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Climate change and *Epichloë coenophiala* association modify belowground fungal symbioses of tall fescue host

Lindsey C. Slaughter [a], [b], [*], Jim A. Nelson [a], Elizabeth Carlisle [a], Marie Bourguignon [c], Randy D. Dinkins [d], Timothy D. Phillips [a], Rebecca L. McCulley [a]

[a] *Department of Plant and Soil Sciences, University of Kentucky, Lexington, KY, USA*
[b] *Department of Plant and Soil Science, Texas Tech University, Lubbock, TX, USA*
[c] *Department of Agronomy, Iowa State University, Ames, IA, USA*
[d] *USDA–ARS, Forage-Animal Production Research Unit, Lexington, KY, USA*

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ABSTRACT

Human alteration of symbiont genetics among aboveground endophytic *Epichloë coenophiala* strains within tall fescue (*Schedonorus arundinaceus*) has led to widespread deployment of novel grass-endophyte combinations, yet little is known about their ecological consequences. In this study, clone pairs (endophyte-infected, endophyte-free) of two tall fescue genotypes received factorial combinations of increased temperature (+3 °C) and precipitation (+30% long-term annual mean) for 2 yr. We measured root arbuscular mycorrhizal fungi (AMF), dark septate endophyte (DSE) colonization, and soil AMF extraradical hyphae (ERH) length. We hypothesized that genetically distinct grass-*E. coenophiala* associations would differentially affect belowground fungi, and that these relationships would be climate-sensitive. Tall fescue genotype, endophyte presence, and climate treatment interactions affected AMF arbuscules, vesicles, and ERH. DSE decreased with *E. coenophiala* presence but increased with warming. Genetically distinct tall fescue-*E. coenophiala* associations may have divergent long-term impacts on other host-symbiont interactions, potentially impacting ecosystem function and response to climate change.

1. Introduction

Plants host ubiquitous above and belowground symbioses with microorganisms (Partida-Martinez and Heil, 2011), whose interactions range along a continuum from parasitic to mutualistic (Carroll, 1988; Bronstein, 1994; Johnson et al., 1997). These interactions can affect ecosystem properties and processes, such as nutrient cycling and productivity. One important type of plant-microbe symbiosis occurs worldwide between cool-season grasses (family Poaceae, subfamily Poöideae) and aboveground fungal endophytes of the genus *Epichloë* (family Clavicipitaceae) (White, 1987; Clay and Schardl, 2002; Schardl et al., 2004). For example, the perennial grass tall fescue (*Schedonorus arundinaceus* = *Lolium arundinaceum* = *Festuca arundinacea*) commonly forms a host-specific constitutive symbiosis with the asexual fungal endophyte *Epichloë coenophiala* [= *Neotyphodium coenophialum* = *Acremonium coenophialum*] (Shelby and Dalrymple, 1987). Residing intercellularly in aboveground plant tissues, *E. coenophiala* commonly defends tall fescue by producing alkaloid compounds that deter mammalian and insect herbivory in exchange for nutrients, shelter, and vertical reproduction and dispersal within plant seeds (Clay, 1988). Presence of *E. coenophiala* has also been associated with increased drought tolerance (Arachevaleta et al., 1989; Bouton et al., 1993; Elmi and West, 1995) and competitive ability (Hill et al., 1991). Ergot alkaloids produced by common toxic strains of *E. coenophiala* (CTE) can harm grazing livestock (Strickland et al., 2011), prompting researchers to identify non-livestock-toxic or ‘novel’ toxic endophyte (NTE) strains of *E. coenophiala* from wild populations and introduce them into improved tall fescue cultivars (Bouton et al., 2002; Hopkins et al., 2011). Both common and novel tall fescue-*E. coenophiala* combinations are heavily utilized worldwide, especially in the United States, New Zealand, and Australia (Young et al., 2013). The pervasiveness of tall fescue and its *Epichloë* symbiont, and the

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[*] Corresponding author. Department of Plant and Soil Science, Campus Box 42122, Texas Tech University, Lubbock, TX 79409, USA.
E-mail address: lindsey.slaughter@ttu.edu (L.C. Slaughter).

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increasing genetic diversity of plant-endophyte combinations (both CTE and NTE) utilized, make understanding these novel symbiotic association effects on ecological processes important (e.g., Rudgers and Clay, 2007; Rudgers et al., 2010; Gundel et al., 2013).

In addition to the widespread occurrence and importance of grass-*Epichloë* symbioses, the roots of most terrestrial plants, including grasses, are frequently colonized by arbuscular mycorrhizal fungi (AMF) (Schüβler et al., 2001; Smith and Read, 2008). AMF can provide increased water and nutrient uptake in host plants (Augé, 2001), help increase soil aggregate size and stability and ecosystem carbon (C) sequestration (Miller and Jastrow, 1990; Wilson et al., 2009; Duchicela et al., 2013), and may even increase pest, pathogen, and allelopathic chemical resistance in host plants (Sikes et al., 2009; Barto et al., 2010; Abhiniti et al., 2013; Tao et al., 2016). Yet, this symbiosis may not always be mutualistic, and is governed by a hierarchy of biotic and abiotic factors operating at multiple ecosystem scales, such as when the plant resource cost outweighs the benefits of mycorrhizal symbiosis under abundant nutrient or reduced light conditions (Johnson et al., 1997).

Additional belowground fungal symbionts are found alongside AMF, such as dark septate endophytes (DSE), which are distinguished from AMF by melanized hyphae and microsclerotia (Melin, 1922) yet may exhibit similar function (Mandyam and Jumpponen, 2005; Wagg et al., 2008; Newsham, 2011; Mayerhofer et al., 2013), such as contributing to N uptake and use efficiency (Alberton et al., 2009; Newsham, 2011). DSE are increasingly reported to colonize plant roots in a variety of ecosystems, and much remains to be determined about their functional roles within plants (Mandyam and Jumpponen, 2014). DSE most likely express a continuum of interactions from parasitic to mutualistic, similar to that of AMF, and host and fungal genotype and environmental conditions can be important in determining host response to DSE (Mayerhofer et al., 2013; Mandyam and Jumpponen, 2014). How these belowground fungi interact with each other, are altered by factors such as host plant genetics and environmental conditions, and impact on ecosystem productivity and processes remains unclear.

Aboveground CTE-symbiosis inhibits AMF colonization rates in tall fescue roots and AMF structures or abundance in soil (Chu-Chou et al., 1992; Guo et al., 1992; Mack and Rudgers, 2008; Buyer et al., 2011), yet the opposite effect has been observed in other grass-*Epichloë* species combinations (Novas et al., 2005, 2009, 2011; Vignale et al., 2016). Presence of CTE + tall fescue litter can inhibit AMF colonization in other plants such as smooth brome grass, yet this effect is not observed when using endophyte-free (E−) or NTE + litter (Antunes et al., 2008). Little is known regarding aboveground *E. coenophiala* effects on DSE, or whether NTE strains differentially affect belowground symbionts. While ergot alkaloids produced by CTE strains may be responsible for some of the observed effects, other factors may influence these interactions, such as availability of host resources or plant C (Liu et al., 2011), differences in host or symbiont genetics, non-ergot alkaloids or other metabolites (Rasmussen et al., 2007, 2008), potential endophyte-related effects on plant genetic expression and signaling (Dinkins et al., 2017), or belowground root exudates (Guo et al., 2015).

Plant-microbe symbioses may be especially important in governing ecosystem responses to climate change (Compant et al., 2010; Kivlin et al., 2013). Yet, how plant-microbe interactions within grassland ecosystems may respond to future climatic alteration is still poorly understood. For example, studies investigating the endophyte-associated alterations in tall fescue drought tolerance or temperature stress response are highly variable, with studies observing positive (Arachevaleta et al., 1989; Elmi and West, 1995), negative (Hill et al., 1996; Assuero et al., 2000), or neutral (MacLean et al., 1993; Elbersen and West, 1996) outcomes, potentially due to genotypic differences in tall fescue × endophyte associations (e.g., Elbersen and West, 1996; Assuero et al., 2000; Malinowski et al., 2000; Yurkonis et al., 2014). A recent study by Bourguignon et al. (2015) found that tall fescue's response to climate change varied depending on host and endophyte genetics, and the symbiotic response was more sensitive to warming than increased precipitation. If host-endophyte responses to climate change vary with genetics, so too may their impact on other symbioses such as AMF or DSE colonization.

Few studies to date have investigated multiple symbiont and host response to climate change, yet research suggests that warming stimulates grass root colonization of both AMF (Rillig et al., 2002; Bunn et al., 2009; Büscher et al., 2012; Kim et al., 2014) and DSE (Olsrud et al., 2010). Conversely, other studies have found little effect of soil warming on root AMF colonization (Heinemeyer and Fitter, 2004; Heinemeyer et al., 2004) or community composition (Yang et al., 2013), but measured significantly increased extraradical AMF mycelium (Heinemeyer and Fitter, 2004) and altered soil AMF communities (Yang et al., 2013). AMF species and allocation to structures may respond differently both to altered temperatures and host characteristics or resources.

Reports of altered moisture effects on AMF and DSE colonization are mixed. Owens et al. (2012) found no effects of altered precipitation on AMF colonization in two warm season grasses. However, summer drought can increase root AMF colonization while decreasing extraradical hyphae (Staddon et al., 2003), and preplanting soil moisture restriction can increase AMF colonization and mycorrhizal responsiveness in tomato (Cavagnaro, 2016). In addition, DSE abundance has been shown to increase in response to water addition (Herrera et al., 2011), but Vandegrift et al. (2015) observed a negative relationship between root DSE colonization and soil water availability. The effects of climate alteration on belowground fungal colonization are complex, and few researchers have further investigated how multiple simultaneous plant-microbe symbioses or plant and fungal genetics interactively govern these responses. Ascertaining how multiple symbionts, such as aboveground *Epichloë* endophytes and belowground AMF and DSE, interact in coordination with global change factors will be key to understanding and predicting ecosystem response to future climatic conditions (Kivlin et al., 2013).

To address this knowledge gap, we used an established long-term manipulative field climate change study to: (1) examine host genotype and endophyte symbiosis controls on root colonization by belowground fungi and associated plant nutrient concentrations and soil properties; and (2) discover how warming and/or added growing season precipitation altered these relationships. We hypothesized that: (1) physiological variability across host and endophyte genotype combinations would differentially affect root colonization by belowground AMF and DSE and associated plant and soil properties, such as lower defensive alkaloid production in NTE + tall fescue enabling greater root AMF and DSE colonization compared to CTE+; and (2) the nature of these interactions would be further impacted by warming and added growing season precipitation, either positively through alleviating or negatively through exacerbating existing temperature or moisture stress, as these environmental factors govern plant carbon fixation and thereby resource availability to symbionts.

2. Materials and methods

2.1. Site description and study design

The study was located at the University of Kentucky Spindletop Research Farm in Lexington, Kentucky (38°06′29.24″ N; 84°29′29.72″ W). This site is 281 m above sea level, receives *c.*