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Seasonality of root fungal colonization in low-alpine herbs

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Abstract Arbuscular mycorrhizal (AM) and dark septate endophytic (DSE) fungal colonization of *Alchemilla glomerulans*, *Carex vaginata*, *Ranunculus acris* ssp. *pumilus* and *Trollius europaeus* growing in low-alpine meadows in the Finnish subarctic were studied at different times during the growing season. Fungal colonization was correlated to soil soluble phosphorus (P) concentration. The influence of flower bud removal on fungal colonization was investigated in *A. glomerulans*, *C. vaginata* and *R. acris* and the correlation between AM and DSE colonization was studied. The fungal colonization patterns were found to be species-specific. *R. acris* maintained a relatively high rate of fungal colonization throughout the summer, while the rates of colonization of *T. europaeus* were lower and decreased towards the end of the season. *A. glomerulans* had constant arbuscular and vesicular colonization throughout the summer, but hyphal and DSE colonization declined towards the end of the season. *C. vaginata* did not form arbuscular mycorrhiza, but was colonized by DSE fungi and hyaline septate hyphae throughout the season. The soil soluble P concentration showed some seasonal variation, but was also highly variable between the study sites. Bud removal decreased arbuscular colonization of *R. acris*, but no unique effects were seen in any other parameters or the other species studied. The root fungal parameters correlated with soil P in some species at some sites, but no consistent trend was found. DSE colonization was positively correlated with root vesicular and hyphal colonization in some cases. The differences in fungal colonization parameters may be related to species-specific phenologies.

Keywords Alpine ecology · Arbuscular mycorrhiza · Dark septate endophytes · Seasonality

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Introduction

In general, mycorrhiza represents a mutualistic partnership involving carbon and nutrient exchange between the symbionts. However, it may also constitute a continuum in which mutualism prevails but parasitic periods also exist (Fitter 1991; Johnson et al. 1997; Lapointe and Molard 1997). Investment in nutrient uptake via mycorrhizal symbiosis implies a considerable carbon cost for the host plant (Douds et al. 1988; Jones et al. 1991; Eissenstat et al. 1993; Smith and Read 1997) which, however, leads to improved nutrient acquisition, largely due to the increased absorption surface provided by the fungal hyphae (Koide 1991). Tuomi et al. (2001) proposed that plants may maximize their growth by optimizing mycorrhizal colonization in their roots. Optimal mycorrhizal colonization should increase plant growth and reproduction and be dependent on the relationship between carbon and nutrient acquisition. Thus, optimal mycorrhizal colonization may change if the availability of soil nutrients and photosynthetic energy fluctuates (Fitter 1991; Tuomi et al. 2001).

Arbuscular mycorrhizal (AM) symbiosis is characterized by short life-cycles of arbuscules (Alexander et al. 1989), rapid colonization of new roots and appearance of vesicles in the oldest colonization units (Smith and Read 1997). Seasonal shifts in AM colonization have been found, but it appears that species-specific variation also exists (Read et al. 1976; Baikalova and Onipchenko 1988; Brundrett and Kendrick 1990; Mullen and Schmidt 1993). Soil moisture conditions have also been

Table 1 The *F*-values of repeated ANOVA for the seasonality of fungal colonization. Treatment, error *df* in parentheses

Plant species	Fungal structure	Within-subject effects		Between-subject effects Site
		Time	Time × Site	
Alchemilla glomerulans	Arbuscules	*F*(3,36)=1.938	*F*(6,36)=0.948	*F*(2,12)=9.436**
	Vesicles[a]	*F*(3,36)=2.055	*F*(6,36)=1.778	*F*(2,12)=13.023**
	Hyphae[a]	*F*(3,36)=3.211*	*F*(6,36)=2.185	*F*(2,12)=8.000**
	DSE	*F*(3,36)=2.937*	*F*(6,36)=1.916	*F*(2,12)=7.016*
Carex vaginata	DSE	*F*(3,27)=2.271	*F*(6,27)=2.064	*F*(2,9)=6.749*
	HS hyphae	*F*(3,27)=1.068	*F*(6,27)=0.525	*F*(2,9)=6.115*
Ranunculus acris ssp. *pumilus*	Arbuscules[a]	*F*(2,24)=0.004	*F*(4,24)=4.367**	*F*(2,12)=7.138**
	Vesicles	*F*(2,24)=0.550	*F*(4,24)=3.300*	*F*(2,12)=0.245
	Hyphae[a]	*F*(2,24)=0.066	*F*(4,24)=1.400	*F*(2,12)=4.577*
	DSE	*F*(2,24)=1.001	*F*(4,24)=1.039	*F*(2,12)=1.197
Trollius europaeus	Arbuscules	*F*(3,30)=3.578*	*F*(6,30)=1.297	*F*(2,10)=1,201
	Vesicles[a]	*F*(3,30)=0.346	*F*(6,30)=1.096	*F*(2,10)=2.561
	Hyphae	*F*(3,30)=3.459*	*F*(6,30)=0.642	*F*(2,10)=3.230
	DSE	*F*(3,30)=4.365*	*F*(6,30)=1.684	*F*(2,10)=5.462*

*$P<0.05$; **$P<0.01$

[a] Variances between the compared groups not homogeneous (Levene's test at $P<0.05$)

proposed to influence root growth and AM colonization (Rabatin 1979; Allen 1983). Seasonal changes in mycorrhizal colonization could indicate that the benefit of mycorrhizal symbiosis for plants changes during the season (Fitter 1986, 1991; Lapointe and Molard 1997). Especially in arcto-alpine conditions, such variation may be connected with periodicity of nutrient availability in soil (Chapin and Bloom 1976; Chapin et al. 1978; Jaeger and Monson 1992; Kielland and Chapin 1992) parallel to the overall photosynthetic energy limitation typical of these ecosystems (Bliss 1971). Mullen and Schmidt (1993) found that alpine *Ranunculus adoneus* maintained arbuscules only for a limited period after flowering and seed set and that plant internal phosphorus (P) concentration peaked soon afterwards. Additionally, Mullen et al. (1998) proposed that nitrogen (N) uptake may occur through root-colonizing dark septate endophytic (DSE) fungi (Jumpponen and Trappe 1998) early in the season before any root growth or AM colonization takes place.

In the present study, the seasonal relationships between soil soluble P and root fungal (AM and DSE) colonization of *Alchemilla glomerulans*, *Carex vaginata*, *Ranunculus acris* ssp. *pumilus* and *Trollius europaeus* were followed in low-alpine conditions. Soil P was chosen as a parameter because it is the main nutrient transferred in AM symbiosis (Smith and Read 1997) and may also be translocated in DSE associations (Jumpponen and Trappe 1998). Flower buds were removed to decrease plant nutrient demand and to study its influence on the AM and DSE colonization rates. Answers were sought to the following questions: (1) Do root fungal colonization and soil soluble P concentration show seasonal variation? (2) Do fungal colonization parameters correlate with soil P concentration? (3) Does bud removal influence the fungal colonization rates? (4) Is colonization by AM and DSE correlated?

Materials and methods

The study was carried out in the Skirhasjohka valley between Mt. Saana and Mt. Jeahkkas at Kilpisjärvi (69°01′ N, 20°50′E) in subarctic Fennoscandia in the summer of 1998. The mean annual temperature at the nearby Kilpisjärvi climatological station in 1961–1985 was –2.5°C, the mean temperature of the warmest month (July) was +10.5°C and mean annual precipitation was 422 mm (Järvinen 1987). The length of the growing season (daily mean temperature >5°C) is about 80 days in the alpine belt and about 95 days in the mountain birch forest belt (Hiltunen 1980). At the tree line (600 m a.s.l.), snow melts in mid-June (Kyllönen 1988).

Three sites in the valley 1 km apart were chosen from the low-alpine belt just above the tree line at 630 m a.s.l. All sites were herb-rich snow-bed meadows dominated by *Deschampsia flexuosa* and characterized by low and tall herbs, such as *Solidago virgaurea* and *Viola biflora*, in addition to the study species. Site 3 had a lower coverage of *D. flexuosa* and was also characterized by ericaceous heath vegetation (*Empetrum nigrum* ssp. *hermaphroditum*, *Vaccinium uliginosum*, *V. vitis-idaea)* and a higher coverage of cyperaceous species. Root samples of *R. acris* ssp. *pumilus* and *T. europaeus* (Ranunculaceae), *A. glomerulans* (Rosaceae) and *C. vaginata* (Cyperaceae) were collected four times during the growing season (25 June, 15 July, 31 July, 18 August), except for *R. acris*, when the first collection was made on 15 July. The plants and their roots were collected with 1 l of soil (5 replicates per site). The samples were brought into the laboratory and stored at +4°C until the roots were cleaned and preserved in 50% alcohol, which was done either immediately or 1–2 days after sampling.

For the bud removal experiment, about 60 plants per species and site were marked and their buds were removed on 25 June (*C. vaginata*) or 15 July (*A. glomerulans* and *R. acris*). On every sampling date, 5 treated replicates per species were collected at each site. The seasonality study specimens served as controls. In the final analysis, some specimens had to be abandoned because the root sample was too small and thus the final number of samples was 15 for *A. glomerulans* and *R. acris* in both the seasonality and bud removal studies, 12 for *C. vaginata* in the seasonality study and 13 in the bud removal study, and 13 for *T. europaeus* in the seasonality study. *T. europaeus* was not included in the bud removal study because it produced only a few flowers at the study sites in 1998.

Root analyses

The roots were stained as described by Väre et al. (1997), with the following exceptions: the bleaching time in alkaline H_2O_2 varied from 10 to 30 min without warming (*T. europaeus* requiring the longest time), acidification in 1% HCl for 2.5 h and staining for 60 min. Fungal colonization was determined by the magnified intersections method (McGonigle et al. 1990) with the following specifications: 50 intersects from about 10 cm of the finest roots were studied. In a few cases, the size of the root sample did not allow 50 intersects to be counted and the lowest number of intersects included in the data was 23. The magnification used was usually ×150, but was ×600 in the cases where a more precise examination was needed. The point to rotate the crosshair of the eyepiece perpendicular to the root was not the point to enter the root but the vascular cylinder of the root because some roots were so broad that it was easier to get the whole root intersect studied this way. The colonization percentages of arbuscules, vesicles, hyphae and DSE were calculated separately. DS microsclerotia (Väre et al. 1992; O'Dell et al. 1993; Fernando and Currah 1996) and DS hyphae were calculated separately, but the occurrence of sclerotia was so occasional that the data were pooled. The colonization percentages are expressed as colonized intersects/total number of intersects × 100.

Soil P analysis

Soil was separated from the root samples in the laboratory and the samples kept at –18°C until analysis (n=35 per date). Soil soluble P was extracted into ammonium acetate as described by Väre et al. (1997) and soluble P was determined using a standard method described by Halonen et al. (1983).

Statistical analyses

Statistical tests were performed with the SPSS software package version 8.0 (SPSS Inc. Chicago). Colonization percentages were Arcsin transformed before the analyses. The Kolmogorov-Smirnov test was used to study the normality of the data and the Levene test to check the homogeneity of the variances. Repeated ANOVA were performed to test the influence of the date (within-subject variable) and site (between-subject variable) on fungal colonization in the seasonality study. Mauchly's sphericity tests gave no significant results (indicating homogeneity of covariance) for any of the studied colonization parameters and thus univariate tests were performed (Potvin et al. 1990). Where repeated ANOVA results indicated that the dates were different, within-subject contrasts were performed by comparing each date mean to the mean of the previous dates. The influence of bud removal on fungal colonization was tested in the same manner, with site and treatment as between-subject variables.

Levene's test indicated slight heterogeneity of variance in some of the studied parameters (marked in Tables 1, 2), but only in one or two of the comparison groups; the variance assumptions were thus presumed not to be strongly violated. When the interactions between time and site became significant (Table 1), one-way ANOVA (or a non-parametric Kruskal-Wallis test in case the variance assumptions were violated) was performed for each site separately in the seasonality study. In the bud removal study, the between-subject factors site and treatment had an interaction in some parameters (Table 2) and, in these cases, a t-test was used to compare the influence of treatment at each site. Where there was interaction between the time, site and treatment (Table 2), two-way ANOVA was performed for each site, with time and treatment as independent variables. At one site, the variances were heterogeneous and, in this case, separate t-tests were performed for each date. Despite this, the variance assumptions were still violated on the other sampling date and a non-parametric Mann-Whitney U-test was performed to study the effect of treatment on this sampling date.

Table 2 The F-values of repeated ANOVA for the influence of flower bud removal. See Table 1 for details

Plant species	Structure	Within-subject effects				Between-subject effects		
		Time	Time × Site	Time × Treatment	Time × Site × Treatment	Site	Treatment	Site × Treatment
A. glomerulans	Arbuscules	$F(1,24)$=0.027	$F(2,24)$=1.960	$F(1,24)$=0.353	$F(2,24)$=0.324	$F(2,24)$=12.383***	$F(1,24)$=0.369	$F(2,24)$ =0.240
	Vesicles[a]	$F(1,24)$=1.770	$F(2,24)$=0.102	$F(1,24)$=0.119	$F(2,24)$=1.385	$F(2,24)$=13.085***	$F(1,24)$=1.871	$F(2,24)$=2.555
	Hyphae[a]	$F(1,24)$=3.317	$F(2,24)$=0.352	$F(1,24)$=0.929	$F(2,24)$ =4.601*	$F(2,24)$=7.638**	$F(1,24)$=0.417	$F(2,24)$=5.125*
	DSE	$F(1,24)$=0.000	$F(2,24)$=0.406	$F(1,24)$=0.007	$F(2,24)$=0.050	$F(2,24)$=11.011***	$F(1,24)$=0.282	$F(2,24)$=5.665*
C. vaginata	DSE	$F(2,38)$=6.628**	$F(2,38)$=0.474	$F(4,38)$=0.038	$F(4,38)$=0.506	$F(1,19)$=0.481	$F(2,19)$=3.116	$F(2,19)$=3.087
	HS hyphae[a]	$F(2,38)$=0.730	$F(2,38)$=0.217	$F(4,38)$=0.501	$F(4,38)$=1.169	$F(1,19)$=0.004	$F(2,19)$=1.310	$F(2,19)$=1.547
R. acris ssp. *pumilus*	Arbuscules[a]	$F(1,24)$=0.000	$F(2,24)$=2.888	$F(1,24)$=0.012	$F(2,24)$=0.598	$F(2,24)$=6.269**	$F(1,24)$=6.636*	$F(2,24)$ =3.097
	Vesicles	$F(1,24)$=0.001	$F(2,24)$=1.708	$F(1,24)$=0.968	$F(2,24)$=0.774	$F(2,24)$=1.548	$F(1,24)$=0.002	$F(2,24)$=5.547*
	Hyphae	$F(1,24)$=0.009	$F(2,24)$=2.125	$F(1,24)$=0.366	$F(2,24)$=0.074	$F(2,24)$=5.042*	$F(1,24)$=1.821	$F(2,24)$=2.276
	DSE	$F(1,24)$=0.074	$F(2,24)$=2.290	$F(1,24)$=0.366	$F(2,24)$=0.445	$F(2,24)$=1.154	$F(1,24)$ =0.352	$F(2,24)$=0.309

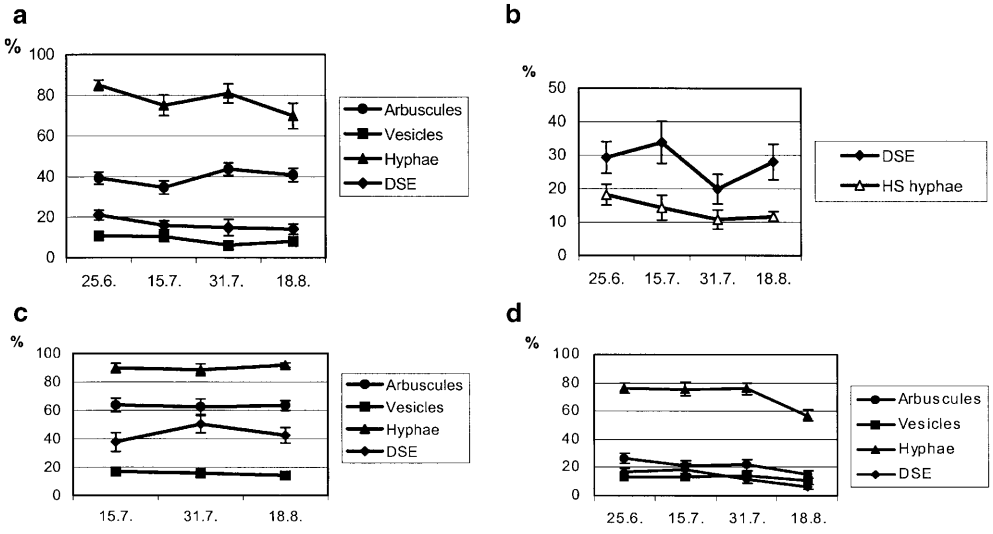

Fig. 1 Fungal colonization percentage (mean±SE) of **a** *Alchemilla glomerulans*, **b** *Carex vaginata*, **c** *Ranunculus acris* ssp. *pumilus*, and **d** *Trollius europaeus* on four sampling dates (three dates for *R. acris*) in the summer of 1998 in Kilpisjärvi. Significant differences from the mean of previous dates: *A. glomerulans* 18 August hyphae ($P<0.05$), 31 July DSE ($P<0.05$); *T. europaeus* 18 August arbuscules ($P<0.05$), 18 August hyphae ($P<0.01$), 31 July and 18 August DSE $P<0.05$) (*Hyphae* all hyaline hyphae, *HS hyphae* hyaline septate hyphae; clearly non-septate hyphae were not detected in *C. vaginata*)

Soil P data were square-root transformed [SQRT (× +0.5)] before analysis to normalize the data. Normality and the homogeneity of the variance was checked as above. Levene's test indicated slightly heterogeneous variance between the comparison groups, even in the soil P data. Because Mauchly's sphericity test indicated heterogeneity of covariance ($W=0.657$, $df = 5$, $P=0.024$) (and the Huynh Feldt/Greenhouse-Geisser epsilon values were similar), we used the corrected Huynh-Feldt values in repeated ANOVA to test whether the soil P concentrations differed on separate dates (within-subject variable) and sites (between-subject variable) (Potvin et al. 1990). Within-subject contrasts were performed, as in the root colonization data above, to find out which dates differed from the others. Pearson's correlation coefficients were used to study the relationships between the soil soluble P concentration and the fungal colonization parameters, as well as the correlations between the fungal colonization parameters. Correlations were calculated separately for each species and site (correlations for separating the study dates in addition to species and sites were also performed, but these results did not differ from the results presented).

Results

Seasonality of fungal colonization

Alchemilla glomerulans, *R. acris* ssp. *pumilus* and *T. europaeus* formed typical AM structures, including arbuscules and vesicles, and DSE colonization was also found frequently in all species (root superficial and internal colonization were not separated). In *C. vaginata*, neither arbuscules nor vesicles were detected. Instead, the roots were colonized by DSE and nearly always by hyaline septate (HS) fungal hyphae. In all plant species, DSE sometimes formed intracellular microsclerotia. Change in percentage fungal colonization during the season is presented in Fig. 1 and the statistical analysis in Table 1.

The arbuscular colonization rate was highest in *R. acris*, with about 64% colonization throughout the growing season without any seasonal variation (Fig. 1c). In *A. glomerulans*, arbuscular colonization was also quite high (35–44%) and no pronounced seasonal trend was detected (Fig. 1a). By contrast, *T. europaeus* showed a clearly decreasing trend in arbuscular colonization during the season (Fig. 1d). For *A. glomerulans* and *R. acris*, the study sites also differed significantly, i.e. temporal colonization trends were similar at each site, but the average colonization at the sites were different. In the case of *R. acris*, the interaction between time and site was significant (Table 1). At site 1, arbuscular colonization decreased significantly [$F_{(2,12)}=5.103$, $P=0.025$] from 15 July to 31 July, while at other sites, where the colonization trends were more or less opposite to that of site 1, no differences between the sampling dates were detected.

The vesicular colonization rate was always lower than arbuscular colonization in all species and no significant seasonality was detected. In *R. acris*, vesicular colonization varied within the narrow range of 14–17% and was thus very stable throughout the season (Fig. 1c). In *A. glomerulans*, the rate of vesicular colonization was 6–11%, being highest in the early part of the summer (Fig. 1a). In *T. europaeus*, vesicular colonization was also lowest late in the growing season and highest in the early season, but there was marked deviation in all cases (Fig. 1d). In *A. glomerulans*, vesicular colonization also differed between the study sites and *R. acris* showed significant interaction between time and site (Table 1). For *R. acris* at site 2, vesicular colonization increased significantly from 15 July to 18 August [$F_{(2,12)}=5.230$, $P=0.023$], while the colonization at the other sites showed no significant changes.

Roots were abundantly colonized by hyaline hyphae of AM type in species forming arbuscular mycorrhiza. The mean percentages were highest in *R. acris* (89–92%) and no seasonal trend was seen (Fig. 1c). However, for *A. glomerulans* and *T. europaeus*, hyphal colonization was lowest in the autumn (Figs. 1a, d; Table 1). Hyphal colonization differed between the study sites for *A. glomerulans* and *R. acris* (Table 1).

DSE colonization in all of the studied species was highly variable, but a general trend of decreasing coloni-

zation towards the end of the season was apparent (Fig. 1a, b, d). *R. acris* showed a relatively high and constant rate of DSE colonization of 38–50% (Fig. 1c). In *A. glomerulans* and *T. europaeus*, DSE colonization was lower and these species also showed a significant decline in DSE colonization during the season (Fig. 1a, d; Table 1). No significant differences were found for *C. vaginata* (Table 1). HS colonization was detected in *C. vaginata*, but no significant seasonal differences were found (Fig. 1b; Table 1). DSE and HS colonization also differed at different sites (Table 1).

For most AM species, hyphal colonization correlated with vesicular and/or arbuscular colonization (data not shown). Root DSE colonization was positively correlated with root hyphal and vesicular colonization in two cases: both vesicular and hyphal colonization correlated with DSE in *T. europaeus* at site 2 (r=0.495, n=20, P=0.026 and r=0.552, n=20, P=0.012, respectively), and hyphal colonization in *R. acris* also correlated at site 2 (r=0.618, n=15, P=0.014). However, no obvious species- or site-specific patterns were detected.

Flower bud removal

Flower bud removal decreased the arbuscular colonization of *R. acris*, but otherwise the treatment had no overall influence on fungal colonization (Fig. 2). In the case of hyphal colonization of *A. glomerulans*, an interaction between time, site and treatment was detected. No significant differences were found at sites 1 and 3, but at site 2 a significant interaction between sampling date and treatment emerged [$F_{(1,20)}$=14.790, P=0.001). Separate t-tests for each date at site 2 were performed to compare the influence of treatment. On 31 July, the cut plants had significantly reduced infection at this site (t=4.074, df=8, P=0.004), but on 18 August there was no significant difference.

In some cases, interactions between site and treatment were also found to be significant, i.e. the influence of the treatment on colonization at these sites differed (Table 2). In the case of *R. acris*, vesicular colonization was reduced in cut plants at site 2 (t=2.649, df=18, P=0.016), while at other sites differences in colonization were not significant. In *A. glomerulans*, hyphal colonization in cut plants was significantly reduced at site 3 (U=26.00, P=0.010), but colonization at the other sites was relatively similar in cut and control plants. Additionally, the DSE colonization in cut *A. glomerulans* plants was significantly reduced at site 2 (t=3.318, df=18, P=0.006), while the differences between cut and control plants at other sites were not significant.

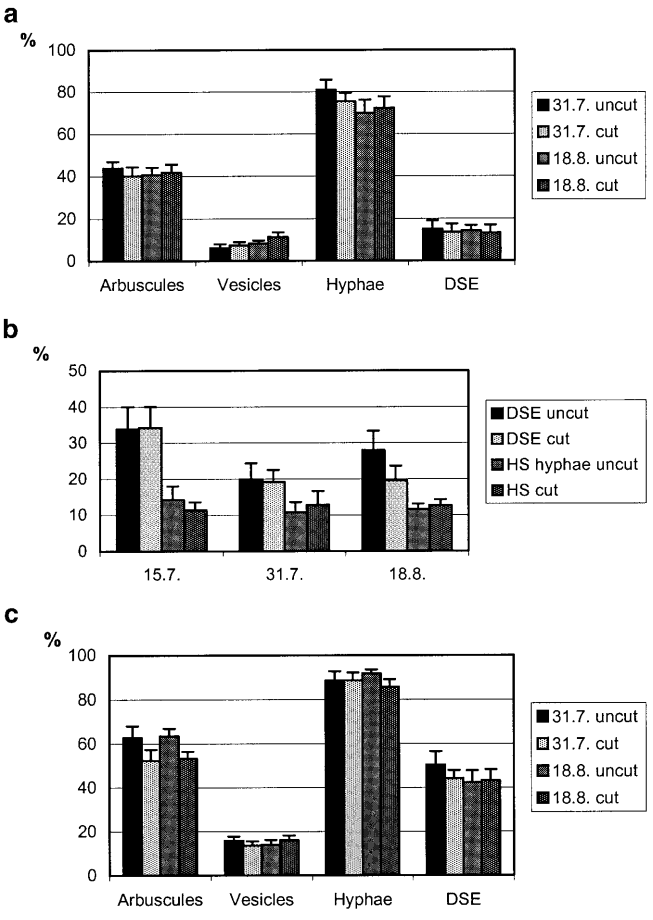

Fig. 2 Influence of bud removal on fungal colonization percentage (mean±SE) in **a** *A. glomerulans*, **b** *C. vaginata* and **c** *R. acris* ssp. *pumilus* in summer of 1998 in Kilpisjärvi. After treatment, samples were collected on three occasions from *C. vaginata* and on two occasions from the other species. Significant influence of bud removal: *R. acris* arbuscules (P<0.05) (*Cut* buds removed, *uncut* control)

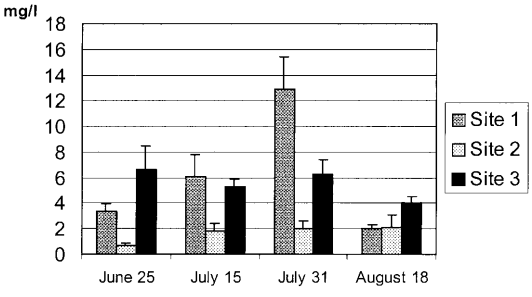

Fig. 3 Mean soil soluble phosphorus concentration (mg/l; mean±SE) at the study sites (numbered 1, 2 and 3) on various collection dates in summer 1998 at Kilpisjärvi (n=35 on each date). Significant differences from the mean of previous dates: 31 July (P<0.05) and 18 August (P<0.05)

Soil P concentration

The mean soil soluble P concentration was lowest in August (2.9 mg/l) and highest at the end of July (8.1mg/l; n=35), but highly variable between the sites (Fig. 3; Table 3). Soil P on 31 July was significantly higher on the preceding dates, whilst the P concentration in mid-August was lower than on the preceding dates (Table 3). Soil P correlated with the fungal parameters in some cases. Arbuscular colonization showed a positive

Table 3 *F*-values of repeated ANOVA for soil soluble P concentration. *Df* and error values of within-subject variables are Huynh-Feldt corrected

Source of variation		SS	*df*	*F*
Within subject	Time	266.9	2.9	6.261**
	Time × Site	344.9	5.8	4.045**
	Error	1364.1	93.2	
Between subject	Site	132.6	2	35.220***
	Error	60.2	32	

*P<0.05, **P<0.01, ***P<0.001

correlation with soil P in *T. europaeus* and *A. glomerulans* at sites 1 and 3 (r=0.576, n=19, P=0.010 and r=0.450, n=20, P=0.047, respectively). However, for *R. acris* at site 3, the root arbuscular colonization was negatively correlated with soil P (r=–0.558, n=15, P=0.031). Hyphal colonization was negatively correlated with soil P concentration in *A. glomerulans* and *R. acris* at sites 2 and 3 (r=–0.504, n=16, P=0.047 and r=–0.630, n=15, P=0.012, respectively). DSE colonization correlated negatively with soil P at site 3 in *T. europaeus* (r=–0.472, n=18, P=0.048).

Discussion

In the present study, AM and DSE colonization of the study plants was relatively constant throughout the growing season, even though significant differences between sample dates were detected. AM and DSE colonization clearly decreased towards the end of the season only in *T. europaeus*, but the differences in percent colonization remained small. AM hyphal and DSE colonization of *A. glomerulans* decreased towards the end of the season. In *C. vaginata*, DSE and HS colonization was highly variable and, although no significant differences between sample dates were found, there was an apparent decreasing trends towards the end of the season. The overall root fungal colonization level appeared higher in *R. acris* than in *A. glomerulans* and *T. europaeus*. *A. glomerulans* and *T. europaeus* had relatively similar hyphal colonization, but arbuscular colonization was lower in *T. europaeus*. Flower bud removal had no general influence on fungal colonization, even though an apparent decrease in arbuscular colonization was found in *R. acris*. For some species and colonization parameters, interactions between sampling time and site, site and treatment and even time, site and treatment were found. It appears that changes in colonization were quite variable between sites. However, the shifts could not be related to any known feature of the study sites; the vegetation at the sites was relatively similar. Even though soluble P concentration showed different time trends at different sites, it was found to correlate with the root colonization parameters only occasionally; both positive and negative correlations with the colonization parameters were detected.

The occurrence of arbuscules has been reported to peak in spring (Rabatin 1979; Allen 1983; Reinhardt and Miller 1990), in summer (Brundrett and Kendrick 1990; Sigüenza et al. 1996) and also in autumn after flowering and seed set (Hayman 1970; Mullen and Schmidt 1993). By contrast, Boerner (1986) and Brundrett and Abbott (1994) observed no variation in AM colonization during the growing season. Seasonal changes in AM symbiosis are probably species-specific and edaphic conditions such as soil temperature and moisture may also influence the development of infection. In alpine conditions, seasonal shifts in mycorrhizal colonization have been studied by Baikalova and Onipchenko (1988), Mullen and Schmidt (1993) and Mullen et al. (1998, for DSE). Mullen et al. (1998) found DSE colonization of high alpine *Ranunculus adoneus* to be highest in spring and proposed that early season uptake of N could take place through these associations. According to Baikalova and Onipchenko (1988), total AM colonization of alpine plants tended to increase slightly towards the end of the season, but arbuscule number decreased and vesicle size increased. In the present study, no significant seasonal variation in root fungal colonization was detected, despite the species-specific patterns and levels of colonization recorded.. In this study, fungal colonization of *R. acris* was the highest, *A. glomerulans* intermediate and *T. europaeus* the lowest; however, the latter two species had relatively similar hyphal colonization. Flower bud excision, to remove the nutrient sink of flowers and seeds, clearly decreased arbuscular colonization of *R. acris.* Ranunculaceae are thought to be a relatively mycotrophic family (Trappe 1987), but in the present study *R. acris* had clearly higher overall colonization levels than *T. europaeus*. Non-treated *R. acris* flowered abundantly, but *T. europaeus* did not flower during the study period. It could be that the higher rates of AM colonization in *R. acris* are related to a high nutrient demand caused by flower and seed formation. DSE colonization was also relatively abundant in this species. Although the relatively low colonization of *T. europaeus* may be due to the absence of flowers, it is possible, for example, that *T. europaeus* was not limited by the nutrients provided by AM.

The highest DSE colonization was found during the first half of the growing season and a more or less significant decline in colonization was found in all of the studied species. This agrees with the findings of Mullen et al. (1998). The functional role of DSE in roots remains unclear, but the possibility of some kind of mutualistic partnership cannot be overlooked (Jumpponen and Trappe 1998). In addition, positive correlations between DSE and AM vesicles and hyphae were found in *R. acris* and *T. europaeus* at one site, even though correlations at the other sites were not significant. This slight co-occurrence of DSE and vesicles might be due to senescing old roots, where DSE fungi may have a saprophytic function (Smith and Read 1997; Jumpponen and Trappe 1998).

In the present study, the soil soluble P concentration was found to be highest at the end of July, while it de-

creased significantly during the first half of August, although the temporal concentration patterns at the study sites differed considerably. In arcto-alpine conditions, the concentrations of soluble nutrients in soil have been found to be high during the short period in spring when snow melts (Chapin et al. 1978; Chapin 1980; Körner 1999) and soluble P may be high also during the senescence of vegetation in the late summer and autumn (Chapin and Bloom 1976). At one site, the highest P concentration occurred soon after snow melt in late June. A high mean P concentration at the end of July does not coincide very well with either the snow melt or the time of vegetation senescence. It may be that the availability of P in these low-alpine grass- and herb-dominated snowbed meadows with continuous vegetation cover does not always coincide exactly with the time of snow melt or the dieback of vegetation (Schmidt et al. 1999).

The fungal colonization parameters did not correlate with soil P in most species and at most sites in this study, even though it was assumed that arbuscular colonization, which allows P translocation into the host plant (Smith and Read 1997), could correlate with soil P. Arbuscules are relatively short-lived structures, with a life-span of only a few days in cultivated plants (e.g. Alexander et al. 1989). In forest ecosystems, however, they can live notably longer, even for months (Brundrett and Kendrick 1990). The observations of Mullen and Schmidt (1993) in high-alpine conditions suggest that arbuscule life-spans are not that long, because arbuscules were only present for a few weeks. However, if the duration of an individual arbuscule is relatively long, it is possible that changes in soil P concentration (which was relatively low all the time) had no consistent influence on the number of arbuscules. In addition, it must be emphasized that the staining method used does not discriminate between living and dead fungal structures and a vital staining method may have given different results (Hamel et al. 1990). One further reason for the lack of correlation could be that the plants took up P from a larger soil volume, at least in the late summer, when part of the fine roots were likely to have reached a larger volume than the sample size. AM colonization also may be affected by plant internal P status. Where this is sufficient, there is no need for effective uptake through mycorrhiza. The plant internal N/P ratio may also influence symbiosis, because if N is a limiting factor, there may be no need to invest in AM, which mainly enhances P uptake (Sylvia and Neal 1990).

In the present study, AM structures were not found in *C. vaginata*. Cyperaceae was thought to represent a mainly non-mycorrhizal strategy (Trappe 1987), but AM has been found associated with several cyperaceous species in relatively recent studies (Miller et al. 1999). However, root studies from arctic and alpine areas have generally not reported AM colonization of *Carex* (e.g. Read and Haselwandter 1981; Väre et al. 1992, 1997).

In conclusion, no consistent seasonal fungal colonization cycles were found in the low-alpine herbs studied, even though some species-specific features of colonization were detected. These may be connected to species-specific phenological rhythms. The soil soluble P concentration showed some seasonal variation, but concentrations at the study sites were also highly variable and no close relationship between the soil P patterns and root colonization was found. The duration of this study was one growing season. Many subarctic plants show between-year variation in flowering, which means that they accumulate nutrient and carbon reserves over long periods (Laine and Henttonen 1983). Thus, our results may have been influenced by this variation. However, the results indicate that variation in fungal colonization during the growing season may be relatively low in low-alpine meadows compared with high-alpine conditions (Mullen and Schmidt 1993; Mullen et al. 1998).

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