LETTER

Plant–fungus mutualism affects spider composition in successional fields

Laura K. Finkes,[1] **Alan B. Cady,**[2] **Juliana C. Mulroy,**[3] **Keith Clay**[4] **and Jennifer A. Rudgers**[5]*

[1]*Department of Parks and Recreation, Slippery Rock University, Slippery Rock, PA 16057, USA*

[2]*Department of Zoology, Miami University, Oxford, OH 45056, USA*

[3]*Department of Biology, Denison University, Granville, OH 43023, USA*

[4]*Department of Biology, Indiana University, Bloomington, IN 47405, USA*

[5]*Department of Ecology and Evolutionary Biology, Rice University, Houston, TX 77005, USA*

Correspondence: E-mail: jrudgers@rice.edu

Abstract

Mutualistic symbionts are widespread in plants and may have strong, bottom-up influences on community structure. Here we show that a grass–endophyte mutualism shifts the composition of a generalist predator assemblage. In replicated, successional fields we manipulated endophyte infection by *Neotyphodium coenophialum* in a dominant, non-native plant (*Lolium arundinaceum*). We compared the magnitude of the endophyte effect with manipulations of thatch biomass, a habitat feature of known importance to spiders. The richness of both spider families and morphospecies was greater in the absence of the endophyte, although total spider abundance was not affected. Thatch removal reduced both spider abundance and richness, and endophyte and thatch effects were largely additive. Spider families differed in responses, with declines in Linyphiidae and Thomisidae due to the endophyte and declines in Lycosidae due to thatch removal. Results demonstrate that the community impacts of non-native plants can depend on plants' mutualistic associates, such as fungal endophytes.

INTRODUCTION

Mutualistic symbionts are widespread and may have strong, bottom-up influences on the structure of communities (Stachowicz 2001; Bruno *et al.* 2003). For example, mycorrhizal fungi associate with an estimated 80% of all Angiosperms (Brundrett 2002) and can affect herbivores of their hosts (Gehring & Whitham 2002). Like mycorrhizal fungi, fungal endophytes are also prevalent in plant communities. Most plant species contain horizontally transmitted, fungal endophytes that form highly localized infections in leaves (Petrini 1991; Stone *et al.* 2000). Further, 20–30% of all grass species are estimated to host 'systemic' fungal endophytes (Leuchtmann 1992). In some cases, endophytes form mutualisms with their hosts, gaining nutrition and protection from the host plant in exchange for the production of bioactive alkaloids that deter herbivores (Clay 1996). Endophytes also can improve host resistance to pathogens, drought tolerance and competition (reviewed by Clay & Schardl 2002, also see Arnold *et al.* 2003). At the community level, mutualistic endophytes can reduce plant diversity (Clay & Holah 1999) and shift the

relationship between plant diversity and ecosystem processes (Rudgers *et al.* 2004, 2005). The strong influence of fungal endophytes on plants and herbivores may cascade to other trophic levels (Rudgers & Clay 2005).

Despite endophytes' influence on herbivores and plant communities, few studies have determined whether endophytes affect trophic dynamics or food web structure (reviewed by Faeth & Bultman 2002). Current evidence suggests a negative effect on predators and parasitoids of herbivores, due in part to the toxic effects of alkaloid bioaccumulation. Endophyte-free *Lolium multiflorum* plants (grown outdoors in pots) supported higher rates of aphid parasitism, more complex food webs, and higher parasitoid species richness than plants with endophytes (Omacini *et al.* 2001). Similarly, laboratory studies have shown that endophytes can reduce the rate of development and pupal mass of individual species of hymenopteran parasitoids (Barker & Addison 1996, 1997; Bultman *et al.* 1997, 2003) as well as increase the mortality of entomopathogenic nematodes (Kunkel & Grewal 2003; Kunkel *et al.* 2004).

Research thus far has focused on specialist predators and parasitoids, and it is unknown how generalist

predators may respond to endophytes. Furthermore, most prior work has been conducted in pots or in the laboratory where community dynamics were greatly simplified. Several mechanisms may underlie potential negative effects of plant mutualists on spiders and other generalist predators, including reductions in the abundance or quality of prey (especially herbivorous insects; Clay 1996; Gehring & Whitham 2002), bioaccumulation of secondary compounds (Gange & West 1994; Bultman *et al.* 1997), changes in plant size (Gange *et al.* 2003) or plant composition (van der Heijden *et al.* 1998; Clay & Holah 1999), or shifts in the composition of the detrital-based food web (Omacini *et al.* 2004; Lemons *et al.* 2005). However, in the only other field study of which we are aware, Davidson & Potter (1995) found no effects of a mutualistic endophyte on several groups of generalist predators (including spiders) in small field plots of *Lolium arundinaceum*.

Here, we investigated whether the symbiotic mutualism between tall fescue grass (*L. arundinaceum*) and a fungal endophyte (*Neotyphodium coenophialum*) affects the composition of spiders in a field setting. Tall fescue is the most abundant and widely distributed endophyte host (Ball *et al.* 1993) and is an important non-native, pest species in the USA (Southern Weed Science Society 1998; Raloff 2003). Although tall fescue is typical of grass–endophyte symbioses that produce high levels of bioactive alkaloids (Clay & Schardl 2002), not all grass–endophyte associations are mutualistic (Saikkonen *et al.* 1998). We use this system as a model for understanding how strong microbial mutualisms involving ecologically dominant host plants can affect food web structure, and not as representative of all plant–endophyte interactions.

To facilitate determination of the ecological importance of the tall fescue–endophyte symbiosis to spiders, we compared the endophyte's effect to an ecological factor with known impacts on spider composition – the abundance of thatch (Riechert & Bishop 1990; Uetz 1991; Halaj *et al.* 2000; Denno *et al.* 2002; Buddle & Rypstra 2003). Often the best test for the consequences of an ecological interaction is to compare the strength of the effect with other factors of known importance (Welden & Slauson 1986). We addressed the following questions: (i) does the abundance of thatch or the presence of the endophyte affect spider abundance or family/morphospecies richness? (ii) Are the effects of endophytes and thatch additive, synergistic or antagonistic? Both thatch and the endophyte affected spider community composition (mainly additively), with variable responses across different spider families. We also investigated one potential mechanism underlying negative effects of the endophyte by asking (iii) does the endophyte reduce herbivore abundance or prey capture rates for spiders?

MATERIALS AND METHODS

Study system

Tall fescue [*L. arundinaceum* (Schreb.) S.J. Darbyshire, formerly *Festuca arundinacea*] is a perennial, cool-season grass introduced to the USA from Eurasia during the 19th century (Ball *et al.* 1993). More than 75% of the tall fescue in the USA is found in association with the fungal endophyte, *N. coenophialum* (Morgan-Jones and Gams) Glenn, Bacon and Hanlin (Ball *et al.* 1993), which grows in the intercellular spaces of aboveground tissues and is exclusively vertically transmitted to the seeds (Clay 1990). Tall fescue is commonly planted for forage and turfgrass but also is widely naturalized in the USA. Field experiments were conducted at the Indiana University Bayles Road Experimental Field, Bloomington, IN, USA (39°13′9″ N, 086°32′29″ W). This site is an old agricultural field dominated by *Ambrosia trifida*, *Cerastium vulgatum*, *Cirsium arvense*, *Conyza canadensis*, *L. arundinaceum*, *Poa pratensis*, *Rumex acetosella*, *Solidago* spp., *Sorghum halepense* and *Trifolium* spp. and previously maintained by mowing.

Endophyte treatment

We examined the effect of the endophyte on spiders by manipulating the presence of *N. coenophialum* in tall fescue plants. Endophyte-infected (E+) and endophyte-free (E−) seeds were obtained from plots of E+ and E− tall fescue that freely cross-pollinated, helping to homogenize the plant genetic background with respect to the endophyte treatment. Tall fescue is self-incompatible, and even cultivars have high genetic variation (Ball *et al.* 1993; Xu *et al.* 1994). Our E− source population was originally established using E+ fescue seeds that had undergone long-term storage to reduce the viability of the endophyte. Seeds used in the experiment were several generations distant from the original endophyte removal treatment. During September 2000, sixteen 30 × 30 m plots (two adjacent rows of eight) were alternately seeded with E+ or E− fescue (sowing rate 45 kg ha^{-1}). Plots were tilled prior to planting, and each plot was bordered by a 1-m-wide strip of unseeded buffer. During the course of the experiment, other plant species recruited into plots from the seed bank, vegetative fragments, and adjacent old fields.

Observations in unmanipulated thatch

Within each of the 16 plots, we chose four 0.5 × 0.5 m subplots representing naturally high thatch cover (75–100% visual cover relative to bare ground) and four 0.5 × 0.5 m subplots representing naturally low thatch

cover (0–25% cover). Thatch primarily consisted of dead tall fescue leaves: E− thatch was 99.2 ± 0.5% tall fescue (dry weight) and E+ thatch was 99.0 ± 0.5% tall fescue, with forbs and other grasses present in very small amounts (Lemons *et al.* 2005). We conducted observations in unmanipulated thatch to assess how they compared with our experimental thatch treatment (see Thatch treatment). Within each subplot, we counted all visible diurnally foraging spiders during 17–26 June 2003. Spiders were censused within a 0.5 × 0.5 m cardboard quadrat with 0.15-m tall sides to minimize escape of the spiders from the subplots during the census period. Census periods lasted *c.* 5 min per subplot to make an exhaustive search. We sifted through the vegetation by hand to flush spiders and to examine all layers of the vegetation. We analysed spider counts using mixed model ANOVA (Proc MIXED, SAS Institute Inc. 2000) with the random effect of plot, which was nested within row and endophyte treatment. *F*-tests for the fixed effects of endophyte, row and endophyte × row were constructed using variation among plots in the denominator to avoid pseudoreplication. Count data were square-root transformed to achieve normality of residuals and equality of variances.

Thatch treatment

We experimentally investigated the effect of thatch on spider distribution and abundance. The thatch treatment was applied to a randomly chosen subset of six 1 × 1 m subplots within each of the 16 experimental field plots. Subplots were randomly assigned to one of three thatch treatments: no thatch (No), mean thatch (Avg), or twice the mean level of thatch (2X). During 26–29 June 2003, thatch was removed from all subplots and taken to the laboratory to determine wet weight for each subplot. We used wet weight because we did not want to alter the chemical composition or microenvironment of the thatch by drying it. Arthropods (> 2 mm) present in the collected thatch were removed. On 3 July 2003, thatch was redistributed according to treatment, with treatment weights calculated on a per plot basis to maintain any differences in thatch associated with each plot. Thatch was returned only to the plot from which it was collected. The presence of the endophyte had no significant effect on the wet weight biomass of thatch in the plots in July 2003 (ANOVA $F_{1,12} = 1.53$, $P = 0.24$; mean thatch biomass (g m^{-2}) ± SE: E+ = 258.1 ± 36.5, $n = 8$ plots, E− = 206.2 ± 18.5, $n = 8$), on thatch dry weight during July and September 2004 (J. A. Rudgers and K. Clay, unpublished data), or on thatch dry weight in a different experiment at a nearby site (Clay & Holah 1999). Thus, the effects of thatch and endophyte treatments on spiders should be additive.

Effectiveness of treatments

On 23 September 2003, endophyte infection was assessed by randomly selecting four tillers of tall fescue from each plot. Infection status was determined via microscopy following staining of thin sections from the inner leaf sheath with lacto-phenol cotton blue (Clark *et al.* 1983). The proportion of tillers showing evidence of endophytes was calculated per plot and angular transformed. Data were analysed using ANOVA with the endophyte treatment, row and endophyte × row as fixed effects (Proc GLM, SAS Institute Inc. 2000). In addition, we conducted a follow-up survey of infection status by collecting 30 tillers per plot during 9–17 June 2005, and used the same data analysis method. Finally, 560 seeds were collected across all plots in July 2003, grown in the greenhouse, and tillers were stained for the presence of the endophyte.

Thatch was recollected and weighed (wet weight) on 26 September 2003. Thatch data were analysed using mixed model ANOVA, as described above for spiders. To compare among the three thatch levels, we conducted *post hoc* pairwise treatment comparisons using a Tukey's honestly significantly different (HSD) test.

Spider censuses in experimental treatments

We counted diurnally foraging spiders once every two weeks from 17 July to 14 September 2003. The cardboard quadrat was placed in the centre of each 1 × 1 m subplot to reduce edge effects. Quadrats may have been small relative to the home ranges of the largest, mobile spiders in our plots (*Hogna hellulo*, *Rabidosa punctulata* and *Schizocosa bilineata*), but we still observed 65 individuals of these species during our censuses (Digital Appendix A). All visible spiders within each quadrat were counted and identified to family and morphospecies in the field. A morphospecies designation was used because of the difficulty in identifying some spiders (immatures) to species. Prior to the censuses, we had gained extensive experience with spider identification by sorting sweepnet and pitfall collections taken from the same plots. Voucher specimens of each species were collected from outside of the experimental treatments and identified to family (and genus/species when possible).

We examined three responses, spider abundance, family richness (total number of families represented) and morphospecies richness (total number of morphospecies). We used repeated measures MANOVA with the fixed effects of endophyte treatment, row, thatch treatment, and all interactions, and the random effect of plot (nested within endophyte × row) (profile analysis, SAS Institute Inc. 2000). We took the conservative approach of assuming an unstructured variance–covariance matrix to avoid restrictive assumptions of sphericity (von Ende 2001). *F*-tests for the

effects of the endophyte, row and endophyte × row were determined using variation among plots in the denominator. Data were square-root transformed to achieve normality and equality of variances. Tukey HSD tests compared the three thatch levels. Results from each census were decomposed using univariate analyses if treatment × time interactions were significant.

Herbivore abundance: sweepnet samples

To assess whether endophyte treatments differed in the general abundance of herbivorous insects (a potential mechanism driving impacts on spiders), we took 200 sweeps
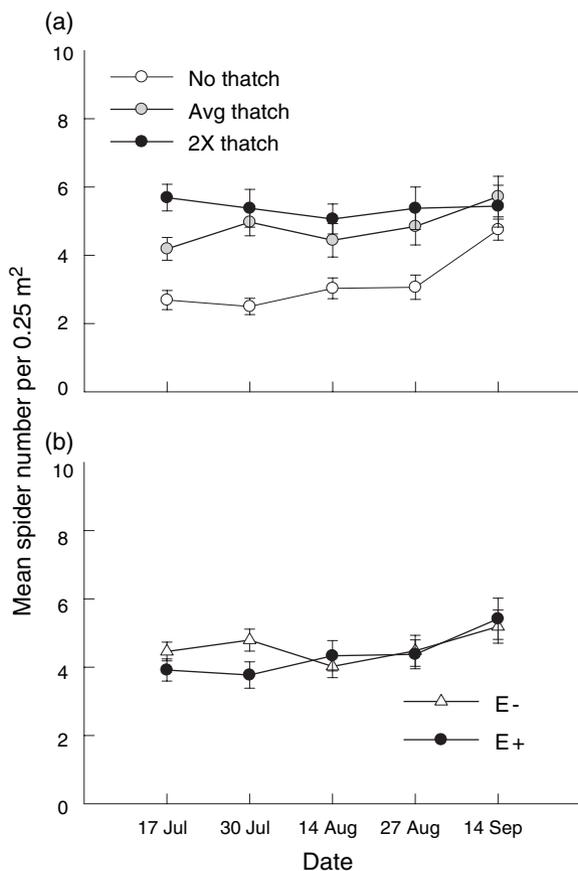

Figure 1 Effect of treatments on the total abundance of spiders. (a) Thatch treatment: No, thatch removal (open circles); Avg, mean thatch biomass (grey circles); 2X, 2X mean thatch biomass (black circles); $n = 16$ plots per treatment; standard errors were calculated from $n = 16$ plots per treatment by first determining the mean values for the two replicate thatch treatments per plot. (b) Endophyte treatment; tall fescue (*Lolium arundinaceum*) infected with endophyte *Neotyphodium coenophialum* (E+, solid circles) or endophyte-free (E−, open triangles), $n = 8$ plots per treatment. Data points represent the mean abundance of spiders and the bars show ± 1 SE.

per plot (standard 37.5-cm diameter sweepnet) on 11 July 2003 and again on 30 September 2003 to sample the entire plot within 1 m of the edge. Herbivore status was determined by identifying all insects to family, and when possible to genus/species. We analysed the effect of the endophyte on total herbivore abundance per plot using repeated measures MANOVA with the fixed effects of endophyte, row and endophyte × row and the repeated effect of time. Data met assumptions of normality and equality of variances.

Aerial web-building spiders: web size and prey capture rates

We also determined whether aerial web spiders caught proportionally more prey per unit area of web in endophyte-free vs. endophyte-infected tall fescue plots. Due to ease of observation, we examined four common species: *Argiope trifasciata* and *Acanthepeira stellata* (Araneidae), *Florinda coccinea* and *Frontinella pyramitela* (Linyphiidae). During 8–24 October 2003, for a randomly selected subset of up to ten webs per species per plot, we measured the length of the major axis (longest diameter) and minor axis (perpendicular to major) of each web to the nearest cm to obtain an estimate of web area with the formula

$$\text{area} = \pi \left(\frac{\text{axis}_{\text{major}} + \text{axis}_{\text{minor}}}{4} \right)^2.$$

Axes for Araneidae were vertical with respect to the ground; axes for Linyphiidae were horizontal. For each web, we counted the number of prey items present. An estimate of prey capture was determined by dividing the number of prey by the area of the web. We conducted mixed model ANOVA on prey number per m^2 of web (angular transformed). Data met assumptions of normality and equality of variances. Fixed factors in the model included the endophyte treatment, row and endophyte × row, and plot (nested within endophyte × row) was considered a random factor.

RESULTS

Observations on spider abundance in unmanipulated thatch

Naturally high thatch cover supported more than twice as many spiders than naturally low thatch cover ($F_{1,108} = 32.2$, $P < 0.0001$; mean number of spiders/0.25 m^2 ± SE: high thatch = 3.89 ± 0.33, $n = 16$ plots; low thatch = 1.72 ± 0.24, $n = 16$). In contrast, the endophyte treatment had no detectable effect on total spider abundance ($F_{1,12} = 0.0$, $P = 0.9$; mean spider number/0.25 m^2 ± SE: E+ = 2.83 ± 0.56, $n = 8$ plots; E− = 2.78 ± 0.57, $n = 8$ plots; thatch × endophyte $F_{1,108} = 0.4$, $P = 0.6$).

Table 1 Analysis of variance examining the effects of the thatch treatment, the endophyte (*Neotyphodium coenophialum*) treatment, row, plot and time on total spider abundance, morphospecies richness, and the abundance of the six most abundant spider families, Araneidae, Linyphiidae, Lycosidae, Salticidae, Theridiidae and Thomisidae in tall fescue (*Lolium arundinaceum*) in Indiana. *P*-values <0.05 are shown in bold.

Effect	d.f.	Spider abundance		Family richness		Morphospecies richness	
		F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Endophyte	1,13	1.17	0.299	14.56	**0.002**	8.44	**0.012**
Row	1,13	0.62	0.447	0.01	0.933	0.14	0.718
Endophyte × row	1,13	0.09	0.763	0.04	0.845	0.01	0.919
Thatch	2,71	22.64	**< 0.001**	5.72	**0.005**	10.07	**< 0.001**
Endophyte × thatch	2,71	1.04	0.359	0.86	0.426	1.23	0.297
Row × thatch	2,71	0.64	0.529	0.74	0.479	0.43	0.649
Endophyte × row × thatch	2,71	2.13	0.127	1.76	0.179	2.16	0.122
Plot (endophyte × row)	13,71	1.37	0.195	0.74	0.720	0.68	0.772
Time	4,68	2.90	**0.028**	2.61	**0.043**	2.24	0.074
Time × endophyte	4,10	1.74	0.218	0.58	0.684	0.78	0.565
Time × row	4,10	3.54	**0.048**	2.29	0.131	2.65	0.096
Time × endo × row	4,10	1.04	0.436	0.78	0.565	0.53	0.717
Time × thatch	8,138	1.97	0.055	3.87	**< 0.001**	3.02	**0.004**
Time × endophyte × thatch	8,138	1.36	0.218	1.53	0.154	1.90	0.064
Time × row × thatch	8,138	0.84	0.572	0.72	0.674	0.69	0.701
Time × endophyte × row × thatch	8,138	1.20	0.301	1.15	0.333	1.45	0.182
Time × plot (endophyte × row)	52,284	1.43	**0.037**	1.27	0.113	81.75	**0.002**

Effect	d.f.	Araneidae		Linyphiidae		Lycosidae	
		F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Endophyte	1,13	1.53	0.237	7.02	**0.020**	0.63	0.441
Row	1,13	0.35	0.566	0.69	0.420	1.04	0.327
Endophyte × row	1,13	0.04	0.852	0.42	0.529	0.24	0.633
Thatch	2,71	1.72	0.187	1.25	0.292	33.87	**< 0.001**
Endophyte × thatch	2,71	1.29	0.282	0.17	0.843	1.48	0.234
Row × thatch	2,71	1.94	0.151	0.03	0.969	1.34	0.268
Endophyte × row × thatch	2,71	3.36	**0.041**	1.59	0.211	0.67	0.516
Plot (endophyte × row)	13,71	1.18	0.310	2.27	**0.015**	2.86	**0.002**
Time	4,68	10.17	**< 0.001**	3.42	**0.013**	11.87	**< 0.001**
Time × endophyte	4,10	0.76	0.572	1.46	0.286	0.56	0.696
Time × row	4,10	1.72	0.223	0.29	0.879	3.36	0.055
Time × endophyte × row	4,10	0.86	0.518	1.76	0.213	0.86	0.520
Time × thatch	8,138	1.20	0.306	1.00	0.438	1.22	0.293
Time × endophyte × thatch	8,138	0.63	0.756	0.52	0.837	1.09	0.372
Time × row × thatch	8,138	1.20	0.302	1.38	0.211	0.76	0.641
Time × endophyte × row × thatch	8,138	0.48	0.868	0.77	0.629	1.22	0.289
Time × plot (endophyte × row)	52,284	1.05	0.387	1.40	0.046	1.33	0.075

Effect	d.f.	Salticidae		Theridiidae		Thomisidae	
		F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Endophyte	1,13	0.44	0.519	3.34	0.091	10.95	**0.006**
Row	1,13	1.59	0.229	0.22	0.644	0.19	0.672
Endophyte × row	1,13	0.22	0.645	0.00	0.963	1.64	0.223
Thatch	2,71	0.35	0.704	2.30	0.107	0.97	0.385
Endophyte × thatch	2,71	0.48	0.621	1.11	0.337	1.44	0.244
Row × thatch	2,71	0.36	0.698	0.20	0.817	0.52	0.597
Endophyte × row × thatch	2,71	0.30	0.744	0.32	0.725	0.05	0.952

Table 1 *Continued*

		Salticidae		Theridiidae		Thomisidae	
		F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Plot (endophyte × row)	13,71	0.65	0.805	2.80	**0.003**	1.25	0.264
Time	4,68	6.96	**< 0.001**	0.66	0.602	1.40	0.243
Time × endophyte	4,10	3.53	**0.048**	1.06	0.426	1.36	0.313
Time × row	4,10	0.81	0.546	0.62	0.662	3.23	0.060
Time × endo × row	4,10	1.55	0.262	0.31	0.867	2.84	0.082
Time × thatch	8,138	1.38	0.209	1.28	0.261	0.85	0.564
Time × endophyte × thatch	8,138	1.36	0.221	1.30	0.247	0.46	0.882
Time × row × thatch	8,138	1.54	0.150	1.30	0.250	0.97	0.463
Time × endophyte × row × thatch	8,138	0.91	0.510	0.83	0.574	0.75	0.644
Time × plot (endophyte × row)	52,284	0.96	0.553	1.45	**0.032**	1.08	0.333

Effectiveness of treatments

Endophyte and thatch treatments remained effective for the duration of the experiment. In September 2003, the percentage of tillers with endophytes was 94 ± 0.04% SE ($n = 8$ plots) in plots with the endophyte when compared with 0 ± 0.00% SE ($n = 8$) in endophyte-free plots ($F_{1,12} = 675.0$, $P < 0.0001$). Similar differences were found in the June 2005 follow-up survey, E+ = 92% ± 0.01 SE ($n = 8$ plots) and E− = 0.1% ± 0.01 SE ($n = 8$) ($F_{1,14} = 3931.1$, $P < 0.0001$), with no significant effect of row in either survey (2003: $F_{1,12} = 3.0$, $P = 0.11$; 2005: $F_{1,12} = 0.1$, $P = 0.8$). Also, plants grown from seeds collected from E+ plots were 94.1% infected, and from E− plots, 0% infected ($n = 280$).

Despite accumulation of thatch during the summer, significant differences in thatch levels were maintained for all subplots when thatch was harvested in September ($F_{2,72} = 52.5$, $P < 0.0001$). Mean thatch biomass (g m^2 ± SE) in the No thatch treatment was 123.2 ± 14.1a ($n = 16$ plots), for Avg thatch, 181.1 ± 14.4b, ($n = 16$) and for 2X thatch, 272.9 ± 24.2c ($n = 16$). Letters indicate significantly different treatments ($P < 0.05$) according to a Tukey HSD test.
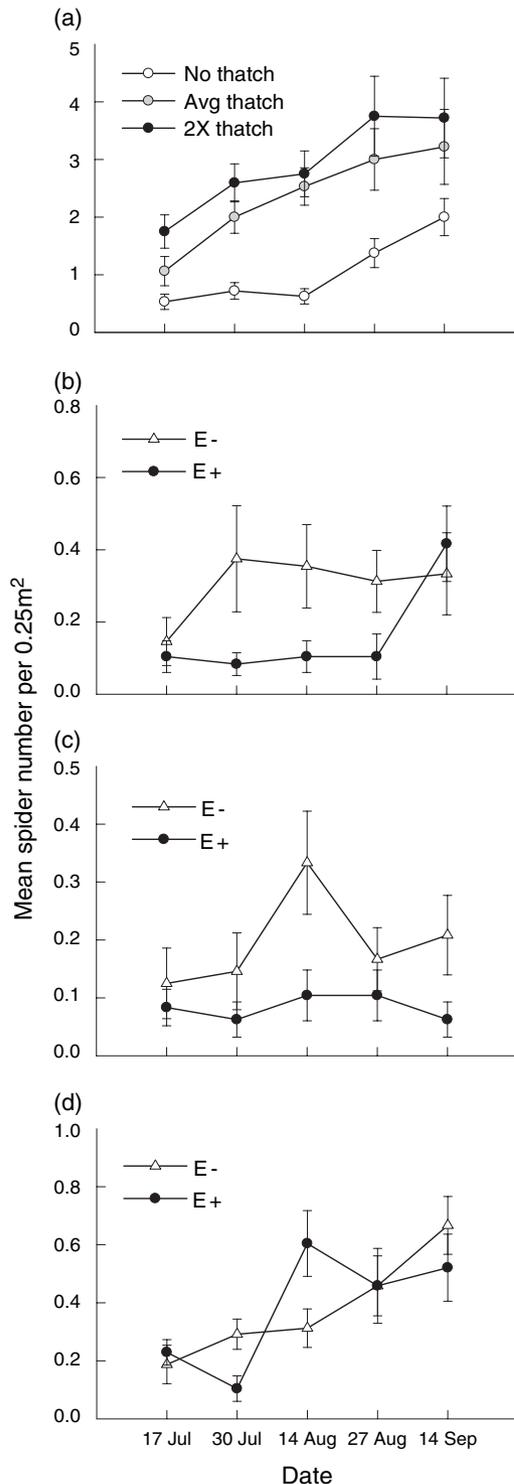
Does thatch or the endophyte affect spider abundance or richness?

Experimental thatch removal decreased total spider abundance (Fig. 1a, $P < 0.001$, Table 1). Average and 2X thatch treatments supported *c.* 60% more spiders than thatch removal over the course of the season. Tukey HSD tests showed that average and 2X thatch treatments had similar numbers of spiders with the exception of the first census, when all treatments differed significantly, and the last census, when no treatments differed significantly. When separated by family, only Lycosidae (wolf spiders) were significantly affected by thatch (Table 1). On average 180% more Lycosidae were found in the 2X thatch than in the thatch removal treatment ($P < 0.001$, Table 1), with no difference between the average and 2X treatments (Fig. 2a).

Experimental removal of the endophyte did not affect the total number of spiders (Fig. 1b, $P = 0.299$, Table 1), a result that was consistent with our initial field observations. However, the presence of the endophyte reduced spider numbers in the families Linyphiidae (sheet-web spiders, Fig. 2b; $P = 0.02$, Table 1) and Thomisidae (crab spiders, Fig. 2c, $P = 0.006$, Table 1), with no difference in the endophyte effect over time (endophyte × time, $P > 0.05$, Table 1). For Linyphiidae, spider abundance was reduced by 47% and for Thomisidae, spiders were reduced by 57%. Finally, Salticidae (jumping spiders), were more abundant in the presence of the endophyte, but only on 14 August (Fig. 2d, endophyte × time, $P = 0.048$, Table 1).

Both thatch and the endophyte affected the family and morphospecies richness of spiders (species listed in Digital Appendix A). The average and 2X thatch treatments did not differ significantly according to a Tukey HSD test (Fig. 3a, b). However, averaged over the season, thatch removal significantly reduced family richness by 15% and morphospecies richness by 24% when compared with average or 2X thatch (Table 1). The effect of thatch removal weakened over time (thatch × time, Table 1), perhaps because thatch accumulated during the season. In addition, spider family and morphospecies richness was significantly lower, 14% and 12%, respectively, in the presence than in the absence of the endophyte, averaged over the course of the experiment (Fig. 3c, d; Table 1). These effects did not significantly vary with time ($P > 0.5$, Table 1). Averaged over time, mean family richness (± SE) was 2.43 ± 0.17 in the presence of the endophyte, and 2.83 ± 0.19 in its absence ($n = 8$ plots over five dates).

(a)

(b)

(c)

(d)

Mean spider number per 0.25m²

17 Jul   30 Jul   14 Aug   27 Aug   14 Sep

Date

were additive (i.e. no significant endophyte × thatch interactions). However, for the Araneidae, the thatch effect depended on the endophyte and block (endophyte × row × thatch, $P = 0.041$, Table 1). Specifically, 2X thatch increased spider number relative to thatch removal, but only in the absence of the endophyte and only in row 2 (Fig. 4).

Does the endophyte reduce herbivore abundance or prey capture rates for spiders?

During July to September 2003, total herbivore abundance was 25–55% lower in plots with the endophyte than in endophyte-free plots (July mean herbivore number ± SE: E+ = 114 ± 24, E− = 153 ± 15, $n = 8$ plots; September: E+ = 70 ± 10, E− = 156 ± 16, $n = 8$ plots; endophyte $F_{1,12} = 10.7$, $P = 0.007$; endophyte × time $F_{1,12} = 3.0$, $P = 0.11$; row and interactions with row, all $P > 0.08$). In addition, the presence of the endophyte reduced prey capture for one common species of aerial web-building spider. *Argiope trifasciata* caught 5.4 times more prey per web unit in endophyte-free plots when compared with endophyte-infected plots ($F_{1,11} = 4.8$, $P = 0.05$; mean number of prey per m² ± SE, E+ = 8.7 ± 6.1; E− = 46.7 ± 29.9, $n = 8$ plots). The endophyte did not affect prey capture for the other species examined (*Acanthepeira stellata* $F_{1,9} = 0.8$, $P = 0.4$; *Florinda coccinea* $F_{1,6} = 0.2$, $P = 0.7$; *Frontinella pyramitela* $F_{1,14} = 0.8$, $P = 0.4$).

DISCUSSION

Experimental manipulations of the presence of *N. coenophialum* in tall fescue grass demonstrated that this fungal endophyte influences the composition of spider communities. To our knowledge, this study is the first to show that a fungal mutualist can affect field populations of this important group of generalist predators. Although the overall abundance of spiders was similar between endophyte-infected and endophyte-free plots, spider family richness and morphospecies richness significantly declined (by 14% and 12% respectively) in plots with *N. coenophialum*
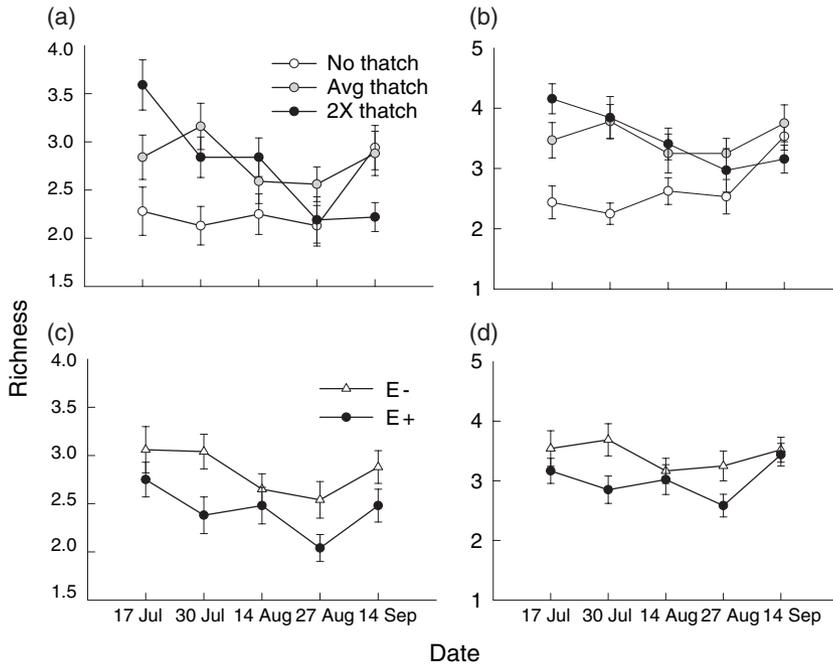
Are the effects of the endophyte and thatch additive, synergistic or antagonistic?

For total abundance, richness and for most families of spiders, the combined effects of endophytes and thatch

Figure 3 Effect of treatments on the richness of spiders. (a) family richness, thatch treatment: No, thatch removal (open circles); Avg, mean thatch biomass (grey circles); 2X, 2X mean thatch biomass (black circles); $n = 16$ plots per treatment. (b) Morphospecies richness, thatch treatment. (c) Family richness, endophyte treatment: tall fescue (*Lolium arundinaceum*) infected with endophyte *Neotyphodium coenophialum* (E+ = solid circles) or endophyte-free (E− = open triangles), $n = 8$ plots per treatment. (d) Morphospecies richness, endophyte treatment. Data points represent the mean richness/0.25 m^2, and the bars show $\pm$ 1 SE.
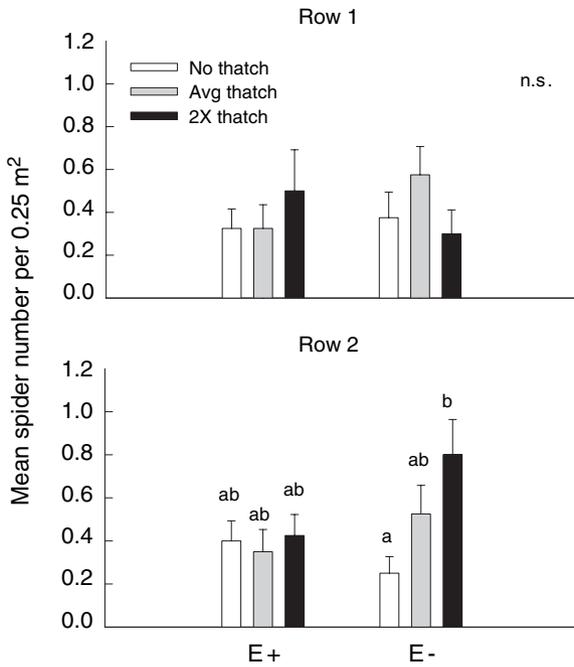

Figure 4 Effect of endophyte treatment [tall fescue (*Lolium arundinaceum*) infected with endophyte *Neotyphodium coenophialum* (E+) or without the endophyte (E−)], the row (or block), and the thatch treatment [No = thatch removal (open bars), Avg = mean thatch biomass (grey bars), 2X = 2X mean thatch biomass (black bars)] on the abundance of spiders in the family Araneidae in Indiana. Data points represent the mean abundance of spiders, and the bars show $\pm$ 1 SE. $n = 8$ plots per treatment. Different letters indicate treatments that differed significantly according to a Tukey HSD test; only treatments in row 2 significantly differed.

compared with endophyte-free plots. Furthermore, community composition shifted, with decreases in the abundances of two families of spiders, Linyphiidae and Thomisidae (by *c.* 50–60%) due to the endophyte.

The effect of the endophyte on spiders was ecologically weaker than the effect of thatch, an important component of spiders' structural habitat that also may influence temperature and humidity levels (Denno *et al.* 2002; Buddle & Rypstra 2003). Thatch removal resulted in *c.* 60% decline in spider abundance and 15–24% decline in spider richness when compared with plots with average levels of thatch. In contrast, the endophyte had no effect on spider abundance and reduced richness 12–14%. In addition, declines in spider abundance because of thatch removal were mainly driven by the response of the family Lycosidae, which did not respond to manipulations of the endophyte. Overall, ambient thatch levels were similar in E+ and E− plots, and there was little evidence that effects of the endophyte depended on the level of thatch (i.e. endophyte × thatch interactions). The only exception was the abundance of Araneidae, which was reduced by the thatch removal treatment, but only in the absence of the endophyte in one block of the experiment.

What mechanisms might underlie the effects of endophytes on spiders? We investigated one hypothesis, that the endophyte affected spiders indirectly by reducing prey abundance. Total herbivore abundance (via sweepnet samples) declined 25–55% in the presence of the endophyte, which could indicate a reduction in prey. Several prior studies in grass-endophyte systems have documented similar, negative impacts of the endophyte

on herbivores (reviewed by Latch 1993; Breen 1994; Clay 1996). However, certain taxa within this trophic group might contribute more to spider diets than others, and more detailed work, including documenting the composition of spiders' diets, would be needed to confirm this mechanism. However, we also observed that prey capture rates of one abundant orb-weaving spider, *Argiope trifasciata*, were reduced more than fivefold in the presence of the endophyte.

Our study was not designed to tease apart the mechanisms underlying the effects of the endophyte, and several additional mechanisms likely contribute to the endophyte effect. First, the endophyte may shift the composition of the prey assemblage. Second, indirect negative effects of the endophyte on spiders may be mediated through changes in the plant assemblage. An 8-year experiment at a nearby site in Indiana demonstrated that *N. coenophialum* in tall fescue reduced the abundance and diversity of plant species, including both forbs and non-fescue grass species (Clay & Holah 1999). Proper architecture for web support is a major factor for habitat selection by web-building spiders (Robinson 1981; Gunnarsson 1990; Halaj *et al.* 2000). Thus, changes in plant composition may contribute to the negative effects of endophytes, particularly on web-building Linyphiidae (for which the endophyte did not reduce prey capture) as well as for plant-dwelling Thomisidae. Third, consumption of alkaloid-laden prey might alter spider prey capture, web building or reproductive behaviours, thereby reducing predation efficiency (e.g. Bultman *et al.* 1997; Kunkel *et al.* 2004). Finally, the endophyte could reduce the nutritional quality of spiders' prey or alter prey behaviour (e.g. reduced foraging time or general inactivity) without directly affecting prey abundance. Further research exploring the relative importance of different mechanisms driving the endophyte's effects is warranted.

CONCLUSION

Here, we show that a plant–microbe mutualism can affect the structure of spider communities by reducing family and morphospecies richness and shifting the relative abundance of taxa. In addition to enhancing basic understanding of the community-level effects of mutualisms, this work is relevant to applied systems, such as turf grass and forage production, where increased use of bio-control agents such as spiders could reduce the need for insecticides. Furthermore, results inform conservation. We show that the ecological impacts of an introduced plant depend on association with a microbial mutualist. Tall fescue with the endophyte had a stronger negative impact on a native spider assemblage than endophyte-free fescue.

REFERENCES

Arnold, A.E., Mejia, L.C., Kyllo, D., Rojas, E.I., Maynard, Z., Robbins, N. *et al.* (2003). Fungal endophytes limit pathogen damage in a tropical tree. *Proc. Natl Acad. Sci. USA*, 100, 15649–15654.

Ball, D.M., Pedersen, J.F. & Lacefield, G.D. (1993). The tall fescue endophyte. *Am. Sci.*, 81, 370–379.

Barker, G.M. & Addison, P.J. (1996). Influence of clavicipitaceous endophyte infection in ryegrass on development of the parasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) in *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae). *Biol. Control*, 7, 281–287.

Barker, G.M. & Addison, P.J. (1997). Clavicipitacedous endophytic infection in ryegrass influences attack rate of the parasitoid *Microctonus hyperodae* (Hymenoptera: Braconidae, Euphorinae) in *Listronotus bonariensis* (Coleoptera: Curculionidae). *Environ. Entomol.*, 26, 416–420.

Breen, J.P. (1994). *Acremonium*/endophyte interactions with enhanced plant resistance to insects. *Annu. Rev. Entomol.*, 39, 402–423.

Brundrett, M.C. (2002). Coevolution of roots and mycorrhizas of land plants. *New Phytol.*, 154, 275–304.

Bruno, J.F., Stachowicz, J.J. & Bertness, M.D. (2003). Inclusion of facilitation into ecological theory. *Trends Ecol. Evol.*, 18, 119–125.

Buddle, C.N. & Rypstra, A.L. (2003). Factors initiating emigration of two wolf spider species (Araneae: Lycosidae) in an agroecosystem. *Environ. Entomol.*, 32, 88–95.

Bultman, T.L., Borowicz, K.L., Schneble, R.M., Coudron, T.A. & Bush, L.P. (1997). Effect of a fungal endophyte on the growth and survival of two *Euplectrus* parasitoids. *Oikos*, 78, 170–176.

Bultman, T.L., McNeill, M.R. & Goldson, S.L. (2003). Isolate-dependent impacts of fungal endophytes in a multitrophic interaction. *Oikos*, 102, 491–496.

Clark, E.M., White, J.F. & Patterson, R.M. (1983). Improved histochemical techniques for the detection of *Acremonium coenophialum* in tall fescue and methods of in vitro culture of the fungus. *J. Microb. Methods*, 1, 149–155.

Clay, K. (1990). Fungal endophytes of grasses. *Annu. Rev. Ecol. Syst.*, 21, 275–297.

Clay, K. (1996). Interactions among fungal endophytes, grasses and herbivores. *Res. Popul. Ecol.*, 38, 191–201.

Clay, K. & Holah, J. (1999). Fungal endophyte symbiosis and plant diversity in successional fields. *Science*, 285, 1742–1744.

Clay, K. & Schardl, C. (2002). Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *Am. Nat.*, 160, S99–S127.

Davidson, A.W. & Potter, D.A. (1995). Response of plant-feeding, predatory, and soil-inhabiting invertebrates to *Acremonium*

endophyte and nitrogen fertilization in tall fescue turf. *J. Econ. Entomol.*, 88, 367–379.

Denno, R.F., Gratton, C., Peterson, M.A., Langellotto, G.A., Finke, D.L. & Huberty, A.F. (2002). Bottom-up forces mediate natural-enemy impact in a phytophagous insect community. *Ecology*, 83, 1443–1458.

von Ende, C.N. (2001). Repeated measures analysis: growth and other time dependent measures. In: *Design and Analysis of Ecological Experiments* (eds Scheiner, S.M. & Gurevitch, J.). Oxford University Press, New York, pp. 134–157.

Faeth, S.H. & Bultman, T.L. (2002). Endophytic fungi and interactions among host plants, herbivores, and natural enemies. In: *Multitrophic Level Interactions* (eds Tscharntke, T. & Hawkins, B.A.). Cambridge University Press, Cambridge, UK, pp. 89–123.

Gange, A.C. & West, H.M. (1994). Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects in *Plantago lanceolata* L. *New Phytol.*, 128, 79–87.

Gange, A.C., Brown, V.K. & Aplin, D.M. (2003). Multitrophic links between arbuscular mycorrhizal fungi and insect parasitoids. *Ecol. Lett.*, 6, 1051–1055.

Gehring, C.A. & Whitham, T.G. (2002). Mycorrhizae-herbivore interactions: population and community consequences. In: *Mycorrhizal Ecology* (eds van der Heijden, M.G.A. & Sanders, I.R.). Springer-Verlag, Heidelberg, Germany, pp. 295–320.

Gunnarsson, B. (1990). Vegetation structure and the abundance and size distribution of spruce-living spiders. *J. Anim. Ecol.*, 59, 743–752.

Halaj, J., Cady, A. & Uetz, G.W. (2000). Modular habitat refugia enhance generalist predators and lower plant damage in soybeans. *Environ. Entomol.*, 29, 383–393.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T. *et al.* (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396, 69–72.

Kunkel, B.A. & Grewal, P.S. (2003). Endophyte infection in perennial ryegrass reduces the susceptibility of black cutworm to an entomopathogenic nematode. *Entomol. Exp. Appl.*, 107, 95–104.

Kunkel, B.A., Grewal, P.S. & Quigley, M.F. (2004). A mechanism of acquired resistance against an entomopathogenic nematode by *Agrotis ipsilon* feeding on perennial ryegrass harboring a fungal endophyte. *Biol. Control*, 29, 100–108.

Latch, G.C.M. (1993). Physiological interactions of endophytic fungi and their hosts: Biotic stress tolerance imparted to grasses by endophytes. *Agric. Ecosyst. Environ.*, 44, 143–156.

Lemons, A., Clay, K. & Rudgers, J.A. (2005). Connecting plant-microbial interactions above and belowground: a fungal endophyte affects decomposition. *Oecologia*, 145, 595–604.

Leuchtmann, A. (1992). Systematics, distribution, and host specificity of grass endophytes. *Nat. Toxins*, 1, 150–162.

Omacini, M., Chaneton, E.J., Ghersa, C.M. & Muller, C.B. (2001). Symbiotic fungal endophytes control insect host-parasite interaction webs. *Nature (Lond.)*, 409, 78–81.

Omacini, M., Chaneton, E.J., Ghersa, C.M. & Otero, P. (2004). Do foliar endophytes affect grass litter decomposition? A microcosm approach using *Lolium multiflorum*. *Oikos*, 104, 581–590.

Petrini, O. (1991). Fungal endophytes of tree leaves. In: *Microbial Ecology of Leaves* (eds Andrews, J.H. & Hirano, S.S.). Springer-Verlag, New York, pp. 179–197.

Raloff, J. (2003). Cultivating weeds: is your yard a menace to parks and wild lands? *Sci. News*, 163, 232.

Riechert, S.E. & Bishop, L. (1990). Prey control by an assemblage of generalist predators: spiders in garden test systems. *Ecology*, 71, 1441–1450.

Robinson, J.V. (1981). The effect of architectural variation in habitat on a spider community: an experimental field study. *Ecology*, 62, 73–80.

Rudgers, J.A. & Clay, K. (2005). Fungal endophytes in terrestrial communities and ecosystems. In: *The Fungal Community* (eds Dighton, E.J., Oudemans, P. & White, J.F.J.). M. Dekker, New York, pp. 423–442.

Rudgers, J.A., Koslow, J.M. & Clay, K. (2004). Endophytic fungi alter relationships between diversity and ecosystem properties. *Ecol. Lett.*, 7, 42–51.

Rudgers, J.A., Mattingly, W.B. & Koslow, J.M. (2005). Mutualistic fungus promotes plant invasion into diverse communities. *Oecologia*, 144, 463–471.

Saikkonen, K., Faeth, S.H., Helander, M. & Sullivan, T.J. (1998). Fungal endophytes: a continuum of interactions with host plants. *Annu. Rev. Ecol. Syst.*, 29, 319–343.

SAS Institute Inc. (2000). *SAS Version 8.1*. SAS Institute, Cary, NC, USA.

Southern Weed Science Society (1998). *Weeds of the United States and Canada, CD-ROM*. Southern Weed Science Society, Champaign, IL.

Stachowicz, J.J. (2001). Mutualism, facilitation, and the structure of ecological communities. *Bioscience*, 51, 235–246.

Stone, J.K., Bacon, C.W. & White, J.F. (2000). An overview of endophytic microbes: endophytism defined. In: *Microbial Endophytes* (eds Bacon, C.W. & White, J.F.). Marcel Dekker, Inc., New York, pp. 3–30.

Uetz, G.W. (1991). Habitat structure and spider foraging. In: *Habitat Structure: the Physical Arrangement of Objects in Space* (eds McCoy, E.D., Bell, S.A. & Mushinsky, H.R.). Chapman and Hall, London, UK, pp. 325–348.

Welden, C.W. & Slauson, W.L. (1986). The intensity of competition vs. its importance: an overlooked distinction and some implications. *Q. Rev. Biol.*, 61, 23–44.

Xu, W.W., Sleper, D.A. & Krause, G.F. (1994). Genetic diversity of tall fescue germplasm based on Rflps. *Crop Sci.*, 34, 246–252.

SUPPLEMENTARY MATERIAL

The following supplementary material is available online for this article from http://www.Blackwell-Synergy.com:

Appendix A List of spider morphospecies detected in non-destructive searches of tall fescue grass (*L. arundinaceum*) plots in an old field community in Bloomington, Indiana.