Ecology, 97(5), 2016, pp. 1159–1169

Native fungal endophytes suppress an exotic dominant and increase plant diversity over small and large spatial scales

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Abstract. Understanding community dynamics and processes, such as the factors that generate and maintain biodiversity, drive succession, and affect invasion susceptibility, is a central goal in ecology and evolution. While most studies of how species interactions affect communities have focused on highly visible macroorganisms, we show that mutualistic microfungal endophytes have community-level effects across their host plant's range and provide the first example of fungal endophytes enhancing plant diversity. A three-year field study in which we experimentally manipulated endophyte abundance in a native Californian grass showed that despite their minute biomass, endophytes dramatically increased plant community diversity (~110% greater increase with endophytes) by suppressing a dominant invasive grass, *Bromus diandrus*. This effect was also detectable, but smaller, across five additional common gardens spanning ecologically diverse habitats, different climates, and >400 km of the host grass' range as well as at microspatial scales within gardens. Our study illustrates that mutualistic microbes, while often hidden players, can have unexpectedly large ecological impacts across a wide range of habitats and scales and may be important for promoting diverse communities and ecosystems.

Key words: biodiversity; Bromus laevipes; Epichloë; *evenness; fungal endophyte; grass; hidden players; invasive species; keystone species; mutualism; richness; symbiosis.*

Introduction

Understanding community dynamics and processes (e.g., what factors generate and maintain biodiversity, drive succession, and affect susceptibility to invasion) is a central goal in ecology and evolution. While most studies of communities and species interactions have focused on macro-organisms, it has become increasingly clear that microorganisms are pervasive and play central roles in the function and structure of all types of communities (Mcfall-ngai 2008, Prakash et al. 2012). For example, pathogens cause disease by shifting animal microbiomes from benign to dysbiotic communities (Hajishengallis et al. 2012). Microbial mutualists, organisms that participate in mutually beneficial interactions, can also have profound impacts upon their communities (Boucher et al. 1982) through many mechanisms, such as producing chemical compounds (e.g., alkaloids and allelochemicals), changing nutrient availability to their hosts, herbivores, and detritivores, and altering competitive hierarchies and dominance of their hosts (Orr et al. 2005, Vázquez-de-Aldana et al. 2013, Keller 2014). While the effects of microbes are typically measured on the host species, or a few associated species, we are gaining an appreciation that host–microbe associations can have very large effects on community structure. For example, suppression of mycorrhizal symbionts altered grassland structure by decreasing the abundance of dominant tall grasses and increasing plant species diversity (Hartnett et al. 1999). By fixing atmospheric nitrogen, rhizobia can influence plant succession (Morris and Wood 1989) and facilitate other major changes to communities, such as invasional meltdown (Vitousek et al. 1987, Simberloff 2006), with important consequences for diversity across trophic levels.

Endophytic fungi are another ubiquitous group of microbial symbionts, occurring in essentially all plant species and across natural, urban, and agroecosystems (Rodriguez et al. 2009), yet our understanding of their consequences for communities is very limited. A growing body of research has shown that systemic Epichloid fungal endophytes, which are found in an estimated 20–30% of grass species (Leuchtmann 1992), can confer substantial benefits to agronomic grass hosts, such as enhanced drought tolerance and nutrient uptake (Malinowski et al. 2000, Clay and Schardl 2002, Clay et al. 2005) and reduced herbivory through the production of alkaloids (Rudgers and Clay 2008, Schardl et al. 2012). In one exotic forage grass, tall fescue, these endophyte-conferred benefits can substantially increase the dominance of their host plants, leading to the invasion of and large impacts on communities. For example, endophytes in tall fescue suppressed secondary succession and reduced plant community diversity and abundance of other species (Rudgers and Clay 2005, Rudgers et al. 2007). Although native

Manuscript received 26 June 2015; revised 1 October 2015; accepted 23 November 2015. Corresponding Editor: J. D. Hoeksema.

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endophyte–plant associations are ubiquitous in nature, it remains untested whether their consequences for plant communities are of the same scale as the effects in agronomic and exotic species. Researchers have argued that the strong impact of endophytes on their host plants, and in turn their communities, observed in introduced turf and forage grasses are pathological, reflecting unnatural low-diversity ecosystems and nonnative species, and thus may not be representative of the magnitude of endophyte effects in diverse native communities (e.g., Faeth 2002, Muller and Krauss 2005, Saikkonen et al. 2006).

Unlike agricultural species that grow in monoculture stands, many endophyte-associating plants are non-dominant species in nature, potentially reducing the opportunity for strong symbiont effects. Further, native plant–endophyte interactions that have experienced long evolutionary histories of biotic and abiotic selection in the presence of their community members may have fundamentally different effects on these communities than newly introduced exotic associations. Recent work showing that endophytes sometimes confer substantial benefits to native hosts (Afkhami and Rudgers 2009, Rudgers et al. 2012, Saikkonen et al. 2013) demonstrates that the potential exists for these endophytes to have important impacts on their communities. However, to our knowledge no studies have examined effects of native endophyte symbioses on natural plant communities.

Here, we explore the community impacts of the fungal endophytes (*Epichloë* spp.) that facultatively associate with a non-dominant, California native grass (*Bromus laevipes*). First, we conducted a detailed three-year field experiment investigating the impact of endophytes on plant community diversity, density, and composition as well as *B. laevipes* performance. Second, we studied the effect of endophytes on plant community diversity across five common gardens spanning a large range of habitats and climatic variation within the *B. laevipes* distribution (~400 km). In the three-year experiment, we found that endophytes in this native grass greatly suppressed an invasive species leading to increased plant diversity; moreover, we found similar (but smaller) increases in diversity associated with endophytes at microspatial scales within our five gardens where this exotic was largely absent. Our research indicates that endophytes may play an important role in community resistance to invasive species and can lead to *increases* in plant diversity, which is a surprising result in light of previous studies that document exotic endophytes suppressing plant diversity. Our results highlight that even small vertically transmitted microbes associated with a non-dominant grass host may have substantial effects on community structure, diversity, and invasion susceptibility.

METHODS

Study system

Bromus laevipes is a native, perennial bunchgrass that typically grows in small, partially shaded populations at forest edges (Hickman 1993). While it is a non-dominant member of the plant community, it occurs commonly throughout California with ~98% of population records from herbaria falling within California and a small buffer around the state borders (Afkhami et al. 2014). In field surveys from 2007 to 2009, we found that *B. laevipes* often associates with systemic endophytes (*Epichloë cabralii* and *Epichloë* sp.) that are vertically transmitted (i.e., passed through the seeds) and in one population with *E. typhina* (not included in this study; Afkhami 2012, Charlton et al. 2014). Previous work has shown that endophytes in this grass species can be mutualists, conferring substantial fitness benefits to their host in both the field and greenhouse, and that while variation in endophyte frequency exists among sites, many *B. laevipes* populations are either highly associated or endophyte-free (i.e., bimodal distribution; Afkhami et al. 2014). *Bromus laevipes* was the only species in the communities we studied that had epichloid fungal endophytes.

Plot-scale experiment

Endophyte manipulation and early plant growth.—Seeds were collected from >30 plants in each of four endophyte-associated populations of *B. laevipes* (85.3% ± 2.99% of the populations consisted of symbiont-associated plants; Appendix S1: Table S1) that predominantly host *E. cabralii* (Charlton et al. 2014). In November 2010, ~300 seeds from each population were germinated in ~630 cm^3 containers with half the seeds from each population on perlite saturated with distilled water (the control treatment) and the other half on perlite with Benomyl fungicide (methyl 1-[butylcarbamoyl]-2-benzim idazolecarbamate) at a concentration of 2 g/L (Latch and Christensen 1982, Afkhami et al. 2014). Seeds were then cold stratified (4°C) for two weeks, and subsequently placed on a sunny lab bench for about five weeks to allow germination. Seedlings were rinsed with deionized water and transplanted into 125-cm^3 pots containing modified University of California Mix potting soil where they received no further exposure to fungicide and were kept in a greenhouse at University of California, Davis (~23°C, watered daily, natural light) prior to field planting.

The advantage of using a fungicide treatment to reduce hyphal density in leaves of E+ plants, rather than using naturally endophyte-free (E-) plants, is that we can control for population-level effects of genotype and environmental effects; a disadvantage of this method is possible unintended effects of fungicide on plant performance (i.e., not caused by changes in endophyte abundance). To test for this, controls in which we added fungicide to plants naturally lacking fungus were included in the range-wide experiment (described in the "Range-wide experiment" methods section), as well as in an experiment reported elsewhere (Afkhami et al. 2014). In both studies, E- plants exposed to fungicide performed indistinguishably from E- plants without fungicide treatment; thus, we have found no detectable effect of seed fungicide treatments. While fungicide effects on other microbes are possible, pesticide reports show that Benomyl degrades rapidly in water and that even with four annual applications residues do not accumulate across years, suggesting that non-target effects on soil microbes are unlikely given our fungicide application occurred only once and before planting, the seeds were rinsed prior to planting, and the experiment ran for three years. However, Benomyl is taken up systemically inside plants, where it is then fairly stable with 48–97% remaining within tissues 21–23 d after application (information from Benoyml MSDS from Rayfull Chemicals; longer term data are not available) meaning that endophytic microbes could be affected. Results from the previous study (Afkhami et al. 2014) and the range-wide experiment described in the "Range-wide experiment" results section, however, show that fungicide-treatment of plants without *Epichloë* endophytes did not substantially impact host performance or community properties, indicating that it is likely *Epichloë*-mediated effects, rather than those from other endophytic microbes, that we observe in this study.

Field planting.—During the Mediterranean growing season (30 January–2 February 2011), we established 12 plots, each of a size similar to a small *B. laevipes* population in nature (1.5 × 1.5 m with 100 *B. laevipes* seedlings) at McLaughlin Natural Reserve (Lake County, California, USA; 38.87428° N, 122.42428° W). The field site contained a naturally endophyte-associated population of *B. laevipes* (100% E+ plants, N = 40 mature individuals). Plant communities were left intact except that natural *B. laevipes* plants were removed from plots; careful removal of these small plants minimized disturbance effects. Total *B. laevipes* biomass removed from the whole experimental area was 6.04 ± 0.96 g/m^2, documenting that it is not a dominant species (total community biomass at harvest = 52.31 ± 8.76 g/m^2 [mean ± s.e.]). Then, we planted 100 one-tiller *B. laevipes* seedlings into each plot: six randomly selected plots received control plants with natural endophyte levels (E+) and six received plants with experimentally reduced endophyte levels (E↓) (total = 1200 seedlings). Within each plot, 25 seedlings from each of the four source populations were randomly assigned to locations in a 10 × 10 grid (~15 cm apart; chosen to reflect natural densities of *B. laevipes* seedlings). Experimental plants were comparable in size to natural seedlings growing at the site. We minimized effects on the local plant community by only excavating a small amount of soil where each seedling was planted.

Data collection.—A week after planting (9 February 2011), we conducted an initial baseline survey of the plant community for each plot. Surveys involved counting all individuals of each plant species in the inner 1 × 1 m of the plot (to reduce edge effects). Non-flowering plant species were assigned temporary morphospecies numbers and were later identified (Appendix S1: Table S2). Many clonal plants could be identified as individuals based on their natural history and/or morphology (e.g., bunch morphology indicated individuals for perennial grasses); for a few more difficult species, we excavated soil around plants outside the plots to help determine the scale of the individual. Diversity, richness, evenness, and density were calculated for each plot (Shannon index; Shannon and Weaver 1949). Surveys and calculations were repeated in April of 2011–2013.

At the end of the rainy season each year (late May/early June), we also collected data on *B. laevipes* performance (live tiller and leaf counts) of randomly selected plants from each population (average of ~100 plants/yr). Endophyte hyphal density was quantified in May 2011 and 2013 (first and last years of the experiment) to determine fungicide treatment efficacy and longevity. Because plants went dormant at the end of each growing season (with no green tissue), tissue sampled in 2013 was two growing seasons removed from the initial fungicide treatment. The outermost healthy leaf blade and sheath were collected from each plant and scored for endophyte density by staining with aniline blue-lactic acid dye. We counted the hyphal intersections under a microscope along four non-overlapping, equally spaced ~1 mm transect lines that were placed perpendicular to the longitudinal axis of the leaf sheath cells (as in Mack and Rudgers 2008, Afkhami et al. 2014).

We destructively harvested the plots after three growing seasons between 25 May and 8 June 2013. All live aboveground biomass was collected for the inner 1 × 1 m quadrat of each plot, and for the center 0.33 × 0.33 m quadrat we separately collected biomass of *B. laevipes* plants. All samples were dried to a constant mass and weighed.

Data analysis.—Changes in plot-level plant community diversity, richness, evenness, and density were analyzed in SAS (2011) using a repeated-measures ANOVA with factors of time and endophyte treatment (control vs. fungicide treatment) and their interaction. For the one-time measurements of total community biomass and biomass of *B. laevipes* collected at harvest, we used ANOVAs with a fixed factor of endophyte treatment. Hyphal density of endophyte in *B. laevipes* as well as host survivorship and growth, tiller and leaf counts, data (growth log-transformed to improve normality) were analyzed using an ANOVA with fixed factors of endophyte-treatment, time, and their interaction as well as population origin as a random factor. Using PC-ORD (McCune and Mefford 2011) we also examined endophyte effects on plant community composition with a nonmetric multidimensional scaling (NMS) ordination (Euclidian distance) and permutational MANOVA (fixed factor of endophyte treatment) on the change in density (i.e., number of plants/m^2) of each plant species. PerMANOVA performed a distance-based multivariate analysis of variance on community composition, evaluating whether endophyte treatments were significantly different with a permutation test rather than assuming a particular underlying distribution.

To further assess how endophyte affected community composition, we regressed the first ordination axis, which represents change in community composition, against the abundance of endophytes in the plots (estimated plot-level hyphal density = average hyphal density per plant × number of surviving plants). We also used Indicator Species Analysis to identify which species were most associated with difference in community composition between treatments. Indicator Species Analysis calculated an indicator value (0, no indication of an endophyte treatment environment, and 100, perfect indication) for each species based on its relative abundance and consistency in E+ and E↓ plots, and the significance of these values was determined with Monte Carlo randomization tests (Dufrêne and Legendre 1997). The most affected species was *Bromus diandrus*, an invasive grass (see *Results*) that does not itself host a fungal endophyte (Afkhami 2012). To determine how the endophytes in *B. laevipes* affected *B. diandrus*, we used a repeated-measures ANOVA with endophyte treatment, time, and their interaction.

Range-wide experiment

As part of other ongoing experiments, we planted five additional common gardens of *B. laevipes* across >400 km of its range. We used these gardens to assess whether impacts of endophytes on local community composition across sites that span a large portion of *B. laevipes'* range and had different resident communities were consistent with those from the above experiment.

Range-wide gardens experimental setup and data collection.—In January 2010, we established five experimental common gardens spanning a wide ecological and climatic gradient (e.g., ~600–2000 mm annual precipitation), and a large geographic range (northern to central California; ~420 km; Appendix S1: Table S4). All sites hosted natural *B. laevipes* populations: three gardens were at sites with naturally endophyte-free (E-) *B. laevipes* populations and two gardens were at sites inhabited by naturally endophyte-associated (E+) plants.

At every site, seeds from nine *B. laevipes* populations originating from northern and central California (Appendix S1: Table S3) were planted: three were E+ (i.e., natural mean endophyte frequency = 89.0% ± 6.68%) and six were E- (i.e., natural endophyte frequency = 0%; Afkhami 2012, Afkhami et al. 2014). Half of the plants in E+ populations were treated with fungicide to generate plants with experimentally reduced endophyte levels and half of the plants in E- populations were also treated to control for any effects of fungicide not caused by its impact on the endophyte. Into each garden, ~270 *B. laevipes* from the nine populations (~1350 plants total) were planted into randomly assigned positions in a grid with 15 cm separation. At planting, seedlings were similar in size to natural *B. laevipes* (one tiller). Endophyte hyphal density was determined as in the first experiment for all live plants to ascertain whether fungicide treatment was effective. In February 2012, three years after garden planting, we surveyed the local plant community within a 10-cm ring surrounding each *B. laevipes* (5 cm radius centered on each plant) by counting the abundance of all morphospecies. In this experiment, because treatments were interspersed rather than at the whole plot level, we analyzed the local community changes around individual plants in different endophyte treatments.

Data analysis.—Richness of the plant community surrounding each *B. laevipes* was analyzed using a mixed-model ANOVA with fixed factors of the source population's endophyte status (i.e., plant from naturally E+ or E- population), endophyte treatment (control or fungicide), garden location, and their interactions. We also included a random factor of population origin nested within endophyte status of the population. A planned contrast between control (naturally E+) and fungicide-treated plants from the same naturally E+ populations was used to determine if endophyte affected richness and to compare to results from the first experiment. A second contrast between control and fungicide-treated plants from naturally E- populations was used to assess whether fungicide treatment of seeds directly impacted species richness. Richness rather than diversity was used for this experiment because there were often only one or a few individuals within the area of the ring (<80 cm^2). The effectiveness of fungicide in reducing hyphal densities in *B. laevipes* from naturally E+ populations was determined using an ANOVA with log transformation of hyphal density, a fixed factor of fungicide, and random factors of population origin and garden location.

RESULTS

Endophyte manipulation

Endophyte hyphal density was reduced by ~40% in the leaves of fungicide-treated plants compared to control plants ($F_{1,189} = 13.19$, $P = 0.0004$) in the plot-level experiment, an effect that was maintained throughout the three-year experiment (treatment × year $F_{1,189} = 0.00$, $P = 0.9701$, Fig. 1A, Appendix S1: Table S5). Across the five common gardens spread throughout the range, hyphal density was reduced by 70% in fungicide-treated plants ($F_{1,327} = 87.45$, $P < 0.0001$, Fig. S1). Thus, in both experiments, fungicide treatment successfully decoupled plant genotype from in planta endophyte abundance.

Plot-scale experiment

Endophytes are mutualists that enhance host plant performance.—Endophytes increased *B. laevipes* survivorship each year by ~35% (at a location where natural *B. laevipes* plants are also endophyte-associated; endophyte

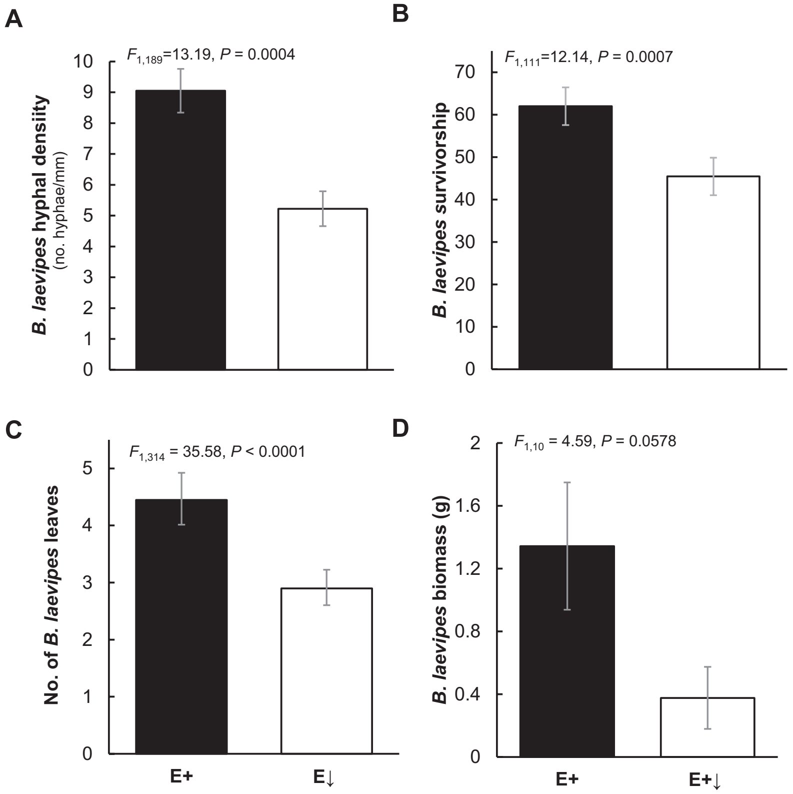

FIG. 1. Endophyte levels and effects on *Bromus laevipes* performance. (A) Endophyte hyphal density was significantly higher for the control plants (solid bars, E+) than for fungicide-treated plants (open bars, E↓), indicating fungicide significantly reduced endophyte level throughout the experiment ($F_{1,189}$ = 13.19, P = 0.0004). (B–D) Endophyte increased *B. laevipes* survivorship by ~35% ($F_{1,111}$ = 12.14, P = 0.0007), the total number of leaves per plant by ~50% ($F_{1,314}$ = 35.58, P < 0.0001), and total biomass by ~250% ($F_{1,10}$ = 4.59, P = 0.0578). Error bars indicate standard error around the mean; survivorship reported as percentage of plants alive.

$F_{1,111}$ = 12.14, P = 0.0007; endophyte × year $F_{2,111}$ = 0.91, P = 0.4051; Fig. 1B, Appendix S1: Table S5). Plants with naturally high endophyte levels (E+) were also larger, with ~50% more leaves (endophyte $F_{1,314}$ = 35.58, P < 0.0001; endophyte × year $F_{2,313}$ = 1.40, P = 0.2473, Fig. 1C, Appendix S1: Table S5) and ~35% more tillers (endophyte $F_{1,314}$ = 26.40, P < 0.0001; endophyte × year $F_{2,313}$ = 1.28, P = 0.2806; Fig. S2) than experimentally reduced (E↓) plants. At harvest, *B. laevipes* made up 19.1% ± 4.29% of the live plant community biomass across all plots. Plots with high levels of endophyte had ~250% more *B. laevipes* biomass than E↓ plots (R^2 = 0.3145, $F_{1,10}$ = 4.59, P = 0.058, Fig. 1D). Taken together, these results show that endophytes were mutualists in this experiment. Further, the increase in *B. laevipes* size could be a mechanism through which endophytes impact other members of the plant community.

Endophyte abundance strongly increased plant diversity and reduced plant density.—Compared to the baseline survey (at the initiation of the experiment in January) diversity increased in all plots by the peak season, however, diversity increased 110% more in E+ plots than E↓ plots ($F_{1,30}$ = 23.3, P < 0.0001, Fig. 2A, Appendix S1: Table S6; endophyte × time $F_{2,30}$ = 1.26, P = 0.2983). Increases in evenness were also significantly greater in E+ plots by ~140% ($F_{1,30}$ = 10.97, P = 0.0024, Appendix S1: Fig. S3 and Table S6; endophyte × time $F_{2,30}$ = 0.66, P = 0.5243), and while richness showed a similar pattern (~50% greater for E+ plots), the effect was not significant ($F_{1,30}$ = 1.80, P = 0.1896, Appendix S1: Fig. S3 and Table S6). These results suggest endophytes enhanced plant community diversity predominately through changes to evenness, with a qualitatively similar but much weaker effect on richness.

Endophyte treatment had a marginally significant effect on the total plant community density (abundance of all species per m^2; $F_{1,30}$ = 3.54, P = 0.0698), such that the total plant density in E↓ plots *increased* by ~70 plants/m^2 while density in E+ plots *decreased* ~40 plants/m^2 (compared to baseline densities at experiment initiation) (Fig. 2B, Appendix S1: Table S6). Endophyte effects on evenness (and in turn diversity) resulted largely from a reduction in abundance of a dominant species, invasive *B. diandrus* (section "endophyte abundance altered community composition"), rather than large increases in the abundance of rare species. Further, we did not observe a significant effect of endophyte treatment on total vegetative biomass alive at harvest

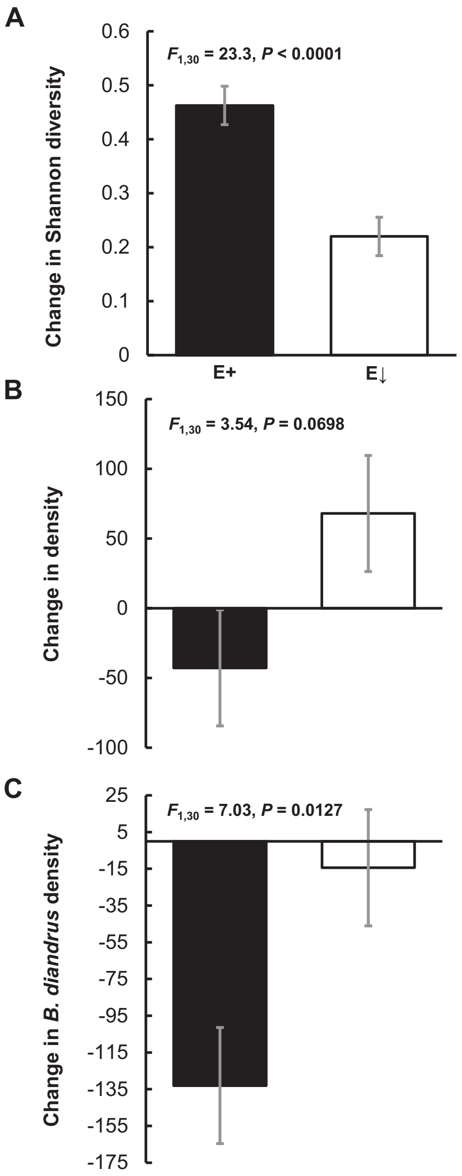

FIG. 2. (A) Endophytes caused an increase in plant Shannon diversity throughout the experiment (endophyte: $F_{1,30} = 23.3$, $P < 0.0001$, endophyte × time: $F_{2,30} = 1.26$, $P = 0.2983$), and (B) reduced plant community density (number of plants per m^2) (marginal significance of endophyte: $F_{1,30} = 3.54$, $P = 0.0698$, endophyte × time: $F_{2,30} = 1.03$, $P = 0.3698$). (C) They also suppressed the dominant and invasive grass *Bromus diandrus* (endophyte: $F_{1,30} = 7.03$, $P = 0.0127$, endophyte × time: $F_{2,30} = 1.37$, $P = 0.2704$). Values on graphs are changes as compared to initial values. High-endophyte (E+) plots represented by solid bars and low-endophyte (E↓) plots represented by open bars (mean ± s.e.).

($F_{1,10} = 0.01$, $P = 0.9131$), indicating that while the endophytes impact plant community diversity, this did not result in a change in productivity.

Endophyte abundance altered community composition.— Nonmetric multidimensional scaling ordination showed that plant community composition diverged between the high- and low-endophyte plots both when we examine changes in composition for the year in which endophytes had the largest effect (permutational MANOVA, $F_{1,10} = 9.09$, $P = 0.0030$) and for the mean across years ($F_{1,10} = 3.54$, $P = 0.0568$) (Fig. 3A). The first ordination axis, which explained ~90% of the variation in community composition, was highly correlated with total endophyte hyphal density in plots (Fig. 3B; $R^2 = 0.63$, $F_{1,9} = 15.44$, $P = 0.0034$), further supporting the conclusion that endophytes strongly influenced composition. While we observed some weak positive correlations between the first ordination axis and individual plant species abundances (e.g., two exotic species: the grass *Cynosurus echinatus* [$r = 0.333$, Kendall's $\tau = 0.334$] and forb *Torilis arvensis* [$r = 0.330$, $\tau = 0.229$]), by far the strongest correlation was a negative relationship with the exotic congeneric grass *Bromus diandrus* ($r = -0.999$, $\tau = -0.962$; Appendix S1: Table S7).

Endophyte effects on plant diversity and community composition appear to be largely caused by their suppression of this invasive grass. *Bromus diandrus* was the dominant species in the plots, comprising 57% of total plant abundance across the experiment. Indicator Species Analysis identified *B. diandrus* as the only species that clearly distinguished E+ and E↓ plots ($P = 0.0040$ from Monte Carlo permutation test; E↓ plots indicator value = 80 vs. E+ plots IV = 20). Further analyses showed that endophytes substantially suppressed the density of *B. diandrus*; decreases in the invasive species were ~800% greater in E+ compared to E↓ plots (endophyte $F_{1,30} = 7.03$, $P = 0.0127$; endophyte × time $F_{2,30} = 1.37$, $P = 0.2704$; Fig. 2C, Appendix S1: Table S8). Moreover, endophyte effects on plant diversity were much weaker when we excluded *B. diandrus* prior to calculating the Shannon diversity index, indicating that the suppression of this dominant by endophytes increased community diversity ($F_{1,30} = 2.84$, $P = 0.1023$, Appendix S1: Table S9). Similarly, divergence in community composition caused by endophytes (Fig. 3) was no longer significant when *B. diandrus* was excluded ($F_{1,10} = 0.33$, $P = 0.6468$).

Range-wide experiment

Local community effects of endophytes across the range.— Our analysis across the five common gardens in which data were collected at the plant rather than plot scale (Fig. 4A) showed that plant species richness was greater around E+ *B. laevipes* compared to *B. laevipes* with experimentally reduced endophyte levels (E↓; fungicide × endophyte status of the population $F_{1,1283} = 5.72$, $P = 0.0169$; contrast of E+ vs. E↓ $F_{1,1283} = 3.31$, $P = 0.069$; Fig. 4B, Appendix S1: Table S10). The effect was marginally significant, however the estimates of endophyte effects on communities in the common gardens are likely conservative because of variation associated with the initial plant community structure (such as woody and

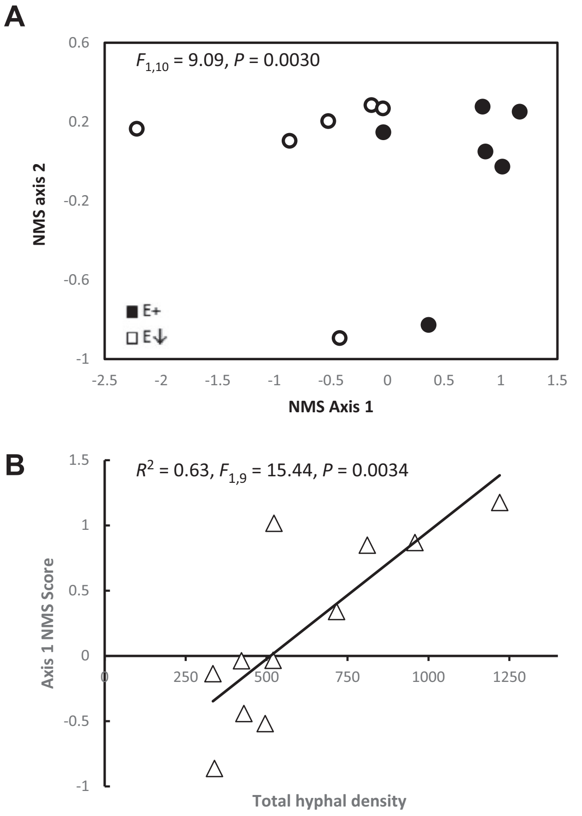

Fig. 3. (A) Nonmetric multidimensional scaling ordination of the change in density (i.e., no./m^2) of each plant species (stress = 1.87). Endophyte treatment explained significant clustering in how the plant community composition changed (permutational MANOVA: $F_{1,10}$ = 9.09, P = 0.0030). First NMDS axis represents ~90% of the variation in community composition (R^2 = 0.897), and the second axis represents an additional ~10% percent (R^2 = 0.094). Solid circles represent high-endophyte plots (E+); open circles represent low-endophyte (E↓) plots. (B) Total endophyte hyphal density of the plots was highly correlated with the first NMDS axis (R^2 = 0.63, $F_{1,8}$ = 15.53, P = 0.0034) further supporting the conclusion that endophyte strongly impacted how plant community composition changed.

large herbaceous perennials present at planting) that we cannot account for without initial community composition data. The lack of difference in community richness between fungicide-treated E- and untreated E- plants suggests that fungicide treatment of seeds did not impact community richness in the field (contrast $F_{1,1283}$ = 2.45, P = 0.118) and if anything there was slightly higher richness around fungicide-treated E- plants. Therefore, the observed higher local richness surrounding E+ plants compared to E↓ was likely due to the reduction in endophyte. This effect was consistent across the range, as we did not detect a three-way interaction with garden location (garden × fungicide × endophyte status of the population $F_{4,1283}$ = 0.81, P = 0.5180, Appendix S1: Table S10), suggesting that the impact of endophytes on richness occurs across geographically and ecologically diverse habitats. We also note that the invasive congener *B. diandrus* was not common at these other sites, suggesting the existence of other mechanisms through which endophytes might increase diversity.

Discussion

We demonstrated that fungal endophytes in the native grass *Bromus laevipes* have striking effects on natural plant communities. Increases in plant diversity were 110% greater for plots with high-levels of endophyte, which is the first documented example of this widespread class of symbiosis enhancing plant biodiversity. Our first experiment also revealed that this increase in diversity arose in large part from endophyte suppression of the dominant invasive *Bromus diandrus.* In our second experiment, we found that these plot-level effects were still visible at microspatial scales around individual plants grown in randomized gardens where plants with and without endophytes were completely interdigitated. Across the five common gardens spanning ecologically diverse habitats, different climates, and separated by >400 km, endophytes fostered similar, but smaller, increases in plant richness. This occurred even at sites where *B. diandrus* was absent, suggesting that in addition to endophyte-mediated suppression of an invasive dominant, other mechanisms through which endophytes increase diversity also might exist. Taken together, these results indicate that fungal endophytes, while invisible to the naked eye, may be important hidden players influencing plant communities in nature. Further, because our studies used "endophyte-reduced" rather than "endophyte-free" plants, the endophytes effects we observed may, in fact, be conservative.

Comparison of community effects with other grass-endophyte systems

The large effects on plant communities attributed to exotic endophyte in the introduced forage grass tall fescue (e.g., ~55% reduction in plant diversity; Clay and Holah 1999) has garnered much attention, emphasizing the significance of endophytes in structuring economically important agronomic plant communities. Further, Saikkonen et al. (2013) found that endophytes in monocultures of another species, meadow fescue (*Scherodonus pratensis*), reduced weed species richness in agricultural fields. However, it has been argued that the substantial benefits conferred by endophytes to forage species, and in turn their large effects on agronomic communities may not be representative of endophyte effects in diverse native communities (e.g., Faeth 2002, Muller and Krauss 2005, Saikkonen et al. 2006). For example, the long evolutionary histories of biotic and abiotic selection in the presence of their community members experienced by native plant–endophyte interactions may cause their effects on communities to differ compared to the effects of newly introduced exotic associations. Further, unlike forage species that often grow in monocultures or as dominants, many

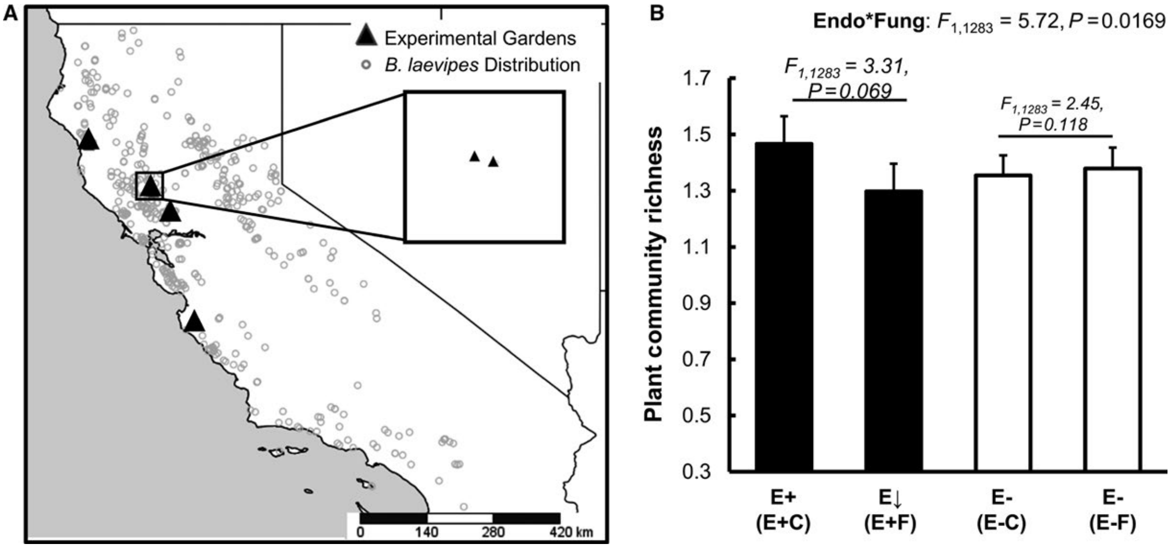

FIG. 4. (A) Location of the five common gardens used in the range-wide experiment. Triangles represent experimental gardens; circles mark *Bromus laevipes* herbarium records. Gardens spanned diverse habitats and ~400 km with a focus on densely inhabited areas. Inset: Two physically close gardens that differed substantially in habitat (serpentine vs. non-serpentine soils/communities) and endophyte status of the natural population (absent vs. present). (B) Endophyte increased plant richness across these gardens ($F_{1,1283}$ = 5.72, P = 0.0169). Plants with high endophyte levels indicated by E+ (control plants from E+ populations); plants with experimentally reduced endophyte levels indicated by E↓ (fungicide-treated plants from E+ populations). Both of these are shown by solid bars. E- represents plants from naturally non-symbiotic populations (open bars), with E–C indicating control plants and E–F indicating fungicide-treated plants. The lack of difference in community richness between E–C and E–F suggests that fungicide treatment did not directly impact community richness ($F_{1,1283}$ = 2.45, P = 0.118) and the difference observed between E+ and E↓ resulted from reductions in endophyte (marginally significance: $F_{1,1283}$ = 3.31, P = 0.069). Values show are mean + SE.

endophyte-associating plants in natural communities are non-dominant species such that increasing their performance could lead to more even communities (as we observed). Our study indicated the consequence of endophytes can be just as strong in natural ecosystems as in agricultural settings, but importantly, the impact on the natural community may be fundamentally different: *enhancing* rather than reducing plant diversity.

What is the mechanism of endophyte-mediated effects on communities?

The beneficial nature of mutualistic interactions is predicted to increase the dominance of their partner species, which could in turn lead to less diverse ecosystems (e.g., Keller 2014). Surprisingly, we found that mutualistic endophytes in *B. laevipes* promoted, rather than suppressed, plant diversity, while simultaneously increasing biomass of this non-dominant grass. Similar to the effects of many keystone predators, endophytes suppressed a dominant competitor, the invasive congener *B. diandrus*, thereby reducing overall plant density in plots and increasing the evenness (and possibly richness) of the community.

Endophytes in *B. laevipes* could cause this increased diversity through several non-exclusive mechanisms. First, endophytes substantially increased *B. laevipes* performance (e.g., higher survival and ~250% more biomass), indicating that endophytes may change the competitive outcomes between *B. laevipes* and other plant species in natural communities. Previous work in agronomic systems has suggested that increased tall fescue dominance (caused by endophyte-provided benefits to hosts) led to reductions in the diversity of the plant community (Clay and Holah 1999), and many studies with agronomic grasses have shown that endophytes can increase their host plant's competitive ability (e.g., Marks et al. 1991). However, in our system, the competitive effects of *B. laevipes* hosting endophytes fell disproportionately on the invasive competitive dominant, *B. diandrus*. Second, some studies have proposed that endophyte-produced alkaloids and other similar compounds may have allelopathic effects on other plant species (Malinowski and Belesky 1999, Orr et al. 2005, Vázquez-de-Aldana et al. 2012), and the endophytes of *B. laevipes* are known to produce several different alkaloids (Charlton et al. 2014). Third, the production of these alkaloids could also impact the other community members through their effects on herbivores (Jani et al. 2010) and/or predators of herbivores (Faeth and Shochat 2010). Herbivores can be deterred or killed by the alkaloids (Rudgers and Clay 2008), which could explain the increase in diversity we observed, if dominants are disproportionately consumed in place of the grass host, as may be the case for a congener.

Endophyte effects on invasions

A growing body of work has investigated the role of soil microbiota in plant invasions (Callaway et al. 2004, Pringle et al. 2009, Seifert et al. 2009, Vogelsang and Bever 2009, Cahill and Cahill 2015), but phyllosphere microbial communities may also have important consequences for invasions. Work over the last few decades has found that fungal endophytes that reside in exotic grass species, especially tall fescue, can increase their hosts' invasiveness and influence on natural communities (Clay and Holah 1999, Lemons et al. 2005, Finkes et al. 2006, Rudgers and Clay 2008). Mutualistic benefits to invasive species, such as enhanced growth and competitive ability, has also been documented for other types of endophytic fungi (i.e., non-epichloid). For example, fungal endophytes in spotted knapweed (*Centaurea stoebe*) increased the competitive ability of this invasive species, with a greater effect on plants from the introduced range than the native range (Aschehoug et al. 2012), in part through intensified allelopathy (Aschehoug et al. 2014). Fungal endophytes in exotic species can even change the relationship between community diversity and resistance to invasions. Rudgers et al. (2005) found that in the absence of endophytes, high initial community diversity reduced the establishment of an invasive grass, but when endophytes were present, establishment was independent of diversity.

These studies all document endophytes increasing the performance of invasive species and/or increased community susceptibility to invasions. However, they also all focus on the endophytes in invasive species, and very little is known about how endophytes in native species impact invasions. One of the only studies we found explicitly examining the role of native endophyte–plant associations on invasions documented that endophyte in the grass *Poa alsodes* increased its host biomass and reduced the biomass of a common invader when they were grown together (Craig et al. 2011). This experiment was conducted in a greenhouse setting and the authors emphasized that future research "in more complex field settings where other important factors, such as herbivores and fluctuating abiotic conditions, come into play" is needed to understand whether endophytes provide invasion resistance to native species. Our study, conducted under field conditions, demonstrated that endophytes in another native, non-dominant plant may enhance resistance to an invasive species, resulting in a more diverse community. These results, in conjunction with the steadily growing literature showing endophytes can enhance plant performance of native hosts under some conditions (Brem and Leuchtmann 2002, Afkhami and Rudgers 2009, Rudgers et al. 2009, Saari and Faeth 2012, Saikkonen et al. 2013), suggest that endophytes suppressing invasive species may be common. We propose that more work explicitly examining how native endophyte-plant associations impact exotic species would be valuable and could shed light on how often endophytes suppress, vs. enhance, invasions.

Role of microbes in restoration

A number of economically and environmentally important "plant" traits (such as drought tolerance and natural enemy resistance) have subsequently been attributed to the microbial symbionts that plants host (reviewed in Friesen et al. 2011). For example, we observed that it was the fungal endophyte in *B. laevipes,* rather than the plant itself, that enhanced community resistance to a dominant exotic grass. While this distinction will not matter in some cases (i.e., when targeting the host also includes the symbionts), it could have important consequences in others, such as when microbe presence is variable or facultative. For example, fungal endophyte frequencies in native grass germplasm may be much lower than in nature (Rudgers and Swafford 2009) owing to negative effects of seed storage protocols on endophyte survival. Given the effects of native endophytes on plant diversity that we observed, paying close attention to the levels of endophyte in natural and stored seed may dramatically alter outcomes of restoration. Our results also suggest that an important role of endophytes in promoting plant diversity may in part come from suppression of dominant invasive grasses like *B. diandrus,* which further emphasizes the potential value of microbial associations in restoration of disturbed habitats.

Acknowledgments

Many thanks especially to J. Rudgers, who gave extensive advice throughout this project. Also, thanks to C. Searcy, J. Stachowicz, K. Rice, M. Stanton, S. Campbell, the Strauss and Stinchcombe labs, two anonymous reviewers, and editor J. Hoeskema for input on content and improvement to the manuscript, C. Lee and G. Reed for help with harvesting and data collection, D. Grossenbacher for assistance with plant identification, and M. Bamford, A. Duong, N. Gaines, L. Hack, C. Liao, K. Matsumoto, N. Sou, and T. Tran for additional assistance in the lab, greenhouse, and/or field. We thank the University of California Natural Reserve System, especially McLaughlin Natural History Reserve and Angelo, Quail Ridge, and Hastings Reserves, for providing protected natural habitats in which to conduct our experiments as well as Mendocino National Forest for allowing seed source collections. We genuinely appreciate the help of the UC Reserve managers: P. Aigner, V. Boucher, J. Clary, L. Johnson, C. Koehler, M. Power, P. Steel, and M. Stromberg. Our work was funded by the NSF Graduate Research Fellowship, the Achievement Rewards for College Scientists (ARCS) Foundation, the Center for Population Biology, and NSF DEB-1011635 to M. E. Afkhami and S. Y. Strauss.

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