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Host genotype overrides endophyte infection effects on growth, physiology, and nutrient content of a native grass, *Achnatherum sibiricum*

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Abstract The effect of infection by the fungal endophyte *Neotyphodium*, host genotype, and their interaction on growth and physiology, as well as photosynthesis, was investigated in the native grass *Achnatherum sibiricum*. We artificially inoculated the endophyte into mature tillers of endophyte-free *A. sibiricum*. Plants were clipped to 5 cm height after recording growth traits, and analyzed for total nonstructural carbohydrates (TNC %), the percentage of nitrogen (N %), and carbon (C %) in leaves before and after clipping. In our study, the prominent host genotype–endophyte infection interactions detected in *A. sibiricum* indicates that, for many growth and storage traits, endophyte infection can impact a little change. However, there is no overriding consistently positive effect of the endophyte on growth or storage in *A. sibiricum* before or after clipping. Our study showed that the interaction between endophyte and host grasses was highly contingent on plant genotypes. We found host genotype overrode fungal endophyte infection in influencing tiller number and photosynthetic properties of *A. sibiricum* before clipping. After clipping, host genotype accounted for more of the variation in regrowth and above-ground biomass of *A. sibiricum* than endophyte infection. Our study showed that host genotype affected the total nonstructural carbohydrates of *A. sibiricum* before and after clipping, whereas endophyte infection increased the carbon content after clipping. Genotype by infection interactions for plant height, leaf mass, total nonstructural carbohydrates, and photosynthetic characteristics indicated genotype-specific effects of endophytes on *A. sibiricum* physiology and photosynthetic capacity. The host genotype–endophyte infection interactions detected in *A. sibiricum* suggest that host genotype overrides fungal endophyte infection on growth, physiology, and nutrient content of this native grass. In contrast, endophyte effects did not appear to positively affect growth, physiology, or photosynthetic capacity before or after clipping.

Keywords Endophyte · *Neotyphodium* · Vertically-transmitted · Growth · Photosynthesis · *Achnatherum sibiricum*

Introduction

Fungal endophytes have been defined as fungi that live for a significant part of their life cycle internally and asymptomatically (without causing any apparent tissue damage) in plants (Saikkonen et al. 1998).

Numerous studies have established that host grasses infected with systemic endophytes have advantages over non-infected ones. These benefits include enhanced drought resistance (Elmi and West 1995;

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Latch et al. 1985), increased resistance to pathogens (McGee et al. 1991; Yue et al. 2000), enhanced competitive abilities (Clay and Holah 1999; Lewis 2004), and increased resistance to herbivory by means of toxic alkaloids produced by the endophyte. Because of these fitness advantages, the grass–endophyte interaction has been described as a mutualism (Clay 1990; Clay and Schardl 2002). However, most of these investigations have focused on agronomic grass/endophyte systems, which are characterized by artificial or anthropogenic selection on the host grass genotypes (Amalric et al. 1999; Clay et al. 2005), and artificial selection may have biased results toward a more mutualistic interaction between grasses and their endophytes (Saikkonen et al. 1998).

Only a few researchers have investigated the relationships between endophytes and their wild host grass species in non-agricultural settings (Brem and Leuchtmann 2001; Craig et al. 2011; Kannadan and Rudgers 2008), where endophyte effects can range from positive to negative (Faeth et al. 2004; Morse et al. 2002). For example, Iannone et al. (2012) studied the associations between *Bromus auleticus* and its endophyte and they found that 3-month-old endophyte-infected plants enhanced growth relative to their endophyte-free counterparts in a greenhouse experiment. However, some studies have indicated that infected native grasses such as Arizona fescue are not more resistant to invertebrate (Lopez et al. 1995; Saikkonen et al. 1999; Tibbets and Faeth 1999) or vertebrate herbivores (Saikkonen et al. 1999). Infection also does not increase germination success (Faeth and Sullivan 2003; Neil et al. 2003), and infection generally decrease host growth and reproduction (Faeth and Sullivan 2003) or regrowth after fire in *Festuca arizonica* (Faeth et al. 2002b).

The effect of the endophyte on host plant performance depends not only on biotic and abiotic factors, but also host genotype (Cheplick 1997, 1998; Clay et al. 1993; Marks et al. 1991). For two important and well-studied forage grasses tall fescue (*Schedonorus arundinaceus*) and perennial ryegrass (*Lolium perenne* L.), physiological responses of the host to endophyte infection in nature depend on both herbivores and competitors (Clay et al. 1993). Moreover, genotypic variation in the response of host grasses to endophyte infection has been described in both *S. arundinacea* (Belesky and Fedders 1996; Elbersen and West 1996; Marks and Clay 1996; Rice et al. 1990) and *L. perenne* (Cheplick 1997, 1998; Cheplick et al. 2000). In another agronomic grass, Johnson-Cicalese et al. (2000) have found that the endophyte in *P. ampla* enhanced panicle and seed production in one host genotype, but has the opposite effect in different host genotypes of the same subspecies. Thus, endophyte infection may have host genotype-specific effects on growth and reproduction.

Endophyte infection may also affect regrowth. For example, Iannone and Cabral (2006) found that *B. auleticus*, infected with *N. pampeanum*, had a higher regrowth rate than endophyte-free plants in the greenhouse. Plant performance during regrowth was measured after clipping which induces the mobilization of carbohydrates, and the regrowth was rapid and vigorous in *L. perenne* (De Visser et al. 1997; Morvan et al. 1997).

The role of endophyte infection in the regrowth ability of host genotypes after clipping is still not well understood (Belesky and Fedders 1996; Cheplick 1998). Although grass responses to clipping are very complex (Ferraro and Oesterheld 2002), regrowth should be related to the ability of a clipped plant to recruit tillers and re-establish leaf area. Carbohydrate pools stored in tiller bases may also be important in grass regrowth (Cheplick and Chui 2001; Danckwerts 1993; Danckwerts and Gordon 1987; Donaghy and Fulkerson 1997, 1998; Volenec 1986). It has reported that regrowth of *L. perenne* after clipping depends on both endophyte infection and host genotype (Cheplick 1998).

Few studies have examined the effects of host genotype and its interaction with fungal infection on plant growth, regrowth, and physiology in native grasses. Here, we have measured growth, storage, and regrowth of host plants with strictly controlled host genotypes to address the following questions: (1) does endophyte influence growth, storage, and regrowth of the important native forage grass *Achnatherum sibiricum*? (2) Does the interaction between endophyte and host grasses depend on plant genotypes?

Materials and methods

Host plant

Achnatherum sibiricum (L.) Keng is a caespitose perennial grass that is widely distributed in the north of

Inner Mongolia steppe, China. High incidences of *Neotyphodium* endophyte infection (86–100 %) in *A. sibiricum* were recorded in seven native populations in our previous study (Wei et al. 2006). Other grasses in this genus, *Achnatherum inebrians* (Hance) Keng ex Tzvelev and *Achnatherum robustum* (Vasey) Barkworth, are notorious for the narcotic effects on livestock, and hence known as "drunken horse grasses" and "sleepy grass," respectively (Bruehl et al. 1994; Petroski et al. 1992). In contrast to *A. inebrians* and *A. robustum*, infected *A. sibiricum* had no obvious herbivore deterring properties according to local records and our own observations. When infected, the host is asymptomatic, but intercellular endophytic hyphae are easily identified via microscopic examination of host leaf sheaths (Hignight et al. 1993; Welty et al. 1986).

Experimental design

Seeds of *A. sibiricum* were collected from natural populations in Hailar in Northeast China (119.67°E, 49.10°N) in August 2008. The annual mean temperature here was around −2 °C, and the annual precipitation was about 367 mm. This meadow steppe belongs to a transitional type of habitat between forest and steppe. We defined seeds of *A. sibiricum* which were collected from different maternal plants had different genotypes. *A. sibiricum* is cross-pollination. To minimize the probability of sampling ramets belonging to the same genet, we sampled plants that were at least 5 m apart. We collected seeds from the sampled plants in August 2008 and stored them at 4 °C. To eliminate the endophyte, we heat treated a subset of randomly chosen seeds in a convection drying oven according to Kannadan and Rudgers (2008). We treated seeds for 30 days at 60 °C, and the temperature treatment had no significant effect on germination rate, germination potential, or germination index (Li et al. 2010).

Seeds of twenty genotypes treated were sown in plastic pots filled with vermiculite in April 2010. The endophyte was isolated and identified from *A. sibiricum*. We then artificially inoculated the endophyte into mature tillers of endophyte-free *A. sibiricum* in March and November 2011. In our study, plants were used for endophyte isolation following the method described by Wei et al. (2007). Isolates were grown on potato dextrose agar medium at a constant temperature 25 ± 1 °C in the dark, and were checked every 2 days. Typical white and cottony colonies of *Neotyphodium* emerged after about 3 weeks and were transferred to new plates immediately. Identification of the endophytic fungi was based on their morphology which had been reported by Zhang et al. (2009). After purification, we stored *Neotyphodium sibiricum* (Zhang et al. 2009) and *Neotyphodium gansuense* (Li et al. 2007) in −4 °C, and purified them again before inoculation. Furthermore, we also purified them in yeast extract-malt extract agar (YM) with shaking method. Each inoculation was performed with a fresh sterile hypodermic needle. Plants were punctured at less than 1 cm height to place the inoculum next to the base of plant, and then injected it with fungus suspension. Each ramet was treated with one fungal strain. After ensuring the endophyte was transferred, five infected genotypes of *A. sibiricum* were cloned manually into ramets after detecting endophyte on December 23, 2011, and marked as G1, G3, G9, G10, and G15, respectively. Individual E+ and E− ramets of *A. sibiricum* genotypes were grown in plastic pots (20 cm diameter, 21 cm deep). Between January and April 2012, 30 E+ and 10 E− ramets of each of the five genotypes were planted individually into 200 plastic pots filled with vermiculite. Plants were watered as needed.

All plants were kept on the plastic pots, and set these pots on the ground of experimental field in Nankai University (39.10°N, 117.16°E) in Tianjin, China. The mean annual precipitation and temperature were 550–680 mm and 12.3 °C, respectively, with most rainfall during summer and highest temperatures in July and early August. In addition, photosynthetically available radiation varied between 600 and 1,800 μmol m^{-2} s^{-1} (Chen et al. 2013).

Tillers number per plant was recorded on May 30, 2012 and the next day, all plants were clipped to 5 cm height. One-half the replicates were completely harvested for carbohydrate analysis. Tiller bases with attached roots were removed from the pots, and soil was rinsed away. After drying for 24 h at 60 °C, the dry mass of tiller bases (stubble) and roots were recorded. On sunny days, gas exchange measurements were taken on the youngest fully expanded attached leaf with a LI-COR 6400 infrared gas analyzer (LI-Cor, Lincoln, NE, USA) before clipping, and we also recorded those before final harvest. Under 400 μmol mol^{-1} CO_2, maximum net photosynthetic rate (P_{max}) was measured

at 1,000 μmol $m^{-2}s^{-1}$ PPFD (photosynthetic photon flux density).

The remainder of the replicates ($n = 100$) was allowed to regrow over the next 4 weeks. Regrowth tillers were counted, and P_{max} was measured on July2 , 2012. Regrowth leaf area was recorded and calculated with Photoshop software immediately. Harvested plants were dried for 24 h at 60 °C. The dry masses of roots, leaves, and tiller bases were recorded.

The 5 cm samples of tiller bases (stubble) that remained after clipping were analyzed for total nonstructural carbohydrates (TNC %) and the percentage of nitrogen (N %) and carbon (C %) in leaves. TNC % was determined using an enzymatic hydrolysis method with the modification of substituting Teles' reagent with dinitrosalicylic acid (Da Silveira et al. 1978). We used vario MACRO CHN analyzer to measure N % and C % in leaves.

Data analysis

Two-way ANOVA was employed to examine the effects of host genotype, endophyte, and their interaction on all response variables of all harvests. All data were analyzed with SPSS16.0.0 (IBM SPSS, Chicago, USA).

Results

Plant growth

Host genotype, but not endophyte presence, affected number of tillers and plant height recorded before clipping (Table 1). Plant growth responses varied among genotypes (Fig. 1). The plant height was greater for only one genotype when endophyte infected. However, the other three genotypes (G1, G9, and G10) showed reduced trend in plant height when infected (Fig. 1a). For three genotypes (G1, G3, and G15), endophyte presence enhanced tiller production (Fig. 1b). Plant height, leaf number, leaf mass, above-ground mass, and leaf area were affected by host genotype after clipping (Table 1). After clipping, the plant height was greater for some genotypes (e.g., G1, G10, and G15) when endophyte infected (Fig. 1c); endophyte-infected genotypes (e.g., G10 and G15) had a greater number of leaves and leaf area than endophyte free (Fig. 1d, e).

Photosynthesis and gas exchange parameters

Host genotype affected all photosynthesis variables, which included P_{max}, stomatal conductance (Gs), intercellular CO_2 concentration (Ci), transpiration rate (Tr), stomatal limitation (Ls), water use efficiency (WUE), and light use efficiency (LUE), recorded before clipping (Table 2). Genotype by infection interactions indicated genotype-specific effects of endophytes on *A. sibiricum* photosynthetic capacity (Table 2). Endophyte infection affected P_{max} and LUE before clipping. However, other photosynthesis variables did not differ between E+ and E− plants (Table 2).

Genotype and infection affected most photosynthesis variables of *A. sibiricum* after clipping, but Tr and WUE did not vary by infection (Table 3). The P_{max}, Gs, Ci, and LUE of E+ plants were higher than E− plants after clipping (Fig. 3).

Before clipping, P_{max} was greater for only one genotype when endophyte infected; however, four genotypes (G3, G9, G10, and G15) showed less P_{max} when infected, and the E− plants had significantly greater P_{max} (15.05 ± 0.33 μmol/(m^{-2} s^{-1})) compared with E− plants (14.14 ± 0.19 μmol/(m^{-2} s^{-1})) (Fig. 2). After clipping, for three genotypes (e.g., G1, G9, and G10), endophyte presence enhanced P_{max}, but other two genotypes did not show this effect (Fig. 2). The Gs and LUE were greater for three genotypes (G1, G9, and G10) when endophyte infected, but other two genotypes did not consistent with this effect at 4 weeks (Fig. 3). The genotypes G1, G9, and G10 had greater Ci when infected, but these three genotypes had lower Ls in E+ plants than that of in E− plants (Fig. 3).

Carbon, nitrogen, and total nonstructural carbohydrates

Significant genotype by infection interaction indicated genotype-specific effects of endophytes on *A. sibiricum* TNC % and nitrogen content (Table 1). C % and TNC % before clipping were affected by host genotype (Table 1). Most genotypes except G1 had greater C % when infected (Fig. 4a). For two genotypes (G1 and G9) showed greater N % when infected (Fig. 4b). Means (±SE) values of C % for E− and E+ plants were 42.71 ± 0.13 and 42.98 ± 0.08 %, respectively. The TNC % was greater for three genotypes (G1, G3, and G9) when endophyte infected, but other two

Table 1 Two-way anova of variables recorded before and after clipping of *A. sibiricum* ramets

Variable	Source	Before				After			
		df	MS	*F*	*P*	df	MS	*F*	*P*
Tiller number	Genotype	4	4.968	2.631	**0.036**	4	0.960	0.743	0.569
	Infection	1	0.360	0.190	0.663	1	1.753	1.357	0.252
	Genotype * Infection	4	1.787	0.947	0.438	4	1.627	1.259	0.304
	Error	167	1.888			36	1.292		
Plant height	Genotype	4	148.810	2.664	**0.034**	4	434.099	6.625	**<0.01**
	Infection	1	9.128	0.163	0.687	1	78.101	1.192	0.282
	Genotype * Infection	4	94.479	1.691	0.154	4	266.882	4.073	**<0.01**
	Error	167	55.859			36	65.529		
Leaf number	Genotype	4	18.267	0.764	0.550	4	36.436	3.096	**0.027**
	Infection	1	33.856	1.415	0.236	1	0.007	0.001	0.980
	Genotype * Infection	4	24.389	1.019	0.399	4	12.913	1.097	0.373
	Error	167	23.924			36	11.769		
Leaf mass	Genotype	4	0.029	0.702	0.593	4	0.132	4.701	**<0.01**
	Infection	1	0.006	0.142	0.708	1	0.040	1.438	0.238
	Genotype * Infection	4	0.074	1.817	0.135	4	0.074	2.638	**0.050**
	Error	73	0.041			36	0.028		
Above-ground mass	Genotype	4	0.059	0.877	0.482	4	0.260	3.126	**0.026**
	Infection	1	0.013	0.191	0.664	1	0.232	2.783	0.104
	Genotype * Infection	4	0.157	2.317	0.065	4	0.069	0.832	0.514
	Error	73	0.068			36	0.083		
Leaf area	Genotype	4	1.460	0.602	0.733	4	35.148	5.611	**<0.01**
	Infection	1	0.022	0.009	0.940	1	1.698	0.271	0.606
	Genotype * Infection	4	3.429	1.415	0.445	4	8.988	1.435	0.242
	Error	1	2.424			36	6.265		
N %	Genotype	4	0.043	1.867	0.131	4	0.527	6.189	**<0.01**
	Infection	1	0.000	0.008	0.930	1	1.698	19.923	**<0.01**
	Genotype * Infection	4	0.479	20.954	**<0.01**	4	0.113	1.321	0.275
	Error	50	0.023			50	0.085		
C %	Genotype	4	0.802	3.102	**0.023**	4	0.229	0.848	0.501
	Infection	1	0.872	3.373	0.072	1	2.495	9.225	**<0.01**
	Genotype * Infection	4	0.095	0.368	0.831	4	0.463	1.714	0.162
	Error	50	0.259			50	0.27		
C/N	Genotype	4	0.820	1.600	0.189	4	16.141	6.454	**<0.01**
	Infection	1	0.227	0.444	0.508	1	51.442	20.570	**<0.01**
	Genotype * Infection	4	9.889	19.304	**<0.01**	4	4.841	1.936	0.119
	Error	50	0.512			50	2.501		
TNC %	Genotype	4	3.826	4.315	**<0.01**	4	3.748	15.739	**<0.01**
	Infection	1	0.017	0.019	0.890	1	0.258	1.082	0.303
	Genotype * Infection	4	2.418	2.728	**0.039**	4	0.834	3.502	**0.013**
	Error	50	0.887			50	0.238		
Leaf area regrowth	Genotype					4	0.015	1.341	0.265
	Infection					1	0.008	0.711	0.402
	Genotype * Infection					4	0.005	0.479	0.751
	Error					61	0.011		

Significant *P* values are in bold

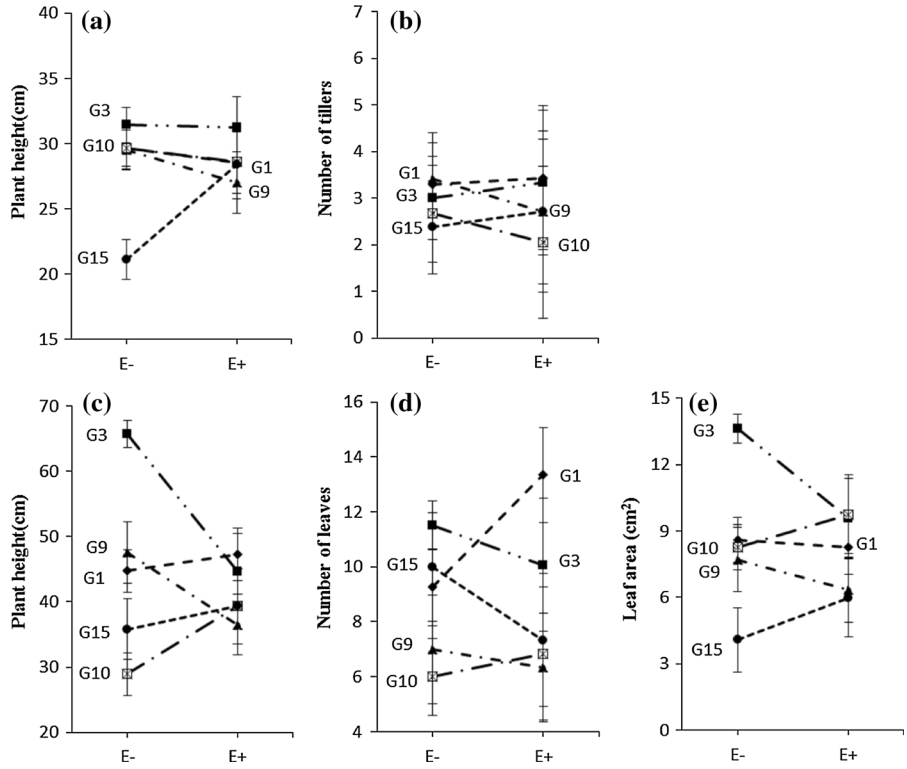

Fig. 1 Genotype–endophyte infection interaction plots for growth variables recorded before (**a** plant height, **b** number of tillers) and after clipping (**c** plant height, **d** number of leaves, **e** leaf area). Different *numbers* and *symbols* denote different genotypes, each connected by a *line* linking E− to E+. Mean ± SE are shown

genotypes did not consistent with this effect. Genotype G15 had the greatest amount of TNC % overall, but differences between E+ and E− replicates for individual genotypes were inconsistent because of a significant interaction of genotype and infection (Fig. 4c). For genotype G15, E− plants had more than 1.4 times as much TNC % as E+ plants, but for genotype G9, E+ plants had more than 1.3 times as much TNC % as E− plants. Differences between E+ and E− were not as extreme for other genotypes, and the overall effect of infection was not significant (Table 1). For four of the five genotypes, levels of TNC % in E+ replicates were at or above that of E− replicates (Fig. 4c). Mean (±SE) values of TNC % for E− and E+ replicates were 3.81 ± 0.24 and 3.86 ± 0.14 %, respectively.

After 4 weeks and clipping, TNC % was significantly affected by host genotype, and C % was increased by infection (Fig. 4d), while the carbon nitrogen ratio (C/N) and N % were affected by both host genotype and infection (Table 1). After clipping, the E+ plants had greater N % (2.93 ± 0.044 %) compared with E− plants (2.54 ± 0.075 %) (Fig. 4e). However, TNC % did not differ between E+ and E− plants (Fig. 4f). The TNC % was greater for two genotypes (G1 and G15) when endophyte infected, but other three genotypes did not consistent with this effect at 4 week. Genotype G9 had the greatest amount of TNC % overall (Fig. 4f).

Regrowth rate and biomass

Leaf area regrowth rates were not significantly affected by genotype and infection (Table 1; Fig. 5a). Also, there was no detectable genotype–infection interaction (Table 1). By the end of the experiment, the above-ground mass and leaf mass were affected by genotype (Table 1). Only one endophyte-infected genotypes G1 showed a higher above-ground mass than endophyte free, as well as G1 and G10 had a consistently higher leaf mass than endophyte free (Fig. 5b, c). The E− plants had greater leaf mass (0.345 ± 0.240 g) compared with E+ plants (0.305 ± 0.170 g) (Fig. 6); the tiller bases mass of E− plants was greater (0.352 ± 0.118 g) than E+ plants (0.298 ± 0.155 g) (Fig. 6). However, root

Table 2 Two-way anova of photosynthetic characteristics recorded before clipping of *A. sibiricum* ramets

	df	P_{max}		Gs		Ci		Tr		Ls		WUE		LUE	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Genotype (G)	4	4.973	**<0.01**	11.565	**<0.01**	9.458	**<0.01**	14.723	**<0.01**	8.970	**<0.01**	9.189	**<0.01**	4.965	**<0.01**
Infection (I)	1	5.751	**0.017**	2.531	0.112	1.305	0.254	2.740	0.098	1.372	0.242	1.114	0.292	5.771	**0.017**
G * I	4	2.214	0.066	2.342	0.054	3.943	**<0.01**	2.542	**0.039**	4.222	**<0.01**	4.901	**<0.01**	2.212	0.066

Significant *P* values are in bold

mass did not differ between E+ and E− plants (Fig. 6). There were genotype-specific effects of endophytes on dry mass, as shown by significant genotype by infection interactions for leaf mass after clipping (Table 1).

Discussion

We found that host genotype effects were stronger than the effects of fungal endophyte infection in influencing tiller number and photosynthetic characteristics of *A. sibiricum* before clipping. Similarly, the effects of host genotype outweigh fungal endophyte infection on the number of tillers of *L. perenne* (Cheplick 2008). Cheplick and Cho (2003) also indicate that the effects of endophyte infection on number of tillers and leaf area are highly dependent on host genotype at the time of defoliation. After defoliation, leaf mass, tiller base mass, and specific leaf area after regrowth are also highly dependent on genotype (Cheplick and Cho 2003). The ability to produce tillers is an important aspect of the population ecology of caespitose grasses (Cheplick 2008); for *A. sibiricum*, endophyte-infected genotypes G1 and G10 showed a higher tiller number and leaf mass than endophyte free; G1 had a higher above-ground biomass than endophyte free, and G10 also had a higher leaf area than endophyte free (Fig. 5b, c). This shows that the tiller number is closely correlated with above-ground mass and leaf area (Cheplick 2004; Gautier et al. 1999; Luxmoore and Millington 1971). Our study shows that host genotype, but not endophyte status, affects tiller numbers and plant height before clipping.

By altering photosynthesis, endophyte infection may affect the temporal dynamics of plant growth (Spiering et al. 2006). It has reported that Arizona fescue plants have lower net photosynthetic rates than uninfected plants on four out of five measuring dates and they tend to produce less above-ground biomass than uninfected plants regardless of treatment or maternal genotype (Morse et al. 2007). However, in our study, we found that host genotype affected all of the photosynthesis variables of *A. sibiricum* before clipping, whereas only P_{max} and LUE varied by infection. These results suggest that the genetic variation found in hosts and endophytes in natural populations is likely to influence growth and physiological parameters.

Table 3 Two-way anova of photosynthetic characteristics recorded after clipping of *A. sibiricum* ramets

	df	P_{max}		Gs		Ci		Tr		Ls		WUE		LUE	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Genotype (G)	4	19.648	**<0.01**	24.876	**<0.01**	112.510	**<0.01**	18.381	**<0.01**	107.291	**<0.01**	61.970	**<0.01**	19.637	**<0.01**
Infection (I)	1	6.876	**<0.01**	5.822	**0.017**	9.144	**<0.01**	3.235	0.073	8.514	**<0.01**	0.917	0.339	6.840	**<0.01**
G * I	4	6.866	**<0.01**	16.954	**<0.01**	24.688	**<0.01**	18.784	**<0.01**	24.337	**<0.01**	22.620	**<0.01**	6.866	**<0.01**

Significant *P* values are in bold

After clipping, host genotype had a larger effect on growth and above-ground biomass of *A. sibiricum* than endophyte infection. Plant biomass is usually used to assess endophyte effects on growth of *L. perenne* (Spiering et al. 2006). Cheplick (2008) found that host genotype effects on biomass of *L. perenne* were greater than fungal endophyte infection. Our study shows that the plant height, leaves number, and leaf area were affected by host genotype, and above-ground mass and leaf mass also depended on genotype at the end of the experiment. Indeed, E− host plants tended to accumulate more above-ground biomass than E+ plants, supporting previous findings that *Neotyphodium* may have antagonistic effects on plant growth in *F.* arizonica, a native grass from North America (Faeth et al. 2004; Faeth and Sullivan 2003). This difference in biomass result from greater tiller base mass and leaf mass of E− plants than E+ plants (Fig. 6) but root mass did not differ. Thus, endophyte infection does not have positive effects on growth in *A. sibiricum* as predicted if the endophyte acts mutualistically. Instead, E− plant are larger than E+ plants due mainly to increases in above-ground growth (Cheplick and Cho 2003; Cheplick et al. 2000).

Many have showed that endophyte-mediated effects on grasses highly depend on environmental conditions and host genotype (Cheplick 2004; Cheplick and Cho 2003; Hesse et al. 2004; Saikkonen et al. 1998). However, even though genotypes varied greatly in tiller number, leaf number, leaf area, leaf mass, and above-ground biomass in the experimental *A. sibiricum*, there were never any genotype by infection interactions (Table 1). This lack of interaction implies that natural selection could differentiate among host genotypes, but that the selection process will be not contingent on whether individuals are infected by endophyte.

For plant carbon content, our study showed that the TNC % of *A. sibiricum* varied among host genotype before and after clipping, whereas endophyte infection affected the C % after clipping. For many growth and storage traits, Cheplick and Cho (2003) reported that the effects of endophyte infection on TNC % of *L. perenne* are highly dependent on host genotype at the time of defoliation. The present study showed that C % and TNC % were affected by host genotype at the time of defoliation, but not by endophyte infection. Accumulation or storage of TNC % might suggest inefficient utilization of carbohydrates for growth in

E− plants, consistent with observations regarding greater mineral nutrient-use efficiency in E+ tall fescue (Malinowski and Belesky 2000). Sullivan et al. (2007) found that damaged E+ hosts had lower foliar nitrogen levels than E− plants and higher C/N, suggesting that E+ tall fescue allocated more nitrogen to defense than growth in *L. arundinaceum*. In our studies, we suggest that C/N and N % were affected by both host genotype and infection, and after clipping, the E+ plants had greater N % compared with E− plants. The differences in costs and benefits of these strategies of E+ and E− grasses may depend on the availability of nitrogen and host genotypes, which may explain the contrasting results found in *A. sibiricum* (Cheplick and Faeth 2009).

Most studies on tall fescue and perennial ryegrass have not reported a consistent influence of endophyte infection on carbohydrate storage (in tiller bases) or on regrowth following defoliation (Belesky and Fedders 1996; Cheplick 1998; Cheplick and Cho 2003; Rasmussen et al. 2008). Cheplick and Cho (2003) suggested that the effects of endophyte infection on regrowth rates are influenced by host genotype, but not by endophyte infection. In general, there was no overriding, consistently positive impact of endophytes on growth, carbohydrate storage, or regrowth following defoliation in *L. perenne* (Cheplick and Cho 2003). Thus, the endophyte–host relationship should not be considered an obligate mutualism (Cheplick 1997; Cheplick et al. 2000; Saikkonen et al. 1998). In our study, we found that leaf area regrowth rates were not affected by genotype and infection in *A. sibiricum*. Therefore, the interaction between the endophyte and host may be highly variable. In agronomic grasses, infection alone often enhances growth and alters physiological parameters (Malinowski et al. 1997; Marks and Clay 1996; Newman et al. 2003). More recent research shows that plant genotype and endophyte strain might influence the outcome of *Neotyphodium* interactions with its host grass (Bony et al. 2001; Cheplick and Cho 2003; Cheplick et al. 2000; Faeth et al. 2002a; Müller and Krauss 2005; Saikkonen et al. 2004).

The genotype–infection interactions recorded for most photosynthesis and storage traits in *A. sibiricum* indicate that endophytic fungi can affect host photosynthesis ability and alter the potential impact of natural selection on genotype-specific plasticity. Indeed, as a result of the genotype–infection effect, a unique sorting of genotypes may occur insofar as discrimination by natural selection would not simply depend on plant genotype as expected, but also on

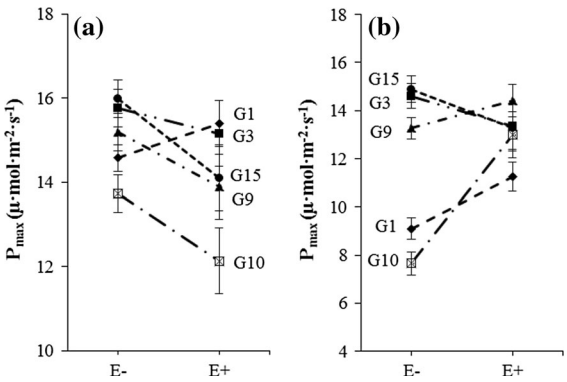

Fig. 2 The maximum net photosynthetic of *A. sibiricum* ramets recorded before (**a**) and after clipping (**b**). Mean ± SE are shown

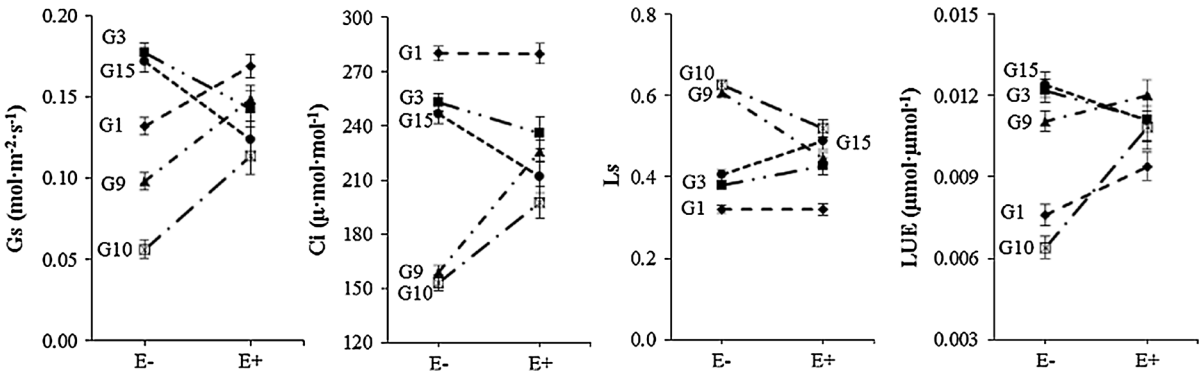

Fig. 3 The photosynthetic indexes of *A. sibiricum* ramets recorded after clipping. Mean ± SE are shown

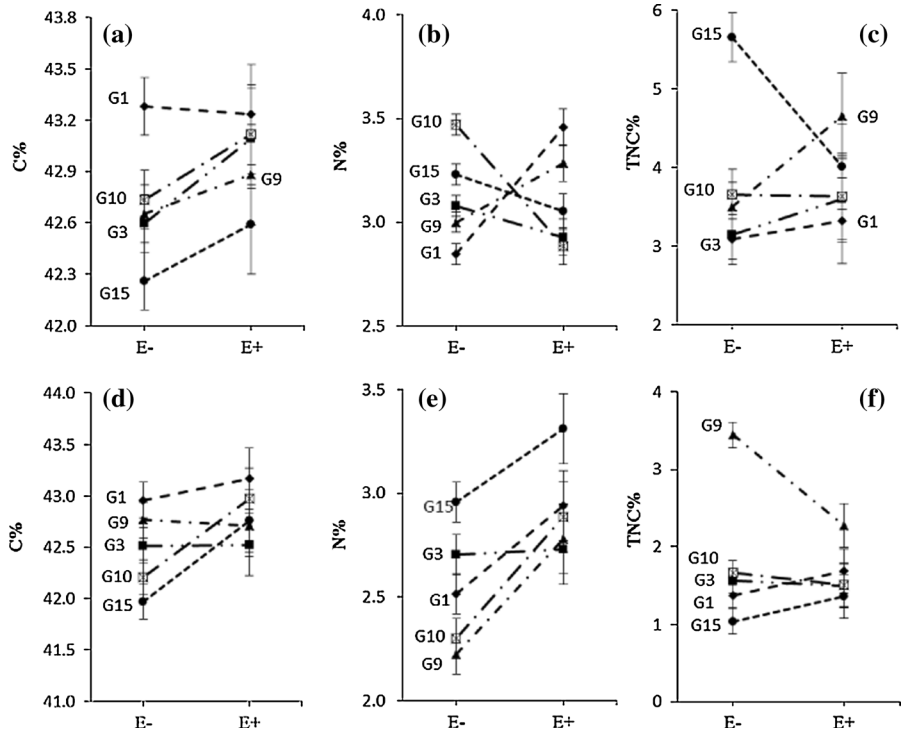

Fig. 4 Genotype–endophyte infection interaction plot for nitrogen content (N %), carbon content (C %) and total nonstructural carbohydrates (TNC %) before (**a**, **b**, **c**) and after (**d**, **e**, **f**) clipping. Mean ± SE are shown

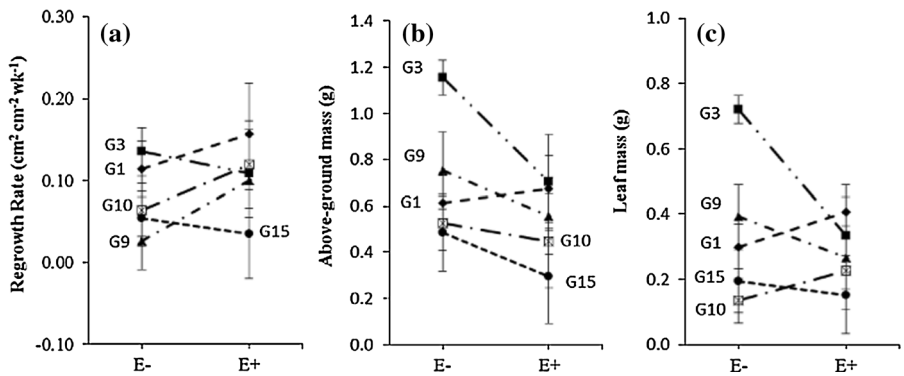

Fig. 5 Genotype–endophyte infection interaction for regrowth rate based on leaf area (**a**), dry mass of above-ground (**b**) and leaves (**c**) recorded 4 week after cliping. Mean ± SE are shown

whether or not the host was infected (Cheplick and Cho 2003). Host genotypic variation in the morphological and physiological response of grasses to endophyte infection has been detected by others ((Belesky et al. 1987; Elbersen and West 1996; Marks and Clay 1996; Meijer and Leuchtmann 2000; Rice et al. 1990). Apparently, the particular host–endophyte combination that is favored by selection is likely to be relied on environmental conditions and which traits are most closely coupled to evolutionary fitness.

In conclusion, the influence of endophyte infection is host genotype-specific in *A. sibiricum*, and the influence of host genotype appears to override endophyte infection, especially for growth, storage traits, and photosynthesis after clipping. Similar to our results, Cheplick (1998) also found genotypic variation in regrowth ability of perennial ryegrass following clipping, but no consistent endophyte effect on regrowth rates (based on leaf mass or leaf area) or on specific leaf area. With the greater genetic diversity in native grass hosts (Saikkonen et al. 2006, 2004), natural populations should exhibit much greater complexities in terms of endophyte–host and endophyte–plant genotype interactions. Based on our highly variable outcomes regarding host performance and physiology, we conclude that this will require a

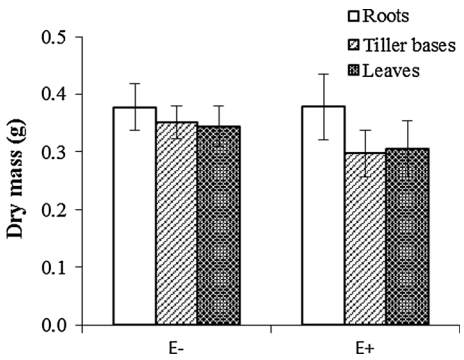

Fig. 6 Final dry mass of leaves, tiller bases, and roots for E− and E+ replicates averaged across all genotypes. Mean ± SE are shown

better understanding of plant genotype, their population biology, and fungal interactions and this can be done with genomics and so many of the modern available tools.

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