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Historical and current climate drive spatial and temporal patterns in fungal endophyte diversity

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ABSTRACT

Horizontally-transmitted foliar endophytic fungi can moderate plant tolerance to abiotic and biotic stress. Previous studies have found correlations between climate and endophyte beta diversity, but were unable to clearly separate drivers related to long-term climate, annual weather, and host plants. To address this, we characterized endophyte communities in the perennial C_4 grass, *Panicum hallii*, across a precipitation gradient in central Texas over 3 years. A total of 65 unique leaf endophytes were isolated and identified based on ITS and LSU regions of rDNA. Mean annual rainfall and temperature were the primary drivers of endophyte richness and community composition, followed by annual weather conditions. In contrast, little explanatory value was provided by plant host traits, vegetation structure, or spatial factors. The importance of historical climate and annual weather in endophyte distributions suggests that species sort by environment and are likely to be affected by future climate change.

1. Introduction

Horizontally-transmitted, Class 3, foliar fungal endophytes (hereafter, 'endophytes') are present in all terrestrial plants examined to date (Carroll, 1988; Rodriguez et al., 2009). Although these heterotrophic fungi use photosynthate carbon from the plant (Siegel et al., 1987; White and Torres, 2010), many endophytes appear to be commensals or mutualists (Carroll, 1988; Eaton et al., 2011). For example, some endophytic fungi ameliorate plant physiological and growth responses to abiotic or biotic stressors, including salt (Rodriguez et al., 2008), heat (Redman et al., 2002), drought (Giauque and Hawkes, 2013), herbivory (Arnold and Lewis, 2005), and pathogens (Arnold et al., 2003). Despite their ubiquity and ecological significance, our understanding of endophyte ecology and evolution remains limited. Improved knowledge of endophyte distributional patterns will allow us to identify potential underlying drivers and will ultimately provide the basis for scaling their ecological impacts.

Unlike free-living organisms, symbionts have hosts as an additional layer of constraint in their spatial distributions. Fungal endophytes rely on their host plant for protection and resources (Rodriguez et al., 2009), and some endophytes are unique to divergent host clades (Higgins et al., 2007), suggesting that host distribution and abundance has some control over endophyte distribution. However, because there is little evidence for endophyte host specificity, particularly among closely related plant species (Del Olmo-Ruiz and Arnold, 2014; Higgins et al., 2014, 2011), important host plant characteristics might relate more to potential incidence (abundance or density) or potential carbon provision (plant size) than to host identity *per se*. Nevertheless, as with free-living organisms (Legendre et al., 2005), the spatial distributions of symbiotic endophytes are also governed by non-host factors. Endophyte communities vary across large latitudinal gradients (Arnold and Lutzoni, 2007) and across habitats (Loro et al., 2012) likely resulting from changes in abiotic and biotic components of the sites. For example, endophyte communities in single host plants were primarily controlled by environmental gradients in both central Texas savanna and the Mauna Loa volcano in Hawaii (Giauque and Hawkes, 2013; Zimmerman and Vitousek, 2012).

Less is known about temporal variation in endophytes, but extrapolating from the drivers of spatial variation suggests that endophytes should track host and climatic factors over time. Within a single year, endophytes can vary seasonally due to increasing plant productivity and other phenological traits

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(Ghimire et al., 2011; Ek-Ramos et al., 2013). For example, endophyte diversity in *Panicum virgatum* peaked with maximum plant biomass and decreased rapidly with plant senescence (Ghimire et al., 2011). Given observed differences in endophytes between wetter and drier sites (Loro et al., 2012; Zimmerman and Vitousek, 2012; Giauque and Hawkes, 2013), it is likely that weather variation and associated environmental stress across years will also play a role either through direct effects on the fungi or a response of fungi to the host plant condition (Garrett et al., 2006). In contrast, in regions such as wet tropical forests where climate varies little between years, interannual differences in endophytes are more likely to be caused by differences in host plant age or life stage (Del Olmo-Ruiz and Arnold, 2014; Higgins et al., 2014).

Current weather must be distinguished from the effects of historical climate, because fungal communities should reflect long-term trends more than short-term annual conditions unless dispersal and species sorting occur on the same time scale as weather variation. Although endophytes have not been considered in this context previously, past drought patterns create legacies that constrain the composition of current soil microbial communities (Evans and Wallenstein, 2012); however, this is not always the case (Rousk et al., 2013). We expect historical climate effects to predominate when communities assembled by environmental filtering over time are difficult to overcome by either resuscitation of endophyte taxa from local dormant pools (Jones and Lennon, 2010) or immigration from regional species pools across years (Kinkel et al., 1989). However, determining the relative importance of these potential drivers of endophyte communities requires studies to capture a range of local abiotic and biotic factors in the context of climate variability over time.

In a previous study, we determined that endophyte community composition and diversity across sites differed largely as a function of the interaction between historical and current precipitation (Giauque and Hawkes, 2013). We could not parse the effect of rainfall any further, having examined only a single time point, and we did not consider other host plant or vegetation characteristics that might be important for symbionts. Here, we investigated temporal variation in endophytes by measuring both spatial and annual variation in potential biotic and abiotic drivers. Specifically, we examined endophyte richness and community composition over 3 years and assessed how these patterns reflected historical climate, annual weather, host plant traits, vegetation structure, and spatial distributions. We hypothesized that interannual variability in rainfall and temperature would modify endophyte communities both directly and through changes in host plant traits or vegetation structure. To address these questions, we characterized annual variation in leaf endophytes of the grass *Panicum hallii* Vasey across a steep rainfall gradient over 3 years. The rainfall gradient allowed us to examine sites with different historical climate conditions, host plant traits, and vegetation structure. By studying endophyte communities at these sites across the 3 years, we could further separate annual weather variation from historical climate conditions and other factors.

2. Materials and methods

2.1. Study sites and field sampling

We sampled fungal endophytes from ten sites located across a steep precipitation gradient in Central Texas. Sampling was restricted to the Edwards Plateau to maintain consistent vegetation and soil types: savanna grasslands on shallow, rocky, calcareous Mollisols. The Plateau spans ~400 km from west to east, with mean annual precipitation (MAP) varying from ~400 to 900 mm, mean annual high temperatures (MHT) from ~24 to 28 °C and mean annual low temperatures (MLT) from ~11 to 13 °C. Sites were selected based on MAP, availability of host plants, and at least 50% grass cover.

We isolated endophytes from *P. hallii*, due to its abundance across the Edwards Plateau. *P. hallii* is a perennial, warm-season grass native to North America. From 2012 to 2014, we sampled plants annually in June in an area of 20 m × 20 m at each site. The month of June was chosen because that is when *P. hallii* typically reaches peak biomass and begins to flower. The same plots were revisited each year, but not the same individual plants. At each site at each sampling date, three tillers were collected from each of four individual *P. hallii* plants. Plants were rinsed in the field with sterile water and stored in plastic bags. Soils were also sampled for gravimetric moisture at each date. Plant leaves were kept on ice for transport back to the lab and refrigerated at 4 °C for no more than 48 h prior to leaf culturing. Additionally, plant characteristics were recorded for each plant, including tiller number, basal area, height, flowering height, and density.

2.2. Fungal culturing

Tillers were sectioned into three 2 mm fragments, which were surface sterilized using 0.5% sodium hypochlorite (2 min), 70% ethanol (2 min), and sterile water (30 s), following Arnold et al. (2000). Sterilized leaf fragments were placed on Petri dishes containing 2% potato dextrose agar (PDA) and 50 ppm ampicillin. The use of PDA is common for fungal-growth media because it minimizes the risk of nutrient limitation and yields large numbers of fungal isolates (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 hyphae emerged from a leaf fragment, the fungus was transferred to a new PDA plate to obtain pure cultures. Once in pure culture, 1 cm × 2 cm fungal fragments were subsampled for (1) long-term storage of three subsamples in 2 ml of RNase/DNase-free water at room temperature (Burdsall and Dorworth, 1994), and (2) immediate DNA extraction of two subsamples.

2.3. Fungal identification

Isolates were initially assigned to morphotypes based on morphological characteristics, as previously described (Arnold et al., 2000; Giauque and Hawkes, 2013). Morphotype identity was confirmed by DNA sequencing, including 195 isolates with at least three representatives of each morphotype. DNA was extracted from fungal tissue using a standard phenol-chloroform-isoamyl procedure modified with bead beating (Griffiths et al., 2000).

For DNA-based Identification, we used both the ITS and LSU regions of rDNA, because these capture both variable and conserved regions to allow for robust alignments (Liu et al., 2012; Porter and Golding, 2012). The ITS region (~500–800 bp) was amplified using the primers ITS1F (5′ CTTGGTCATTTAGAGGAAGTAA 3′) and ITS4 (5′ TCCTCCGCTTATTGATATGC 3′) (Gardes and Bruns, 1993; White et al., 1990). The D1/D2 region of the LSU (~650 bp) was amplified using the general fungal primers NL1 (5′ GCATATCAATAAGCGGAGGAAAAG 3′) AND NL4 (5′ GGTCCGTGTTTCAAGACGG3 3′) (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^{-1} $MgCl_2$, 200 μmol l^{-1} dNTPs, and 0.5 μmol l^{-1} 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 ABI3730XL DNA Analyzer (Applied Biosystems, Carlsbad, California, USA) at the DNA Sequencing Facility at the University of Texas at Austin. Sequences