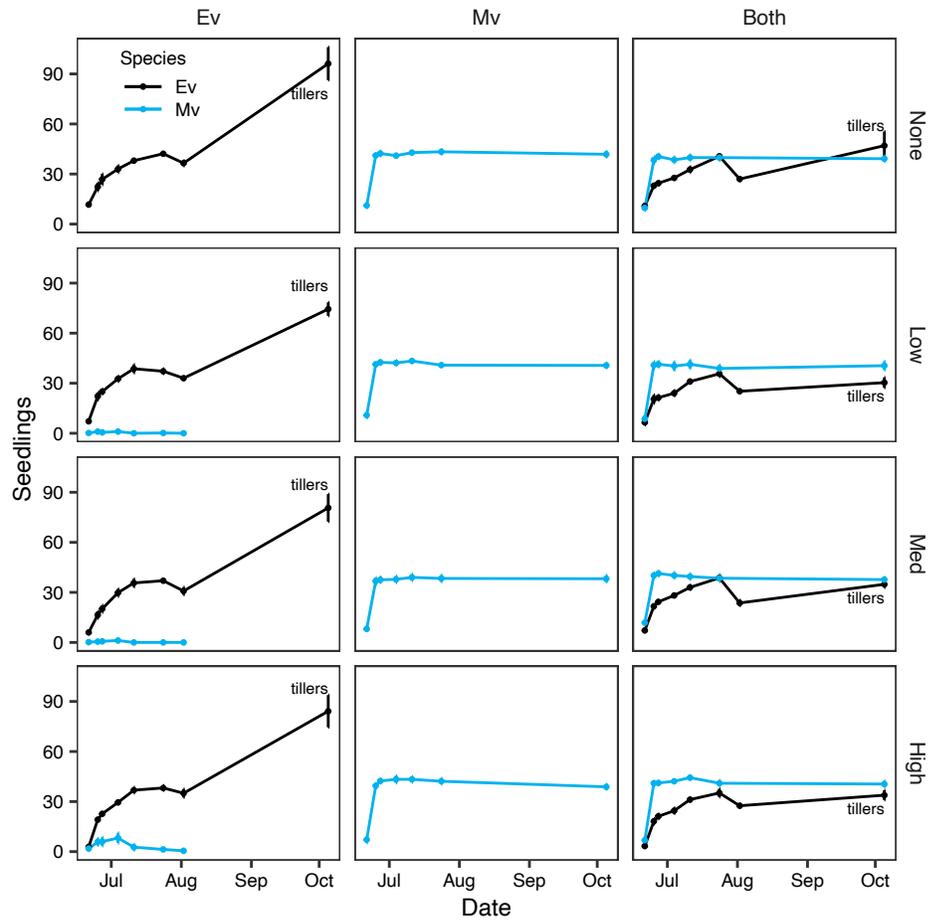
Figure S1. The number of *Microstegium vimineum* (Mv) and *Elymus virginicus* (Ev) seedlings (mean ± 1SE) in each planting treatment (columns) and litter treatment (rows). Tillers of *E. virginicus* were counted instead of seedlings on the last date (post-harvest). *Microstegium vimineum* in the Ev treatment were unintentional (likely from cleistogamous seeds in the litter) and were therefore removed after counting starting on July 4, 2018. Seedling counts recorded on the fifth date (for *M. vimineum*) and the sixth date (for *E. virginicus*) were used in analyses of establishment.

Appendix S3 for "Invasive grass litter suppresses a native grass species and promotes disease" by Liliana Benitez, Amy E. Kendig, Ashish Adhikari, Keith Clay, Philip F. Harmon, Robert D. Holt, Erica M. Goss, and S. Luke Flory
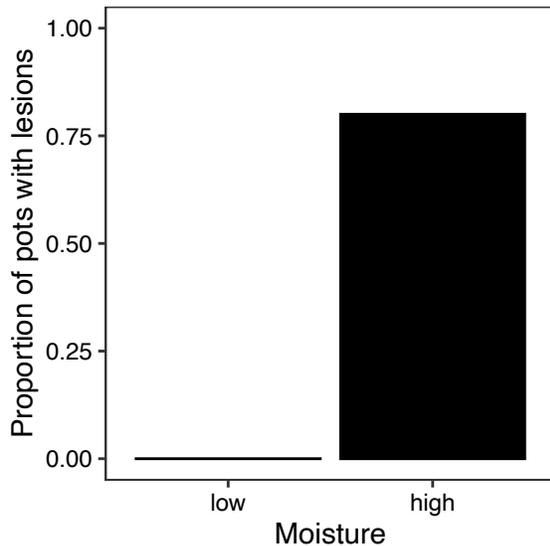*Ecosphere*

Figure S1. The effects of moisture on litter-induced disease of *Microstegium vimineum*. These results are from a greenhouse experiment in which five replicate pots of 40 seeds per pot covered with *M. vimineum* litter received either a low moisture treatment or a high moisture treatment. For the high moisture treatment, we misted plants with tap water for five seconds after litter had been added to pots and at two-hour intervals from 9:30 AM to 1:30 PM for the first three days of the experiment. Low moisture pots were misted once per day for the second and third days from 9:30 AM to 1:30 PM. Pots were considered infected if at least two brown lesions were observed on leaf surfaces at the end of the experiment. The high moisture treatment significantly increased the number of pots with foliar lesions compared to the low moisture treatment (Fisher's Exact Test, odds ratio = 0, 95% CI = 0–0.98, $P = 0.047$).

For more information on this experiment, please see the data repository for this publication: https://doi.org/10.6073/pasta/3688be04a98697c95efa5747eb3c9513