

Environmental conditions and host plant origin override endophyte effects on invertebrate communities

Salla-Riikka Vesterlund · Marjo Helander ·
Stanley H. Faeth · Terho Hyvönen · Kari Saikkonen

Received: 15 December 2010 / Accepted: 5 January 2011 / Published online: 29 January 2011
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Abstract Systemic fungal endophytes of grasses can produce high concentrations of alkaloids that are known to deter invertebrate herbivores and reduce their abundance, especially in agronomic grasses. Grass endophytes may also influence invertebrate community diversity and composition. Here, we examined in a common garden experiment with wild tall fescue plants and the agronomic cultivar Kentucky 31, whether infection by *Neotyphodium coenophialum*, the genetic background (origin) of the host plant, abiotic factors, and their interactions affected the invertebrate communities living on tall fescue. We collected a total of 18650 invertebrates from the 480 tall fescue plants, identified them to 97 morphological taxa (mainly to family level) representing five feeding guilds (herbivores, detritivores, omnivores, predators and parasitoids). In contrast to past literature, endophyte infection did not affect abundances of any taxon or feeding guild, or taxonomic diversity and the composition of the invertebrate community. Instead, the invertebrate community of native tall fescue appears to be primarily driven by environmental conditions and niche differentiation among taxonomical groups of invertebrates. We propose that community approaches are required to understand of the role of endophytes on arthropod abundances and diversity in nature.

Keywords Endophytic fungi · Tall fescue · Invertebrate diversity and community structure · Functional guild · Herbivory · Genetic variation

Introduction

Asexual *Neotyphodium* endophytes (family Clavicipitaceae) form symbiotic relationships with many cool-season grasses belonging to the sub-family Pooidae (Clay 1988, 1990). Infections are systemic and the endophyte is transmitted vertically to the next generation through seeds (Schardl et al. 2004; Clay and Schardl 2002). Tall fescue (*Schedonorus phoenix* (Scop. Holub.) [= *Lolium arundinaceum* (Schreb.) Darbysh. = *Schedonorus arundinaceus* (Schreb.) Dumort]) has been widely used as forage and turf grass in the United States for decades (Ball et al. 1993). Thus, one of the most studied grass–endophyte associations is the *N. coenophialum* and tall fescue symbiosis (Saikkonen et al. 2006, 2010). Tall fescue cultivars are dominated by a widely-adapted cultivar named “Kentucky 31” (hereafter referred to as K-31), which has a long growing season and is resistant to pests, drought, poor soil conditions, and variations in soil pH (Ball et al. 1993). Based on the research of this grass–endophyte system, the relationship between the endophytic fungus and its host has generally been thought to be mutualistic (Clay 1988; Clay et al. 1993; Saikkonen et al. 2006; Schardl and Phillips 1997). Recent studies have shown, however, that this relationship can vary from mutualism to antagonism, depending on the genotype of the fungus and the host as well as environmental conditions, especially in native grasses (Cheplick et al. 1989; Cheplick and Faeth 2009; Faeth 2002; Faeth and Saikkonen 2007; Faeth and Sullivan 2003). Saikkonen et al. (1998, 2004, 2006) therefore proposed that the

S.-R. Vesterlund (✉) · M. Helander
Section of Ecology, Department of Biology, University of Turku,
Turku FI-20014, Finland
e-mail: salla.vesterlund@utu.fi

S. H. Faeth
Department of Biology,
University of North Carolina at Greensboro,
Greensboro, NC 27402, USA

T. Hyvönen · K. Saikkonen
MTT Agrifood Research Finland, Plant Protection,
Jokioinen FI-31600 Finland

prevailing concept of endophytes as mutualists is likely historical and system based rather than based on evidence from natural populations.

In the case of the tall fescue–*N. coenophialum* symbiosis, much of the research has been done in the United States on agronomic cultivars such as K-31 (Saikkonen et al. 2006), although the origins of this grass are in Eurasia. In these agronomic cultivars planted outside their native distributional range, *Neotyphodium* is widely known to cause detrimental effects (e.g., toxicosis) on vertebrate grazers in high-nutrient agronomic environments (Ball et al. 1993; Clay 1989, 1990; Saikkonen et al. 2006, 2010; Schardl and Phillips 1997). These effects are related to high concentrations of alkaloids (Clay 1990; Lyons et al. 1986), which are known to deter both vertebrate and invertebrate herbivores (Bacon 1995; Bacon et al. 1977; Bazely et al. 1997; Siegel and Bush 1996, 1997; Vicari et al. 2002). Because alkaloids are nutrient-rich compounds, their synthesis has cost to other basic plant growth and reproductive functions (Faeth 2002; Faeth and Bultman 2002; Faeth and Fagan 2002). These costs may outweigh the benefits of the endophyte infection in most environments, but particularly so in nutrient-poor environments in nature (Ahlholm et al. 2002; Faeth 2002; Lehtonen et al. 2005). Thus, in its native habitat, infected wild tall fescue may produce lower levels and fewer types of alkaloids than its cultivated and selective-bred varieties in nutrient-rich environments in the introduced range (Saikkonen et al. 1998, 2010; Siegel and Bush 1996; but see Piano et al. 2005).

Recent evidence supports this idea: (1) the levels and composition of alkaloids produced varies among fungal species and genotypes (e.g., Piano et al. 2005; Siegel and Bush 1997), also (2), alkaloid types and levels in natural populations vary more than those in agronomic grasses (Bony et al. 2001; Faeth and Saikkonen 2007), (3) the number of non-toxic endophyte-infected grasses exceed toxic ones (Faeth 2002), and (4) in some cases, infection decreased, rather than increased, the herbivore resistance of the host plant (Faeth and Shochat 2010; Jani et al. 2010; Saikkonen et al. 1998; Schulthess and Faeth 1998).

Although well-studied in agronomic cultivars such as K-31 in introduced areas, the interactions between tall fescue and *Neotyphodium* endophytes are still largely ignored in their native range in Europe (Saari et al. 2010; Zabalgoeazcoa and Bony 2005), probably because tall fescue is not a preferred livestock forage grass (Niemeläinen et al. 2001) and livestock toxicosis is rare (Zabalgoeazcoa and Bony 2005). The nature and ecological importance of the tall fescue–*N. coenophialum* symbiosis may be different in its native range (Saikkonen 2000; Saikkonen et al. 1998; Siegel and Bush 1996).

We examined whether the *N. coenophialum* endophyte infection and the origin of the host plant as well as abiotic

factors and their possible interactions affect the invertebrate community living on tall fescue. Besides herbivores, fungal endophytes may also affect detritivores (e.g., Lemons et al. 2005) and the natural enemies of herbivores (Faeth and Shochat 2010; Hartley and Gange 2009; Jani et al. 2010; Omacini et al. 2001) or render herbivores more or less susceptible to natural enemies by affecting their attack rates (Benrey and Denno 1997; Saari et al. 2010) and delaying herbivore development (e.g. Breen 1994; Clay et al. 1985; Popay and Rowan 1994). However, there are only a few studies that have considered the impact of grass endophytes on arthropod communities or functional groups (e.g., Afkhami and Rudgers 2009; Faeth and Shochat 2010; Jani et al. 2010). In this study, we used a whole-invertebrate community survey of a controlled common garden experiment to test how invertebrate diversity and community structure, and the number of individuals in functional invertebrate taxa and guilds differs between (i) endophyte infected (E+), endophyte free (E-), and manipulatively endophyte-free (ME-) tall fescue, (ii) host plants of different origin (wild populations from Åland, Gotland, coastal Sweden and one agronomical cultivar, K-31 from USA), and (iii) host plants growing in different abiotic environments (nutrient and water treatments). Based on the past studies on defensive endophyte-grass mutualism (Saikkonen et al. 2010) and the few recent studies on how endophytes structure arthropod communities in tall fescue (Rudgers and Clay 2008), we predicted that: (1) endophyte infection decreases invertebrate diversity and community structure, (2) the abundances of plant feeding invertebrates (herbivores, omnivores and detritivores) are highly variable, but in general, lower in endophyte infected (E+) host plants compared with endophyte-free plants, particularly in the fertilized plants, (3) the agricultural cultivar (K-31) shows strong deterrence to plant feeding invertebrates, and (4) endophyte infection may both positively and negatively affect the natural enemies of herbivores (predators and parasitoids).

Materials and methods

Plant and seed material

To test the effect of infection, host plant origin, and environmental factors (water and nutrient treatments), in August 2005, we collected seeds from multiple natural tall fescue populations by the Baltic Sea in localities that were geographically separated from each other by approximately 500 km. These were the island of Åland (8 populations), the island of Gotland (9 populations), and the west coast of Sweden (6 populations). 10 to 50

individuals were collected from each population, and three seeds from each plant individual were stained for microscopic examination of the endophyte infection status (Saha et al. 1988). *Neotyphodium coenophialum* infectivity varied between 85–100% in all tall fescue populations from the three locations. Uninfected (E-) and infected (E+) seeds were combined separately from populations within each of the three study areas (Åland, Gotland, and coastal Sweden). In other words, we pooled all E- seeds and then all E+ from the populations within each location to create three batches of E- seeds and three batches of E+ seeds that represented the three geographic origins. In addition to plants from natural tall fescue populations, we used E+ and E- K-31 (from T. Phillips, University of Kentucky) cultivar seeds in our experiment. To test the role of the endophyte on invertebrate communities while controlling for plant genotypic background, we experimentally removed the endophyte from portion of E+ seeds (manipulatively endophyte-free plants = ME-). To kill the fungus while the seeds remained viable, the E+ seeds were heat-treated by keeping the seeds in warm water (56–57°C) for 10–20 min.

All tall fescue seeds from natural populations, K-31 cultivar and endophyte-removed seeds were germinated on moist tissue paper in Petri-dishes in a greenhouse and planted 7 days after germination to individual pots with sand and peat mixture.

Table 1 Invertebrate taxa collected from the experimental plants

Taxon	Number of individuals	Feeding guild
Diptera	1393	herbivorous
	704	detritivorous
	328	omnivorous
	25	predatory
	3	parasitic
Hymenoptera	46	herbivorous
	606	parasitic
Collembola	8360	detritivorous
	197	herbivorous
Hemiptera	51	predatory
	37	herbivorous
Homoptera	28	herbivorous
	379	predatory
Coleoptera	589	detritivorous
	281	predatory
Araneae (Arachnida)	281	predatory
Acari (Arachnida)	4017	omnivorous / parasitic
Thysanoptera	62	(guild not identified)

Table 2 The effects of endophyte status (E+ = endophyte infected, E- = endophyte-free, and manipulatively endophyte free = ME-), water and nutrient treatments (C = control, N = nutrient, W = water, and WN = water + nutrient), plant origin (A = Åland, G = Gotland, and S = coastal Sweden; K = cultivar “Kentucky 31”) and plant biomass on the abundances of dipterans, mites (Acari), Hymenopterans, collembolas and Coleopterans

Taxon	Feeding guild	Endophyte status (E)		Treatment (TRT)		Plant origin (PO)		E*TRT		E*PO		TRT*PO		Plant biomass	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p
Diptera	herbivorous	0.20	0.8202	2.34	0.0727	2.15	0.0931	2.30	0.0337	0.59	0.7402	2.57	0.0070	9.21	0.0026
	detritivorous	0.84	0.4317	11.62	<0.0001	3.04	0.0291	0.92	0.4807	1.06	0.3846	2.36	0.0133	5.47	0.0199
	omnivorous	1.04	0.3540	0.97	0.4091	1.29	0.2791	3.04	0.0063	0.90	0.4948	1.18	0.3058	1.59	0.2077
	parasitic	0.06	0.9398	0.97	0.4072	1.63	0.1820	1.40	0.2122	0.99	0.4289	0.77	0.6458	5.75	0.0169
	predatory	1.52	0.2190	2.57	0.0537	1.07	0.3628	1.30	0.2541	0.45	0.8420	0.68	0.7289	0.31	0.5761
Acari	omnivorous & parasitic	1.16	0.3141	3.76	0.0110	0.07	0.9743	0.41	0.8735	1.69	0.1220	0.61	0.7885	4.66	0.0315
	parasitic	2.13	0.1204	0.68	0.5659	4.76	0.0028	0.51	0.7970	0.73	0.6279	1.48	0.1518	0.59	0.4446
Araneae	predatory	0.47	0.6260	1.95	0.1213	1.16	0.3255	0.64	0.6975	1.05	0.3911	0.93	0.5025	4.13	0.0429
	detritivorous	0.97	0.3785	11.91	<0.0001	3.14	0.0253	2.68	0.0146	0.29	0.9404	0.75	0.6660	10.39	0.0014
Collembola	detritivorous	0.16	0.8514	23.63	<0.0001	3.10	0.0268	1.95	0.0716	0.31	0.9322	2.51	0.0084	0.07	0.7964
	predatory	2.67	0.0708	18.81	<0.0001	1.28	0.2792	0.68	0.6669	1.60	0.1455	1.77	0.0730	2.85	0.0923

Field experiment

To test the role of endophyte infection, plant geographic origin and environmental factors, a common garden field experiment was established at Botanical Garden, University of Turku, Finland in 2004. The study site is at the edge of the northern distribution range of natural tall fescue populations and has been in cultivation in the past. It was tilled in the summer 2004 without nutrient application. The experimental area was fenced to prevent large vertebrates (e.g., rabbits, deer) from browsing the plants. However, smaller vertebrates (e.g., voles) and invertebrates were allowed to freely access the area. The space between experimental plants was either mowed, hand weeded or sprayed with herbicide two times during the growing season to prevent interspecific competition in the field.

The experimental design was a randomized block consisting of 10 blocks, each divided to 4 plots. The four plots in each block were randomly designated to one of the four treatments: (i) control (C) receiving only ambient water and nutrients, (ii) water treatment (W) with 3 litres of water applied to each plant separately three times a week from June to August, (iii) nutrient treatment (N) where 1dl of N-P-K-fertilizer (Nurmen Y2, Kemira KnowHow,[N-P-K/20-6-6])/plant was applied two times during the growing season, and (iv) water–nutrient treatment (WN) combining both water and nutrient applications. The treatments were applied during the period of 2005–2006.

Tall fescue plants with 2–3 tillers were planted in August 2004 about 0.5 meters apart from each other and from the edge of the plot. Forty plants from each origin (natural populations A = Åland, G = Gotland, and S = coastal Sweden; cultivars K = “Kentucky 31”) and infection status (E+, E-, ME-) were randomly chosen. Thus, there were 12 plants in each of the 40 plots for a total of 480 plants used in the present study. The infection status of all individual

plants was confirmed in 2006 via seed staining (Saha et al. 1988). The biomass of the above-ground plant parts was removed, dried and weighed in autumn at the end of the growing season 2006.

Collection and identification of invertebrates

Invertebrates were collected from each plant individual with an Insect Vortis Vacuum® sampler (Burkard Ltd., UK) in July 2006. Every plant was vacuumed in the same way for 10 s from the middle of the plant. The samples were placed into reclosable plastic bags and frozen immediately after sampling. Invertebrates were then later counted, identified to family level under a microscope, and assigned to the following five feeding guilds based on the key family and species characteristics in literature: herbivores, omnivores, detritivores, predators and parasitoids (Table 1).

Statistical analyses

We used ANCOVA (with plant biomass as a covariate) in the Mixed model procedure of SAS statistical software (SAS Utilities 9.1) to analyze the effects of endophyte status (E+, E-, and ME-), water and nutrient treatments (W, N, WN, and C), plant origin (A, G, K, S), and block (1–10) on the abundances of invertebrates in the feeding guilds (herbivores, omnivores, detritivores, predators and parasitoids) and taxonomical groups with sufficient sample sizes for meaningful statistical analyses (herbivorous, detritivorous, omnivorous, parasitic, and predatory dipterans; Acari; Hymenoptera; spiders; collembolas, and detritivorous and predatory Coleoptera). Treatment, origin, endophyte, and their possible interactions were considered to be fixed factors in all models, whereas block was a random factor. Plant biomass was used as a covariate, because plant size may influence invertebrate abundan-

Table 3 The effects of endophyte status (E+ = endophyte infected, E- = endophyte-free, and manipulatively endophyte-free = ME-), water and nutrient treatments (C = control, N = nutrient, W = water, and

WN = water + nutrient), plant origin (A = Åland, G = Gotland, and S = coastal Sweden; K = cultivar “Kentucky 31”) and plant biomass on abundances of herbivores, detritivores and predators

	df	Herbivores		Detritivores		Omnivores		Parasitoids		Predators	
		F	p	F	p	F	p	F	p	F	p
Endophyte status (E)	2	0.35	0.7036	0.80	0.4484	0.29	0.8330	2.14	0.1192	2.31	0.1007
Treatment (TRT)	3	3.10	0.0268	15.05	<0.0001	0.71	0.5471	0.63	0.5987	15.38	<0.0001
Plant origin (PO)	3	1.61	0.1870	3.99	0.0080	0.52	0.5932	4.59	0.0036	1.04	0.3730
E * TRT	6	2.62	0.0169	2.63	0.0165	0.50	0.8089	0.55	0.7674	0.68	0.6681
E * PO	6	0.74	0.6199	0.26	0.9565	0.87	0.5156	0.75	0.6119	1.04	0.3987
TRT * PO	9	1.94	0.0449	0.72	0.6885	0.44	0.9142	1.46	0.1591	1.45	0.1662
Plant biomass	1	9.67	0.0020	10.28	0.0015	0.04	0.8338	0.78	0.3781	3.22	0.0734

Table 4 Means and standard errors (SE) of taxonomic groups of invertebrates showing statistically significant (a) interactive effects of water and nutrient treatments (C = control, N = nutrient, W = water, and WN = water + nutrient) and endophyte status (E+ = endophyte

infected, E- = endophyte-free, and manipulatively endophyte-free = ME-), (b) effects of plant origin (A = Åland, G = Gotland, and S = coastal Sweden; K = cultivar “Kentucky 31”) and (c) interactive effects of water and endophyte status (see Table 2)

		Taxon									
a	Treatment	Endophyte status	n	Herbivorous Diptera		Omnivorous Diptera		Collembola			
				mean	SE	mean	SE	mean	SE		
	C	E+	39	2.7	2.7	1.2	0.37	9.4	1.76		
		E-	39	3.4	3.4	0.5	0.14	10.2	2.03		
		ME-	40	3.7	3.7	0.6	0.12	11.7	2.54		
	W	E+	39	3.2	3.2	0.7	0.15	20.7	3.27		
		E-	40	2.6	2.6	0.6	0.13	14.3	2.31		
		ME-	39	2.1	2.1	0.8	0.25	11.4	1.81		
	N	E+	32	2.4	2.4	0.6	0.14	21.8	3.36		
		E-	37	2.4	2.4	0.5	0.13	28.7	5.10		
		ME-	34	3.6	3.6	0.6	0.13	25.9	3.66		
	WN	E+	38	3.9	3.9	0.7	0.18	33.7	6.22		
		E-	34	4.6	4.6	1.6	0.36	18.8	3.87		
ME-		34	3.3	3.3	0.5	0.14	22.0	3.80			
b	Plant origin	n	Detritivorous Diptera		Hymenoptera		Collembola		Coleoptera		
			mean	SE	mean	SE	mean	SE	mean	SE	
	Åland	118	1.9	0.24	1.8	0.17	24.3	2.34	1.9	0.33	
	Gotland	113	1.65	0.19	1.2	0.17	17.7	2.06	1.2	0.26	
	K-31	99	1.1	0.14	0.96	0.12	13.9	1.78	0.8	0.14	
	Sweden	115	1.6	0.17	1.4	0.13	18.4	2.10	1.3	0.18	
c	Treatment	Plant origin	n	Herbivorous Diptera		Detritivorous Diptera		Coleoptera			
				mean	SE	mean	SE	mean	SE		
	C	Åland	30	2.8	0.47	1.0	0.25	0.3	0.10		
		Gotland	29	3.3	0.60	1.2	0.25	0.3	0.11		
		K-31	29	3.1	0.44	0.9	0.20	0.4	0.15		
		Sweden	30	3.6	0.32	1.0	0.26	0.4	0.12		
	W	Åland	28	2.9	0.53	1.8	0.39	0.5	0.15		
		Gotland	30	1.9	0.31	2.0	0.37	0.4	0.09		
		K-31	30	2.7	0.45	1.0	0.25	0.5	0.16		
		Sweden	30	3.1	0.64	1.6	0.35	0.7	0.22		
	N	Åland	30	2.9	0.47	1.1	0.22	2.2	0.58		
		Gotland	26	2.8	0.40	1.2	0.31	1.7	0.40		
		K-31	19	2.6	0.63	1.1	0.27	1.7	0.45		
		Sweden	28	2.8	0.44	1.3	0.27	1.7	0.33		
	WN	Åland	30	6.1	0.76	3.9	0.72	4.5	1.00		
Gotland		28	3.6	0.65	2.2	0.52	2.7	0.89			
K-31		21	2.2	0.71	1.4	0.38	1.0	0.33			
Sweden		27	3.3	0.71	2.6	0.37	2.4	0.53			

ces. Plant size was significantly increased by watering and fertilization ($df=3$, $F=17.07$, $p<0.0001$)(C: mean=395 g, SE=16.4; N: mean=414 g, SE=22.1; W: mean=422 g, SE=15.2; WN: mean=587 g, SE=24.2) except in the case of the K-31 cultivar. Results on plant growth and performance will be reported and discussed in more detail elsewhere.

The effects of endophyte status (E+, E-, and ME-), water and nutrient treatments (W, N, WN, and C), plant origin (A, G, K, S) and plant biomass on taxonomic invertebrate diversity were examined in two ways. First, we tested the effects of the explanatory factors and their interactions on species numbers and the Shannon diversity index by a mixed model analysis of covariance

(ANCOVA) with plant biomass as a covariate, using the Mixed procedure of SAS statistical software (SAS Utilities 9.1). The plant-specific Shannon index value (H') was calculated as follows: $H' = -\sum_i p_i \ln(p_i)$ where p_i is the proportion of individuals in the i the taxonomical groups in the experimental plants. Compared to species number or richness, the advantage of the Shannon index is that it incorporates the number of taxonomical groups and their evenness. Second, to examine the amount of variation (%) that endophyte status, water and nutrient treatments and plant origin explained in the invertebrate community composition, we used a partial Canonical Correspondence Analysis CCA (Borcard et al. 1992) with CANOCO 4 software (Ter Braak and Šmilauer 1998). Only the variation explained by statistically significant environmental variables was partitioned (Økland 1999). The default options of CANOCO (except $\log x + 1$ data transformation and downweighting of rare species) were used. The significance of the first CCA axis and the CCA model, as well as each environmental variable was evaluated by Monte Carlo permutation tests (500 permutations) in all analyses. Nutrient and water treatments along with plant biomass appeared to be significant ($p < 0.01$) in CCA.

Results and discussion

Recent literature indicates that fungal endophytes alter invertebrate communities in both agronomic and wild grass populations (Rudgers and Clay 2007; Benrey and Denno 1997; Faeth and Shochat 2010; Hartley and Gange 2009; Jani et al. 2010; Lemons et al. 2005; Omacini et al. 2001; Saari et al. 2010). However, the *Neotyphodium* endophyte infection failed to influence the abundances of the taxa or feeding guild, or overall taxonomic diversity and composition of the invertebrate community in our experimental

common garden study of wild tall fescue plants collected across the northern distribution range of the species and the well studied tall fescue K-31 cultivar. Instead, the invertebrate community of native tall fescue in this experiment appears to be primarily driven by environmental conditions interacting with plant geographic origin.

Invertebrate abundance and community composition

A total of 18650 invertebrates were collected and identified to family level from the experimental plants. Springtails (Collembola), mites (Acari), and flies and midges (Diptera) comprised 48%, 23% and 14% of the individual invertebrates, respectively (a total of 85%) (Table 1). The rest 15% of the invertebrates were Coleopterans (6%), Hymenopterans (4%), spiders (2%), and Hemipterans (2%). Only one percentage of species remained unidentified. 56% and 24% of the invertebrate community consisted of detritivores and parasitoids, respectively, because of the high number of detritivorous Collembola and Acari mites and parasitic Hymenopterans in our samples (Table 1). Only 10% of all invertebrates were herbivores, but this feeding guild was taxonomically the most diverse comprising of 42 identified taxa.

E+ plants did not differ from E- and ME- plants in the abundance of any taxonomic invertebrate group (Table 2) or feeding guild (Table 3). However, endophyte infection affected the abundance of herbivorous and omnivorous dipterans, and collembolas interactively with water and nutrient treatments. For example, the abundance of herbivorous dipterans was higher on watered and fertilized E- and E+ plants compared to the other treatment and infection combinations, whereas the abundance of omnivorous dipterans was highest on watered and fertilized E- plants, second highest on untreated E+ plants, and lowest on fertilized E- plants (Table 4a). In contrast to dipterans, detritivorous Collembola (springtails) were much more

Fig. 1 The effects of endophyte status (E+, E-, and ME-) and water and nutrient treatments (W, N, WN, and C) on the total number of herbivores (a) and detritivores (b)

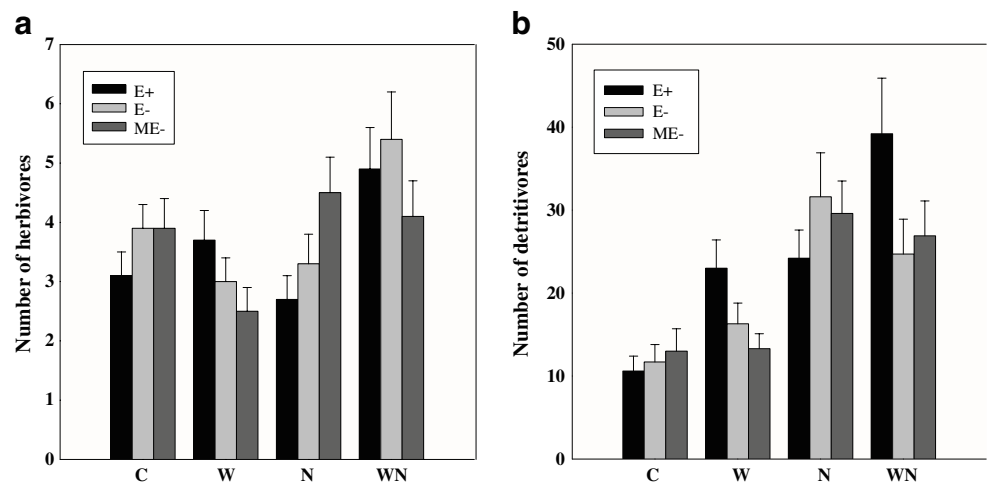


Table 5 The effects of endophyte status (E+ = endophyte infected, E- = endophyte-free, and manipulatively endophyte-free = ME-), water and nutrient treatments (C = control, N = nutrient, W = water, and WN = water + nutrient), plant origin (A = Åland, G = Gotland, and S = coastal Sweden; K = cultivar “Kentucky 31”) and plant biomass on identified taxon numbers and the Shannon diversity index

	df	Taxon number		Shannon diversity index	
		F	p	F	p
Endophyte status (E)	2	0.88	0.4148	0.37	0.6931
Treatment (TRT)	3	11.05	<0.0001	6.07	0.0005
Plant origin (PO)	3	1.52	0.2086	0.80	0.4923
E * TRT	6	1.95	0.0714	0.60	0.7268
E * PO	6	1.25	0.2815	1.29	0.2605
TRT * PO	9	1.12	0.3456	1.03	0.4159
Plant biomass	1	12.23	0.0005	4.38	0.0369

abundant and appeared to prefer watered and fertilized E+ plants (Table 4a; see also Faeth and Shochat 2010). Likewise, the total number of herbivores and detritivores did not show a common trend of preference or avoidance of E+ or E- plants in either low or high nutrient environments (Table 2, Fig. 1).

Plant origin significantly affected the abundances of detritivorous Diptera, Hymenoptera, Collembola and Coleoptera (Table 2), as their mean abundances was highest on plants collected from Åland and lowest on the cultivar “Kentucky 31” in all groups (Table 4b). In the cases of Coleoptera and both herbivorous and detritivorous Diptera, abundances varied among plant origins interactively with water and nutrient treatment (Table 2), but were highest on plants from Åland and lowest on the K-31 when the plant was watered and fertilized (Table 4c). This indicates differences in resistance among plant genotypes in different environments.

Plant size appears to be positively related to invertebrate abundance. Plant biomass explained significantly the numbers of herbivorous, detritivorous and parasitic dipterans, spiders (Araneae), and mites (Acari) (Table 2), and the abundances of these taxa were positively correlated with plant size except in the case of parasitic dipterans (herbivorous Diptera: $n=445$, $r=0.21$, $p<0.0001$; detritivorous: $n=445$, $r=0.26$, $p<0.0001$; parasitic Diptera: $n=445$, $r=0.06$, $p<0.2035$; Collembola: $n=445$, $r=0.24$, $p<0.0001$; Araneae: $n=445$, $r=0.13$, $p=0.0074$). Likewise, the total number of both

herbivores and detritivores positively correlated with plant biomass (Herbivores: $n=445$, $r=0.22$, $p<0.0001$; detritivores: $n=445$, $r=0.26$, $p<0.0001$).

Invertebrate richness

Invertebrate richness followed the same trends as taxonomic groups. Neither the number of taxa nor Shannon diversity index varied by the infection status of the plant (Table 5). Instead, invertebrate richness was positively correlated with plant size (plant biomass—the number of taxa: $n=444$, $r=0.27$, $p<0.0001$; plant biomass—the Shannon diversity index: $n=444$, $r=0.15$, $p=0.0016$) and it was significantly higher on fertilized plants (Table 5, Fig. 2).

Invertebrate community structure

Canonical Correspondence Analysis (CCA) suggests that invertebrate community well mirrors abiotic environmental conditions and the size of the plant. Most of the variation in the taxonomical composition was highly dependent on nutrient (Axis 1 in Fig. 3a) and water (Axis 2 in Fig. 3a) availability in the soil. The sum of all canonical eigenvalues was 0.131. The first axis explained 3.2% of taxon variation and 57.6% of the variation of the taxon-environment relationship. In the Monte Carlo test, the significance for the first axis was $P=0.002$ ($F=14.2$) and for all axes $P=0.002$ ($F=2.8$). Treatment explained 73.3% of the variation,

Fig. 2 Mean (\pm SE) number of taxa (a) and the Shannon diversity index in water and nutrient treatments

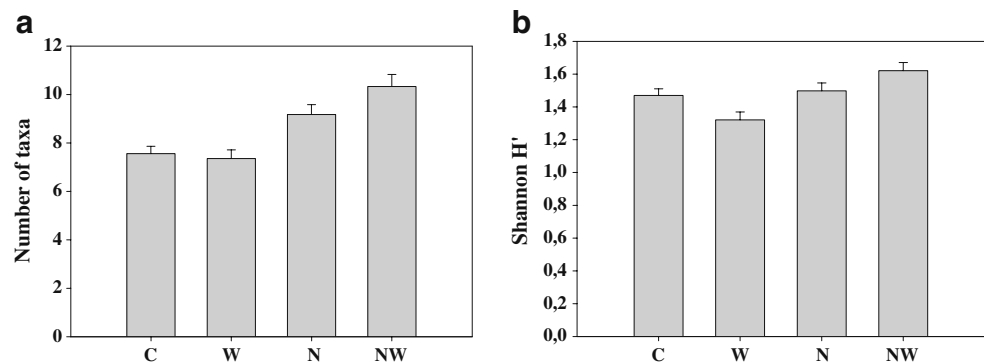
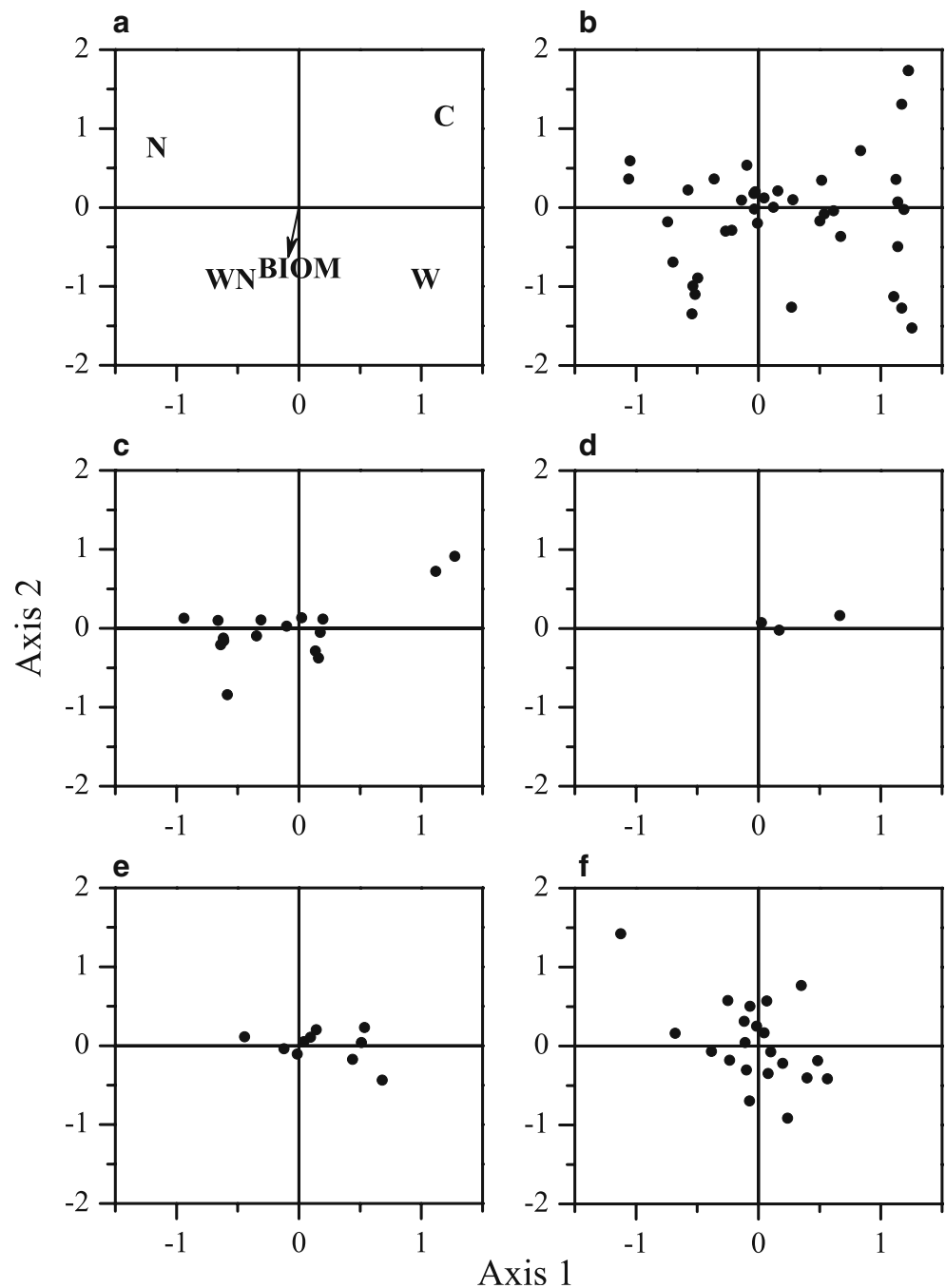


Fig. 3 Canonical Correspondence Analysis (CCA) of the relationship between taxonomical groups and examined biotic (endophyte status of the plant, plant origin and plant biomass) and abiotic (water and nutrient treatments) environmental factors. Significant environmental variables (a) (W = water, N = nitrogen, WN = water and nitrogen, C = control) and plant biomass (BIOM) are shown with five taxonomical invertebrate groups: herbivores (b), detritivores (c), omnivores (d), parasitoids (e) and predators (f). Eigenvalue for the first axis was 0.171 and for the second axis 0.056



whereas the proportion of the other factors remained smaller (plant origin 9.9%, endophyte status 7.6%, plant biomass 6.9%) and statistically insignificant (C: $F=7.0$, $P=0.002$; W: $F=5.5$, $P=0.002$; N: $F=8.1$, $P=0.002$; NW: $F=3.8$, $P=0.002$; Biomass of the plant: $F=1.986$, $P=0.002$; E+: $F=1.161$, $P=0.2196$; E-: $F=0.815$, $P=0.7884$; ME-: $F=0.955$, $P=0.5250$; A: $F=1.083$, $P=0.3593$; G: $F=0.902$, $P=0.6727$; S: $F=0.729$, $P=0.9022$; K: $F=0.884$, $P=0.6966$).

However, there was no common structure in the invertebrate community related to endophyte status, plant origin or water and nutrient treatments across the taxonomical groups or

feeding guilds (Fig. 3). In contrast, CCA indicates that each taxonomical group in a feeding guild appears to prefer different environments and the taxonomical diversity of a feeding guild is in relation to specialization in resource use according to the idea of niche partitioning (Elton 1927; Hutchinson 1961; Richards et al. 2000; Diehl 2003). For example, the herbivore feeding guild was taxonomically most diverse (42 taxa), but the place of herbivore taxa in the experimental water and nutrient environments were not identical (Fig. 3b) In other words, the species clearly do not occupy exactly the same host type.

Conclusions

Our results demonstrate that (1) the taxonomical diversity and complexity of an invertebrate community can be very high even in relatively simple plant communities, and (2) the diversity is commensurate with primary production and environmental factors that interact with plant origin rather than endophyte infections. Furthermore, invertebrate community, particularly the most diverse feeding guild, herbivores, showed strong differentiation along the examined water and nutrient gradients. This may drive the community structure of invertebrate herbivores in a patchy environment. The lack of increased or decreased herbivore resistance might be partly explained by the fact that alkaloids in native European tall fescue are not of the type or level that reduce (Afkhani and Rudgers 2009) or promote (Faeth and Shochat 2010; Jani et al. 2010) plant feeding invertebrates. However, such differences in alkaloid profiles and other plant characteristics due to differences among plant or endophyte genotypes fails to explain the lack of taxon, feeding guild and community level responses with the cultivar K-31.

We propose that empirical whole-community approaches are required to understand the importance of endophytes and other mechanisms driving plant populations and invertebrate communities feeding on them. Accumulating evidence from endophyte mediated interactions has revealed that endophytes can negatively affect plant feeding herbivores (Saikkonen et al. 2010). However, the accumulating evidence also indicates that diversity in results and interpretations of the general importance of endophytes in grassland communities increases as new model systems appear. Current literature appears to be strongly biased by two model species, tall fescue and perennial ryegrass and their few cultivars such as K-31, in introduced and agronomic environments, and this has distracted the literature (Saikkonen et al. 2006, 2010). By using wild tall fescues in their native continent, we were able to show that environmental conditions and host plant origin override endophyte effects on invertebrate diversity, community structure, and feeding guilds.

Acknowledgements This study was funded by the Academy of Finland (Project no. 110658).

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References

- Afkhani ME, Rudgers JA (2009) Endophyte-mediated resistance to herbivores depends on herbivore identity in the wild grass *Festuca subverticillata*. *Environ Entomol* 38:1086–1095
- Ahlholm JU, Helander M, Lehtimäki S, Wäli P, Saikkonen K (2002) Vertically transmitted fungal endophytes: different responses of host-parasite systems to environmental conditions. *Oikos* 99:173–183
- Bacon CW (1995) Toxic endophyte-infected tall fescue and range grasses: historic perspectives. *J Anim Sci* 73:861–870
- Bacon CW, Porter JK, Robbins JD, Luttrell ES (1977) *Epichloë typhina* from toxic tall fescue grasses. *App Environ Microb* 34:576–581
- Ball DM, Pedersen JF, Lacefield GD (1993) The tall-fescue endophyte. Evolution meets economics in the tale of the nation's most popular planted grass, which owes many of its qualities to a fungus toxic to livestock. *Am Sci* 81:370–379
- Bazely DR, Vicari M, Emmerich S, Filip L, Lin D, Inman A (1997) Interactions between endophyte-infected *Festuca rubra* from the Scottish islands of St Kilda, Benbecula and Rum. *J Appl Ecol* 34:847–860
- Benrey B, Denno RF (1997) The slow-growth-high-mortality hypothesis: a test using the cabbage butterfly. *Ecology* 78:987–999
- Bony S, Pichon N, Ravel C, Durix A, Balfourier C, Guillaumin J-J (2001) The relationship between myotoxin synthesis in fungal endophytes of *Lolium perenne*. *New Phytol* 152:125–137
- Borcard D, Legendre P, Drapeau P (1992) Partialling out the spatial component of ecological variation. *Ecology* 73:1045–1055
- Breen JP (1994) *Acremonium* endophyte interactions with enhanced plant resistance to insects. *Annu Rev Entomol* 39:401–423
- Cheplick GP, Faeth SH (2009) Ecology and evolution of the grass-endophyte symbiosis. Oxford University Press
- Cheplick GP, Clay K, Marks S (1989) Interactions between infection by endophytic fungi and nutrient limitation in the grasses *Lolium perenne* and *Festuca arundinaceae*. *New Phytol* 111:89–97
- Clay K (1988) Fungal endophytes of grasses: a defensive mutualism between plants and fungi. *Ecology* 69:10–16
- Clay K (1989) Clavicipitaceous endophytes of grasses: their potential as biocontrol agents. *Mycol Res* 92:1–12
- Clay K (1990) Fungal endophytes of grasses. *Annu Rev Ecol Syst* 21:275–297
- Clay K, Schardl CL (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *Am Nat* 160:99–127
- Clay K, Hardy TN, Hammond AM Jr (1985) Fungal endophytes of grasses and their effects on an insect herbivore. *Oecologia* 66:1–6
- Clay K, Marks S, Cheplick GP (1993) Effects of insect herbivory and fungal endophyte infection on competitive interactions among grasses. *Ecology* 74:1767–1777
- Diehl S (2003) The evolution and maintenance of omnivory: dynamic constraints and the role of food quality. *Ecology* 84:2557–2567
- Elton CS (1927) Animal ecology. Sidgwick and Jackson, London
- Faeth SH (2002) Are endophytic fungi defensive plant mutualists? *Oikos* 99:200–200
- Faeth SH, Bultman TL (2002) Endophytic fungi and interactions among host plants, herbivores and natural enemies. In: Tschamtker T, Hawkins BA (eds) Multitrophic level interactions. Cambridge University Press, pp 89–123
- Faeth SH, Fagan WF (2002) Fungal endophytes: common host plant symbionts but uncommon mutualists. *Integr Comp Biol* 42:360–368
- Faeth SH, Saikkonen K (2007) Variability is the nature of the endophyte-grass interaction. Proceedings of the 6th International Symposium on Fungal Endophytes of Grasses “From Lab to Farm”. Christchurch, New Zealand March 25–28, 2007, Popay AJ, Thom ER (eds) Grassland Research and Practice Series No. 13. New Zealand Grassland Association, Dunedin, NZ, ISSN 0110-8581
- Faeth SH, Shochat E (2010) Inherited microbial symbionts increase herbivore abundances and alter arthropod diversity on a native grass. *Ecology* (in press)

- Faeth SH, Sullivan TJ (2003) Mutualistic asexual endophytes in a native grass are usually parasitic. *Am Nat* 16:310–325
- Hartley SE, Gange AC (2009) Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. *Annu Rev Entom* 54:323–342
- Hutchinson GE (1961) The paradox of the plankton. *Am Nat* 95:137–145
- Jani A, Faeth SH, Gardner DR (2010) Asexual endophytes and associated alkaloids alter community structure and increase herbivore abundances on a native grass. *Ecol Lett* 13:106–117
- Lehtonen P, Helander M, Saikkonen K (2005) Are endophyte-mediated effects on herbivores conditional on soil nutrients? *Oecologia* 142:38–45
- Lemons A, Clay K, Rudgers JA (2005) Connecting plant–microbial interactions above and belowground: a fungal endophyte affects decomposition. *Oecologia* 145:595–604
- Lyons PC, Plattner RD, Bacon CW (1986) Occurrence of peptide and clavine ergot alkaloids in tall fescue grass. *Science* 232:487–489
- Niemeläinen O, Jauhiainen L, Miettinen E (2001) Yield profile of tall fescue (*Festuca arundinacea*) in comparison with meadow fescue (*F. pratensis*) in Finland. *Grass Forage Sci* 56:249–258
- Økland RH (1999) On the variation explained by ordination and constrained ordination axes. *J Veg Sci* 10:131–136
- Omacini M, Chaneton EJ, Ghersa CM, Müller CB (2001) Symbiotic fungal endophytes control insect host–parasite interaction webs. *Nature* 409:78–81
- Piano E, Bertoli FB, Romani M, Tava A, Riccioni L, Valvassori M, Carroni AM, Pecetti L (2005) Specificity of host-endophyte association in tall fescue populations from Sardinia, Italy. *Crop Sci* 45:1456–1463
- Popay AJ, Rowan DD (1994) Endophytic fungi as mediators of plant–insect interactions. In: Bernays EA (ed) *Insect–plant interactions*. CRC, Boca Raton, pp 83–103
- Richards SA, Nisbet RM, Wilson WG, Possingham HP (2000) Grazers and diggers: exploitation competition and coexistence among foragers with different feeding strategies on a single resource. *Am Nat* 155:266–279
- Rudgers JA, Clay K (2007) Endophyte symbiosis with tall fescue: how strong are the impacts on communities and ecosystems? *Fung Biol Rev* 21:107–124
- Rudgers JA, Clay K (2008) An invasive plant–fungal mutualism reduces arthropod diversity. *Ecol Lett* 11:831–840
- Saari S, Helander M, Faeth S, Saikkonen K (2010) The effects of endophytes on seed production and seed predation of tall fescue and meadow fescue. *Microbial Ecol* 60:928–934
- Saha DC, Jackson MA, Johnson-Cicalese JM (1988) A rapid staining method for detection of endophytic fungi in turf and forage grasses. *Phytopathol* 78:237–239
- Saikkonen K (2000) Kentucky 31, far from home. *Science* 287:1887a
- Saikkonen K, Faeth SH, Helander M, Sullivan TJ (1998) Fungal endophytes: a continuum of interactions with host plants. *Annu Rev Ecol Syst* 29:319–343
- Saikkonen K, Helander M, Faeth SH (2004) Fungal endophytes: Hitchhikers of the green world. In: Gillings M, Holmes A (eds) *Plant microbiology*. BIOS Scientific Publishers Limited, Oxford, pp 79–97
- Saikkonen K, Lehtonen P, Helander M, Koricheva J, Faeth S (2006) Model systems in ecology: dissecting the endophyte–grass literature. *Trends Plant Sci* 9:1360–1386
- Saikkonen K, Saari S, Helander M (2010) Defensive mutualism between plants and endophytic fungi? *Fung Div*. doi:10.1007/s13225-010-0023-7
- Schardl CL, Phillips TD (1997) Protective grass endophytes. Where are they from and where are they going? *Plant Dis* 81:430–438
- Schardl CL, Leuchtman A, Spiering MJ (2004) Symbioses of grasses with seedborne fungal endophytes. *Annu Rev Plant Biol* 55:315–340
- Schulthess FM, Faeth SH (1998) Distribution, abundance, and associations of the endophytic fungal community of Arizona fescue (*Festuca arizonica*). *Mycologia* 90:569–578
- Siegel MR, Bush LP (1996) Defensive chemicals in grass–fungal endophyte associations. *Recent Adv Phytochem* 30:81–118
- Siegel MR, Bush LP (1997) Toxin production in grass/endophyte associations. In: Carroll GC, Tudzynski P (eds) *The mycota V. Part A. Plant relationships*. Springer-Verlag, Berlin, Heidelberg, pp 185–207
- Ter Braak CJF, Šmilauer P (1998) *CANOCO reference manual and user's guide to Canoco for windows: Software for canonical community ordination (Ver 4)*. Microcomputer Power, Ithaca
- Vicari M, Hatcher PE, Ayres PG (2002) Combined effect of foliar and mycorrhizal endophytes on an insect herbivore. *Ecology* 83:2452–2464
- Zabalgoageazcoa I, Bony S (2005) *Neotyphodium* research and application in Europe. In: Roberts CA, West CP, Spiders DE (eds) *Neotyphodium* in cool-season grasses. Blackwell Publishing, Ames, pp 23–33