Potential for endophyte symbiosis to increase resistance of the native grass *Poa alsodes* to invasion by the non-native grass *Microstegium vimineum*

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Abstract Microbial symbionts can improve the competitive ability and stress tolerance of plant hosts and thus may enhance native plant resistance against invaders. We investigated whether symbiosis between a native grass, *Poa alsodes*, and a fungal endophyte (*Neotyphodium* sp.) improved the grass's ability to compete against *Microstegium vimineum* (Japanese stiltgrass), a common invader in the eastern USA. We challenged naturally endophyte-symbiotic and experimentally endophyte-free *P. alsodes* plants with the invader. In the first experiment, we manipulated symbiosis and water availability to test for context-dependency in symbiont benefits. In the second experiment, we manipulated symbiosis and *M. vimineum* diversity (the number of invader populations), since greater intraspecific diversity is expected to improve invasion success and might alter the efficacy of symbiosis in invasion resistance. In both experiments, presence of the endophyte reduced the per plant biomass of *M. vimineum* and increased *P. alsodes* biomass. We found no evidence that benefits of the symbiont depended on water availability, and population-level diversity had a minor influence on *M. vimineum*: inflorescence number showed a parabolic relationship with increasing numbers of *M. vimineum* populations. Overall, symbiosis in the native grass had stronger effects on invader growth than either water availability or invader genetic diversity. Our results suggest that endophyte symbioses in native plants can increase host performance against an invader, although this conclusion needs confirmation in more complex field settings where other important factors, such as herbivores and fluctuating abiotic conditions, come into play.

Keywords Fungal endophyte · Genetic diversity · Population ecology · Symbiosis · Invasive species · Japanese stiltgrass

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1 Introduction

Invasive species can pose substantial threats to native communities and can be an important cause of declining biodiversity (Mack et al. 2000; Ehrenfeld 2003; Mack and D'Antonio 2003; Adams and Engelhardt 2009). The significant ecological and economic costs associated with invaders (Pimentel et al. 2005; Simberloff 1996) make it important to understand how these species affect native communities. In addition, deciphering the factors that influence the ability of native species to resist invasion, while less studied, can improve the management of invasions in natural areas (Levine et al. 2004; Whitney and Gabler 2008).

One factor that has often been overlooked is the potential for beneficial symbiota to increase native species' resistance to invasion (Richardson et al. 2000). Most plants host microbial symbionts, such as mycorrhizal fungi, nitrogen-fixing bacteria, or fungal endophytes. These microorganisms can mitigate nutrient and water limitation, improve competitive ability, and protect hosts against herbivores and pathogens (Auge 2001; Cheplick and Faeth 2009; Smith

and Read 1997; Barbosa et al. 1991; Clay et al. 2005; Rudgers et al. 2005). When symbionts aid plant hosts in coping with stress, it is possible that they could also influence native species' abilities to resist and compete with invaders.

Recent work suggests that symbiotic microbes play important roles in interactions between native and invasive plant species. Several studies have revealed that invasive plants alter soil microbial community composition and function (e.g., Kourtev et al. 2003; Hawkes et al. 2005; Batten et al. 2006). For example, presence of the invasive plant *Centaurea maculosa* (spotted knapweed) shifted the composition of the arbuscular mycorrhizal fungi community, suppressed fungal diversity, and reduced fungal hyphal lengths by >20% in invaded sites (Mummey and Rillig 2006). Other work suggests that *C. maculosa* can manipulate the microbial community to its advantage in competition with native plant species (Callaway et al. 2004). A similar disruption has been reported for invasive *Alliaria petiolata* (garlic mustard), which suppressed the symbiosis between native forest tree seedlings and below-ground mycorrhizal fungi communities (Stinson et al. 2006). However, the majority of prior work has focused on how microbial communities change following invasions (Ehrenfeld et al. 2001; Kourtev et al. 2002) or interact with invasive species (Klironomos 2002; Seifert et al. 2009). Less is known about whether native symbioses alter the competitive abilities of native plants against invaders; such studies would require manipulations of native symbioses prior to the introduction of an invader.

Here, we investigate whether the symbiosis between a native grass and a systemic fungal endophyte (*Neotyphodium* sp.) affects resistance to an invasive plant by increasing the native species' competitive ability and reducing invader performance. Fungal endophytes can promote host defense against natural enemies, tolerance of drought, and nutrient uptake (Muller and Krauss 2005). Although a significant body of literature has investigated symbioses between fungal endophytes and domesticated turf and forage grasses, fewer studies have tested for benefits of endophytes in native, wild grasses (reviewed by Cheplick and Faeth 2009). To our knowledge, our work is the first to test whether native endophyte symbioses can improve a native host plant's competitive ability against an invader.

We challenged both naturally endophyte-infected and experimentally endophyte-disinfected *Poa alsodes* plants with the invader *Microstegium vimineum* (Japanese stiltgrass), which commonly co-occurs with *P. alsodes* in forest understory habitats in North America (personal observations; Hunt and Zaremba 1992). *M. vimineum* is native to Asia, was first recorded in 1919 in Tennessee (Fairbrothers and Gray 1972), and has rapidly spread to >20 eastern states (USDA-NRCS 2009). Negative impacts of *M. vimineum* on native herbaceous communities have been documented in both observational and experimental studies (Adams and Engelhardt 2009; Flory and Clay 2009; Oswalt et al. 2007). *M. vimineum* can reduce native herbaceous plant biomass up to 64% and diversity up to 38%; invaded areas also have significantly different native plant species composition than control areas, lower arthropod abundance and diversity, and suppressed tree regeneration (Flory 2010; Flory and Clay 2010; Simao et al. 2010). Removing the invader from field sites with a grass-specific herbicide allowed for the return of native graminoids and forbs and stimulated up to 123% more native tree regeneration than in areas where invasions were left intact (Flory and Clay 2009).

In addition to manipulating symbiosis, we subjected competing plants to two levels of water availability to examine the context-dependency of symbiont benefits. For many symbioses, the strengths of benefits conferred by symbionts depend on external factors. For example, high soil nutrient levels can cause mycorrhizal fungi to switch from acting as mutualists to acting as parasites of plant hosts (Johnson et al. 1997). Context-dependency of symbiont benefits may be particularly critical in light of predicted changes in climate, which include higher temperatures, altered rainfall patterns, and increased frequencies or durations of drought (IPCC 2007). For example, symbiotic mutualists, such as endophytes that confer drought tolerance, may more strongly enhance the competitive ability of plant partners when water is more limiting (Kannadan and Rudgers 2008).

Because characteristics of the invading population can influence the success of species invasions (Baker 1974; Whitney and Gabler 2008), we also evaluated the relative importance of symbiosis in the native species against the amount of population-level diversity in the invading species. Genetic diversity of founder populations varies extensively (reviewed in Dlugosch and Parker 2008), but can equal or exceed levels found in native habitats (Kolbe et al. 2004; Genton et al. 2005). Distinct invader genotypes can differ considerably in performance (Vellend et al. 2010), and populations of colonizers with high levels of genetic diversity may show greater efficiencies of resource utilization and improved establishment in novel environments (Ahlroth et al. 2003). For example, higher genetic diversity within populations of the weedy herb *Arabidopsis thaliana* resulted in significantly higher seedling emergence, biomass, flowering duration, and reproduction, which all could increase colonization success in novel habitats (Crawford and Whitney 2010). Population-level diversity may be important in explaining invasive success of our focal invader, *M. vimineum*, as previous work suggests that phenotypic diversity in drought tolerances and in responses to light availability may allow for *M. vimineum* invasions across a wide range of habitat conditions (Droste et al. 2010).

We addressed three questions linking symbiosis and invasion: (1) Does fungal symbiosis increase the resistance of a native plant to invasion? (2) Does reduced water availability increase the strength of the symbiont's benefits? (3) What is the relative influence of symbiosis in the native plant vs. population diversity in the invading plant in affecting invader performance?

2 Materials and methods

2.1 Study species

Poa alsodes (grove bluegrass, Poaceae) is a perennial C3 grass native to moist woodland habitats in northeastern and central North America (Gleason and Cronquist 1991). It is commonly associated with an unnamed fungal endophyte, *Neotyphodium* sp., a hybrid species of *Neotyphodium* known to produce loline alkaloids (P. Nagabhyru and C. Schardl, unpubl. data), compounds that can function in defense against insect herbivores (Schardl et al. 2007; Crawford et al. 2010). The endophyte is vertically transmitted from host plants to seeds and is not known to reproduce sexually or spread contagiously. *P. alsodes* is classified as a rare species in Indiana and endangered in neighboring Illinois, but occurs commonly in northeastern states where it can be a dominant member of the forest understory (pers. obs.; USDA-NRCS 2009). We collected seeds from natural populations in the Indiana University Research and Teaching Preserve (IURTP) at Lilly Dickey Woods, Nashville, Indiana, USA (39°14′29″N, 86°13′07″W, May–June 2006 and 2007) and in private landholdings near Bridgewater, Vermont (43°39′28″N, 72°39′05″W, 18 June 2007).

Microstegium vimineum (Japanese stiltgrass, Poaceae) is an annual C4 grass native to Asia (Winter et al. 1982). *M. vimineum* has colonized much of the eastern U.S. in extensive, dense patches in edge habitat and throughout the forest understory (Fairbrothers and Gray 1972; Barden 1987) where it co-occurs with *P. alsodes* (Plate 1). *M. vimineum* performs best with high light and high soil moisture but can survive at relatively low (<15%) soil moisture (Droste et al. 2010; Touchette and Romanello 2010).

2.2 Experiment 1: symbiosis and water availability

Endophyte treatment To remove the endophyte from *P. alsodes*, a subset of randomly chosen seeds was heat treated in a convection drying oven (VMR International 1390FM, Sheldon Manufacturing Inc, Oregon) for 7–10 days at 60°C (based on previous endophyte elimination trials, Kannadan and Rudgers 2008). Endophyte-infected and disinfected seeds were planted individually into Conetainer

pots (Stuewe & Sons, Inc, Corvalis, OR) filled with Metromix 200 (Sun Gro Horticulture Inc., Bellevue, WA) on 2–5 July 2007.

To assess endophyte presence, we made several thin sections of the inner leaf sheaths of each plant, stained these with lactophenol cotton blue, and examined tissue under a compound brightfield microscope (Leica Microsystems, Wetzlar, Germany) at 200–400X (Bacon and White 1994). For the Indiana ("IN") population, 92.6% of control seedlings had the endophyte (121 plants) and 43% of seedlings in the heat-treatment retained the endophyte (151 plants). For the Vermont ("VT") population, the percentage of symbiotic plants was 100% (61 plants) in the control and 33% (87 plants) in the heat treatment. Only successfully disinfected plants ("E-") or naturally infected controls ("E+") were used in the experiment. Sample sizes were as follows: E+ IN: 60 plants, E- IN: 60 plants, E+ VT: 60 plants, E- VT: 58 plants.

Greenhouse set up We used a 2×2×2 factorial design, including *P. alsodes* population of origin (IN or VT), the endophyte treatment (E+ or E-), and a water treatment (high or low water availability). Plants were grown in the Rice University Greenhouse, Houston, Texas, USA (29°43′07″N, 95°24′10″W). Greenhouse temperature was maintained at ~21–24°C with no supplemental light. From 13–15 August 2007, seedlings were transferred into Regal Standard Round pots (15 cm diam. × 14.4 cm deep, Hummert International, Earth City, MO) filled with Pro-Mix BX soil mix (BWI, Quakertown, PA). From 5–7 December 2007, we cloned material from all plants by removing 5 tillers, and replanted these tillers using the same pot type and soil mix. This cloning procedure allowed the assessment of plant performance to be separated from the initial heat treatment (or control) by a round of vegetative reproduction. Cloned plants were randomly assigned to either the high or low water treatments. Pots were arranged in a randomized order in the greenhouse. To mimic the reduced light levels of forested habitats, we installed shade structures (constructed of standard vinyl window screen over a PVC frame) on 12 November 2007. The screen reduced light levels by 54% (AccuPAR Linear PAR Ceptometer, Decagon Devices, Inc., Pullman WA).

Invader addition We collected seeds of *M. vimineum* from a single source population in the IURTP at Lilly Dickey Woods, Nashville, Indiana, USA (39°15′18″N, 86°12′32″W). *M. vimineum* seeds were germinated on wet paper towels in sealed plastic containers. Six *M. vimineum* seedlings were added to each pot containing a single *P. alsodes* clone during 29 November–1 December 2007. The density of *M. vimineum* varies widely among sites (personal observations), and we utilized a conservative density of 6 plants per pot

(equivalent to 340 seedlings/m^2). Field density at a nearby site (Morgan-Monroe State Forest, Martinsville, Indiana) reached as high as 12,362 seedlings/m^2 in June 2005 (unpubl. data).

Water treatment We imposed an experimental drought from 17 January–3 April 2008 (11 weeks). Pots in the high water treatment were given tap water twice daily with automatic drip emitters (Rain Bird, San Diego, CA). Low water treatment plants received the same water regimen, but were watered every other day. To test the effectiveness of the drought treatment, we measured gravimetric water content during 13 March–18 March 2008 by taking a soil core from each pot (3.12 cm^3 metal tube). Soil was weighed fresh, dried for 5 d in a convection oven (60°C), and reweighed dry.

Response variables To assess *P. alsodes* growth non-destructively, we counted the number of tillers per plant on 15–16 January 2008 (prior to initiation of the water treatment) and on 3–12 April 2008 (at harvest). Tiller number at harvest was positively correlated with above-ground biomass (Pearson $r=0.63$, $P<0.0001$, $n=241$ plants). We counted the number of *M. vimineum* individuals per pot on 14 January 2008 (before drought), 21–27 February 2007 (mid-drought), and 3–12 April 2008 (at harvest). Non-destructive measures of *M. vimineum* size included plant height and the number of leaves, quantified on 21–27 February 2007 and 27–30 March 2008. *M. vimineum* plants were harvested before seed maturation to ensure that seeds of this invader did not contaminate the local site; however, we counted the number of *M. vimineum* inflorescences per plant as a measure of reproductive fitness. Finally, above-ground biomass was harvested during 3–12 April 2008 and dried for 3 d in a convection oven (60°C) to constant mass. It was not possible to separate the roots of the two species.

Statistical analysis Data were analyzed with mixed model ANOVA, or when variables were measured repeatedly, with repeated measures ANOVA (SAS Institute Inc. 2009). Models included the fixed factors of endophyte treatment (E+ or E-), water treatment (high or low), *P. alsodes* population (IN or VT) and all interaction terms. A significant endophyte × water treatment interaction would demonstrate context-dependency in endophyte benefits. We also included the spatial location of plants in the greenhouse as a covariate in the models. Analyses met assumptions of normality of residuals and homogeneity of variances following log transformation of *M. vimineum* above-ground mass per plant and square-root transformation of the number of *M. vimineum* individuals.

2.3 Experiment 2: symbiosis and invader diversity

Endophyte treatment We used *P. alsodes* seeds that were collected from experimental field plots planted at Lilly Dickey Woods, Nashville, Indiana during May 2009. The plots were created two years previously (September 2007) by generating endophyte-free plants by heat-treating endophyte-symbiotic seeds from Lilly Dickey Woods, as described in Experiment 1. During the second year of reproduction in the field, we collected seeds for experiment 2, and propagated seedlings in 10 cm square plastic pots. Thus, plants for experiment 2 were one generation removed from the original endophyte elimination treatment. We randomly chose a total of 18 endophyte-symbiotic genotypes and 18 endophyte-free genotypes from the pool of plants. We split each plant into 5 equally sized clones, such that each *P. alsodes* genotype was represented across every level of *Microstegium* diversity (see next section).

Invader diversity treatment Eighteen populations of *M. vimineum* from sites in the eastern United States in October 2008 were used as seed sources for the diversity manipulation (Table 1). We created five levels of diversity (0, 1, 2, 4, or 8 populations) by drawing populations from this pool. We treated source populations as distinct ecotypes. Preliminary microsatellite work suggested the existence of unique alleles within populations (Novy, Culley, & Flory, unpubl. data). The 0 treatment contained no *M. vimineum* and provided a baseline for *P. alsodes* performance in the absence of the invader. The 1 population treatment was replicated twice for each of the 18 *M. vimineum* populations (36 replicates) to create replicate monocultures. All other treatment levels had 18 replicates.

To minimize problems associated with the non-independence of replicates within treatments and the increasing similarity among treatment levels as diversity increases (Huston and McBride 2002), the 2, 4, and 8 diversity treatment levels had populations randomly chosen from the pool of 18. Randomly chosen combinations of populations were discarded (and new combinations generated randomly) to meet the following criteria: (a) replicates of the 2-population treatment were allowed only 1 population in common, (b) replicates of the 4- population treatment were allowed only 2 populations in common, and (c) replicates of the 8- population treatment could share no more than 5 populations in common. EstimateS software (v. 8.2, Colwell 2009) was used to calculate similarity indices for each replicate. Similarity estimates were low, and were similar to or smaller than those in other recent diversity experiments (Crawford and Whitney 2010; Weltzin et al. 2003): the average Jaccard coefficients of similarity (which measures the similarity of sample sets)

Table 1 Sources of *M. vimineum* seeds for Experiment 2

Genotype ID	Nearest city	Site	Latitude	Longitude
1	Newark, DE	Judge Morris Estate	39.71044°N	75.70450°W
2	Newark, DE	Possum Hill	39.72455°N	75.74694°W
3	Delaware City, DE	Fort Dupont	39.57281°N	75.58062°W
4	Wilmington, DE	Alapocas Run	39.77338°N	75.56390°W
5	Newark, DE	Creole Road	39.69781°N	75.75356°W
6	Greenville, DE	Brandywine Creek	39.80739°N	75.57592°W
7	Bear, DE	Lums Pond	39.55667°N	75.71722°W
8	Chapel Hill, NC	Mason Farm	35.89000°N	79.01528°W
9	Chapel Hill, NC	New Hope Creeel	35.97917°N	79.00111°W
10	Queenstown, MD	833 Stagwell Road	38.92667°N	76.15205°W
11	Bushkill, PA	Bushkill Road	41.09611°N	75.00278°W
12	Whitman, MD	Chesapeake Bay	38.79389°N	76.29417°W
13	Hopkins, SC	Conagree NP	33.80750°N	80.86528°W
14	Oceanville, NJ	EB Forsyth NWR	39.49028°N	74.42750°W
15	Morgantown, WV	Mongahila River Floodplain	39.66250°N	79.98333°W
16	Bloomington, IN	Indiana Univ. Preserve	39.21944°N	86.54167°W
17	Madison, IN	Big Oaks NWR, area 28	38.98722°N	85.37944°W
18	Madison, IN	Big Oaks NWR, area 4	39.05306°N	85.38750°W

NP, National Park; NWR, National Wildlife Refuge

within 2-, 4- and 8- population treatments were 0.05, 0.12 and 0.28, respectively. Between adjacent treatments, the average Jaccard similarity coefficient was 0.09 for 2- and 4- population treatments, and 0.19 for 4- and 8- population treatments.

Greenhouse set-up Each *P. alsodes* clone was planted into a 15 cm diameter × 12.5 deep plastic pot filled to 9 cm with Pro-mix BX soil (Plate 1a, b). *P. alsodes* clones were allowed to establish in the pots for three weeks before *M. vimineum* seeds were added. At the time of *M. vimineum* planting (25 October 2009), we counted the number of tillers per *P. alsodes* plant as a pre-diversity treatment measure of plant size. In each pot, we added 160 *M. vimineum* seeds (derived from 1, 2, 4 or 8 source populations) to achieve a density of 0.75 seeds/cm^2, which is within the range of densities occurring in nature (pers. obs.). The pots were hand-watered daily. Positions of the pots in the greenhouse were randomized every 2 weeks to allow for uniform growing conditions.

Response variables After 13 weeks, beginning 15 January 2010, we harvested all vegetation at the soil surface. We counted the total number of *M. vimineum* plants and inflorescences per pot. *P. alsodes* plants did not reproduce sexually during the experiment, but we counted the final number of tillers as a measure of vegetative growth. Above-ground material of both species was dried in a convection oven (60°C) to achieve constant mass and then weighed. It was not possible to separate the roots of the two species to assess below-ground biomass.

Statistical analyses Data were analyzed with two-way ANOVA including the fixed factors of the endophyte treatment and diversity treatment as well as the diversity × endophyte interaction. A significant interaction would indicate that the effect of symbiosis on the biomass of the invader (or target native) depended on the diversity of the invader. *P. alsodes* genotype identity (clone) and *P. alsodes* initial tiller number (covariate) were included in models if they explained significant variation in the response variables. Response variables for *P. alsodes* were the final number of tillers (log-transformed) and above-ground biomass per individual (square-root transformed). For *M. vimineum*, we analyzed the final number of plants per pot, above-ground biomass per plant (log-transformed), and the total number of inflorescences produced per pot (power (0.4) transformed). Transformations were applied to meet assumptions of homogeneity of variances and normality of residuals for ANOVA.

## 3 Results

3.1 Experiment 1

Treatment effectiveness The low water treatment significantly reduced soil moisture by 24%, as measured by gravimetric water content (mean ± s.e., high: 42%±1%; low: 32% ± 1%; $F_{1,232}=42.4$, $P<0.001$). There was no significant effect of endophyte presence on soil gravimetric water

content ($F_{1,232}$=0.5, P=0.466; endophyte × water $F_{1,232}$= 0.1, P=0.762).

Native Poa alsodes Symbiotic (E+) plants produced 12–20% more tillers than symbiont-free (E-) plants, depending on the water treatment, with the stronger difference at low water availability. However, there was no significant interaction between the endophyte and water treatments (Fig. 1a, Table 2), suggesting that direct growth benefits of the endophyte were not conditional on water availability in this experiment. The two *P. alsodes* populations differed in the strength of the endophyte effect: E+ plants from Vermont produced 28% more tillers than E- plants (mean ± s.e., E+: 44.8±1.5; E-: 35.1±1.5), compared to a 5% difference for the Indiana population (E+: 39.1±1.6; E-: 37.2±1.5; Endophyte × Population, P=0.010, Table 2). Above-ground biomass responded less to our treatments than tiller number, with no significant increase due to endophyte presence or higher water availability (Table 2). However, across treatments, plants from the Indiana population were larger, on average, than those from Vermont (mean mass (g) ± s.e., IN: 2.2±0.6; VT: 1.6±0.4).

Invasive Microstegium vimineum Endophyte symbiosis in *P. alsodes* significantly reduced the growth of the invader. Per plant biomass was 28% lower in the presence of the endophyte than when grown with endophyte-free plants (Fig. 1b). This negative effect was also evident in marginally significant endophyte-mediated reductions in *M. vimineum* leaf number (P=0.067, Table 3, mean ± s.e., February E+: 8.73±0.08; E-: 9.02±0.10; March E+: 8.85±0.10; E-: 9.06±0.12) and height per plant (P=0.068, Table 3, February E+: 16.8±0.3; E-: 17.9±0.5; March E+: 19.5±0.6; E-: 20.9±0.8 (cm)).

Fig. 1 Experiment 1: Responses (means ± 1 s.e.) of the native *Poa alsodes* and its competitor, invasive *Microstegium vimineum*, to the presence of the endophyte in *P. alsodes* (E- vs. E+) and the soil water treatment (low vs. high). **a** Number of tillers per plant for *P. alsodes*. **b** Above-ground biomass per plant for *M. vimineum*. Full statistical results presented in Tables 2 and 3
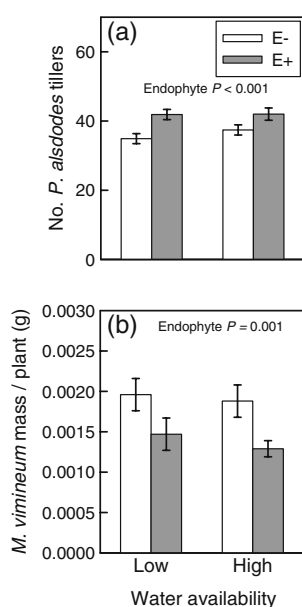
Despite reductions in *M. vimineum* growth, the endophyte did not affect the number of *M. vimineum* individuals in each pot (Table 3). However, the two *P. alsodes* populations differed in their effects on *M. vimineum* survival, with a greater number of plants surviving in competition with Vermont *P. alsodes* than with plants from the Indiana population (Table 3, mean number of plants per pot in April ± s.e., VT: 3.8±0.1; IN: 2.8±0.2); this effect corresponded with the differences in *P. alsodes* biomass between the populations. The number of inflorescences produced by *M. vimineum* was also higher when *M. vimineum* was in competition with the smaller sized plants of the Vermont population than with plants from Indiana (mean inflorescence number ± s.e., VT: 3.7±0.1; IN: 2.6±0.2).

Water availability had few effects on *M. vimineum*, but did reduce plant height by ~2 cm on the March measurement date (Time × Water, Table 3; mean ± s.e. (cm); high: 21.2± 0.7, low: 19.1±0.6). Neither the endophyte nor water treatment significantly influenced reproduction of the invader (Table 2).

3.2 Experiment 2

Native Poa alsodes The presence of the endophyte increased the growth of the native host plant. Prior to the addition of the invader, the endophyte increased *P. alsodes* tiller number by 57% (mean ± s.e., E-: 6.4±0.6, E+: 10.1± 0.6, $F_{1,35}$=19.0, P<0.001). At the termination of the experiment, this difference was stronger with, on average, 107% more tillers per E+ plant compared to E- (Fig. 2a, $F_{1,41}$=38.4, P<0.001). Above-ground biomass of *P. alsodes* was also significantly greater, with E+ plants attaining 118% greater biomass than E- plants (Fig. 2c, $F_{1,41}$=61.4, P<0.001), a larger difference than in Experiment 1.

In contrast to experiment 1, experiment 2 allowed us to evaluate the competitive effect of *M. vimineum* on *P. alsodes* by comparing the "0" diversity level (absence of *M. vimineum*) to all other levels. The presence of *M. vimineum* reduced *P. alsodes* tiller number by 36% relative to the "0" diversity level (Fig. 2b, Diversity $F_{4,170}$=4.1, P= 0.004) and reduced above-ground biomass by 23% (Fig. 2d, $F_{4,170}$=5.1, P<0.001), indicating that the two species are competitors. However, when *M. vimineum* was present, the number of *M. vimineum* populations (diversity treatment) had no additional effect on *P. alsodes* growth (Fig. 2b,d).

Invasive Microstegium vimineum Symbiosis in the native grass reduced growth of the invader, as in experiment 1. Competition from symbiotic *P. alsodes* reduced the total above-ground biomass of *M. vimineum* per pot by 18% compared to the effects of endophyte-free plants (mean ± s.e., E-: 4.5±0.12 g, E+: 3.7±0.12 g, $F_{1,172}$=19.7, P<0.001). This

Table 2 Experiment 1: Statistical results from ANOVA

Effect	df	P. alsodes number of tillers		P. alsodes above-ground mass/plant (g)		M. vimineum above-ground mass/plant (g)		M. vimineum total inflorescences	
		F	P	F	P	F	P	F	P
Endophyte	1, 232	15.7	**<0.001**	1.2	0.164	11.1	**0.001**	0.1	0.812
Water	1, 232	0.9	0.358	0.8	0.381	1.3	0.255	2.1	0.146
Population	1, 232	1.2	0.283	64.2	**<0.001**	0.1	0.798	25.5	**<0.001**
Endophyte × Water	1, 232	0.5	0.466	0.7	0.421	0.0	0.990	2.6	0.109
Endophyte × Population	1, 232	6.8	**0.010**	0.2	0.682	3.4	0.065	0.1	0.802
Water × Population	1, 232	0.1	0.779	3.4	0.067	1.4	0.244	0.0	0.865
Endophyte × Water × Population	1, 232	1.2	0.162	0.3	0.578	0.2	0.662	0.0	0.947
Order	1, 232	5.4	**0.022**	2.7	0.105	29.5	**<0.001**	0.6	0.427

Results for the effects of the endophyte treatment, water treatment, and *Poa alsodes* population of origin on performance of the native *P. alsodes* and the invasive *Microstegium vimineum*. Order was included as a covariate to account for the spatial location of plants in the greenhouse. *P*-values<0.05 are shown in bold face type

effect was driven by a reduction in the above-ground mass per *M. vimineum* individual, which was 13% lower when the endophyte was present in *P. alsodes* (Fig. 3e, $F_{1,172}$=13.3, P<0.001). Despite these effects on *M. vimineum* growth, there was no significant endophyte effect on the number of *M. vimineum* per pot (Fig. 3a, $F_{1,172}$=1.1, P=0.290) or the number of inflorescences produced per pot (Fig. 3c, $F_{1,172}$=0.1, P=0.803).

The number of populations affected *M. vimineum* reproduction but had no effect on the number of plants per pot (Fig. 3b, $F_{3,172}$=0.2, P=0.915), total above-ground biomass ($F_{3,172}$=1.3, P=0.269), or above-ground mass per *M. vimineum* plant (Fig. 3f, $F_{3,172}$=0.8, P=0.478). The number of inflorescences produced showed a humped shaped relationship with diversity, with the highest reproduction at intermediate diversity (Fig. 3d, $F_{3,172}$=3.4,

Table 3 Experiment 1: Statistical results from repeated measures ANOVA for *Microstegium vimineum* responses

Effect	df	Leaf number/plant		Height/plant (cm)		Number of individuals		
		F	P	F	P	df	F	P
Endophyte	1,232	3.4	0.067	3.4	0.068	1,232	0.5	0.466
Water	1,232	2.9	0.091	4.2	**0.042**	1,232	0.4	0.533
Population	1,232	14.6	**<0.001**	20.4	**<0.001**	1,232	21.5	**<0.001**
Endophyte × Water	1,232	2.9	0.088	0.9	0.334	1,232	1.1	0.299
Endophyte × Population	1,232	0.9	0.340	0.3	0.560	1,232	0.2	0.686
Water × Population	1,232	0.6	0.423	0.0	0.996	1,232	0.6	0.427
Endophyte × Water × Population	1,232	0.1	0.814	0.2	0.684	1,232	0.0	0.993
Order	1,232	2.8	0.095	0.2	0.631	1,232	18.6	**<0.001**
Time	1,232	14.1	**<0.001**	60.9	**<0.001**	3,230	18.5	**<0.001**
Time × Endophyte	1,232	1.2	0.278	0.3	0.606	3,230	0.2	0.889
Time × Water	1,232	0.2	0.667	5.1	**0.025**	3,230	1.5	0.217
Time × Population	1,232	4.5	**0.034**	20.9	**<0.001**	3,230	7.5	**<0.001**
Time × Endophyte × Water	1,232	0.1	0.711	0.7	0.396	3,230	1.9	0.130
Time × Endophyte × Population	1,232	0.0	0.837	0.2	0.702	3,230	0.2	0.907
Time × Water × Population	1,232	0.3	0.589	0.0	0.944	3,230	1.1	0.339
Time × Endophyte × Water × Population	1,232	0.1	0.782	3.2	0.075	3,230	1.1	0.366
Time × Order	1,232	11.0	**0.001**	4.4	**0.038**	3,230	8.6	**<0.001**

Results for the effects of the endophyte treatment, water treatment, and native *Poa alsodes* population of origin on performance of the invasive *Microstegium vimineum*. Order was included as a covariate to account for the spatial location of plants in the greenhouse. Leaf number and height were assessed on two dates; the number of individuals was counted on four dates. *P*-values<0.05 are shown in bold face type
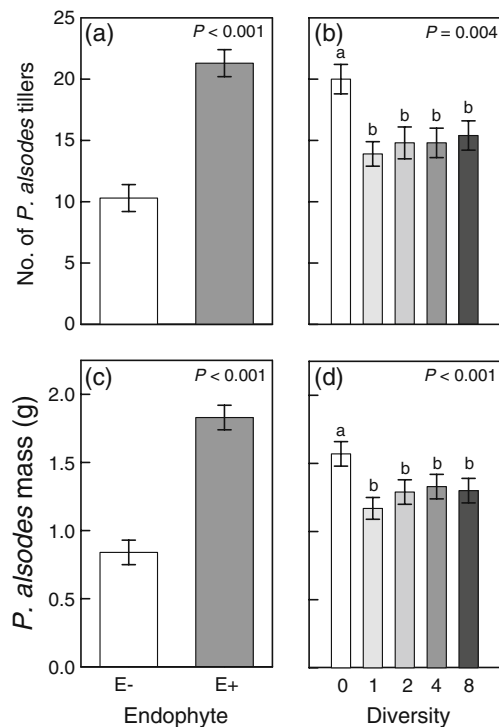
Fig. 2 Experiment 2: Responses (mean ± 1 s.e.) of the native plant *Poa alsodes* to the presence of its endophyte symbiont (E- vs. E+) and to the population diversity of invasive *Microstegium vimineum* (1, 2, 4, or 8 populations; the zero treatment level had no *M. vimineum*). **a, b** Number of tillers per plant. **c, d** Above-ground biomass per plant (g). Full statistical results presented in the Section 3.2 text

$P = 0.019$); this finding was also supported by a significant quadratic term for diversity when added to the linear model ($F_{1,176} = 4.0$, $P = 0.048$). However, the effect size for population-level diversity was not large: there was an 11% difference between the highest (diversity=2: 236.2± 22.2 inflorescences) and lowest (diversity=1: 213.2±15.7 inflorescences) values for inflorescence number (Fig. 3d).

Relative importance of symbiosis versus population-level diversity Overall, the effects of the native symbiosis were stronger than the effects of invader diversity, as indicated by the significant reduction in *M. vimineum* above-ground biomass when grown with symbiotic *P. alsodes* in both experiments; in contrast, the number of populations of *M. vimineum* did not affect its biomass and had only minor effects on reproduction. For the most part, symbiosis and diversity effects were additive. The only significant interactive effect occurred for *P. alsodes* above-ground biomass (Fig. 4, endophyte × diversity, $F_{4,170} = 2.5$, $P = 0.047$). In the presence of *M. vimineum*, the difference between E+ and E- plants was strongest under the "1" population treatment level—a 140% increase in mass due to the endophyte—and weakest at the "8" diversity level, with a 90% increase (Fig. 4). Effects of the endophyte were even stronger (160% increase

in *P. alsodes* above-ground biomass) in the absence of *M. vimineum*, suggesting that the presence of the non-native competitor weakened the relative benefits received from endophyte symbiosis.

4 Discussion

(1) Does fungal symbiosis increase native plant resistance to invasion?

Results from two greenhouse experiments confirmed that fungal endophyte symbiosis in a native host plant can
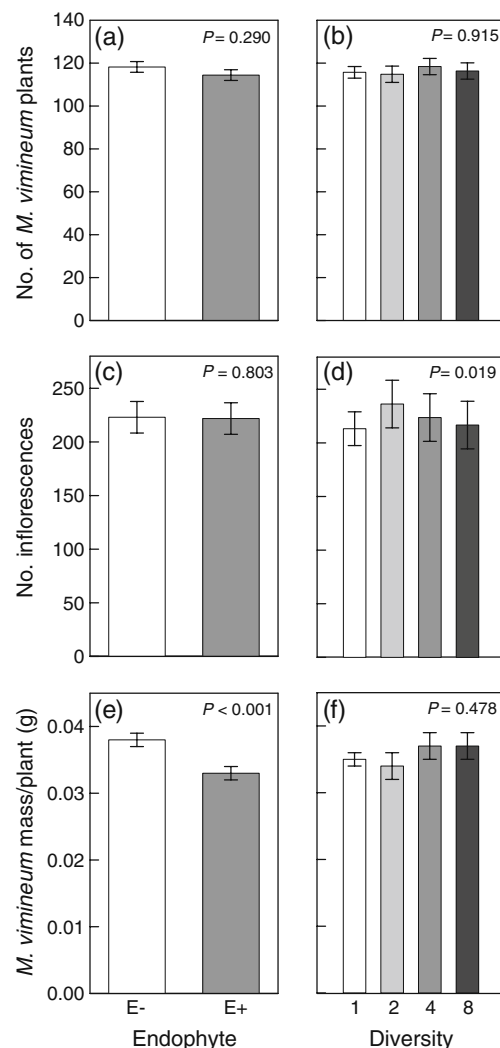

Fig. 3 Experiment 2: Responses (mean ± 1 s.e.) of the invasive *Microstegium vimineum* to endophyte symbiosis in the native plant *Poa alsodes* (E- vs. E+) and to its own population diversity (1, 2, 4, or 8 populations; the zero treatment level had no *M. vimineum*). **a, b** Number of plants per pot. **c, d** Number of inflorescences per pot. **e, f** Above-ground biomass per plant (g). Full statistical results presented in the Section 3 text
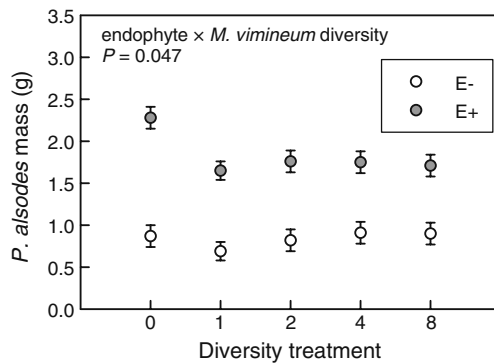
Fig. 4 Experiment 2: Interactive effects of endophyte symbiosis in *Poa alsodes* (E- vs. E+) and population diversity of *Microstegium vimineum* (1, 2, 4, or 8 populations; the zero treatment level had no *M. vimineum*) on above-ground biomass (g) of *P. alsodes*. Symbols show means ± 1 s.e. Full statistical results presented in the Section 3 text

reduce the growth of a competing invasive plant. These effects occurred mainly through reductions in biomass of the invader, rather than in germination or survival. Endophyte-mediated reductions in *M. vimineum* biomass did not translate into diminished inflorescence production, raising the question of whether symbiosis effects are strong enough to affect invasion potential. In natural habitats, total *M. vimineum* seed production per area was positively correlated with plant biomass ($r^2 = 0.52$, S.L. Flory *unpublished data*, see also (Cheplick 2008)). Thus, substantial reductions in invader biomass could result in reduced seed output or other unmeasured components of reproductive success (e.g., seed mass, seeds per inflorescence). To our knowledge, this is the first study to suggest that endophyte symbiosis in a native

plant can negatively impact a competing invader, but further work, including field studies to understand consequences for populations of invaders, will be needed to assess the potential for invasion resistance.

Effect sizes of symbiosis demonstrated here were within the range of magnitudes that have been reported in other experimental studies of environmental factors on *M. vimineum*. In our work, the presence of the endophyte in native *P. alsodes* reduced *M. vimineum* biomass up to 28% (expt. 1) and 18% (expt. 2) compared to the treatment with the endophyte experimentally removed. In comparison, Droste et al. (2010) found that drought reduced *M. vimineum* biomass by 22% and shade (17% ambient light) reduced biomass by 38%. Competition with a dominant invasive vine (*Lonicera japonica*) resulted in ~30% reduction in *M. vimineum* biomass (Belote and Weltzin 2006), and competition with commonly co-occurring native grasses, sedges, and forbs reduced invader biomass by 61% under shaded forest conditions (Flory et al. 2007). However, there was no significant effect of leaf litter depth on the survival or biomass of *M. vimineum* (Schramm and Ehrenfeld 2010).

The presence of the fungal endophyte positively affected its native grass host, *Poa alsodes*, by increasing tiller production (both experiments) and by increasing above-ground biomass (experiment 2). Benefits to the native grass were particularly strong in experiment 2, and the endophyte benefit was larger in the absence of *M. vimineum* than when *M. vimineum* was present (Fig. 4). Further experiments would be needed to decipher whether endophyte benefits generally weaken in the presence of competitor plants.
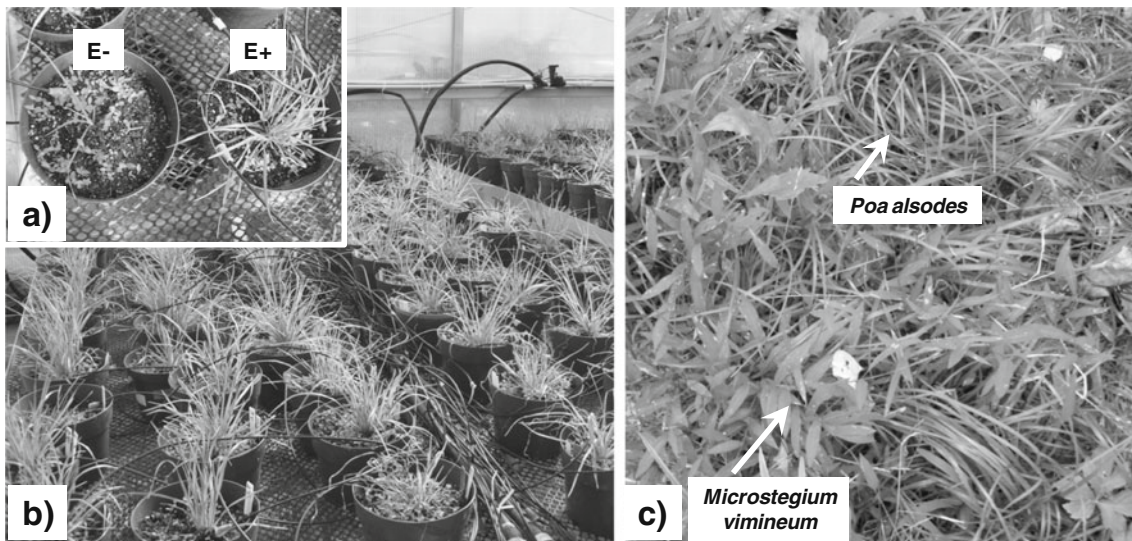

Plate 1 Photographs of *Poa alsodes* and *Microstegium vimineum*. **a** Endophyte-free and endophyte-symbiotic *P. alsodes* growing with seedlings of the invader, *M. vimineum*, Experiment 2, Rice University Greenhouse, Houston, Texas, USA. **b** Partial view of pots for

Experiment 2. **c** *P. alsodes* and *M. vimineum* growing naturally in forest understory habitat, Lilly Dickey Woods Preserve, Nashville, Indiana, USA

To understand the applied implications for restoration, our greenhouse results need confirmation in more complex field settings where other important factors, such as herbivores and fluctuating abiotic conditions, come into play. One goal of restoration ecology is to create communities that will resist invasion (Levine et al. 2004). Selection of native species with beneficial endophytes (such as *P. alsodes*) could help to achieve this goal. These considerations also highlight the need for proper seed storage for restoration material; seed storage under hot, humid conditions (and even at room temperature) can reduce endophyte viability (Siegel et al. 1984; Welty et al. 1987) and potentially limit restoration success.

(2) Does reduced water availability increase the benefits of endophyte symbiosis?

We found no evidence that benefits of the endophyte were contingent on water availability. This result was unexpected, as a prior greenhouse experiment (Kannadan and Rudgers 2008) showed strong context-dependency of endophyte benefits for *P. alsodes* grown in the absence of competitors, with significant effects of the endophyte on plant performance under low, but not high, water availability. Experiment 1 and Kannadan and Rudgers (2008) imposed similar reductions in water availability (24%) and had comparable durations (11–12 weeks); thus, the lack of context-dependency in endophyte benefits in experiment 1 may have resulted from the presence of *M. vimineum* as a competitor. Supporting this hypothesis, in experiment 2, we found stronger benefits of the endophyte to *P. alsodes* in the absence of *M. vimineum* than in the presence of this competitor.

(3) What is the relative influence of symbiosis in the native plant vs. population-level diversity in the invading plant in affecting invader performance?

Overall, symbiosis in the native plant had a stronger effect on the invasive plant than the number of populations of the invader. In fact, we found little support for the hypothesis that diversity promotes the performance of *M. vimineum*. While diversity had weak effects on inflorescence production of this invader, this was due to a small (11%) difference between the '1' and '2' diversity levels, rather than a steady increase in inflorescence number with increasing diversity. In general, these results contrast with other studies finding that genetically diverse colonizing populations outperform less diverse ones (Ahlroth et al. 2003; Crawford and Whitney 2010; but see Vellend et al. 2010). Possibly, our source populations of *M. vimineum* are weakly divergent, causing our diversity treatments to represent only a shallow gradient of phenotypic diversity. However, phenotypic differences in response to light and moisture have been found among *M. vimineum* populations sampled from a relatively small region (southern Indiana;

Droste et al. 2010), making this explanation less likely. Alternatively, it may be that the conditions in our experiment were not ideal for the expression of intraspecific diversity effects. For example, niche partitioning among genotypes (e.g., allowing more complete usage of available resources) is a common mechanism by which genotypic mixtures outperform monocultures (Hughes et al. 2008). This mechanism requires that individuals of different genotypes are at high enough densities or grow large enough to compete strongly. Perhaps a longer-term field experiment would have allowed *M. vimineum* plants to compete more strongly, and thus express more niche partitioning among genotypes.

4.1 Summary

Our results demonstrate that endophyte symbiosis can improve native plant competitive ability against an invader. The benefits of symbiosis to the native plant were largely consistent across levels of water availability and variation in the number of invader populations, supporting the hypothesis that benefits of native symbiosis are not highly context-dependent. Our results further suggest that restoration efforts may be aided by the preservation of symbiotic relationships in the native species that are used to construct restored communities.

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