The Effects of Nutrient Availability on *Schedonorus pratensis* Infected With *Epichloë uncinata* in an Old Field Community

by

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ABSTRACT

THE EFFECTS OF NUTRIENT AVAILABILITY ON SCHEDONORUS PRATENSIS INFECTED WITH EPICHLOË UNCINATA IN AN OLD FIELD COMMUNITY

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Early field studies working with tall fescue have demonstrated that endophyte infected tall fescue can reduce species richness and become dominant in natural communities, however some studies have observed that endophyte infected grasses under nutrient limited conditions are not successful at invading natural communities and endophyte advantages are not always prominent. My research is aimed at answering two questions: (a) how does the plant community alter when exposed to Schedonorus pratensis over time (b) how does nutrient availability affect the symbiosis between S. pratensis and E. uncinata. Plant communities were sampled in unmanaged plots, the plots were either E+ S. pratensis plots, E- S. pratensis plots or unseeded control plots. My research suggests that S. pratensis abundance is decreasing with time. Species abundances and measures of community richness, Simpson’s diversity and evenness fluctuated with year Fertilizer did not have a significant effect on endophyte concentration. Due to the experimental site being nutrient poor when S. pratensis plots were established it may be that S. pratensis cannot overcome years of exposure to nutrient low conditions or it is possible that meadow fescue and its fungal endophyte are not behaving mutualistically together.
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TABLE OF CONTENTS

ABSTRACT .................................................................................................................. ii
ACKNOWLEDGEMENTS ............................................................................................. iii
TABLE OF CONTENTS ............................................................................................. iv
LIST OF TABLES .......................................................................................................... vi
LIST OF FIGURES ...................................................................................................... vii

CHAPTER 1: *Schedonorus pratensis* and its *Epichloë* endophyte ........................................ 1
1.1 Introduction ............................................................................................................. 1
1.2 History of *Schedonorus pratensis* ......................................................................... 1
1.3 *Schedonorus pratensis* and its endophytic symbiont *Epichloë uncinata* ............... 2
1.4 Benefits endophytes provide their hosts ................................................................. 3
1.5 Variables that can affect grass-endophyte symbiosis ............................................... 3
1.6 Invasiveness of meadow and tall fescue in natural communities .......................... 4
1.7 Objective ................................................................................................................. 5

CHAPTER 2: The effect of *Schedonorus pratensis* infected with the endophyte
*Epichloë uncinata* on an old field plant community ....................................................... 6
2.1 Introduction ............................................................................................................. 6
2.2 Method .................................................................................................................... 8
2.2.1 Study site and experimental set-up ...................................................................... 8
2.2.2 Fertilizer Treatment ......................................................................................... 9
2.2.3 Soil Sample ....................................................................................................... 9
2.2.4 Vegetation survey ........................................................................................... 9
2.2.5 Statistics ......................................................................................................... 9
2.3 Results .................................................................................................................. 11
2.3.1 Species Abundances ..................................................................................... 11
2.3.2 Soil Samples ................................................................................................. 12
2.3.3 Diversity and *Schedonorus pratensis* abundance data (2011-2014, 2017) ........ 12
2.3.4 Diversity and *Schedonorus pratensis* abundance data (2017) ....................... 14
2.3.5 Weather data ............................................................................................... 15
2.4 Discussion ............................................................................................................ 15

CHAPTER 3: The effect of nutrient availability on endophyte concentration .................. 41
3.1 Introduction ........................................................................................................... 41
3.2 Method .................................................................................................................. 42
3.2.1 Study site and experimental set-up .................................................................... 42
3.2.2 Fertilizer Treatment ...................................................................................... 43
3.2.3 Tiller Collection ............................................................................................ 43
3.2.4 Estimation of endophyte concentration .......................................................... 43
3.2.5 Statistics ........................................................................................................ 44
3.3 Results ................................................................................................................ 44
3.4 Discussion .......................................................................................................... 45
CHAPTER 4: Conclusions

4.1.1 Summary of result

4.1.2 Dominance over time

4.1.3 Invader

4.1.4 Cultivar effect

4.1.5 Endophyte concentration

4.2 Perspective and prospects

References
LIST OF TABLES

Table 2.1a: ANOVA results from partial redundancy analyses of vegetation composition for all Schedonorus pratensis cultivars (E+, E-, unseeded control) ....................... 19

Table 2.1b: ANOVA results from partial redundancy analyses of vegetation composition from 2017 for all Schedonorus pratensis cultivars (E+, E-, unseeded control) .............. 19

Table 2.2a: Species list of grasses, forbs, legumes and shrubs that occurred in multiple years as well as 2017 used in the partial redundancy analyses .......................... 23

Table 2.2b: Species list of grasses, forbs, legumes and shrubs that occurred in multiple year as well as 2017 used in the biodiversity analyses ........................................ 24

Table 2.3a: Paired Samples Test results of soil samples from fertilized and unfertilized plots measured for Nitrate, Phosphorus and Ammonium .......................... 25

Table 2.3b: Mean, standard deviation and variance for soil samples taken from fertilized and unfertilized plots measuring Nitrate, Phosphorus and Ammonium .................. 25

Table 2.4: One-way ANOVA results of the effect of endophyte on species Richness, Simpson’s diversity, and Evenness for 2011-2014, 2017 ................................. 26

Table 2.5: Mixed Model ANOVA where the main response variables were tested in a split-plot analysis of repeated measures for the effects of year, endophyte and the interaction endophyte x year on species Richness, Diversity, and Evenness from 2011-2014 and 2017 ................................................................. 30

Table 2.6: Mixed Model ANOVA where the main response variables were tested in a split-plot analysis of repeated measures for the effects of year, cultivar and the interaction cultivar x year on species Richness, Diversity, and Evenness from 2011-2014 and 2017 ................................................................. 30

Table 2.7: Mixed Model ANOVA results for the effects of year, endophyte and the interaction endophyte x year on Schedonorus pratensis abundance from 2011-2014 and 2017 ................................................................. 32

Table 2.8: Mixed Model ANOVA results for the effects of year, cultivar and the interaction cultivar x year on Schedonorus pratensis abundance from 2011-2014 and 2017 ................................................................. 34

Table 2.9: One-way ANOVA results of the effect of endophyte on species Richness, Simpson’s diversity, and Evenness for 2017 ................................................................. 35

Table 2.10: Univariate ANOVA results for the effects of fertilizer, endophyte and the interaction fertilizer x endophyte on species Richness, Diversity and Evenness for 2017 ................................................................. 35

Table 2.11: Univariate ANOVA results for the effects of fertilizer, cultivar and the interaction fertilizer x cultivar on species Richness, Diversity and Evenness for 2017 ................................................................. 36
Table 2.12: Univariate ANOVA results for the effects of fertilizer, endophyte and the interaction endophyte x fertilizer on *Schedonorus pratensis* abundance for 2017

Table 2.13: Univariate ANOVA results for the effects of fertilizer, cultivar and the interaction fertilizer x cultivar on *Schedonorus pratensis* abundance for 2017

Table 2.14: Univariate ANOVA results for the effect of Year, Season and the interaction Year x Season on total weekly precipitation from 2011-2017

Table 2.15: Univariate ANOVA results for the effects of Year, Season and the interaction Year x Season on daily mean temperatures from 2011-2017

Table 3.1: Univariate ANOVA results for the effects of fertilizer, endophyte and the interaction fertilizer x endophyte on endophyte concentration (Gene Copies ng⁻¹ Total gDNA)

Table 3.2: Univariate ANOVA results for the effects of fertilizer, cultivar and the interaction fertilizer x cultivar on endophyte concentration (Gene Copies ng⁻¹ Total gDNA)

Table 3.3: Data from the soil cores taken from six fertilized plots and six unfertilized plots
LIST OF FIGURES

Figure 2.1a: Biplots based on a partial redundancy analysis of the vegetation composition with respect to the effect of year ......................................................... 18

Figure 2.1b: Biplots based on a partial redundancy analysis of the vegetation composition with respect to the effect of cultivar ................................................................. 20

Figure 2.1c: Biplots based on a partial redundancy analysis of the vegetation composition from 2017 with respect to the effect of cultivar ................................................................. 21

Figure 2.1d: Biplots based on a partial redundancy analysis of the vegetation composition from 2017 with respect to the effect of fertilizer treatment ......................................................... 22

Figure 2.2a: Species richness in response to endophyte status. Richness data from 2011-2014 and 2017 .................................................................................................................. 27

Figure 2.2b: Simpson’s diversity response to endophyte status. Simpson’s diversity data from 2011-2014 and 2017 ........................................................................................................ 28

Figure 2.2c: Evenness in species abundance in response to endophyte status. Evenness data from 2011-2014 and 2017 ........................................................................................................ 29

Figure 2.3a: Richness between five sampling years. Irrespective of cultivar and endophyte treatments applied to the plots .................................................................................................. 31

Figure 2.3b: Simpson’s diversity response between five sampling years. Irrespective of cultivar and endophyte treatments applied to the plots ........................................................................ 31

Figure 2.3c: Evenness of plant communities between five sampling years ................................................................. 32

Figure 2.4: The effect of year on *Schedonorus pratensis* abundance across five sampling years ........................................................................................................................... 33

Figure 2.5: The effect of the interaction endophyte x year on *Schedonorus pratensis* abundance across five sampling years .................................................................................................. 33

Figure 2.6: The effect of cultivar on *Schedonorus pratensis* abundance across five sampling years .......................................................................................................................... 34

Figure 2.7: Effect of Year on weekly precipitation from 2011-2017 .............................................................................. 38

Figure 2.8a: Effects of year on daily mean temperature from 2011-2017 ........................................................................ 38

Figure 2.8b: Effects of the interaction year x season on daily mean temperature from 2011-2017 ....................................................... 39

Figure 2.9a: The effect of fertilizer on species richness ........................................................................................................ 39

Figure 2.9b: The effect of fertilizer on Simpson’s Biodiversity .......................................................................................... 40

Figure 2.9c: The effect of fertilizer on species evenness .......................................................................................... 40

Figure 3.1a: The effect of endophyte on endophyte concentration (Gene Copies ng⁻¹ Total gDNA) ................................................................. 48
Figure 3.1b: The effect of endophyte x fertilizer on endophyte concentration (Gene Copies ng⁻¹ Total gDNA)........................................................................................................ 49

Figure 3.3: The effect of cultivar on endophyte concentration (Gene Copies ng⁻¹ Total gDNA)........................................................................................................ 50
CHAPTER 1

Schedonorus pratensis and its Epichloë endophyte

1.1 Introduction

Symbiotic relationships exist throughout the world on a continuum from mutualistic to parasitic. The nature of each relationship depends on interactions between organisms and changes in external variables, and costs and benefits of the relationship can change during an organism's ontogeny (Skelton et al. 2016). Mutualistic symbiosis is the exchange of beneficial products and services between a symbiont and its host (Schardl et al. 2004). Mutual costs can exist in the partnership; however, the benefits must outweigh the costs for both partners (Schardl et al. 2004). An example of a mutualistic relationship is the one that exists between ants in the Pseudomyrmex ferrugineus group and swollen thorn acacias (Vachellia species) (Kautz et al. 2008; Ward et al. 2017). The ants receive shelter in the swollen stipular thorns and feed on the floral nectar the tree provides (Kautz et al. 2008; Ward et al. 2017). In turn, ants provide the plant with protection from grazing herbivores and other competing plants (Kautz et al. 2008; Ward et al. 2017). Another example of a mutualistic relationship is the one between flowering plants and the animals they rely on for pollination and seed dispersal, such as honeybees, butterflies, and ants (Heil 2008) The plant receives a chance to reproduce and the pollinator receives a food source (Martin Heil 2008). In parasitic symbioses, one organism receives benefits at the expense of the other organism, such that the relationship can be harmful or fatal to the host (Saggiomo et al. 2007). Some species of wasps exhibit parasitic behavior on insect larvae by using the larvae as a vessel and nutrient source for its eggs (Pashalidou et al. 2014; Ullah et al. 2016). For example, the wasp species Cotesia glomerata L. lay their eggs in Pieris brassicae (cabbage butterfly) larvae (Pashalidou et al. 2014; Ullah et al. 2016). The cabbage butterfly larvae act as a food source for the wasp larvae but die when the wasp eggs hatch (Pashalidou et al. 2014; Ullah et al. 2016).

1.2 History of Schedonorus pratensis

Meadow fescue (Schedonorus pratensis (Hudson) P. Beauvois) is an understudied grass in North America; its congeneric grass, tall fescue (Schedonorus arundinacea Scherb), and another cool season grass, perennial rye grass (Lolium perenne), have been studied extensively especially in North America and New Zealand (Clay & Schardl 2002; Schardl et al. 2004). I will refer to studies on tall fescue when information on meadow fescue is not available. Meadow fescue and tall fescue are cool season C3 grasses, from the family Poaceae (Schardl et al. 2004). Meadow fescue is distributed through Europe and is commonly found in Nordic and alpine pastures where is used for its winter hardiness and forage quality (Duncan et al. 2013). Currently meadow fescue has expanded its range eastward into Central and Western Asia, and it has also been introduced to North America, Japan, Australia, and New Zealand (Fjellheim et al. 2006). In North America specifically, meadow fescue was introduced into midwestern USA as a pasture species (Duncan et al. 2013). The distributional changes and introduction success meadow fescue has experienced is likely due to its ability to thrive in a variety of natural (i.e. riverbanks, grasslands, and meadows) and human managed (i.e. pastures, fields, and disturbed areas) habitats. It is shade tolerant and can grow in moist, sandy soil despite its preference for rich, deep soils (Fjellheim et al. 2006). Both meadow fescue and tall fescue are used as pasture and turf
grasses in North America and Europe because of the numerous benefits they exhibit (Saikkonen et al. 1998) such as increased drought tolerance (Scharld & Phillips 1997; Malinowski & Belesky 2000; Scharld et al. 2004; Malinowski & Belesky 2006), increased resistance to pests (insects and mammals) (Clay 1996; Scharld & Phillips 1997; Clay & Scharld 2002; Rudgers et al. 2006; Yurkonis et al. 2012), and increased tolerance to grazing (Scharld et al. 2004; Saari et al. 2009; Saari et al. 2010). Both species of fescue are used in grazing agriculture; however, tall fescue is used more often because it tested superior in biomass capacity, robustness and resistance to Crown Rust caused by \textit{Puccinia coronate} compared to meadow fescue (Fjellheim et al. 2007).

The toxicity related herbivores that grazed on tall fescue was not confirmed until the 1970s (Saikkonen et al. 1998). Livestock that fed on tall fescue often suffered from a variety of ailments, sheep and cattle especially showed signs of weight loss, rapid breathing, gangrene of the extremities, elevated body temperature, decrease in reproductive performance, and reduced feed intake (Chestnut et al. 1991; Schmidt & Osborn 1993; Prestidge 1993). The three main disorders that affected livestock were called Fescue foot, Bovine fat necrosis, and Fescue toxicosis (Chestnut et al. 1991; Schmidt & Osborn 1993). The connection between livestock poisoning and the advantages exhibited by tall fescue as forage grass was determined to be caused by systemic endophytic fungi that can be found living within the intracellular spaces of the host grass’ aboveground tissues (leaves, stem and tillers) (Clay 1990; Saikkonen et al. 1998; Clay & Scharld 2002; Schaardl et al. 2004; Rudgers et al. 2006).

\subsection{Schedonorus pratensis and its endophytic symbiont \textit{Epichloë uncinata}}

The endophytic fungus that is found within meadow fescue is called \textit{Epichloë uncinata} (Gams, Petriini and Schmidt; Glenn, Bacon, and Hanli) formerly classified as \textit{Neotyphodium uncinatum} (Leuchtmann et al. 2014). The fungus found in tall fescue is called \textit{Epichloë coenophiala} (Morgan-Jones & W. Gams) formally known as \textit{Neotyphodium coenophialum} (Leuchtmann et al. 2014). \textit{Neotyphodium} endophytes were reclassified within the genus \textit{Epichloë} because classification using the name \textit{Neotyphodium} did not accurately represent the divergent evolutionary histories, life histories or host interactions within the clade \textit{Epichloë} (Leuchtmann et al. 2014). Leuchtmann et al. (2014) suggest that classification within the genus \textit{Epichloë} focuses on life history traits, host interactions and various strains for each species of \textit{Epichloë}. \textit{Epichloë} endophytes can reproduce sexually (horizontal transmission), asexually (vertical transmission) or both depending on the species (Saikkonen et al. 1998). Sexually reproducing endophytes tend to be parasitic by causing abortion of the host inflorescence, which is classified as choke disease. The fungi produce fruiting bodies or spores that inhibit host’s reproductive organs (Clay & Scharld 2002). Asexual endophytes use vertical transmission to disseminate to the next generation by penetrating the host’s seeds with hyphae (Saikkonen et al. 1998). Asexual endophytes have arisen separately from their sexual ancestors through interspecific hybridization (Clay & Scharld 2002). \textit{Epichloë uncinata} is a hybrid of \textit{Epichloë bromicola} and \textit{Epichloë typhina} (Moon et al. 2004). \textit{Epichloë uncinata} is one of the three hybrids that produce high levels of loline alkaloids, which protect the host from a broad range of insects by deterring eating (Saikkonen et al. 2016).
1.4 Benefits endophytes provide their hosts

*Epichloë* endophytes provide their hosts with a variety of advantages to abiotic and biotic stresses. One major benefit endophytic fungi provide is drought resistance and tolerance (Malinowski & Belesky 2000). Under drought conditions, endophyte infected (E+) grasses are often more likely to succeed than endophyte free (E-) grasses (Malinowski & Belesky 2000). For example, endophyte-infected meadow fescue and tall fescue have been known to survive and recover from long periods of drought relative to their endophyte free counterparts (Arachevaleta et al. 1989; Bacon 1993; West et al. 1993; Elbersen & West 1996; Hill et al. 1996; Malinowski et al. 1997; Malinowski & Belesky 2000; Kulda & Bacon 2008; Roderiguez et al. 2008). Endophyte infected grasses can experience this increased drought survival because the fungal endophytes increase the number of compounds that the host plant uses to provide protection for enzymatic plant functions and from heat stress, such as nitrogen, nitrates, and prolines (Bacon 1993; Belesky & Bacon 2009). Drought-stressed plants that are endophyte infected have been known to increase their root hair length and decrease their root diameter making water and mineral acquisition easier during periods of drought (Elbersen & West 1996; Malinowski & Belesky 2000; Kulda & Bacon 2008; Roderiguez et al. 2008). Some studies have also shown that endophyte infected plants have higher stomatal resistance relative to endophyte free plants, which can help regulate water loss during drought periods (Belesky et al. 1987; Malinowski et al. 1997). Additional evidence suggests that endophyte infected meadow and tall fescue decrease stomatal conduction faster than endophyte free meadow and tall fescue, causing more rapid stomatal closure and helping reduce water loss (Belesky et al. 1987; Elbersen et al. 1994; Elmi & West 1995; Malinowski et al. 1997; Malinowski & Belesky 2000). Endophyte infected grasses have also exhibited better regrowth when periods of drought have ended compared to their uninfected counterparts (Arachevaleta et al. 1989; Bacon 1993; Malinowski et al. 1997; Malinowski & Belesky 2000).

Several studies have shown that both endophyte infected fescue species are excellent inter- and intraspecific competitors (Marks et al. 1991; Malinowski & Belesky 2000; Brem & Leuchtmann 2002; Clay & Schardl 2002; Schardl et al. 2004; Lemons et al. 2005). Endophyte infected plants can receive beneficial competitive abilities, such as increased shoot and root growth (Latch et al. 1985; Malinowski et al. 1997; Brem & Leuchtmann 2002; Takai et al. 2010) and increased seed production (Marks et al. 1991; Brem & Leuchtmann 2002), and they can also experience increased resistance and deterrence to herbivory from insects and mammals due to alkaloid production compared to their uninfected counterparts (Siegel et al. 1990; Arechavaleta et al. 1991; Bush et al. 1997; Lowell et al. 1997; Schardl & Phillips 1997; Clay & Schardl 2002; Breen 1994; Scharld et al. 2004; Faeth et al. 2006). Endophytes tend to produce alkaloids from four main groups: peramines, lolines, ergots, and lolitrems (Siegel et al. 1990; Lowel et al. 1997).

1.5 Variables that can affect grass-endophyte symbiosis

Natural grass populations can comprise plants that are all endophyte infected, plants that are all endophyte free, or a mixture of endophyte infected and endophyte free plants (Clay 1990). Seedborne endophytes can cause complete infection of all individuals in a host’s population, which suggests that there is a stronger selective advantage for infected plants; however, infection can be lost by the inability of the fungus to penetrate the host’s seeds or death of the fungus by extended seed dormancy (Clay 1990; Scharld et al. 2004). Endophyte infection frequency in
individual tillers of tall fescue and meadow fescue can range from 0 to 100% infected (Clay et al. 2005; Saari et al. 2008). Endophyte infected grasses have been known to be competitively superior in nutrient rich plant communities compared to their uninfected counterparts (Clay & Holah; Saikkonen 2000; Saikkonen et al. 2006; Rudgers et al. 2007; Rudgers et al. 2010; Gundel et al. 2013; Dirihan et al. 2014).

The mutualistic symbiosis between fungal endophytes and their hosts can be affected by environmental factors such as nutrient availability (Rasmussen et al. 2008). For example, a high supply of nitrogen can reduce endophyte concentration in some cool season grasses, such as perennial ryegrass (*Lolium perenne*) (Lane et al. 1997; Rasmussen et al. 2006; Rasmussen et al. 2008). Rasmussen et al. (2008) exposed endophyte infected perennial ryegrass to high amounts of nitrogen and found that the endophyte concentration decreased by 40%. In a similar study by Liu et al. (2011), endophyte infected perennial ryegrass that was exposed to high nitrogen treatments showed a reduction in endophyte concentration by 50%. However, endophyte infected tall fescue exposed to high nitrogen treatments experienced an increase in endophyte concentration (Ryan et al. 2014). It has been suggested that endophyte concentration can decrease because nitrogen can dilute the endophyte concentration by stimulating plant growth more than endophytic growth (Lane et al. 1997; Rasmussen et al. 2008). Ryan et al. (2013) suggests that grass-endophyte symbiosis and their responses to nutrient availability may not be generalizable across *Epichloë* species.

### 1.6 Invasiveness of meadow and tall fescue in natural communities

Not all Pooid-*Epichloë* symbiotic relationships are generalizable. External variables such as site conditions, vegetation composition, nutrient availability and climate can also determine how successful infected grasses thrive in unmanaged landscapes (Shukla et al. 2015). The literature has shown that, in most cases, tall fescue, a species closely related to meadow fescue, tends to be an invasive species and is even more so when infected with its fungal endophyte *Epichloë coenophiala* (Morgan-Jones & W. Gams). In field studies, Clay & Holah (1999) observed that endophyte infected tall fescue dominated plots four years after planting and these plots had a lower species richness. Rudgers and colleagues (2010) observed a similar trend when working with tall fescue and different strains of *E. coenophilala* and determined that the common toxic endophyte strain caused a reduction in plant species richness. Additionally, Rudgers et al. (2007) found that endophyte infected tall fescue played a role in the reduction of tree abundance and richness at two different field locations, suggesting that tall fescue can affect the landscape as it transitions from grassland to forest and can be difficult to remove from natural communities (Rudgers et al. 2007). Similarly, meadow fescue and tall fescue can affect biodiversity by increasing (Shukla et al. 2015) or decreasing (Clay & Holah 1999) species richness. Takai et al. (2010) found that endophyte infected meadow fescue had higher proportions in monocultures and mixed cultures, suggesting that endophyte infection influenced the persistence, vegetative growth and competitive ability of meadow fescue. Shukla et al. (2015) observed vastly different results in a field-based study of seven cultivars of meadow fescue, in which they investigated the influence of endophyte infected meadow fescue on promoting host grass abundance and effects on plant diversity and composition. They found that both meadow and tall fescue increased species richness relative to unseeded control plots and determined that this effect was stronger in E+ meadow fescue plots. They also determined that endophyte infection did not allow either fescue species to achieve high abundance or become dominant in the plant community. Ultimately, Shukla et al. (2015) suggest that site-specific
effects such as climate, nutrient availability may have a stronger influence on the establishment of endophyte infected meadow fescue in natural communities than species, cultivar and endophyte effects.

1.7 Objective

My research is aimed at answering the question of: how does nutrient availability and the presence of grasses that are infected with systemic vertically transmitted fungi alter plant community composition and endophyte concentration. The study organism I used in my research was meadow fescue (*Schedonorus pratensis* (Hudson) P. Beauvois) and its fungal endophyte *Epichloë uncinata* (Gams, Petrini and Schmidt) (Glenn, Bacon, and Hanli). This mutualistic grass-endophyte symbiosis has been predominately studied in and from an agronomic perspective, with most of the current literature focusing on tall fescue, perennial ryegrass and meadow fescue. Many beneficial properties have been linked to endophyte infected grasses, such as drought tolerance, increased competition and increased resistance to herbivory as I discussed in in section 1.4. Meadow and tall fescue are highly valued grasses within the agronomic industry, but their expansion into natural communities as an invader has varying responses. It is well known that external environmental factors can help or hinder endophyte infected grasses in invading natural communities. The focus of this thesis is to determine how nutrient availability in this case application of fertilizer effects meadow fescue’s relative abundance in the plant community (CHAPTER 2) and if it influences endophyte concentration (CHAPTER 3). I am also examining the effect meadow fescue has on plant community diversity over time and to determine if endophyte concentration has changed with time. Multiple cultivars of meadow fescue have been used to manipulate the presence of the endophyte to account for differences in genetic variability of meadow fescue. Based on the literature as I discussed in section 1.5 I expect the E+ cultivars of meadow fescue that were fertilized to have a greater relative abundance of meadow fescue and lower plant diversity compared to E- cultivars. Based on the limited research done on the effect nutrient availability has on endophyte concentration I expect endophyte concentration in meadow fescue to decrease in fertilized plots even though tall fescue a comparable species had endophyte concentration increase at elevated CO2 level under high nitrogen levels (Ryan et al 2014).
CHAPTER 2

The effect of *Schedonorus pratensis* infected with the endophyte *Epichloë uncinata* on an old field plant community

2.1 Introduction

Invasive plant species can be detrimental to natural communities and native plant species (Hayes & Holzmueller 2012). In unmanaged landscapes invasive species can alter ecosystem functions such as nutrient cycling, thereby decreasing the productivity of other species (Hayes & Holzmueller 2012). Invasive species can also decrease biodiversity by outcompeting and replacing native plant species (Schmilz & Simberloff 1997; Wilcove et al. 1998; Gaertner et al. 2009; Hayes & Holzmueller 2012). An example of an invasive plant species that was introduced to North America in 1900s from Asia is *Rosa multiflora*, also known as multiflora rose, which is a perennial shrub used as an ornamental plant in landscaping (Banasiak & Meiners 2009; Hayes & Holzmueller 2012; Jones 2012). The multiflora rose was able to spread from domestic settings to wooded areas and grasslands because it possesses a variety of qualities that allow it to be a successful invader. These invasive qualities include: rapid growth (Hayes & Holzmueller 2012), shade and drought tolerance (Hayes & Holzmueller 2012), its ability to be pollinated by a variety of different insects (Banasiak & Meiners 2009; Hayes & Holzmueller 2012), and seed dispersal by birds, which allowed it to be broadly dispersed deeper into forests (Banasiak & Meiners 2009; Hayes & Holzmueller 2012). The multiflora rose is a major conservation issue in the United States with 31 states reporting it as invasive since 2006 (Banasiak & Meiners 2009). In herbaceous communities the multiflora rose has been shown to reduce species richness by suppressing local colonization rates of other species (Yurkonis et al. 2015). The multiflora rose is an example that demonstrates how an invasive species can reduce the species richness due to its invasive qualities (Banasiak & Meiners 2009; Jones 2012).

Meadow and tall fescue are cool season C$_3$ grasses that are important forage grasses in North America (Clay 1988). Two forms of meadow and tall fescue can exist in natural populations: one form involves the infection of meadow or tall fescue with their specific mutualistic fungal endophyte, and the other is an uninfected form (Clay 1990). These fungal endophytes belong to the genus *Epichloë* and are asexual, vertically-transmitted fungi (Wani et al. 2015). The endophytes in meadow and tall fescue are transmitted via the seeds of the grass host (Wani et al. 2015). These fungi exist in the intercellular spaces of the host plant and extend hyphae up into the host plant’s developing seeds (Wani et al. 2015). The host plant provides the fungus with nutrients, shelter and a chance to disseminate to the next generation (Wani et al. 2015). Systemic fungal endophytes have been known to provide their host with certain advantages, some of which include increased plant vigor (Marks et al. 1991; Brem & Leuchtmann 2002), resistance to herbivores and pathogens, (Clay 1996; Schardl & Phillips 1997; Clay & Schardl 2002; Rudgers et al. 2006; Yurkonis et al. 2012) and tolerance to environmental stressors such as drought (Schardl & Phillips 1997; Malinowski & Belesky 2000; Schardl et al. 2004; Malinowski & Belesky 2006), salinity and heavy metals (Malinowski and Belesky 2000; Vila-Aiub et al. 2003). It has been suggested that the benefits provided by the fungus to the host plant have helped the grasses to establish themselves in natural communities more successfully (Saikkonen et al. 2006).
Early field studies demonstrated how endophyte infected tall fescue could invade natural communities and reduce species richness (Clay & Holah 1999; Rudgers et al. 2007; Rudgers et al. 2010). In field plots seeded with infected and uninfected tall fescue, infected plots were dominated by tall fescue and suppressed grasses and dicots relative to uninfected plots (Clay & Holah 1999). Infected tall fescue plots had lower species richness compared to uninfected plots (Clay & Holah 1999). Spyreas et al. (2001) examined the effects of mowing and fertilizer treatments on plant community diversity in tall fescue plots and they observed that species richness decreased with endophyte infection frequency in plots there were not mowed. Rudgers et al. (2007) also found across two experimental sites endophyte infected tall fescue reduced tree species richness by 60%, and endophyte infection reduced tree abundance between 60-80%. Similarly, another study examined the affect endophyte genotype had on the symbiosis between tall fescue and its endophyte and how genotypes affected the natural plant community (Rudgers et al. 2010). Endophyte infected tall fescue suppressed species richness and reduced other plant species such as forbs and graminoids (Rudgers et al. 2010).

In contrast to the studies discussed above, others have demonstrated that endophyte infection does not have a strong negative effect on species richness. Yurkonis et al. (2012) found that endophyte infected tall fescue forage cultivars had a slight decrease in species richness compared to uninfected forage cultivars, however the differences were not consistent between specific endophyte infected and uninfected cultivars. In a study by Ahlholm et al. (2002), endophyte infection reduced tiller and root biomass in Festuca pratensis, thus hindering plant growth. Shukla et al. (2015) found that endophyte infected meadow fescue did not achieve high abundance in an old field community regardless of cultivar. Neither Schedonorus species regardless of endophyte status became dominant in the old field community and both meadow and tall fescue increased local species richness relative to unseeded control plots (Shukla et al. 2015). This effect was stronger in E+ plots compared to E- meadow fescue plots (Shukla et al. 2015). The grass cultivar and fungal endophyte strain used can result in different physical and physiological responses between the endophyte and the plant. Rasmussen et al. (2007) worked with two cultivars of perennial ryegrass and found differing responses between cultivars in endophyte-produced alkaloids and plant growth. Shukla et al. (2015) suggests that site specific effects such as climate, soil moisture and nutrient availability may be stronger than species, cultivar and endophyte effects, and thus future studies should be replicated in a variety of locations. The genetic compatibility of the host grass and endophyte along with land management practices such as fertilizer application can make the mutualistic symbiosis between grass and endophyte unpredictable in natural plant communities (Rasmussen et al. 2007). A meta-analysis by Saikkonen et al. (2006) suggested that much of the research to date, such as the examples I have discussed thus far, concern the response of endophyte infected plants on herbivory, or they have been conducted using the same two agricultural grasses (tall fescue and perennial ryegrass) primarily in agronomic settings or former agricultural fields. However, Saikkonen et al. (2006) report a low number of studies that examine endophyte-plant interactions in nutrient-poor environments, the results of which can aid in determining how plant-endophyte symbiosis responds in nutrient poor plant communities.
In this chapter I discuss my research on how the infection of *Schedonorus pratensis* with its fungal endophyte, *Epichloë uncinata*, affects plant assemblage in an old field community and how endophyte advantages are more prominent under nutrient rich conditions compared to nutrient poor conditions. Other studies have conducted similar research, however my work and the work done by Shukla et al. (2015) is unique because multiple replicates of meadow fescue cultivars were used to account for and examine the genetic variability of these different populations of meadow fescue. The study site is considered a nutrient poor environment. My work includes 7 different cultivars of meadow fescue, a control and fertilizer treatment. I predict that: (a) meadow fescue will be more abundant in fertilized plots compared to unfertilized plots because the additional nutrients added to meadow fescue should allow endophyte infected meadow fescue to provide more nutrients (glucose) to its fungal endophyte thus increasing the advantages the fungal endophyte provides, and (b) this effect will be larger in high endophyte infected plots compared to low endophyte infected plots (i.e. a significant interaction). In observing multiple genetic varieties of meadow fescue, I hope to produce some insight on how external variables such as nutrient availability can affect the relative abundance of endophyte infected meadow fescue in a natural plant community.

2.2 Methods

2.2.1 Study site and experimental set-up

The study site was previously an apple orchard that was maintained with the occasionally mowing for 20 plus years prior to establishing our experimental site, which is located at the University of Guelph Turfgrass Institute and Environmental Research Center (Guelph, ON, Canada; 43° 32’ 56” N, 80° 12’ 39” W). The land is composed mainly of sandy loam (Brunisolic Gray-Brown Luvisol) soil developed on a loam till. Prior to conducting this study and the studies done by Yurkonis et al. (2012) and Shukla et al. (2015) the plant community consisted of non-native species such as *Elymus repens* L. Gould, *Poa pratensis* L., *Taraxacum officinale* F.H. Wigg, and *Cirsium arvense* L. Scop (Yurkonis et al. 2012; Shukla et al. 2015). The experimental site was established in 2008 and consists of an 826 m² area divided into 80 2x2 meter plots, separated by 0.5-meter mowed boundaries in a completely randomized block design (Yurkonis et al. 2012; Shukla et al. 2015).

The eighty plots were tilled twice and divided into ten blocks with eight plots in each block. Seven plots per block were randomly selected and seeded with one of the seven cultivars of *S. pratensis* (meadow fescue) at a rate of 5g m² (Clay and Holah 1999; Yurkonis et al. 2012; Shukla et al. 2015) and one plot was unseeded and served as a control. The *S. pratensis* seeds were obtained from Finland (Niemeläinen et al. 2001; Fjellheim et al. 2007; Saari et al. 2009) and stored at -16°C until sowing. The 80 plots were watered until germination and left to grow undisturbed and unmanaged. Within each block, four of the meadow fescue cultivars (Antti, Fure, Ilmari, Kalevi) had no or a low endophyte frequency, and three cultivars (Kasper, Salten, Inkeri) had a high endophyte frequency.
2.2.2 Fertilizer Treatment

In 2015, seven years after establishment, a fertilizer treatment was added to the experiment. The 10 blocks were combined to make 5 blocks that consisted of 16 plots in each block and one replicate of each cultivar treatment was randomly assigned to be fertilized or not. Fertilized plots were treated with 25:4:10 N:P:K (Greenskeeper, Brussels Agromart, Brussels, Ontario), for which the recommended amount for turf grass = 2kg product/100m² = 80g of product per 2m² (~80ml by volume). The plots were fertilized May 12, 2015, August 26, 2015, May 2, 2016, September 19, 2016, June 16, 2017 and September 18, 2017. The fertilizer was applied by broadcasting by hand in a rectangular shape in the center of the plots to avoid edge effects.

2.2.3 Soil Sample

Soil cores were taken in June 2017 from six fertilized and six unfertilized plots, each randomly selected from the total of 80 plots. Four cores were taken from the center of each of the selected plots in a rectangular pattern, to a depth of approximately 15 cm. The four cores were placed in Ziplock bags and thoroughly mixed in order to obtain a homogenous mixture. The soil cores were taken to a commercial lab, SGS Agrifood, to be tested for nitrate, ammonium and phosphorus concentrations. Concentrations of nitrate, ammonium, and phosphorus were measured in parts per million (ppm).

2.2.4 Vegetation Survey

The vegetation survey was conducted on all 80 plots in July 2017 using non-destructive point intercept sampling (Jonasson 1988; Bråthen & Hagberg 2004; Yurkonis et al. 2012; Shukla et al. 2015). The sampling method uses an elevated one-meter long PVC pipe with eight steel pins dropped vertically through the pipe and into the vegetation. The pins were located 10 cm apart, with the first and last pin located 0.5 m into the plot to account for associated edge effects. Vegetation that touched each pin was identified to species or genus level and the number of times each species touched the pins was recorded.

2.2.5 Statistics

The species count data from multiple years (2011-2014, 2017) was imported into an ordination statistical program (Canoco 5; Microcomputer Power, Ithaca, NY) where it was log (X + 1) transformed and centered for the analyses (Shukla et al. 2015). Multiple partial redundancy analyses were performed in Canoco 5. The species composition variation was examined by multiple factors (Block, Cultivar, Year and the interaction between Cultivar and Year). Hierarchal permutations were used in order to maintain split plot form of the repeated measures to test the effects of cultivar and year on species abundance. Species that occurred less than five times across the five years were omitted from the analyses. *Schedonorus pratensis* was excluded as a response variable because it is confounded with cultivar treatment but was included as a supplementary explanatory variable for visualization in biplots.

The species count data from 2017 was imported into an ordination statistical program (Canoco 5; Microcomputer Power, Ithaca, NY) where it was log(X + 1) transformed and centered for the analyses (Shukla et al. 2015). Multiple partial redundancy analyses were performed in Canoco 5. The species composition variation was examined by multiple factors (Block, Cultivar, Fertilization and the interaction between Cultivar and Fertilization).
\textit{Schedonorus pratensis} was excluded as a response variable because it is confounded with cultivar treatment but was included as a supplementary explanatory variable for visualization in biplots.

Measures of community richness (S), evenness (S/(1/D)), and Simpson’s diversity from 2011-2014 and 2017 (1/D) were box-cox transformed in R 3.4.4 (R Core Team 2017) to meet assumptions of normality and homogeneity of the error variance. The three measurements of biodiversity were run in R 3.4.4 (R Core Team 2017) in a mixed model ANOVA where the main response variables were tested in a split-plot analysis of repeated measures. Endophyte was nested in plot (the whole plot factor) and crossed factored with year (the subplot factor). Simpson’s diversity is the combined measurement of richness and evenness and was determined using the Simpson’s Evenness diversity index (1/D). The measures of community richness, evenness and Simpson’s diversity were examined for significant differences in response to endophyte, year and the interaction between endophyte x year. Factors that were found to have a significant effect on richness, evenness, and Simpson’s diversity were subjected to post-hoc analysis using Tukey HSD (honestly significant difference) test. A one-way ANOVA with pre-planned contrasts was performed in SPSS (IBM Corp. Released 2017, version 25.0) to examine the effect of endophyte status (E+, E-, Control) on plant community richness, evenness, and Simpson’s diversity. Endophyte was the only factor in the analysis with the pre-planned contrasts specifically looking at E+ vs. E- plots, E+ vs. control plots and E- vs control plots.

\textit{Schedonorus pratensis} abundance (species counts) was box-cox transformed and run in R 3.4.4 (R Core Team 2017) in a mixed model ANOVA where the main response variables were tested in a split-plot analysis of repeated measures. Endophyte was nested in plot (the whole plot factor) and crossed factored with year (the subplot factor). \textit{S. pratensis} abundance examined for significant differences in response to endophyte, year and the interaction between endophyte x year. Factors that were found to have a significant effect on \textit{S. pratensis} abundance were subjected to post-hoc analysis using Tukey HSD (honestly significant difference) test.

Richness, evenness, and Simpson’s diversity measurements for 2017 were box-cox transformed and run in R 3.4.4 (R Core Team 2017) using a univariate ANOVA that examined for significant differences in response to endophyte, fertilizer and the interaction between endophyte x fertilizer. Factors that were found to have a significant effect on richness, evenness, and Simpson’s diversity were subjected to post-hoc analysis using Tukey HSD (honestly significant difference) test. A one-way ANOVA with pre-planned contrasts was performed in SPSS (IBM Corp. Released 2017, version 25.0) to examine the effect of endophyte status (E+, E-, Control) on plant community richness, evenness, and Simpson’s diversity. Endophyte was the only factor in the analysis with the pre-planned contrasts specifically looking at E+ vs. E- plots, E+ vs. control plots and E- vs control plots.

\textit{Schedonorus pratensis} abundance (species counts) for 2017 were box-cox transformed and run in R 3.4.4 (R Core Team 2017) using a univariate ANOVA that examined for significant differences in response to endophyte, fertilizer and the interaction between endophyte x fertilizer. Factors that were found to have a significant effect on richness, evenness, and Simpson’s diversity were subjected to post-hoc analysis using Tukey HSD (honestly significant difference) test. A one-way ANOVA with pre-planned contrasts was performed in SPSS (IBM Corp. Released 2017, version 25.0) where a paired samples test was performed.
Weekly precipitation measurements (mm) from 2011-2017 were collected from the Elora and Turfgrass weather stations, box-cox transformed and run in R 3.4.4 (R Core Team 2017) using a univariate ANOVA that examined for significant differences in response to year, season, and the interaction between year x season. Factors that were found to have a significant effect on weekly precipitation were subjected to post-hoc analysis using Tukey HSD (honestly significant difference) test. Daily mean temperature measurements (°C) from 2011-2017 were box-cox transformed and run in R 3.4.4 (R Core Team 2017) using a univariate ANOVA that examined for significant differences in response to year, season, and the interaction between year x season. Factors that were found to have a significant effect on daily mean temperature were subjected to post-hoc analysis using Tukey HSD (honestly significant difference) test.

2.3 Results

2.3.1 Species Abundances

The effect of year (multiple years) on species abundances was analyzed using a partial redundancy analysis. Year explained nearly three times the amount of variation in the vegetation composition compared to cultivar (13% vs. 3.7%; Table 2.1a). The effect of year on vegetation composition and abundances was significant (F=14.1, P=0.002; Table 2.1a) In the year biplot axis 1 separated 2011 and axis 2 separated 2017 from the other three years (Figure 2.1a). *Taraxacum officinale* (Dandelion) and *Schedonorus pratensis* (Meadow Fescue) were highly associated with 2011 compared to the other four years. *Aster lanceolatus* (Lance-Leaf Aster), *Solidago canadensis* (Goldenrod), *Poa pratensis* (Kentucky Blue Grass), *Vicia cracca* (Tufted Vetch) abundances were associated more with 2017 than the other four years. *Schedonorus arundinacea* (Tall Fescue) was not correlated with either of the first two ordination axis whereas *Schedonorus pratensis* (Meadow Fescue) was correlated with axis 2 (Figure 2.1a). *S. pratensis* abundances were negatively correlated with *Aster lanceolatus* (Lance-Leaf Aster), *Solidago canadensis* (Goldenrod), *Poa pratensis* (Kentucky Blue Grass), *Vicia cracca* (Tufted Vetch) abundances.

The effect of cultivar on species abundances was not significant. In the cultivar biplot (multiple years) axis 1 separated Inkeri, Fure, Ilmari, Kasper and Kalevi plots from Control, Antti, and Salten plots and axis 2 separated Antti, Salten, Ilmari, Kalevi and Kasper plots from Inkeri, Fure, and Control plots (Figure 2.1b). *Schedonorus pratensis* abundance was associated with Ilmari, Kalevi and Kasper plots, but was not associated with Control plots. There was no clear separation of cultivars with respect to endophyte status. *Dactylis glomerate* (Orchard Grass) abundance was highly associated with Salten plots. *Schedonorus arundinacea* (Tall Fescue) and *Elymus repens* (Quack Grass), and *Convolvulus arvensis* (Field Bindweed) abundances were associated with Control plots. *Schedonorus pratensis* abundances were negatively associated with *Schedonorus arundinacea* (Tall Fescue), *Elymus repens* (Quack Grass), and *Convolvulus arvensis* (Field Bindweed) abundances.

The effect of fertilizer (2017 data) on species abundances was analyzed using a partial redundancy analysis. Cultivar and fertilizer did not significantly differ in variation in the vegetation composition (6.5% vs. 7.9%; Table 2.1b). The effect of fertilizer on vegetation composition and abundances was significant (F=5.7, p=0.002; Table 2.1b). In the cultivar biplot axis 1 separated Inkeri, Salten, and Kalevi plots and axis 2 separated Kasper and Antti plots from Fure, Control and Ilmari plots (Figure 2.1c). There was no clear separation of cultivars with
respect to endophyte status. *Elymus repens* (Quack Grass), *Sonchus arvensis* (Sow Thistle), and *Convolvulus arvensis* (Field Bindweed) abundances were associated with Fure, Control and Ilmari plots. *Schedonorus pratensis* abundance was associated with Fure and Ilmari plots. *Schedonorus pratensis* abundance was negatively associated with *Poa pratensis* (Kentucky Blue Grass) abundance. *Solidago canadensis* (Goldenrod), *Vicia cracca* (Tufted Vetch), *Bromus inermus* (Brome Grass), and *Solanum dulcamara* (Bitter nightshade) abundances were associated with Antti and Kasper plots. *Dactylis glomerate* (Orchard Grass) abundance was weakly associated with Salten plots.

*Schedonorus pratensis* abundance was not correlated with either fertilizer treatment. In the fertilizer treatment biplot fertilized and not fertilized fell along axis 2 (Figure 2.1d). *Schedonorus pratensis* abundance was negatively associated with *Dactylis glomerate* (Orchard Grass) and *Linaria vulgaris* (Yellow Toadflax) abundances. *Cirsium arvense* (Canadian Thistle), *Elymus repens* (Quack Grass), *Convolvulus arvensis* (Field Bindweed), and *Silene vulgaris* (Bladder Campion) abundances were associated with fertilized plots. *Poa pratensis* (Kentucky Blue Grass) abundance was weakly correlated with nonfertilized plots.

### 2.3.2 Soil Samples

The paired samples test for soil samples nitrate, phosphorus and ammonium concentrations were compared between fertilized and unfertilized plots. There was a significant difference between fertilized and unfertilized plots in respect to nitrate ($t(5) = 4.497, p=0.006$), phosphorus ($t(s) = -7.08, p=0.001$), and ammonium ($t(s) = -5.12, p=0.004$; Table 2.3) concentrations.

### 2.3.3 Diversity and *Schedonorus pratensis* abundance data (2011-2014, 2017)

The effect of endophyte was significant for two measurements of plant community biodiversity. A one-way ANOVA was performed to test the effect of endophyte status on three measures of diversity (richness, Simpson’s diversity, and evenness). Pre-planned contrasts were conducted to see if species richness, evenness, and Simpson’s diversity measurements were affected by endophyte status (E+, E-, and Control). Endophyte status had a significant effect on species richness ($F_{(2,397)} = 6.738, p=0.001$; Table 2.4) and Simpson’s diversity ($F_{(2,397)} = 4.740, p=0.009$), but did not significantly affect species evenness ($F_{(2,397)} = 1.522, p=0.220$).

The effect of year and endophyte on species richness was significant. Pre-planned contrasts suggests that species richness in E+ plots did significantly differ from control plots ($t_{(397)} = 2.745, p=0.006$) and E- plots ($t_{(397)} = 3.294, p=0.001$; Figure 2.2a). Species richness in E-plots did not significantly differ from control plots ($t_{(397)} = 0.585, p=0.559$). Plots seeded with highly infected cultivars (E+) of *Schedonorus pratensis* were more species rich than control and low endophyte infected cultivars (E-). In a univariate ANOVA with endophyte, year and the interaction endophyte x year species richness was significantly affected by year ($F_{(4,308)} = 10.3840, p=6.676e-08$; Table 2.5). A tukey honestly significant difference post hoc test on year found that 2011 species richness significant differed from species richness in 2012 ($t_{(308)} = 5.263, p=0.0001$), 2013 ($t_{(308)} = 2.970, p=0.02$), and 2014 ($t_{(308)} = 5.642, p=0.0001$; Figure 2.3a). Species richness was also significantly different between 2014 and 2017 ($t_{(308)} = 3.00, p=0.02$).

Endophyte also had a significant effect on richness ($F_{(2,70)} = 6.099, p=0.003$). A tukey honestly significant difference post hoc test on endophyte found a significant difference between E- vs E+ plots ($t_{(72)} = -3.138, p=0.006$) and E+ vs control plots ($t_{(68)} = 2.538, p=0.035$).
between endophyte and year did not have a significant effect on species richness ($F_{(8,308)} = 0.509$, $p=0.8492$).

The effect of year on species richness was significant. In a separate univariate ANOVA with cultivar, year, and the interaction cultivar x year, cultivar ($F_{(7,63)} = 1.679$, $p=0.130$) and cultivar x year ($F_{(28,288)} = 1.220$, $p=0.210$) did not have a significant effect on species richness. Year did have a significant effect on species richness ($F_{(4,288)} = 14.90$, $p=4.397 \times 10^{-11}$; Table 2.6). A tukey honestly significant difference post hoc test on year found that 2011 species richness significantly differed from 2012 ($t_{(288)} = 6.446$, $p=0.0001$), 2013 ($t_{(288)} = 3.037$, $p=0.02$), 2014 ($t_{(288)} = 6.579$, $p=0.0001$), and 2017 ($t_{(288)} = 3.455$, $p=0.005$). Species richness was significantly different between 2012 and 2013 ($t_{(288)} = -3.408$, $p=0.006$), as well as 2012 and 2017 ($t_{(288)} = -2.991$, $p=0.02$). There was also a significant difference in species richness between 2013 and 2014 ($t_{(288)} = 3.542$, $p=0.004$), and 2014 and 2017 ($t_{(288)} = -3.124$, $p=0.016$).

The effect of year on Simpson’s diversity was significant. The effect of endophyte was only significant in the pre-planned contrasts. Pre-planned contrasts of Simpson’s diversity suggests that E+ plots significantly differ from E- plots ($t_{(397)} = -3.077$, $p=0.002$; Figure 2.2b), but there was no significant difference between E- and control plots ($t_{(397)} = -1.017$, $p=0.310$), as well as E+ and control plots ($t_{(397)} = 1.050$, $p=0.294$). Plots seeded with E+ cultivars were more diverse than control and E- plots. In a univariate ANOVA with endophyte, year, and endophyte x year, year had a significant effect on Simpson’s diversity ($F_{(4,308)} = 8.559$, $p=1.464 \times 10^{-6}$; Table 2.5). A tukey honestly significant difference post hoc test revealed that Simpson’s diversity in 2011 differed from 2012 ($t_{(308)} = 3.165$, $p=0.014$), 2013 ($t_{(308)} = 3.313$, $p=0.009$), 2014 ($t_{(308)} = 5.701$, $p=0.0001$), and 2017 ($t_{(308)} = 3.985$, $p=0.0008$; Figure 2.3b). Endophyte ($F_{(2,70)} = 3.046$, $p=0.053$) and the interaction endophyte x year ($F_{(8,308)} = 0.9116$, $p=0.506$) did not have a significant effect on Simpson’s diversity.

The effect of year had a significant effect on species richness. In a separate univariate ANOVA with cultivar, year, and the interaction cultivar x year, cultivar ($F_{(7,63)} = 1.743$, $p=0.115$) and cultivar x year ($F_{(28,288)} = 0.760$, $p=0.806$) did not have a significant effect on Simpson’s diversity. Year did have a significant effect on Simpson’s diversity ($F_{(4,288)} = 11.18$, $p=1.887 \times 10^{-8}$; Table 2.6). A tukey honestly significant difference post hoc test on year found that 2011 Simpson’s diversity significantly differed from 2012 ($t_{(288)} = 4.424$, $p=0.0001$), 2013 ($t_{(288)} = 3.634$, $p=0.003$), 2014 ($t_{(288)} = 6.452$, $p=0.0001$), and 2017 ($t_{(288)} = 4.496$, $p=0.0001$). Simpson’s diversity was significantly different between 2013 and 2014 ($t_{(288)} = 2.819$, $p=0.04$).

The effect of year had a significant effect on evenness. Pre-planned contrasts of species evenness suggest that there are no significant differences between endophyte status. E+ versus E- plots ($t_{(397)} = 0.712$, $p=0.477$), E+ versus control plots ($t_{(397)} = 1.744$, $p=0.082$), and E- versus control plots ($t_{(397)} = 1.315$, $p=0.189$; Figure 2.2c). In univariate ANOVA with endophyte, year, and endophyte x year, year had a marginal effect on evenness ($F_{(4,308)} = 2.419$, $p=0.04$), but endophyte ($F_{(2,70)} = 1.1891$, $p=0.3105$) and endophyte x year ($F_{(8,308)} = 1.0040$, $p=0.429$ Table 2.5) did not have a significant effect on evenness. A tukey honestly significant difference post hoc test was performed on year, but no significant differences were found between years.
The effect of year had a significant effect on evenness. In a separate univariate ANOVA with cultivar, year, and cultivar x year, year had a marginal effect on evenness (F(4,288) = 2.536, p= 0.040: Table 2.6). A tukey honestly significant difference post hoc test was performed on year, but no significant differences were found between years. Evenness was not significantly affected by cultivar (F(7,63) =1.098, p=0.375 or cultivar x year (F(28,288) = 1.095, p=0.342).

*Schedonorus pratensis* abundance was significantly affected by year, endophyte and cultivar. In a univariate ANOVA with endophyte, year, and endophyte x year, year (F(4,308) =19.63, p= 2.082e-14), endophyte (F(2,68) =23.23, p= 2.041e-08; Table 2.7), and endophyte x year (F(8,308) =2.026, p=0.043) all had a significant effect on *Schedonorus pratensis* abundance. A tukey honestly significant difference post hoc test was performed on all three factors (Figure 2.4 & 2.5). In a separate univariate ANOVA with cultivar, year, and cultivar x year, year (F(4,288) =42.36, p=2.2e-16), cultivar (F(7,63) =16.29, p=4.843e-12), and cultivar x year (F(28,288) =2.744, p=1.303e-05; Table 2.8) all had a significant effect on *Schedonorus pratensis* abundance. A tukey honestly significant difference post hoc test was performed on all three factors (Figure 2.6).

### 2.3.4 Diversity and *Schedonorus pratensis* abundance data (2017)

The effect of endophyte was not significant for all three measurements of plant community biodiversity. A one-way ANOVA was performed to test the effect of endophyte status on three measurements of diversity (richness, Simpson’s diversity, and evenness) Pre-planned contrasts were conducted to see if species richness, evenness, and Simpson’s diversity measurements were affected by endophyte status (E+, E-, and Control). Endophyte status did not have a significant effect on species richness (F(2,77) =0.233, p=0.793; Table 2.9), Simpson’s diversity (F(2,77) =0.163, p=0.850) and evenness (F(2,77) =0.105, p=0.900).

The effect of endophyte and fertilizer did not have a significant effect on species richness. Pre-planned contrasts suggests that there are no significant differences in species richness regardless of endophyte status; E- versus control plots (t(77) =-0.219, p=0.827), E+ versus control plots (t(77) =0.239, p=0.812), and E+ versus E- plots (t(77) =-0.682, p=0.497. In a univariate ANOVA with fertilizer (F(1,70) =0.476, p=0.492; Table 2.10), endophyte (F(2,70) =0.241, p=0.785), and the interaction fertilizer x endophyte (F(2,70) =0.316, p=0.729) none of the factors had a significant effect on species richness.

The effect of cultivar and fertilizer did not have a significant effect on species richness. In a separate univariate ANOVA with cultivar (F(7,60) =0.417, p=0.887; Table 2.11), fertilizer (F(1,60) =1.556, p=0.217), and fertilizer x cultivar (F(7,60) =0.392, p=0.903) none of these factors had a significant effect on species richness.

The effect of endophyte did not have a significant effect on Simpson’s biodiversity, however the effect of fertilizer was significant. Pre-planned contrasts suggests that there are no significant differences in Simpson’s diversity regardless of endophyte status; E- versus control plots (t(77) =-0.201, p=0.983), E+ versus control plots (t(77) =0.344, p=0.732), and E+ versus E- plots (t(77) =-0.551, p=0.583). In a univariate ANOVA with fertilizer, endophyte and the interaction fertilizer x endophyte, fertilizer had a significant effect on Simpson’s diversity (F(1,70) =32.29, p= 1.985e-07; Table 2.10 ), but endophyte (F(2,70) =0.298, p=0.742) and fertilizer x endophyte (F(2,70) =1.671, p=0.195) did not have a significant effect on Simpson’s diversity.
The effect of cultivar did not have significant effect on Simpson’s diversity. In a separate univariate ANOVA with cultivar, fertilizer, and fertilizer x cultivar, fertilizer had a significant effect on Simpson’s diversity ($F_{(1,60)} = 36.86, p = 9.312e-08; \text{Table 2.11}$), but cultivar ($F_{(7,60)} = 0.734, p = 0.643$) and fertilizer x cultivar ($F_{(7,60)} = 0.940, p = 0.482$) did not significantly affect Simpson’s diversity.

The effect of endophyte did not have a significant effect on evenness, however the effect of fertilizer was significant. Pre-planned contrasts suggests that there are no significant differences in evenness regardless of endophyte status; E- versus control plots ($t_{(77)} = 0.367, p = 0.714$), E+ versus control plots ($t_{(77)} = 0.110, p = 0.913$), and E+ versus E- plots ($t_{(77)} = -0.371, p = 0.711$). In a univariate ANOVA with fertilizer, endophyte, and fertilizer x endophyte, fertilizer had a significant effect on evenness ($F_{(1,70)} = 11.26, p = 0.001; \text{Table 2.10}$), but endophyte ($F_{(2,70)} = 0.126, p = 0.881$) and fertilizer x endophyte ($F_{(2,70)} = 0.060, p = 0.941$) did not have a significant effect on evenness.

The effect of cultivar did not have significant effect on evenness. In a separate univariate ANOVA with cultivar, fertilizer, and fertilizer x cultivar, fertilizer had a significant effect on evenness ($F_{(1,60)} = 8.662, p = 0.004; \text{Table 2.11}$), but cultivar ($F_{(7,60)} = 0.484, p = 0.841$) and fertilizer x cultivar ($F_{(7,60)} = 0.587, p = 0.763$) did not have a significant effect on evenness.

*Schedonorus pratensis* abundance was not significantly affected by endophyte, fertilizer and cultivar. In a univariate ANOVA testing the effect of fertilizer ($F_{(1,74)} = 0.312, p = 0.577$), endophyte ($F_{(2,74)} = 2.972, p = 0.057$), and fertilizer x endophyte ($F_{(2,74)} = 0.600, p = 0.941$) on *Schedonorus pratensis* abundance neither factor was found to significantly affect *S. pratensis* abundance. In a separate univariate ANOVA testing the effect of fertilizer ($F_{(1,64)} = 0.727, p = 0.396; \text{Table 2.12}$), cultivar ($F_{(7,64)} = 1.974, p = 0.072$), and cultivar x fertilizer ($F_{(7,64)} = 0.311, p = 0.946; \text{Table 2.13}$) on *Schedonorus pratensis* abundance neither factor was found to significantly affect *S. pratensis* abundance.

2.3.5 Weather data

I examined the effect of year, season and the interaction year x season on total weekly precipitation from 2011 to 2017 in a univariate ANOVA and found that year had a significant effect on weekly precipitation ($F_{(6,353)} = 2.479, p = 0.023; \text{Table 2.14}$). A tukey honestly significant difference post hoc test on year found that there was a significant difference between 2012 and 2017 in weekly precipitation ($t_{(353)} = -3.311, p = 0.017; \text{Figure 2.7}$). In a univariate ANOVA I examined the effects of year, season and the interaction year x season on daily mean temperature from 2011 to 2017. Year ($F_{(6,2471)} = 6.734, p = 4.34e-07; \text{Table 2.15}$), season ($F_{(1,2471)} = 3199.29, p = 2e-16$), and year x season ($F_{(6,2471)} = 3.436, p = 0.00221; \text{Table}$) all had a significant effect on daily mean temperature. A tukey honestly significant difference post hoc test was performed on year (Figure 2.8a), season and the interaction year x season (Figure 2.8b).

2.4 Discussion

I was interested in how endophyte infected grasses effected old field communities’ years after being established. I was also interested in the effect of fertilizer application on the relative abundance of *Schedonorus pratensis* and how it would affect plant community assemblage. Experiments that have studied the performance of endophyte infected *Schedonorus pratensis* have had varying results. Takai et al. (2010) found that endophyte infected *S. pratensis* was more
abundant in monocultures and mixtures compared to uninfected *S. pratensis* grown in the same conditions. Two genetically different E+ *S. pratensis* cultivars grown with *Dactylis glomerate* (Orchard grass) and *Trifolium repens* (White clover) had increased abundances and endophyte frequencies compared to E- *S. pratensis* grown under the same conditions (Takai et al. 2010). Niemeläinen et al. (2001) also found that endophyte infected *S. pratensis* abundances increased more than endophyte uninfected abundances when grown with *Phleum pretense* (Timothy grass) and *Trifolium pratense* (Red Clover). These studies suggest that *S. pratensis* can perform well in mixed vegetative communities, however Shukla et al. (2015) found that *S. pratensis* did not achieve high abundance in an old field community regardless of endophyte status. E+ *S. pratensis* plots increased species richness more than unseeded control plots (Shukla et al. 2015). Shukla et al. (2015) suggests that *S. pratensis* became incorporated into the community by displacing other dominant plant species instead of becoming a dominant species itself.

In this study E+ *S. pratensis* were more species rich than E- and control plots in the one-way ANOVA (Figure 2.2a). In a univariate analysis I performed *S. pratensis* abundance fluctuated in the years 2011, 2012, and 2013, but started decreasing in 2014 and 2017. In the partial redundancy analysis with year *S. pratensis* abundance was negatively correlated with *Poa pratensis* (Kentucky Bluegrass), *Vicia cracca* (Tufted Vetch), and *Solidago canadensis* (Canada Goldenrod) abundances. With *P. pratensis* abundances being positively correlated with the year 2017 suggesting that *P. pratensis* abundances are increasing over time (Figure 2.1a). I think it important to state that this field site already had established *P. pratensis* naturally occurring in the plant community (Shukla et al. 2015) and it being deemed an invasive species (Saikkonen et al. 1998; Saikkonen et al. 2000) could suggest that it is out competing or masking the competitive advantages of endophyte infected *S. pratensis*. This suggests that community richness and certain species maybe able to influence the success of *S. pratensis* in a natural community (Niemeläinen et al. 2001; Takai et al. 2010). In my study Simpson’s diversity did not have the same results as richness suggesting that richness is one of the main responses driving changes in plant community biodiversity.

Long term field studies of endophyte infected *S. pratensis* in grazed and non-grazed pastures grown alongside *P. pratensis*, *Lolium perenne*, and *D. golmerata* found that *S. pratensis* was abundant across pastures and had the highest infection frequencies in the oldest (21 years) non-grazed pastures (Saari et al. 2010). *Schedonorus pratensis* was the most dominant grass in the plant communities (Saari et al. 2010). This was not the case for the study done by Shukla et al. (2015) they found that *S. pratensis* was not able to become abundant in the plant community, however the duration of establishment was significantly lower (3 years) than Saari et al. (2010) (4-21 years). My study site being the same site as Shukla et al. (2015), however the duration of my study was 5 years and an additional treatment of fertilizer was added. *S. pratensis* abundance after 8 years is still low in the plant community. Multiple studies have suggested that in natural plant community’s endophyte infection frequencies can change within a plant population and these changes can depend upon local selection pressures as well as grass species (Saikkonen et al. 2000; Saari et al. 2009). Infection frequencies can change in populations if the endophyte inside the seed dies before germination, if the endophyte is not successful in colonizing the host plant seeds, or if the metabolic cost of having an endophyte becomes too high for the host plant (Saari et al. 2010). Cultivars of *S. pratensis* are quite commonly infected however infection frequencies can range from high to low infection (Saari et al. 2009). The infection frequency of a cultivar is presumed to be relatively constant due to the endophyte being vertically transmitted
via host plant seeds (Saari et al. 2009). Saari et al. (2009) found that *S. pratensis* endophyte infection frequencies varied from high to low and this varied among and within cultivars. Varying infection frequencies were seen in *S. pratensis* cultivars Kasper and Salten, whereas the cultivar Inkeri maintained high infection frequencies (Saari et al. 2009). Shukla et al. (2015) found that *S. pratensis* cultivars Inkeri and Kasper had the highest infection rates than all other cultivars. The infection rate for *S. pratensis* fluctuated with year, with 2011 having the higher infection frequencies compared to 2010 and 2013 (Shukla et al. 2015). This could suggest that endophyte infection frequencies may decrease over time. I wonder if other dominant plant species along with poor nutrient conditions could change the endophyte infection frequencies of a plant population.

Shukla et al. (2015) has suggested that site specific effects such as nutrient availability and climate may have stronger effects on *S. pratensis* durability within natural communities than species, cultivar and endophyte effects. Niemeläinen et al. (2001) found that in the field endophyte infected *S. pratensis* did not differ from endophyte uninfected *S. pratensis* under nutrient limited, low light, and dry soil conditions. Ahlholm et al. (2002) also found no differences between endophyte infected and endophyte uninfected *S. pratensis* grown under nutrient limited conditions. Increasing evidence suggests that low nutrient availability can affect the symbiosis between endophytes and their plant host (Cheplick 2007). Dirihan et al. (2009) found that *S. pratensis* grown in monoculture and mixtures only showed endophyte infected advantages under high nutrient conditions.

In my study *S. pratensis* abundance is decreasing with time. Meadow fescue abundance fluctuated from 2011 to 2013 and started declining in 2014. The fluctuation in meadow fescue abundance could be related to environmental factors such as precipitation and temperature. Weekly precipitation fluctuated from 2011 to 2017 with the average weekly precipitation being 17.89mm across the seven years. Year had a significant effect on weekly precipitation with the year 2012 being different than 2017 (Figure 2.7). The average yearly temperature also fluctuated with year. With the average temperature across the seven years being 7°C (Figure 2.8a). Fertilizer was applied in between sampling years with 2014 being the last year before fertilizer was applied for three more years. The data collected from 2017 suggests that fertilizer did not have a significant effect on species richness but did have a significant effect on evenness and Simpson’s diversity. It would be interesting to see how well infected *S. pratensis* populations would fair in a community that started with high nutrient levels that decreased over time. Overall, I think all the experiments I have discussed have lent claim to the importance of studying the symbiosis between endophytes and their host under different environmental conditions, over longer periods of time and in natural plant communities to understand how endophyte infected grasses can be incorporated into natural plant communities. In this study *S. pratensis* and its endophyte *Epichloë uncinata* did not achieve high levels of abundance and the effect of fertilizer was marginally seen on the plant community. The effect of fertilizer might not have fully been seen due to the study site being considered nutrient poor, it might take several more years for the effect of fertilizer to be seen in the plant community.
Figure 2.1a: Biplots based on a partial redundancy analysis of the vegetation composition with respect to the effect of year. *Schedonorus pratensis* was used as a supplementary variable in the ordination model.
Table 2.1a: ANOVA results from partial redundancy analyses of vegetation composition for all *Schedonorus pratensis* cultivars (E+, E-, unseeded control). Bold font indicates significant effects.

<table>
<thead>
<tr>
<th>Community</th>
<th>Source</th>
<th>df</th>
<th>Total SS</th>
<th>Pseudo-F</th>
<th>P</th>
<th>%variation¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. pratensis</em></td>
<td>Block</td>
<td>9</td>
<td>0.104305</td>
<td>2.1</td>
<td>0.392</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>Cultivar</td>
<td>7</td>
<td>0.0328024</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>4</td>
<td>0.111868</td>
<td>14.1</td>
<td>0.002</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>C x Y</td>
<td>28</td>
<td>0.0375992</td>
<td>0.7</td>
<td>0.748</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Residual</td>
<td></td>
<td>0.713426</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Percent variation in community abundance data explained by the model term.

Table 2.1b: ANOVA results from partial redundancy analyses of vegetation composition from 2017 for all *Schedonorus pratensis* cultivars (E+, E-, unseeded control). Bold font indicates significant effects.

<table>
<thead>
<tr>
<th>Community</th>
<th>Source</th>
<th>df</th>
<th>Total SS</th>
<th>Pseudo-F</th>
<th>P</th>
<th>%variation¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. pratensis</em></td>
<td>Block</td>
<td>4</td>
<td>0.072414</td>
<td>0.7</td>
<td>0.952</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>Cultivar</td>
<td>7</td>
<td>0.0606284</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fertilizer</td>
<td>1</td>
<td>0.0683051</td>
<td>5.7</td>
<td>0.002</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>C x F</td>
<td>7</td>
<td>0.0926718</td>
<td>1.1</td>
<td>0.218</td>
<td>11.6</td>
</tr>
</tbody>
</table>

¹ Percent variation in community abundance data explained by the model term
Figure 2.1b: Biplots based on a partial redundancy analysis of the vegetation composition with respect to the effect of cultivar. *Schedonorus pratensis* was used as a supplementary variable in the ordination model. Triangle = low-endophyte frequency (E-), + = high-endophyte frequency (E+).
Figure 2.1c: Biplots based on a partial redundancy analysis of the vegetation composition from 2017 with respect to the effect of cultivar. *Schedonorus pratensis* was used as a supplementary variable in the ordination model. Triangle = low-endophyte frequency (E-), + = high-endophyte frequency (E+).
Figure 2.1d: Biplots based on a partial redundancy analysis of the vegetation composition from 2017 with respect to the effect of fertilizer treatment. *Schedonorus pratensis* was used as a supplementary variable in the ordination model.
Table 2.2a: Species list of grasses, forbs, legumes and shrubs that occurred in multiple years as well as 2017 used in the partial redundancy analyses.

<table>
<thead>
<tr>
<th>Plant Type</th>
<th>Plant species 2011-2014, 2017</th>
<th>Plant species 2017</th>
</tr>
</thead>
</table>
| Grasses    | *Schedonorus pratensis* (Meadow Fescue)  
*Schedonorus arundinacea* (Tall Fescue)  
Poa pratensis  (Kentucky Blue Grass)  
*Elymus repens*  (Quack Grass)  
*Dactylis glomerate* (Orchard Grass) | *Schedonorus pratensis* (Meadow Fescue)  
*Schedonorus arundinacea* (Tall Fescue)  
Poa pratensis  (Kentucky Blue Grass)  
*Elymus repens*  (Quack Grass)  
*Dactylis glomerate* (Orchard Grass)  
*Bromus inermis*  (Brome Grass) |
| Forbs      | *Cirsium arvense* (Canadian Thistle)  
*Sonchus arvensis* (Sow Thistle)  
*Taraxacum officinale*  (Dandelion)  
*Aster lanceolatus*  (Lance-Leaf Aster)  
*Solidago canadensis* (Goldenrod)  
*Lychnis alba*  (Chickweed)  
*Linaria vulgaris*  (Yellow Toadflax)  
*Convolvulus arvensis*  (Field Bindweed) | *Cirsium arvense* (Canadian Thistle)  
*Sonchus arvensis* (Sow Thistle)  
*Taraxacum officinale*  (Dandelion)  
*Aster lanceolatus*  (Lance-Leaf Aster)  
*Solidago canadensis* (Goldenrod)  
*Linaria vulgaris* (Yellow Toadflax)  
*Convolvulus arvensis* (Field Bindweed)  
*Solanum dulcamara*  (Bitter Nightshade)  
*Silene vulgaris*  (Bladder Campion)  
*Asclepias syriaca*  (Common Milkweed) |
| Legumes    | *Vicia cracca*  (Tufted Vetch) | *Vicia cracca*  (Tufted Vetch) |
| Shrubs     | | *Ribes triste*  (Redcurrant) |
Table 2.2b: Species list of grasses, forbs, legumes and shrubs that occurred in multiple year as well as 2017 used in the biodiversity analyses.

<table>
<thead>
<tr>
<th>Plant Type</th>
<th>Plant Species 2011-2014, 2017</th>
<th>Plant Species 2017</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grasses</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Schedonorus pratensis</em> (Meadow Fescue)</td>
<td><em>Schedonorus pratensis</em> (Meadow Fescue)</td>
</tr>
<tr>
<td></td>
<td><em>Schedonorus arundinacea</em> (Tall Fescue)</td>
<td><em>Schedonorus arundinacea</em> (Tall Fescue)</td>
</tr>
<tr>
<td></td>
<td><em>Poa pratensis</em> (Kentucky Blue Grass)</td>
<td><em>Poa pratensis</em> (Kentucky Blue Grass)</td>
</tr>
<tr>
<td></td>
<td><em>Elymus repens</em> (Quack Grass)</td>
<td><em>Elymus repens</em> (Quack Grass)</td>
</tr>
<tr>
<td></td>
<td><em>Dactylis glomerate</em> (Orchard Grass)</td>
<td><em>Dactylis glomerate</em> (Orchard Grass)</td>
</tr>
<tr>
<td></td>
<td><em>Bromus inermis</em> (Brome Grass)</td>
<td><em>Bromus inermis</em> (Brome Grass)</td>
</tr>
<tr>
<td></td>
<td><em>Phleum pretense</em> (Timothy Grass)</td>
<td></td>
</tr>
<tr>
<td>Forbs</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Cirsium arvense</em> (Canadian Thistle)</td>
<td><em>Cirsium arvense</em> (Canadian Thistle)</td>
</tr>
<tr>
<td></td>
<td><em>Sonchus arvensis</em> (Sow Thistle)</td>
<td><em>Sonchus arvensis</em> (Sow Thistle)</td>
</tr>
<tr>
<td></td>
<td><em>Taraxacum officinale</em> (Dandelion)</td>
<td><em>Taraxacum officinale</em> (Dandelion)</td>
</tr>
<tr>
<td></td>
<td><em>Aster lanceolatus</em> (Lance-Leaf Aster)</td>
<td><em>Aster lanceolatus</em> (Lance-Leaf Aster)</td>
</tr>
<tr>
<td></td>
<td><em>Solidago canadensis</em> (Canada Goldenrod)</td>
<td><em>Solidago canadensis</em> (Canada Goldenrod)</td>
</tr>
<tr>
<td></td>
<td><em>Lychnis alba</em> (Chickweed)</td>
<td></td>
</tr>
<tr>
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<td><em>Linaria vulgaris</em> (Yellow Toadflax)</td>
<td><em>Linaria vulgaris</em> (Yellow Toadflax)</td>
</tr>
<tr>
<td></td>
<td><em>Convolvulus arvensis</em> (Field Bindweed)</td>
<td><em>Convolvulus arvensis</em> (Field Bindweed)</td>
</tr>
<tr>
<td></td>
<td><em>Solanum dulcamara</em> (Bitter Nightshade)</td>
<td><em>Solanum dulcamara</em> (Bitter Nightshade)</td>
</tr>
<tr>
<td></td>
<td><em>Medicago lupulina</em> (Black Medick)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Symphyotrichum novae-angliae</em> (New England Aster)</td>
<td><em>Symphyotrichum novae-angliae</em> (New England Aster)</td>
</tr>
<tr>
<td></td>
<td><em>Nepeta cataria</em> (Catnip)</td>
<td></td>
</tr>
<tr>
<td>Legumes</td>
<td><em>Vicia cracca</em> (Tufted Vetch)</td>
<td><em>Vicia cracca</em> (Tufted Vetch)</td>
</tr>
<tr>
<td>Shrubs</td>
<td><em>Ribes triste</em> (Redcurrent)</td>
<td><em>Ribes triste</em> (Redcurrent)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Viburnum</em></td>
</tr>
</tbody>
</table>

*We were only able to identify to genus the one shrub (Viburnum).
**Table 2.3a:** Paired Samples Test results of soil samples from fertilized and unfertilized plots measured for Nitrate, Phosphorus and Ammonium. Response variables were box-cox transformed for the analysis. Bold font indicates significant effects.

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>95% Confidence Interval</th>
<th>T</th>
<th>df</th>
<th>Sig 2-tailed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilized Nitrate vs Unfertilized Nitrate</td>
<td>7.94063</td>
<td>4.32497171</td>
<td>1.76566231</td>
<td>3.401 - 12.47</td>
<td>4.49</td>
<td>5</td>
<td><strong>0.006</strong></td>
</tr>
<tr>
<td>Fertilized Phosphorus vs Unfertilized Phosphorus</td>
<td>-0.5048</td>
<td>0.01744369</td>
<td>0.00712135</td>
<td>-0.0687 - 0.032</td>
<td>-7.08</td>
<td>5</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Fertilized Ammonium vs Unfertilized Ammonium</td>
<td>-0.3055</td>
<td>0.01458917</td>
<td>0.00595600</td>
<td>-0.0458 - 0.0152</td>
<td>-5.12</td>
<td>5</td>
<td><strong>0.004</strong></td>
</tr>
</tbody>
</table>

**Table 2.3b:** Mean, standard deviation and variance for soil samples taken from fertilized and unfertilized plots measuring Nitrate, Phosphorus and Ammonium.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilized Nitrate</td>
<td>6</td>
<td>15.667</td>
<td>8.8568</td>
<td>78.443</td>
</tr>
<tr>
<td>Fertilized Phosphorus</td>
<td>6</td>
<td>25.33</td>
<td>15.135</td>
<td>229.067</td>
</tr>
<tr>
<td>Fertilized Ammonium</td>
<td>6</td>
<td>5.016</td>
<td>1.2006</td>
<td>1.442</td>
</tr>
<tr>
<td>Not Fertilized Nitrate</td>
<td>6</td>
<td>1.9333</td>
<td>.57504</td>
<td>.331</td>
</tr>
<tr>
<td>Not Fertilized Phosphorus</td>
<td>6</td>
<td>19.3333</td>
<td>8.16497</td>
<td>66.667</td>
</tr>
<tr>
<td>Not Fertilized Ammonium</td>
<td>6</td>
<td>3.2500</td>
<td>.39370</td>
<td>.155</td>
</tr>
</tbody>
</table>
Table 2.4: One-way ANOVA results of the effect of endophyte on species Richness, Simpson’s diversity, and Evenness for 2011-2014, 2017. Response variables were box-cox transformed for the analysis. Bold font indicates significant effects.

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Richness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>11.139</td>
<td>2</td>
<td>5.570</td>
<td>6.738</td>
<td>.001</td>
</tr>
<tr>
<td>Within Groups</td>
<td>328.171</td>
<td>397</td>
<td>.827</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>339.310</td>
<td>399</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Simpson’s Diversity (1/D)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>.141</td>
<td>2</td>
<td>.070</td>
<td>4.740</td>
<td>.009</td>
</tr>
<tr>
<td>Within Groups</td>
<td>5.902</td>
<td>397</td>
<td>.015</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6.043</td>
<td>399</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Evenness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>.934</td>
<td>2</td>
<td>.467</td>
<td>1.522</td>
<td>.220</td>
</tr>
<tr>
<td>Within Groups</td>
<td>121.776</td>
<td>397</td>
<td>.307</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>122.710</td>
<td>399</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.2a: Species richness in response to endophyte status. Richness data from 2011-2014 and 2017) E+ endophyte treatments consist of data collected from plots containing high endophyte infected Schedonorus pratensis cultivars Inkeri, Salten, and Kasper and E- endophyte treatments consisted of data collected from low endophyte infected S. pratensis cultivars Antti, Kalevi, Fure, and Ilmari. The control plots were unseeded to represent the natural uninvaded community background. Pre-planned contrasts suggest that richness in E+ plots significantly differed from richness in control plots (p=0.006) and E- plots (p=0.001). Richness was highest in E+ plots compared to control and E- plots. The untransformed data is plotted. The letters above the bars represent the results from the pre-planned contrast analysis indicating that different letters are significantly different.
Figure 2.2b: Simpson’s diversity response to endophyte status. Simpson’s diversity data from 2011-2014 and 2017) E+ endophyte treatments consist of data collected from plots containing high endophyte infected Schedonorus pratensis cultivars Inkeri, Salten, and Kasper and E- endophyte treatments consisted of data collected from low endophyte infected S. pratensis cultivars Antti, Kalevi, Fure, and Ilmari. The control plots were unseeded to represent the natural uninvaded community background. Pre-planned contrasts suggest that Simpson’s diversity in E+ plots significantly differed from Simpson’s diversity in E- plots (p=0.002) but did not significantly differ from control plots. Simpson’s diversity was highest in E+ plots compared to control and E- plots. The untransformed data is plotted. The letters above the bars represent the results from the pre-planned contrast analysis indicating that different letters are significantly different.
Figure 2.2c: Evenness in species abundance in response to endophyte status. Evenness being how even species abundances were distributed in the plant community. Evenness data from 2011-2014 and 2017) E+ endophyte treatments consist of data collected from plots containing high endophyte infected Schedonorus pratensis cultivars Inkeri, Salten, and Kasper and E- endophyte treatments consisted of data collected from low endophyte infected S. pratensis cultivars Antti, Kalevi, Fure, and Ilmari. The control plots were unseeded to represent the natural unininvaded community background. Pre-planned contrasts suggest that there are no significant differences in evenness between endophyte treatments. The untransformed data is plotted.
**Table 2.5:** Mixed Model ANOVA where the main response variables were tested in a split-plot analysis of repeated measures for the effects of year, endophyte and the interaction endophyte x year on species Richness, Diversity, and Evenness from 2011-2014 and 2017. The response variables were Box-Cox transformed for the analysis. Bold font indicates significant effects.

<table>
<thead>
<tr>
<th></th>
<th>Richness</th>
<th>Simpson’s Diversity</th>
<th>Evenness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>Year</td>
<td>4,308</td>
<td>10.38</td>
<td>6.67e-08</td>
</tr>
<tr>
<td>Endophyte</td>
<td>2,70</td>
<td>6.09</td>
<td>0.003</td>
</tr>
<tr>
<td>Endophyte x Year</td>
<td>8,308</td>
<td>0.50</td>
<td>0.849</td>
</tr>
</tbody>
</table>

**Table 2.6:** Mixed Model ANOVA where the main response variables were tested in a split-plot analysis of repeated measures for the effects of year, cultivar and the interaction cultivar x year on species Richness, Diversity, and Evenness from 2011-2014 and 2017. The response variables were Box-Cox transformed for the analysis. Bold font indicates significant effects.

<table>
<thead>
<tr>
<th></th>
<th>Richness</th>
<th>Simpson’s Diversity</th>
<th>Evenness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>Year</td>
<td>4,288</td>
<td>14.90</td>
<td>4.397e-11</td>
</tr>
<tr>
<td>Cultivar</td>
<td>7,63</td>
<td>1.67</td>
<td>0.13</td>
</tr>
<tr>
<td>Cultivar x Year</td>
<td>28,288</td>
<td>1.22</td>
<td>0.21</td>
</tr>
</tbody>
</table>
Figure 2.3a: Richness between five sampling years. Irrespective of cultivar and endophyte treatments applied to the plots. The effect of year on species richness was significant ($p=6.676 \times 10^{-08}$). 2011 species richness was highest out of five years. The letters above the bars are the results of a pairwise Tukey’s Honestly Significant Difference (HSD) post hoc test with bars not sharing the same letters being significantly different. The untransformed data is plotted.

Figure 2.3b: Simpson’s diversity response between five sampling years. Irrespective of cultivar and endophyte treatments applied to the plots. The effect of year on Simpson’s diversity was significant ($p=1.464 \times 10^{-06}$). 2011 Simpson’s diversity was highest out of five years. The letters above the bars are the results of a pairwise Tukey’s Honestly Significant Difference (HSD) post hoc test with bars not sharing the same letters being significantly different. The untransformed data is plotted.
**Figure 2.3c:** Evenness of plant communities between five sampling years. Evenness being how even species abundances were distributed in the plant community. The effect of year on evenness was significant ($p=0.04$), however a pairwise Tukey’s Honestly Significant Difference (HSD) post hoc test did not reveal any significant differences between years. The untransformed data is plotted.

**Table 2.7:** Mixed Model ANOVA results for the effects of year, endophyte and the interaction endophyte x year on *Schedonorus pratensis* abundance from 2011-2014 and 2017. The response variable was Box-Cox transformed for the analysis. Bold font indicates significant effects.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>4,308</td>
<td>19.6</td>
<td>2.082e-14</td>
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<tr>
<td>Endophyte</td>
<td>4,308</td>
<td>23.2</td>
<td>2.041e-08</td>
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<tr>
<td>Endophyte x Year</td>
<td>4,308</td>
<td>2.02</td>
<td>0.04</td>
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Figure 2.4: The effect of year on *Schedonorus pratensis* abundance across five sampling years. Year had a significant effect ($p=2.082\times10^{-14}$). The letters above the bars are the results of a pairwise Tukey’s Honestly Significant Difference (HSD) post hoc test with bars not sharing the same letters being significantly different. The untransformed data is plotted.

Figure 2.5: The effect of the interaction endophyte x year on *Schedonorus pratensis* abundance across five sampling years. Endophyte x year had a significant effect ($p=0.043$).
Table 2.8: Mixed Model ANOVA results for the effects of year, cultivar and the interaction cultivar x year on *Schedonorus pratensis* abundance from 2011-2014 and 2017. The response variable was Box-Cox transformed for the analysis. Bold font indicates significant effects.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
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<td>42.36</td>
<td>2.2e-16</td>
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<tr>
<td>Cultivar</td>
<td>7,288</td>
<td>16.29</td>
<td>4.843e-12</td>
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<tr>
<td>Cultivar x Year</td>
<td>28,288</td>
<td>2.74</td>
<td>1.303e-05</td>
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</table>

Figure 2.6: The effect of cultivar on *Schedonorus pratensis* abundance across five sampling years. Cultivar had a significant effect (p=4.843e-12). The letters above the bars are the results of a pairwise Tukey’s Honestly Significant Difference (HSD) post hoc test with bars not sharing the same letters being significantly different. The untransformed data is plotted.
Table 2.9: One-way ANOVA results of the effect of endophyte on species Richness, Simpson’s diversity, and Evenness for 2017. Response variables were box-cox transformed for the analysis.

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
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<tr>
<td>Richness</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>.366</td>
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<td>.183</td>
<td>.233</td>
<td>.793</td>
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<tr>
<td>Within Groups</td>
<td>60.585</td>
<td>77</td>
<td>.787</td>
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<tr>
<td>Total</td>
<td>60.951</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simpson’s Diversity</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>.008</td>
<td>2</td>
<td>.004</td>
<td>.163</td>
<td>.850</td>
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<tr>
<td>Within Groups</td>
<td>1.907</td>
<td>77</td>
<td>.025</td>
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<tr>
<td>Total</td>
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<tr>
<td>Evenness</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
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<td>.023</td>
<td>.105</td>
<td>.900</td>
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<tr>
<td>Within Groups</td>
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<td>77</td>
<td>.218</td>
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<tr>
<td>Total</td>
<td>16.799</td>
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</table>

Table 2.10: Univariate ANOVA results for the effects of fertilizer, endophyte and fertilizer x endophyte on species Richness, Diversity and Evenness for 2017. The response variables were Box-Cox transformed for the analysis. Bold font indicates significant effects.

<table>
<thead>
<tr>
<th></th>
<th>Richness</th>
<th>Simpson’s Diversity</th>
<th>Evenness</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>1.70</td>
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<td>0.49</td>
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<tr>
<td>Endophyte</td>
<td>2.70</td>
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<td>0.78</td>
</tr>
<tr>
<td>Fertilizer x Endophyte</td>
<td>2.70</td>
<td>0.31</td>
<td>0.72</td>
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</table>
Table 2.11: Univariate ANOVA results for the effects of fertilizer, cultivar and fertilizer x cultivar on species Richness, Diversity and Evenness for 2017. The response variables were Box-Cox transformed for the analysis. Bold font indicates significant effects.

<table>
<thead>
<tr>
<th></th>
<th>Richness</th>
<th></th>
<th>Simpson’s Diversity</th>
<th></th>
<th>Evenness</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>p</td>
<td>df</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>1,60</td>
<td>1.55</td>
<td>0.21</td>
<td>1.60</td>
<td>36.86</td>
<td><strong>9.312e-08</strong></td>
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<tr>
<td>Cultivar</td>
<td>7,60</td>
<td>0.41</td>
<td>0.88</td>
<td>7,60</td>
<td>0.73</td>
<td>0.64</td>
</tr>
<tr>
<td>Fertilizer x Cultivar</td>
<td>7,60</td>
<td>0.39</td>
<td>0.90</td>
<td>7,60</td>
<td>0.94</td>
<td>0.48</td>
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Table 2.12: Univariate ANOVA results for the effects of fertilizer, endophyte and endophyte x fertilizer on *Schedonorus pratensis* abundance for 2017. The response variable was Box-Cox transformed for the analysis.

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<tr>
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<tr>
<td>Fertilizer</td>
<td>1,74</td>
<td>0.31</td>
<td>0.57</td>
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<tr>
<td>Endophyte</td>
<td>2,74</td>
<td>2.97</td>
<td>0.05</td>
</tr>
<tr>
<td>Fertilizer x Endophyte</td>
<td>2,74</td>
<td>0.06</td>
<td>0.94</td>
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</table>

Table 2.13: Univariate ANOVA results for the effects of fertilizer, cultivar and fertilizer x cultivar on *Schedonorus pratensis* abundance for 2017. The response variable was Box-Cox transformed for the analysis.

<table>
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<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilizer</td>
<td>1,64</td>
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<td>0.39</td>
</tr>
<tr>
<td>Cultivar</td>
<td>7,64</td>
<td>1.97</td>
<td>0.07</td>
</tr>
<tr>
<td>Fertilizer x Cultivar</td>
<td>7,64</td>
<td>0.31</td>
<td>0.94</td>
</tr>
</tbody>
</table>
Table 2.14: Univariate ANOVA results for the effect of Year, Season and the interaction Year x Season on total weekly precipitation from 2011-2017. The response variable was Box-Cox transformed for the analysis.

<table>
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<tr>
<th></th>
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<th>Mean Sq.</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
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<td>121.5</td>
<td>20.253</td>
<td>2.479</td>
<td>0.0232</td>
</tr>
<tr>
<td>Season</td>
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<td>0.0</td>
<td>0.025</td>
<td>0.003</td>
<td>0.9562</td>
</tr>
<tr>
<td>Year x Season</td>
<td>6</td>
<td>45.8</td>
<td>7.638</td>
<td>0.935</td>
<td>0.4699</td>
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<tr>
<td>Residuals</td>
<td>353</td>
<td>2884.1</td>
<td>8.170</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.15: Univariate ANOVA results for the effects of Year, Season and the interaction Year x Season on daily mean temperatures from 2011-2017. The response variable was Box-Cox transformed for the analysis. Bold font indicates significant effects.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Sum Sq.</th>
<th>Mean Sq.</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>6</td>
<td>9820</td>
<td>1637</td>
<td>6.734</td>
<td>4.34e-07</td>
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<td>Season</td>
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<td>777653</td>
<td>777653</td>
<td>3199.294</td>
<td>&lt;2e-16</td>
</tr>
<tr>
<td>Year x Season</td>
<td>6</td>
<td>5011</td>
<td>835</td>
<td>3.436</td>
<td>0.00221</td>
</tr>
<tr>
<td>Residuals</td>
<td>2471</td>
<td>600626</td>
<td>243</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.7: Effect of Year on weekly precipitation from 2011-2017. The factor year tested significantly (p=0.023). The letters above the bars are the results of a pairwise Tukey’s Honestly Significant Difference (HSD) post hoc test with bars not sharing the same letters being significantly different. The untransformed data is plotted.

Figure 2.8a: Effects of year on daily mean temperature from 2011-2017. The factor year tested significantly (p=4.34e-07). The letters above the bars are the results of a pairwise Tukey’s Honestly Significant Difference (HSD) post hoc test with bars not sharing the same letters being significantly different. The untransformed data is plotted.
Figure 2.8b: Effects of the interaction year x season on daily mean temperature from 2011-2017. Overwinter = October–March; Growing season = April–September. The factor year x season tested significantly (p=0.002). The letters above the bars are the results of a pairwise Tukey’s Honestly Significant Difference (HSD) post hoc test with bars not sharing the same letters within growing season and overwinter being significantly different. The untransformed data is plotted.

Figure 2.9a: Fertilizer did not have a significant effect on species richness. There was no difference in species richness between fertilized and unfertilized plots. The untransformed data is plotted.
Figure 2.9b: The effect of fertilizer on Simpson’s biodiversity was significant (p=1.985e-07). Fertilized plots were more species diverse than unfertilized plots. The untransformed data is plotted.

Figure 2.9c: Fertilizer had a significant effect on species evenness (p=0.001). Fertilized plots had more species evenness than unfertilized plots. The untransformed data is plotted.
CHAPTER 3

The effect of nutrient availability on endophyte concentration

3.1 Introduction

Asexual *Epichloë* endophytes are obligate mutualists (Breen 1994). They provide their host with a plethora of advantages such as increased growth rates, germination, biomass production, drought tolerance, and herbivore resistance in exchange for shelter, nutrients, and transmission to the next generations (Malinowski & Belesky 2000; Gundel et al. 2013). It has been shown that endophyte infection frequency can change within a population because of local selection factors (Saari et al. 2009). The changes in infection frequencies within a population can be caused by the endophyte dying before germination, unsuccessful colonization of the hosts seeds by the endophyte and the metabolic costs of hosting an endophyte becoming too high (Saikkonen et al. 2000; Saari et al. 2009). Studies have also shown that endophyte infection frequencies are not the only thing that can change within a grass population. There is evidence that suggests the mutualistic relationship between endophytic fungi and their host plant can depend on host genotype, the strain of endophyte, herbivory load, competition from other fungi and environmental conditions such as nutrient availability and climate, which has been examined using endophyte infected perennial ryegrass and tall fescue (Cheplick 2004; Malinowski & Belesky 2006; Saikkonen et al. 2006; Rasmussen et al. 2007; Lui et al. 2011; Ryan et al. 2015).

Studies using quantitative PCR (qPCR) to quantify endophyte infection have found that multiple factors, both physiological and environmental, can affect endophyte concentration. Internal factors include: the plant leaf carbohydrate content, and the interaction between host-plant and endophyte genotypes (Rasmussen et al. 2007, 2008; Tian et al. 2013; Ryan et al. 2015). External factors include soil nitrogen supply (Rasmussen et al. 2007, 2008, Ryan et al. 2015), climate (Fuchs et al. 2017), atmospheric CO2 concentrations (Ryan 2014, Hunt et al. 2005) and competition with arbuscular mycorrhizal fungi (Liu et al. 2011). All of these factors that affect endophyte concentration have the potential to change the efficiency of the symbiosis between the endophyte and host through changes in anti-herbivory characteristics, toxicity and the invasive potential of endophyte infected plants (Hume et al. 2016).

Much of the experimental support for the effects of these external factors comes from greenhouse, laboratory or growth chamber experiments. Fuchs et al. (2017a), working in the field in central Germany, showed that fungal and alkaloid concentrations in perennial ryegrass were affected by season or temperature (confounded in this common garden experiment). The highest endophyte concentration occurred in the warm summer months with the lowest concentrations occurring during the cold winter months (Fushs et al. 2017a). In another garden experiment working with perineal ryegrass Fuchs et al. (2017b) examined the effect different forms of herbivory had on endophyte concentrations and observed that herbivore treatments marginally affected endophyte concentrations. Endophyte infected plants that were clipped had higher endophyte concentrations than unclipped control plants (Fushs et al. 2017b). A field experiment using *Festuca paniculate* (East Alpine Violet Fescue) infected with *Epichloë* sp. examined the effect mowing had on endophyte concentrations (Binet et al. 2017). *Festuca paniculate* leaves that were unmown had an average quantity of 2.5 times more endophyte DNA compared to mown plants (Binet et al. 2017).
In this chapter I discuss my research on how the infection of *Schedonorus pratensis* with its fungal endophyte, *Epichloë uncinata*, is affected by soil fertility. I specifically look at the effect of soil fertility on endophyte concentration. Other studies have conducted similar research looking at endophyte and alkaloid concentrations in perennial ryegrass (Rasmussen et al. 2008) and tall fescue (Ryan et al. 2014) under different nitrogen conditions, however my work is unique because I looked at the effect of nutrient availability on the symbiosis between meadow fescue and its fungal endophyte, in a field experiment where the target plants were embedded in a mature multispecies old field community. Using multiple replicate old field communities that were invaded with different meadow fescue cultivars (to account for and examine the genetic variability of these different populations of meadow fescue) I examined the effect of fertilizer on these communities (CHAPTER 2) and, in this chapter, on endophyte concentrations. I predict based on the limited research (a) that endophyte concentrations will be reduced in fertilized plots regardless of high endophyte infection or low endophyte infection based on the dilution effect where nitrogen is stimulating plant growth more than fungal growth and (b) that endophyte concentrations will be reduced more in high endophyte infected cultivar (E+) compared to low endophyte infected cultivar (E-) because the plant will allocate more nutrients (glucose) to plant functions and processes than to the endophyte and this will be seen more in E+ cultivars because endophyte concentrations tend to be higher in these cultivars.

3.2 Methods

3.2.1 Study site and experimental set-up

The study site was previously an apple orchard that was maintained with the occasionally mowing for 20 plus years prior to establishing our experimental site, which is located at the University of Guelph Turfgrass Institute and Environmental Research Center (Guelph, ON, Canada; 43° 32’ 56” N, 80° 12’ 39” W). The land is composed mainly of sandy loam (Brunisolic Gray-Brown Luvisol) soil developed on a loam till. Prior to conducting this study and the studies done by Yurkonis et al. (2012) and Shukla et al. (2015) the plant community consisted of non-native species such as *Elymus repens* L. Gould, *Poa pratensis* L., *Taraxacum officinale* F.H. Wigg, and Cirsium arvense L. Scop (Yurkonis et al. 2012; Shukla et al. 2015). The experimental site was established in 2008 and consists of an 826 m² area divided into 80 2x2 meter plots, separated by 0.5-meter mowed boundaries in a completely randomized block design (Yurkonis et al. 2012; Shukla et al. 2015).

The eighty plots were tilled twice and divided into ten blocks with eight plots in each block. Seven plots per block were randomly selected and seeded with one of the seven cultivars of *S. pratensis* (meadow fescue) at a rate of 5g m² (Clay and Holah 1999; Yurkonis et al. 2012; Shukla et al. 2015) and one plot was unseeded and served as a control. The *S. pratensis* seeds were obtained from Finland (Niemeläinen et al. 2001; Fjellheim et al. 2007; Saari et al. 2009) and stored at -16°C until sowing. The 80 plots were watered until germination and left to grow undisturbed and unmanaged. Within each block, four of the meadow fescue cultivars (Antti, Fure, Ilmari, Kalevi) had no or a low endophyte frequency, and three cultivars (Kasper, Salten, Inkeri) had a high endophyte frequency.
3.2.2 Fertilizer Treatment

In 2015, seven years after establishment, a fertilizer treatment was added to the experiment. The 10 blocks were combined to make 5 blocks that consisted of 16 plots in each block and one replicate of each cultivar treatment was randomly assigned to be fertilized or not. Fertilized plots were treated with 25:4:10 N:P:K (Greenskeeper, Brussels Agromart, Brussels, Ontario), for which the recommended amount for turf grass = 2kg product/100m² = 80g of product per 2m² (~80ml by volume). The plots were fertilized May 12, 2015, August 26, 2015, May 2, 2016, September 19, 2016, June 16, 2017 and September 18, 2017. The fertilizer was applied by broadcasting by hand in a rectangular shape in the center of the plots to avoid edge effects.

3.2.3 Tiller Collection

To estimate endophyte concentration, meadow fescue tillers were harvested in August 2017. Tillers were cut at the surface of the soil and included the grass sheath. DNA was extracted from the sheath and blade tissues because the endophyte is found in higher concentrations in these tissues. Due to the low abundances of meadow fescue plants in the communities, the total number of tillers collected differed between plots, with ten tillers being the maximum tillers taken. A total of 259 tillers were collected. Tillers were placed in labelled pill boxes, flash frozen in liquid nitrogen and stored until endophyte concentration could be determine. The concentration of the endophyte E. uncinata was estimated based on the quantification of the number of copies of endophyte specific genes found in the plant and expressed per ng of total genomic DNA.

3.2.4 Estimation of endophyte concentration

Tissue samples were removed from the freezer and ground by placing them in 5 ml tubes with 9.5 mm ball bearing and ground using a 2010 Geno/Grinder® (SPEX® SamplePrep, USA) tissue homogenizer. Genomic DNA was extracted from 20 mg of ground sheath tissue using DNeasy®Plant Mini Kit (Qiagen, Valencia, CA, USA), following the manufacture’s protocol for the kit, and following the automated sample prep instructions for the QIAcube® (Qiagen Inc., Toronto, Canada). Each sample’s total genomic DNA (plant and fungal) was measured by placing 2 μL of sample on a NanoDrop® 2000. Each sample was tested 3 times to determine an average total of gDNA. The gDNA was diluted to a working concentration of 0.5 ng total gDNA/μL using autoclaved Millipore water. The qPCR reactions were set up with a total volume of 15 μL. Each reaction consisted of 9 μL of PCR mix (forward and reverse primers (0.75 μL each, 0.5 μmol concentration), abm® EvaGreen 2X qPCR MasterMix (7.5 μL, 2x concentration)), and 3 ng of gDNA (6 μL gDNA, 0.5 ng/μL concentration). Each sample was tested in triplicates. Dilutions and plating were carried out using an automated PCR robot QIAgility® (Qiagen Inc., Toronto, Canada). PCR reactions were performed on a LightCycler® 480 Instrument II (Roche, Canada). The PCR thermocycling conditions were as follows: initial denaturation for one cycle at 95 °C for 5 min, followed by amplification for 45 cycles of 95 °C for 10 s, 64 °C for 10 s, and 72 °C for 10 s.
Primers

Forward Primer: 5’- CACGTACTGACTGAAGCGTAGC -3’

Reverse Primer: 5'- CGA ACT TCT CGA TGG TAC GCT TGT C -3'

The tefA gene of *Epichloë uncinata* was compared to *Epichloë coenophiala* tefA gene and the standard tefA used in the Newman Lab Protocol in order to determine a new reverse primer. *Epichloë uncinata* and *E. coeno* phiala have slightly different base pairs in their tefA gene. An Oligo sequence analyzer was used to produce the complementary strand for the reverse primer that was used for *Schedonorus pratensis* samples.

3.2.5 Statistics

In order to permit data transformation, I add 0.1 to the number of gene copies per ng of total gDNA (endophyte concentration) for each sample. I performed a univariate ANOVA, on the Box-Cox transformed date (to homogenize the residual variance) using R 3.4.4 (R Core Team 2017). Due to the unbalanced experimental design I conducted the analysis using two models. One univariate ANOVA contained the factors: endophyte, fertilizer, the interaction fertilizer x endophyte, and the random factor block (Newman et al. 1997). The other univariate ANOVA contained the factors: cultivar, fertilizer, the interaction fertilizer x cultivar, and the random factor block. The first ANOVA isolates the difference between those cultivars with high endophyte concentrations and those with low endophyte concentrations. The second ANOVA examines differences between the cultivars irrespective of endophyte status.

3.3 Results

In a univariate ANOVA with endophyte, fertilizer, and fertilizer x endophyte, the tillers from high endophyte cultivars had higher concentrations of endophyte than tillers from cultivars with low endophyte concentrations ($F_{(1,35)} =7.915$, $p=0.007$; Table 3.1 & Figure 3.1). Fertilizer ($F_{(1,35)} =2.589$, $p=0.116$) and fertilizer x endophyte ($F_{(1,36)} =1.350$, $p=0.257$) did not have a significant effects on endophyte concentration (Figure 3.2).

In the univariate ANOVA with cultivar, fertilizer, and fertilizer x cultivar, cultivar had a significant effect on endophyte concentration ($F_{(6,28)} =3.190$, $p=0.016$; Table 3.2). A tukey honestly significant difference post hoc test was performed on the factor cultivar, but no significant differences were seen between cultivars (Figure 3.3). Fertilizer ($F_{(1,28)} =2.286$, $p=0.141$), and fertilizer x cultivar ($F_{(6,28)} =1.604$, $p=0.1828$) did not have a significant effect on endophyte concentration.
3.4 Discussion

In this study I measured the endophyte concentrations of seven *Schedonorus pratensis* cultivars half of which had fertilizer applied to them. To determine if (a) nutrient availability affects endophyte concentrations in *Schedonorus pratensis* (b) if endophyte concentrations differ amongst cultivars and fertilizer treatments. I found that generally endophyte concentrations were higher in E+ cultivars relative to E- cultivars, but increasing soil fertility did not translate to changes in endophyte concentration. This suggests that soil fertility did not affect endophyte concentration and it did not affect high endophyte versus low endophyte cultivars differently.

Lui et al. (2011) found that high nitrogen supply in a high sugar cultivar (AberDove) of *L. perenne* was able to reduce endophyte concentration by 50% compared to the control. These results under high nitrogen supply support the results from Rasmussen et al. (2007) and Ryan et al. (2015) who also found that high sugar cultivars of *L. perenne* had a decrease in endophyte concentrations under high nitrogen supply. High phosphorus supplies also reduced endophyte concentrations in the high sugar cultivar of *L. perenne* (Lui et al. 2011). These studies that have examined the effect resource supply has on endophyte concentration have mainly focused on how nitrogen can affect endophyte concentration (Rasmussen et al. 2007; Ryan et al. 2015). However, in my study, fertilizer did not have a significant effect on endophyte concentration which could be due to the type of fertilizer (25:4:10 N:P: K) and whether it effect may depend on whether the soil is nitrogen or phosphorus limited or both. It is possible that low nutrient levels at the experimental site were limiting the growth of *S. pratensis* and by using a fertilizer with multiple nutrients (i.e., both nitrogen and phosphorus) that these nutrients affected each other’s ability to be used by the plant. Nitrogen and phosphorus concentrations in plant tissues and in the soil are determined by multiple factors such as plant uptake of nutrients, carbon assimilation and loss of carbon, nitrogen, and phosphorus (Güsewell 2004). Increases in nitrogen availability can affect plant metabolism and the relative availability of other nutrients such as phosphorus by, for example, changing how plant roots absorb phosphorus (Grunes 1959). If the nitrogen to phosphorus ratio is high (as in my fertilizer mix), this could potentially result in phosphorus depletion in the plant (Duff et al. 1994; Li et al. 2012). Plants deficient in nitrogen will increase nitrogen uptake while reducing phosphorus uptake and plants that are phosphorus deficient will increase phosphorus uptake and reduce nitrogen uptake (Güsewell 2004). I think my study site was limited in both nitrogen and phosphorus and the fertilizer use N:P: K 25:4:10 helped with nitrogen limitation more than phosphorus limitation based on the variation in phosphorus concentrations within fertilized and unfertilized plots (Table 3.3). This suggests that a change in nutrient supply along with cultivar traits or specificity between the endophyte and its host can affect the symbiosis between an endophyte and its host (Saikkonen et al. 2004; Rasmussen et al. 2009; Lui et al. 2011). Studies how found that using different grass endophyte combinations can result in different physical and physiological responses between the plant and the endophyte (Saikkonen et al. 2004; Rasmussen et al. 2009; Lui et al. 2011). One particular study working with different cultivars of infected perennial ryegrass found different responses in endophyte produced alkaloids and plant growth (Tian et al. 2013).
In a study done by Ryan et al. (2014) on *Schedonorus arundinaceus* a species comparable to *Schedonorus pratensis* found that under elevated CO2 (800ppm) and under high nitrogen conditions endophyte concentrations increased in infected *S. arundinaceus*. In my study endophyte status did have a significant effect on endophyte concentration with high endophyte infected cultivars having higher endophyte concentrations than low endophyte infected cultivars. This could be due to the fact that high endophyte infected cultivars of meadow fescue can have endophyte infection rates ranging from 60-100% (Saari et al. 2009). Ryan et al. (2014) suggests that nitrogen fertilization increases endophyte concentration, but that has not been the case in *L. perenne*, which suggests that this is not generalizable across *Epichloë* species. Soil fertility did not have a significant effect on endophyte concentration in my study. Endophyte infection can compete with other plant functions for limited nutrients which could explain why endophyte infected grasses do well in high nutrient environments, however in poor nutrient environment endophytes enhance nutrient uptake in their host by increasing nutrient uptake in the roots (Li et al. 2012). It has been suggested that in high nitrogen treatments a dilution effect can cause the plant to be stimulated to grow more than the fungus thus effecting endophyte concentrations, however plant biomass can explain less than 5% of the variation in endophyte concentration (Rasmussen et al. 2007). Ryan et al. (2015) found that cultivar only accounted for 2% of the variability in endophyte concentration whereas host genotype accounted for half and endophyte strain accounted for a third of the variability in endophyte concentration. Ryan et al. (2015) concluded that genetic compatibility between the plant and fungus can explain the largest amount of variation in endophyte concentrations.

In my study soil fertility did not have a significant effect on endophyte concentrations. This could be due to the experimental site still being a nutrient poor environment even after fertilizer application. In fertilized plots that had soil cores taken phosphorus and nitrate levels varied between plots (Table 3.3). Phosphorus levels ranged from low to high, nitrate levels ranged from medium to high and ammonium levels were considered typical based on soil test guidelines (Espinoza et al. 2006). It could be possible that the effect of fertilizer was not seen due to the experimental site still being nutrient poor even after multiple applications of fertilizer. I think future experiments should examine the effect of fertilizer over a longer period of time. Cultivar did have a significant effect on endophyte concentration, however after further examination no differences were seen between cultivars. This could be due to the small sample size (n =42) as well as the uneven number of samples for each cultivar. There were more tillers collected from the high endophyte infected cultivars compared to the low endophyte infected cultivars. There were also more tillers collected from fertilized plots compared to unfertilized plots. The uneven sample sizes could potential affect how the statistical test was run by placing more significance on the factors with larger n values. The reason cultivar had a significant effect on endophyte concentration could be due to the fact that cultivars Kasper and Salten are known to have varying infection frequencies whereas the cultivar Inkeri tends to have high infection frequencies (Saari et al. 2009). It could be that within certain plots of each cultivar endophyte concentrations may vastly differ. Meadow fescue abundance appears to be decreasing over time which could suggest that meadow fescue is not at optimal performance in the plant community. It is possible that endophyte concentrations maybe decreasing along with meadow fescue abundance. I think it would have been worthwhile to measure endophyte concentration each year. Decreasing endophyte concentration could be the reason why meadow fescue abundances are decreasing. If endophyte concentrations are decreasing this could inhibit meadow fescue’s
ability to be successful in a natural community. The advantages the endophyte provide may not be able to be seen.

Overall, I think all the experiments I have discussed have added to the importance of examining the effect different external factors such as soil nitrogen supply, climate, atmospheric CO2 concentrations and competition with arbuscular mycorrhizal fungi have on endophyte concentration. I think it is also important to observe the effect these factors have on endophyte concentration under a wide range of experimental environments. I think more studies need to be conducted in natural plant communities in order to observe if endophyte concentrations are affected by competition from other species. In this study soil fertility did not have a significant effect on endophyte concentration this could be because during the initial establishment of meadow fescue the study site was nutrient poor and it is possible that even with endophyte infection meadow fescue was not able to overcome being exposed to poor nutrients over time.
Table 3.1: Univariate ANOVA results for the effects of fertilizer, endophyte and fertilizer x endophyte on endophyte concentration (Gene Copies ng⁻¹ Total gDNA). The response variable was Box-Cox transformed for the analysis. Bold font indicates significant effects.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilizer</td>
<td>1.35</td>
<td>2.589</td>
<td>0.116</td>
</tr>
<tr>
<td>Endophyte</td>
<td>1.35</td>
<td>7.915</td>
<td><strong>0.007</strong></td>
</tr>
<tr>
<td>Fertilizer x Endophyte</td>
<td>1.36</td>
<td>1.350</td>
<td>0.252</td>
</tr>
</tbody>
</table>

Figure 3.1a: The effect of endophyte on endophyte concentration (Gene Copies ng⁻¹ Total gDNA). Endophyte has a significant effect (p=0.007). E+ endophyte treatments consist of data collected from plots containing high endophyte infected *Schedonorus pratensis* cultivars Inkeri, Salten, and Kasper and E- endophyte treatments consisted of data collected from low endophyte infected *S. pratensis* cultivars Antti, Kalevi, Fure, and Ilmari. The untransformed data is plotted.
Figure 3.1b: The effect of endophyte x fertilizer on endophyte concentration (Gene Copies ng\(^{-1}\) Total gDNA). E+ endophyte treatments consist of data collected from plots containing high endophyte infected *Schedonorus pratensis* cultivars Inkeri, Salten, and Kasper and E- endophyte treatments consisted of data collected from low endophyte infected *S. pratensis* cultivars Antti, Kalevi, Fure, and Ilmari. The untransformed data is plotted.

Table 3.2: Univariate ANOVA results for the effects of fertilizer, cultivar and fertilizer x cultivar on endophyte concentration (Gene Copies ng\(^{-1}\) Total gDNA). The response variable was Box-Cox transformed for the analysis. Bold font indicates significant effects.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilizer</td>
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<td>2.286</td>
<td>0.141</td>
</tr>
<tr>
<td>Cultivar</td>
<td>6,28</td>
<td>3.190</td>
<td><strong>0.016</strong></td>
</tr>
<tr>
<td>Fertilizer x Cultivar</td>
<td>6,28</td>
<td>1.604</td>
<td>0.182</td>
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</table>
Figure 3.3: The effect of cultivar on endophyte concentration (Gene Copies ng⁻¹ Total gDNA). E+ endophyte treatments consist of data collected from plots containing high endophyte infected Schedonorus pratensis cultivars Inkeri, Salten, and Kasper and E- endophyte treatments consisted of data collected from low endophyte infected S. pratensis cultivars Antti, Kalevi, Fure, and Ilmari. Cultivar has a significant effect (p=0.016), however a pairwise Tukey’s Honestly Significant Difference (HSD) post hoc test did not reveal any significant differences between cultivars. The untransformed data is plotted.

Table 3.3: Data from the soil cores taken from six fertilized plots and six unfertilized plots. Soil samples were tested for Nitrate, Phosphorus and Ammonium levels and were measured in parts per million (ppm).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nitrate (ppm)</th>
<th>Phosphorus (ppm)</th>
<th>Ammonium (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilized</td>
<td>16.30</td>
<td>16</td>
<td>5.50</td>
</tr>
<tr>
<td>Fertilized</td>
<td>30.30</td>
<td>48</td>
<td>4.60</td>
</tr>
<tr>
<td>Fertilized</td>
<td>17.20</td>
<td>19</td>
<td>4.60</td>
</tr>
<tr>
<td>Fertilized</td>
<td>15.10</td>
<td>41</td>
<td>4.40</td>
</tr>
<tr>
<td>Fertilized</td>
<td>12.20</td>
<td>13</td>
<td>7.20</td>
</tr>
<tr>
<td>Fertilized</td>
<td>2.90</td>
<td>15</td>
<td>3.80</td>
</tr>
<tr>
<td>Not Fertilized</td>
<td>1.50</td>
<td>18</td>
<td>3.90</td>
</tr>
<tr>
<td>Not Fertilized</td>
<td>1.90</td>
<td>33</td>
<td>3.30</td>
</tr>
<tr>
<td>Not Fertilized</td>
<td>1.80</td>
<td>13</td>
<td>2.80</td>
</tr>
<tr>
<td>Not Fertilized</td>
<td>2.50</td>
<td>15</td>
<td>3.40</td>
</tr>
<tr>
<td>Not Fertilized</td>
<td>2.70</td>
<td>12</td>
<td>3.20</td>
</tr>
<tr>
<td>Not Fertilized</td>
<td>1.20</td>
<td>25</td>
<td>2.90</td>
</tr>
</tbody>
</table>
CHAPTER 4
Conclusions

4.1.1 Summary of results

The main purpose of my thesis was to attempt to observe how an old field community responded years after the initial establishment of Schedonorus pratensis as well as observing how the plant community and S. pratensis responds to soil fertility. I observed the changes in S. pratensis abundance over time, S. pratensis responses to soil fertility and how the vegetative community responded to high endophyte (E+) infected cultivars and low endophyte (E-) infected cultivars. This study along with Shukla et al. (2015) is rare due to the fact that they are field based and embedded in a multispecies community. It is also rare to study meadow fescue in North America considering that most studies use perennial ryegrass or tall fescue. Multiple cultivars of meadow fescue were used to replicate the differences in genetic variability in a population of meadow fescue. My study was unique because I observed the different cultivars of S. pratensis response to soil fertility and how fertilizer can impact endophyte concentrations in the 7 different cultivars of S. pratensis.

Previous studies have shown that endophyte infected grasses are stronger competitors than their uninfected counter parts (Clay & Holah 1999; Rudgers et al. 2007; Rudgers et al. 2010). Infected plants have a negative effect on natural communities by decreasing species richness (Clay & Holah 1999; Rudgers et al. 2007; Rudgers et al. 2010). In field plots seeded with infected and uninfected tall fescue, infected plots were dominated by tall fescue and suppressed grasses and dicots relative to uninfected plots (Clay & Holah 1999). Rudgers et al. (2007) also found across two experimental sites endophyte infected tall fescue reduced tree species richness by 60%, and endophyte infection reduced tree abundance between 60-80%. Similarly, Rudgers et al. (2010) observed that endophyte infected tall fescue suppressed species richness and reduced other plant species such as forbs and graminoids. Studies have emerged that suggest that endophyte infected grasses may not be as successful as invaders and that endophyte advantages may not always be prominent (Ahlholm et al. 2002; Yurkonis et al. 2012; Shukla et al. 2015). In a study by Ahlholm et al. (2002), endophyte infection reduced tiller and root biomass in S. pratensis, thus hindering plant growth. Yurkonis et al. (2012) found that endophyte infected tall fescue plots had a slight decrease in species richness compared to uninfected forage cultivars, however the differences were not consistent between specific endophyte infected and uninfected cultivars. Shukla et al. (2015) found that S. pratensis did not reach high abundance in the plant community 3 years after the initial establishment, and that E+ plots had higher species richness than E- and unseeded control plots. After a further 8 years post invasion, my work suggests that S. pratensis abundance has been decreasing with time and that E+ plots still have higher species richness compared to E- and unseeded control plots. The motivation for my study was that the study site used by myself, Yurkonis et al. (2012) and Shukla et al. (2015) had poor soil fertility compared to the soil fertility in studies done by Clay & Holah (1999), Rudgers et al. (2006), Rudgers & Clay (2007) and Rudgers & Clay (2008). We thought by increasing soil fertility we would achieve results similar to the studies I listed above, however in my study the application of fertilizer did not have a significant effect on the abundance of S. pratensis. Fertilizer application also did not have a significant effect on
endophyte concentrations. Below, I discuss potential reasons why three years of nutrient conditioning did not affect meadow fescue abundance and endophyte concentrations.

4.1.2 Dominance over time

Saari et al. (2010) found that endophyte infection frequencies in different aged pastures increased with time. Meadow fescue was able to become the dominant grass species regardless of the age of the pasture (Saari et al. 2010). Saikkonen et al. (2000) and Saari et al. (2009) measured endophyte infection frequencies in pastures that range in age from 2-11 years old and also found that infection frequencies were high in all pastures regardless of age. Shukla et al. (2015) only conducted a study for 3 years and found that S. pratensis did not become a dominant species in the plant community and did not reach high levels of abundance. My study was conducted 8 years after Shukla et al. (2015) collected their data and S. pratensis abundance is decreasing with time and has still not achieved high abundance in the plant community. It seems that even with 3 years of fertilizer application S. pratensis was not able to reach high abundance or become a dominant species in the plant community. It is possible with further exposure to fertilizer application and more time S. pratensis could become more abundant within the plant community, however it could be that S. pratensis may not be able overcome being exposed to a low nutrient soil since its initial establishment. Ahlholm et al. (2002) found that endophyte infected meadow fescue grown under nutrient limited conditions had reduced root and tiller biomass compared to uninfected meadow fescue. Another study was only able to observe endophyte advantages in meadow fescue grown under a high nutrient supply (Dirihan et al. 2015).

4.1.3 Invader

Saari et al. (2010) found that S. pratensis was able to become a dominant species within many different aged pastures when grown with similar species found at my study site (Poa pratensis and Dactylis glomerate). Schedonorus pratensis grown in mixtures alongside Dactylis glomerate Trifolium repens, and Trifolium pretense increased S. pratensis abundance and its performance as a competitor. Whereas the field site prior to my and Shukla et al. (2015) use was a 20-year-old orchard field community where Poa pratensis was already well established. This could suggest that infected S. pratensis was not able to compete against another dominant grass species or that the advantages endophyte provide their infected host were masked. It is also possible that field sites conditions such as location (latitudes), daylength, and temperature influenced meadow fescue because they vastly differ from Saari et al. (2010) field site conditions. I think the study done by myself and Shukla et al. (2015) suggests that S. pratensis is not a successful invader in Southern Ontario, however more studies done under natural conditions or in natural communities could determine the role S. pratensis plays in natural communities. These experiments should also examine S. pratensis in natural communities under a range of different environmental conditions to see if the endophyte advantages can still be observed.
4.1.4 Cultivar effect

Endophyte infected *S. pratensis* in long term field studies has been observed to be an invasive species (Fjellheim *et al.* 2009; Saari *et al.* 2010; Saikkonen *et al.* 2010). Cultivars of endophyte infected *S. pratensis* can have endophyte infection frequencies ranging from high to low (Saari *et al.* 2009). Saari *et al.* (2009) observed endophyte infection frequencies ranging from high to low and varied among and within cultivars. Infection frequencies varied in *S. pratensis* cultivar Kasper and Salten, however the cultivar Inkeri consistently had high endophyte infection frequencies (Saari *et al.* 2009). Shukla *et al.* (2015) found that *S. pratensis* cultivars Inkeri and Kasper had the highest infection rates of all the cultivars used in this experiment. The infection rate for *S. pratensis* fluctuated with year, with 2011 having higher infection frequencies compared to 2010 and 2013 (Shukla *et al.* 2015). In my study *S. pratensis* cultivars Kasper and Inkeri had the highest abundance of *S. pratensis* across the five years (2011-2014, 2017). Overall *S. pratensis* abundance appears to be decreasing regardless of cultivar or endophyte status and may eventually be completely outcompeted and excluded from the community, or perhaps continue to persist at relatively low abundances especially for the cultivars Kasper and Inkeri that had the highest abundance of meadow fescue.

4.1.5 Endophyte concentration

Studies using q-PCR have been able to estimate the concentration of endophytic fungi within a host plant (Rasmussen *et al.* 2007; Rasmussen *et al.* 2008; Rasmussen *et al.* 2009; Tia *et al.* 2013; Ryan *et al.* 2014; Ryan *et al.* 2015). With this technique, more studies have emerged that observe how different environmental factors such as the plant leaf carbohydrate content, nitrogen supply and competition from other non-foliar fungi can affect endophyte concentrations (Rasmussen *et al.* 2007; Rasmussen *et al.* 2008; Rasmussen *et al.* 2009; Tia *et al.* 2013; Ryan *et al.* 2014; Ryan *et al.* 2015; Fuchs *et al.* 2017). Fuchs *et al.* (2017) discovered that fungal concentrations in perennial ryegrass were affected by season or temperature with endophyte concentrations increasing in the summer with higher ambient temperatures. High sugar cultivars of perennial ryegrass exposed to high nitrogen supply showed a decrease in endophyte concentrations (Rasmussen *et al.* 2007; Rasmussen *et al.* 2008; Ryan *et al.* 2015). Most studies agree that host genotype, endophyte strain and genetic compatibility between plant and fungi can explain the largest amount of variation in endophyte concentrations (Tia *et al.* 2013; Ryan *et al.* 2015). In my study fertilizer did not have a significant effect on endophyte concentrations, however cultivar did have a significant effect, but a multi-comparison test was not able to separate this effect. This could be due to the small sample size as well as the uneven number of samples for each cultivar. E+ cultivars had higher endophyte concentrations compared to E- cultivars which could be due to E+ cultivars having higher infection rates that can range from 60-100% (Saari *et al.* 2009). More studies observing the effect environmental factors have on *S. pratensis* endophyte concentrations should be carried out because it is unclear if *S. pratensis* will mimic *S. arundinaceus* or *L. perenne* when exposed to different levels of nutrients. I think the results from my study further supports the claim that not all endophyte-grass symbiotic relationships are generalizable, and not all endophyte-grass combinations are highly invasive, or at least not so in every location.
4.2 Perspective and prospects

There is a general perception that all asexual Epichloë symbiotic relationships are mutualistic and that Epichloë endophyte infected grasses have been suggested to be highly invasive species that can impact natural plant communities by decreasing species richness, however most of the literature is based on two grasses, tall fescue and perennial ryegrass, which have been studied in current or previous agricultural settings (fields, pastures; Saikkonen et al. 2006). Some researchers have suggested that asexual Epichloë species may not be or may not always be mutualist (Ahlholm et al. 2002). More studies have shown that the symbiosis between endophyte and plant can depend on environmental conditions such nutrient availability (Shukla et al. 2015). The results of my study support the results of Shukla et al. (2015) who found that S. pratensis was not a particularly good invader and that S. pratensis abundance started to decline years after establishment. It would be interesting to see if continued fertilization would eventually show case the advantages endophytes provide their hosts. Future studies should continue to look at endophyte infected grasses in natural plant communities and expose these plants to different environmental conditions.
References


