Endophytes inconsistently affect plant communities across *Schedonorus arundinaceus* hosts

Kathryn A. Yurkonis · Kruti Shukla · Jessica Holdenried ·
Heather A. Hager · Kim A. Bolton · John N. Klironomos ·
Hafiz Maherali · Jonathan A. Newman

Abstract Fungal endophytes in cool-season grasses may affect communities at multiple trophic levels. However, it is unclear whether community-scale endophyte effects arise due to the endophyte itself or as a result of unique, endophyte–host interactions. We used a long-term field experiment to test whether common-toxic (CT) and non-ergot alkaloid-producing (novel) endophytes in *Schedonorus arundinaceus* (tall fescue) forage cultivars consistently affect communities across tall fescue hosts. Tilled plots (2 × 2 m; Guelph, ON) were seeded with

Georgia 5 and Jesup cultivars containing either the CT or AR542 (novel) endophyte and allowed to be re-colonized by plant species from the local propagule pool. Non-seeded control plots were included to assess effects of seeding the non-native grass. We assessed plant, invertebrate, soil moisture, and soil nutrient responses to the endophyte–cultivar treatments after four growing seasons. Seeding tall fescue affected plant species abundances, but not richness, and did not consistently alter soil moisture and nutrient pools. Endophyte identity in the tall fescue cultivars affected the communities, but effects were not consistent between cultivars. Within Georgia 5, the AR542 endophyte reduced tall fescue abundance and altered the invertebrate community relative to CT plots. Within Jesup, the AR542 endophyte reduced species evenness and decreased soil moisture during dry periods relative to CT plots. Endophyte effects were not consistent between cultivars, and it is probable that the community-scale effects of endophyte infection in tall fescue cultivars arise due to unique interactions between cultivar and endophyte.

K. A. Yurkonis
Department of Biology, University of North Dakota, Stop 9019, Grand Forks, ND 58202, USA

K. Shukla · J. Holdenried · H. A. Hager ·
K. A. Bolton · J. A. Newman (✉)
School of Environmental Sciences, University of Guelph, Guelph, ON N1G 2W1, Canada
e-mail: jnewma01@uoguelph.ca

J. N. Klironomos
Department of Biology, University of British Columbia – Okanagan, Kelowna, BC V1V 1V7, Canada

H. Maherali
Department of Integrative Biology, University of Guelph, Guelph, ON N1G 2W1, Canada

Introduction

Fungal endophytes in cool-season grasses may affect community structure and composition at multiple

trophic levels (Saikkonen et al. 2006; Rudgers and Clay 2007). These endophytes typically produce secondary metabolites, primarily lolines, and ergot alkaloids that negatively affect invertebrate and vertebrate herbivores, respectively, and can indirectly affect other aspects of host physiology and growth (Hesse et al. 2003; Rasmussen et al. 2009; Saikkonen et al. 2013). While a substantial body of literature supports the idea that endophyte effects are dependent on interactions among endophytes, their hosts, and the environment, controlled studies are still needed to determine the community-scale effects of these interactions (Cheplick 2008; Saikkonen et al. 2011).

To determine whether interactions between the host and endophyte affect communities, we need to assess effects of specific endophytes across multiple plant populations. Insertion of so-called "novel" endophytes into commonly seeded grass cultivars provides an opportunity to conduct such a test (as in Timper et al. 2005; Rudgers et al. 2010) on grasses that would commonly be encountered in and adjacent to former pastureland. To produce cultivars with endophyte-derived invertebrate and pathogen resistance and reduced detrimental effects on livestock, naturally occurring endophyte strains that do not produce vertebrate-toxic alkaloids have been inserted into high-yielding proprietary cultivars (Latch et al. 2000; Bouton et al. 2002). In the *Schedonorus arundinaceus* Schreb. Dumort (tall fescue) forage cultivars—Georgia 5 and Jesup, the "common-toxic" (CT) *Neotyphodium coenophialum* (Morgan-Jones & Gams) Glenn, Bacon, and Hanlin endophyte strains were replaced with the AR542 endophyte strain (Latch et al. 2000; Bouton et al. 2002).

Since alkaloid production differs between cultivars with the CT and AR542 endophytes, we would expect that introduction of grasses with the same plant profile, but different endophyte genotypes would differentially affect local community structure and function. Aboveground, the AR542 endophyte may alter local plant community structure by affecting plant growth (Hunt and Newman 2005) and competitive ability (Tozer et al. 2007; Rudgers et al. 2010). Despite predictions that altering the ergot alkaloid composition would only affect vertebrates, it appears that the AR542 endophyte may also affect invertebrate herbivory and composition (Hunt and Newman 2005; Bultman et al. 2009; Keathley and Potter 2012). Belowground, the AR542 endophyte may affect plant

root exudate composition and nutrient acquisition (Malinowski et al. 2005; Belesky et al. 2008), decomposition (Antunes et al. 2008), soil biota (Popay and Jensen 2005; Timper et al. 2005), root mycorrhizal colonization (Antunes et al. 2008), and nutrient cycling (Iqbal et al. 2013). Although the AR542 endophyte appears to have distinct ecological effects in SA, because hosts can uniquely interact with their endophytes (reviewed in Saikkonen et al. 2013), it is unclear whether such effects are consistent across plant populations (cultivars).

Only a handful of studies have systematically compared effects of endophyte identity across cultivars and they have found mixed results. Endophytes uniquely interacted with cultivars to affect plant physiology and growth in the study of Assuero et al. (2000), but it is unclear whether these effects would also occur under field conditions. AR542 had weaker effects on plant species richness and the cover of co-occurring plant species than the CT endophyte, and effects of endophyte identity were relatively consistent between cultivars in a six-year field study (Rudgers et al. 2010). AR542 also had weaker effects on plant parasitic soil nematodes in Jesup and Georgia 5 cultivars than the CT endophyte in a controlled greenhouse study (Timper et al. 2005). Endophyte type may affect plant physiology and growth in excess of differences among cultivars, but whether this translates to effects on above- and below-ground interactions in communities needs further investigation under field conditions.

The availability of cultivars with the same plant profile containing two-endophyte strains provides a unique opportunity to test for the consistency of community-scale endophyte effects across plant genotypes in a non-native species. We used a field experiment to test whether similar ecological effects occurred between plant genotypes containing CT and novel endophytes at multiple levels of community structure and function. We tested the predictions that CT tall fescue would be more abundant and more strongly reduce other plants and invertebrates than cultivars containing the AR542 endophyte due to the presence of the complete suite of endophyte metabolites. We isolate the effects of plant genotype and endophyte identity in tall fescue to advance our understanding of the conservation implications of seeding novel endophyte cultivars (Gundel et al. 2013) and to test the generality of community-scale endophyte effects (Saikkonen et al. 2006; Rudgers and Clay 2007).

Methods

Experimental design

Plots (2 × 2 m; 0.5 m spacing) were seeded in June 2007 with Jesup or Georgia 5 tall fescue seed (harvested from the University of Georgia seed production plots; stored dry at −17 °C upon arrival) containing either the CT or AR542 endophyte. Plots were established in an old-field at the University of Guelph Turfgrass Institute (Guelph, ON, CA; 43°32′56″ N, 80°12′39″ W), which is located within the moderate to well suitable tall fescue suitability zone (Fribourg et al. 2009). Plots were arranged in a randomized complete block design with 10 blocks as part of the larger, Long-term effects of fungal endophytes experiment (for the purposes of this study: 2 cultivar types × 2 endophyte types × 10 blocks = 40 plots plus 10 non-seeded control plots). The area had been maintained by occasional mowing for at least 20 years prior to our study and was dominated by perennial, non-native species (i.e., *Poa pratensis* L., *Elymus repens* (L.) Gould, *Taraxacum officinale* F. H. Wigg., *Cirsium arvense* (L.) Scop.). Prior to seeding, the area was tilled twice to break apart the existing vegetation. Plots were mostly bare soil after the tilling preparation, and an herbicide application was not deemed necessary in this process.

Tall fescue was hand seeded (5 g m^{-2}) onto the soil in excess of regionally (southern Ontario) recommended pasture seeding rates (2–3 g m^{-2})(Leahy and Robinson 2000). Plots were watered every 2–3 days through July 2007 to ensure the establishment and were not otherwise disturbed (i.e., no weeding, mowing, grazing, or herbicide applications). Since propagules were allowed to establish from the local pool, additional tilled, but not seeded, plots were included to test for the effects of seeding tall fescue. Little vegetative recruitment occurred from the pre-existing vegetation, and a majority of the plants that established likely did so from the seed we added or the local seed bank. Aisles among plots were mowed as needed. A complete description of the site, experimental design, and sampling is provided by Yurkonis et al. (2012).

Seed and field tillers were tested for endophyte presence in June 2010 with immunoblot assay kits (Agrinostics Ltd. Co., Watkinsville, GA, US). Both cultivars had a high frequency (Georgia 5 E+: 81 %; Georgia 5 AR542: 76 %; Jesup E+: 87 %; Jesup AR542: 77 %) of endophyte infection (described in Yurkonis et al. 2012). We did not test tissue alkaloid concentrations as part of this study, but presumably they were high given results from other studies (Rudgers et al. 2010).

Vegetation sampling

Plant species were recorded in each plot and species relative abundances were quantified at peak biomass in the fourth (August 2010) and fifth (August 2011) growing seasons using non-destructive point-intercept sampling, a proxy for species biomass (Jonasson 1988; Brathen and Hagberg 2004). Vegetation was sampled by recording the identity of each leaf and stem touching (intercepting) a narrow rod (a point) placed vertically through the vegetation at 10 cm intervals along the diagonal of each plot (described in Yurkonis et al. 2012). Vegetation was not sampled within 0.5 m of plot edges. Species relative abundances (p_i = number of touches for species i/total number of point intercept touches for a plot) and Simpson's evenness ([1/D]/number of species recorded during point-intercept sampling, where $D = \sum p_i^2$) were calculated from point-intercept sampling data and were inclusive of SA. In 2011, one plot (Jesup CT) was inadvertently missed during vegetation sampling, and sample sizes are thus reduced for this year.

Invertebrate sampling

Above-ground invertebrates were collected in the late June 2010 and mid-July 2011 (between 10 am and 4 pm) by moving a Vortis insect suction sampler (Burkard Manufacturing Co. Ltd., Rickmansworth, UK) through the vegetation for 1 min. Samples were stored at −18 °C, and invertebrates were subsequently identified to order and, when possible, to family. Invertebrate Simpson's diversity (1/D) was calculated based on the relative abundances of invertebrates in each order because not all invertebrates were identified to family. The proportion of total individuals from primarily herbivorous, saprophagous, predatory, and parasitoid families (determined from Daly et al. 1998; Marshall 2006) was calculated to assess differences in invertebrate trophic composition among plot types.

Soil sampling

Soil volumetric water content (soil moisture; Campbell Scientific HydroSense Soil Water Measurement System; Edmonton, AB, CA) was sampled to 20 cm in the late May, early June, and weekly through July and August in 2008. Soil moisture was sampled weekly in 2009, in alternate weeks in 2010, and monthly in 2011. Over the four-year period, soil moisture in control plots was less than 15 % on 16 sample dates ranging from May to September. This translates to approximately half of the field capacity of a sandy loam soil (Ratliff et al. 1983) and reflects occasions when plants could be negatively affected (Shortt et al. 2011). Soil moisture was analyzed separately among growing seasons and, because we were particularly interested in endophyte effects during dry periods, across the 16 sample dates.

Three soil cores (15 cm × 2.5-cm diameter) were collected from the center of each plot in the late August 2008 (second growing season) and 2010 (fourth growing season) for soil % C and % N content analysis (approximately 1 g of homogenized sample, vario Max CN analyzer, Elementar Analysesysteme Gmbh, Hanau, DE). Plant-available nitrate (PAN) production was estimated using the Plant Root Simulator (PRS)^{TM} probes (Western Ag Innovation Inc., Saskatoon, SK, CA) in root exclusion cylinders with probes inserted continuously throughout the growing season (described in Yurkonis et al. 2012). In 2010, PAN was measured with probes inserted over four-week periods from May to September. In 2011, PAN was measured over two, 9-week periods: June to August and August to mid-October. Values from each period were summed to estimate growing-season total PAN for each year.

Data analysis

Vegetation and soil responses were analyzed with repeated measures ANOVA (proc glm, SAS 9.3, SAS Institute, Cary, NC, USA) with block as a fixed effect and group (five levels: control, CT and AR542 in Georgia 5, CT and AR542 in Jesup), year, and their interactions as model terms. Invertebrate diversity and proportions of invertebrates in each feeding guild were analyzed with ANOVA (proc glm; SAS 9.3; SAS Institute, Cary, NC, USA) with block as a fixed effect and group as model terms. Contrasts were used to test

for differences between non-seeded and seeded plots, between endophyte types, and between cultivar types. Least Significant Difference post hoc tests ($\alpha = 0.05$ = probability of a Type I error for any one comparison) were used to infer pairwise differences among plot types. The proportion of forbs and the proportion of invertebrates in each trophic group were arcsin square-root transformed to meet normality assumptions. Season total plant available nitrate was natural-log transformed to meet normality assumptions and analyzed separately by year because of different sampling periods and frequencies between years.

Results

Vegetation

After four growing seasons (August 2010), tall fescue was present in all seeded plots (4–60 % of the leaf touches) and in three non-seeded plots (<12 %). In 2011, tall fescue was present in all seeded plots (2–41 % of the leaf touches) and in one non-seeded plot (not encountered during vegetation sampling). In addition to tall fescue, the grasses *P. pratensis* L. and *E. repens* (L.) Gould, the forbs *C. arvense* (L.) Scop., and *T. officinale* F. H. Wigg. and *Linaria vulgaris* Mill. were present in all plots in 2010 and in a majority of the plots in 2011. Although woody species (*Cornus* spp. L., *Vitis* spp. L. and *Acer negundo* L.) were present, these were not encountered during point-intercept sampling in either year. *P. pratensis* was the most abundant plant species, composed 20–86 % of the leaf touches across all plot types and years.

Seeding tall fescue affected species evenness, but not richness (Table 1; Fig. 1). Species richness was lower in 2011 than in 2010 ($F_{1,35} = 5.52$; $P < 0.05$; all other terms and contrasts n.s.) and was similar across plot types in both years. In pairwise comparisons, Jesup CT plots were less species rich than non-seeded plots in 2010, but not in 2011 (Fig. 1). Three of the four seeded plot types were more even than non-seeded plots in 2010, but in 2011 only Jesup CT plots were more even than non-seeded plots (Fig. 1). In both years, the proportion of forbs was similar and the proportion of other grasses was greater in non-seeded plots (Table 1; Fig. 2). Tall fescue was equally abundant in all seeded plot types in 2010 and was

Table 1 Results from repeated measures ANOVA of treatment effects on plant species Simpson's evenness and the relative abundance of *S. arundinaceus* (SA; tall fescue) and other grasses in 2010 and 2011

Model term	df	Evenness	% SA	% Non-SA grass
Block (B)	9,35	0.42	1.64	2.52*
Group (G)	4,35	5.58**	22.97***	19.36***
Contrasts				
(1) Non-seeded vs. Seeded	1,35	14.31***	83.16***	70.15***
(2) Endophyte	1,35	5.89*	$3.15^{\dagger}$	0.44
(3) Cultivar	1,35	0.43	1.77	4.14*
Year (Y)	1,35	17.47***	54.39***	55.30***
Y × B	9,35	0.43	0.75	0.46
Y × G	4,35	$2.63^{\dagger}$	4.58**	4.14**
Contrasts				
Y × 1	1,35	4.64*	10.92**	8.95**
Y × 2	1,35	1.54	5.73*	6.61*
Y × 3	1,35	$3.23^{\dagger}$	0.13	0.10

Values are *F*-values. Plots were either non-seeded or seeded with Georgia 5 or Jesup cultivars that contained either the CT or AR542 endophyte

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; $^{\dagger} P < 0.10$

more abundant in Georgia 5 CT plots than in the remaining seeded plots in 2011.

Presence of the AR542 endophyte within Georgia 5 and Jesup affected plant community structure, but effects were not consistent between cultivars. Jesup AR542 plots were less even than CT plots, but had similar functional composition (Table 1; Figs. 1, 2). In contrast, Georgia 5 AR542 and CT plots were similarly even across years, but AR542 plots contained marginally less tall fescue, and a greater proportion of other grasses and forbs (endophyte contrast $F_{1,35} = 3.13$; $P = 0.08$, all other contrasts n.s.) than AR542 plots in 2011 (Table 1; Figs. 1, 2).

Invertebrates

The above-ground invertebrate community contained representatives from ten insect orders, five arachnid orders, and a single order each of entognathans and gastropods. Samples primarily consisted of hemipterans (2010: 42 ± 1.4 %; 2011: 21 ± 1.0 %) and dipterans (2010: 28 ± 1.2 %; 2011: 18 ± 0.7 %). Herbivorous invertebrates (2010: 61.7 ± 1.4 %; 2011: 52 ± 1.1 %)
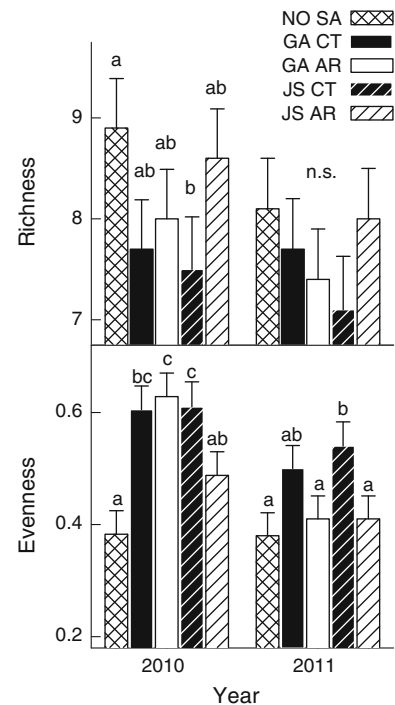

Fig. 1 Effects of the CT and AR542 (AR) endophyte on plant species richness and Simpson's evenness (mean ± SE) after four (2010) and five (2011) growing seasons. Plots were either non-seeded (NO SA) or seeded with the *S. arundinaceus* (SA; tall fescue) cultivars Georgia 5 (GA) and Jesup (JS). Categories with different letters are significantly different within years

dominated the samples, and most were in the hemipteran families Cicadellidae and Delphacidae. Gastropods were collected from six plots in 2010 and two plots in 2011 and comprised not more than 0.5 % of a sample. A small percentage (2010: 3.0 ± 0.4 %; 2011: 6.4 ± 0.6 %) of the specimens were unidentifiable. More invertebrates were collected and the samples were more diverse in July 2011 than in June 2010 (668 ± 34 individuals sample^{-1} in 2011; 325 ± 35 individuals sample^{-1} in 2010; Fig. 3).

Endophyte type affected the invertebrate community in Georgia 5, but not in Jesup. Invertebrates were less diverse in non-seeded and Georgia 5 AR542 plots than in Georgia 5 CT plots in June 2010, and were more diverse in Georgia 5 AR542 plots than in non-seeded and Georgia 5 CT plots in July 2011 (Year: $F_{1,36} = 398.96$, $P < 0.0001$; Date × group: $F_{4,36} = 6.63$, $P = 0.0004$; Date × Endophyte contrast: $F_{1,36} = 18.45$, $P = 0.0001$; Fig. 3). Georgia 5 AR542 plots were more dominated by herbivorous
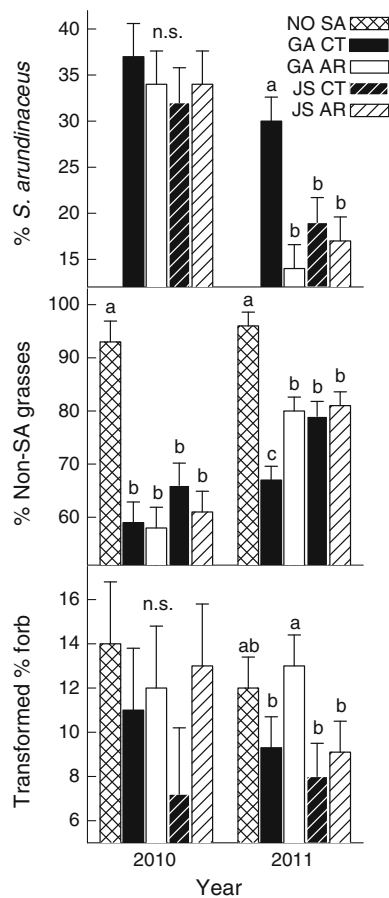
Fig. 2 Effects of the CT and AR542 (AR) endophyte on the proportion of tall fescue (SA), the proportion of co-dominant (non-SA) grasses, and the proportion of forb (arcsin square root transformed) leaf touches (mean ± SE) after four (2010) and five (2011) growing seasons. Plots were either non-seeded (NO SA) or seeded with the cultivars Georgia 5 (GA) and Jesup (JS). Categories with different letters are significantly different within years
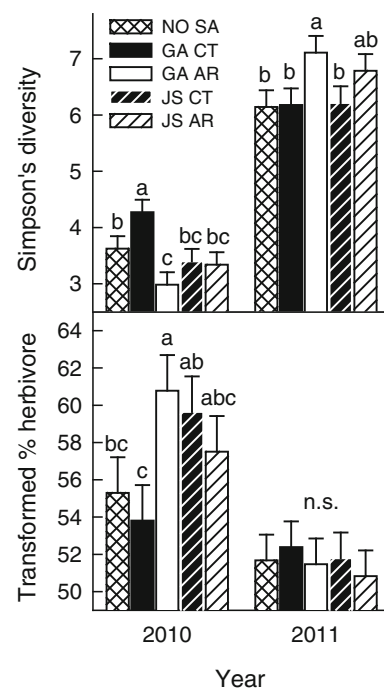

Fig. 3 Effects of the CT and AR542 (AR) endophyte on invertebrate Simpson's diversity (order), and the proportion of invertebrates (arcsin square-root transformed) classified in predominately plant-feeding families (mean ± SE) after four (2010) and five (2011) growing seasons. Plots were either non-seeded (NO SA) or seeded with the cultivars Georgia 5 (GA) and Jesup (JS). Categories with different letters are significantly different within years

invertebrates in June 2010, but not in July 2011 (Year: $F_{1,36} = 37.03$, $P < 0.0001$; Date × Group: $F_{4,36} = 2.29$, $P = 0.0781$; Fig. 3). The total number of invertebrates and the percentage of invertebrates from primarily predatory and parasitic families were similar among all plot types within years.

Belowground

Soil moisture varied across the growing season in three of the four growing seasons (2008: $F_{10,360} = 382.32$, $P < 0.001$; 2009: $F_{12,432} = 371.13$, $P < 0.001$; 2010: $F_{20,270} = 330.22$, $P < 0.001$; and 2011:

$F_{3,108} = 174.38$, $P < 0.001$), but was similar among groups (all other contrasts n.s.). Average soil moisture ranged from 8.1 ± 0.2 to 37.5 ± 0.6 % and generally declined to the lowest levels in July, with occasional dry periods in the early spring and late fall. In 2010, seeded plots were inconsistently wetter and drier than control plots throughout the season (Date × Seeded contrast $F_{20,270} = 2.17$, $P < 0.05$), and AR542 plots were marginally drier than CT plots (Endophyte contrast $F_{1,35} = 4.06$, $P = 0.051$).

In dry periods when there were pairwise differences between seeded and non-seeded plots, CT plots were always wetter (two occasions) than non-seeded plots, and AR542 plots were always drier than non-seeded plots (two occasions; Fig. 4). Endophyte identity affected soil moisture during some of the dry periods (Date × Endophyte contrast $F_{15,540} = 2.06$, $P < 0.05$; Online Resource 1). When endophyte identity affected soil moisture, AR542 plots were always drier
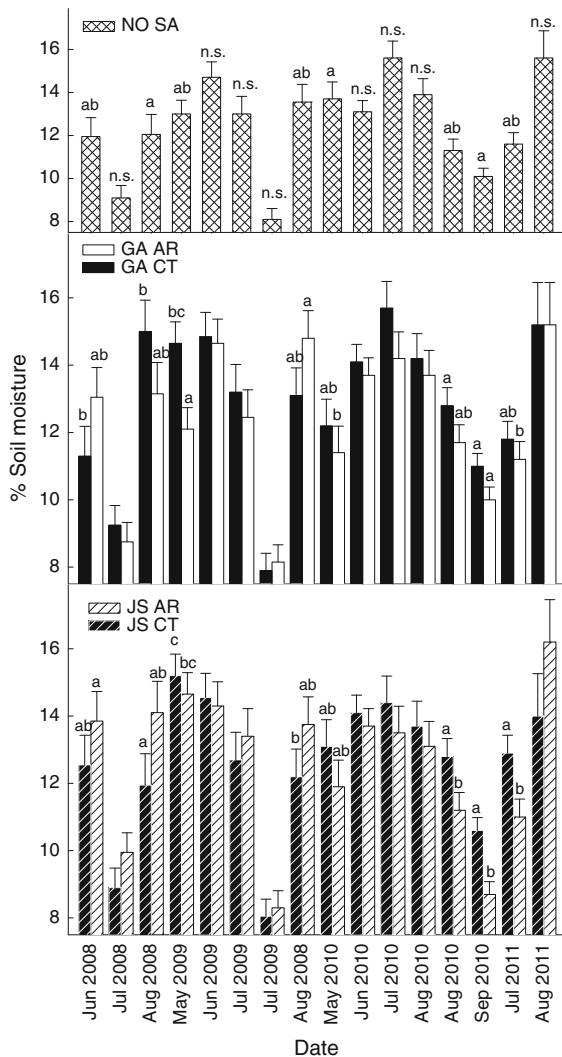
Fig. 4 Effects of the CT and AR542 (AR) endophyte on soil moisture (mean ± SE) in the second (2008) to the fifth (2011) growing seasons. Plots were either non-seeded (NO SA) or seeded with the cultivars Georgia 5 (GA) and Jesup (JS). *Letters* indicate differences among plot types (among panels) at each sample date, letters should not be compared across dates
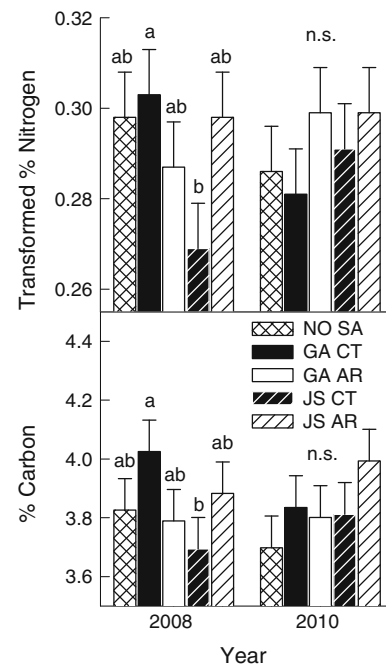

Fig. 5 Effects of the CT and AR542 (AR) endophyte on soil nitrogen (ln-transformed) and soil carbon (mean ± SE) in the second (2008) and fourth (2010) growing seasons. Plots were either non-seeded (NO SA) or seeded with the cultivars Georgia 5 (GA) and Jesup (JS). Categories with *different letters* are significantly different within years

than CT plots, and this occurred more so in Jesup than in Georgia 5 plots (Fig. 4).

In 2010, although PAN changed across the season (Date $F_{4,140} = 46.63$, $P < 0.001$), total PAN was similar between non-seeded and seeded plots (mean = 234.8 ± 34.5 mg NO_3^- 10 cm^{-2} 140 days^{-1}). In July 2010, PAN was lower in Georgia 5 CT plots (7.5 ± 1.9 mg NO_3^- 10 cm^{-2} 28 days^{-1}) than in non-seeded plots ($P < 0.05$; 34.4 ± 15.4 mg NO_3^- 10 cm^{-2} 28 days^{-1}). However, PAN was similar

among groups in the remaining months. In 2011, season total PAN was marginally higher in AR542 plots (126.5 ± 36.6 mg NO_3^- 10 cm^{-2} 111 days^{-1}) than in CT plots ($F_{1,35} = 3.66$, $P = 0.064$; 53.03 ± 14.1 mg NO_3^- 10 cm^{-2} 131 days^{-1}), but this was not reflected in pairwise comparisons (all n.s.).

Seeding tall fescue marginally affected total soil nitrogen (Date × Group $F_{4,36} = 2.33$, $P = 0.074$; all other model terms and contrasts n.s.) and carbon (Date × Group $F_{4,36} = 2.30$, $P = 0.078$, Date × Cultivar contrast $F_{1,36} = 4.88$, $P < 0.05$; all other model terms and contrasts n.s.) pools (Fig. 5). Shortly after planting, total soil carbon and nitrogen pools were greater in Georgia 5 CT plots than in Jesup CT plots, but this effect disappeared in 2010 (Fig. 5).

Discussion

Seeding the non-native grass tall fescue into a disturbed landscape altered plant composition after four growing seasons, but did not consistently affect

other aspects of community structure and function relative to communities established from the local propagule pool. Differences occurred among cultivar types, but these effects were not consistently attributable to a particular cultivar or endophyte. Within cultivars, the novel AR542 endophyte affected plant community evenness, soil moisture, and invertebrate diversity and composition either directly or as a result of effects on the co-dominant species. However, as with previous studies (Assuero et al. 2000; Hesse et al. 2003; Timper et al. 2005; Rudgers et al. 2010; Iqbal et al. 2013), these effects were cultivar specific. This finding indicates that we cannot reasonably extend endophyte effects from within a single cultivar (i.e., KY-31) to other tall fescue forage cultivars or across cool-season grass species (Saikkonen et al. 2006).

Effects associated with seeding E+ tall fescue may be similar to those of other, non-native grasses in the background propagule pool. Tall fescue did not affect overall plant community structure and function relative to the dominant grass *P. pratensis*, which may also harbor fungal endophytes (Wei et al. 2006) and is similar in phenology and litter production (Cully et al. 2003; Vellend et al. 2010). Although tall fescue can reach high abundances in native and disturbed landscapes (Clay and Holah 1999; Cully et al. 2003), it is quite possible that this species is a passenger rather than a driver of community change in non-native dominated systems (MacDougall and Turkington 2005). In our case, where tall fescue was able to reduce co-occurring species abundances, tall fescue may have been a better competitor than *P. pratensis* for sites previously occupied by *P. pratensis*, which could reflect a legacy of the presence *P. pratensis* (Matthews and Clay 2001; Kardol et al. 2007) more so than the invasive potential of tall fescue. The impact of seeding tall fescue may be worse in native or restored systems (Cully et al. 2003; but see Garrison and Stier 2010) with few established cool-season grasses.

The type of endophyte present in a cultivar affects multiple aspects of community structure and function, but these effects are inconsistent between cultivars. It is not surprising that endophyte type within a cultivar affected invertebrate diversity and composition (as shown in Hunt and Newman 2005; Bultman et al. 2009; Rudgers et al. 2010; Keathley and Potter 2012). Other studies have found increased food-web complexity (Bultman et al. 2009) and herbivory (Hunt and Newman 2005; but see Keathley and Potter 2012) in the presence of novel, non-ergot producing endophytes, effects which may arise indirectly due to effects on plant composition or directly due to altered alkaloid composition in novel endophyte plants. Effects of endophyte identity on plant community composition may be attributable to direct effects of alkaloids on plant growth (explained in Hesse et al. 2003), herbivory (Matthews and Clay 2001), or indirect effects on below-ground interactions among plants (Omacini et al. 2012). Belowground, Iqbal et al. (2013) also found stronger endophyte identity effects during dry periods (fall), and these effects may arise from indirect effects on community composition or direct effects on tall fescue rooting depth (De Battista et al. 1990) and associated hydraulic lift (Armas et al. 2012) or on stomatal closure and transpiration losses (Malinowski and Belesky 2006). As with Iqbal et al. (2013), we found that the AR542 endophyte did not have a lasting effect on soil nutrients relative to the common-toxic endophyte, indicating that these particular endophytes may similarly affect below-ground processes. However, this may not be the case for other possible endophyte–host combinations (Iqbal et al. 2013).

Inconsistent effects of endophyte type among cultivars most likely reflect unique endophyte–plant interactions. Georgia 5 and Jesup are genetically distinct cultivars (Mian et al. 2002) developed from different source populations (Bouton et al. 1993; Bouton et al. 1997). These cultivars could potentially differ in their traits and chemical profile which, in conjunction with environmental conditions and the plant nutritional status, could affect endophyte expression (Hesse et al. 2003; Cheplick 2008; Saikkonen et al. 2011; Saikkonen et al. 2013). While we cannot rule out the possibility that responses differed because CT and novel plants of the same cultivar are genetically distinct, as E+ and E− populations may diverge quickly during the seed production process (Vaylay and van Santen 2002), this explanation is less compelling given that this grass is an obligate out-crosser. It is also unlikely that the variation in the effects of the same endophyte between cultivars results from genetic variation in the endophyte because the endophyte is restricted to asexual, vertical transmission in SA.

Conclusions

This study builds on previous community-scale tests of the effects of endophyte identity in an agriculturally

important grass (Rudgers et al. 2010; Vesterlund et al. 2011; Iqbal et al. 2013) by considering effects on the plant, invertebrate, and soil communities. While the novel endophyte in this study was selected based on a suite of desired traits important in the agronomic context (i.e., consistent passage to seeds and high transmission to seeds), effects of other strains (Christensen and Latch 1991) should be similarly variable among cultivars. Additional investigations are needed to determine how each endophyte uniquely interacts with each host to contribute to such community-scale endophyte–plant interactions (Kuldau and Bacon 2008; Saikkonen et al. 2013). Finally, because endophyte effects are inconsistent between cultivars, efforts to commercialize endophytes with desired effects must be done in the context of particular cultivars (Funk et al. 1993), and effects of endophyte identity from one cultivar should not be considered indicative of all potential effects (Antunes et al. 2008; Saikkonen et al. 2011; Gundel et al. 2013) when considering effects of their presence in areas managed for conservation purposes.

References

Antunes PM, Miller J, Carvalho LM, Klironomos JN, Newman JA (2008) Even after death the endophytic fungus of *Schedonorus phoenix* reduces the arbuscular mycorrhizas of other plants. Funct Ecol 22:912–918

Armas C, Kim J, Bleby T, Jackson R (2012) The effect of hydraulic lift on organic matter decomposition, soil nitrogen cycling, and nitrogen acquisition by a grass species. Oecologia 168:11–22

Assuero SG, Matthew C, Kemp PD, Latch GCM, Barker DJ et al (2000) Morphological and physiological effects of water deficit and endophyte infection on contrasting tall fescue cultivars. N Z J Agric Res 43:49–61

Belesky DP, Burner DM, Ruckle JM (2008) Does endophyte influence resource acquisition and allocation in defoliated tall fescue as a function of microsite conditions? Environ Exp Bot 63:368–377

Bouton JH, Gates RN, Hill GM, Owsley M, Wood DT (1993) Registration of 'Georgia 5' tall fescue. Crop Sci 33:1405

Bouton JH, Duncan RR, Gates RN, Hoveland CS, Wood DT (1997) Registration of 'Jesup' tall fescue. Crop Sci 37:1011–1012

Bouton JH, Latch GCM, Hill NS, Hoveland CS, McCann MA et al (2002) Reinfection of tall fescue cultivars with non-ergot alkaloid producing endophytes. Agron J 94:567–574

Brathen KA, Hagberg O (2004) More efficient estimation of plant biomass. J Veg Sci 15:653–660

Bultman TL, Rodstrom JL, Radabaugh KR, VanDop JD, Librizzi JM et al (2009) Influence of genetic variation in the fungal endophyte of a grass on an herbivore and its parasitoid. Entomol Exp Appl 130:173–180

Cheplick GP (2008) Host genotype overrides fungal endophyte infection in influencing tiller and spike production of *Lolium perenne* (Poaceae) in a common garden experiment. Am J Bot 95:1063–1071

Christensen MJ, Latch GCM (1991) Variation among isolates of *Acremonium* endophytes (*A. coenophialum* and possibly *A. typhinum*) from tall fescue (*Festuca arundinacea*). Mycol Res 95:1123–1126

Clay K, Holah J (1999) Fungal endophyte symbiosis and plant diversity in successional fields. Science 285:1742

Cully AC, Cully JF, Hiebert RD (2003) Invasion of exotic plant species in tallgrass prairie fragments. Conserv Biol 17:990–998

Daly HV, Doyen JT, Purcell AH (1998) Introduction to insect biology and diversity. Oxford University Press, Oxford

De Battista JP, Bouton JH, Bacon CW, Siegel MR (1990) Rhizome and herbage production of endophyte-removed tall fescue clones and populations. Agron J 82:651–654

Fribourg HA, Hannaway DB, West CP (2009) Tall fescue for the twenty-first century. American Society of Agronomy, Madison

Funk CR, White RH, Breen JP (1993) Importance of *Acremonium* endophytes in turf-grass breeding and management. Agric Ecosyst Environ 44:215–232

Garrison MA, Stier JC (2010) Cool-season turfgrass colony and seed survival in a restored prairie. Crop Sci 50:12

Gundel PE, Perez LI, Helander M, Saikkonen K (2013) Symbiotically modified organisms: nontoxic fungal endophytes in grasses. Trends Plant Sci 18:420–427

Hesse U, Schoberlein W, Wittenmayer L, Forster K, Warnstorff K et al (2003) Effects of *Neotyphodium* endophytes on growth, reproduction and drought-stress tolerance of three *Lolium perenne* L. genotypes. Grass Forage Sci 58:407–415

Hunt MG, Newman JA (2005) Reduced herbivore resistance from a novel grass–endophyte association. J Appl Ecol 42:762–769

Iqbal J, Nelson J, McCulley R (2013) Fungal endophyte presence and genotype affect plant diversity and soil-to-atmosphere trace gas fluxes. Plant Soil 364:15–27

Jonasson S (1988) Evaluation of the point intercept method for the estimation of plant biomass. Oikos 52:101–106

Kardol P, Cornips NJ, van Kempen MML, Bakx-Schotman JMT, van der Putten WH (2007) Microbe-mediated plant-soil feedback causes historical contingency effects in plant community assembly. Ecol Monogr 77:147–162

Keathley CP, Potter DA (2012) Arthropod abundance in tall fescue, *Lolium arundinaceum*, pastures containing novel 'safe' endophytes. J Appl Entomol 136:576–587

Kuldau G, Bacon C (2008) Clavicipitaceous endophytes: their ability to enhance resistance of grasses to multiple stresses. Biol Control 46:57–71

Latch GCM, Christensen MJ, Tapper BA, Easton HS, Hume DE et al (2000) Tall fescue endophytes. United States Patent No. 6,111,170, Washington, DC

Leahy M, Robinson S (eds) (2000) Pasture production. Ontario Ministry of Agriculture, Food and Rural Affairs [Publication 19], Toronto

MacDougall AS, Turkington R (2005) Are invasive species the drivers or passengers of change in degraded ecosystems? Ecology 86:42–55

Malinowski DP, Belesky DP (2006) Ecological importance of Neotyphodium spp. grass endophytes in agroecosystems. Grassl Sci 52:1–14

Malinowski DP, Zuo H, Belesky DP, Alloush GA (2005) Evidence for copper binding by extracellular root exudates of tall fescue but not perennial ryegrass infected with Neotyphodium spp. endophytes. Plant Soil 267:1–12

Marshall SA (2006) Insects: their natural history and diversity. Firefly, Buffalo

Matthews JW, Clay K (2001) Influence of fungal endophyte infection on plant-soil feedback and community interactions. Ecology 82:500–509

Mian MAR, Hopkins AA, Zwonitzer JC (2002) Determination of genetic diversity in tall fescue with AFLP markers. Crop Sci 42:944–950

Omacini M, Semmartin M, Perez LI, Gundel PE (2012) Grass-endophyte symbiosis: a neglected aboveground interaction with multiple belowground consequences. Appl Soil Ecol 61:273–279

Popay AJ, Jensen JG (2005) Soil biota associated with endophyte-infected tall fescue in the field. N Z Plant Prot 58:117

Rasmussen S, Parsons A, Newman J (2009) Metabolomics analysis of the Lolium perenne—Neotyphodium lolii symbiosis: more than just alkaloids? Phytochem Rev 8:535–550

Ratliff LF, Ritchie JT, Cassel DK (1983) Field-measured limits of soil water availability as related to laboratory-measured properties. Soil Sci Soc Am J 47:770–775

Rudgers JA, Clay K (2007) Endophyte symbiosis with tall fescue: how strong are the impacts on communities and ecosystems? Fungal Biol Rev 21:107–124

Rudgers JA, Fischer S, Clay K (2010) Managing plant symbiosis: fungal endophyte genotype alters plant community composition. J Appl Ecol 47:468–477

Saikkonen K, Lehtonen P, Helander M, Koricheva J, Faeth SH (2006) Model systems in ecology: dissecting the endophyte-grass literature. Trends Plant Sci 11:428–433

Saikkonen K, Wali PR, Helander M (2011) Genetic compatibility determines endophyte-grass combinations. PLoS ONE 5:e11395

Saikkonen K, Gundel P, Helander M (2013) Chemical ecology mediated by fungal endophytes in grasses. J Chem Ecol 39:962–968

Shortt R, Eng P, Verhallen A, Fisher P (2011) Monitoring soil moisture to improve irrigation decisions. Ontario Ministry of Agriculture, Food and Rural Affairs Factsheet, Toronto

Timper P, Gates RN, Bouton JH (2005) Response of Pratylenchus spp. in tall fescue infected with different strains of the fungal endophyte Neotyphodium coenophialum. Nematology 7:105–110

Tozer KN, Lucas RJ, Edwards GR (2007) Suppression of annual grass weeds by AR542 endophyte infection in dryland tall fescue pastures. N Z Plant Prot 60:164–167

Vaylay R, van Santen E (2002) Application of canonical discriminant analysis for the assessment of genetic variation in tall fescue. Crop Sci 42:534–539

Vellend M, Drummond E, Tomimatsu H (2010) Effects of genotype identity and diversity on the invasiveness and invasibility of plant populations. Oecologia 162:371–381

Vesterlund S-R, Helander M, Faeth S, Hyvonen T, Saikkonen K (2011) Environmental conditions and host plant origin override endophyte effects on invertebrate communities. Fungal Divers 47:109–118

Wei YK, Gao YB, Xu H, Su D, Zhang X et al (2006) Occurrence of endophytes in grasses native to northern China. Grass Forage Sci 61:422–429

Yurkonis KA, Maherali H, Bolton KA, Klironomos JN, Newman JA (2012) Cultivar genotype, application and endophyte history affects community impact of Schedonorus arundinaceus. J Appl Ecol 49:1094–1102