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Root fungal symbionts interact with mammalian herbivory, soil nutrient availability and specific habitat conditions

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Abstract Herbivory, competition and soil fertility interactively shape plant communities and exhibit an important role in modifying conditions for host-dependent fungal symbionts. However, field studies on the combined impacts of natural herbivory, competition and soil fertility on root fungal symbionts are rare. We asked how mammalian herbivory, fertilization, liming and plant–plant competition affect the root colonization of arbuscular mycorrhizal fungi (AMF) and dark septate endophytic (DSE) fungi of the dicot herb, *Solidago virgaurea.* The 2-year full-factorial experiment was conducted in two contrasting habitats: non-acidic and acidic mountain tundra. We found that herbivory increased arbuscular colonization (i.e. the site of resource exchange) at fertile non-acidic sites, where vegetation was rich in species having AMF symbionts, whereas at infertile acidic sites, where plants having AMF symbiont are scarce, the response was the opposite. Herbivory of the host plant negatively affected DSE hyphal and sclerotial colonization in unfertilized plots, possibly due to reduced carbon flow from the host plant while there was no effect of herbivory in fertilized plots. DSE colonization was highest in unfertilized exclosures where soil nutrient concentrations were also lowest. Liming had a negative effect on DSE hyphal colonization, and its effect also interacted with herbivory and the habitat. Biomass removal of the neighboring plants did not affect the root colonization percent of either arbuscules or DSE. Our results show that the impacts of aboveground mammalian herbivory, soil nutrient availability and specific habitat conditions on belowground root fungal symbionts are highly dependent on each other. Arbuscule response to herbivory appeared to be regulated by specific habitat conditions possibly caused by differences in the AMF availability in the soil while DSE response was associated with availability of host-derived carbon. Our result of the relationship between herbivory and soil nutrients suggests an important role of DSE in ecosystem processes.

Keywords Arbuscular mycorrhiza · Carbon limitation · Dark septate endophytes · Grazing · Soil fertility

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Introduction

Biotic and abiotic factors determining plant performance are likely to exhibit a concordant impact on belowground plant symbiotic associations. Herbivory, plant–plant competition and soil nutrient availability play key roles in shaping plant performance and community dynamics (e.g., Grace and Tilman 1990; Crawley 1997) and should, therefore, play important roles in modifying the conditions for host-dependent fungal symbiosis, such as mycorrhiza (Smith and Read 2008). On the other hand, mycorrhiza and other members of the soil are considered important drivers of aboveground ecosystem function and diversity (Reynolds et al. 2003; Bennett et al. 2006; Van der Heijden et al. 2008).

In theory, herbivory, plant–plant competition and high nutrient availability could exhibit a negative impact on the mycorrhizal symbiosis (Marschner et al. 1996; Gehring and Whitham 1994, 2002; Smith and Read 2008). This is due to the bidirectional nature of the plant–fungal relationship, where the fungal component provides nutrients to the plant

in exchange for carbon, and both carbon limitation and surplus of soil nutrients, either alone or in interaction, can lead to an imbalance of the symbiosis (Johnson et al. 1997). Both the loss of foliar biomass to herbivores or reduced growth caused by plant-neighborhood competition may lead to carbon limitation. Findings of empirical studies of herbivore impacts on mycorrhizal symbiosis range from positive to negative depending on host plant species, study system and intensity of herbivory; however, most studies report negative effects (reviewed by Gehring and Whitham 1994, 2002; see also Wallace 1987; Eom et al. 2001; Gange et al. 2002; Lugo et al. 2003; Grigera and Oesterheld 2004; Kula et al. 2005; Wearn and Gange 2007). A recent meta-analysis by Barto and Rillig (2010), however, challenged this view and argued that herbivory has only a minor impact on mycorrhizal colonization. Experiments on responses of mycorrhizal colonization to plant–plant competition also show mixed outcomes: positive (Urcelay et al. 2003), negative (McHugh and Gehring 2006) and neutral effects on mycorrhizal colonization (Hartley and Amos 1999; Titus and Leps 2000) have been reported.

High nutrient levels are often associated with low or decreased mycorrhizal colonization in experimental systems (e.g., Johnson et al. 1997, 2003, 2010; Hartley and Amos 1999; Cornwell et al. 2001; Smith and Read 2008). In nutrient-rich conditions, the dependency of the host plant on mycorrhizal compartment may decrease because it is more cost-efficient to obtain nutrients directly from the soil than via carbon-demanding mycorrhizal fungi (Tuomi et al. 2001). In contrast, a number of field studies have also reported fertilization to have no impact on mycorrhizal colonization (Heijne et al. 1994; Caporn et al. 1995; Michelsen et al. 1999; Johansson 2000; Urcelay et al. 2003). Soil nutrient availability is also closely associated with soil pH (Kinzel 1983; Eskelinen et al. 2009), which, in turn, affects microbial communities (Madigan et al. 2003; Högberg et al. 2007; Eskelinen et al. 2009). The occurrence of different mycorrhizal fungal types coincides with changes in soil pH (Read 1991), and colonization by AM fungi has been found to decrease along with a decreasing pH gradient (Postma et al. 2007). However, although both soil nutrient availability and pH are strongly linked to each other and may have individual and joint effects, we are unaware of studies experimentally separating their effects on root symbiotic fungi.

Both theory and empirical studies show that the impacts of herbivory and competition on plant performance and community diversity change along soil fertility gradients (e.g., Grime 1973; Oksanen et al. 1981; Louda et al. 1990; Bakker et al. 2006; Eskelinen 2008). Similarly, it could be anticipated that herbivory and competition effects on a plant's mycorrhizal symbionts would depend on habitat fertility and soil nutrient availability. Gehring and Whitham (2002) hypothesized that, although, on average, increasing intensity of herbivory decreases mycorrhizal colonization, herbivory also interacts with the level of abiotic environmental harshness: high herbivory pressure should have a more negative impact on mycorrhizal colonization in low-productivity habitats with low resource availability and harsh environmental conditions compared to more benign environments. At a low level of herbivory, an opposite pattern is predicted: mycorrhizal colonization should be higher in habitats of high abiotic stress (in terms of low resource availability) compared to habitats of low abiotic stress (with high resource availability). These discrepancies are predicted to arise from differences in host plant carbon allocation to their mycorrhizal symbionts. Plants in resource-poor habitats may reduce their carbon allocation to mycorrhizal fungi more quickly when they are subjected to a high level of herbivory, compared to plants in resource-rich habitats, whereas, at a low level of herbivory, plants in resource-poor sites may allocate relatively more carbon to mycorrhiza and maintain higher mycorrhizal colonization to meet the nutritionally more severe conditions, compared to the plants in more favorable sites (Gehring and Whitham 2002). However, although it is widely accepted that the functioning of mycorrhizal symbiosis depends on both biotic and abiotic contexts (e.g., Hoeksema et al. 2010), and despite relatively well-known interactive effects of herbivory, nutrient availability and plant–plant competition on host plant performance, experimental field studies on their combined effects on mycorrhizal colonization, the basic measure of mycorrhizal symbiosis and function, are surprisingly still absent.

We carried out a factorial field experiment to test the interactive effects of herbivory, plant–plant competition and soil fertility on root fungal colonization of the dicot herb *Solidago virgaurea* in a mountain tundra ecosystem. We investigated the colonization of arbuscular mycorrhiza (AM), which facilitates plant nutrient uptake, both nitrogen and phosphorus (Read 1991), and colonization of dark septate endophytic fungi (DSE), which are globally ubiquitous root-associates of plants, occurring abundantly across various ecosystems and plant taxa. Several studies have shown root DSE colonization to be associated with improved plant growth and nitrogen uptake (reviewed by Jumpponen and Trappe 1998; Jumpponen 2001; Smith and Read 2008; Rodriguez et al. 2009). Usuki and Narisawa (2007) were the first to demonstrate that carbon fixed by the host plant was given to a root-associated DSE fungus, but the mechanistic details on improved nutrient uptake of the host are still unknown. Despite a plethora of studies on impacts of different factors on root colonization percent in arbuscular mycorrhizal fungi (AMF) (Smith and Read 2008 and references therein) and DSE (Mandyam and Jumpponen 2005), existing data are rarely based on factorial field experiments.

Since soil fertility can be affected by both nutrient concentrations and soil pH, and these may have separate effects on microbial community, we used both fertilization and liming to experimentally manipulate soil fertility. We also replicated all treatments in a full-factorial design in two different habitat types, fertile non-acidic heaths and infertile acidic habitats, which allowed us to separately test natural differences in habitat fertility and experimentally imposed changes in soil fertility. Our specific objectives were to (1) determine the individual and interactive effects of above-ground herbivory, soil fertility and plant–plant competition on fungal colonizations in plant roots, and (2) to assess the individual and joint effects of different soil fertility factors (soil pH, nutrient availability, and habitat fertility) on fungal colonization rates.

Materials and methods

Study system and sites

The experiment was conducted on Mt. Saana in Kilpisjärvi (69°03′N, 20°50′E), north-western Finland. The tree line formed by mountain birch (*Betula pubescens* ssp. *czerepanowii*) lies at ca. 600–700 m a.s.l. In the area, the mean annual temperature is −2.6°C and the mean annual precipitation is 420 mm (measured at Kilpisjärvi climatological station at 483 m a.s.l.; Järvinen 1987). The main mammalian herbivores are semi-domesticated reindeer (*Rangifer tarandus*), which graze on Mt. Saana in July, microtine rodents such as grey-sided voles (*Clethrionomus rufocanus*) and mountain hares (*Lepus timidus*), which are encountered occasionally. No signs of insect herbivory were seen on *S. virgaurea* or other plants in the experimental plots during the two study years (A. Eskelinen, personal observation).

On the SW and NE slopes of Mt. Saana, the bedrock consists of a mosaic of dolomitic and siliceous rocks, resulting in non-acid and relatively fertile soils characterized by herb- and graminoid-rich *Dryas* heaths, and acid barren soils dominated by dwarf shrub-rich *Empetrum* heaths, respectively. Depending on the small-scale heterogeneity in underlying bedrock material, these two heath types form mosaics where non-acidic and acidic heath vegetation patches alternate within short distances (i.e. within a few tens of meters). In autumn 2004, five non-acidic and five acidic heath vegetation patches (hereafter called sites) that were interspersed with each other within a distance of 5 km were chosen. The sites represented as similar moisture and topographical conditions as possible and were located at altitudes ranging from 720 to 800 m a.s.l. Acidic heath sites were dominated by dwarf shrubs (*Empetrum nigrum* ssp. *hermaphroditum*, *Vaccinium* sp. and *Betula nana*), which were significantly more abundant in acidic than in non-acidic sites at the beginning of the experiment (Eskelinen et al. 2009). Graminoids (e.g., *Calamagrostis lapponica*, *Carex bigelowii*, *Festuca ovina*) and forbs (e.g., *Bistorta vivipara*, *Pedicularis lapponica*) occurred sparsely. Typical species of non-acidic heath sites were arctic-alpine forbs (e.g., *Astragalus frigidus*, *Dryas octopetala*, *Saussurea alpina*, *Saxifraga oppositifolia*, *Silene acaulis*, *Thalictrum alpinum*), which were significantly more abundant in non-acidic than in acidic sites (Eskelinen et al. 2009). Dwarf shrubs preferring high pH habitats (e.g., *Cassiope tetragona*, *Rhododendron lapponicum*, *Salix reticulata*) and graminoids (e.g., *Carex rupestris*, *Carex vaginata*) were also abundant, and most of the species of acidic heaths occurred commonly. Before the application of the experimental treatments, soil pH and NH_4–N content were significantly higher in non-acidic than in acidic heaths (Eskelinen et al. 2009).

Experimental design and treatments

The experimental design and treatments follow an earlier experiment conducted in the same area and sites (Eskelinen 2008). In this experiment, plantlets of *S. virgaurea* L. (hereafter referred to as *Solidago*) had been transplanted into different sites (acidic and non-acidic) and treatments (herbivore exclusion, fertilization, liming and biomass removal) on Mt. Saana, and plant survival, growth and flowering in response to these treatments were reported in Eskelinen (2008). Here, we report the arbuscular and dark septate (DSE) fungal colonization of *Solidago* roots in response to the same experimental treatments and habitat.

At each of the ten chosen sites, eight 50 × 25 cm plots were established, resulting in 80 plots in total. Each plot was further split into two 25 × 25 cm subplots, and one of these was randomly assigned to the neighbor removal treatment. Given the height of vegetation (5–15 cm) and the small size of plants at the studied habitats (up to 19 vascular species per 25 × 25 cm subplot), this plot size was suitable for our experiment. There were 160 subplots in the experiment. The transplants of *Solidago* were later planted into these subplots, either with or without neighboring vegetation. The main plots (consisting of two subplots) were randomly assigned to three treatments in a factorial design: (1) herbivore exclusion, (2) fertilization, and (3) liming, or control. This design resulted in one replicate of each treatment combination per site.

In autumn 2004, seeds of *Solidago* were collected from the study area, cold-stratified for 5 months and grown in a greenhouse for 3.5 months. Two seedlings of *Solidago* were planted per small pot (5 × 5 cm in diameter and 7 cm deep) and these were transplanted into 25 × 25 cm subplots (one pot per subplot) at Mt. Saana at the beginning

of June 2005. Plants were removed from the pots just before planting. The plots were fertilized, limed and watered (500 ml water from nearby brooks) for the first time just after the planting.

The neighbor removal treatment was accomplished by removing all above-ground plant biomass from an area of 25 × 25 cm. Roots were left intact to avoid disturbance of the soil. For the grazer exclusion treatment, 80–100 cm fences were established on half of the experimental plots in late autumn 2004. The exclosures were circular and ca. 1.5 m in diameter. They were made of galvanized net (mesh size 1.2 × 1.2 cm) and dug into the soil to a depth of 10 cm to prevent grazing by all mammalian herbivores, including voles. To manipulate soil nutrient availability, commercial fast-dissolving NPK fertilizer (16-9-22) was applied to the fertilization treatment plots twice per growing season, a total of 9.6 g N m^{-2}, 5.4 g P m^{-2}, 13.2 g K m^{-2} per year. To manipulate soil pH, dolomite lime ($CaMg(CO_3)_2$) was applied to the liming treatment plots at a total amount of 300 g/m^2 in 2005 (at the same time when fertilizer was applied) and 600 g/m^2 in 2006, as the first application of lime did not alter soil pH sufficiently. All experimental blocks were watered with 500 ml of water from nearby brooks immediately following fertilization and lime application. Both applications were made to a 15-cm-wide zone around each experimental plot.

Sampling and root colonization analysis

In mid-August 2006, at the time of harvesting of the above-ground biomass of *Solidago* individuals (see Eskelinen 2008), root samples were sampled with a soil corer (diameter 3 cm) from one of the two *Solidago* individuals in each subplot. We first cut the above-ground part of the stem of the host plant. Immediately after that, we placed the soil corer around the remaining base of the stem in the soil and took the soil sample. By following the base of the *Solidago* stem we were able to separate fine roots originating from *Solidago* from other plants' roots in the soil. Earlier observations from the study area have indicated that one sampling is a good estimate of root fungal colonization for whole growing season (Ruotsalainen et al. 2002). Soil samples were kept in a freezer (−18°C) until preparation. To analyze the fungal colonization of the roots, the soil cores were thawed and the roots were cleaned by subsequent washings with tap water. Roots were stained by using a modified Phillips and Hayman (1970) method as follows: KOH 10% overnight, rinsing with tap water, alkaline H_2O_2 20 min, rinsing with tap water, 1% HCl 2.5 h, staining with 0.01% trypan blue in lactoglycerol in 80°C for 1 h. After staining, roots were preserved in lactoglycerol at room temperature. Colonization percent of the fungal structures (arbuscules and vesicles for AM fungi, DSE hyphae and DSE sclerotia) were quantified by using the magnified intersections method (McGonigle et al. 1990). AMF vesicles are not reported further; they occurred in less than 8% of the samples, average colonization being as low as 3.4% in those samples. Analysis of AMF hyphae was omitted because their identification was considered unreliable due to the light staining of the samples. Although it would have been informative to obtain data on other AMF structures too (Johnson et al. 2003), arbuscules are considered the sites of nutrient exchange between the fungi and host plant, and are therefore good indicators of functional symbiosis (Smith and Read 2008), and they were reported for each sample. DSE hyphal colonization was analyzed although their function is unknown (Jumpponen and Trappe 1998; Mandyam and Jumpponen 2005). Sclerotia are assumed to serve as survival and dispersal structures of the DSE fungi (Currah et al. 1993; Jumpponen and Trappe 1998; Ruotsalainen et al. 2002; A.L. Ruotsalainen, unpublished data) and these structures are also included in our counts of DSE colonization.

Data analysis

We used linear mixed effects models (Pinheiro and Bates 2000; Crawley 2007) to investigate the habitat and treatment effects on arbuscular and DSE colonization in *Solidago* roots. In the models, percentages of arbuscules and dark septate structures (hyphae, sclerotia) were used as dependent variables, and the habitat, herbivore exclusion, fertilization, liming, and biomass removal were used as explanatory variables (fixed factors). To account for the hierarchical structure in the experimental set-up, biomass removal treatment (subplots) was nested within plot-level treatments (herbivore exclusion, fertilization and liming) which were nested within sites.

All colonization data were arcsine-transformed before the analysis to improve the homogeneity of variances (Zar 1999; Crawley 2007). Model fits were inspected using model diagnostic plots (Crawley 2007), and these data fulfilled the homogeneity assumptions. The analyses were restricted to three-way interactions. The R statistical environment was used to perform all the analyses (R Development Core Team 2007).

Results

Arbuscular colonization

The arbuscular colonization of *Solidago* roots was significantly affected by an interaction between the habitat type and herbivore exclusion (Table 1). In fertile non-acidic habitats, exclusion of mammalian herbivores had a

Table 1 Results from linear mixed effects models of the impacts of habitat, exclosure, fertilization and liming on arbuscular mycorrhiza (*arbuscules*) and dark-septate (*DSE*) hyphal and sclerotial colonizations (%) in roots of *Solidago virgaurea*

Source of variation	Arbuscules		DSE hyphae		DSE sclerotia	
	F_{df}	P	F_{df}	P	F_{df}	P
Habitat	$0.029_{1,8}$	0.8688	$1.624_{1,8}$	0.2382	$3.239_{1,8}$	0.1096
Exclosure	$2.771_{1,55}$	0.1017	$4.690_{1,55}$	**0.0347**	$3.258_{1,55}$	0.0765
Fertilization	$0.221_{1,55}$	0.6400	$2.587_{1,55}$	0.1135	$2.453_{1,55}$	0.1230
Liming	$0.426_{1,55}$	0.5167	$5.980_{1,55}$	**0.0177**	$1.525_{1,55}$	0.2221
Competition	$0.003_{1,44}$	0.9585	$0.318_{1,44}$	0.5754	$0.135_{1,44}$	0.7146
Habitat × exclosure	$8.230_{1,55}$	**0.0058**	$0.055_{1,55}$	0.8153	$1.304_{1,55}$	0.2584
Exclosure × fertilizer	$0.578_{1,55}$	0.4503	$6.941_{1,55}$	**0.0109**	$9.083_{1,55}$	**0.0039**
Habitat × exclosure × fertilization	$0.812_{1,55}$	0.3715	$0.015_{1,55}$	0.9035	$6.008_{1,55}$	**0.0174**
Habitat × exclosure × liming	$1.073_{1,55}$	0.3048	$5.633_{1,55}$	**0.0211**	$0.990_{1,55}$	0.3240

In the models, fertilizer, liming and exclosure treatments were nested within the habitat. All main effects but only significant interactions are reported: $P < 0.05$ in bold

negative impact on arbuscular colonization, whereas in infertile acidic habitats, herbivore exclusion had a positive effect on arbuscular colonization (Fig. 1). Other treatments, fertilization, liming and neighbor removal, had no significant main or interactive effects on arbuscular colonization.

DSE colonization

The hyphae and sclerotia of DSE fungi in *Solidago* roots showed clear and strong responses to most of the experimental treatments (except for the neighbor removal treatment, of which DSE colonization was independent; Table 1) and to the habitat. Herbivore exclusion had a positive main effect on DSE hyphal colonization and its effect on both DSE hyphae and sclerotia also depended on fertilization, as shown by a significant interaction between herbivore exclusion and fertilization (Table 1). In unfenced plots accessible to herbivores, the DSE colonization was slightly positively influenced by fertilizer addition, while inside exclosures the colonization was much higher in unfertilized than in fertilized plots (Figs. 2, 3). Overall, the colonization was highest in unfertilized exclosures. The responses of DSE sclerotia to herbivores and fertilization also varied depending on the habitat type as indicated by a three-way interaction between the habitat, exclosure and fertilization (Table 1). In infertile acidic habitats, where the colonization of DSE sclerotia was much higher than in fertile non-acidic habitats, the negative impact of fertilization inside exclosures was much more pronounced than in non-acidic habitats (Fig. 3). Liming had a negative main effect on DSE hyphal colonization (Table 1; Fig. 2). There was also a significant three-way interaction between the habitat, exclosure and liming (Table 1): in fertile non-acidic habitats, liming had a negative impact on DSE hyphae only outside exclosures, whereas in infertile acidic habitats, liming decreased DSE colonization in both fenced and unfenced plots, the negative impact of liming being much greater in fenced plots not accessible to grazers (Fig. 2).

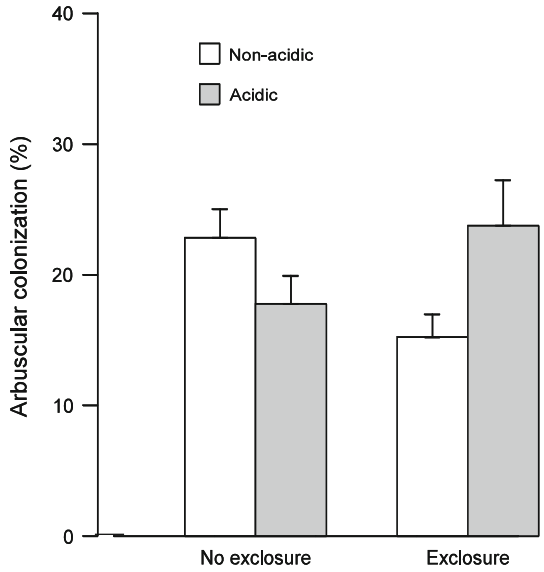

Fig. 1 Root arbuscular colonization of transplanted *Solidago virgaurea* in relation to herbivore exclusion treatment and habitat (non-acidic or acidic). *Bars* show pooled mean + SE across other treatments (biomass removal, fertilization and liming) to show significant treatment interactions

Discussion

Our results show that herbivory and soil fertility have strong interactive effects on both arbuscular and DSE colonization of *Solidago*, but the detailed responses of these fungal groups also exhibit marked differences. These

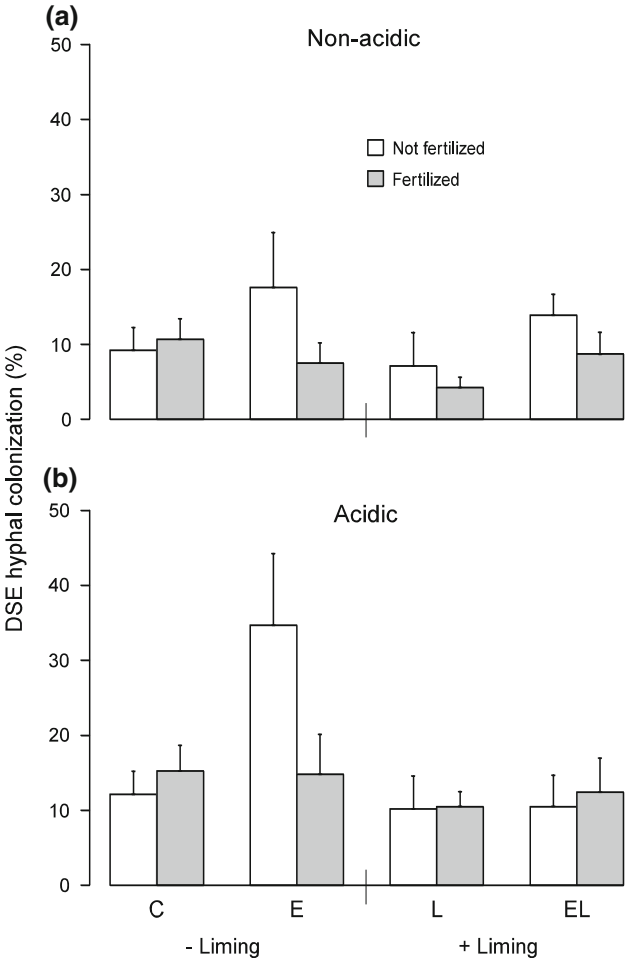

Fig. 2 Root DSE hyphal colonization of transplanted *Solidago virgaurea* in **a** non-acidic and **b** acidic habitats in response to fertilization in control (*C*), herbivore exclusion (*E*) and liming (*L*) treatments. *Bars* show pooled mean + SE across biomass removal treatment

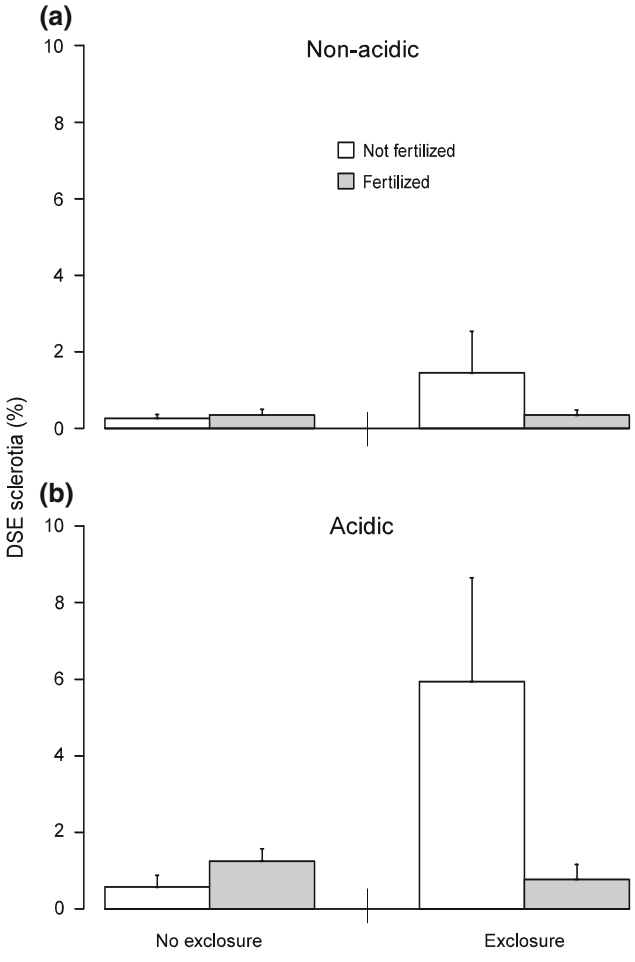

Fig. 3 Root colonization of DSE sclerotia of transplanted *Solidago virgaurea* in **a** non-acidic and **b** acidic habitats in relation to herbivore exclusion and fertilization treatments. *Bars* show pooled mean + SE across biomass removal and liming treatments

findings are concordant with the biomass losses in response to herbivory and increased growth in response to fertilization of the host plant under the same experimental setup (Eskelinen 2008). Plant–plant competition was not found to affect root fungal colonization either as a main factor or in interaction with other treatments, which concurs with the negligible impact of neighborhood competition on the performance of the host plant (Eskelinen 2008). In general, our results are somewhat contradictory to the meta-analysis of Barto and Rillig (2010) which suggested only a minor effect of herbivory on mycorrhizal colonization. On the basis of our findings, we argue that their results may be confounded by pooling all different habitats and ecosystems together, thereby being unable to detect the dependency of herbivory effects on habitat characteristics. Furthermore, we show that herbivory and soil nutrient availability impose a strong interactive impact on DSE fungal colonization, a group of root symbiotic fungi that has been largely ignored by ecological studies but may possess a greater functional role in plant–fungal interactions than previously thought.

Responses of arbuscular colonization

Effects of herbivory by mammalian grazers on arbuscular colonization differed between the habitats: in fertile non-acidic habitats, herbivory had a positive impact, and in nutrient-poor acidic habitats, the response was negative. This pattern is qualitatively in accordance with the hypothesis of Gehring and Whitham (2002); given that our nutrient-poor habitat represents their definition of abiotic stress and our exclosure treatment is comparable to their expression of low herbivory intensity. However, their hypothesis also assumed a decrease in mycorrhizal colonization with increasing herbivory pressure both in high-stress and low-stress environments—in our experiment, the

presence of herbivory did not cause a general reduction in colonization. Furthermore, we did not detect a similar interaction between experimental nutrient addition and herbivory on arbuscular colonization. Differences in pH-dependent availability of phosphorus (Frank 2008) could also have played a role in dictating habitat-specific responses to herbivory. However, despite a significant difference in pH between limed and non-limed plots (Eskelinen 2008), liming did not have any effect on arbuscular colonization. Therefore, contrary to the hypothesis of Gehring and Whitham (2002), it seems that nutrient availability in soil does not directly explain our findings.

We suggest that habitat-specific differences in soil biotic environment, related to plant community composition rather than soil nutrient availability, could explain the different responses of arbuscular colonization to herbivory in the two habitat types. Vegetation in non-acidic habitats is dominated by forbs and graminoids, which are typical AMF species (Trappe 1987). In contrast, acidic habitats are characterized by ericoid dwarf shrubs that support ericoid mycorrhizal symbionts, and arbuscular mycorrhizal plant species occur very sporadically in this habitat type (Eskelinen et al. 2009). AMF seedlings receive their mycorrhizal colonization either from spores in the soil or hyphae connected to established vegetation (Read et al. 1976; Simard and Durrall 2004), which can facilitate seedling establishment (Hartnett et al. 1994; reviewed by Van der Heijden and Horton 2009). Although we did not measure the availability of AMF in the soil, it is likely to be higher in non-acidic habitats due to the markedly higher coverage of AMF plant species in vegetation, which may play an important role in dictating the functioning of plant–fungal relationships. We suggest that a high abundance of AMF in the rhizosphere and in the surrounding soil may maintain and buffer against changes in root fungal colonization despite a severe reduction in aboveground host biomass. This may explain why herbivory did not decrease arbuscular colonization in fertile non-acidic habitats. In contrast, in barren acidic habitats, grazing by mammalian herbivores seems to lead to decreased arbuscular colonization, potentially indicating both lower carbon supply to the fungal symbiont under grazing (in agreement with Gehring and Whitham 1994; 2002) and lower availability of fungal symbionts in this habitat.

Given that AMF hyphal abundance in the soil can correlate with AMF species richness (van der Heijden et al. 2008), non-acidic habitats could also support more diverse fungal assemblages. Diverse fungal communities are more likely to include a highly beneficial fungal species which can tolerate lower carbon supply and increase host plant's tolerance to herbivory (Gange et al. 2005; Bennett and Bever 2007). Differences in fungal species identity and diversity between the two habitats could therefore also contribute to the observed responses (Eom et al. 2001; Murray et al. 2010).

The finding that in non-acidic habitats herbivory increased arbuscular colonization while the colonization decreased in acidic habitats could partly mirror the distinct grazing intensity in these two habitats: the loss of the above-ground biomass of the host plant, *Solidago*, was much greater in non-acidic than in acidic habitats (Eskelinen 2008). Some recent studies emphasize that the intensity of grazing may be a key determinant of the direction of plant–fungal relationship (Wearn and Gange 2007). If the intensity of grazing and concurrent loss of a host plant's above-ground biomass is proportional to the post-defoliation carbon allocation to the roots and exudation of labile carbon from the roots (Holland et al. 1996), it could attract mycorrhiza and lead to a subsequent increase in colonization in the roots of heavily grazed plants. This would be beneficial to the host plant, allowing acquisition of extra nutrients and storage of the nutrients to the below-ground rhizome, which will enable compensatory regrowth during the following growing season (McNaughton 1983). However, as the fungal response to carbon flow to the roots may also depend on the abundance and diversity of fungi (including both spores and hyphae) in the soil, an increase in colonization being possible only in environments where suitable fungi are available in the rhizosphere, our suggested importance of the availability and abundance of AMF in the soil in determining the observed colonization patterns will hold.

Responses of DSE colonization

DSE fungal colonization of *Solidago* responded to herbivory, nutrient availability and habitat in a contrasting manner compared to AMF fungi. Consistent with the host plant response to reindeer grazing (Eskelinen 2008), herbivory had a direct negative effect on DSE hyphal colonization. In open plots accessible to grazers, where *Solidago* individuals were eaten by reindeer to a great extent (Eskelinen 2008), DSE hyphal colonization in plant roots also decreased. Our results contradict those of Medina-Roldán et al. (2008), which is the only other study, to our knowledge, where the impact of natural herbivory on DSE has been studied. Decreased DSE hyphal colonization in association with the loss of a host's aboveground biomass suggests that DSE are limited by photosynthate availability and carbon flux to the fungi in a manner comparable to mycorrhizal fungi (Daft and El Giahmi 1978; Gehring and Whitham 1994, 2002; Smith and Read 2008). This interpretation is further supported by the strong interaction between herbivory and fertilization on both DSE hyphae and sclerotia. In grazed plots, there was no difference in DSE colonization between fertilized and

unfertilized plots, whereas in ungrazed plots, fertilization had a negative impact on DSE colonization. These fungal responses coincided with soil nutrient concentrations, which were lowest in unfertilized exclosures and highest in fertilized exclosures (Eskelinen 2008). The decrease of DSE due to fertilization in the absence of herbivory is in accordance with its potential role as a nutrient acquiring symbiont whose colonization can be expected to decrease when the soil nutrient availability is high and the dependency of plant nutrient acquisition on the fungal compartment is low (Johnson et al. 1997, 2003, 2010; Hartley and Amos 1999; Cornwell et al. 2001; Smith and Read 2008). DSE colonization was highest inside unfertilized exclosures, where nutrient concentrations were also lowest (Eskelinen 2008), indicating the host plant's greater reliance on and allocation to the fungal symbiont in nutrient-poor conditions. Above-ground biomass of vegetation seemed higher inside exclosures (A. Eskelinen, personal observation) which, in the absence of nutrient enrichment, may have reduced soil nutrient pools and intensified plant dependence on fungi in nutrient acquisition. Furthermore, in plots accessible to grazers neither soil nutrient concentrations (Eskelinen 2008) nor DSE fungal colonization seemed to be affected by nutrient amendment, which is likely due to the indirect impact of herbivory on soil nutrients through the consumption of the increased plant biomass in nutrient enriched plots.

Usuki and Narisawa (2007) documented carbon delivery to a DSE symbiont in host plant roots, but there is no unequivocal evidence of direct, fungal-mediated nutrient transfer to the host plant. Absence of specific intraradical structures for nutrient transfer between the host and DSE fungi suggests that other mechanisms of nutrient exchange may be involved. Ability to utilize organic nutrient sources in extraradical environment is well-documented for DSE (Caldwell et al. 2000; Rodriguez et al. 2009). Production of extracellular enzymes by DSE is likely to be facilitated by plant-derived labile carbon, and mineralized nutrients could be assimilated by the host either directly from the soil solution or via DSE connections. Our results of DSE responses to interactions between fertilization and herbivory are consistent with a nutrient for carbon exchange relationship between DSE and host plant. However, the mechanistic details of functioning of the DSE symbiosis, especially those related to nutrient uptake, remain to be solved in future studies.

Our results contradict those of Mandyam and Jumpponen (2008) and Medina-Roldán et al. (2008) who did not find effects of fertilization or defoliation on root DSE colonization, respectively. Their results may be confounded by the lack of treatments, as our results suggest that herbivore and fertilization effects strongly rely on each other. Furthermore, given that the few existing studies reporting significant impacts of defoliation and fertilization on DSE have been conducted on tundra (the present study; Pietikäinen et al. 2005; see also Ruotsalainen et al. 2002), it is possible that DSE plays an especially important role in these barren ecosystems. In tundra, where major nutrient pools are bound to recalcitrant organic matter, a symbiont possessing capability to access these pools could be extremely beneficial. Since vegetation in tundra areas are currently facing dramatic changes due to climate warming imposed increase in nutrient availability (e.g., Post et al. 2009; Wookey et al. 2009) and intensive grazing (e.g., Gough et al. 2007; Aunapuu et al. 2008; Eskelinen 2008), we urgently call for more studies investigating the role of DSE in plant-root symbiotic interactions and ecosystem functioning.

Liming had a direct negative impact on DSE hyphal colonization. Our experimental results are similar to those of Postma et al. (2007), who found a negative correlation between soil pH and DSE colonization. However, contrary to Postma et al. (2007) we did not find a concurrent increase in AMF colonization, suggesting that fungal competition did not inflict a decline in DSE colonization. Nor did we find any impact of liming on the host plant (Eskelinen 2008), which implies that liming had a direct negative effect on DSE hyphae independent of the host plant performance. This finding corresponds to the general notion that the majority of soil fungi favor relatively low pH (Madigan et al. 2003). The impact of liming also depended on grazing and the habitat, suggesting that the negative impact of pH is contingent upon the carbon received from the host-plant and the original acidity of the environment. In non-acidic heaths, where soil pH was initially much higher, pH rise was especially harmful to DSE hyphae in association to grazing which may reflect a combined negative effect of high pH and decreased carbon supply from the host (Usuki and Narisawa 2007). In acidic heaths with much lower initial pH, the negative impact of liming was not that pronounced in general. However, liming still counteracted the benefits of excluding grazers, which emphasizes that environmental conditions can be a major determinant of fungal responses to above-ground herbivory. To conclude, our results suggest that DSE hyphal colonization is more sensitive to increase in soil pH than AMF colonization and that some negative physiological responses to higher pH may be behind this relationship.

Conclusions

Our results highlight the importance of mammalian herbivory in mediating relationships between plants and their root-associated fungal symbionts in tundra ecosystems. For the first time, our results can separate the effects between

experimentally manipulated soil nutrient availability and pH, and natural soil fertility gradient on herbivore–plant–fungi interactions, at the level of root colonization. The same treatments could also have influenced AM fungal community composition (Murray et al. 2010); however, community level responses cannot be evaluated with the present data. Our findings suggest that arbuscular colonization in host plant roots may be more regulated by the habitat-specific differences in soil AMF availability and abundance that depend on vegetation and correlate with habitat fertility, than resource supply per se. In contrast, DSE seems to be more directly dependent on host-derived carbon and soil nutrient concentrations. Our results indicate a tight relationship between herbivory, soil nutrient availability and DSE fungal colonization, and imply an important, yet very little studied, role of DSE in plant–fungal interactions and ecosystem processes.

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