**LETTER**

## Mutualist-mediated effects on species' range limits across large geographic scales

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### Abstract

Understanding the processes determining species range limits is central to predicting species distributions under climate change. Projected future ranges are extrapolated from distribution models based on climate layers, and few models incorporate the effects of biotic interactions on species' distributions. Here, we show that a positive species interaction ameliorates abiotic stress, and has a profound effect on a species' range limits. Combining field surveys of 92 populations, 10 common garden experiments throughout the range, species distribution models and greenhouse experiments, we show that mutualistic fungal endophytes ameliorate drought stress and broaden the geographic range of their native grass host *Bromus laevipes* by thousands of square kilometres (~ 20% larger) into drier habitats. Range differentiation between fungal-associated and fungal-free grasses was comparable to species-level range divergence of congeners, indicating large impacts on range limits. Positive biotic interactions may be underappreciated in determining species' ranges and species' responses to future climates across large geographic scales.

### Keywords

Drought, *Epichloë*, facilitation, fungal endophyte, grass, mutualism, niche, range, species distribution model, symbiosis.

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## INTRODUCTION

Accounting for species' responses to climate change requires a mechanistic understanding of the processes determining range limits. Many recent studies have emphasised the critical role of biotic interactions in shaping species' distributions, but have also pointed out that these effects have rarely been investigated on large geographic scales (Van der Putten *et al.* 2010; Dawson *et al.* 2011; Wiens 2011; Wisz *et al.* 2013; HilleRis-Lambers *et al.* 2013). The few existing studies have focused on the range-limiting forces of negative interactions, such as competition and predation (Anderson *et al.* 2002; Harley 2011; Aragón & Sánchez-Fernández 2013; Ettinger & HilleRisLambers 2013). In contrast, positive species interactions like facilitation or some mutualisms have the unique potential to ameliorate abiotic stressors, and may result in organisms using a unique and/or broader range of habitats (Fig. 1) (Bruno *et al.* 2003; Rodriguez-Cabal *et al.* 2012; Stachowicz 2012). While a few studies suggest that lack of appropriate obligate mutualists can limit persistence across species ranges (Nuñez *et al.* 2009; Mueller *et al.* 2011), variation in facultative mutualisms (*e.g.* pollination, seed dispersal) may also influence species distributions, and potentially in different ways. For example, if individuals with mutualists have different environmental tolerances from those without, they could occupy unique portions of the range, resulting in a larger species range (Fig. 1). Given the ubiquity of such facultative mutualisms in nature (Bronstein 1994; Bruno *et al.* 2003), studying their effects is essential to developing a deeper understanding of the processes determining range limits, especially in light of more stressful, future climates (Kivlin *et al.* 2013).

We used a combination of field surveys, species distribution models and field common garden and greenhouse experiments to explore the effect of fungal endophytes on the geographic range of a California-native grass, *Bromus laevipes*. Species distribution models demonstrated that endophyte-associated plants uniquely occupied drier habitats and that fungal-associated range divergence was comparable to range differentiation among species of congeners from the same region. A series of common gardens documented the fitness consequences of associating with endophytes across a ~ 1400 mm precipitation gradient in the field, and a greenhouse experiment examined the drought tolerance of grass hosts in which we experimentally manipulated fungal association, water levels and host population origin.

## MATERIALS AND METHODS

### Study system

Endophytic fungi are extremely widespread, occurring in every major plant lineage (Rodriguez *et al.* 2009) with systemic fungal endophytes of the genera *Neotyphodium* and *Epichloë* (Clavicipitaceae) residing in the aboveground tissue of an estimated 20–30% of the approximately 10 000 grass species (Poaceae) (Leuchtmann 1992). In agronomic systems, fungal endophytes are often mutualistic, conferring drought tolerance, resistance to herbivory and pathogens, enhanced nutrient uptake and increased competitive ability to their hosts (Clay & Schardl 2002). In exchange, the fungi gain nutrition in the form of carbon (Thrower & Lewis 1973). While less work has explored the role of these symbionts in natural systems, recent studies indicate that endophytes can also benefit
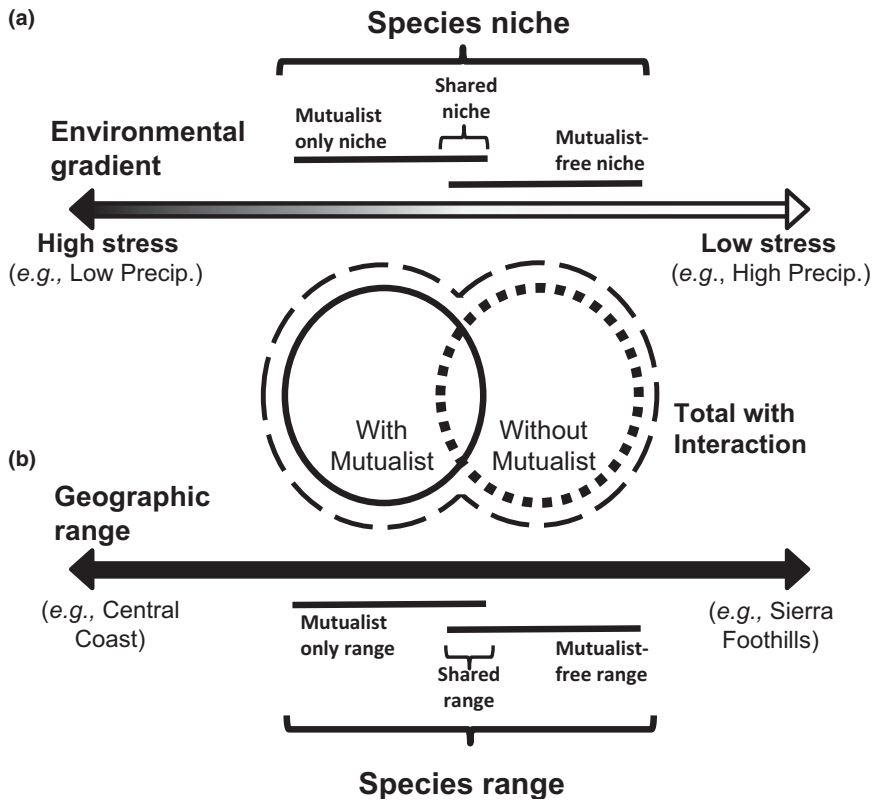
**(a)**



**Figure 1** Mutualist-mediated niche and range effects. (a) Mutualists can ameliorate abiotic stressors, which may alter the range of habitats their partners can occupy (*e.g.* able to persist in drier habitats). These same interactions can become costly, grading into parasitism, under some conditions (*e.g.* in low stress habitats, plants no longer need amelioration of environmental stress but still experience the cost of providing photosynthetic carbon to their partners). (b) If organisms with and without mutualists have different environmental tolerances, they could each occupy unique portions of the range, resulting in a larger species-level range when both are considered. Dotted line indicates niche/range without mutualists, solid line indicates the niche/range with mutualists and dashed line indicates total niche/range with mutualisms (sum of with and without mutualists).

native, wild grasses (*e.g.* Saikkonen *et al.* 2013). Like many mutualisms, the association is facultative (from the perspective of the plant) and the costs and benefits of hosting endophytes have been documented to vary in time and across environments (Ahlholm *et al.* 2002), and may thus provide opportunities for ranges of mutualist-associated and mutualist-free plants to differ.

*Bromus laevipes* (Chinook brome) is a perennial C$_3$ bunchgrass, widespread throughout California. While a few populations of this native grass have been documented in Washington, approximately 98% of population records of this grass fall within California and southern Oregon (*California Consortium of Herbaria* and the *Consortium of Pacific Northwest Herbaria*). *B. laevipes* typically grows in partial shade, favouring forest edges, and populations are often small and patchily distributed (Hickman 1993). In field surveys of California's native grasses conducted in 2007–2009, we found that *B. laevipes* commonly associates with systemic endophytes, which are vertically transmitted (*Epichloë* spp.) (Afkhami 2012), but may be lost in certain environments, or over time in dormant seeds (Clay & Schardl 2002; Afkhami & Rudgers 2008).

**Survey of natural populations**

We surveyed 92 natural populations of *B. laevipes* in northern and central California for endophyte frequency in 2009 and 2010 (~ 20 plants per population; > 1800 total; Table S1, Fig. S1). Each plant was examined for fungal hyphae by staining with aniline blue-lactic acid dye and examining tissue at 200× under a compound microscope, which gives similar results to PCR methods (SI Methods: Survey).

**Species distribution models across the range**

To test how mutualisms impact species distributions, we used the survey data in combination with climate data to build two species distribution models (SDMs): one for E+ (fungal-associated) and one for E− (fungal-free) *B. laevipes*. Models were generated using Maxent v3.3.1 (Phillips *et al.* 2006) with default parameter settings and logistic output values. For locality data, we grouped the surveyed *B. laevipes* populations according to fungal status. The 35 populations used to create the E+ model were 18 populations with ≥ 90% infected and another 17 populations of intermediate infection frequency (Table S1). The E− model was created using 57 populations with ≤ 10% of individuals infected, plus the 17 intermediate infection populations. By including intermediate frequency populations in both E+ and E− models, we increased the degree of model overlap, and thus any estimates of climatic or range differences were conservative (see Analysis S1).

For climate information, we used four axes from a principle component analysis (PCA) of the 19 'Bioclim' variables (1950–2000; http://www.worldclim.org; Hijmans *et al.* 2005), which explained ~ 94% of the climatic variation within California (per cent variation in Table 1 and loadings of climate variables on axes in Table S2). PCA was employed to minimise the effect of intercorrelation between variables and avoid overfitting our models (Warren *et al.* 2008). PC1 represented a broad statewide cline from wet, cold areas (*e.g.* northern California) to hot, dry areas (*e.g.* desert/Central Valley), whereas PC2 represented a cline from Continental to Mediterranean weather patterns. PC3 was strongly associated with increasing precipitation as well as temperature seasonali-

**Table 1** Niche breadths and overlap values for E+ and E− *Bromus laevipes*

| Axis | % Variance[1] | Overlap[2] | Breadth[3] | |
|---|---|---|---|---|
| | | | E+ | E− |
| Geographic | | | | |
|   *D* | – | 0.659** | 0.268 | 0.273 |
|   *I* | – | 0.875** | – | – |
| PC1 | 50.6 | 0.936 | 0.558 | 0.579 |
| PC2 | 28.7 | 0.861 | **0.438** | **0.368** |
| PC3 | 8.1 | 0.797* | **0.588** | **0.488** |
| PC4 | 6.3 | 0.749** | **0.547** | **0.761** |

[1]Per cent of variance in California's statewide climate that each axis explains. We included four axes in our models based on a predetermined cumulative cut-off of including 90% of the variation in the climate. Fungal endophyte was associated with significant changes to the climatic niche across PC axes 2–4 that explained *c.* 45% of the variation in California's climate, but not PC1.
[2]Overlap of E+ and E− plant significantly less than expected by chance (randomisation method) indicated by *P < 0.05 and **P < 0.001. Overlaps for all axes were calculated using Schoener's D (*D*). Modified Hellinger distance (*I*) was also calculated for the geographic axis.
[3]Bold indicates that E+ and E− plants have significantly different breadths (observed E+ breadth does not fall within 95% CI of E−, and E− breadth does not fall within CI of E+).

ty, and PC4 was associated with increasing precipitation of the warmest quarter, increasing mean temperature of wettest quarter and decreasing mean diurnal temperature range.

All environmental layers consisted of California and a 45-km buffer zone around the state borders and were at a 30″ scale (~ 1 km² geographic cells at the equator). The geographic boundaries were selected to encompass the majority of the species range (*c.* 98% of population records from the *California Consortium of Herbaria* and the *Consortium of Pacific Northwest Herbaria* fall within the modelled area). Models were evaluated using bootstrapped AUC scores. E+ and E− ecological models had mean test AUC scores of 0.972 (95% CI: 0.941, 0.992) and 0.957 (95% CI: 0.930, 0.978), respectively, indicating that these models are much better than random at predicting where each group occurs (Phillips *et al.* 2006). For details on AUC bootstrapping methods, see SI Methods: Model Evaluations.

**Range-wide overlap and breadth statistics**

Niche overlap, the similarity of resources/environments utilised by two species or groups, was used to determine differentiation between fungal-associated and fungal-free plants. We calculated overlap using Schoener's D across geographic and climatic niche axes (Schoener 1968). Overlap ranges from 0 to 1 (no to complete overlap respectively) and is calculated as D $(p_X, p_Y) = 1 - \frac{1}{2} \sum |p_{X,i} - p_{Y,i}|$, where $p_{X,i}$ represents the suitability assigned by the distribution model to a specific location *i* for fungal-associated *B. laevipes* and $p_{Y,i}$ represents the suitability assigned by the distribution model to the same location for fungal-free *B. laevipes*. Range overlap was calculated by comparing suitability values between geographic cells across the landscape. Climatic overlap (across each PC axis) was calculated by comparing the summed suitability at all locations with a particular climate value *i* for fungal-associated and fun-

gal-free *B. laevipes*. We then used randomisation methods to compare observed overlaps to random distributions of 1000 overlap values generated with R and Maxent (R Development Core Team 2011), evaluating if the observed overlaps were significantly less than expected by chance (as in Warren *et al.* 2008) (see SI Methods: Overlap and Breadth for details).

We also examined whether the range divergence between E+ and E− *B. laevipes* was biologically meaningful by comparing it with range differentiation observed between species of *Bromus* native to the same region. We created distribution models for eight *Bromus* species and calculated the range overlap between all pairwise combinations of species. We then calculated a two-tailed 95% CI around the overlap of E+ and E− *B. laevipes* via bootstrapping. The proportion of between-species overlaps greater than or comparable to (*i.e.* fell within the bootstrapped 95% CI) the geographic overlap between E+ and E− *B. laevipes* was determined (SI Methods: Overlap and Breadth).

Niche breadth characterises the range of resources/environments utilised by a species or group. If endophytes influenced the niche or range breadth of their host, fungal-associated plants could have significantly different breadth values than fungal-free plants for one or more axis. We calculated breadths across geographic and climatic niche space using proportional similarity (following Feinsinger *et al.* 1981) as described for overlap, but in this case $p_{X,i}$ represented the suitability assigned by the distribution model to an environment *i* for the group whose breadth we are calculating (either E+ or E− plants) and $p_{Y,i}$ represents the proportion of environmental values in the landscape that occur in category *i*. We then calculated two-tailed 95% CIs around E+ and E− breadths via bootstrapping methods (1000 replicate data sets and models for E+ and E− populations separately) using R and Maxent (SI Methods: Overlap and Breadth). The robustness of the overlap and breadth statistics results (to unequal E+ and E− sample sizes, clustering of populations, etc.) was confirmed with SI Analyses 1–4.

**Field common garden experiments**

We collected seeds from three naturally E+ populations (mean freq = 100%) and three naturally E− populations (mean freq = 0%; Table S3). To examine the effects of fungal association on plant performance across a range of climatic conditions, in December 2009 we planted 30 E+ and 30 E− seeds (10 per population) into 10 common gardens at field sites spanning > 400 km and a mean annual precipitation gradient from ~ 550 to 1950 mm (PRISM: Wang *et al.* 2012; http://www.prism.oregonstate.edu; Table S4) (Wright *et al.* 2006). Sites received ~ 400–1000 mm during the course of the experiment (PRISM). The precipitation range of the 10 gardens includes 87.5% of the precipitation gradient experienced by the populations we surveyed and ~ 67% of all *B. laevipes* herbarium records from the *California Consortium of Herbaria*. Seeds were planted in a grid with 10 cm spacing (an observed natural field density). For each site, we scored seedling establishment/survival 3 months after planting (February 2010) and calculated per cent survival of E+ and E− seeds. In other field experiments with *B. laevipes*, we have found that

performance at 3 months after planting is strongly associated with performance at 2 years (growth: $F_{1,488} = 15.25$, $P = 0.0001$, mortality: $\chi_1^2 = 49.56$, $P < 0.0001$; Table S5). We regressed the relative survival of E+ vs. E− seeds across sites against December–February precipitation (PRISM) in R (Ågren & Schemske 2012).

## Manipulative drought experiment

In a greenhouse experiment, we directly tested for benefits of fungi under water limitation using grasses from three naturally E+ populations (mean freq = 96.7%) and four naturally E− populations (mean freq = 0%; Table S6). By manipulating water level directly, we decoupled water-mediated effects from other correlated variables (*e.g.* temperature, surrounding vegetative biomass and other community members such as mycorrhizal fungi) that could have been present in the field experiment and observations. We also experimentally treated half of the E+ seeds (E+↓) with 2 g L$^{-1}$ Benomyl fungicide (Latch & Christensen 1982) to control for inherent or genotypic differences in drought tolerance between fungal-associated and fungus-free populations. Half of the E− seeds received the same treatment to control for direct fungicide effects on host performance (November 2010; SI Methods: Fungicide Treatment). Seeds were cold stratified at 4 °C for 2 weeks, then placed on a sunny laboratory bench for ~ 5 weeks to allow germination and initial growth.

In January 2011, ~ 50 fungicide-treated and ~ 50 control seedlings from each population (~ 700 total) were transplanted into pots (~ 700 mL; Deepots, Stuewe & Sons, Tangent, OR, USA) containing modified University of California Mix potting soil and placed into randomly assigned positions in a greenhouse on the campus of University of California, Davis (~ 23–25 °C; no supplemental light). Density of fungal hyphae in plant leaves was measured to confirm that fungicide treatment reduced endophyte levels (SI Methods: Fungicide Treatment) (Mack & Rudgers 2008). The fungicide treatment successfully reduced endophyte hyphal densities by *c.* 40% in E+↓ plants (Fig. S2).

After exposure to a wet period (November–April; water daily) to simulate rainy Mediterranean winters/early spring, we imposed a drought period (May–July) to reflect natural patterns of summer drying in Mediterranean climates. Plants from each fungicide-population combination were assigned to 10 watering levels chosen to span the extremes of annual precipitation experienced by the populations over 30 years: 180–1980 mm increasing in 200-mm increments (determined using 1980–2009 downscaled PRISM data from ClimateWNA; Wang *et al.* 2012; Fig. S3). We calculated the per cent of annual precipitation typically experienced early in the dry period (May) and divided it equally among weeks, which resulted in a weekly water addition of 5–50 mL in 5-mL increments. Plants were watered once per week to mimic storm events. We measured volumetric soil moisture to confirm that our treatments resulted in differing water availability to plants (Fig. S4).

We harvested the experiment at the end of July 2011, recording mortality and number of live leaves (> 60% green) and clipping aboveground tissue at the soil surface. We also washed the roots of ~ 200 randomly selected plants through a 1-mm sieve (US Standard Sieve No. 18; Soil Test Inc., Lake Bluff, IL, USA). Above- and belowground biomass were dried to constant mass (at 60 °C) and weighed to the nearest 0.001 g. All performance data were analysed using mixed model ANCOVAs in SAS (SAS Institute 2011) with fixed categorical factors of the endophyte status of the population (E+ or E−) and fungicide treatment (control or fungicide), a continuous covariate of precipitation treatment (axis of 10 water levels) and all interactions. We included a random factor of population origin nested within endophyte status of the population. Logarithmic transformations improved normality of aboveground biomass and leaf production data. We also used two orthogonal planned contrasts. The first tested whether the effect of endophyte on plant performance depended on water availability, comparing the response to decreasing watering for plants with the highest level of endophyte (E+) vs. all other treatments [plants with no (E−) and experimentally reduced endophyte (E+↓)] (Littell *et al.* 2006). The second compared performance of E+ vs. all other treatments without considering water level.

## RESULTS

### Survey of natural populations

Endophyte frequency across *B. laevipes* populations was bimodally distributed with ~ 80% of the populations either having endophyte in 100% of plants or 0% of plants (Fig. S1a). When endophytes were present, fungal association within grass populations ranged from 25 to 100% of plants, but most were at high frequency, especially on the coast and in the Coast Range (Fig. S1).

### Species distribution models across the range

Species distribution models indicated that E+ and E− populations shared many of the same environmental attributes as reflected in their undifferentiated scores on PC1 (overlap = 93.6%; Table 1). For example, neither E+ nor E− *B. laevipes* populations occupy the Central Valley and Mojave Desert and both do occur in the Coast Range (Fig. 2). Thus, E+ and E− populations respond similar to PC1, which represents a statewide cline from wet/cold (*e.g.* Northern California) to hot/dry areas (*e.g.* Mojave Desert/Central Valley).

However, endophyte was associated with significant differences in the climatic niche of *B. laevipes* across the other three PC axes (PC2–4) that together explained *c.* 45% of the variation in California's climate. First, E+ populations occupied a 20% broader climatic niche than E− populations across PC axes 2 and 3, occurring in a broader range of habitats with Continental to Mediterranean weather patterns (PC2), water availabilities and temperature seasonalities (PC3) (Tables 1, S2, S7). In contrast, the climatic range of E− plants was significantly broader for PC4 (~ 30%; Table 1, S2, S7; axis represents precipitation of the warmest quarter, temperature of wettest quarter and diurnal temperature range), suggesting that endophytes do not broaden all aspects of their host's niche.
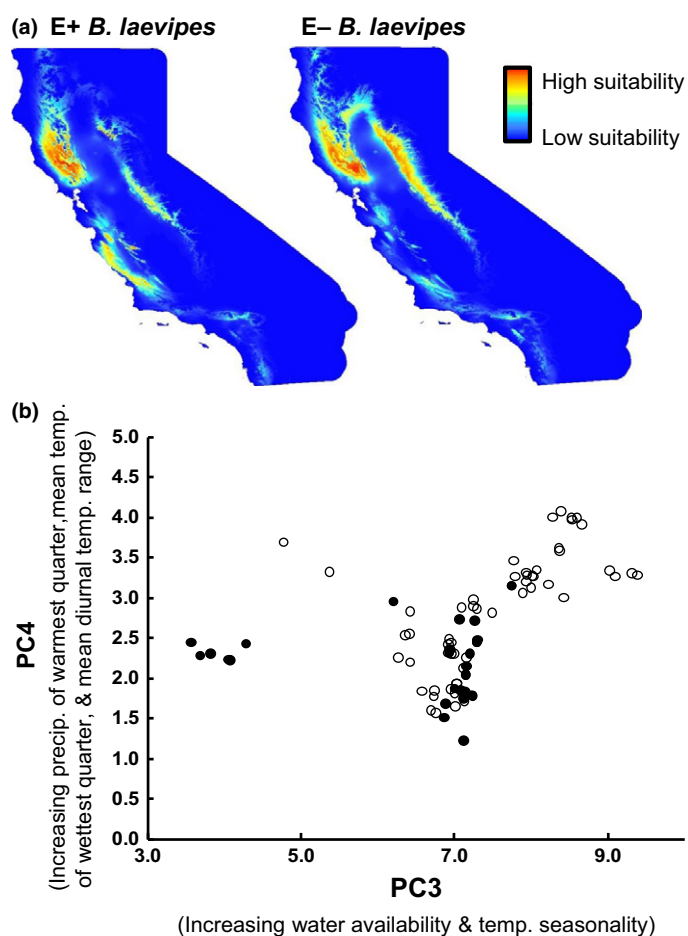
## (a) E+ *B. laevipes*     E− *B. laevipes*



High suitability

Low suitability

## (b)



PC4
(Increasing precip. of warmest quarter, mean temp. of wettest quarter, & mean diurnal temp. range)

PC3
(Increasing water availability & temp. seasonality)

**Figure 2** (a) Species distribution models demonstrated the geographic differentiation between fungal-associated and fungal-free *B. laevipes*. Habitat suitability for *B. laevipes* ranged from high (red) to low/ unsuitable (blue). (b) E+ *B. laevipes* occupied a different climatic niche than E− populations. Only E+ *B. laevipes* occupied habitats with low values of PC3 (strongly associated with low water availability and temperature seasonality), whereas only E− populations occupied habitats with high values of PC3 and PC4. PC1 predominately described aspects of the climatic niche shared by both E+ and E− plants (*e.g. B. laevipes* does not in the desert or Central Valley), while endophyte-associated changes to the climatic niche occurred along PC2–4, which explained *c.* 45% of the variation in California's climate. Filled circles represent E+ populations (endophyte freq. > 50%, *n* = 27), and open circles represent E− populations (freq. < 50%; *n* = 65).

Second, the climatic niche overlap between E+ and E− plants was significantly less than expected by chance – 79.7% and 74.9% for PC axes 3 and 4 respectively (Schoener's D; Table 1; $P = 0.020$, $P < 0.001$). Only E+ populations occupied habitats with low water availability and temperature seasonality (low values of PC3 such as the Central Coast) (Fig. 2, Table S2). Our results also suggest a possible cost of mutualism as E− *B. laevipes* uniquely occupied wetter, more temperature-variable areas with higher summer precipitation (high values of PC3 and PC4 such as the northern Sierra Nevada) (Fig. 2, Table S2). Observed differentiation between fungal-associated and fungal-free plants may explain how variation in symbiosis is maintained in nature.

These climate differences translated into significant geographic range divergence between E+ and E− populations. Range overlap was significantly less than expected by chance: 65.9% (Schoener's D) and 87.5% (Hellingers distance) (Table 1; $P < 0.001$, $P < 0.001$; confirmed with conventional MANOVA statistics in Analysis S4) (Schoener 1968; Warren *et al.* 2008). Furthermore, 19–22% of the *B. laevipes* species range, as much as $\sim 25\,277$ km$^2$, was only suitable for E+ plants (Table S8; exact added range size also depends on other non-climate niche factors such as herbivore pressure). Thus, endophytes were associated with a species-level geographic range increase of $\sim 20\%$, relative to the range occupied by E− populations.

To better understand whether the range divergence observed between fungal-associating and fungal-free plants was biologically meaningful, we compared the extent of range overlap between E+ and E− populations to that between *B. laevipes* and other California-native species of *Bromus*. Approximately half of the *Bromus* species (3–4 species of 8) had statistically comparable or more overlap with *B. laevipes* than E+ and E− *B. laevipes* had with each other (Table 2). Similarly, 9–12 of the 28 possible pairs of California *Bromus* species ($\sim 30$–40%) had comparable or more overlap between them than E+ and E− *B. laevipes* had with each other (Table 2). Thus, the extent of range differentiation generated by endophyte association is of comparable magnitude to range divergence among many native California brome species, and hence is biologically significant.

**Table 2** Geographic niche overlap among species of *Bromus* native to California

|  | *B. laevipes* | | *B. arizonicus* | | *B. carinatus* | | *B. ciliatus* | | *B. grandis* | | *B. orcuttianus* | | *B. suksdorfii* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | D | I | D | I | D | I | D | I | D | I | D | I | D | I |
| *Bromus arizonicus* | 0.405 | 0.664 | – | – | – | – | – | – | – | – | – | – | – | – |
| *B. carinatus* | **0.734** | **0.936** | 0.409 | 0.659 | – | – | – | – | – | – | – | – | – | – |
| *B. ciliatus* | 0.370 | 0.677 | 0.052 | 0.207 | 0.500 | **0.791** | – | – | – | – | – | – | – | – |
| *B. grandis* | **0.568** | **0.848** | 0.440 | 0.716 | **0.559** | **0.842** | 0.166 | 0.430 | – | – | – | – | – | – |
| *B. orcuttianus* | 0.492 | **0.792** | 0.088 | 0.313 | 0.507 | **0.810** | **0.605** | **0.857** | 0.295 | 0.588 | – | – | – | – |
| *B. suksdorfii* | 0.329 | 0.627 | 0.037 | 0.161 | 0.460 | 0.743 | **0.879** | **0.987** | 0.143 | 0.384 | **0.563** | **0.832** | – | – |
| *B. vulgaris* | **0.624** | **0.891** | 0.235 | 0.499 | **0.574** | **0.869** | 0.391 | 0.709 | 0.399 | 0.703 | **0.568** | **0.818** | 0.351 | 0.652 |

Niche overlap was calculated using Schoener's D (*D*) and a version of Hellinger distance equation (*I*). Overlap values greater than or comparable to the niche overlap calculated between E+ and E− *B. laevipes* are shown in bold. Comparable overlap was defined as overlaps falling within the 95% CI around the overlap between E+ and E− *B. laevipes*. CI around *D* was (0.519, 0.751), and CI around *I* was (0.753, 0.937).

## Field common garden experiments

We followed these observational and modelling projects with experimental approaches that allowed us to more fully attribute our observed range and climate distributions to association with endophytes. Across the 10 common gardens that spanned ~ 90% of the precipitation gradient and > 400 km, the relative survivorship of E− plants was higher in wet areas and that of E+ plants was higher in drier parts of the range (Fig. 3a, Fig. S5; $R_{adj} = 0.45$, $F_{1,8} = 8.29$, $P = 0.021$), suggesting that endophytes confer advantages in drier habitats and perhaps a cost under wetter conditions.

## Manipulative drought experiment

In the greenhouse experiment, we directly tested for benefits of fungi under water limitation when water-mediated effects were decoupled from other correlated variables that could have been present in the field experiment/observational studies and when fungal associations were experimentally reduced. While survivorship of plants in all treatments was similar at higher water levels, when water availability was low, survivorship of the plants with high endophyte levels (E+) was significantly greater than plants with no (E−) and experimentally reduced endophyte (E+↓) (Fig. 3b, Table S9; contrast of E+ vs. other treatments' response to water: $T_{1,127} = -2.27$, $P = 0.025$). This result was further supported by testing the response of only E+ and E− control plants to water level, which again showed that E+ plants performed better in low water and E+ and E− plants performed similarly in high water (Table S10; water × endophyte: $F_{1,61} = 6.49$, $P = 0.0134$). Aboveground biomass and leaf production were strongly reduced by water limitation, but not affected by endophyte or fungicide treatments, and belowground biomass was unaffected by all treatments (Tables S11–S13).

## DISCUSSION

Our results demonstrate that positive biotic interactions can result in greater species-level geographic ranges when mutualists alter the environmental tolerances and niche of their partners. By ameliorating environmental stresses, mutualists open possibilities for response to changing climates, for new ecological interactions and for ensuing evolutionary trajectories.

## Effects of endophyte mutualism on grass range and niche

Mutualists may alter their host's range through at least two types of changes to the host niche. First, a mutualistic partner could cause an organism to have a wider range of tolerances by ameliorating stressors (Bruno *et al*. 2003). Consistent with this pathway, we found that endophyte-associated plants had a 20% broader climatic niche than E− plants across several climate axes (PC2 and 3). Second, a mutualistic interaction could result in a larger species range (*e.g.* Fig. 1) if partner-associated individuals occupy a unique area of niche space compared with individuals that do not
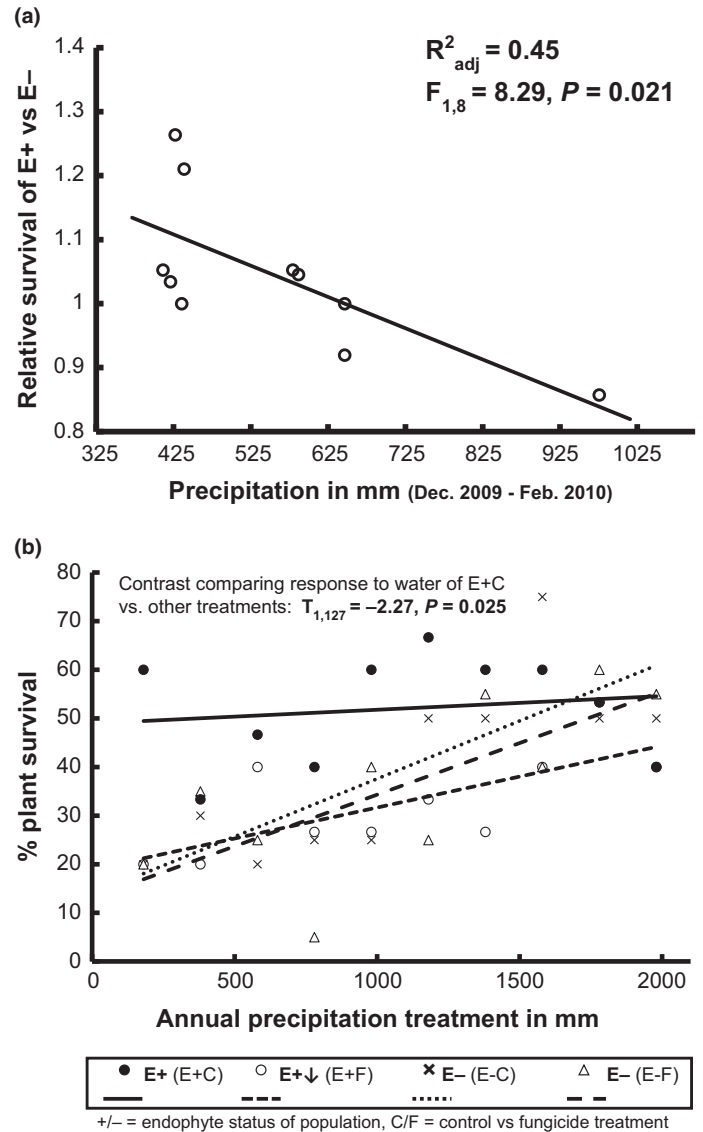


**Figure 3** Field and greenhouse experiments demonstrated how endophytes affect species distributions along a water availability axis. (a) Across 10 field common gardens, more E+ seeds established/survived than E− seeds at low precipitation sites, whereas at high precipitation sites establishment/survival of E− seeds was higher. Relative survival equals the ratio of E+ to E− seed survival (under equal survival, ratio = 1). (b) Greenhouse drought experiment: In low water treatments, plants with high endophyte levels (E+C) have greater survivorship than plants with low (E+F) or no (E−C and E−F) endophyte and in high water have similar survivorship.

participate in the interaction. In our study, E+ plants uniquely inhabited drier areas compared to the wetter areas solely occupied by E− plants (20–25% differentiation across PC3 and 4); comparison with other native *Bromus* species demonstrated that endophyte-associated range divergence was not only statistically, but also biologically, meaningful. As a result of these change to the niche, endophyte-associated populations occupied thousands of square kilometres of habitat where endophyte-free grass was absent, resulting in a 20% larger species range.

### Endophyte-mediated drought tolerance

Endophyte-conferred drought tolerance has been found in other grass species in greenhouse experiments and surveys (Oliveira & Castro 1998; Clay & Schardl 2002; Morse *et al.* 2002; Novas *et al.* 2007; Davitt *et al.* 2011). Physiological mechanisms underlying this benefit include: alteration of root morphology (Malinowski & Belesky 2000), changes to sensitivity for stomatal closures (Elmi & West 1995) and/or accumulation of endophyte-produced metabolites (Nagabhyru *et al.* 2013). Our common garden experiments and greenhouse study showed that endophytes were important for plant fitness in dry environments both in nature and under controlled conditions. The association of endophyte-conferred drought tolerance with patterns of host occupancy across the geographic range may reflect an ongoing response to recent weather, strong selective effects of occasional severe weather events (*e.g.* severe drought) and/or past climate conditions. By experimentally demonstrating increased drought tolerance for grasses with endophytes in the field and greenhouse, we link endophyte-enhanced performance in dry environments to the observed habitat occupancy differences, climate niche differences and range differentiation in models of endophyte-associated and endophyte-free plants for the first time.

### Alternative hypotheses

Alternative hypotheses to explain these results must also be considered. First, it is possible that endophytes are acquired from other host plant species in the E+ *B. laevipes* range and that these hosts might be absent in the E− range. Afkhami (2012) surveyed 3800 plants across 207 populations and 36 species of grasses from California, finding only two other species that host this type of endophyte, both of which were vertically transmitted from maternal plant to seed (Afkhami 2012). Furthermore, of those two species, one currently has a completely non-overlapping distribution with *B. laevipes*, and endophyte-infected plants of the other species were only found in a population that was in the non-symbiotic portion of the *B. laevipes* range, thus this source of the observed pattern seems unlikely.

A second class of explanations of the observed pattern relies on either imperfect vertical transmission (loss of endophytes in offspring of symbiotic parents) under high humidity conditions (Afkhami & Rudgers 2008) or that endophytes are parasitic or neutral symbionts that thrive in dry region and perform poorly in wet areas (and thus are lost from the latter). While these alternatives are hard to refute or support from our data, we know that 1) *in planta* hyphal density of the fungal endophyte (a proposed proxy for transmission success and thus endophyte performance) was similar across *B. laevipes* plants in five common gardens that varied substantially in climate (2 years after planting; $F_{1,233} = 1.43$, $P = 0.2235$, Table S14), suggesting that differential transmission may not be source of these patterns. We also note that 2) our greenhouse and field experiments demonstrated that endophytes increased performance of their hosts under drought conditions, so explanations relying on a lack of benefit to the host in dry conditions are not parsimonious. However, endophytes could also act as parasites or neutral symbionts in wet environments where the cost of providing them with photosynthetic carbon would still exist but the 'benefit' of endophyte-enhanced drought tolerance would be of little or no value.

Finally, while we hypothesised that by gaining mutualistic endophytes *B. laevipes* was able to move into drier, more stressful habitats, it is also possible that possessing endophyte is the ancestral condition and a loss of a symbiosis has allowed endophyte-free grass to use novel mesic habitats. The lack of endophyte in other native *Bromus* spp. from the region (Afkhami 2012 and Afkhami *unpub. data*) suggests that endophyte was an addition (rather than a loss), but without historical data on the distribution of endophyte-associated and endophyte-free plants, these alternative hypotheses are difficult to evaluate. Regardless of whether the use of new habitats resulted from a gain or loss of the endophyte, *B. laevipes*'s broader environmental tolerances and larger overall species range have likely resulted from participation in this facultative mutualism, since there are climatic tolerances/areas of the range uniquely associated with E+ and E− plants.

### Interaction-mediated effects on ranges

Very few existing studies document large-scale effects of biotic interactions of any type on the ranges of native species (Van der Putten *et al.* 2010; Dawson *et al.* 2011; Wiens 2011; HilleRisLambers *et al.* 2013; Wisz *et al.* 2013). Despite the fact that little work has *quantified* the effects of positive interactions on species ranges, larger ranges resulting from positive interactions are probably common, given the ubiquity of mutualism and facilitation in nature. Previous studies on obligate mutualisms with leafcutter ants have shown that northern range boundaries of the ant were limited by cold tolerance of their obligate fungal cultivar (Mueller et al. 2011), and other work has demonstrated that the lack of an obligate mutualist may limit the growth of organisms in habitats at the local scale (*e.g.* Nuñez *et al.* 2009). However, facultative mutualisms are far more common and represent a somewhat different case than obligate associations, which require a partner for persistence in all habitat types and parts of the species range. Facultative mutualists have been shown to speed range expansion of exotics recently arrived to new geographic areas (*e.g.* Parker *et al.* 2006; Nuñez *et al.* 2009; Hynson *et al.* 2013). Our study, however, provides some of the first insight into how facultative mutualists of *native* species influence the geographic distribution and ranges of species that have had many millennia to disperse and occupy habitats. Moreover, the nature of facilitative interactions that ameliorate environmental stress is quite different from facilitative interactions with dispersers, and could be expected to have different impacts on habitat use (*e.g.* important for persisting in new habitats vs. for arriving in new habitats).

### Importance of interaction-mediated effects on ranges

Alterations to the range caused by positive interactions have the potential to play an important role in the evolution and diversification of species. Mutualist-generated differentiation could pave the way for speciation, if divergent selection across

niches leads to reduced gene flow (Thompson 1987). For example, under drought, associating with fungal endophytes caused another grass, *Agrostis hyemalis*, to flower earlier than fungal-free conspecifics (Davitt *et al.* 2011), providing pathways for endophyte-linked assortative mating, reproductive isolation and possible speciation in dry habitats when considered in conjunction with the results presented here.

Furthermore, our rapidly warming climate will require species to adapt to novel conditions *in situ* or to shift their ranges to more suitable areas (Moritz & Agudo 2013). Climate change scenarios for California generally predict mean annual temperatures to increase by $\sim$ 2–6 °C across the state by 2070–2100. Although precipitation projections are more variable, the cascading effects of increased temperature (early snow melt, higher evapotranspiration) are generally predicted to result in decreased water availability and increased water stress (Flint *et al.* 2013; Neelin *et al.* 2013). This suggests that in future, *B. laevipes* may need fungal endophytes for persistence in key habitats, such as the Sierra foothill, which currently are mesic and contain non-symbiotic plants. In general, our results suggest that mutualists that ameliorate abiotic stressors can allow persistence under drier conditions and could be incorporated into management schemes to provide a second route to *in situ* persistence of plant biodiversity. Because grasses are important in many ecosystems (estimated to cover more than one-fifth of land on Earth; Shantz 1954) and are often the 'workhorses' of restoration, our study also indicates that endophyte-associated grass seed may increase the chances of restoration success of native communities under drier climates.

## CONCLUSIONS

We show that an integrative approach utilising field surveys, species distribution modelling and field and greenhouse experiments can provide a mechanistic understanding of how biotic interactions may affect range limits across large geographic scales. While some studies have documented niche/range reductions caused by competition or predation, mutualisms can significantly broaden or differentiate partner niches, generating larger species ranges and exposing partners to novel environments. Our work shows the importance of considering positive interactions in the predictions of range limits, as well as in predictions of species responses to climate change.

## AUTHORSHIP

MEA and PJM constructed distribution models and related statistics. MEA and SYS designed the field gardens and greenhouse experiments. MEA conducted these experiments and field surveys. All authors contributed to writing/editing.

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