CAN INCREASING GRASS-FUNGAL ENDOPHYTE SYMBIOTIC DIVERSITY ENHANCE GRASSLAND ECOSYSTEM FUNCTIONING?

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Dr. Mark Coyne, Director of Graduate Studies
CAN INCREASING GRASS-FUNGAL ENDOPHYTE SYMBIOTIC DIVERSITY ENHANCE GRASSLAND ECOSYSTEM FUNCTIONING?

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Agriculture, Food and Environment at the University of Kentucky

By

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Lexington, Kentucky

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2018

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ABSTRACT OF THESIS

CAN INCREASING GRASS-FUNGAL ENDOPHYTE SYMBIOTIC DIVERSITY ENHANCE GRASSLAND ECOSYSTEM FUNCTIONING?

The relationship between biodiversity and ecosystem functioning is important in maintaining agroecosystem sustainability. Plant-microbe symbioses, such as exists between the grass tall fescue (Schedonorus arundinaceum) and the asexual fungal endophyte Epichloë coenophiala, can be utilized to enhance agroecosystem functions, such as herbivore resistance. “Novel” E. coenophiala strains that vary in the production of mammal- and insect-toxic compounds have been identified, inserted into tall fescue cultivars, and are planted in pastures globally. Novel fungal endophyte-tall fescue associations may have divergent ecosystem function effects. This study assessed effects of different fescue-endophyte symbiotic combinations on pasture ecosystem function, including aboveground (fescue biomass, plant species richness, alkaloid synthesis, arthropod abundance) and belowground (soil microbial biomass, soil enzyme activity, trace gas fluxes) parameters. Results showed no significant effects of increasing symbiotic diversity within a fescue stand on aboveground measurements, bar arthropod abundance and alkaloid synthesis. Most soil parameters quantified had significant symbiotic diversity effects. For example, soil microbial biomass decreased whereas soil enzyme activity increased with increasing symbiotic diversity. Overall, our results suggested that increasing symbiotic diversity had weak to moderate effects on aboveground processes and stronger effects on certain belowground processes, indicating that symbiotic diversity can impact ecosystem functions and warrants further research.

KEYWORDS: Biodiversity, Epichloë coenophiala, extracellular enzyme activity, grassland ecosystem functioning, tall fescue, trace gas fluxes

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July 12th, 2018
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به مادر و پدرم که امیریکا را به من دادند؛
و به ستاره، که با نورش من را هدایت میکند.
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Chapter One: Introduction

1.1 Biodiversity as a Regulator of Ecosystem Function

Global biodiversity, the variety of life forms present on Earth, has rapidly declined in the past decades due to anthropogenic influences, including expanding industrialized agriculture and growing human populations that deplete natural ecosystems (Matson et al., 1997; Sala et al., 2000). The biodiversity of an ecosystem has traditionally been synonymous with “species richness” (number of species present), though there are many elements to biodiversity (Figure 1.1).

Figure 1.1. The different components of biodiversity: structure, composition, and function. Adapted from Noss (1990).

Biodiversity is comprised of three primary components (Figure 1.1): structure, function, and composition (Noss, 1990). The variation in organization of an ecosystem or
the pattern of a system describes the structural diversity; it can be measured by physical patterns such as those formed by plant species responding to landscape variation, or variation within populations (e.g., sex ratios). Functional attributes include the variation of ecological processes carried out at different hierarchal levels, such as gene flow (population-level) and nutrient cycling (ecosystem-level). Composition involves the identity and variety of organisms at a given organizational level; this can range from the intra- and interspecies level to variation at the level of ecosystems and biomes (Tilman et al., 1997; Diaz and Cabido, 2001). As global populations expand, the consequences of anthropogenically-influenced biodiversity loss on ecosystem functionality are of growing concern.

Ecological literature has long debated and attempted to quantify the relationship between biodiversity and ecosystem function (BEF); theories and hypotheses concerning ecosystem function dependence on biodiversity have become points of dispute. The notion that increasing biodiversity positively influences ecosystem function can be traced back to Darwin (1872), who posited that ecosystem productivity is higher in grass plots with “several distinct genera of grasses.” Many ecologists have since built off of Darwin’s original hypothesis and developed diversity-ecosystem function theories that state increasing positive interspecies interactions will increase ecosystem stability by providing alternative pathways for nutrient and energy flow in the case of disturbance terminating or damaging primary pathways (Odum, 1953; MacArthur, 1955; Elton, 1958). Terrestrial plant community research has shown high-diversity systems maintain ecosystem services with time. In a seven-year study in Minnesota, primary production was higher (up to 22%) in species-rich polycultures (16 grassland perennials) than
monocultures (Tilman et al., 2001). Empirical evidence has also shown biodiversity maintains or enhances drought tolerance (Tilman and Downing, 1994) and nutrient capture (van der Heijden et al., 1998) (e.g., the relationship illustrated by line 1 in Figure 1.2).

**Figure 1.2.** Three common hypothetical relationships between biodiversity (species richness, in this case) and ecosystem process: (1) linear; (2) saturating; and (3) flat [Vitousek and Hopper, 1993 in (Tilman, 1997)].

Increasing ecosystem function with increasing biodiversity can result from two underlying mechanisms: 1) niche complementarity, where increasing the number of coexisting species more fully captures the entirety of available resources, either by utilizing different resources or using the same resource at different times, resulting in reduced interspecific competition and increased production; or 2) facilitation, typically positive interactions between organisms that benefit at least one of the participants and harms neither, often categorized by mutualistic or commensalistic interactions. The frequency of facilitative interactions between species has the potential to increase with increasing species diversity (Trenbath, 1974; Petchey, 2000). However, positive species
interactions as a result of increased diversity do not always unequivocally impact ecosystem response; other mechanisms, such as interference competition (where one organism bars another access to a resource), also impact competitive success and do not maximize productivity (Grime, 2001). Such interactions may elicit saturating or flat diversity-functioning responses (lines 2 and 3, respectively, in Figure 1.2).

Ecologically, not all species are equal and competitive abilities vary across time and space. Theories that predict positive linear relationships between diversity and ecosystem function assume species singularity, that each species lost or gained is equal in influence. However, the role of a species can range from “drivers,” where their removal invokes a cascade effect in the ecosystem, to “passengers,” whose elimination incites little effect [sensu (Walker, 1992)]. The type of species (i.e., “drivers” or “passengers”) lost or gained might drive ecosystem processes rather than the overall change in species richness per se (Petchey, 2000; Hooper et al., 2005).

In a 2006 meta-analysis, Cardinale et al. reported that the magnitude of ecosystem function response is largely determined by the particular species going extinct and the type of ecosystem function being measured. Some functions may be more sensitive to species change than others. In contrast, others argue that the main drivers of ecosystem functioning are key functional traits of dominant species and the array of functional types present (Mikola and Setälä, 1998). Replication of certain species or functional groups (groups of species that share a similar response to environmental change, or similarly influence ecosystem function) provide what is called “species redundancy” (Walker, 1992); redundancy manifests itself as a saturation point on asymptotic diversity-ecosystem function response curves. Saturation points vary depending on the degree of
niche overlap (Petchey, 2000). Communities containing high degrees of redundancy are theorized to experience little loss of function when species loss or extinction occurs [see Figure 1 in (Petchey, 2000)]. For this reason, Naeem (1998) argues that species redundancy is a critical ecosystem attribute for long-term stability; declining species richness, and thereby potentially species redundancy, could have serious implications for ecosystem functioning.

Diversity is no guarantee of enhanced ecosystem functioning, and relationships can be context dependent (Risser, 1995; Johnson et al., 1996; Chapin et al., 1998; Cardinale et al., 2000). Empirical support has illustrated flat diversity-functioning responses characterizing some ecosystem processes, including primary production (Hooper and Vitousek, 1997) and decomposition (Wardle et al., 1997), the latter study demonstrating that the presence or absence of plants impacted soil respiration, rather than their diversity. Second, the diversity-function relationship at local scales cannot necessarily be projected to the regional scale. Island area studies, for example, have illustrated that in smaller, high-diversity island areas, ecosystem processes rates (e.g., N acquisition) were lower than larger, less diverse areas (Wardle et al., 1997), suggesting that other environmental differences determining biological community composition, not diversity per se, are the main drivers of ecosystem processes. Finally, changes in temporal and spatial environmental variables can additionally impact ecosystem resource partitioning; such effects have an overpowering potential to alter the type and magnitude of ecosystem response to diversity, potentially resulting in a flat BEF response (Tilman et al., 1997; Cardinale et al., 2000).
Differences between large-scale, observational and small-scale, experimental approaches evaluating diversity-functionality responses have further deepened the BEF debate (Loreau et al., 2001). Experimental data, typically gathered from manipulating species richness under specific environmental conditions in temperate grasslands, demonstrates a positive diversity-function relationship (Tilman, 1997). The Cedar Creek biodiversity experiment in Minnesota, USA (Tilman et al., 1996) and the BIODEPTH experiment conducted across multiple European sites (Hector et al., 1999) are both examples of long-term, robust data sets demonstrating similar results: a log-linear increase of above-ground biomass (a common productivity measure for grasslands) as species richness increases. Observational data, however, generally evaluates spatial diversity across environmental gradients (e.g., soil fertility), and depicts hump-backed response curves where diversity-function is driven primarily by environmental conditions (Huston, 1994; Grime, 2001).

The stark contrast in observational and experimental results drew criticism from opposing sides. Critical responses deemed the Cedar Creek and BIODEPTH experiments to be inducing the “sampling effect,” in which the probability of including a key [per Tilman (1997), “highly productive”] species increases with greater diversity treatments, and therefore, the relationship discovered is simply a function of the experimental design (Huston, 1997). Reconciling differences between large- and small-scale experiments required inspecting the primary mechanisms driving the disparity (Loreau et al., 2001). Observational experiments, which factor in spatial patterns, can highlight relationships between diversity and ecosystem function driven by environmental conditions, whereas smaller scale experimental studies can uncover the influence of species functional traits
and diversity on ecosystem function once significant amounts of spatial heterogeneity have been reduced. In an attempt to link theoretical, experimental, and observational studies, scientists working on the BEF framework have reached the following consensus:

1) functional traits of species and interspecific interactions are potentially more influential than species richness itself; 2) feedbacks between abiotic variables (e.g., climate) and biodiversity can influence ecosystem processes and functional relationships; and 3) diversity effects and mechanisms can differ by scale (i.e., temporal and spatial) and hierarchal level (e.g., species- versus population-level) (Hooper et al., 2005).

1.2 Biodiversity-Ecosystem Function in Agroecosystems

One type of land use that is known to significantly reduce biodiversity is the intensification and augmentation of cultivated land (Matson et al., 1997). Agricultural intensification was stimulated by “the Green Revolution,” a movement beginning in the 1960s that prescribed to increased usage of high-yielding crop varieties, chemicals (e.g., N-based fertilizer and pesticides), and irrigation to ultimately enhance total crop yields (Matson et al., 1997). As a result of the Green Revolution, today’s agroecosystems are typically large acreage monocultures of a single or possibly a few species, depending on management practices (Aguilar et al., 2015). Furthermore, these species monocultures often harbor reduced intra-species genetic diversity due to breeding and seed industry practices that often select for high-performing varieties. Such agroecosystems, characterized by low species richness and genetic uniformity, are known to be vulnerable to disturbances, such as extreme climactic events and pest outbreaks (Lin, 2011).

Following BEF theory, agroecologists have hypothesized that improving species and
genetic diversity within agroecosystems will improve cropping system resilience and function (Altieri, 1999).

While the functional role of biodiversity will likely differ across agroecosystems, which vary in age, environmental conditions, management, and structure, there is evidence that higher-diversity systems can promote beneficial ecosystem services, like increased crop yield (Bullock et al., 2007). Further examples include integrated pest management, or IPM, a widely adopted agroecosystem management practice that uses polycultures to lower insect pest population densities by encouraging natural enemies species diversity (Altieri, 1999; Ehler, 2006). In the pursuit to expand the breadth of sustainable management practices, agroecological research is exploring the use of functional trait diversity to support ecosystem function. For example, research has shown that diversifying rotational legume/grass cover crop systems, which uses complementary crop functional traits, across environmental conditions can enhance agriculturally beneficial functions, such as soil N retention (Blesh, 2018). Because agricultural systems tend to experience biological simplification, the diversity of species functional traits present can diminish (Moonen and Barberi, 2008). Through diversification of functional traits in the landscape, farmers can harness beneficial ecological interactions (e.g., facilitation and complementarity) to enhance and support agroecosystem functions, like biological nitrogen fixation, weed and pest suppression, and organic matter accumulation (Shennan, 2008).

Several functional traits that are of particular interest to agricultural production arise from host-specific, symbiotic plant-microbe interactions. Biological N-fixation, for example, is a functional trait that results from a host-specific symbiosis formed between
leguminous plants and rhizobia bacteria (van der Heijden et al., 2006). Legume-rhizobia symbioses are known to wield considerable influence on large-scale ecosystem processes, including the carbon and nitrogen cycles [see Table 1 in (van der Heijden et al., 2008)], making them of high-interest to certain producers. However, because of the degree of host specificity required to establish a successful association between plant and microbe, increasing the diversity of edaphic rhizobia does not necessarily result in higher legume grain yields, nor increased N$_2$-fixation (Koskey et al., 2017). Some rhizobia strains may have host compatibility issues and cannot produce root nodules (where N$_2$-fixing rhizobia are housed), or negative microbial interactions can deter function (Martinez-Romero, 2003). However, cultivating mixtures of legume species and their associated N-fixers has the potential to enhance ecosystem functioning by increasing intra-specific functional group diversity. Studies have also evaluated the synergistic effects of tri-partite symbiotic associations between AMF (arbuscular mycorrhizal fungi) and rhizobia on legume function, finding enhanced plant productivity due to complementary nutrient acquisition (i.e., plants received P from AMF and N from rhizobia) (van der Heijden et al., 2008).

Another important plant-microbe interaction that often equates into agricultural functions of interest (e.g., herbivore deterrence and abiotic stress tolerance) is that which occurs between cool season grasses and certain fungal endophytes. Endophytic interactions are characterized by the inhabitation of one organism by another, typically a fungal or bacterial endophyte, within the plant host’s tissue during all or parts of the endophyte’s life cycle (Wilson, 1995). Fungal endophytes are of particular interest because they reside in all major lineages of terrestrial plants, making them one of the most diverse groups of plant-associated symbionts (Arnold, 2007). Like legume-rhizobia
symbioses, successful grass-endophyte complexes are determined by host-strain compatibility. Furthermore, the nature and ecological impacts of the association are susceptible to changing environmental conditions and resource availability (Malinowski and Belesky, 2006).

Fungal produced bioactive alkaloid compounds are commonly considered to be the mechanisms by which grass-endophyte ecological effects are observed. Different grass species (or cultivars of a single grass species) forming associations with different endophyte species (or strains) produce qualitatively and quantitatively distinct alkaloid profiles [see Table 1 in (Bush et al., 1997)]. Plant tissue type, season, and other abiotic environmental conditions can likewise influence alkaloid production. Alkaloids and other endophyte-produced secondary metabolites are known to confer influential functional traits, such as deterring insect and mammalian herbivory and enhancing nutrient uptake (Clay, 1988). Examining the relationship between symbiosis-driven functional traits, such as alkaloid profiles, and ecosystem function may provide useful insight into developing novel biodiversity approaches for sustainably managing agroecosystems such as pastures.

1.3 Grass-Fungal Endophyte Relationships

While there is significant interest amongst the agricultural community to utilize plant-microbe symbioses to improve crop production and sustainability, often plant-microbe interactions are difficult to reliably manipulate, especially in long-term field studies. However, one plant-microbe interaction that has shown to be receptive to long-term manipulation is that of grasses and their symbiotic fungal endophytes.
Fungal endophytes are categorized into two distinct functional groups based on taxonomy, plant hosts, and ecological function: Clavicipitaceae (C-endophytes) and Non-Clavicipitaceae (NC-endophytes) (Rodriguez et al., 2009). Shoot- and foliar-specific fungal endophytes of the family Clavicipitaceae (Class 1) form highly specialized, above-ground interactions with certain agricultural grasses, making them of significant ecological and economical importance, which is further reflected by the considerable amount of agroecosystem research conducted on grass-endophyte symbiota (Clay, 1990).

Certain Class 1 endophytes are most often known to form mutualistic interactions with and confer several functional traits to their grass-hosts, such as tolerance to drought, heat, and saline conditions (Clay, 1990). Mutualism, however, is not universally experienced by all grass-endophyte associations; furthermore, endophyte-associated functional traits can depend on host and endophyte genetics, environmental conditions, and interactions thereof (Saikkonen et al., 1999; Faeth et al., 2006). The type of grass-endophyte interaction is also contingent upon the endophyte’s life cycle, which further divides C-endophytes into three camps: Type I, II, and III (Clay and Schardl, 2002; Johnson et al., 2013) (Table 1.1).
Obligate sexual fungal endophytes (Type I) represent one end of the spectrum; infection is horizontally transmitted (contagious to external individuals) via production of ascospores. This pathogen-like infection can express antagonistic traits, such as choke disease, where fungal infection stunts plant growth by impeding seed head development (Saikkonen et al., 1998). Grass interactions with Type II endophytes are mixed; Type II endophytes are known as “pleiotropic symbionts” (Scharld et al., 1997), meaning that their dynamic lifecycle encompasses both sexual and asexual reproductive mechanisms. Type II endophytes can provide host benefits such as increased growth (Clay, 1986), but the fungus may still express virulence towards its host.

On the other end of the spectrum is Type III inhabitation, which is systemic, non-contagious, and occurs for the entirety of the host’s life cycle (Clay and Scharld, 2002). Obligate asexual endophytes vertically transmit lineages of single fungal genotypes through hyphal growth into seed embryos. Due to their dependence on host-mediated infection-transmission, asexual endophytes have co-evolved in association with specific species of grass hosts (Clay and Scharld, 2002). Unlike Type I (and sexually reproducing

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**Table 1.1.** From Clay and Scharld (2002) illustrating differences between Type I, II, and III grass-endophytes associations.

<table>
<thead>
<tr>
<th>Fungus:</th>
<th>Symptomatic (Type I)</th>
<th>Mixed (Type II)</th>
<th>Asymptomatic (Type III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproduction</td>
<td>Sexual</td>
<td>Both</td>
<td>Clonal</td>
</tr>
<tr>
<td>Transmission</td>
<td>Horizontal</td>
<td>Both</td>
<td>Vertical</td>
</tr>
<tr>
<td>Propagule</td>
<td>Ascospores</td>
<td>Both</td>
<td>Seeds</td>
</tr>
<tr>
<td>Host:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproduction</td>
<td>Sterile/clonal</td>
<td>Partial sterility</td>
<td>Sexual</td>
</tr>
<tr>
<td>Interaction</td>
<td>Pathogenic</td>
<td>Intermediate</td>
<td>Mutualistic</td>
</tr>
<tr>
<td>Infection frequency</td>
<td>Low-moderate</td>
<td>Intermediate</td>
<td>High</td>
</tr>
<tr>
<td>Taxonomy</td>
<td>Entire grass family</td>
<td>C3 pooid grasses</td>
<td>C3 pooid grasses</td>
</tr>
</tbody>
</table>

---

12
Type II), asexual endophytes asymptptomatically reside in plant tissue [though not always, see (Faeth and Sullivan, 2003)] and tend to have higher endophyte infection frequencies within host populations because of the relatively high efficiency of vertical transmission. Type III fungal endophytes provide a unique model for agroecological research and manipulation based on their non-virulent, obligate, and systemic association. Furthermore, associations between grasses and these types of endophyte tend to increase host fitness and are frequently mutualistic in nature (Takach and Young, 2014). Exploring the chemotypic diversity in alkaloid production among and between certain asexual fungal endophytes and their relationship with aspects of ecological functional traits is of high interest to agroecosystem research (McNear Jr and McCulley, 2012; Iqbal et al., 2013; Yurkonis et al., 2014; Guo et al., 2015).

1.4 The Tall Fescue-\textit{Epichloë coenophiala} Model

Grasses of the \textit{Poaceae} family, subfamily \textit{Poödieae}, are known to form symbiotic associations with clavicipitaceous fungal endophytes, such as the genus \textit{Epichloë} (anamorph = \textit{Neotyphodium}) (Clay, 1988; Bacon, 1995). \textit{Epichloë coenophiala} (Morgan-Jones and W. Gams; Shelby and Dalrymple, 1987), for example, has, over time, formed an intimate symbiosis with tall fescue \textit{[Schedonorus arundinaceus (Schreb.) Dumort]}, an agronomically dominant cool-season pooid bunchgrass native to Eurasia and North Africa, which now occupies Australia, New Zealand, and over 15 million ha in the United States (Buckner \textit{et al.}, 1979; Schardl and Phillips, 1997; Young \textit{et al.}, 2013). In the United States, 95\% of all tall fescue pastures are infected with \textit{E. coenophiala} (Shelby and Dalrymple, 1987). Several studies have documented the adaptive abilities that \textit{Epichloë}-infected tall fescue exhibits (Clay, 1988; Latch, 1997; Belesky and West, 2009).
One of the first tall fescue cultivars to come to prominence was that of “Kentucky-31,” discovered in Eastern Kentucky in 1931, where it was noted that the grass had competitively established itself over a span of diverse climates and soil types (typically in areas prone to drought and nutrient-deficiency) (Hoveland, 2009). Kentucky-31 soon grew to become the dominant forage grass of the east-central and mid-southern United States, where, at the time, no other cool-season perennial grass was able to persist in pastures due to hot, frequently water-stressed summers. Kentucky-31 persistence was accredited to its endemic asexual symbiont, *E. coenophiala*, conferring several resistance traits to its host, including enhanced nutrient acquisition, climactic tolerance, and resistance to herbivory (Christensen and Voisey, 2009). Despite the grass’ lauded capabilities, livestock grazing in Kentucky-31 endophyte infected pastures experienced deteriorating health and worsening performance (Hoveland, 1993). Mammal-related toxicity problems were ultimately traced back to neurotoxic ergot alkaloid compounds produced by a strain (i.e., genotype) of *E. coenophiala* now referred to as the “common toxic endophyte” (Bacon, 1995).

Endophyte-associated toxicity increased interest in identifying and utilizing naturally occurring, non-mammal-toxic *E. coenophiala* genotypes, or so-called “novel endophytes” (NE). NE strains, which retain beneficial resistance traits, like producing insect-active compounds, but little to no mammal-toxic ergot alkaloids (Bouton et al., 2002), can be isolated from their plant hosts and inserted into commercial tall fescue lines (Latchs and Christensen, 1985). For example, the seed company Pennington Seed® (Madison, GA) markets the fescue cultivar Jesup™ (owned and developed by the University of Georgia, U.S.A.) exclusively containing novel endophyte strain, AR-542
(owned by Grasslanz Technology Ltd., New Zealand, whose subsidiary, AgResearch Ltd., New Zealand, developed the strain). When AR-542 is in endophytic association with Jesup™ it goes by the trade name MaxQ™, commercializing the grass-endophyte “package” as Jesup MaxQ™. There are several such “packages” on the market, having been developed and released over the past 20-30 years (Table 1.2). Producers, however, frequently purchase and cultivate one type of “package” in a pasture, thereby creating monocultures of one fescue cultivar – endophyte strain association.

**Table 1.2.** Examples of novel endophyte and tall fescue products currently commercially available (Smith and Phillips, 2016).

<table>
<thead>
<tr>
<th>Year of First Sale</th>
<th>Cultivar</th>
<th>Tall Fescue Owner</th>
<th>Endophyte Brand</th>
<th>Strain I.D.</th>
<th>Endophyte Owner</th>
<th>Seed Marketer</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>Jesup</td>
<td>University of Georgia</td>
<td>MaxQ™</td>
<td>AR-542</td>
<td>Grasslanz</td>
<td>Pennington Seed®</td>
</tr>
<tr>
<td>2007</td>
<td>BarOptima</td>
<td>Barenbrug</td>
<td>Plus E34™</td>
<td>E34</td>
<td>Barenbrug</td>
<td>Barenbrug USA®</td>
</tr>
<tr>
<td>2011</td>
<td>Texoma</td>
<td>Noble Foundation</td>
<td>MaxQII™</td>
<td>AR-584</td>
<td>Grasslanz</td>
<td>Pennington Seed®</td>
</tr>
</tbody>
</table>

To date, novel endophyte research has primarily focused on maintaining fescue production and insect resistance, while reducing grazing animal toxicity, but has tended to disregard any additional ecosystem services grass- novel endophyte associations may provide. Because different endophyte strains have divergent alkaloid profiles and possibly functional attributes, it is possible to test whether increasing endophyte genotypic diversity within a tall fescue community can improve above- and belowground ecosystem functioning.
1.5 Aboveground effects of Tall Fescue-E. coenophiala symbiosis

Because tall fescue-E. coenophiala associations are highly specialized and non-contagious, and because they are sold as “packages,” it is possible to manipulate fescue cultivar-endophyte strain combinations to assess grass-endophyte effects on community and agroecosystem dynamics. Thus far, grass-endophyte research has focused on identifying endophyte presence effects within a single cultivar. For example, many studies have been conducted evaluating endophyte-free (E-) and common toxic endophyte-infected (CTE+) Kentucky-31. Fewer studies have evaluated differences between E- and novel endophyte-infected material [though, see (Rudgers and Clay, 2007; Iqbal et al., 2013)]. Results from these studies have shown that endophyte presence tends to promote aboveground plant productivity, compared to E- populations, and can alter plant community structure, citing that E- stands often support greater plant species richness compared to endophyte-infected communities (Clay and Holah, 1999; Rudgers et al., 2009; Iqbal et al., 2013). Endophyte presence can directly impact host competitive ability (Hunt and Newman, 2005; Yurkonis et al., 2014) by stimulating host growth and dominance (Hesse et al., 2003; Iqbal et al., 2013; Guo et al., 2015), or via indirect mechanisms, such as shifts in invertebrate community composition and associated herbivory (Miathews and Clay, 2001; Keathley and Potter, 2012) that, in turn, change plant community composition (Rudgers et al., 2010).

Fungal-produced alkaloid compounds confer grass hosts resistance to herbivorous insects in part through two main classes of broadly insecticidal metabolites: lolines and peramine (Afkhami and Rudgers, 2009). In their 2008 study, Rudgers and Clay found that the endophyte-grass mutualism altered community structure by a 70% reduction in
arthropod abundance and a 20% decrease in arthropod diversity in CTE+ plots compared to E- stands. In contrast, Keathley and Potter (2012) argued that invertebrate guilds were more diverse in E- tall fescue stands because of increases in plant species diversity, instead of direct alkaloid effects per se. Community structure studies quantifying generalist predator abundance, such as spiders, have found reduced richness of both spider families and morphospecies in CTE+ tall fescue, but no differences in abundance compared to E- stands (Finkes et al., 2006). Laboratory studies have also measured adverse, albeit subtle, tri-trophic effects on natural enemies feeding on or within hosts ingesting endophytic grass (Bultman et al., 1997; Bixby-Brosi and Potter, 2012). Though, in such studies it is often difficult to isolate the indirect effects of the host itself experiencing harmful effects of endophytic grass consumption from the direct effects of parasitoid exposure to host-ingested alkaloids.

Pasture ecosystem studies have also examined the effects of endophyte presence and strain on aboveground plant performance and plant-insect relationships. Generally, in planta CTE endophytes can exert stronger ecological pressures than their NE counterparts, due to well-established promotion of host competitive abilities, as outlined above (Rudgers et al., 2010). Iqbal et al. (2013), using a single tall fescue cultivar (Texoma) infected with either CTE, one of two novel endophytes, AR-542 or AR-584, or endophyte-free, and an additional treatment, hereafter referred to as the “mix” treatment, containing 25% of all four endophyte statuses, found that NE-infected populations tended to have intermediate levels of plant diversity and were less dominated by tall fescue compared to CTE-infected stands. Interestingly, while differences in fescue biomass were not statistically significant across treatments, “mix” treatment plots measured a 47%
fescue cover increase compared to E-, suggesting that fescue stands containing diverse endophyte strains increase the competitive ability of grass host populations. Endophyte infection is able to increase tall fescue persistence and fitness possibly due to decreases in herbivory, shifts in soil microbial community compositions enhancing nutrient cycling, or production of allelopathic compounds (Iqbal et al., 2013; Orr et al., 2005).

Because of their different alkaloid profiles and concentrations, different endophyte strains have divergent effects on invertebrate communities (Keathley and Potter, 2012). When foliar-associated, grass-adapted invertebrates are exposed to genetically different endophyte strains (i.e., NE versus CTE), responses vary by insect species and pasture conditions. Across four different fescue-endophyte treatments, including Kentucky-31 CTE-infected, two cultivars containing different NE strains (Jesup infected with AR-542 and cultivar KYFA9301 infected with AR-584), and a KYFA9301 E- control, Keathley and Potter (2012) reported, with a few exceptions, no difference in chewing (grasshoppers, crickets, caterpillars) and sucking insects (leafhoppers) across CTE+ and NE+ pastures. Only Aphrodes spp. leafhoppers were most abundant in CTE+ plots, compared to certain species that experienced no difference (Endria inimica) (Davidson and Potter, 1995; Keathley and Potter, 2012). In the field, Keathley and Potter (2012) additionally found no significant differences in natural enemy populations of spiders, ground beetles, lady beetles, rove beetles, oribatid mites, or parasitism of leafhoppers across different endophyte statuses (i.e., E- compared to NEAR-584+ and CTE+). Insect-active compounds can indirectly influence insect herbivores by altering plant growth by inducing selective grazing (Bultman et al., 1997),
or by indirectly affecting soil invertebrates through endophyte-associated differences in soil composition (Rudgers and Orr, 2009; Siegrist et al., 2010).

While the effects of CTE+ versus E- on plant productivity, plant species diversity, and arthropod abundance are well-documented, little work has been done to date concerning the aboveground effects of chemotypically diverse alkaloid synthesis, which has potential to differentially impact that the persistence and competitive abilities of tall fescue communities. Shifts in aboveground plant and insect communities may lead to concomitant belowground consequences.

1.6 Belowground consequences of Tall Fescue-\textit{E. coenophiala} symbiosis

While there have been numerous studies documenting the influences of \textit{E. coenophiala} symbioses on aboveground ecological processes, there has been less work examining effects on belowground parameters. However, studies have shown endophyte presence to influence root exudate production, soil microbial activity and community composition, organic matter decomposition and nutrient pools [see Table 1 in (McNear and McCulley, 2012)]. Endophyte-associated shifts in aboveground plant biomass, soil microclimate, plant community composition, and/or the degree of aboveground herbivory can also influence belowground parameters (Omacini et al., 2005). The brunt of belowground studies, however, has been concentrated on evaluating the effects of CTE+ tall fescue versus E-.

Endophyte-altered soil processes can arise from several factors. For instance, plant litter of endophyte-infected grasses has been found to contain loline and ergot alkaloids (Siegrist et al., 2010; Franzluebbers and Hill, 2005), which may prove to be
harmful to soil microfauna as alkaloids leach into the soil (McNear and McCulley, 2012) and lead to changes in microbial community composition (Franzluebbers and Studemann, 2002). Antunes et al. (2008) provided a mechanism for such occurrences, documenting 10% less mycorrhizal spore germination in aqueous extracts of CTE-infected Georgia-5 shoot tissue compared to E- material. Changes in rhizosphere chemistry from altered root exudate composition can further impact microbial community composition, particularly in the rhizosphere (Rojas et al., 2016). Endophyte presence is also known to stimulate soil microbial activity as indicated by increased soil respiration (Van Hecke et al., 2005); however, results vary by soil microbial community and activity, and can differ between in situ field experiments and laboratory incubations. For example, studies have found that CTE+ infections increase soil microbial biomass compared to E- stands (Handayani et al., 2011; Iqbal et al., 2012), and other studies have documented lowered microbial biomass and soil respiration in high CTE-infected fescue pastures (65-94%) compared to low infection (0-29%) (Franzluebbers et al., 1999).

Endophyte presence has also been known to influence belowground ecosystem processes that moderate nutrient cycling and soil organic matter turnover (Guo et al., 2016). Due to increased root growth (Richardson et al., 1990), and decreases in microbial biomass and basal soil respiration, soils of CTE+ stands can retain more belowground inputs, such as higher soil organic C (SOC) and nitrogen (N), than E- stands (Franzluebbers and Studemann, 2005). Reduced plant litter decomposition can further abate C turnover and loss, and lead to greater ecosystem C sequestration (Handayani et al., 2011). Land-use and site management are equally important when assessing the strength of endophyte effects on nutrient pools. For example, Iqbal et al. (2012)
determined that sites used to study cattle performance and grazing on CTE+ vs. E-pastures had the greatest differences in soil C and N pools.

In evaluating endophyte strain effects (Guo et al., 2016), a study reported C and N concentrations (in particulate organic matter) were significantly higher under CTE+ and NEAR-584+ infected tall fescue compared to E- plots. It is possible that tissue chemistry (i.e., alkaloid content) can influence soil nutrient (particularly C and N) cycling by hindering decomposition (Horner et al., 1988; Omancini et al., 2004; Walela et al., 2014). It has been argued (Siegrist et al., 2010), however, that alterations in tissue chemistry, aside from alkaloid composition, may be of equal importance. Concerning microbial activity, McNear and McCulley (2012) posited that endophyte status of a tall fescue plant can mediate bioactivity by way of whole root exudate production, reporting that NEAR-542+-tall fescue combinations produced less growth-inhibiting exudates vis-à-vis E- and CTE+ pairs. In a similar vein, soil-to-atmosphere trace gas fluxes can also be impacted by endophyte infection. For example, under field conditions, Iqbal et al. (2013) found that soil CO$_2$ and N$_2$O fluxes were more susceptible to endophyte identity than presence; the highest rates of CO$_2$ and N$_2$O fluxes were measured in fescue stands infected with NEAR-542, and the lowest rates occurred in NEAR-584 tall fescue (Iqbal et al., 2013). Again, changes in soil microclimate, nutrient concentrations, and trace gas fluxes under genetically diverse endophytic grasses may occur in response to alkaloid-driven aboveground differences (e.g., plant litter inputs or plant community composition) affecting substrate availability and microbial community composition, though additional research is needed to further elucidate these mechanisms.
To date, there has been no work [bar the inclusion of a mix treatment in (Iqbal et al., 2013)] assessing how mixtures of fescue cultivar – endophyte strains impact above- and belowground ecological characteristics and processes. Results of the 2013 Iqbal et al. study suggested that a mix of endophyte statuses, of which contained ~25% of the common-toxic form, within a tall fescue population invokes similar mechanisms to those by which stands dominated by CTE+ tall fescue have been shown to alter ecosystem services; these findings encourage the need for additional research exploring the potential ecological attributes provided by functionally diverse mixtures of endophyte strains within grassland ecosystems.

1.7 Justification and Hypotheses

The purpose of this study was to investigate the ecological effects of manipulating community- and ecosystem-level symbiotic diversity between *Epichloë coenophiala* and its cool-season grass host, tall fescue. These types of symbioses are of significant ecological dominance, influencing global biogeochemical cycles, and are economically important in agronomic grasslands that support global industries, such as animal production. Once established, the presence of asexual *E. coenophiala* is maintained for the duration of its host’s life cycle, and strain identification and presence are easily verified, making grass-endophyte treatments relatively amenable to long-term ecosystem assessment (Takach et al., 2012). The current body of work has typically focused on common-toxic *Epichloë* strain effects on mainly aboveground ecosystem characteristics (such as biomass yield and plant species diversity), and some belowground investigations. Furthermore, given the functional diversity of fungal endosymbionts conferred by divergent alkaloid profiles, biodiversity-ecosystem functioning research has
yet to explore the impact of genetic manipulation of symbiont diversity on ecosystem functions, such as greenhouse gas emissions. To help fill this gap in knowledge, I explored the following question: how does increasing aboveground symbiotic diversity within the tall fescue-\textit{Epichloë} interaction impact grassland ecosystem function? Overall ecosystem function was measured in terms of aboveground (plant species diversity, tall fescue yield, alkaloid production, and arthropod abundance) and belowground parameters (greenhouse gas emissions, soil microbial biomass, and extracellular enzyme activity).

**Hypothesis 1:** I predicted, per BEF theory, that mixtures of functionally dissimilar endophyte strains within a tall fescue stand would enhance resource utilization by filling more ecological niche space, thereby promoting net aboveground plant productivity over endophyte-free (symbiotic diversity – 0) and endophyte-monoculture (symbiotic diversity – 1) stands.

**Hypothesis 2:** Foliar, grass-adapted invertebrates, such as leafhoppers can be susceptible to endophytic grasses. Therefore, I hypothesized that herbivorous insect populations would experience declines in symbiotically diverse plots versus E- and symbiotic monocultures, because symbiotically diverse plots will produce a broader range of chemotypically diverse insecticidal-alkaloid compounds.

**Hypothesis 3:** As more niche spaces are occupied with increasing symbiotic diversity, and resource use increases, concomitant changes in aboveground ecosystem properties, such as plant productivity and species diversity, could in turn impact belowground ecosystem services. I predicted that increasing endophyte strain diversity would alter belowground parameters, such changes in trace gas fluxes and soil microbial biomass, over endophyte-free (symbiotic diversity – 0) and endophyte-monoculture (symbiotic
diversity – 1) stands. Because soil microbial activity can be stimulated by endophyte-presence by way of increased C and N inputs, I further hypothesized that soils under increasing symbiotic diversity would support increased soil enzymatic activity, though responses might vary by enzyme (i.e., variability in enzyme substrate availability or microbial nutritive demands might lead to such differences).
Chapter Two: Material and Methods

2.1 Site Description

This study was conducted at University of Kentucky’s Spindletop Farm Research and Education Center (38°08'03" N, 84°29'56" W) in Lexington, KY. Over a 30-year observation period (1978-2008), the site averaged 114.7 cm precipitation per year with an average wintertime air temperature of 1.6°C and a mean summer temperature of 23.8°C (Ferreira et al., 2010). The field site’s soil series is a Bluegrass-Maury silt loam complex with two to six percent slope. This series is a well-drained, fine-silty, mixed, active, mesic Typic Paleudalf that developed from thin fine-silty non-calcareous loess overlaying clayey residuum resulting from weathered phosphatic limestone (Sims et al., 1968).

2.2 Experimental design

The symbiotic diversity project was established in Fall 2016. The study included 80 plots (each 8 x 8 m) consisting of 16 fescue-endophyte treatments, each replicated five times (Figure 2.1), that ranged in stand-level endophyte symbiotic diversity from 0 to 4 (Table 2.1). Prior to experiment establishment, Roundup Ready® Alfalfa was grown at the site from 2009-2016. From 2009-2015, vegetation was annually treated with Roundup®. In 2016, remnant alfalfa was tilled (August 15th) into the field to prepare the seed bed for planting. Once completed, a field cultivator was taken once over the field (September 13th), followed by a culti-packer (twice over the field), and plots were flagged (September 15th). Tall fescue-endophyte diversity seed mixes were created by obtaining cultivars containing the desired endophyte strain from grass
breeders (Dr. Mike Trammell at The Noble Foundation provided the KY-31 CTE+; Dr. Tim Phillips at UK provided the KY-31 E- seed) and seed companies (Joe Schmidlen of Barenburg USA® provided BarOptima E34+; Chris Agee of Pennington Seed® provided the Jesup MaxQ+ and Texoma MaxQII+ seed). Seed packets containing various endophyte diversity treatments (Table 2.1) were hand-mixed. Tall fescue seeds were hand-broadcasted at the rate of 28.02 kg live seed ha\(^{-1}\) in early Fall (September 26\(^{th}\), 2016) followed by one pass of a culti-packer to facilitate seed-to-soil contact.

### 2.3 Pasture management

Following planting, plots were irrigated twice in the Fall (October 2016). To mimic common hay production procedures, plots were mowed to a height of 10 cm biannually in the Summer (June 21\(^{st}\), 2017) and again in the Fall (November 15\(^{th}\), 2017), with all the biomass removed. In Fall (November 29\(^{th}\) 2017, Urea (46-0-0) was applied to the field at a rate of 67.25 kg/ha.
Symbiotic Diversity Study

**Figure 2.1.** Randomized complete blocking design of the Symbiotic Diversity Study located at the University of Kentucky Spindletop Research Farm in Lexington, KY. Sixteen fescue-endophyte treatments were created by hand-mixing seeds from different proprietary cultivars (each containing a unique strain of *Epichloë coenophiala*): Kentucky-31 endophyte-free (KY-), Kentucky-31 common toxic endophyte (KY+), Jesup MaxQ (J), Texoma MaxQ II (T), and BarOptima (B). Due to limitations in field dimensions, three columns from the fifth block were cultivated separately from the fourth column.
Table 2.1. Sixteen fescue-endophyte treatments were created by hand-mixing seeds of several proprietary cultivars: Kentucky-31, Jesup MaxQ, BarOptima, and Texoma MaxQII. Each cultivar contains a unique strain of *Epichloë coenophiala* or is endophyte-free, in the case of Kentucky-31 E-. CTE+ = common toxic endophyte; NE+ = novel endophyte. NE584+ and NE542+ are two different novel endophyte strains. The endophyte symbiotic diversity of the seed mixtures ranges from 0 to 4. Color codes match those shown in Figure 1.1.

<table>
<thead>
<tr>
<th>Endophyte Symbiotic Diversity</th>
<th>Seed Mix for Each Treatment</th>
<th>Treatment #</th>
<th>Abbreviation</th>
<th>Fescue-Endophyte Treatment</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100%</td>
<td>1</td>
<td>KY-</td>
<td>Kentucky-31 E-</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>100%</td>
<td>2</td>
<td>KY+</td>
<td>Kentucky-31 CTE+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>J</td>
<td>Jesup MaxQ NE542+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>B</td>
<td>BarOptima NE34+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>T</td>
<td>Texoma MaxQII NE584+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>50/50%</td>
<td>6</td>
<td>KY+/T</td>
<td>KY-31 CTE+/ Texoma MaxQII</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>KY+/B</td>
<td>KY-31 CTE+/ BarOptima</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>KY+/J</td>
<td>KY-31 CTE+/ Jesup MaxQ</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>T/B</td>
<td>Texoma MaxQII/ BarOptima</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>T/J</td>
<td>Texoma MaxQII/Jesup MaxQ</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>B/J</td>
<td>BarOptima/ Jesup MaxQ</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>33/33/33%</td>
<td>12</td>
<td>KY+/T/B</td>
<td>KY-31 CTE+/ Texoma MaxQII/BarOptima</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>KY+/T/J</td>
<td>KY-31 CTE+/ Texoma MaxQII/Jesup MaxQ</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>KY+/B/J</td>
<td>KY-31 CTE+/BarOptima/Jesup MaxQ</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>T/B/J</td>
<td>Texoma MaxQII/BarOptima/Jesup MaxQ</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>25/25/25/25%</td>
<td>16</td>
<td>KY+/T/B/J</td>
<td>KY-31 CTE+/ Texoma MaxQII/Jesup MaxQ/BarOptima</td>
<td></td>
</tr>
</tbody>
</table>
2.4 Verification of Endophyte Diversity Treatments

In the field, immediately prior to planting, randomly collected subsamples of seed (n=30 seeds) were taken from each treatment’s seed packet and sent to Dr. Carolyn Young of the Noble Research Institute (Ardmore, OK) to verify endophyte identify and infection frequency (Table 2.2). Endophyte infection frequency and identification were performed using a high-throughput PCR-based marker system (Takash et al., 2012; Young et al., 2014). For additional endophyte verification and alkaloid analyses, 50 tillers per plot were harvested in spring (May 23rd – June 5th, 2017) and fall (October 5th, 2017) of the first full growing season. Tillers were selected at random in each plot, cut at ground-level with a razor blade, placed in a plastic bag, and immediately placed on ice. Half the pseudo-stem was cut from each tiller using a razor blade and kept at -18°C until shipped to Dr. Young for endophyte strain identification and verification. The remaining biomass was freeze-dried, ground, weighed and sent to Dr. Huihua Ji, University of Kentucky for alkaloid analysis.
Table 2.2. Endophyte infection frequency (% EIF) results from seed tests (sub-samples taken prior to planting) performed by Dr. Carolyn Young.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment #</th>
<th>% EIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>KY-31 E-</td>
<td>1</td>
<td>0%</td>
</tr>
<tr>
<td>KY-31+</td>
<td>2</td>
<td>99%</td>
</tr>
<tr>
<td>Jesup MaxQ</td>
<td>3</td>
<td>83%</td>
</tr>
<tr>
<td>BarOptima</td>
<td>4</td>
<td>75%</td>
</tr>
<tr>
<td>Texoma MaxQ II</td>
<td>5</td>
<td>93%</td>
</tr>
<tr>
<td>KY+/Texoma</td>
<td>6</td>
<td>94%</td>
</tr>
<tr>
<td>KY+/BarOptima</td>
<td>7</td>
<td>80%</td>
</tr>
<tr>
<td>KY+/Jesup</td>
<td>8</td>
<td>79%</td>
</tr>
<tr>
<td>Texoma/BarOptima</td>
<td>9</td>
<td>91%</td>
</tr>
<tr>
<td>Texoma/Jesup</td>
<td>10</td>
<td>90%</td>
</tr>
<tr>
<td>BarOptima/Jesup</td>
<td>11</td>
<td>77%</td>
</tr>
<tr>
<td>KY+/Texoma/BarOptima</td>
<td>12</td>
<td>91%</td>
</tr>
<tr>
<td>KY+/Texoma/Jesup</td>
<td>13</td>
<td>91%</td>
</tr>
<tr>
<td>KY+/BarOptima/Jesup</td>
<td>14</td>
<td>82%</td>
</tr>
<tr>
<td>Texoma/BarOptima/Jesup</td>
<td>15</td>
<td>81%</td>
</tr>
<tr>
<td>KY+/Texoma/BarOptima/Jesup</td>
<td>16</td>
<td>83%</td>
</tr>
</tbody>
</table>
**Figure 2.2.** Endophyte infection frequency (EIF) by strain from grass seeds collected prior to field cultivation.

### 2.5 Plant Parameters

#### 2.5.1 Plant Species Diversity & Richness

Plot-level plant species diversity was measured using Shannon’s Diversity index (1948):

$$ H = - \sum_{i=1}^{N} \frac{n_i}{N} \ln \left( \frac{n_i}{N} \right) $$

Species diversity was calculated using relative abundance, which was quantified using the biomass of individual species ($n_i$) and total biomass ($N$) per harvested quadrat.
Total aboveground biomass was harvested twice in 2017 (June and October); vegetation was clipped to 7 cm from two randomly placed 50 x 50 cm$^2$ quadrats per plot. Vegetation was individually sorted to species, which varied between plots but in general consisted of: tall fescue (*Schedonorus arundinaceus*), pansy (*Viola bicolor*), speedwell (*Veronica* spp.), unknown forb (UNK forb), mouse-ear chickweed (*Cerastium vulgatum*), cheatgrass (*Bromus tectorum*), orchard grass (*Dactylis glomerata*), buckhorn plantain (*Plantago lanceolata*), crab grass (*Digitaria* spp.), and yellow wood sorrel (*Oxalis stricta*) (Table 2.3).

**Table 2.3.** Relative abundances of aboveground plant species averaged across plots harvested in June and October 2017.

<table>
<thead>
<tr>
<th>Species</th>
<th>Relative Abundance</th>
<th>Species</th>
<th>Relative Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tall fescue</td>
<td>0.982137</td>
<td>Tall fescue</td>
<td>0.989682</td>
</tr>
<tr>
<td>Pansy</td>
<td>0.006649</td>
<td>Orchard grass</td>
<td>0.005729</td>
</tr>
<tr>
<td>UNK forb</td>
<td>0.005032</td>
<td>Crabgrass</td>
<td>0.003825</td>
</tr>
<tr>
<td>Orchard grass</td>
<td>0.003075</td>
<td>Plantain</td>
<td>0.000745</td>
</tr>
<tr>
<td>Cheatgrass</td>
<td>0.001237</td>
<td>Sorrel</td>
<td>4.81E-06</td>
</tr>
<tr>
<td>Chickweed</td>
<td>0.000529</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veronica</td>
<td>0.000223</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plantain</td>
<td>9.67E-05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorrel</td>
<td>2.25E-05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 2.5.2 Fescue Production

Following biomass sorting, tall fescue samples were oven-dried at 60°C for three days and then weighed to calculate total fescue yield (production). A grab sample was taken from each plot and stored at room temperature for future analysis of dry plant matter, such as total C and N.
2.5.3 Alkaloid Concentrations

Sub-samples of freeze-dried, ground tillers, which came from endophyte verification sampling, from each harvest period (June and October 2017) were sent to Dr. Huihua Ji, University of Kentucky and analyzed for loline, peramine, and ergot alkaloid concentrations (see Table 2.3). Ergot alkaloids, ergovaline and ergovalinine, concentrations were quantified using high performance liquid chromatography (HPLC) methods detailed in Yates and Powell (1988). Extraction was performed by mixing 80% methanol with 0.1 g ground sample and mechanically shaking for two hours. Using a syringe, the mixture was then processed through a PreSep column (SPE, C18 disposable columns 100 mg/mL) and a 0.2 μm polystyrene filter (PTFE). The 1st and 2nd mL of extractant were disposed as waste, while the 3rd mL was placed into separately labeled HPLC vials and analyzed via HPLC (Perkins Elmer Series 2000), equipped with an autosampler and fluorescence detector. The elution was created using the following two solutions: 1) 0.1 M ammonium acetate:acetonitrile, 97:3 v/v and 2) 10 % acetonitrile. Samples were separated at a flow rate of 1.2 mL/min using a reverse phased Kinetex XB-C18 column (100 mm x 4.6 mm with 2.6 μm particle size (Phenomenex, USA)). A gradient concentration was created by increasing 22 % mobile phase B to 35 % in 20 minutes to 58 % in 8 minutes to 100 % B and hold for 5 minutes. Then, it was reduced to 22 % B and hold for 9 minutes to regain equilibrium. Ergot alkaloids were detected with excitation at 310 nm and emission at 420 nm.

Loline alkaloids, N–acetylnorloline (NANL), N–formyloline (NFL), and N–acetylloline (NAL), were quantified using gas chromatography (GC) methods detailed in Blankenship et al. (2001). Extractions were conducted by adding sodium bicarbonate and
methylene chloride, which contained 15 μg/mL quinoline as an internal standard, to ground samples and shaken for 1 hour. Samples were then filtered by kimwipe and transferred to amber vials. Analysis was performed using a GC (PerkinElmer Clarus 500) with an autosampler, flame ionization detection (FID) detector, and SPB-1 fused silica capillary column (15 m x 0.53 m, 0.5 μm film thickness) from SUPELCO, USA. The GC temperature regiment was as follows: 80 °C to 160 °C at 20 °C/min, hold for 2 minutes, then ramp at 45°C/min to 290 °C, and hold for 5 minutes. The injector was kept at 250 °C and the detector was held consistent at 275 °C.

Peramine was extracted in two stages using methods detailed in Tapper et al. (1989). Ground plant material was added to 3 mL of methanol:chloroform (1:1, v/v) for 30 min at 18 °C in polyethylene-capped vials and mixed, followed by an addition of 3 mL of hexane:water (1:1, v/v) and mixed for an additional 30 min. The two solvent phases were separated using centrifugation; the lower phase extract was aspirated, eluted through a CBA column, and measured using a reverse-phase HPLC with a C18 column (5 μm particle size, 100 x 8 mm). Peramine detection was set at a fixed-wavelength UV detector at 280 nm.
Table 2.4. Summary of alkaloid compounds synthesized by the different *Epichloë coenophiala* strains used in this study (synthesis capability is indicated with “✓”) (Popay and Tapper, 2006; Johnson *et al.*, 2013; Barenbrug, personal communication). Producers do not report concentrations of ergovaline.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Common Toxic Endophyte</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>AR542 (Jesup MaxQ)</td>
<td>✓</td>
<td>-</td>
<td>-</td>
<td>✓</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AR584 (Texoma MaxQ II)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+E34 (BarOptima)</td>
<td>No published information</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

2.5.4 Insect Abundance

Fall sweep-net samples were used to determine the abundance of active foliar-associated arthropods. Sampling occurred along a single, diagonal transect in each plot, using a 40 cm wide sweep-net with 20 sweeps per plot. Grasshoppers were sampled on September 21st, 2017 and October 2nd, 2017, and leafhoppers were sampled on October 2nd, 2017. Samples were inverted from the sweep-net into sample bags, stapled shut, and immediately placed on ice. In lab, grasshopper and leafhopper samples were sorted and stored in 70% ethanol in 20 ml scintillation vials until they could be identified to the genus level and counted. All grasshoppers were from the family *Acrididae*; and leafhopper were from four genera: *Draeculacephala*, *Graminella*, *Empoasca*, and *Forcipata*. Identification was aided by Drs. Dan Potter and Paul Freytag (University of Kentucky, Entomology Department).
2.6 Soil Sampling and Analyses

Several sets of soil samples were collected during this study. A Giddings Soil Probe was used to collect two 30 cm deep soil samples per plot in Winter 2017 (February 13th-15th, 2017). Each core was sectioned into depth increments of 0-10 cm, 10-20 cm, and 20-30 cm. To calculate bulk density, air-dried core samples were weighed, with a subset of each sample oven-dried at 105°C for 48 hours, and then weighed to determine oven-dried soil water content. Air-dried samples were sieved to 2 mm to homogenize the sample and remove roots, and a sub-sample was sent to Soil Testing Regulatory Services, University of Kentucky to be analyzed for pH, soil texture (including sand, silt, clay fractions), cation exchange capacity, base saturation, exchangeable cations (K, Ca, Mg, Na), macronutrients (P, K, Ca, Mg) and micronutrients (Zn) (Soil and Plant Analysis Council Inc, 2000). Roots separated from sieved samples were stored for further analysis.
For enzyme and microbial analysis, soil samples taken from a sub-set (40 plots) of all treatments (80 plots) were collected once during each season: April 24\textsuperscript{th}, 2017 (Spring), July 18\textsuperscript{th}, 2017 (Summer), October 25\textsuperscript{th}, 2017 (Fall), and January 29\textsuperscript{th}, 2018 (Winter). Five 1.5 cm diameter cores from each plot were taken to a depth of 10 cm and composited. With each seasonal collection, a subsample of roughly 7 g was taken for storage at -80 °C until phospholipid fatty acid analysis could be performed (data not included in thesis) and the remaining soil was bagged and placed on ice in a cooler and immediately transported to the University of Toledo. Extracellular enzyme analysis and chloroform fumigation extractions were performed on transported material.

2.6.1 Soil Microbial Biomass

Soil microbial biomass was measured using a modified chloroform fumigation extraction method [(Scott-Denton \textit{et al.}, 2006) developed from Brookes \textit{et al.} (1985)]. Extraction was performed on fumigated and non-fumigated samples, including three soil-free blanks. For non-fumigated samples, extraction was performed by adding 25 mL of 0.5M K\textsubscript{2}SO\textsubscript{4} to 5 g of fresh soil (or a blank) in a 50 mL tube. Samples were placed on an orbital shaker table for 1 hour, then filtered through 47 mm filter paper using a vacuum filter apparatus and freezing at -20 °C until total organic carbon and total organic nitrogen could be measured. For fumigated samples, 2 mL of ethanol-free chloroform was added to a 250 mL Erlenmeyer flask containing 5 g of fresh soil and stoppered immediately. Samples were then placed in a fume hood and incubated at room temperature for 24 hours. Following incubation, flasks were left uncorked for 30 minutes or until the chloroform evaporated, and then extractions were performed as described above. A Shimadzu organic carbon (TOC-VCPN) analyzer was used to measure total dissolved
organic carbon (DOC). The difference between DOC in fumigated and non-fumigated samples yielded extractable microbial biomass carbon (MBC), which is expressed as μg-C dry soil⁻¹.

2.6.2 Extracellular Enzyme Activity

Extracellular enzyme activity was assessed using methods described in Saiya-Cork et al. (2002) and Weintraub et al. (2007). Six enzymes were assayed using 96-well microplates: 1) β- 1,4 Glucosidase (BG); 2) Leucine amino peptidase (LAP); 3) β- 1,4-N-Acetyl-glucosaminidase (NAG); 4) Phosphatase (PHOS); 5) Phenol oxidase (Phenox); and 6) Peroxidase (Perox). Hydrolytic enzymes (BG, LAP, NAG, PHOS) were fluorometrically assayed using black microplates. Oxidative enzymes (Phenox and Perox) were colorimetrically assayed on clear microplates (Weintraub et al., 2007).
Table 2.5. Extracellular enzymes assayed, associated functions, and respective substrates that were used to quantify their activity. Adapted from Weintraub et al. (2007).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Function</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>β- 1,4-Glucosidase (BG)</td>
<td>Facilitates the hydrolysis of 1,4-linked β-D-glucose residues from compounds such as cellobiose, a short chain cellulose oligomers, to release β-D glucose.</td>
<td>4-MUB- β-D-glucoside</td>
</tr>
<tr>
<td>Leucine aminopeptidase (LAP)</td>
<td>Broad spectrum enzyme that enables the hydrolysis of leucine and amino acid residues from N-terminus of peptides, strongly targeting leucine, and hydrolyzing amino acid amides and methyl esters.</td>
<td>L-Leucine-7-amino-4-methylcoumarin</td>
</tr>
<tr>
<td>B-1,4-N-acetyl-glucosaminidase (NAG)</td>
<td>Facilitates the hydrolysis of N-acetyl-β-D-glucosaminidase from 1,4-β residues in chitin-derived oligomers.</td>
<td>4-MUB- N-acetyl-β-D-glucosaminidase</td>
</tr>
<tr>
<td>Phosphatase (PHOS)</td>
<td>Facilitates release of mineralized organic P from hydrolysis of phosphoric ester bonds.</td>
<td>4-MUB-phosphate</td>
</tr>
<tr>
<td>Phenol oxidase (Phenox)</td>
<td>Uses oxygen to degrade aromatic carbon compounds like benzenediols into semiqinnones (free radicals).</td>
<td>L-DOPA</td>
</tr>
<tr>
<td>Peroxidase (Perox)</td>
<td>Reduces H₂O₂ to catalyze oxidation reactions; Perox is also considered a lignolytic enzymes due to its ability to degrade irregularly-structured molecules.</td>
<td>L-DOPA</td>
</tr>
</tbody>
</table>
For each sample, 1 g of fresh, hand-homogenized soil was weighed into a 125 mL plastic Nalgene bottle to make soil slurries; 125 mL of 50 mM sodium bicarbonate buffer (calibrated to match soil pH at harvest, 7.1) was added to bottles and blended for 1 minute using a Biospec Tissue Tearer. Sample slurries were then placed in a 13 °C incubator until samples were ready to plate. During plating, 200 μL of samples were pipetted from continuously-stirred slurries using large orifice tips into the corresponding columns of the 96-well plates with 16 replicate wells for each sample and enzyme. For fluorometric enzymes (BG, LAP, NAG, PHOS), 50 μL of 50 mM substrate solution was added to each sample well. Blank wells for each sample and enzyme received only 200 μL of soil slurry and 50 μL of sodium bicarbonate buffer solution. Negatives control wells for each sample and enzyme received 50 μL of the corresponding substrate and 200 μL of buffer solution. Quench standard wells, to correct for how much fluorescence was blocked by soil particles, received 50 μL of standard, 10 mM 4-methylumbelliferone (for BG, NAG, PHOS) or 50 μL of 7-amino-4-methylcounarin (LAP), and 200 μL of soil slurry. Reference standard wells received 200 μL of buffer solution and 50 μL of standard. Blank, control, and quench standard wells had 8 replications each.

Once plated, fluorometric plates were incubated at 13 °C for 5 hours; fluorescence was then quantified using a Bio-Tek Synergy HT microplate reader at 365 nm excitation and 460 nm emission filters. Enzyme activity is expressed as nmol activity h⁻¹ g soil⁻¹ after values are corrected using quench and negative control values. For colorimetric assays (Phenox, Perox), sample and substrate were added in a similar fashion as fluorometric assays, except 25 mM L-DOPA was used as the substrate, and Perox received an additional 10 μL 0.3% H₂O₂ solution in substrate, blank, and negative control
wells. Colorimetric plates were then incubated at 13 °C for 3 hours, after which absorbance was measured on the Bio-Tek Synergy HT microplate reader with 460 nm emission filters. Phenox and Perox activities were expressed as μmol activity h⁻¹ g soil⁻¹. The difference between Phenol oxidase activity and Peroxidase activity represented net Peroxidase activity, which was reported as Perox (Weintraub et al., 2007).

2.6.3 Greenhouse Gas Emissions

Trace gas fluxes (NH₃, N₂O, and CO₂) were measured biweekly between 9:00 to 14:00 from March 28th, 2017 to April 17th, 2018. Measurements were taken from permanently fixed chambers using a 1412 Infrared Photoacoustic Spectroscopy (PAS) gas analyzer (Innova Air Tech Instruments, Ballerup, Denmark) (Castellano et al., 2010; Iqbal et al., 2013). Twenty-four hours prior to the first reading (March 27th, 2017), chambers were randomly installed in each plot. Chambers consisted of a polyvinyl chloride (PVC) tube (10 cm in diameter and 10 cm tall) sunk 5 cm in the ground. A PVC lid of equal dimensions sealed with the base during measurements, with two sampling ports fixed atop the lid leading to the gas analyzer. Harvest-associated tractor activity in the plots required chamber relocation in half the plots, which occurred on June 26th, 2017. Prior to each measurement period, if necessary, vegetation in the chambers was cut to roughly 4 cm. Sampling periods lasted five minutes, with one-minute sampling intervals at a rate of 1.8 Lmin⁻¹. The gas fluxes were calculated by using the following equation:

\[ F = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \rho \times \alpha \]

\( F \) represents the total gas production rate (mg m⁻² h⁻¹), \( \Delta C/\Delta t \) is the increase or decrease of gas concentration (C) in the chamber over time (t), \( V \) represents the volume
of the chamber (m$^3$), $A$ is the chamber cross-sectional surface area (m$^2$), $\rho$ signifies density of gas at 20°C, and $\alpha$ denotes the gas mass conversion coefficient.

Soil moisture and temperature measurements were both taken concurrently near each chamber at approximately 10 cm depth using a digital thermometer and a ML2 ThetaProbe Soil Moisture Sensor (Delta-T Devices, Cambridge, UK).

2.7 Statistical Analysis

This study evaluated the effects of increasing above-ground grass-endophyte symbiont diversity on above- and below-ground ecosystem function. A randomized complete block design (RCBD) with repeated measures ANOVA generalized linear mixed model was used with SAS 9.4 (Cary, NC) to evaluate time, treatment, and symbiotic diversity main effects and interactions. Treatment (16 grass-endophyte treatments) and Symbiotic Diversity (SymDiv) level (0-4) were treated as fixed effects and blocking (five blocks) was treated as a random effect. Overall, time, which was designated as a repeated measure, varied by parameter: extracellular enzyme activity and soil microbial biomass samples were collected seasonally (April, July, October, and January); trace gas measurements were collected bi-weekly; plant diversity and fescue production were harvested twice a year (June and October); and insect abundance was sampled in August (grasshoppers) and September (grasshoppers and leafhoppers).

I performed the following transformations, when needed, to meet statistical assumptions of normality: eleven measurements [grasshopper abundance, alkaloid concentrations (peramine, total loline, NAL, and NFL), certain enzyme activities (BG, PHOS, and LAP), MBC, temperature and CO$_2$ fluxes] received square-root
transformations; four parameters were log(1+)-transformed (ergovaline, ergovalinine, POX, and PER); and finally, NAG was log-transformed. Statistical analyses were conducted (and p-values reported) on transformed data of listed measurements.

Covariance structures were primarily unstructured, unless a different structure improved model fit. A Tukey’s HSD was used as a post-hoc evaluation of fixed effects means; if significant interactions were detected, a Least Squares Means (LSMEANS) statement was used to compare separate treatment, SymDiv, and time effect means. P-values < 0.05 were considered significant.
Chapter Three: Results

3.1 Aboveground Parameters

3.1.1 Tall Fescue Production

There were no significant main effects of individual treatment or symbiotic diversity level for tall fescue production, though there was a significant time effect (Table 3.1). Overall, biomass production was 1.7 times higher in June than October. Although there were no significant interactions between time and treatment, or symbiotic diversity, fescue production across treatments varied over the two time periods. In the summer, the zero symbiotic diversity treatment (KY-) yielded the lowest biomass and the BarOptima (SymDiv = 1) treatment had the highest yield (Figure 3.1A). In the fall, Jesup (SymDiv1) and BarOptima/Jesup (SymDiv2) treatments had the lowest yield, while KY+/Jesup (SymDiv2) had the highest (Figure 3.1B).

3.1.2 Plant Species Richness

Plant species richness also varied across time, but not across individual treatments or symbiotic diversity (Table 3.1). Generally, species diversity was slightly higher in June (Shannon’s Diversity Index = 0.341 averaged across treatments) than October (0.327).
Table 3.1. Significance tests for fescue production and plant species diversity over seasonal harvests (time), individual treatments (Trmt), and symbiotic diversity (SymDiv) levels. Bold indicates statistical significance (P < 0.05).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Fescue Production</th>
<th>Plant Species Diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n, d, F, P</td>
<td>n, d, F, P</td>
</tr>
<tr>
<td>Trmt</td>
<td>15,122 1.31 0.2086</td>
<td>15,124 0.30 0.9950</td>
</tr>
<tr>
<td>Time</td>
<td>1,122 405.81 &lt;0.0001</td>
<td>1,124 3.49 0.0642</td>
</tr>
<tr>
<td>Trmt*Time</td>
<td>15,122 0.60 0.8692</td>
<td>15,124 0.27 0.9967</td>
</tr>
<tr>
<td>SymDiv</td>
<td>4,144 1.52 0.1982</td>
<td>4,146 0.33 0.8560</td>
</tr>
<tr>
<td>Time</td>
<td>1,144 238.21 &lt;0.0001</td>
<td>1,146 5.36 0.0220</td>
</tr>
<tr>
<td>SymDiv*Time</td>
<td>4,144 0.46 0.7684</td>
<td>4,146 0.64 0.6339</td>
</tr>
</tbody>
</table>
Figure 3.1. Tall fescue production for individual treatments in June (A) and October (B) 2017. Symbiotic diversity level means ($x_n=...$) are shown above bars. Time was significant ($P < 0.0001$); Treatment and symbiotic diversity, including respective interactions with time, were not significant. Error bars represent ± 1 S.E.
Figure 3.2. Individual treatment and symbiotic diversity effects on plant species richness in June (A) and October (B) 2017. Symbiotic diversity level means ($x_n=\ldots$) are shown above bars. Error bars represent ± 1 S.E.
3.1.3 Alkaloid Production

3.1.3a Peramine

For both harvests, alkaloid production was quantified for each grass-endophyte treatment to assess differences in the production of insect- and mammalian-toxic alkaloid compounds. For peramine, an insect-active secondary metabolite, a significant interaction between treatment and time was detected (Table 3.2). Overall, peramine concentrations were about two times higher in June (averaged across treatments = 3.0 µg/g) than in October (1.4 µg/g). All but three treatments produced higher concentrations of peramine in June versus October: KY- (SymDiv0), BarOptima (SymDiv1), and Texoma (SymDiv1) did not have significantly different peramine levels across the two time points (Figure 3.3). Treatments containing Texoma and BarOptima tended to have lower concentrations of peramine in plant tissue. Additionally, peramine production was significantly affected by symbiotic diversity level (Table 3.2). During both June and October, SymDiv1-4 treatments had higher production levels than SymDiv0 treatments, but there were no discernable trends discovered across SymDiv1-4 (Figure 3.3).

3.1.3b Lolines

On an individual loline compound basis, there was a significant interaction detected between time and treatment for N-acetylnorloline (NANL) production (Table 3.3). Treatments did not significantly vary across time, except four that had higher production levels in June than October: Jesup (SymDiv1), Texoma/Jesup (SymDiv2), BarOptima/Jesup (SymDiv2), and Texoma/BarOptima/Jesup (SymDiv3) (Figure 3.4 A & B). In both June and October, Jesup (SymDiv1) had the highest concentrations of NANL detected, while BarOptima and Texoma (both SymDiv1) consistently had the lowest.
While there was no significant symbiotic diversity effect, in both June and October SymDiv$_{1-4}$ were higher than SymDiv$_0$. There was also a significant treatment and time interaction for N-acetyllolline (NAL) production (Table 3.3), reflecting the fact that all but three treatments had greater NAL concentrations in October: KY- (SymDiv$_0$), Jesup (SymDiv$_1$), and Texoma/Jesup (SymDiv$_2$) had no significant differences between the two time points (Figure 3.4 C & D). Like NANL, there was no significant symbiotic diversity effect, though SymDiv levels 1-4 were higher in production than SymDiv$_0$. There were significant treatment and symbiotic diversity effects detected for N-formylolline (NFL) production (Table 3.3). Overall, KY+ (SymDiv$_1$) produced the greatest levels of NFL (averaged across time = 237 µg/g), and Jesup (SymDiv$_1$) had the lowest levels (2 µg/g). SymDiv$_{1-4}$ had higher levels of NFL production than SymDiv$_0$, though there were no significant differences across SymDiv$_{1-4}$ (Figure 3.5).

There was a significant treatment by time interaction observed for total loline concentrations (Table 3.3). Total loline concentrations were slightly higher in the fall (averaged across treatments = 242 µg/g) compared to the spring (223 µg/g) (Figure 3.6). Individual treatments did not significantly differ in total loline concentrations across time, bar three treatments: KY+/Texoma and KY+/BarOptima (both SymDiv$_2$ treatments) had greater production in the fall, whereas Texoma/Jesup (also SymDiv$_2$) had higher spring concentrations. Endophyte treatments containing KY+ tended to have higher levels of total loline concentrations (Figure 3.6 A, B). There also was a symbiotic diversity effect detected; SymDiv$_{1-4}$ produced higher concentrations of total lollines than SymDiv$_0$, though there were no differences between SymDiv$_{1-4}$ (Figure 3.6).
3.1.3c Ergots

There were significant treatment by time interactions for individual ergots compounds, ergovaline and ergovalinine (Table 3.4). Production for both ergots alkaloids were about two times higher in June [averaged across treatments = 0.1 (ergovaline) and 0.08 (ergovalinine)] than October [0.05 µg/g (ergovaline) and 0.03 µg/g (ergovalinine)]. There were lower ergovaline and ergovalinine concentrations in treatments containing novel endophytes compared to treatments with KY+ (Figure 3.7 B, C, E, F). A significant symbiotic diversity by time interaction was detected for ergovalinine production (Table 3.4). Within a season, SymDiv levels 1-4 were higher than SymDiv0. While June tended to have greater ergovalinine concentrations than October, this temporal trend was only significant for SymDiv3 and SymDiv4 (Figure 3.7 C, F).

There was a significant treatment by time interaction also identified for total ergots concentrations (Table 3.4). Levels were roughly two times higher in June (averaged across treatments = 0.19 µg/g) than in October (0.08 µg/g). There were no significant treatment differences detected within October; however, in June, treatments containing KY+ tended to have higher ergot concentrations than monocultures and mixtures containing novel endophytes (Figure 3.7 A, D).
Table 3.2. Significance tests for peramine production by treatment (Trmt) and Symbiotic Diversity (SymDiv) level over seasonal harvests (Time). Bold indicates statistical significance (P < 0.05).

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>Peramine</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n, d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trmt</td>
<td>15,120</td>
<td>4.81</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>1,120</td>
<td>172.89</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Trmt*Time</td>
<td>15,120</td>
<td>2.23</td>
<td>0.0087</td>
<td></td>
</tr>
<tr>
<td>SymDiv</td>
<td>4,142</td>
<td>4.48</td>
<td>0.0019</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>1,142</td>
<td>77.78</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>SymDiv*Time</td>
<td>41,442</td>
<td>1.30</td>
<td>0.2713</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.3. Individual treatments effects on peramine concentrations during June (A) and October (B) 2017. Symbiotic diversity level means ($x_n\ldots$) are shown above bars. Uppercase letters represent significant treatment differences within a season. Inset graph (units are similar to main figures) depicts symbiotic diversity (SymDiv) level effects on peramine concentrations averaged across time. Lowercase letters within bars represent significant SymDiv differences. Error bars represent average ± 1 S.E.
Table 3.3. Significance tests on N-acetylnorloline (NANL), N-formyloline (NFL), N-acetylloline (NAL), and total lolines (sum of NANL, NFL, NAL concentrations) production by treatment (Trmt), symbiotic diversity (SymDiv) level, and harvest season (Time). Bold indicates statistical significance (P < 0.05).

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF n, d</th>
<th>NANL F P</th>
<th>NFL F P</th>
<th>NAL F P</th>
<th>Total Lolines F P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trmt</td>
<td>15,120</td>
<td>4.87 &lt;0.0001</td>
<td>8.13 &lt;0.0001</td>
<td>8.22 &lt;0.0001</td>
<td>6.26 &lt;0.0001</td>
</tr>
<tr>
<td>Time</td>
<td>1,120</td>
<td>12.26 0.0007</td>
<td>2.59 0.1104</td>
<td>250 &lt;0.0001</td>
<td>0.89 0.3470</td>
</tr>
<tr>
<td>Trmt*Time</td>
<td>15,120</td>
<td>2.12 0.0132</td>
<td>0.83 0.6383</td>
<td>3.33 0.0001</td>
<td>1.75 0.0493</td>
</tr>
<tr>
<td>SymDiv</td>
<td>4,142</td>
<td>2.25 0.0666</td>
<td>3.00 0.0205</td>
<td>2.42 0.0512</td>
<td>3.66 0.0072</td>
</tr>
<tr>
<td>Time</td>
<td>1,142</td>
<td>7.63 0.0065</td>
<td>1.64 0.2025</td>
<td>83.06 &lt;0.0001</td>
<td>0.09 0.7691</td>
</tr>
<tr>
<td>SymDiv*Time</td>
<td>4,142</td>
<td>0.18 0.9477</td>
<td>0.76 0.5551</td>
<td>1.46 0.2163</td>
<td>0.14 0.9686</td>
</tr>
</tbody>
</table>
A) NANL (ug/g)

$X_0 = 40.40 \quad X_1 = 70.53 \quad X_2 = 75.40 \quad X_3 = 63.30 \quad X_4 = 79.60$

B) Symbiotic Diversity Level

- 0
- 1
- 2
- 3
- 4

$X_0 = 11.80 \quad X_1 = 46.55 \quad X_2 = 50.93 \quad X_3 = 46.60 \quad X_4 = 47.80$

C) Treatment

<table>
<thead>
<tr>
<th>KY-</th>
<th>KY+</th>
<th>Jesup</th>
<th>BarOptima</th>
<th>Texoma</th>
<th>KY+/Tex</th>
<th>KY+/BarO</th>
<th>KY+/Jes</th>
<th>Tex/BarO</th>
<th>KY+/Tex/BarO</th>
<th>KY+/Tex/Jes</th>
<th>KY+/BarO/Jes</th>
<th>Tex/BarO/Jes</th>
<th>KY+/Tex/BarO/Jes</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>AB</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>ABC</td>
<td>ABC</td>
<td>A</td>
<td>C</td>
<td>ABC</td>
<td>A</td>
<td>ABC</td>
<td>C</td>
<td>A</td>
</tr>
</tbody>
</table>

D) NANL (ug/g)

$X_0 = 10.0 \quad X_1 = 79.0 \quad X_2 = 82.7 \quad X_3 = 79.6 \quad X_4 = 72.4$
Figure 3.4. Individual treatments effects on N – acetylnorloline (NANL) and N – acetylloline (NAL) production during June (A and C) and October (B and D) 2017. Symbiotic diversity (SymDiv) level means ($\mu_n = \ldots$) are shown above bars. Uppercase letters represent significant treatment differences within a time point. Significant Treatment*Time interactions are represented by symbol (*) above bar. Error bars represent average ± 1 S.E.
**Figure 3.5.** Individual treatments effects on N – formyloline (NFL) concentrations averaged across time. Symbiotic diversity (SymDiv) level means ($x_0=...$) are shown above bars. Uppercase letters represent significant treatment differences. Lowercase bolded letters indicate significant SymDiv level differences. Error bars represent average ± 1 S.E.
Figure 3.6. Individual treatments effects on total loline concentrations during June (A) and October (B) 2017. Symbiotic diversity (SymDiv) level means (\(x_n=\ldots\)) are shown above bars. Uppercase letters represent significant treatment differences within a time point. Inset graph (units are similar to main figures) depicts SymDiv effects on total loline concentrations averaged across time; lowercase letters represent significant SymDiv level differences. Error bars represent average ± 1 S.E.
Table 3.4. Significance tests for ergovaline, ergovalinine, and total ergots (sum of ergovaline and ergovalinine concentrations) production by treatment (Trmt) and symbiotic diversity (SymDiv) level. Bold indicates statistical significance (P < 0.05).

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF n, d</th>
<th>Ergovaline</th>
<th></th>
<th>Ergovalinine</th>
<th></th>
<th>Total Ergots</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Trmt</td>
<td>15,120</td>
<td>7.34</td>
<td>&lt;0.0001</td>
<td>8.24</td>
<td>&lt;0.0001</td>
<td>7.72</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time</td>
<td>1,120</td>
<td>54.54</td>
<td>&lt;0.0001</td>
<td>20.76</td>
<td>&lt;0.0001</td>
<td>52.83</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trmt*Time</td>
<td>15,120</td>
<td>4.48</td>
<td>&lt;0.0001</td>
<td>4.04</td>
<td>&lt;0.0001</td>
<td>4.93</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SymDiv</td>
<td>4,142</td>
<td>1.44</td>
<td>0.2228</td>
<td>1.71</td>
<td>0.1516</td>
<td>1.33</td>
<td>0.2600</td>
</tr>
<tr>
<td>Time</td>
<td>1,142</td>
<td>17.97</td>
<td>&lt;0.0001</td>
<td>7.98</td>
<td>0.0054</td>
<td>16.71</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SymDiv*Time</td>
<td>4,142</td>
<td>1.39</td>
<td>0.2403</td>
<td>3.82</td>
<td>0.0056</td>
<td>1.42</td>
<td>0.2295</td>
</tr>
</tbody>
</table>
**Figure 3.7.** Individual treatments effects on concentrations of ergovaline, ergovalinine alkaloids, and their sum (total ergots) during June (A-C) and October (D-F) 2017. Symbiotic diversity (SymDiv) level means ($x_\bar{n}$=…) are shown above bars. Uppercase letters above bars (B) represent significant treatment differences within a time point. Significant Treatment*Time interactions are represented by symbol (*) above bar. Inset graph (units are similar to main figures) in (F) represents SymDiv effects on ergovalinine concentration; significant SymDiv*Time interactions are represented by symbol (*) above bar. Error bars represent ± 1 S.E.
3.1.4 Insect Population Abundance

3.1.4a Leafhoppers

There was a significant treatment effect on leafhopper abundance (Table 3.5). The lowest leafhopper abundance was measured in the Texoma/Jesup (SymDiv$_2$) treatment, and the highest was measured in the BarOptima monoculture (SymDiv$_1$). Clear trends associated with specific symbiotic treatments were not observed (Figure 3.8). For example, all mixtures containing BarOptima did not always support high leafhopper numbers (e.g., Texoma/BarOptima). Although average leafhopper abundance increased from SymDiv$_0$ to SymDiv$_4$ (Figure 3.8), this pattern did not prove statistically significant (Table 3.5). Leafhopper abundances did not exhibit high correlation ($r^2 < 0.3$) to alkaloid concentrations (graph not shown).

3.1.4b Grasshoppers

Grasshopper abundance was significantly affected by treatment and time, but not their interaction (Table 3.5). Overall, populations were roughly two times higher in September (averaging across treatments n=20) than in August (n=9; data not shown). KY+/Texoma/Jesup (SymDiv$_3$) contained the highest numbers of grasshoppers, and the lowest were measured in KY+/BarOptima/Jesup (also a SymDiv$_3$ treatment) (Figure 3.9). No apparent symbiotic diversity trends were observed in either August or September. Grasshopper abundances did not exhibit high correlation ($r^2 < 0.3$) to alkaloid concentrations (graph not shown).
**Table 3.5.** Significance tests for leafhopper and grasshopper abundance by individual treatment (Trmt) and symbiotic diversity (SymDiv) levels. Leafhoppers were only sampled for once. Significance tests for grasshopper abundance by time additionally shown. Bold indicates statistical significance (P < 0.05).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Leafhopper Abundance</th>
<th>Grasshopper Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n, d</td>
<td>F</td>
</tr>
<tr>
<td><strong>Trmt</strong></td>
<td>15,60</td>
<td>1.94</td>
</tr>
<tr>
<td><strong>Time</strong></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Trmt*Time</strong></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>SymDiv</strong></td>
<td>4,71</td>
<td>0.84</td>
</tr>
<tr>
<td><strong>Time</strong></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>SymDiv*Time</strong></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 3.8. September 2017 leafhopper (*Cicadellidae*) abundance across individual treatments. Symbiotic diversity level means ($x_n =$...) are shown above bars. There was a significant treatment effect ($P < 0.05$); however, Tukey’s HSD means separation tests failed to identify significant differences across individual treatments. Error bars represent ± 1 S.E.
Figure 3.9. Treatment effects on grasshopper populations averaged across both sample periods (August and September 2017). Symbiotic diversity level means ($x_0 = \ldots$) are shown above bars. There was a significant treatment effect ($P < 0.05$); however, Tukey’s HSD means separation tests failed to identify significant differences across individual treatments. Error bars represent ± 1 S.E.
3.2 Belowground Parameters

3.2.1 Soil Microbial Biomass

There were significant main effects of symbiotic diversity and time for microbial biomass as measured by chloroform fumigation extraction, but no significant interaction (Table 3.6). Microbial biomass C (MBC), on average, was 1.4 times higher in April and October compared to July and January (inset graph in Figure 3.10). This pattern was consistent across symbiotic diversity levels. SymDiv\(_0\) had the highest MBC, roughly four times the measured biomass of the lowest treatment –SymDiv\(_3\). SymDiv\(_1\) and SymDiv\(_2\) had intermediate levels of MBC (Figure 3.10).
Table 3.6. Significance tests for microbial biomass carbon (MBC) by individual treatment (Trmt) and symbiotic diversity (SymDiv) levels across time. Bold indicates statistical significance (P < 0.05).

<table>
<thead>
<tr>
<th>Effect</th>
<th>MBC</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n, d</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Trmt</td>
<td>7,121</td>
<td>1.46</td>
<td>0.1872</td>
</tr>
<tr>
<td>Time</td>
<td>3,121</td>
<td>17.46</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trmt*Time</td>
<td>21,121</td>
<td>1.19</td>
<td>0.2740</td>
</tr>
<tr>
<td>SymDiv</td>
<td>3,141</td>
<td>4.03</td>
<td>0.0088</td>
</tr>
<tr>
<td>SymDiv*Time</td>
<td>9,141</td>
<td>1.04</td>
<td>0.4098</td>
</tr>
<tr>
<td>Time</td>
<td>3,141</td>
<td>13.57</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Figure 3.10. Symbiotic Diversity (SymDiv) level effects on microbial biomass carbon (MBC). Lowercase letters represent significant differences between SymDiv levels. Inset graph (units similar to main figure) depicts MBC differences over time; lowercase letters represent significant seasonal differences. Error bars represent ± 1 S.E.
3.2.2 Extracellular Enzyme Activity

All of the extracellular enzymes assayed were significantly affected by time (Table 3.7), though seasonal patterns varied by enzyme. In direct contrast to MBC seasonal trends, β-1, 4, Glucosidase (BG) levels were highest during July and January and lower in April and October (inset Figure 3.11B). For all other measured enzymes, October tended to be highest with either April [phosphatase (PHOS), β-1, 4-N-Acetyl-glucosaminidase (NAG), phenol oxidase (POX), peroxidase (PER)] or the lowest in January [leucine amino peptidase (LAP)] (Figures 3.12B, 3.13, 3.14).

Significant treatment effects, which were only detected for hydrolytic enzymes, varied by enzyme; only BG and LAP experienced significant treatment by time interactions (Table 3.7). During April and July, BG activity significantly differed by treatment, but no treatment differences were identified during October and January (Figure 3.11A). Generally, KY+/Texoma/Jesup (SymDiv3) had the greatest BG activity (Figure 3.11A). For LAP, significant treatment differences were detected during April and October. In April, KY+/Texoma (SymDiv2) had the lowest recorded activity (0.10 ± 0.04 nmol activity h\(^{-1}\) μg-C\(^{-1}\)) and KY+/Texoma/Jesup (SymDiv3) had the highest (0.20 ± 0.03 nmol activity h\(^{-1}\) μg-C\(^{-1}\); Figure 3.12A). Conversely, during October, KY+/Texoma/Jesup (SymDiv3) had the lowest measured activity (0.20 ± 0.10 nmol activity h\(^{-1}\) μg-C\(^{-1}\)) and KY+ (SymDiv1) had the highest (1.30 ± 0.40 nmol activity h\(^{-1}\) μg-C\(^{-1}\); Figure 3.12A).

Both NAG and PHOS were significantly affected by treatment with no significant interactions with time (Table 3.7). The KY+/Texoma/Jesup treatment (SymDiv3) had the
highest recorded activity for PHOS (0.92 ± 0.20 nmol activity h⁻¹ μg-C⁻¹; Figure 3.13A), and the second highest activity for NAG (0.32 ± 0.10 nmol activity h⁻¹ μg-C⁻¹; Figure 3.13B). KY+/Texoma had the highest recorded NAG activity with 0.34 ± 0.20 nmol activity h⁻¹ μg-C⁻¹, but also had significant variability across replicates.

Symbiotic diversity (SymDiv) level significantly affected all assayed hydrolases and one oxidoreductase, peroxidase (POX) (Table 3.7). LAP and POX activity had a significant SymDiv interaction with time. Within a season, both July and October LAP and POX activities significantly differed by treatment (no differences detected for April and January) (Figures 3.12B and 3.14 A). For both LAP and POX, seasonal trends varied by SymDiv level. Activities of both enzymes peaked in July for SymDiv₃, while the other SymDiv levels tended to peak in October. There were significant SymDiv level effects for BG, PHOS, and NAG activity; for all three exoenzymes, SymDiv₃ was significantly higher than SymDiv₀, SymDiv₁, and SymDiv₂ (Figure 3.11B; Figure 3.13 A, B).
Table 3.7. Significance tests for extracellular enzyme activities BG (β-1, 4, Glucosidase), NAG (β-1, 4-N-Acetyl-glucosaminidase), PHOS (phosphatase), LAP (leucine amino peptidase), PER (peroxidase), and POX (phenol oxidase) by treatment (Trmt) and symbiotic diversity (SymDiv) level. Bold indicates statistical significance (P < 0.05).

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF n, d</th>
<th>BG</th>
<th></th>
<th>NAG</th>
<th></th>
<th>PHOS</th>
<th></th>
<th>LAP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Trmt</td>
<td>7,121</td>
<td>3.58</td>
<td><strong>0.0015</strong></td>
<td>7.16</td>
<td><strong>&lt;0.0001</strong></td>
<td>6.72</td>
<td><strong>&lt;0.0001</strong></td>
<td>2.74</td>
<td><strong>0.0113</strong></td>
</tr>
<tr>
<td>Time</td>
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<td>104.11</td>
<td><strong>&lt;0.0001</strong></td>
<td>113.02</td>
<td><strong>&lt;0.0001</strong></td>
<td>93.53</td>
<td><strong>&lt;0.0001</strong></td>
<td>72.56</td>
<td><strong>&lt;0.0001</strong></td>
</tr>
<tr>
<td>Trmt*Time</td>
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<td>1.74</td>
<td><strong>0.0330</strong></td>
<td>1.32</td>
<td>0.1766</td>
<td>1.35</td>
<td>0.1599</td>
<td>3.69</td>
<td><strong>&lt;0.0001</strong></td>
</tr>
<tr>
<td>SymDiv</td>
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<td>12.47</td>
<td><strong>&lt;0.0001</strong></td>
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<td><strong>&lt;0.0001</strong></td>
<td>2.76</td>
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</tr>
<tr>
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<td><strong>&lt;0.0001</strong></td>
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<td><strong>&lt;0.0001</strong></td>
<td>46.60</td>
<td><strong>&lt;0.0001</strong></td>
</tr>
<tr>
<td>SymDiv*Time</td>
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<td>0.1632</td>
<td>0.94</td>
<td>0.4923</td>
<td>0.85</td>
<td>0.5736</td>
<td>5.05</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF n, d</th>
<th>PER</th>
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<th>POX</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Trmt</td>
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<td>0.09</td>
<td>1.17</td>
<td>0.325</td>
</tr>
<tr>
<td>Time</td>
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<td>3.15</td>
<td><strong>0.0273</strong></td>
</tr>
<tr>
<td>Trmt*Time</td>
<td>21,124</td>
<td>1.05</td>
<td>0.41</td>
<td>1.49</td>
<td>0.0941</td>
</tr>
<tr>
<td>SymDiv</td>
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<td>0.6649</td>
<td>3.04</td>
<td><strong>0.0312</strong></td>
</tr>
<tr>
<td>Time</td>
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<td><strong>&lt;0.0001</strong></td>
<td>4.41</td>
<td><strong>0.0054</strong></td>
</tr>
<tr>
<td>SymDiv*Time</td>
<td>9,140</td>
<td>0.99</td>
<td>0.455</td>
<td>2.75</td>
<td><strong>0.0054</strong></td>
</tr>
</tbody>
</table>
**Figure 3.11.** Extracellular enzyme activities for BG (β-1, 4, Glucosidase) normalized by microbial biomass carbon by (A) individual treatment and (B) symbiotic diversity level (SymDiv). Uppercase letters indicate significant treatment differences within a time point, if there were significant differences (only in April and July). Lowercase letters within bars represent significant differences in SymDiv levels. Inset graph (units similar to main figures) in (B) shows seasonal BG activity averaged across SymDiv level; lowercase letters within bars represent significant seasonal differences. Error bars represent average ± 1 S.E.
Figure 3.12. Extracellular enzyme activities for LAP (leucine amino peptidase) normalized by microbial biomass carbon by (A) individual treatment and (B) symbiotic diversity level (SymDiv). Lowercase letters represent significant treatment differences across time; if there were significant treatment differences within a time point, these are represented by uppercase letters (April and October). Lowercase letters represent significant SymDiv differences across time; if there were significant SymDiv differences within a time point, these are represented by uppercase letters (July and October). Error bars represent average ± 1 S.E.
A) PHOS

B) NAG

Symbiotic Diversity Level

Treatment

nmol h⁻¹ ug-C⁻¹

0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4

April July October January

nmol h⁻¹ ug-C⁻¹

0.0 0.1 0.2 0.3 0.4 0.5 0.6

April July October January

nmol h⁻¹ ug-C⁻¹

KY- KY+ Jesup Texoma KY+/Texoma KY+/Jesup Texoma/Jesup KY+/Jesup/Texoma

0.1 0.2 0.3 0.4 0.5 0.6

KY- KY+ Jesup Texoma KY+/Texoma KY+/Jesup Texoma/Jesup KY+/Jesup/Texoma

A

B

A

B

A

B
**Figure 3.13.** Extracellular enzyme activities normalized by microbial biomass carbon across individual treatment and Symbiotic Diversity (SymDiv) levels. (A) PHOS = Phosphatase; (B) NAG = β-1, 4-N-Acetyl- glucosaminidase. Inset graphs (units similar to main figures) in (A) and (B) depict seasonal enzyme activity averaged across treatment; small letters represent significant seasonal differences. Both treatment and SymDiv were significant; small letters within bars represent significant treatment differences, while bolded uppercase letters above bars indicate significant differences in SymDiv level. Error bars represent average ± 1 S.E.
Figure 3.14. Extracellular enzyme activities normalized by microbial biomass carbon across individual treatment and Symbiotic Diversity (SymDiv) levels. (A) POX = phenol oxidase; (B) PER = peroxidase. Inset graph (units are similar to main figure) in (B) depicts seasonal enzyme activity averaged across treatment; small letters represent significant seasonal differences. Uppercase letters (A) represent significant SymDiv differences within a time point (only July and October). Error bars represent average ± 1 S.E.
3.2.3 Trace Gas Fluxes

Significant seasonal trends were observed for soil moisture, soil temperature, CO₂, and N₂O (but not for NH₃; Tables 3.8). Soil moisture experienced a low of 17.1 % VWC ± 1.0 (July 2017) and a high of 40.0 % VWC± 1.8 (March 2018), and soil temperature had a low of 1.58 °C ± 0.2 (January 2018) and a high of 23 °C ± 0.3 (July 2017) (Figure 3.18). The warm, wet spring and summer months supported the greatest CO₂ and N₂O fluxes (Figures 3.15, 3.16, 3.18). Carbon dioxide levels experienced maxima emission in early April 2017 (309.9 ± 31.3 mg C m⁻²h⁻¹ measured in treatment KY+/BarOptima, SymDiv₂; Figure 3.15) and lows in late January 2018 (9.5 ± 4.0 mg C m⁻²h⁻¹ measured in BarOptima/Jesup treatment, SymDiv₂; Figure 3.15). Seasonal patterns were not as apparent for N₂O; emission maxima were recorded during early May 2017 (410.4 ± 149.9 μg N m⁻²h⁻¹ in KY+/Texoma/BarOptima, SymDiv₃; Figure 3.16) and minima in mid-March 2018 (-167.5 ± 97.3 μg N m⁻²h⁻¹ in BarOptima, SymDiv₁; Figure 3.16).

Although no main effect of treatment was observed for soil moisture, soil temperature, CO₂, N₂O, or NH₃, symbiotic diversity was significant for NH₃ and CO₂ had significant SymDiv by time interactions (Table 3.8). SymDiv₀ tended to have the lowest CO₂ emissions when overall CO₂ fluxes were high (spring and summer); however, in the winter, this trend reversed (Figure 3.15). For NH₃, SymDiv₀ had lower fluxes compared to SymDiv₃ and SymDiv₂ (Figure 3.17). SymDiv₁ and SymDiv₄ had intermediate NH₃ fluxes (Figure 3.17).
Table 3.8. Significance tests for the effect of treatment (Trmt), symbiotic diversity (SymDiv) level, time, and their interaction on carbon dioxide, nitrous oxide, and ammonia fluxes measured during 2017-2018. Bold indicates statistical significance (P < 0.05).

<table>
<thead>
<tr>
<th>Effect</th>
<th>CO₂</th>
<th></th>
<th>N₂O</th>
<th></th>
<th>NH₃</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n, d</td>
<td>F</td>
<td>P</td>
<td>n, d</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Trmt</td>
<td>15;64</td>
<td>0.40</td>
<td>0.9733</td>
<td>15;59.64</td>
<td>0.93</td>
<td>0.5389</td>
</tr>
<tr>
<td>Time</td>
<td>21;1334</td>
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<td>21;1336</td>
<td>18.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trmt*Time</td>
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<td>0.80</td>
<td>0.9924</td>
<td>315;1336</td>
<td>0.87</td>
<td>0.9361</td>
</tr>
<tr>
<td>SymDiv</td>
<td>4.77.37</td>
<td>0.16</td>
<td>0.9590</td>
<td>4;831.1</td>
<td>0.97</td>
<td>0.4210</td>
</tr>
<tr>
<td>Time</td>
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<td>304.12</td>
<td>&lt;0.0001</td>
<td>21;138.8</td>
<td>15.48</td>
<td>&lt;0.0001</td>
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<tr>
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<td>84;1565</td>
<td>1.32</td>
<td>0.0309</td>
<td>84;135.2</td>
<td>1.01</td>
<td>0.4688</td>
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</table>
Figure 3.15. Average soil-to-atmosphere carbon dioxide fluxes by symbiotic diversity (SymDiv) level measured over 2017-2018. Symbols (*) represent significant SymDiv effects within a time point. Error bars represent average ± 1 S.E.
Figure 3.16. Average soil-to-atmosphere nitrous oxide fluxes by symbiotic diversity (SymDiv) levels measured over 2017-2018. Bars represent average ± 1 S.E.
Figure 3.17. Average soil-to-atmosphere ammonia fluxes by symbiotic diversity (SymDiv) level measured over 2017-2018. Inset graph (units are similar to main figure) depicts SymDiv effects on ammonia fluxes; lowercase letters represent significant SymDiv level differences. Bars represent average ± 1 S.E.
Table 3.9. Significance tests for moisture and temperature by treatment (Trmt), time (Time), and symbiotic diversity (SymDiv) level. Bold indicates statistical significance (P < 0.05).

<table>
<thead>
<tr>
<th>Effect</th>
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<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>F</td>
</tr>
<tr>
<td>Trmt</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>Time</td>
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</tr>
<tr>
<td>SymDiv*Time</td>
<td>76;1492</td>
<td>0.49</td>
</tr>
</tbody>
</table>
Figure 3.18. Soil temperature (°C) and soil volumetric water content (% VWC) averaged across treatment over time. Bars represent ± 1 S.E.
Chapter Four: Discussion

Biodiversity-ecosystem functioning studies have generally agreed upon three guiding tenets: functional trait diversity and species interactions may be more influential than species richness; abiotic feedbacks require consideration; and biodiversity effects and mechanisms may experience spatiotemporal variability, along with differences across ecologic organizational levels (Hooper et al., 2005). This study attempted to quantify the role of tall fescue-\textit{E. coenophiala} symbiotic diversity in grassland ecosystem functioning. Overall, our results suggested that increasing symbiotic diversity had weak to moderate effects on aboveground processes (e.g., tall fescue biomass and insect abundance), and stronger effects on certain belowground processes, such as soil microbial biomass and enzyme activity.

4.1 Symbiotic Diversity and Aboveground Ecosystem Functions

My first hypothesis predicted, following biodiversity-ecosystem function theory, that net aboveground plant productivity would increase with increasing symbiotically diverse mixtures due to functionally diverse endophyte genotypes occupying broader niche spaces and additionally improving plant fitness through a range of bio-protective alkaloid compounds. Alkaloid data collected from this study generally correlated with the known synthesis pathways of each endophyte strain (Table 2.4). Concentrations of these various alkaloids varied dramatically across treatments (Figures 3.3 - 3.7), suggesting that if alkaloids were important in driving aboveground functional responses, patterns across the measured parameters would be observed.

Contrary to my prediction, there were no significant differences amongst treatments in aboveground biomass or plant species richness, indicating that neither
endophyte presence nor symbiotic diversity (or associated alkaloid profile differences) strongly influenced plant productivity and species diversity at this early stage in the life of the stands (first growing season). These results are in direct contrast to related studies, where CTE+ infected tall fescue more strongly suppressed plant species diversity than NE+ and E- plots (Rudgers et al., 2010; Iqbal et al., 2013). Iqbal et al. (2013), however, similarly found no statistical differences in plant biomass across endophyte treatments, including a “mix” treatment that contained equal parts of three endophyte statuses (CTE+, NEAR-542, and NEAR-584) and E- tall fescue.

The strength of the endophyte symbiosis can vary across time and has been shown to be susceptible to plant and endophyte genetic identity, as well as biotic and abiotic pressures, meaning symbiont-driven mechanisms may require time to further manifest (Clay, Holah, & Rudgers, 2005; Rudgers et al., 2010). For example, mammalian and insect herbivory pressures, over a six year manipulative field study, were found to increase endophyte infection frequency within the fescue community over time, a common endophyte symbiota response to stressful conditions, and led to accompanying shifts in plant community structure (Clay, Holah, & Rudgers, 2005). Endophyte infection is known to improve tall fescue fitness and competition through several mechanisms, including: suppressing vertebrate and invertebrate herbivory (Hoveland, 2009); conferring climactic resilience (Malinowski and Belesky, 2000); and inducing shifts in belowground microbial communities that might impact plant nutrient availability and/or plant-soil feedbacks (McNear and McCulley, 2012).

Due to symbiont-associated differences in the quantity of insecticidal alkaloid compounds measured in this study, I also hypothesized that increasing endophyte strain
diversity within a tall fescue community would adversely affect foliar-associated chewing (grasshopper) and sucking (leafhopper) invertebrate population abundances. While both grasshopper and leafhopper abundances were significantly different across treatments, neither feeding guild had a high correlation with the two main classes of insect-active metabolites, lolines [N-acetylnorloline (NANL), N-formyloline (NFL), N-acetylloiline (NAL)] and peramine, indicating that alkaloids did not directly influence population abundance. Keathley and Potter (2012) similarly found no significant differences in grasshopper populations in response to endophyte infection (CTE+ and NE+) compared to E- stands. Leafhoppers, however, being a grass-adapted species, can be inconsistently affected by endophyte presence (Muegge et al., 1991); several studies have quantified their responses to endophyte infection, determining variability to arise from pasture conditions (e.g., amount of precipitation versus drought conditions experienced during sampling season) and species-specific adaptions (possibly through physiological strategies invoking specialized ingestion of plant sap) (Chougule and Bonning, 2012; Keathley and Potter, 2012).

It is possible that endophyte-driven effects were not fully captured in the single sampling of leafhopper populations; a typical growing season in Kentucky can experience three to four leafhopper generations, therefore it may be that continuous sampling throughout the growing season (May-August) is needed (Muegge et al., 1991), or endophyte effects may be stronger and more likely observed at only certain times of the year. Moreover, due to the species-related nuances, assessing endophyte effects on invertebrate populations may best be evaluated on a species-level. Changes in leaf tissue palatability, including silica content, water- and nutrient-availability, and leaf toughness,
can additionally influence insect herbivores (Bernays and Barbehnn, 1987; Keathley and Potter, 2011) and were not quantified in this study. It is possible that variation in these characteristics across the fescue-endophyte packages drove the observed treatment differences. Finally, some of the taxa \((Draeculacephala, Graminella, \text{ and } Forcipata)\) that were identified in this study were also found in abundance in three surveys conducted on tall fescue in the United States (Quisenberry et al., 1979; Kirfman et al., 1986; Muegge et al., 1991). These taxa may have become adapted to feeding on common strain endophytic grasses found in North America, an adaptation that is possibly translated to novel endophytes, making these organisms less impacted by \textit{Epichloë} produced alkaloids (Keathley and Potter, 2012).

**4.2 Symbiotic Diversity and Belowground Ecosystem Functioning**

In my third hypothesis, I predicted that differences in alkaloid profiles in tall fescue stands infected with mixtures of endophyte strains would induce differential shifts in aboveground parameters (e.g., insect pressure), and thereby exert divergent influences on belowground processes compared to E- (SymDiv\(_0\)) and monoculture (SymDiv\(_1\)) plots. Soil microbial activity is known to be stimulated by endophyte-altered root exudates and increased C and N inputs, though endophyte-presence effects can vary [see Table 1 in (McNear and McCulley, 2012)]. In this study, increasing symbiotic diversity tended to stimulate extracellular enzyme activity (EEA), which was measured using key indicator enzymes (e.g., BG, NAG, LAP, PHOS) that are responsible for releasing assimilable nutrients from organic C, N and P sources (Sinsabaugh and Follstad Shah, 2012). Interestingly, however, microbial biomass carbon (MBC) experienced a negative relationship with symbiotic diversity levels in contrast to EEA.
Common enzyme-based models assume that enzyme activity is biomass-specific; that is, all enzyme production is constitutive, and hence, proportional to microbial biomass (Schimel and Weintraub, 2003). However, the results of this study refute this model, displaying a negative relationship between certain hydrolytic enzymes (BG, PHOS, and NAG) and MBC, suggesting that perhaps instead of constitutive enzyme production, conditions associated with increased symbiotic diversity led to inducible enzyme production (Moorhead et al., 2013). It is also possible that when MBC was highest (SymDiv0), microbial acquisition of litter C was occurring without extracellular hydrolysis (Moorhead et al., 2013). Franzleubbers et al. (2005) similarly found a decrease in MBC under CTE+ endophytic stands, and conjectured that this response was possibly due to endophyte byproducts from plant residue leaching throughout the surface soil profile or decreases in carbohydrates in root exudates. I did not assess substrate availability and use, which may have contributed insight into C mineralization, and has been shown to be modified by endophyte presence (Van Hecke et al., 2005; Iqbal et al., 2012). Furthermore, MBC was quantified using chloroform fumigation extraction (CFE), which is the most common methodology measuring cytoplasmic microbial C in soil (Brookes et al., 1985), but does not distinguish between active and dormant biomass pools (Paul and Clark, 1996). Another microbial biomass metric is phospholipid fatty acid (PLFA) analysis, which measures the amount of active microbial biomass (Zak et al., 1996) and may have yielded dissimilar results to CFE [though, see (Zelles et al., 1992)]. Additionally, this study quantified EEA from bulk soils, whereas related studies (McNear and McCulley, 2012) have also examined EEA under bulk and rhizosphere soils, finding significant differences between the two. It is possible that different
techniques and/or additional measurements would have aided in elucidating the mechanisms driving the observed MBC and EEA responses.

I also hypothesized that different fescue-endophyte associations with divergent alkaloid profiles would differentially impact soil-to-atmosphere trace gas fluxes. Previous studies have found soil CO\textsubscript{2} and N\textsubscript{2}O fluxes to be sensitive to endophyte identity (Iqbal et al., 2013). My results found no significant differences in N\textsubscript{2}O fluxes; however, CO\textsubscript{2} fluxes were significantly impacted by symbiotic diversity levels interacting with time. Treatment differences were more apparent in the early growing season (March 2017 and April 2018) compared to rest of the year (Figure 3.15). While soil moisture levels were relatively similar (Figure 3.18) during the early growing season, soil temperature was slightly warmer during March 2017 (12.9 ± 0.03 °C) compared to April 2018 (7.7 ± 0.04°C). There were no significant differences among and between symbiotic diversity levels in soil temperature and moisture, which are two abiotic drivers of soil trace gas fluxes, yet, it is possible that differences in plant and soil microclimates were more pronounced earlier in the season, possibly due to plant growth trends of cool-season grasses, which tend to experience increased productivity during spring and fall months (Hannaway et al., 2009). Soil NH\textsubscript{3} generally experienced volatilization under more symbiotically diverse tall fescue stands and tended to be consumed by soil microbiota under endophyte-free plots.

Patterns in soil trace gas fluxes may be due to several factors: changes in microbial community composition, rhizosphere deposits, plant and/or soil microclimate, substrate availability, plant community composition, and/or endophyte byproducts leaching (Van Hecke et al., 2005; Iqbal et al., 2013). The data collected from this
experiment does not examine most of the possible driving factors, though soil microclimate and plant community composition were assessed, albeit with no significant differences detected across symbiotic diversity levels. Variances in CO$_2$ fluxes across symbiotic diversity were more pronounced during the early growing season; paired with the April 2017 maxima in microbial biomass C and significant differences in biomass with increasing symbiotic diversity, the significant CO$_2$ fluxes may have reflected changes in substrate quality or quantity, such as total soil organic C (SOC). Changes in SOC can lead to fluctuations in C mineralization, and thereby differences in soil-to-atmosphere CO$_2$ fluxes (Iqbal et al., 2012). Additionally, the inverse relationship between EEA and MBC may indicate that the differences in soil respiration are due to changes in microbial biomass-resource relationships, which regulate the allocation of enzymes to acquire C, N, and P (Moorhead et al., 2013). Moreover, in mixture, it is not yet known whether fungal endophyte genotypes differ in root exudate profiles, which can additionally cause alterations in active soil microbial populations, one of the main biotic regulators of soil trace gas fluxes, through litter inputs and decomposition. The results of this study indicate that symbiotic diversity in aboveground grass-endophyte associations invoke significant belowground effects that may have broader implications for biogeochemical processes.

4.3 The Role of Symbiotic Diversity in Grassland BEF Dynamics

Results of this study partially supported predictions that increasing community-level genetic diversity within a grass-fungal endophyte symbiosis would enhance ecosystem functioning. It is possible that due to the young age of the stand (first growing season), stronger symbiotic diversity level effects may manifest with additional time.
(Franzluebbers and Stuedemann, 2002). For example, in a similar field study (Iqbal et al., 2013), experimental plots had been established for six years prior to data collection. Previous studies (Clay, Holah, & Rudgers, 2005) have also documented temporal variability in community-level fescue-endophyte effects. Furthermore, tall fescue- *E. coenophiala* symbiota are known to be strengthened by adverse climates (Malinowski and Belesky, 2006), high herbivore/pathogen pressure (Clay et al., 2005), and stronger under nutrient poor conditions (Cheplick et al., 2009), none of which were likely experienced by our experimental plots during the first year of the study. Lack of biotic and abiotic stress was evidenced by nutrient data gathered from the field site during the first growing season, which did not significantly differ across treatments (Appendix 2), and mean annual precipitation (MAP = 149.5 cm; 30% greater than norm) and temperature (MAT = 13.9 °C) aligned with long-term conditions for annual central Kentucky climatological norms (MAP = 114.7 cm; MAT = 13.1 °C). Despite this, beneficial ecological interactions (e.g., positive plant-soil feedbacks) may have been shaping ecosystem responses, like elevated enzymatic activity, to increasing symbiotic diversity in grassland communities.

Aboveground parameters, such as plant biomass, remained unaffected by genetically diverse mixtures of tall fescue- *E. coenophiala* symbiota, and while insect populations significantly differed across treatments, no statistical significance was observed at the symbiotic diversity level. It is possible that weak aboveground responses are due to symbiotic functional redundancy (Walker, 1992). Meaning, functionally, different endophyte strains behaved similarly in mixtures and monocultures alike (i.e., filling the same niche space), and therefore, increasing strain diversity within a
community was simply replicating functional traits, not invoking new ones. However, indicators of belowground processes, such as soil microbial biomass and extracellular activity, displayed stronger responses to increasing levels of symbiotic diversity. Despite the lack of aboveground response across symbiotic diversity levels, differences in alkaloid identity and concentrations, and subsequent differences in functionality, could have induced synergistic effects between mixtures of grass-endophyte packages and belowground processes, thereby resulting in significant differences within belowground parameters (McNear and McCulley, 2012). Quantifying and tracking additional belowground parameters, such as substrate availability, particulate and non-particulate organic matter, edaphic microbial community composition, root exudate composition, and/or root biomass, could assist in interpreting symbiotic diversity effects. Furthermore, due to fluctuations in climate trends, herbivore and pathogen pressure, and/or nutrient cycling over time, potential niche spaces may diversify and enable symbiotic diversity treatment effects to manifest. For this reason, continuous long-term research, which would capture such changes, is warranted to further establish mechanisms at work.
Chapter Five: Conclusions

This study assessed whether increasing grass-fungal endophyte symbiotic diversity affects grassland ecosystem functioning. Increasing symbiotic diversity had stronger effects on certain belowground processes (soil microbial biomass and enzyme activity) than aboveground parameters (tall fescue biomass and arthropod abundance). Tall fescue biomass and plant species diversity did not significantly differ across symbiotic diversity levels. While leafhopper and grasshopper populations significantly responded to treatments, there was no correlation between invertebrate abundances and insect-active alkaloids, lolines and peramine. Conversely, soil microbial biomass and enzyme activity (β-1,4, Glucosidase, BG; β-1,4-N-Acetyl-glucosaminidase, NAG; and Phosphatase, PHOS) responded differentially to increasing symbiotic diversity; dissimilar to common enzyme models, which depict extracellular enzyme activity as constitutive to soil microbial biomass, the former decreased under increasing symbiotic diversity, and the latter experienced a linear increase. My data suggested that certain enzymatic activities (BG, NAG, and PHOS) and microbial biomass pools can be independent of each other. Trace gas fluxes, specifically CO\textsubscript{2} and NH\textsubscript{3}, experienced modest but significant differences across symbiotic diversity levels. Differences in CO\textsubscript{2} trends were more pronounced during the early growing season, potentially linked to cultivar activity or rhizodeposit trends during cool, wet spring months. Soil NH\textsubscript{3} generally experienced greater volatilization under more symbiotically diverse tall fescue stands, possibly due to or reflecting differences in soil microbial communities, which are one of the main regulators of trace gas fluxes.
Prior biodiversity-ecosystem function studies have documented that temporal and spatial environmental factors alter the type and magnitude of ecosystem response to changing diversity, sometimes resulting in a flat BEF response (Tilman et al., 1997; Cardinale et al., 2000), such as was observed in this study for several parameters (e.g., fescue biomass). For example, certain studies have found less diverse areas to have higher rates of ecosystem processes (e.g., N acquisition) (Wardle et al., 1997), suggesting that environmental differences, such as nutrient gradients, determining biological community composition were the main drivers of ecosystem processes. Due to the lack of aboveground effects of manipulating tall fescue- *Epichloë coenophiala* symbiotic diversity, it is possible that environmental conditions (i.e., lack of climactic-, herbivore-, and nutritive-stress) experienced during the first growing season did not elicit a strong interaction between grass and fungal symbiont. As previous studies (Clay, Holah, & Rudgers, 2005) have shown, the relative strength of grass-fungal endophyte interactions within a grassland community is sensitive to extrinsic conditions, such as herbivory pressure, which can increase endophyte presence within a tall fescue pasture over time. Therefore, symbiota-driven mechanisms and effects may require time to manifest, and as such, require long-term monitoring.

Increasing symbiotic diversity effects on soil microbial activity was evident despite no main effects on host biomass and plant species diversity. This pronounced sensitivity indicates that, initially, edaphic microbial communities may be key indicators of aboveground, chemical or biological shifts induced by symbiotically diverse tall fescue communities. It is possible that mixtures of fungal symbionts within a fescue stand excrete unique profiles of rhizodeposits, or allocated exudates differentially to other
belowground pools (i.e., root biomass), compared to monocultures of tall fescue-*E. coenophiala* associations, and in turn, stimulated soil microbial activity more so. Previous studies have documented divergent effects of different endophyte statuses and fescue cultivars on root exudate composition (Guo *et al.*, 2015). Increasing symbiotic diversity may have additionally increased the likelihood of positive facilitative interactions occurring between fescue communities and soil microbiota (i.e., plant-soil feedbacks), leading to increased soil microbial activity. Chemically-induced (i.e., rhizodeposits or litter input) shifts in soil microbial communities, and other abiotic and biotic regulators, such as plant activity or substrate availability, may have led to differences in certain trace gas fluxes (CO₂ and NH₃). The belowground effects of increasing symbiotic diversity within a fescue community may serve as early indicators of changes in soil activity and quality.

As agricultural systems continue to face the effects of human-induced biodiversity loss, examining the relationships between symbiosis-driven functional traits, such as herbivore resistance, and ecosystem function may provide beneficial insight into expanding novel biodiversity approaches for sustainably managing grassland agroecosystems and beyond. Long-term research is required to elucidate relationships between biodiversity and agroecosystem functional dynamics. This type of experimental research is instrumental in teasing out nuanced ecological feedbacks between human-manipulated biodiversity and long-term agroecosystem sustainability.
Appendix 1. Bulk density of field soil by depth (0-10 cm, 10-20 cm, 20-30 cm), data from deep soil core sampling in February 2017. Bars represent ± 1 S.E.
<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>1 M KCl pH</th>
<th>Sikora pH</th>
<th>P</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avg. (SE)</td>
<td>n,d</td>
<td>Avg. (SE)</td>
<td>n,d</td>
</tr>
<tr>
<td>0-10</td>
<td>5.83 (0.024)</td>
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<td>0.0498</td>
<td>6.94 (0.010)</td>
</tr>
<tr>
<td>10-20</td>
<td>5.36 (0.029)</td>
<td>15,140</td>
<td>0.2496</td>
<td>6.79 (0.013)</td>
</tr>
<tr>
<td>20-30</td>
<td>5.19 (0.029)</td>
<td>15,140</td>
<td>0.0754</td>
<td>6.73 (0.012)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ca</th>
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<th>Zn</th>
<th>Sand %</th>
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</thead>
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<td>Avg. (SE)</td>
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<td>P</td>
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<td>10-20</td>
<td>-</td>
<td>-</td>
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<td>20-30</td>
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<td>0.1778</td>
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**Appendix 2.** Regulatory Services Data by depth (0-10 cm, 10-20 cm, 20-30 cm) from deep soil core sampling in February 2017 averaged across treatments. Bold indicates statistical significance (P < 0.05).
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EDUCATION

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B.S. Biological Sciences
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HONORS AND AWARDS

- Doyle E. Peaslee Award for outstanding graduate student in Plant and Soil Sciences at University of Kentucky, 2017-2018 academic year winner.

- ASA, CSA, SSSA Future Leaders in Science Award. 2018

- First Place Poster, December Tracy Farmer Institute for Sustainability and the Environment Poster Session. Poster Title: "Can increasing grass-fungal symbiotic diversity in fescue pastures improve long-term sustainability?" 2017

- First Place Speaker, April University of Kentucky Integrated Plant and Soil Sciences (IPSS) Graduate Student Research Symposium. Presentation Title: "Can manipulating plant-microbe genetic diversity improve tall fescue pasture sustainability?" 2017

- Kerri Casner Environmental Science Fellowship. 2017

- Graduate Student Winner, Intercultural Awareness Day Essay Contest. 2016