

2016

Nonsystemic fungal endophytes increase survival but reduce tolerance to simulated herbivory in subarctic *Festuca rubra*

Published version

James S. Santangelo

Peter M. Kotanen

Santangelo, J. S., and P. M. Kotanen 2016. Nonsystemic fungal endophytes increase survival but reduce tolerance to simulated herbivory in subarctic *Festuca rubra*. *Ecosphere* 7(5):e01260.
[doi:10.1002/ecs2.1260](https://doi.org/10.1002/ecs2.1260)



This work is licensed under the Creative Commons Attribution 3.0 License.
To view a copy of this license, visit <https://creativecommons.org/licenses/by/3.0/>

HOW TO CITE TSPACE ITEMS

Always cite the published version, so the author(s) will receive recognition through services that track citation counts, e.g. Scopus. If you need to cite the page number of the TSpace version (original manuscript or accepted manuscript) because you cannot access the published version, then cite the TSpace version **in addition to** the published version using the permanent URI (handle) found on the record page.

Nonsystemic fungal endophytes increase survival but reduce tolerance to simulated herbivory in subarctic *Festuca rubra*

JAMES S. SANTANGELO^{1,†} AND PETER M. KOTANEN²

¹Department of Biology, University of Toronto Mississauga, 3359 Mississauga Road, Mississauga, Ontario L5L 1C6 Canada

²Department of Ecology and Evolutionary Biology, University of Toronto Mississauga,
3359 Mississauga Road, Mississauga, Ontario L5L 1C6 Canada

Citation: Santangelo, J. S., and P. M. Kotanen. 2016. Nonsystemic fungal endophytes increase survival but reduce tolerance to simulated herbivory in subarctic *Festuca rubra*. *Ecosphere* 7(5):e01260. 10.1002/ecs2.1260

Abstract. Plant–microbial symbioses are widespread in nature and can shape the ecology and evolution of hosts and interacting symbionts. Fungal endophytes—fungi that live asymptotically within plant tissues—are a pervasive group of symbionts well known for their role in mediating host-responses to biotic and abiotic stresses. However, they also may become pathogenic and often impose metabolic costs on hosts. Here, we examine the role of fungal endophytes in mediating responses of the host grass red fescue (*Festuca rubra*) to salt and herbivore stress. We collected 38 red fescue genotypes from within its native range on Akimiski Island, Nunavut, Canada, where it occurs in the supratidal region on the northern part of the island and is heavily grazed by nesting and brood-rearing snow geese (*Chen caerulescens caerulescens*) and Canada geese (*Branta canadensis*). We screened all plants for the presence of the systemic endophyte *Epichloë festucae* and sequenced the nonsystemic endophytic community on a subset of these plants. While we did not detect *E. festucae*, our plants instead were host to a diverse array of nonsystemic fungal endophytes. We then conducted a fully factorial greenhouse experiment where we crossed plant genotype (4 levels) with simulated grazing (clipped or unclipped), endophyte status (present or absent) and salinity (0, 32 or 64 ppt) to examine the ecological role of this endophytic community. Overall, the presence of nonsystemic endophytes increased plant survival, but only in the absence of salt or clipping. On the other hand, these endophytes reduced plant tolerance to simulated herbivory by 69% but had no effect on aboveground plant growth. Thus, our results identify a potential nonsystemic endophyte-mediated trade-off in host plant survival and tolerance to herbivory and suggest this trade-off may be altered by stressful abiotic conditions.

Key words: Akimiski Island; Canada geese; *Festuca rubra*; herbivory; nonsystemic endophytes; plant–fungal interactions; red fescue; snow geese.

Received 20 August 2015; revised 18 September 2015; accepted 28 September 2015. Corresponding Editor: D. P. C. Peters.

Copyright: © 2016 Santangelo and Kotanen. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

† **E-mail:** james.santangelo37@gmail.com

INTRODUCTION

Plant–microbial interactions are ubiquitous in nature and can have profound effects on the ecology and evolution of both plants and symbionts (Larimer et al. 2010, Yang et al. 2013). Furthermore, the tremendous diversity of plant

symbionts—both bacterial and fungal—means their effects on host fitness likely depend on the interactive effects of multiple partners (Larimer et al. 2010). Thus, by examining the effects of whole symbiotic communities on plant fitness, we may gain insight into their ecological role in natural host populations.

Fungal endophytes—including both mutualistic and pathogenic fungi that live within plant tissues—are a well-studied and pervasive group of plant symbionts in nature (Clay and Saikkonen et al. 1998, Clay and Schardl 2002, Schardl et al. 2004, Sánchez Márquez et al. 2012, Rodríguez et al. 2009). Research into the distribution and ecological role of endophytes has been especially intensive in the family Clavicipitaceae, where the asexual genus *Neotyphodium* and closely related species of the sexual genus *Epichloë* have provided model systems. These fungi systemically colonize the aboveground tissues of many cool-season grasses (Subfamily: Pooideae) and are vertically transmitted through seeds (Clay and Schardl 2002, Schardl et al. 2004). However, plants also are host to a broad array of horizontally transmitted, nonclavicipitaceous endophytes that generally form nonsystemic localized infections within plant tissues (Sánchez Márquez et al. 2012). These species form a highly diverse community within aboveground plant tissues, although this diversity shows latitudinal clines, being lowest in arctic and boreal biomes and highest in the tropics (Arnold and Lutzoni 2007, Sánchez Márquez et al. 2012). Systemic endophytes typically are considered plant mutualists, increasing resistance, and tolerance to a range of biotic and abiotic conditions such as herbivory (Clay 1988, Bazely et al. 1997, Clay et al. 2005), competition (Marks et al. 1991), drought (Kane 2011, Nagabhyru et al. 2013) and soil salinity (Reza Sabzalilian and Mirlohi 2010, Yin et al. 2014) to name a few, although antagonistic interactions also occur (Saikkonen et al. 1998, Faeth 2002, 2009, Gorischek et al. 2013). In contrast, studies of nonsystemic fungal symbionts, although fewer in number, show potentially important but highly variable effects on plant fitness and thus their ecological significance is less well understood (Rodríguez et al. 2009, Sánchez Márquez et al. 2012).

Red fescue (*Festuca rubra*; Subfamily: Pooideae) is commonly infected with both systemic and nonsystemic fungal endophytes (Zabalgogezcoa et al. 2006, 2013). It is a perennial grass with a holarctic distribution and is a staple forage plant for nesting and brood-rearing colonies of snow and Canada geese in coastal Canadian subarctic ecosystems (O et al. 2005, 2006, Kotanen and Abraham 2013). Geese in these systems can have

pronounced effects on the dynamics of plant communities, greatly reducing the abundance of forage grasses in addition to increasing salinity, temperature, and soil anoxia which limit plant recruitment and survival in revegetated areas (Jefferies et al. 2003, 2006, Abraham et al. 2005, Kotanen and Abraham 2013). Given the ability of fungal endophytes to influence host survival (Rudgers et al. 2012) and preference and performance of grazing herbivores (Conover and Messmer 1996, Bazely et al. 1997, Koh and Hik 2007), they may play an important role in shaping the response and performance of *F. rubra* to biotic and abiotic stresses in the Canadian subarctic. However, the endophytic fungal community of *F. rubra* has never been characterized in the Canadian subarctic and thus its potential importance in mediating interactions between nesting geese and their food plants and in structuring northern plant populations remain unknown. We used a combination of microscopic examination and high-throughput sequencing of the ITS2 region of nuclear rDNA to describe the systemic and nonsystemic fungal endophytes of *Festuca rubra* collected from Akimiski Island, Nunavut Territory, Canada where it occurs on saline, supratidal soils, and is heavily grazed by lesser snow and Canada geese (Martini and Glooschenko 1984, O et al. 2006). We then conducted a fully factorial greenhouse experiment where we manipulated the presence/absence of endophytes, simulated herbivory, salt concentrations, and plant genotype to address the following questions: (1) Which fungal endophytes are present? (2) Do fungal endophytes influence plant survival? (3) Do endophytes influence plant growth and/or tolerance to simulated herbivory? (4) Do the costs and benefits of endophytes vary with soil salinity?

METHODS

Study system

Akimiski Island (53 °N 81 °W), Nunavut Territory, Canada is the largest island in James Bay and characterized by a cold, yet humid subarctic climate (Martini and Glooschenko 1984, O et al. 2005). This island is an important site for staging and nesting of several species of geese. In particular, lesser snow geese (*Chen caerulescens caerulescens* [Linnaeus]) nest in a

relatively dense colony of about 4000 birds along a 35-km stretch of the north shore of this island (Abraham et al. 1999, Kotanen and Abraham 2013), while Canada geese (*Branta canadensis* (Linnaeus) *interior* Todd) nest at lower densities throughout the island (Leafloor et al. 1996, 2000). Following hatch, these species intensively graze leaves of forage plants in intertidal and supratidal areas, as well as in nearby freshwater fens (O et al. 2006, Kotanen and Abraham 2013). Geese have had pronounced effects on the composition of intertidal vegetation communities at this site, replacing the forage grass *Puccinellia phryganodes* and sedge *Carex subspathacea* with devegetated mudflats in heavily grazed areas (Jefferies et al. 2006, Kotanen and Abraham 2013). Such vegetation changes typically are associated with highly altered soil properties, including increased salinity, temperature, and anoxia (Iacobelli and Jefferies 1991, Srivastava and Jefferies 1995, 1996, McLaren and Jefferies 2004). *Festuca rubra* is the most important forage species in supratidal areas, where it forms extensive stands containing a mosaic of short, heavily grazed or tall, infrequently grazed patches interspersed among devegetated and degraded mudflats (O et al. 2005, 2006, Kotanen and Abraham 2013). While the reasons for the differences between these patches are not well understood, they may reflect geese's preferences for feeding on short-statured grasses—due to easier handling and better nutrient content—so that once tall patches are grazed, they are maintained that way.

In August 2014, 38 plugs (~2 cm × 2 cm) of *Festuca rubra* were collected from nine sites on the north coast of Akimiski Island, from areas used for grazing by postnesting snow and/or Canada geese and goslings. Because *Festuca rubra* grows vegetatively through the production of stolons and rhizomes, resulting in the formation of large clonal patches (Grime et al. 2007), we ensured sampling locations were at least 20 m apart to avoid sampling the same individual. While plugs may contain multiple individuals, the vigorous clonal growth of *F. rubra* suggests this is unlikely and thus henceforth we refer the plugs as distinct genotypes. *Festuca rubra* is commonly infected asymptotically with the systemic fungal endophyte *Epichloë festucae* (Wäli et al. 2006, Zabalgoeazcoa et al. 2006), which lives intercellularly

in the sheaths, culms, and rhizomes of the plant and is strictly vertically transmitted through seeds (Rodriguez et al. 2009). In addition, *F. rubra* is also colonized by a diverse community of vertically and/or horizontally transmitted, non-systemic, fungal endophytes with potentially diverse ecological roles (Zabalgoeazcoa et al. 2013).

Detection of endophytes

The plugs from Akimiski Island were transported to the University of Toronto Mississauga and transplanted into 10-cm pots containing a mixture of potting mix and field soil and maintained in the greenhouse. In September 2014, we determined the presence of systemic, clavicipitaceous endophytes in all plants using a modification of the methods described in Clark et al. (1983). From each of 39 plants, we excised all leaf sheaths from a single, fully formed tiller and boiled them for 10 min in a solution containing 0.1% lactophenol aniline blue and mounted on them microscope slides with cover slips. We then examined each sheath in its entirety at 100×–200× for the presence or absence of sparsely branching, septate, intercellular hyphae characteristic of *Neotyphodium/Epichloë* endophytes (Clark et al. 1983). If endophytes were not seen during this time, they were presumed absent. To confirm results from the staining procedure, we used a commercially available endophyte detection kit (Phytoscreen Immunoblot Kit #ENDO7973, Agrinostics, Watkinsville, Georgia, USA, <http://www.agrinostics.com>). These methods were able to reliably detect *Epichloë festucae* in infected test samples of *F. rubra*.

Hyphae not matching the typical growth pattern of *E. festucae* were considered potential nonsystemic endophytes. To identify nonsystemic endophytes, we sent four plant samples (one from near the Ministik River: N53.190, W81.216, one from near the Thompson River: N53.212, W81.310, and two from a goose-banding location, ASO: N53.203, W81.388) with unidentified hyphae for next-generation sequencing of fungal ITS2 genetic markers (Canadian Centre for DNA Barcoding, Biodiversity of Ontario, University of Guelph). We queried returned sequences against known fungal ITS2 regions using the BLAST algorithm in NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). We assigned species identities

when the top match was at least 99% similar to the queried sequence. We then cross-referenced species identities with published literature; if they were known to act as endophytes in other systems, they were presumed to be endophytic. We acknowledge that four samples are insufficient to accurately characterize the diverse community of nonsystemic endophytes in any system. However, our goal here is to make a first step toward understanding of the ecological role these symbionts are playing in this system rather than conducting a comprehensive survey of nonsystemic endophytes.

Experimental design

Using three of the sequenced plants from above (one from ASO, one from the Thompson River, and one from the Ministik River) and one unsequenced plant (from the Ministik River), we set up a fully factorial greenhouse experiment at the University of Toronto Mississauga. We manipulated the presence or absence of endophytes (*i.e.*, E+ or E-), simulated herbivory (*i.e.*, clipped or unclipped), salt concentration (*i.e.*, 0 ppt, 32 ppt, or 64 ppt), and genotype (*i.e.*, four plant genotypes, each with its associated endophytes) for a total of 48 treatments. To generate endophyte-free clones, we split experimental plants into four large ramets in late November 2014 and planted them individually into 10-cm round pots containing potting soil. We then treated two of these ramets with three applications of 4 g/L propiconazole, a systemic fungicide (BUMPER, Mana Canada Inc.), at 10-d intervals with the first and third doses applied to the soil and the second given as a foliar application (Zabalgogezcoa et al. 2006), while control plants received an equivalent amount of sterile distilled water. Between fungicide treatments, all plants were watered as needed with distilled water. Propiconazole inhibits the synthesis of ergosterol, a hormone required for the formation of fungal cell walls, and has been successfully used to eliminate systemic (Faeth 2009, Reza Sabzalain and Mirlohi 2010) systemic and nonsystemic nonsystemic (Canals et al. 2014) fungal assemblages in other systems. Plants were grown for 2 months post-fungicide application to minimize the effects of the fungicide on plant performance and to ensure that they were producing new tissue before being used in the experiment. Treated

and untreated ramets did not differ in growth rate throughout fungicide application (repeated measures ANOVA, $F_{1,20} = 2.521$, $P = 0.128$; Appendix S1: text and Fig. S1) and none of the fungicide-treated plants showed signs of endophyte infection, as determined using the staining procedure described above.

In late January 2015, we generated 70 E+ and 70 E- tillers from each plant genotype using the ramets described above and planted them individually into 8.75-cm square pots filled with potting soil. We used tillers formed from new clonal growth postfungicide application to minimize the effects of the fungicide on host growth and performance. We let tillers grow for 1 week prior to starting experimental treatment applications to minimize the effects of transplant shock and to allow us to remove any plants that failed to establish. At the start of the experiment, we had at least 10 replicates per treatment for a total of 506 plants. We recorded initial plant biomass by measuring the diameter and height to the longest leaf of each plant and calculating the volume of a cylinder (*i.e.*, $V = \pi r^2 h$), which is highly correlated with aboveground biomass in this (Santangelo et al. 2015) and closely related species (Faeth and Sullivan 2003). At days 0 and 30 we clipped all plants in the clipping treatment to 1 cm above the soil surface using a sharp razor blade as a proxy for grazing herbivores, as has been successfully done to measure *F. rubra*'s tolerance to herbivory in other systems (Turley et al. 2013, Didiano et al. 2014, Santangelo et al. 2015). We watered plants weekly with 50–100 mL of distilled water containing 0, 32, or 64 parts-per-thousand sea salt, corresponding to control, seawater, and twice seawater treatments, within the range of salt concentrations expected in similar systems (Earle and Kershaw 1989, Iacobelli and Jefferies 1991, Srivastava and Jefferies 1995, McLaren and Jefferies 2004), though high compared to values measured at this site by O et al. (2005).

We recorded survival of plants at day 30 and day 60 as 1 (alive) or 0 (dead). Above and belowground biomass of each of the surviving unclipped plants was harvested after 60 d, oven-dried for 48 h at 60 °C and weighed to the nearest 0.1 mg. Clippings taken at 30 and 60 d were oven-dried for 48 h at 60 °C and weighed to the nearest 0.1 mg. Given that plants were clipped at day 0, the sum of the amount of tissue regrown

from 0 to 30 d and from 30 to 60 d can be used as a measure of plant tolerance to herbivory.

Statistical analyses

To examine differences in survival among plants, we fit a logistic regression using endophyte status (*i.e.*, E+ or E-), clipping (*i.e.*, clipped or unclipped), salinity (*i.e.*, 0 ppt, 32 ppt, or 64 ppt) and genotype (*i.e.*, four genotypes) as predictors of plant survival. We began with a global model including all main effects and interactions. We then used the “dredge” function in R’s “MuMIn” package (Bartoń 2013) to compare 167 possible models and, among all models with a $\Delta\text{AIC} < 2$, we selected the one with the best fit containing the interactions of *a priori* interest (Appendix S1: Table S1); this model did not include any three- or four-way interactions and results were qualitatively similar to a global model containing all main effects and interactions, and similar to those obtained when parameter estimates are based on averaging of all models with a $\Delta\text{AIC} < 2$. We then fitted this model using the “glm” function in the “lme4.0” package (Bates et al. 2013) and obtained *P*-values for model terms using the “ANOVA” function, which performs likelihood ratio tests by testing the reduction in deviance caused by addition of each term against a chi-squared distribution with degrees of freedom equal to $\text{df}_{\text{larger model}} - \text{df}_{\text{reduced model}}$. Where significant interactions were found, we performed Tukey HSD *post hoc* tests to control for multiple comparisons and examine differences among treatment levels. Given the qualitatively different results for survival after 30 and 60 d, we chose to run these analyses separately.

To examine differences in tolerance to herbivory, we fit a one-way ANOVA to plants in the clipping treatment using endophyte status as a predictor of tissue regrowth (in mg) over the 60 d of the experiment. We could not examine how tolerance is mediated by salt concentrations due to high plant mortality at 32 ppt and 64 ppt salt resulting in reduced power to detect differences in tolerance at these levels. We log-transformed tissue regrowth values to improve normality and homogeneity of variance. To examine differences in above and belowground biomass production, we fit a one-way ANOVA using endophyte status as a predictor of final log-transformed aboveground biomass or untransformed below-

ground biomass (to meet assumptions of normality) on the final sampling data for unclipped plants only. Log-transformed initial plant volume was used as a covariate in the analysis of aboveground biomass. All statistical analyses were performed in R version 3.0.1 (R Core Team 2014).

RESULTS

Endophyte communities

Microscopic examination of leaf sheaths revealed that 73% of plants contained hyphae of potential endophytes. *Epichloë festucae* was not detected by means of immunoblotting, staining, or ITS sequencing in any plants, but sequencing of four plant samples identified the presence of 12 presumed nonsystemic endophytes (Table 1), with two of these (*Microdochium nivale* and *Plectosphaerella cucumerina*) occurring in all four plants.

Plant survival

After 30 d, plants with nonsystemic endophytes had 72% greater survival than plants treated with fungicide (deviance = 47.82, *df* = 1, *P* < 0.001), although this depended on salt concentrations (endophyte × salt interaction, deviance = 7.28, *df* = 2, *P* = 0.021, Fig. 1a). Specifically, endophytes increased survival by 53% at 0 ppt salt (Tukey HSD *P* < 0.001, Fig. 1a), 90% at 32 ppt (Tukey HSD, *P* < 0.001, Fig. 1a) and 90% at 64 ppt salt, although the latter was nonsignificant (Tukey HSD, *P* = 0.477, Fig. 1a). The effects of simulated herbivory on plant survival depended on salt concentrations (clipping × salt interaction, deviance = 7.21, *df* = 2, *P* = 0.027), although clipping had no significant overall effect on survival (deviance = 0.91, *df* = 1, *P* = 0.341). Plant genotypes differed in survival (deviance = 25.73, *df* = 3, *P* < 0.001), although this effect again depended on salt concentrations (genotype × salt interaction, deviance = 21.79, *df* = 6, *P* = 0.001) and the presence of endophytes (genotype × endophyte interaction, deviance = 10.59, *df* = 3, *P* = 0.014). For each genotype, endophytes either increased survival or had no significant effect (Appendix S1: Fig. S2a). None of the best models included a significant three-way interaction, meaning that the effects of endophytes on salt and herbivore tolerance were not statistically plant genotype-dependent.

Table 1. Identification of nonsystemic endophytic fungi present in four *Festuca rubra* plant samples.

Species	Plants†	Putative role	Reference‡
<i>Acremonium alternatum</i>	AS02-4-4	Mutualist	Raps and Vidal 1998
<i>Alternaria</i> sp.	AS02-4-4, T-2-2, MS-5-3	Latent saprotroph	Zabalgogazcoa et al. 2013
<i>Chrysosporium</i> sp.	MS-5-3	Produces gibberellins	Hamayun et al. 2009a
<i>Cladosporium sphaerospermum</i>	AS01-1-1, AS02-4-4, T-2-2, MS-5-3	Latent saprotroph/mutualist	Hodgson et al. 2014
<i>Cladosporium cladosporioides</i>	MS-5-3	Saprotroph/produces allelochemicals	Waqas et al. 2013
<i>Cryptococcus flavescens</i>	MS-5-3	Unknown	Ellsworth et al. 2013
<i>Epicoecum nigrum</i>	MS-5-3	Saprotroph	Sánchez Márquez et al. 2012
<i>Fusarium avenaceum</i>	AS02-4-4	Pathogen	Varvas et al. 2013
<i>Lewia</i> sp.	AS02-4-4	Mutualist	Cruz-Hernández et al. 2013
<i>Microdochium nivale/majus</i>	AS01-1-1, MS-5-3, T-2-2	Pathogen	Zabalgogazcoa et al. 2013
<i>Microdochium bolleyi</i>	AS01-1-1	Pathogen	An et al. 1993
<i>Paecilomyces inflatus</i>	AS02-4-4	Entomopathogen	Vega et al. 2008
<i>Plectosphaerella cucumerina</i>	AS01-1-1, AS02-4-4, T-2-2, MS-5-3	Pathogen	Herrero et al. 2009

† Labels refer to distinct plant genotypes and are separated by commas.

‡ Reference suggesting species may be endophytic.

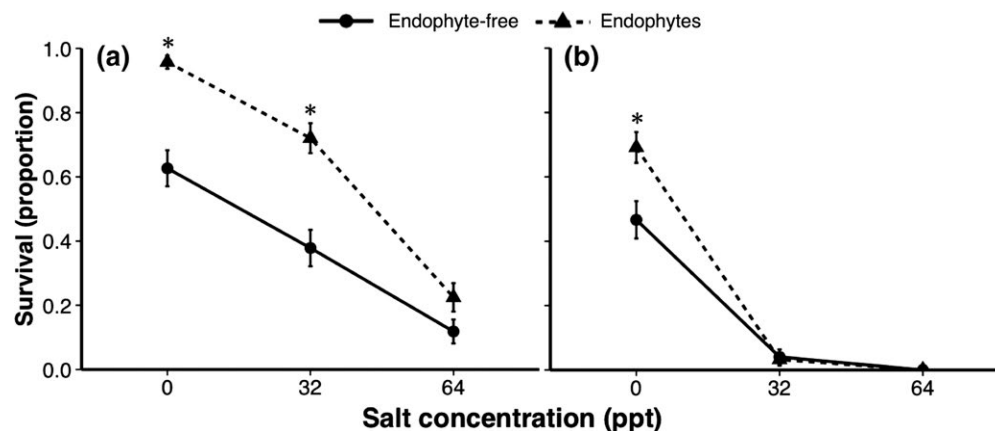


Fig. 1. Mean proportion of surviving *Festuca rubra* plants after (a) 30 d and (b) 60 d either infected or uninfected with non-systemic endophytes in the presence of 0 ppt ($n_{\text{infected}} = 94$, $n_{\text{uninfected}} = 75$), 32 ppt ($n_{\text{infected}} = 93$, $n_{\text{uninfected}} = 74$) or 64 ppt ($n_{\text{infected}} = 89$, $n_{\text{uninfected}} = 76$) sea salt. All points represent means $\pm$ 1SE. Asterisks denote significant differences (Tukey HSD, $P < 0.05$) between infected and uninfected plants within each level of the salt treatment.

After 60 d, the presence of nonsystemic endophytes increased plant survival by 45% (deviance = 7.17, $df = 1$, $P = 0.007$), although this depended on salt concentrations (endophyte $\times$ salt interaction, deviance = 6.74, $df = 2$, $P = 0.034$, Fig. 1b). Specifically, endophytes increased survival by 48% at 0 ppt salt (Tukey HSD, $P = 0.025$, Fig. 1b), while reducing survival by 20% at 32 ppt, although this effect was not significant (Tukey HSD, $P = 0.999$, Fig. 1b). Endophytes had no effect on survival at 64 ppt; instead, there was

complete mortality of infected and uninfected plants (Fig. 1b).

Interestingly, final survival in response to simulated herbivory was differentially influenced by the presence of endophytes (endophyte $\times$ clipping interaction, deviance = 8.65, $df = 1$, $P = 0.003$, Fig. 2), a result that was not apparent after 30 d. Specifically, infected plants had 110% higher survival than uninfected plants when unclipped (Tukey HSD, $P = 0.019$, Fig. 2) but there was no significant effect of endophytes on plant survival

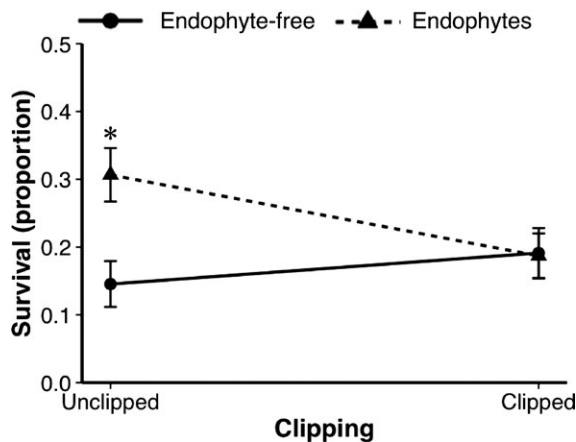


Fig. 2. Mean proportion of surviving *Festuca rubra* plants after 60 d either infected or uninfected with nonsystemic endophytes in the presence ($n_{\text{infected}} = 137$, $n_{\text{uninfected}} = 115$) or absence ($n_{\text{infected}} = 137$, $n_{\text{uninfected}} = 110$) of clipping to simulate herbivore damage. All points represent means $\pm$ 1SE. Asterisks denote significant differences (Tukey HSD, $P < 0.05$) between infected and uninfected plants within each level of the clipping treatment.

in the presence of clipping (Tukey HSD, $P = 0.999$, Fig. 2). Furthermore, plant genotypes differed in survival (deviance = 12.30, $df = 3$, $P = 0.003$), although this effect depended on the presence of endophytes (genotype $\times$ endophyte interaction, deviance = 20.75, $df = 3$, $P < 0.001$). Again, endophytes either increased survival or had no significant effect, depending on the plant genotype (Appendix S1: Fig. S2b), and the best fit model did not include a significant 3-way interaction.

Plant biomass production and tolerance to herbivory

The presence of nonsystemic endophytic fungi reduced tolerance to simulated herbivory by 69% ($F_{1,44} = 6.39$, $P = 0.015$, Fig. 3a). Endophytes in unclipped plants had no significant effect on plant aboveground biomass production ($F_{1,55} = 0.742$, $P = 0.393$, Fig. 3b) but reduced final belowground biomass by 35.7% ($F_{1,56} = 5.285$, $P = 0.025$, Fig. 3c).

DISCUSSION

Endophytic community of *Festuca rubra*

Although we found ample evidence of infection by nonsystemic endophytes, we found no evidence of systemic infection by *Epichloë festucae*

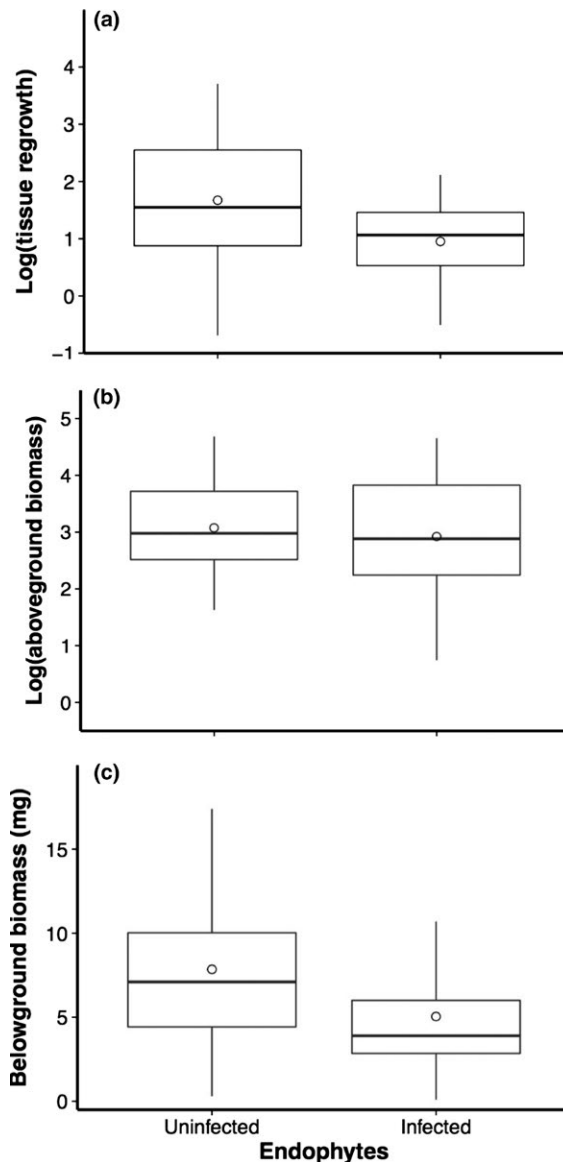


Fig. 3. Boxplots showing (a) tolerance to herbivory, (b) aboveground, and (c) belowground biomass of *Festuca rubra* plants infected or uninfected with nonsystemic endophytes. Note log-transformed response variables in (a) and (b). Original data in (a) and (b) in mg. Boxplots show medians (horizontal line), means (open circles), interquartile range (IQR) as 25th and 75th percentiles (boxes), and $1.5 \times$ IQR (whiskers).

in any sampled *F. rubra* populations, while the 73% of plants with nonsystemic endophytes reported here is likely conservative given the limited number of tissue samples examined. While the frequency of the grass-*Epichloë* symbiosis varies

widely in nature (Leuchtman 1992, Iannone et al. 2011), our result is consistent with a previous survey finding a greater proportion of uninfected *Festuca* populations at higher latitudes (Semmartin et al. 2015) and suggests that grass–*Epichloë* interactions are impaired by the cold conditions of the Canadian subarctic. In fact, colder temperatures can reduce the fidelity of *Epichloë* transmission (Ju et al. 2006), which may result in lower endophyte frequencies in natural populations and shape endophyte–host population dynamics (Afkhami and Rudgers 2008, Gundel et al. 2011a,b, Semmartin et al. 2015). Furthermore, geese can significantly increase the availability of nitrogen in grazed areas, in part due to the addition of goose droppings (Bazely and Jefferies 1985, 1989). High nitrogen concentrations may favor uninfected plants resulting in selection against grass–*Epichloë* symbioses (Faeth and Fagan 2002). Finally, their absence may result from isolation: Akimiski is an offshore island and likely has never been connected to the mainland.

In contrast with these results, microscopic analysis and next-generation sequencing revealed a complex community of nonsystemic endophytes. Nonsystemic fungal endophytes (including pathogens) are ubiquitous, although species-level diversity and abundance tend to decrease toward poles (Arnold and Lutzoni 2007, Sánchez Márquez et al. 2012). While the relatively low diversity reported here corroborates this view, one finding is of particular interest. A recent survey of nonsystemic endophytes of *F. rubra* in northern Finland found no evidence of infection by species of *Alternaria* or *Cladosporium*, two species commonly infecting temperate grass populations (Sánchez Márquez et al. 2012), and the authors suggest these genera may be uncommon at arctic latitudes (Zabalgogea et al. 2013). While our study populations are not as far north as theirs, our detection of both these genera in our plant samples suggests their distributions stretch at least as far north as the Canadian subarctic. In line with the previous study, we also found evidence of *Microdochium nivale* in our study populations, a common pathogenic snow mold at higher latitudes (Zabalgogea et al. 2013). Two endophytes, *Cladosporium sphaerospermum* and *Plectosphaerella cucumerina*, were found in all plant samples, potentially

reflecting host colonization in a common greenhouse environment.

Ecological role of nonsystemic endophytes

While the ecological and evolutionary consequences of grass symbioses with vertically transmitted systemic endophytes are well documented (Clay and Schardl 2002, Saikkonen 2004, Saikkonen et al. 2006), little is known about the potential role that the more diverse community of nonsystemic endophytes may play in natural populations (Sánchez Márquez et al. 2012). Here, we have shown that nonsystemic endophytes from the Canadian subarctic mediate a trade-off in plant performance by increasing survival of *F. rubra*, but reducing tolerance to simulated herbivory. The positive effects on survival suggest the fungi we detected are not simply pathogens, although their effects are also not simply beneficial. While we cannot attribute these effects to the role of any one fungal taxon, our results suggest that these symbionts serve important ecological functions in natural systems despite often being overlooked.

Moderate grazing by nesting and brood-rearing geese can result in the production of grazing lawns, where the quantity and quality of forage are maintained by the herbivores that are feeding on it (Cargill and Jefferies 1984, Hik and Jefferies 1990, Jefferies et al. 2003, O et al. 2006). However, such effects are often unstable (Abraham et al. 2005, Jefferies 1988, Jefferies and Rockwell 2002, Jefferies et al. 2003). Over time, grazing and grubbing by geese can reduce vegetation cover, increasing temperatures and evaporation rates and ultimately result in hypersaline soils that reduce plant survival and limit plant recruitment (Iacobelli and Jefferies 1991, Srivastava and Jefferies 1996, O et al. 2005). Our results suggest that nonsystemic endophytes can play a role in enhancing plant survival under otherwise favorable conditions; unfortunately, this initial benefit was lost in clipped and saline treatments, suggesting it may be of limited value in sites severely grazed by or devegetated by geese. Still, endophytes may at least delay plant mortality in saline soils, as evidenced by the greater survival of infected plants in all salt treatments after 30 d—an effect that disappeared by the end of the experiment. As well, although the salt concentrations we used (32 ppt and 64 ppt) are not

unusual in systems severely degraded by snow geese (Iacobelli and Jefferies 1991, Srivastava and Jefferies 1995, McLaren and Jefferies 2004), they are much higher than the values reported by O et al. (2005) for revegetated soils at our study location (<5 g/L). These values are point estimates based on limited sample sizes in patchily damaged areas, and certainly are low compared to many more severely damaged sites. Nonetheless, they indicate that salinity may not immediately reach high values in revegetated areas; in sites such as these, endophytes may increase survival of remaining plants, even if this benefit ultimately is lost as salt continues to accumulate.

While nonsystemic endophytes seem to increase plant survival in our system, we found they reduce regrowth following simulated herbivory suggesting an endophyte-mediated trade-off between plant performance and herbivore tolerance. Endophyte-mediated trade-offs have been reported for systemic endophytes (i.e., *Epichloë/Neotyphodium*), where they can reduce host survival but increase population growth (Rudgers et al. 2012), increase reproductive biomass but reduce reproductive effort (Gundel et al. 2013), and increase resistance to herbivores at the cost of reduced tolerance (Bultman et al. 2004). Tolerance—defined as a plant's ability to maintain fitness in the presence of herbivores (Rosenthal and Kotanen 1994, Strauss and Agrawal 1999)—is a complex plant trait subject to both environmental and genetic variation and can alter patterns of resource allocation following damage (Rosenthal and Kotanen 1994, Núñez-Farfán et al. 2007). Thus, given the metabolic demands of harboring symbionts, nonsystemic endophytes may alter patterns of resource allocation thereby reducing *F. rubra*'s ability to regrow lost tissue in the presence of herbivores. Similarly, the reduced belowground biomass production of infected plants detected here suggests endophytes may have a role in reducing their hosts' ability to assimilate resources required for tissue regrowth. This may also explain why the survival benefits imparted by endophytes were only detected in the absence of clipping, where plants do not have to expend additional resources regrowing damaged tissues. Finally, the production of costly nitrogen-rich alkaloids by systemic endophytes (Leuchtman et al. 2000), while increasing resistance, may

explain their reduced tolerance to herbivores (Bultman et al. 2004). While we did not quantify resistance here, nonsystemic endophytes have been shown to increase host resistance to herbivores in other systems. For example, nonsystemic endophytes of *Carex brevicollis* produce alkaloids that are toxic to cattle (Canals et al. 2014) and *Cladosporium sphaerospermum*, an endophyte detected here, can produce alkaloids and other compounds (e.g., phenolics) that may explain the reduced survival of *Spodoptera litura* caterpillars fed infected cauliflower (Thakur et al. 2013). If endophytes in our system also produce herbivore-deterrent alkaloids, this may in part explain the reduced tolerance of infected grasses.

While endophytes reduced plant tolerance to herbivory, they had no effect on aboveground biomass production. This is interesting given the role many nonsystemic endophytes, including *Cladosporium sphaerospermum*, *Fusarium* sp., and *Chrysosporium* sp., have in producing gibberellins and other plant growth hormones (Hamayun et al. 2009a,b, Rai et al. 2014). However, it is possible that antagonistic interactions of beneficial symbionts with known plant pathogens, such as the snow mold *Microdochium nivale*, outweighed any potential benefits on growth conferred by potential mutualists. This result highlights our need to better understand the role that individual symbiotic taxa are playing, a goal that could not be achieved here. Controlled inoculation experiments with some of the nonsystemic endophytes detected here are thus required to tease apart the ecological effects of each and provide insight into their role in subarctic *F. rubra* populations. Nonetheless, it is clear that this often overlooked community of nonsystemic endophytes has important consequences on plant performance that are contingent upon interactions with both biotic and abiotic stresses.

ACKNOWLEDGMENTS

This research was supported by NSERC with assistance from the Ontario Ministry of Natural Resources. Numerous members of the Akimiski Field Station provided assistance, and we would especially like to thank Sarah Hagey (OMNR) for organizing the collection of plant samples, and Ken Abraham (OMNR) for commenting on a draft

of the manuscript. Marc Vicari kindly provided endophyte-containing *Festuca* for use as positive controls. Cheyenne Cameron helped apply treatments and provided logistical support throughout the experiment.

LITERATURE CITED

- Abraham, K. F., J. O. Leafloor, and H. G. Lumsden. 1999. Establishment and growth of the lesser snow goose nesting colony on Akimiski Island, James Bay, Northwest Territories. *Canadian Field-Naturalist* 113:245–250.
- Abraham, K. F., R. L. Jefferies, and R. F. Rockwell. 2005. Goose-induced changes in vegetation and land cover between 1976 and 1997 in an arctic coastal marsh. *Arctic, Antarctic, and Alpine Research* 37:269–275.
- Afkhami, M. E., and J. A. Rudgers. 2008. Symbiosis lost: imperfect vertical transmission of fungal endophytes in grasses. *American Naturalist* 172:405–416.
- An, Z. Q., M. R. Siegel, W. Hollin, H. F. Tsai, D. Schmidt, and C. L. Schardl. 1993. Relationships among non-*Acremonium* sp. fungal endophytes in five grass species. *Applied and Environmental Microbiology* 59:1540–1548.
- Arnold, A. E., and F. Lutzoni. 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? *Ecology* 88:541–549.
- Bartoń, K. 2013. MuMIn: multi-model inference. R package version 1.9.5.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2013. lme4: linear mixed effects models using Eigen and S4. R package version 1.0.5.
- Bazely, D. R., and R. L. Jefferies. 1985. Goose faeces: a source of nitrogen for plant growth in a grazed salt marsh. *Journal of Applied Ecology* 22:693–703.
- Bazely, D. R. and R. L. Jefferies. 1989. Lesser snow geese and the nitrogen economy of a grazed salt marsh. *Journal of Ecology* 77:24–34.
- Bazely, D. R., M. Vicari, S. Emmerich, L. Filip, D. Lin, and A. Inman. 1997. Interactions between herbivores and endophyte-infected *Festuca rubra* from the Scottish islands of St. Kilda, Benbecula and Rum. *Journal of Applied Ecology* 34:847–860.
- Bultman, T. L., G. Bell, and W. D. Martin. 2004. A fungal endophyte mediates reversal of wound-induced resistance and constrains tolerance in a grass. *Ecology* 85:679–685.
- Canals, R. M., L. San-Emeterio, S. Sánchez Márquez, I. Ruiz de los Mozos, P. Pujol and Í. Zabalgoeazcoa. 2014. Non-systemic fungal endophytes in *Carex brevicollis* may influence the toxicity of the sedge to livestock. *Spanish Journal of Agricultural Research* 12:623.
- Cargill, S. M., and R. L. Jefferies. 1984. The effects of grazing by lesser snow geese on the vegetation of a sub-arctic salt marsh. *Journal of Applied Ecology* 21:669–686.
- Clark, E. M., J. F. White, and R. M. Patterson. 1983. Improved histochemical techniques for the detection of *Acremonium coenophialum* in tall fescue and methods of in vitro culture of the fungus. *Journal of Microbiological Methods* 1:149–155.
- Clay, K. 1988. Fungal endophytes of grasses: a defensive mutualism between plants and fungi. *Ecology* 69:10–16.
- Clay, K., and C. Schardl. 2002. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *American Naturalist* 160:S99–S127.
- Clay, K. K., J. J. Holah, and J. A. J. Rudgers. 2005. Herbivores cause a rapid increase in hereditary symbiosis and alter plant community composition. *Proceeding of the National Academy of Sciences* 102:12465–12470.
- Conover, M. R., and T. A. Messmer. 1996. Feeding preferences and changes in mass of Canada geese grazing endophyte-infected tall fescue. *Condor* 98:859–862.
- Cruz-Hernández, A., A. Tomasini-Campocoso, L. J. Pérez-Flores, F. J. Fernández-Perrino, and M. Gutiérrez-Rojas. 2013. Inoculation of seed-borne fungus in the rhizosphere of *Festuca arundinacea* promotes hydrocarbon removal and pyrene accumulation in roots. *Plant and Soil* 362:261–270.
- Didiano, T. J., N. E. Turley, G. Everwand, H. Schaefer, M. J. Crawley, and M. T. J. Johnson. 2014. Experimental test of plant defence evolution in four species using long-term rabbit exclosures. *Journal of Ecology* 102:584–594.
- Earle, J. C., and K. A. Kershaw. 1989. Vegetation patterns in James Bay coastal marshes. 3. Salinity and elevation as factors influencing plant zonations. *Canadian Journal of Botany* 67:2967–2974.
- Ellsworth, K. T., T. N. Clark, C. A. Gray, and J. A. Johnson. 2013. Isolation and bioassay screening of medicinal plant endophytes from eastern Canada. *Canadian Journal of Microbiology* 59:761–765.
- Faeth, S. H. 2002. Are endophytic fungi defensive plant mutualists? *Oikos* 99:200.
- Faeth, S. H. 2009. Asexual fungal symbionts alter reproductive allocation and herbivory over time in their native perennial grass hosts. *American Naturalist* 173:554–565.
- Faeth, S. H., and W. F. Fagan. 2002. Fungal endophytes: common host plant symbionts but uncommon mutualists. *Integrative and Comparative Biology* 42:360–368.
- Faeth, S. H., and T. J. Sullivan. 2003. Mutualistic asexual endophytes in a native grass are usually parasitic. *American Naturalist* 161:310–325.

- Horischek, A. M., M. E. Afkhami, E. K. Seifert, and J. A. Rudgers. 2013. Fungal symbionts as manipulators of plant reproductive biology. *American Naturalist* 181:562–570.
- Grime, J. P., J. G. Hodgson, and R. Hunt. 2007. *Comparative Plant Ecology: A functional approach to common British species*. Castlepoint Press, Colvend, Dalbeattie, Kirkcudbrightshire, Scotland.
- Gundel, P. E., L. A. Garibaldi, M. A. Martínez-Ghersa, and C. M. Ghersa. 2011a. *Neotyphodium* endophyte transmission to *Lolium multiflorum* seeds depends on the host plant fitness. *Environmental and Experimental Botany* 71:359–366.
- Gundel, P. E., J. A. Rudgers, and C. M. Ghersa. 2011b. Incorporating the process of vertical transmission into understanding of host-symbiont dynamics. *Oikos* 120:1121–1128.
- Gundel, P. E., L. A. Garibaldi, M. Helander, and K. Saikkonen. 2013. Symbiotic interactions as drivers of trade-offs in plants: effects of fungal endophytes on tall fescue. *Fungal Diversity* 60:5–14.
- Hamayun, M., S. A. Khan, I. Iqbal, C.-I. Na, A. L. Khan, Y.-H. Hwang, B.-H. Lee, and I.-J. Lee. 2009a. *Chrysosporium pseudomerdarium* produces gibberellins and promotes plant growth. *Journal of Microbiology* 47:425–430.
- Hamayun, M., et al. 2009b. *Cladosporium sphaerospermum* as a new plant growth-promoting endophyte from the roots of *Glycine max* (L.) Merr. *World Journal of Microbiology and Biotechnology* 25:627–632.
- Herrero, N., S. Sánchez Márquez, and Í. Zabalgoitia. 2009. Mycoviruses are common among different species of endophytic fungi of grasses. *Archives of Virology* 154:327–330.
- Hik, D. S., and R. L. Jefferies. 1990. Increases in the net above-ground primary production of a salt-marsh forage grass: a test of the predictions of the herbivore-optimization model. *Journal of Ecology* 78:180–195.
- Hodgson, S., C. de Cates, J. Hodgson, N. J. Morley, B. C. Sutton, and A. C. Gange. 2014. Vertical transmission of fungal endophytes is widespread in forbs. *Ecology and Evolution* 4:1199–1208.
- Iacobelli, A., and R. L. Jefferies. 1991. Inverse salinity gradients in coastal marshes and the death of stands of *Salix* - the effects of grubbing by geese. *Journal of Ecology* 79:61–73.
- Iannone, L. J., J. F. White, L. M. Giussani, D. Cabral, and M. Victoria Novas. 2011. Diversity and distribution of *Neotyphodium*-infected grasses in Argentina. *Mycological Progress* 10:9–19.
- Jefferies, R. L. 1988. Pattern and process in arctic coastal vegetation in response to foraging by lesser snow geese. Pages 341–369 in L. D. Gottlieb, and S. K. Jain, editors. *Plant evolutionary biology*. Chapman and Hall, London, UK.
- Jefferies, R. L., and R. F. Rockwell. 2002. Foraging geese, vegetation loss and soil degradation in an Arctic salt marsh. *Applied Vegetation Science* 5:7–16.
- Jefferies, R. L., R. F. Rockwell, and K. F. Abraham. 2003. The embarrassment of riches: agricultural food subsidies, high goose numbers and loss of Arctic wetlands – a continuing saga. *National Research Council Canada, Environmental Reviews* 11:193–232.
- Jefferies, R. L., A. P. Jano, and K. F. Abraham. 2006. A biotic agent promotes large-scale catastrophic change in the coastal marshes of Hudson Bay. *Journal of Ecology* 94:234–242.
- Ju, H.-J., N. S. Hill, T. Abbott, and T. Ingram. 2006. Temperature influences on endophyte growth in tall fescue. *Crop Science* 46:404–412.
- Kane, K. H. 2011. Effects of endophyte infection on drought stress tolerance of *Lolium perenne* accessions from the Mediterranean region. *Environmental and Experimental Botany* 71:337–344.
- Koh, S. S., and D. S. D. Hik. 2007. Herbivory mediates grass-endophyte relationships. *Ecology* 88:2752–2757.
- Kotanen, P. M., and K. F. Abraham. 2013. Decadal changes in vegetation of a subarctic salt marsh used by lesser snow and Canada geese. *Plant Ecology* 214:409–422.
- Larimer, A. L., J. D. Bever, and K. Clay. 2010. The interactive effects of plant-microbial symbionts: a review and meta-analysis. *Symbiosis* 51:139–148.
- Leafloor, J. O., K. F. Abraham, D. H. Rusch, R. K. Ross, and M. R. J. Hill. 1996. Status of the Southern James Bay Population of Canada geese. Pages 103–108 in J. Ratti, editor. *Proc 7th International Waterfowl Symposium*. Ducks Unlimited, Memphis, Tennessee.
- Leafloor, J. O., M. R. J. Hill, D. H. Rusch, K. F. Abraham and R. K. Ross. 2000. Nesting ecology and gosling survival of Canada geese on Akimiski Island, Northwest Territories. Pages 109–116 in K. M. Dickson, editor, *Towards conservation of the diversity of Canada geese (Branta canadensis)*. Vol. 103. Canadian Wildlife Services Occasional Paper, Ottawa, Ontario.
- Leuchtmann, A. 1992. Systematics, distribution, and host specificity of grass endophytes. *Natural Toxins* 1:150–162.
- Leuchtmann, A., D. Schmidt, and L. P. Bush. 2000. Different levels of protective alkaloids in grasses with stroma-forming and seed-transmitted *Epichloë/Neotyphodium* endophytes. *Journal of Chemical Ecology* 26:1025–1036.
- Marks, S., K. Clay, and G. P. Cheplick. 1991. Effects of fungal endophytes on interspecific and intraspecific competition in the grasses *Festuca arundinacea*

- and *Lolium perenne*. *Journal of Applied Ecology* 28:194–204.
- Martini, I. P., and W. A. Glooschenko. 1984. Emergent coasts of Akimiski Island, James Bay, Northwestern Territories, Canada: geology, geomorphology, and vegetation. *Sedimentary Geology* 37:229–250.
- McLaren, J. R., and R. L. Jefferies. 2004. Initiation and maintenance of vegetation mosaics in an arctic salt marsh. *Journal of Ecology* 92:648–660.
- Nagabhyru, P., R. D. Dinkins, C. L. Wood, C. W. Bacon, and C. L. Schardl. 2013. Tall fescue endophyte effects on tolerance to water-deficit stress. *BMC Plant Biology* 13:1.
- Núñez-Farfán, J., J. Fornoni, and P. L. Valverde. 2007. The evolution of resistance and tolerance to herbivores. *Annual Review of Ecology, Evolution, and Systematics* 38:541–566.
- O, P. C., P. M. Kotanen, and K. F. Abraham. 2005. Survival and growth of the forage grass *Festuca rubra* in naturally and artificially revegetated sites in a sub-arctic coastal marsh. *Écoscience* 12:279–285.
- O, P. C., P. M. Kotanen, and K. F. Abraham. 2006. Geese and grazing lawns: responses of the grass *Festuca rubra* to defoliation in a subarctic coastal marsh. *Botany-Botanique* 84:1732–1739.
- Rai, M., D. Rathod, G. Agarkar, M. Dar, M. Brestic, G. M. Pastore, and M. R. M. Junior. 2014. Fungal growth promotor endophytes: a pragmatic approach towards sustainable food and agriculture. *Symbiosis* 62:63–79.
- Raps, A., and S. Vidal. 1998. Indirect effects of an unspecialized endophytic fungus on specialized plant-herbivorous insect interactions. *Oecologia* 114:541–547.
- R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Reza Sabzalain, M., and A. Mirlohi. 2010. *Neotyphodium* endophytes trigger salt resistance in tall and meadow fescues. *Journal of Plant Nutrition and Soil Science* 173:952–957.
- Rodriguez, R. J., J. F. Jr White, A. E. Arnold, and R. S. Redman. 2009. Fungal endophytes: diversity and functional roles. *New Phytologist* 182:314–330.
- Rosenthal, J. P., and P. M. Kotanen. 1994. Terrestrial plant tolerance to herbivory. *Trends in Ecology & Evolution* 9:145–148.
- Rudgers, J. A. J., T. E. X. T. Miller, S. M. S. Ziegler, and K. D. K. Craven. 2012. There are many ways to be a mutualist: endophytic fungus reduces plant survival but increases population growth. *Ecology* 93:565–574.
- Saikkonen, K. 2004. Evolution of endophyte-plant symbioses. *Trends in Plant Science* 9:275–280.
- Saikkonen, K., S. H. Faeth, M. Helander, and T. J. Sullivan. 1998. Fungal endophytes: a continuum of interactions with host plants. *Annual Review of Ecology and Systematics* 29:319–343.
- Saikkonen, K., P. Lehtonen, M. Helander, J. Koricheva, and S. H. Faeth. 2006. Model systems in ecology: dissecting the endophyte-grass literature. *Trends in Plant Science* 11:428–433.
- Sánchez Márquez, S., G. F. Bills, N. Herrero, and Í. Zabalgogea. 2012. Non-systemic fungal endophytes of grasses. *Fungal Ecology* 5:289–297.
- Santangelo, J. S., N. E. Turley, and M. T. J. Johnson. 2015. Fungal endophytes of *Festuca rubra* increase in frequency following long-term exclusion of rabbits. *Botany-Botanique* 93:1–9.
- Schardl, C. L., A. Leuchtmann, and M. J. Spiering. 2004. Symbioses of grasses with seedborne fungal endophytes. *Annual Review of Plant Biology* 55:315–340.
- Semmartin, M., M. Omacini, P. E. Gundel, and I. M. Hernández-Agramonte. 2015. Broad-scale variation of fungal-endophyte incidence in temperate grasses. *Journal of Ecology* 103:184–190.
- Srivastava, D. S., and R. L. Jefferies. 1995. Mosaics of vegetation and soil salinity: a consequence of goose foraging in an arctic salt marsh. *Canadian Journal of Botany* 73:75–83.
- Srivastava, D. S., and R. L. Jefferies. 1996. A positive feedback: Herbivory, plant growth, salinity, and the desertification of an Arctic salt-marsh. *Journal of Ecology* 84:31–42.
- Strauss, S. Y., and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology & Evolution* 14:179–185.
- Thakur, A., S. Kaur, A. Kaur, and V. Singh. 2013. Enhanced resistance to *Spodoptera litura* in endophyte infected cauliflower plants. *Environmental Entomology* 42:240–246.
- Turley, N. E., W. C. Odell, H. Schaefer, G. Everwand, M. J. Crawley, and M. T. J. Johnson. 2013. Contemporary evolution of plant growth rate following experimental removal of herbivores. *American Naturalist* 181:S21–S34.
- Varvas, T., K. Kasekamp, and B. Kullman. 2013. Preliminary study of endophytic fungi in timothy (*Phleum pratense*) in Estonia. *Acta Mycologia* 48:41–49.
- Vega, F. E., F. Posada, M. Catherine Aime, M. Pava-Ripoll, F. Infante, and S. A. Rehner. 2008. Entomopathogenic fungal endophytes. *Biological Control* 46:72–82.
- Wäli, P. R., J. U. Ahlholm, M. Helander, and K. Saikkonen. 2006. Occurrence and genetic structure of the systemic grass endophyte *Epichloë festucae* in fine fescue populations. *Microbial Ecology* 53:20–29.
- Waqas, M., A. Khan, L. Ali, S.-M. Kang, Y.-H. Kim, and I.-J. Lee. 2013. Seed germination-influencing

- bioactive secondary metabolites secreted by the endophyte *Cladosporium cladosporioides* LWL5. *Molecules* 18:15519–15530.
- Yang, T., Y. Chen, X.-X. Wang, and C.-C. Dai. 2013. Plant symbionts: keys to the phytosphere. *Symbiosis* 59:1–14.
- Yin, L., A. Ren, M. Wei, L. Wu, Y. Zhou, X. Li, and Y. Gao. 2014. Neotyphodium coenophialum-infected tall fescue and its potential application in the phytoremediation of saline soils. *International Journal of Phytoremediation* 16:235–246.
- Zabalgogezcoa, Í., A. G. Ciudad, B. R. Vázquez de Aldana, and B. G. Criado. 2006. Effects of the infection by the fungal endophyte *Epichloë festucae* in the growth and nutrient content of *Festuca rubra*. *European Journal of Agronomy* 24:374–384.
- Zabalgogezcoa, Í., P. E. Gundel, M. Helander, and K. Saikkonen. 2013. Non-systemic fungal endophytes in *Festuca rubra* plants infected by *Epichloë festucae* in subarctic habitats. *Fungal Diversity* 60:25–32.

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.1260/supinfo>