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SPECIAL INVITED PAPER—GLOBAL BIOLOGICAL CHANGE

CLIMATE AFFECTS SYMBIOTIC FUNGAL ENDOPHYTE DIVERSITY AND PERFORMANCE[1]

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- *Premise of the study:* Fungal endophytes are symbionts that inhabit aboveground tissues of most terrestrial plants and can affect plant physiology and growth under stressed conditions. In a future faced with substantial climate change, endophytes have the potential to play an important role in plant stress resistance. Understanding both the distributions of endophytes and their functioning in symbiosis with plants are key aspects of predicting their role in an altered climate.
- *Methods:* Here we characterized endophytes in grasses across a steep precipitation gradient to examine the relative importance of environmental and spatial factors in structuring endophyte communities. We also tested how 20 endophytes isolated from drier and wetter regions performed in symbiosis with grass seedlings under high and low soil moisture in the greenhouse.
- *Key results:* Environmental factors related to historical and current precipitation were the most important predictors of endophyte communities in the field. On average, endophytic fungi from western sites also reduced plant water loss in the greenhouse compared to fungi from eastern sites. However, there was substantial variability in how individual endophytic taxa affected plant traits under high and low water availability, with up to two orders of magnitude difference in the plasticity of plant traits conferred by the different fungal taxa.
- *Conclusions:* While species sorting appears to largely explain local endophyte community composition, their function in symbiosis is not predictable from local environmental conditions. The development of a predictive framework for endophyte function will require further study of individual fungal taxa and genotypes across environmental gradients.

Key words: drought; *Panicum*; perennial C_4 grass; precipitation gradient.

Water availability is a primary controller of plant growth and survival (Lauenroth and Sala, 1992; Knapp and Smith, 2001), and thus the ability of plants to resist drought can have large impacts on primary production, diversity, and distributions (Tilman and Haddi, 1992; Knapp et al., 2002; Archaux and Wolters, 2006; Craine et al., 2013). Plant drought resistance is likely to become even more critical given future climate predictions for widespread increases in drought frequency and intensity (Meehl et al., 2007; Seager et al., 2007; Solomon et al., 2007; Schoof et al., 2010). Given its importance in plant productivity, understanding the mechanisms underlying drought resistance can be used to improve plant productivity under current and future environments where water is limited. Although most studies of plant drought resistance have been largely focused on the physiology and genetics of the plant in its abiotic environment (e.g., Juenger et al., 2005; Rampino et al., 2006; Tuberosa and Salvi, 2006), there is mounting evidence that microbial symbionts can also play a role in mediating plant responses to drought and other stresses (e.g., Augé, 2001; Márquez et al., 2007; Xu et al., 2008).

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Fungal endophytes are ubiquitous plant symbionts that can directly influence plant drought resistance, including effects on strategies for avoiding drought by increasing water uptake or decreasing transpiration rates and tolerating drought by osmotic adjustment (Malinowski and Belesky, 2000; Waller et al., 2005; Rodriguez et al., 2009, 2010; Morsy et al., 2010). Drought-stressed host plants colonized by endophytes can have increased biomass production, lower stomatal conductance, and lower overall water loss relative to plants without endophytes (Elmi and West, 1995; Kannadan and Rudgers, 2008; Rodriguez et al., 2008; Kane, 2011). Not all endophytes are mutualists, however, and colonization by some endophytes results in reduced biomass and increased rate of leaf water loss (Cheplick, 2004; Arnold and Engelbrecht, 2007; Kleczewski et al., 2012). Variation in endophyte function can occur across fungal taxa, genotypes, and habitats (Cheplick, 2004; Morse et al., 2007; Rodriguez and Redman, 2008), suggesting that there are complex drivers of endophyte effects.

As in macroecological communities, the distributions of fungal endophytes and their associated functions are likely to be driven by a combination of spatial, ecological, and evolutionary forces (Leibold et al., 2004). Although microbial dispersal is often thought to be widespread, evidence for local dispersal limitation is accumulating (Waldrop and Firestone, 2006; Peay et al., 2010; Kivlin et al., 2011; Martiny et al., 2011). Limited dispersal can lead to spatial structure and species turnover; for example, endophyte community similarity in two grasses declined with distance in coastal Spain (Sánchez Márquez et al., 2008). If dispersal is sufficient in areas with heterogeneous habitats, species can sort by environment (Leibold et al., 2004). Using community data from 158 studies, Cottenie (2005) found

that 44% of communities were structured by species sorting, 29% by a combination of species sorting and dispersal effects, and 8% by spatial factors presumably reflecting neutral processes or patch dynamics. No terrestrial or symbiotic microbial communities were included in this meta-analysis. Here we will focus on common nonclavicipitaceous endophytes of aboveground plant tissues, which are generally horizontally transmitted from the surrounding environment (e.g., soil, other plants, Rodriguez et al., 2009). Horizontally transmitted endophytes are unlikely to have tight associations with host plant species as can happen in vertically transmitted endophytes passed from parent to offspring via seed (Rodriguez et al., 2009; Higgins et al., 2011). Instead, some combination of environmental factors and spatial processes are more likely to be important for endophytes. For example, latitude was the best predictor of endophyte diversity across 28 host species from arctic tundra to tropical forest (Arnold and Lutzoni, 2007), which undoubtedly reflects both dispersal and habitat constraints.

How the distribution of endophytes relates to their functional capabilities is key to the development of a predictive framework for endophyte function in symbiosis. A combination of species sorting via environmental filtering and local adaptation may play a role in their distributions based on the association of endophyte function with some stressful habitats. For example, endophytes isolated from saline and geothermal environments conferred salt and heat tolerance, respectively, on several plant species (Redman et al., 2002; Rodriguez et al., 2008). When considering drought stress, we must consider that both long-term historical patterns of drought and current levels of drought are likely to act as environmental filters (Evans and Wallenstein, 2012). Long-term drought stress might affect the available species pool, while recent moisture conditions can affect the assembly of current endophyte communities. In contrast, if dispersal is the primary controller of endophyte distributions independent of environmental factors, then endophytes are likely to be distributed according to the spatial arrangement of sites with little or no relationship to function. Understanding the importance of environmental factors (species sorting) relative to spatial processes (neutral or mass effects) in endophyte community distributions may help us to better predict their function in plants under different environmental conditions. In particular, understanding the distribution of endophyte function in plant drought resistance may improve our ability to predict plant responses to drought under future climate change scenarios.

In this study, we addressed (1) how endophyte community distributions relate to underlying environmental factors and spatial processes and (2) whether the functional role of endophytes in symbiosis reflects local environmental conditions. We focused on moisture as an environmental driver. To assess endophyte distributions, we sampled endophyte communities in two grasses across a steep precipitation gradient where most other factors were similar across sites or not correlated with the rain gradient. We expected to find endophyte communities that varied from drier to wetter regions, with environmental sorting as the dominant factor (Saunders et al., 2010). Given the distance of sites across the gradient, however, dispersal limitation might also play a role (Peay et al., 2010). Second, we took endophytes isolated from the wettest and driest areas of the gradient and paired them with plants under high and low soil moistures in the greenhouse. We focused on early establishment of grasses, which can be a critical stage for plant survival and fitness in drought-prone environments. We hypothesized that endophytes from drier sites would be more likely to confer drought tolerance on plants than those from wetter regions (Conover et al., 2009). We also expected variation in function among endophyte taxa within regions, and our design allowed us to look for endophyte by environment interactions and functional plasticity.

MATERIALS AND METHODS

***Study sites and field sampling*—**Our sampling took place across an ~50-cm precipitation gradient spanning 400-km on the Edwards Plateau in Central Texas. Across the Plateau, annual rainfall varies from ~40 to 90 cm west to east, with a change of ~10 cm in mean annual precipitation every 40–50 km. All sampling sites were savanna grasslands on shallow, rocky, calcareous Mollisols. Sites were selected from those we could access based on historical mean annual precipitation, availability of host plants, and at least 50% grass cover. The sites were geographically clumped into three precipitation regions: west (42–62 cm), central (70–81 cm), and east (82–90 cm).

We focused on two grasses in the same genus, *Panicum hallii* Vasey and *P. virgatum* L. (Poaceae), to minimize potential host effects while maximizing the chance of finding hosts across the entire gradient. This decision was further supported by a preliminary study in which we found no differences among endophytes of four grass species (*P. virgatum*, *Andropogon gerrdii*, *Leptochloa dubia*, and *Sorghastrum nutans*) at one site on the gradient (H. Giauque and C. V. Hawkes, unpublished data). Host generalism has been found within tropical grasses as well (Higgins et al., 2011). Both *Panicum* species are perennial, warm-season grasses native to North America, with *P. hallii* often found locally in drier sites compared with *P. virgatum*.

From late May to July 2011, three tillers from three individual plants were collected from each of 12 sites across the gradient, with four sites in each rainfall region. The two hosts were never found at the same sites. After examining preliminary sampling effort curves, we added an additional site and three additional plants to the wettest eastern region to ensure that we sampled sufficiently. Plants were rinsed in the field and stored in plastic bags on ice for to transport to the laboratory.

***Fungal culturing*—**From each plant tiller, three 2-mm sections without visible signs of disease were removed and surface sterilized in 0.5% sodium hypochlorite (2 min) and 70% ethanol (2 min), as previously described by Arnold et al. (2000). Once sterilized, each leaf fragment was placed on a petri dish containing 2% potato dextrose agar (PDA) and 50 ppm ampicillin. We chose PDA because it is a general growth medium commonly used in endophyte studies and known to yield large numbers of endophytic isolates (e.g., Ghimire et al., 2011; Loro et al., 2012; Orlandelli et al., 2012). Plates were incubated at room temperature and assessed daily for fungal growth. When hyphal tips emerged from a leaf segment, the fungus was subcultured to new 2% PDA + ampicillin plates to obtain pure colonies. Once in pure culture, 1-mm diameter × 2-mm deep fungal fragments were placed in 2 mL of RNase/DNase-free water at room temperature for long-term storage (Burdsall and Dorworth, 1994) and at −80°C for long-term DNA storage. Additional fungal fragments of this size were used for immediate DNA extraction.

***DNA-based identification of fungal isolates*—**Each fungal isolate was assigned to an initial morphotype based on eight morphological characteristics: spore production, colony color, medium color, surface texture, margin texture, margin color, underside color, and growth rate (Arnold et al., 2000). To identify morphotypes, we sequenced DNA from 120 representative cultures, including three of each morphotype. DNA was extracted from the fungal tissue using a standard phenol–chloroform–isoamyl procedure modified with bead beating (Griffiths et al., 2000). The D1/D2 region of the ribosomal large subunit (LSU) was amplified using the general fungal primers NL1 (5′GCATATCAATAAGCGGAGGAAAAG3′) and NL4 (5′GGTCCGTGTTTCAAGACGG3′) (O'Donnell, 1993). Each 25-μL PCR reaction contained approximately 10 ng of fungal DNA, 0.75 U *Taq* polymerase, 1× PCR buffer, 2 mmol/L $MgCl_2$, 200 μmol/L dNTPs, and 0.5 μmol/L each of primers. Thermal cycling reactions used the following conditions: 1 cycle of 95°C for 2 min; 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 2 min; 1 cycle of 72°C for 5 min. Amplified products were sequenced on an ABI 3730XL DNA Analyzer (Applied Biosystems, Carlsbad, California, USA) at the DNA Sequencing Facility at the University of Texas at Austin. Sequences have been deposited into NCBI GenBank under accession numbers KC582560–KC582600. Operational taxonomic

units (OTUs) were defined with UCLUST (Edgar, 2010). Sequences associated with each OTU were screened in GenBank using BLAST (Altschul et al., 1990), and the resulting best matches (similarity >98%, E value = 0.0, Max ident > 98%) are listed in Appendix 1.

***Experimental tests of plant–endophyte interactions*—**To assess how endophyte region of origin and current soil moisture affected plant performance, we grew *P. virgatum* with endophytes isolated from eastern or western sites, or with no fungi, under high (15%) or low (3%) soil moisture conditions in a full factorial design. Ten fungal isolates were tested from each region (Appendix 1). Of these 20, we included two isolates (one from each region) that ultimately clustered to the same OTU and thus are likely independent genotypes rather than taxa (Appendix 1). Each treatment combination was replicated five times, and an additional 30 controls were included with no fungi for a total of 230 plants. To estimate evaporative water losses, we included 10 replicates without plants with five for each moisture treatment. Details of the set up and treatments are further described below.

Panicum virgatum seeds were a local upland variety purchased from Native American Seed (Junction, TX). Seeds were surface sterilized in 0.5% sodium hypochlorite and 70% ethanol, rinsed in sterile water, and germinated on dampened, sterile filter paper. Any germinating seeds with evidence of fungal growth were discarded. Seedlings showing no fungal outgrowth were transplanted into plant culture boxes (Magenta GA-7; Magenta Corp., Chicago, Illinois, USA) containing sterile sand. The planting boxes were constructed of clear plastic and consisted of a bottom pot filled with sand and a top cover attached to the bottom by a ring. The tops were modified with two, 3-cm diameter holes covered in 20-μm nylon mesh to allow for gas and water exchange while reducing the probability of fungal contamination. Because the planting medium was sterile sand, nutrients were provided as 5 mL of filtered 1/4 strength Hoagland's solution (Hoagland and Arnon, 1950). Plants were kept in an isolated greenhouse at ambient light and temperatures ranging from 27°–35°C (day vs. night). Plants were allowed to grow for 3–4 wk before fungal inoculation, when seedlings were at least 4 cm tall. Although patterns of endophyte colonization of *P. virgatum* seedlings in the field are not known, we wanted to ensure that seedlings were established before inoculation (as many seedlings do not survive in the first 2–3 wk in the incubator).

To inoculate the plants with target endophytes, we cultured the fungi in PD broth (PDB) and diluted the culture with water as needed to obtain 10^5 spores·mL^{-1}. Each plant received 1 mL of inoculum, which was pipetted onto the shoot (Rodriguez et al., 2008). Control plants were mock-inoculated with the same volume of sterile water. After inoculation, plants were grown for 1 wk to allow the fungi sufficient time to colonize the plant (Rodriguez et al., 2008) before the drought treatment was imposed. To ensure the inoculation was successful and that nontarget fungi did not contaminate the plants, leaf tissue was taken from a subset of replicate plants, surface sterilized, and recultured on PDA to compare with expected morphotypes.

In the weeks before the water treatment, all plants were grown in soils maintained at 15% gravimetric soil moisture, which was near saturation for the sand soils. When the low moisture treatment was imposed, soils were allowed to dry to 3% soil moisture (7–10 d). The low soil moisture treatment was expected to create extreme drought stress for *P. virgatum* (Barney et al., 2009), a condition that is common at the western end of the Edwards Plateau precipitation gradient. Moisture levels were checked every 3 d and adjusted to maintain the treatments. All moisture manipulations were based on the mass of the entire planting box.

***Measurements of plant response to fungal endophytes*—**To assess the effects of fungal endophytes on plant activity and growth, we measured whole-plant water loss, number of wilt-free days, plant height, and number of tillers at 3-d intervals (days 1, 4, 7, 10, 13, 16, 19). Whole-plant water loss was measured as the loss of water by mass from each planting box at each time, adjusted for average water loss from plant-free controls during the same time period (Meurs and Stanghellini, 1992). The average rate of water loss was calculated over time (g water·d^{-1}). Relative growth rate was calculated based on the change in plant height over time (cm·d^{-1}). On day 19, all plants were harvested; shoots were clipped, and roots were separated from sand with tweezers, rinsed with DI water, dried at 40°C for 48 h, and weighed.

***Statistics for characterization of endophyte communities across the field gradient*—**We estimated total species richness based on our sampling effort for each region using Chao2 in the program EstimateS v8.2 (Colwell, 2013). Site-level richness, calculated as the number of OTUs observed at a site, was analyzed with univariate ANOVA as a function of region and host. Beta diversity variation was estimated as the total number of taxa in the region divided by the average site-level richness (Whittaker, 1972).

To test for differences in endophyte community composition across the regions of the precipitation gradient and the two plant hosts, we used multiple response permutation procedures (MRPP) (Mielke and Berry, 2007). Because site was our level of replication, the number of isolates found in tillers and plants were collapsed into a single site-level measurement to avoid pseudoreplication. Differences among communities were visualized using nonmetric multidimensional scaling (NMS) with Bray–Curtis distances. The relative contributions of environmental and spatial factors to patterns along the gradient were assessed with partial redundancy analysis (partial RDA) run with environmental variables (average annual precipitation, current spring precipitation, average maximum temperature, average minimum temperature), spatial variables (latitude, longitude, elevation), and both (Borcard et al., 1992; Legendre et al., 2005). Specifically, we calculated total explained variation, environmental variation, spatial variation, environmental variation independent of space, and spatial variation independent of environment (Borcard et al., 1992; Cottenie, 2005; Legendre et al., 2005). Randomization tests were used to determine significance in the partial RDA. For significant factors, partial RDA was run separately for the individual variables to determine their contributions to the overall effects. If endophyte community structure was driven purely by dispersal from a regional pool, then only spatial factors should relate to endophyte distribution patterns; in contrast, species sorting by environment would be evidenced by effects of the environment independent of spatial factors (Cottenie, 2005). If both environmental and spatial factors are independently related to endophyte distributions, then a combination of species sorting and dispersal via mass effects are likely (Cottenie, 2005). Environmental data were obtained from the PRISM Climate Group (Oregon State University, http://prism.oregonstate.edu, created 25 October 2012). The MRPP, NMS, and RDA were run in the program PC-Ord v6.08 (McCune and Mefford, 2011); the regression was run in SPSS v19.0.0.1 (IBM SPSS, Armonk, New York, USA).

***Statistics for experimental tests of plant–endophyte function*—**Univariate ANOVAs were used to examine whole-plant water loss, wilt-free days, shoot biomass, root biomass, final tiller number, and relative height growth rate as a function of soil moisture treatment (low, high), endophyte region of origin (east, west, none), and their interaction, as well as endophyte identity. Soil moisture treatment and endophyte origin were fixed factors; endophyte identity was included as a random factor nested in region of origin. We set our significance level at 0.0083 using a Bonferroni correction for the univariate ANOVAs run on the six different plant measurements. When fixed factors were significant, posthoc Ryan–Einot–Gabriel–Welsch F tests were used to examine differences among endophyte origins. When the nested factor was significant, additional univariate ANOVAs were run to examine how the response variables were affected by endophyte identity and soil moisture treatment separately for each region of origin (west, east).

Plasticity for each OTU was calculated with the phenotypic plasticity index (PI_v), which ranges from 0 to 1 and is calculated as the difference between the maximum and minimum treatment means standardized by the maximum mean value (Valladares et al., 2000, 2006). Bivariate correlations were examined for plasticity values among traits. Mean plasticity was calculated as the average PI_v across the six response variables (water loss, wilt-free days, shoot biomass, root biomass, relative height growth rate, final tiller number). All ANOVA, correlation, and regression analyses were run in SPSS v19.0.0.1.

RESULTS

***Endophyte communities across the field gradient*—**A total of 179 fungal isolates were cultured from 351 leaf segments. These were grouped into 40 initial morphotyes representing 41 OTUs at 98.5% similarity (Appendix 1). Only one morphotype was split into two OTUs. Our sampling captured between 80–100% of the expected richness based on Chao2 estimates (west = 100%, central = 98.29%, and east = 80.20%). The total number of fungal taxa varied at the regional level, with 12, 18, and 26 in the west, central, and east regions, respectively. Beta diversity was 5.47 overall, suggesting that turnover was similar to average site-level richness (7.31 ± 1.00), which did not vary across regions ($F_{2,7} = 4.85$, $P = 0.686$). Richness also was unrelated to host plant ($F_{1,7} = 5.08$, $P = 0.059$) or the interaction of region and host plant ($F_{2,7} = 0.57$, $P = 0.592$).

Endophyte community composition varied along the gradient (MRPP test: $A = 0.155$, $T = -3.879$, $P = 0.002$), with communities in the drier west different from those in the wetter central and eastern regions (Fig. 1). Endophyte communities did not differ between *Panicum* host plants (MRPP test: $A = -0.017$, $T = 0.615$, $P = 0.692$). The central region included only one site with *P. virgatum*, so the interaction of region and host could not be tested.

Using partial RDA, a total of 65.6% of the total variance observed in endophyte community composition was explained. Environmental factors independent of the spatial arrangement of sites explained 34.8% ($P = 0.022$). When the environmental variables were considered individually, current spring rainfall explained 11.5% ($P = 0.005$) and mean annual precipitation explained 5.4% ($P = 0.046$) of the variation in endophytes; most of the variation ($r^2 = 21.9\%$) was in their interaction. The average maximum (24.8–27.3°C) and minimum (10.6–13.2°C) temperature did not contribute to the observed pattern. Total environmental variation not controlling for space ($r^2 = 11.8\%$) and purely spatial factors independent of environmental variation ($r^2 = 18.9\%$) were not significant sources of variation ($P > 0.05$). A total of 34.4% of the variance in endophytes was unexplained.

Experimental tests of plant–endophyte function— Whether an endophyte originated in the drier west or wetter east end of the gradient significantly affected whole-plant water loss ($F_{2,14} = 7.01$, $P = 0.008$; Fig. 2A). Wilting was unaffected by fungal origin ($F_{2,12} = 0.623$, $P = 0.553$; Fig. 2C). Water loss more than doubled under 15% soil moisture ($F_{1,199} = 321.12$, $P < 0.001$; Fig. 2B), but wilting did not occur for an additional 3 d on average ($F_{1,206} = 21.18$, $P < 0.001$; Fig. 2D). There were no interactions of fungal origin and soil moisture on whole-plant water loss ($F_{2,199} = 1.585$, $P = 0.208$) or wilting ($F_{2,206} = 0.492$, $P = 0.612$). No plant size or growth metrics were affected by either endophyte region of origin or soil moisture treatment.

The specific identity of the endophytes played a role in all measured plant responses except tiller production (Fig. 3). When plants were colonized by endophytes isolated from the drier western sites, wilting ($P = 0.001$) was controlled by the interaction of identity and moisture treatment, and water loss ($P = 0.012$), shoot biomass ($P = 0.015$), and root biomass ($P = 0.034$) had similar trends, such that the reaction norms were not parallel among taxa. In plants colonized by endophytes isolated from wetter eastern sites, a similar interaction was only observed for whole-plant water loss; measures of biomass were affected by fungal identity, but their relative effects were the same in wet and dry soil treatments. The reaction norms for shoot and root biomass of taxa from eastern and western regions are provided as an example in Fig. 3. Relative height growth rate was affected by fungal identity in both regions, independent of soil moisture treatment.

To further quantify the interaction of endophyte taxa and moisture treatment, we used the phenotypic plasticity index (PI_v). The plasticity of plant traits associated with endophyte taxa in 3% and 15% soil moisture treatments was highly variable, with the rank order of taxa changing for every trait (Fig. 4). On average, however, plasticity across all traits was ~10% higher in endophytes from western than eastern regions ($F_{2,18} = 1.147$, $P < 0.001$). Average plasticity with endophytes was also nearly double that of control plants without endophytes, but this varied substantially among endophytic taxa and traits (Fig. 4). Plasticity among the six traits was not correlated. For example, plants colonized by one OTU from the east (E10) had the highest plasticity for whole-plant water loss and the lowest for wilt-free days; similarly, one OTU from the west (W2) had the highest plasticity for root biomass and the lowest for relative height growth rate.

DISCUSSION

Species sorting of endophytic taxa between drier and wetter regions appears to dominate this system, which could make their distributions more predictable. However, endophyte origin across the gradient did not directly translate into predictable functioning in symbiosis with plants. All endophytes reduced water loss in plants, with a slight advantage for endophytes from drier sites. Otherwise, endophyte identity was more important than origin in explaining effects on plant traits. Habitat filtering for endophytes may be based on environmental factors that directly affect fungal survival and reproduction when outside the plant (e.g., soil moisture), yet do not necessarily translate into a direct role in stress responses of the host plant when in symbiosis. The drivers of endophyte function are likely to be more complex outside of extreme environments, and a predictive framework will require more extensive studies across population and community scales.

The primary role for environmental factors such as historical and current rainfall in endophyte distributions is consistent with endophytes sorting among heterogeneous habitats in this region

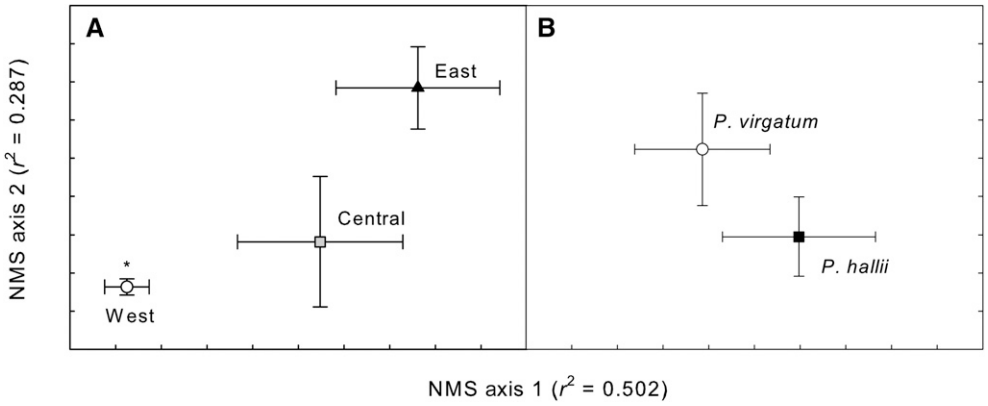

Fig. 1. Nonmetric multidimensional scaling of endophyte communities by (A) region and (B) host plant. Endophyte communities from the drier western region differed from those in the central and eastern regions. There were no differences in endophytes between host plants. Bars are ±1 SE.

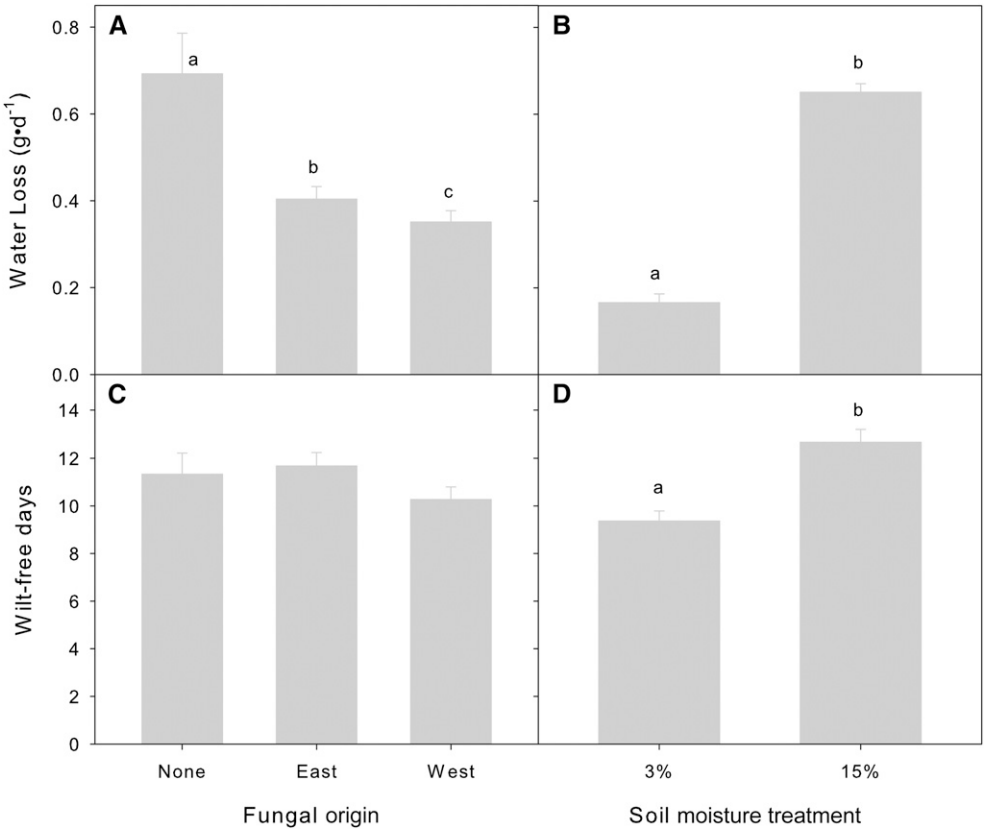

Fig. 2. Effects of (A, C) fungal origin and (B, D) soil moisture treatment on (A, B) plant water loss and (C, D) number of wilt-free days. Different letters indicate significant differences among groups. Bars are +1 SE.

(Cottenie, 2005). Current spring rain likely affects plant productivity (Knapp, 1984) as well as fungal dispersal (Kauserud et al., 2011). For endophytes, we suspect that the historical drought regime affects locally available species pools (Chase, 2007). It is also possible that past drought patterns create legacies that constrain current community and ecosystem properties (Evans and Wallenstein, 2012; Sala et al., 2012). Together, historical mean annual and current spring rainfall explained ~35% of the variation in endophyte community composition. Only 10% of the environmental variation was confounded with the spatial arrangement of sites, which was surprising given the linear nature of the historical rainfall gradient. There may be a threshold effect between the very dry west and the other sites, rather than a linear effect of decreasing rain. Consistent with our results, rainfall and elevation together explained 53% of the variation in leaf endophyte distributions associated with the tree, *Metrosideros polymorpha*, in Hawaii (Zimmerman and Vitousek, 2012).

The occurrence of habitat-associated adaptation would allow simple prediction of endophyte function in symbiosis under similar conditions. For example, in very stressful thermal or saline environments, endophytes have been isolated that confer heat or salt resistance on a variety of plant hosts, including taxa as divergent as grasses and tomato (Rodriguez et al., 2008). In another group of fungal symbionts, the arbuscular mycorrhizas, Antunes et al. (2011) found that isolates of the same taxon from different climatic regions affected plants differently, but the regions were geographically very distant (e.g., Toronto, Ontario, Canada; and Gainesville, Florida, USA). Over smaller scales where the stress is less extreme, selection for functional divergence may not be as strong. Indeed, we found that plant traits in symbiosis with endophytes were largely unpredictable from endophyte origin, with the exception of water loss. Lack of predictability could result from a weak habitat filter or a disconnect between the filter and function in symbiosis. Plant performance in this symbiosis is not necessarily linked to fungal fitness. Some fungal endophytes are thought to be commensals while the plant is alive and decomposers once tissues senesce (Müller et al., 2001; Promputtha et al., 2010). Gaining priority in decomposition by inhabiting a plant host no doubt has different selection pressures compared to cases where fungi are mutualists dependent on sugars from plant photosynthate (Vargas et al., 2009; Rasmussen et al., 2012). Other endophytes appear to be parasitic, reducing plant biomass with no clear benefit to the plant (Cheplick, 2004).

Plasticity can allow individuals to tolerate and grow in new environmental conditions (Bradshaw, 1965). In symbiosis, the majority of endophytic taxa in this study increased the plasticity of plant traits compared to plants without symbionts, and this was slightly greater in taxa from the drier western region of the gradient. That some endophytes can facilitate more flexible plant responses to the environment may provide a substantial advantage given future climate change. Plasticity may be adaptive if the new phenotype is in the direction favored by selection in the new environment (Ghalambor et al., 2007). When moved from lower to higher soil moisture, for example, we might expect larger, more active plants; this was true in 2/3 of plants associated with endophytic fungi isolated from drier western sites. The converse was true for 80% of plants associated with endophytes from wetter eastern environments, which were smaller and less active under low soil moisture

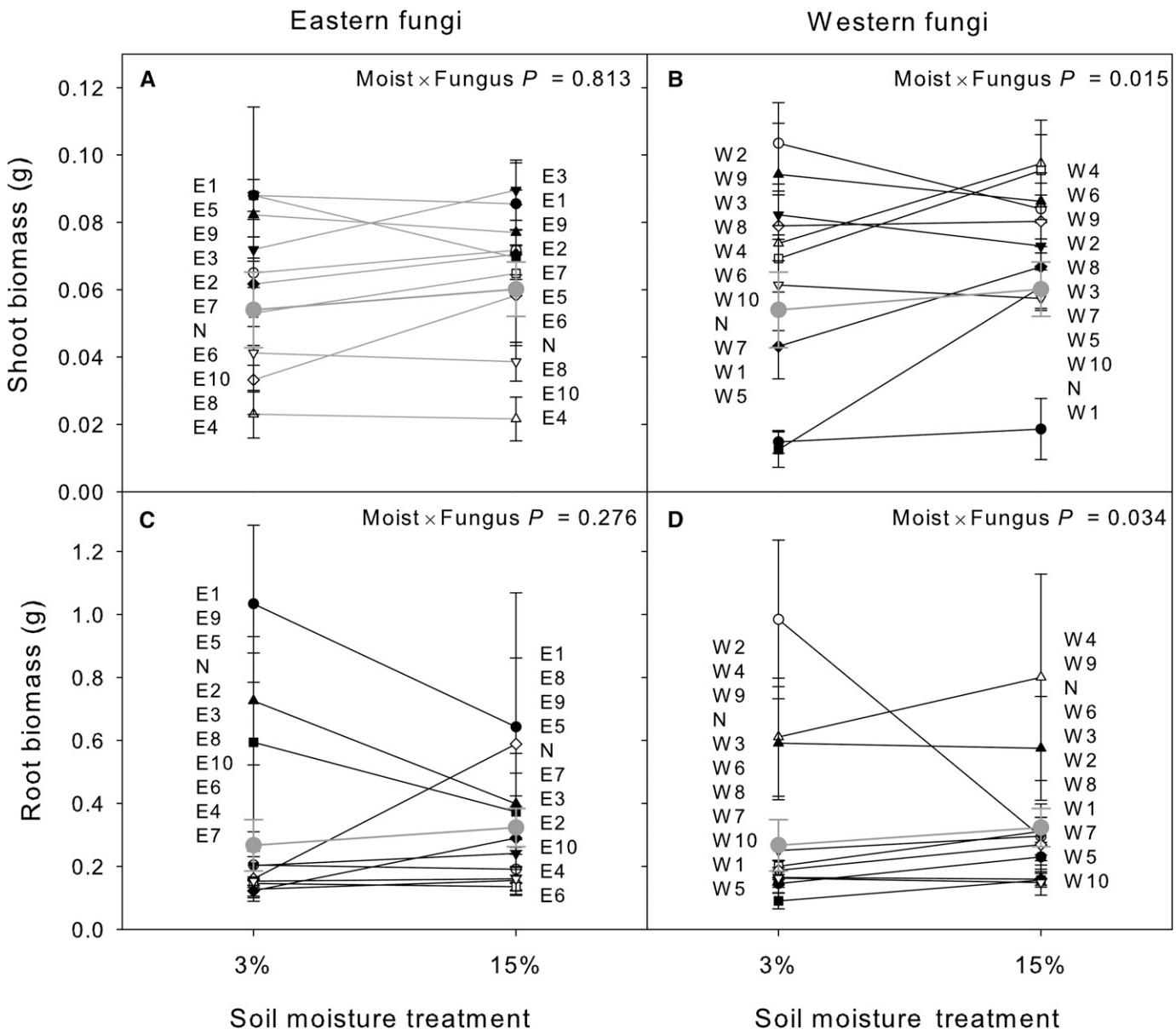

Fig. 3. Differences in (A, B) shoot and (C, D) root biomass produced by plants grown with endophytic taxa from (A, C) eastern and (B, D) western regions of the gradient at 3% and 15% soil moisture. Significance levels for the interaction of moisture treatment ("Moist") and fungal identity ("Fungus") are listed in each panel. Individual taxa are designated by different symbols, and for clarity, their OTU designations are listed in rank order for each treatment; control plants without fungal endophytes are indicated by gray circles and "N." Bars are ±1 SE.

conditions. Adaptive plasticity should be more likely in variable environments with reliable cues, selection for different phenotypes in different environments, and no single superior phenotype (Ghalambor et al., 2007), which is consistent with the precipitation gradient that we sampled. In some cases, however, endophytes substantially reduced plant plasticity relative to control plants without fungi, suggesting that some endophytes may actually limit a plant's ability to respond to changes in the environment.

Plant responses to changes in soil moisture may also be constrained by local adaptation of the fungal symbiont. Some fungal taxa may be adapted to local conditions, such that their effects on plant traits are optimized for the environment from which they were isolated (Conover et al., 2009). In a literature survey of reciprocal transplant experiments of many different organisms, the frequency of local adaptation was 0.71, and local populations had 45% greater fitness on average than nonlocal populations (Hereford, 2009). When endophytes were lumped by origin in the present study, only water loss had the potential for local adaptation. However, significant interactions of endophyte identity and moisture treatment of fungi from the drier western region also support local adaptation of some fungal taxa. The best-performing plants under low moisture conditions were colonized by taxa originating from the drier western region for four out of six traits and under high moisture conditions were colonized by taxa from the wetter eastern region for three out of six traits. We can also evaluate local adaptation by comparing performance of endophytes in home vs. away conditions with our common garden reciprocal moisture design.

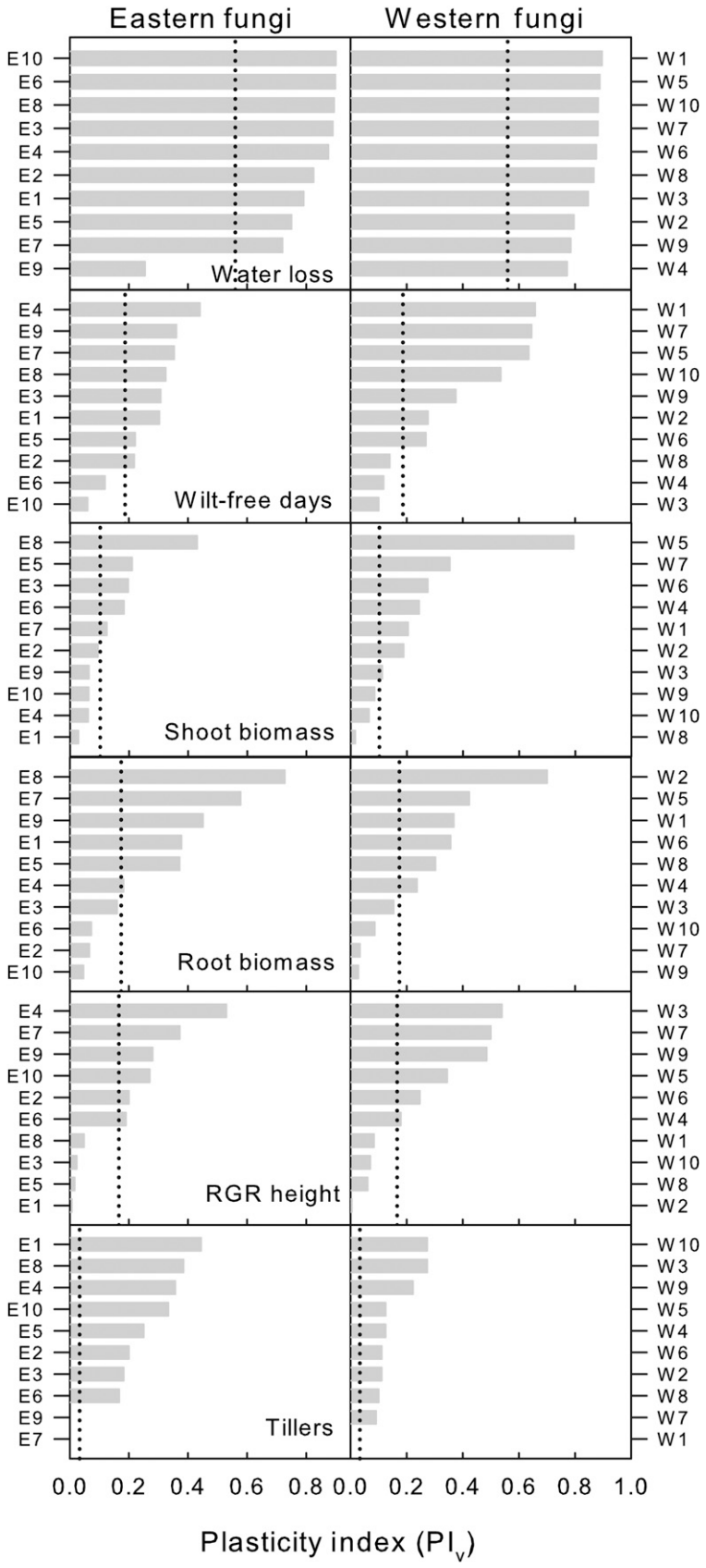

Fig. 4. Plasticity (PI_v) for plant traits conferred by endophytes isolated from eastern or western regions of the rain gradient. Individual OTUs are indicated on the *y*-axes, ordered from low to high plasticity for each trait. Dotted lines indicate PI_v for control plants lacking symbionts.

Although home vs. away comparisons can confound differences in selection and habitat quality in the field (Kawecki and Ebert, 2004), here the only environmental factor that varied was soil moisture. Averaged across all traits, plants in symbiosis with endophytes from the drier west had an advantage in home conditions in 53% of cases, and those in symbiosis with endophytes from the wetter east in 63% of cases. Local adaptation may be exploited in endophytes isolated for use in management, but may create a lag in response to climate change in situ.

The results of this study have several limitations. Only two grass species in one genus were sampled, and these may not be representative of endophyte distributions in other species in the same sites (Wearn et al., 2012) despite our preliminary study of four tallgrass species with no apparent host effects (see methods). We also only sampled once, targeting the time of maximum host plant growth. Endophytes can vary temporally throughout the growing season (Ghimire et al., 2011), across seasons (Suryanarayanan et al., 2002; Sánchez Márquez et al., 2012), and between years (Kleczewski et al., 2012), such that one snapshot may not be representative. Our experimental tests of symbiont effects on plant traits were short-term and limited to the seedling stage; while this is often a critical time for plant survival, the results are unlikely to represent interactions with adult plants. We also only tested one endophytic fungus at a time, whereas in nature fungal communities in a single leaf can be highly diverse (Lodge et al., 1996) and may interact with other symbionts throughout the plant (Jumpponen and Trappe, 1998; Mack and Rudgers, 2008). We did not control for host plant genotype, although it could affect the outcome of interactions with endophytes (Cheplick, 2004). Finally, we did not take a phylogenetic approach to understanding endophyte effects on drought resistance given our limited number of taxa, although a phylogenetic signal associated with environmental response could facilitate prediction (Allison and Martiny, 2008).

We found strong evidence that fungal endophytes sort by environment across a local precipitation gradient, with extensive variation in the effects of individual taxa on plant traits in symbiosis that were generally not related to origin. The high degree of variation in plant plasticity when associated with endophyte taxa, as well as interactions of endophyte taxon by environment, warrant a greater focus in the future on endophyte population ecology and evolution. In addition, a more mechanistic approach to plant–endophyte interactions will facilitate a greater understanding of why some endophytes are beneficial for plant drought resistance while others are not. Although the ability to predict their effects on plant traits such as drought resistance will require more extensive work, the potential role of endophytes in mediating how plants respond to climate change is clear.

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APPENDIX 1. Endophytic fungi isolated from the field gradient (OTU no.) and used in experimental tests (Experiment ID).

OTU no.	Experiment ID	Region[a]	Grass[b]	Percentage of samples	Accession no.	Best BLAST match	ID of best BLAST match
1	E1	E, C	Ph, Pv	5.76	KC582563	JF773659.1	*Alternaria* sp. *CHTAM37*
2	E2	E	Pv	0.72	KC582564	JX535499.1	*Aspergillus niger*
3	E3	E	Ph	1.44	KC582568	AY342076.1	*Cladosporium cladosporoides*
4	E4	E	Pv	0.72	KC582575	HE599562.1	*Fusarium oxysporum*
5	E5	E	Pv	1.44	KC582584	JN938953.1	*Penicillium crustosum*
6	E6, W10[c]	E, C, W	Ph, Pv	7.91	KC582590	JN642222.1	*Penicillium solitum*
7	E7	E	Pv	0.72	KC582587	JN940834.1	*Pestalotiopsis foedans*
8	E8	E	Pv	1.44	KC582588	JQ318010.1	*Phoma foliacephila*
9	E9	E	Ph, Pv	2.88	KC582591	GU238102.1	*Phoma medicaginus*
10	E10	E	Pv	0.72	KC582582	FN868847.1	*Preussia* sp. *BLE35*
11	W1	W	Ph, Pv	2.16	KC582562	AB474751.1	*Acremonium cellulolyticus*
12	W2	W	Ph, Pv	4.32	KC582560	AB667802.1	*Alternaria alternata*
13	W3	W	Ph	0.72	KC582561	AB517941.1	*Alternaria tenuissima*
14	W4	W	Ph	1.44	KC582565	JX174046.1	*Aspergillus terreus*
15	W5	W	Ph, Pv	2.16	KC582567	AJ312101.1	*Chaetomium bostrychodes*
16	W6	W	Ph	1.44	KC582570	JN941506.1	*Cochliobolus kusanoi*
17	W7	W	Ph, Pv	4.32	KC582580	GU048592.1	*Nigrosopra* sp. *CHTAR07*
18	W8	W	Ph, Pv	8.63	KC582586	JN938952.1	*Penicillium expansum*
19	W9	W	Ph, Pv	5.76	KC582595	HM469418.1	*Penicillium pinophilum*
20		C	Pv	0.72	KC582578	JN712560.1	*Leptosphaerulina australis*
21		C	Pv	0.72	KC582597	JF772180.1	*Penicillium italicum*
22		C	Ph, Pv	2.88	KC582589	HQ876766.1	*Penicillium funiculosum*
23		C	Ph, Pv	5.76	KC582596	JF710188.1	*Penicillium* sp. *PSR2*
24		E	Pv	0.72	KC582566	AY176750.1	*Byssochlamys nivea*
25		E	Pv	0.72	KC582571	JX256399.1	*Cochliobolus lunatus*
26		E	Pv	0.72	KC582572	JF499855.1	*Cladosporium perangustum*
27		E	Pv	0.72	KC582583	AY283542.1	*Myrothecium roridum*
28		E, C	Ph, Pv	2.88	KC582576	GQ168842.1	*Gibberella moniliformis*
29		E, C	Pv	1.44	KC582574	GU237979.1	*Epicoccum sorghi*
30		E, C	Pv	1.44	KC582577	AB669037.1	*Khuskia* sp. *11BG11*
31		E, C	Pv	1.44	KC582583	AY999102.1	*Podospora cupiformis*
32		E, C	Pv	2.16	KC582592	FN868847.1	*Preussia minima*
33		E, C	Pv	2.16	KC582598	AY999096.1	*Schizothecium curvisporum*
34		E, C	Ph, Pv	4.32	KC582581	GQ328855.1	*Nigrospora oryzae*
35		E, C	Pv	2.16	KC582585	AY999099.1	*Podospora curvicola*
36		E, C	Ph, Pv	5.04	KC582593	AY213618.1	*Penicillium minioluteum*
37		E, C	Pv	1.44	KC582594	AF510496.1	*Penicillium verruculosum*
38		E, C	Pv	2.16	KC582599	FR774290.1	*Sordaria macrospora*
39		E, C	Pv	2.16	KC582600	HQ130664.1	*Sporormiella* sp. *WF105*
40		W	Pv	0.72	KC582569	JN941528.1	*Curvularia geniculata*
41		W	Ph, Pv	2.88	KC582573	JN859487.1	*Curvularia* sp. *REF154*

[a] Regions are east (E), central (C), and west (W).

[b] Grasses are *Panicum hallii* (Ph) and *P. virgatum* (Pv).

[c] Two separate isolates of this OTU from east and west were used.