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ORIGINAL PAPER

Polygonum viviparum mycobionts on an alpine primary successional glacier forefront

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Abstract *Polygonum viviparum* is one of the first ectomycorrhizal (EM) plant species colonising primary successional sites at the Rotmoos glacier forefront (Tyrolean Alps, Austria). On a site with soil development of about 150 years (2,400 m above sea level), mycobionts of *P. viviparum* were identified by morphotyping and fungal ribosomal deoxyribonucleic acid internal transcribed spacer sequencing. For studying seasonal dynamics and spatial heterogeneity, ectomycorrhizae were sampled on five plots during all seasons. *P. viviparum* root tips were always EM. In total, 18 mycobiont taxa of the following genera were identified: *Cenococcum* (1), *Cortinarius* (2), *Helvella* (1), *Inocybe* (3), *Russula* (1), *Sebacina* (2), *Thelephora* (2) and *Tomentella* (6). All were non-specific EM partners of EM plants. As early as 2 weeks after spring snow melt, EM were well developed, vital and showed high mycobiont diversity. The relative abundance of senescent root tips was lowest in spring and increased throughout the year, with a maximum in winter (frozen soil). Thus, mycobiont growth and physiological activity obviously start when soil is still under snow cover: We speculate that water availability is one important initiation factor for mycorrhizal development under snow cover, when temperatures still range around the freezing point. Irrespectively of the season, most abundant mycobionts at this primary successional site belonged to the genera *Tomentella*, *Sebacina* and *Cenococcum*, also in frozen soil. Spatial heterogeneity was high when considering species composition and diversity indices. Overall mycobionts species richness was restricted at this site, probably because of the limited availability of fungal partners. We regard the presence/absence of fungal partner and limiting abiotic impacts of the environment as key factors for the symbiotic status of *P. viviparum*.

Keywords *Bistorta vivipara* · Ectomycorrhiza · Primary succession · Seasonal dynamics · Spatial heterogeneity

Introduction

Polygonum viviparum L. is an ectomycorrhizal (EM) herbaceous plant with wide-spread distribution in circumpolar arctic–alpine habitats (Dormann et al. 2002) and in peri-glacial regions. In the Austrian central Alps, it occurs between 1,000 and 2,600 m above sea level (Polatschek 2000). The EM symbiotic association of *P. viviparum* is exceptional within Polygonaceae: Besides *Coccoloba uvifera* in Central America and *P. capitatum* and *P. weyrichii* both in Asia, this is the only species of Polygonaceae forming EM (Wang and Qiu 2006).

The EM colonisation of *P. viviparum* fine roots was early evidenced by studies carried out in arctic–alpine and boreal habitats (e.g. Hesselman 1900; Fontana 1977; Haselwandter and Read 1980; Read and Haselwandter 1981; Lesica and Antibus 1986; Harley and Harley 1987; Blaschke 1991; Treu et al. 1996). Recently, also dual (EM and arbuscular) mycorrhizal infection of *P. viviparum* (*Bistorta vivipara*) was described (Eriksen et al. 2002). Some studies, however, reported *P. viviparum* not to be EM in alpine (Nespiak 1953) or in arctic habitats (Bledsoe et al. 1990; Väre et al. 1992).

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The diversity of *P. viviparum* ectomycorrhizae was first documented by morphological approaches: Fontana (1977) described 16 morphotypes (MT), Haselwandter and Read (1980) and Read and Haselwandter (1981) observed a *Cenococcum*-like MT, and Massicotte et al. (1998) distinguished several MTs that were not further described. These and other previous studies on EM mycobionts of *P. viviparum* are summarised by Gardes and Dahlberg (1996) and Massicotte et al. (1998). Applying molecular methods, Moreau et al. (2006) compared polymerase chain reaction (PCR) restriction fragment length polymorphism patterns of *Alnicola* fruitbodies with EM root tips.

Fruitbody occurrence was also often used as indicator for potential symbiotic associations of *P. viviparum* to EM fungal genera like *Amanita*, *Inocybe* or *Russula* (e.g. Kühner 1972; Senn-Irlet et al. 1990; Graf 1994).

However, none of the abovementioned studies generally focused on EM of this plant, and *P. viviparum* mycobionts have never been systematically investigated with molecular approaches. The aim of this study was to identify the mycorrhizal partners of *P. viviparum* at a well-characterised primary successional alpine habitat. Seasonal dynamics and spatial distribution of mycobionts were monitored.

Materials and methods

Study site

Sampling was performed on the Rotmoos glacier forefront, a primary successional site in the Tyrolean Alps (Ötz valley, Austria): this glacier forefront in the Rotmoos valley was situated at 2,280 to 2,450 m above sea level. The sampling area was at the moraine of 1858 (46°50′N, 11°01′E), thus with approximately 150 years of soil development. The study site had a total area of about 100×200 m. Soil composition was very heterogeneous because of the dependence on microhabitats. The mean content of organic matter was about 7% (Erschbamer et al. 1999). The vegetation of the sampling area was described in detail by Erschbamer et al. (1999): Briefly, the plant community was an initial grassland community with *Carex curvula*, *C. sempervirens*, *Poa alpina* and *Trifolium pallescens* dominating (Raffl and Erschbamer 2004; Raffl et al. 2006). Other abundant EM plants were *P. viviparum*, *Salix* spp. and *Kobresia myosuroides*. Plant cover varied between 50 and 90%.

Sampling

Five sampling plots (1×1 m; plots 1 to 5) were selected, on which *P. viviparum* occurred as one of the dominating plants. At least five plants were randomly selected for each sampling plot and were excavated with a small scoop including their roots and surrounding soil. The resulting holes measured approximately 5×5×5 cm. The plants were stored until further treatment for no longer than 2 weeks in the original soil at 4°C. Sampling was carried out four times in 2005: 2 weeks after snow melt (June 15, spring), twice during the vegetation period (August 4, summer; September 15, fall) and under an approximately 40-cm-high snow cover (December 14, winter; Fig. 1).

To obtain reference sequences for species-level identification of mycorrhizal fungi, all occurring fruitbodies in the

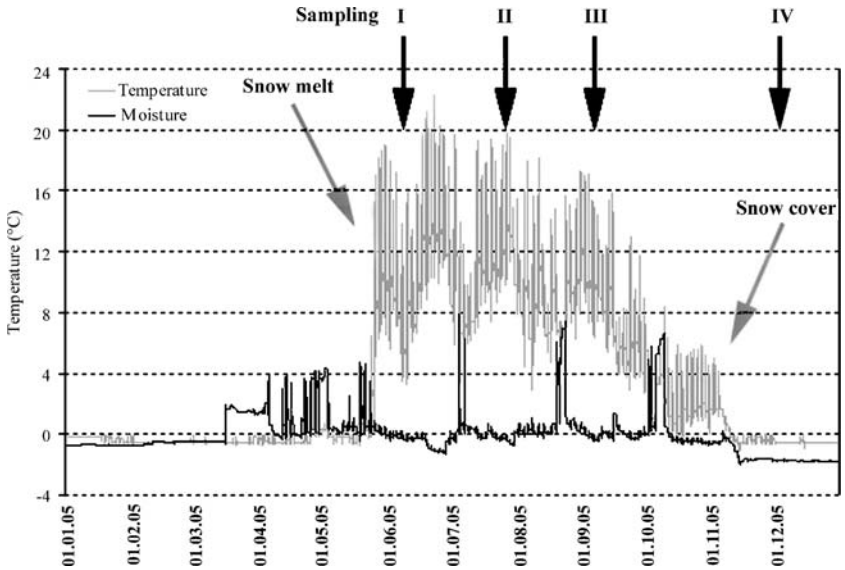

Fig. 1 Temperature in degrees Celsius (*grey*) and moisture as relative values (*black*) in 10-cm soil depth during 2005: *Black arrows* indicate the sampling dates (*I–IV*). *Grey arrows* highlight the dates when snow cover ended or started. The soil moisture values are constant as soon as soil is frozen

study site during 2005 and 2006 were harvested. Voucher material was deposited in the Herbarium IB (University of Innsbruck).

Environmental data

Soil temperature and relative values of moisture data were kindly provided by Dr. Rüdiger Kaufmann (University of Innsbruck, Institute of Ecology). Data Lockers were buried at a soil depth of 10 cm at the study site.

Sample processing

After careful washing, roots were isolated from *Polygonum* bulbils. One hundred mycorrhizal root tips were randomly selected for each sampling plot, resulting in a total of 500 root tips for each sampling date. Root tips were examined at 10- to 100-fold magnification and sorted into MTs based on colour, emanating elements, mantle layer and hyphal anatomy (Agerer 1991: supplementary Table 1).

At least three representatives of each MT were transferred into cetyl trimethyl ammonium bromide (CTAB) buffer and stored at −20°C until molecular investigations. Each MT was analysed at least three times. If only one sequence pattern (operational taxonomical unit [OTU]) was obtained, the MT was referred to be this OTU. Otherwise, additional root tips of this MT were sequenced. The most abundant OTU was then regarded as the mycorrhizal partner.

Molecular methods

Deoxyribonucleic acid (DNA) was extracted according to Southworth (2000). Briefly, EMs were ground in 1.5 ml Eppendorf tubes containing 50 µl CTAB buffer using a micropestle. After adding 550 µl CTAB buffer (final concentration per sample: 12.5 mg hexa-decyltrimethylammonium bromide, 10 mM Tris–HCl pH 8, 1.4 M NaCl, 20 mM ethylenediamine tetraacetic acid [EDTA], 0.2% β-mercaptoethanol), the samples were incubated at 65°C for 40–60 min. After centrifugation for 7 min at maximum speed (16,000×*g*), the supernatant was precipitated using an equal volume of chloroform and repeatedly centrifuged for 15 min at a speed of 16,000×*g*. The upper phase was transferred into a new Eppendorf tube containing 750 µl cold (−20°C) isopropyl alcohol 98% and stored at −20°C for 30 min or overnight for precipitation. After 30 min centrifugation at 16,000×*g*, the pellet was washed with 200 µl cold (−20°C) 70% ethanol and centrifuged for 5 min at 4,500×*g*. Dried DNA pellets were resuspended in 50 µl Tris–EDTA buffer.

Five microliters of diluted DNA extracts were combined with 20 µl PCR mix, containing 10× buffer S (10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$), 5× enhancer (10 mM Tris–HCl, 50 mM KCl, 0.1 mM EDTA and 50% glycerine), deoxyribonucleotide triphosphate, peqGOLD *Taq* DNA polymerase (all Peqlab, Erlangen, Germany) and primers. The final concentrations of these components in 25 µl reaction mix were: 200 µM of each of 2′-deoxyadenosine 5′-triphosphate, 1.25 U of *Taq* polymerase, 0.4 mM of each primer, 0.64 mM Tris–HCl, 53.2 mM KCl, 1.5 $MgCl_2$, 6.4 µM EDTA and 0.8 µl glycerine.

PCR was performed with a Primus 96 advanced thermocycler (Peqlab) with the following conditions: 94°C for 5 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 55 s (annealing time increasing 3 s each cycle) and extension at 72°C for 45 s. Final extension was at 72°C for 6 min.

The following primer combinations were used: ITS1F×LR15, ITS1F×NL4 and ITS1F×ITS4. Some DNA extracts were amplified with ITS1F×ITS2 (Vilgalys 2005). To obtain PCR products of the ITS region and of a part of the large subunit, we also used the primer pair ITS1F and LR21.

DNA isolation and sequencing protocols for fruitbody processing followed those of a previous study (Peintner et al. 2001). Primers used for PCR amplification and sequencing of the ITS region of fruitbodies were ITS1 paired with ITS4, LR15, LR21 or NL4.

Purified PCR products were sequenced by MWG AG Biotech with the primers ITS1. Resulting sequences were edited and checked using Sequencher (version 4.6; Gene Codes Inc. Ann Arbor, MI).

Sequence analyses

Blast searches were carried out against the public sequence databases National Center for Biotechnology Information and UNITE (Kõljalg et al. 2005). OTUs were defined based on phylogenetic analyses (Bacher 2006). Sequences with at least 97% similarity were defined as one OTU and regarded as belonging to one species.

Statistical analyses

All statistical analyses were carried out with relative abundances of MTs. Thus, we regarded MTs as synonymous for OTUs in these analyses.

To analyse seasonal and spatial variation of the EM fungal communities associated with *Polygonum*, we calculated species richness (*S*), evenness (*E*) (distribution of species) and Simpson's (*H*) and Shannon (*D*) diversity indices according to McCune and Grace (2002). To categorise samples based on species composition of EM fungi, we used detrended correspondence analysis (DCA)

and hierarchical cluster analyses (Sørensen distance measure with flexible Beta, group average and nearest-neighbour linkage methods). Diversity indices, DCA and cluster analyses were calculated with PC-ORD Version 5.0 (McCune and Mefford 1999).

Analysis of variance (ANOVA) calculation was performed using SigmaStat Software (SigmaStat for Windows 3.5, 2006, Systat Software): As the dependant variable, we used either Shannon diversity indices or relative abundances of the most frequent MTs (MT 3, MT 6, MT 14 and MT 2) and the pooled abundances of the remaining MTs. After passing the normality test, two-way ANOVA was calculated with the two independent variables 'season' and 'plot.' The pairwise multiple comparison procedure (Tukey Test) was applied to detect groups differing significantly from the others ($p>0.050$).

To minimise underestimations of species richness (because of the sampling methodology itself), several richness estimators were performed: (1) abundance-based coverage estimator of species richness (ACE) relies on the abundances of rare species for estimation of the true species richness; (2) incidence-based coverage estimator of species richness (ICE) is based on presence/absence data; (3) Chao estimators (Chao1 and Chao2) use only singletons and doubletons to estimate the number of missing species and (4) the second-order Jackknife richness estimator (Jack2) is very sensitive to the number of rare species and can perform poorly with small a sample size. All estimations were calculated in EstimateS 8.0 (Colwell 2006).

Results

All *P. viviparum* root tips examined were EM. Neither arbuscular mycorrhizae nor dark-septae fungi were observed.

Based on morphological analyses, 19 MTs were distinguished out of a total of 2,000 root tips (Table 1, supplementary Table S1). MT 2 was a black MT with a gaunt, unhealthy appearance. Molecular analyses of this MT yielded a number of different sequences, among them soil fungi like *Exophiala* and *Phoma*, which are not potentially EM (data not shown). Therefore, we considered the representatives of MT 2 as senescent root tips and the resulting OTUs as secondary infections and not as mycorrhizal symbionts.

Three MTs were the clearly dominating mycorrhizal symbionts of *P. viviparum*: MT 6 (22.4%), MT 14 (20.5%) and MT 3 (18.5%) together colonised more than 60% of all root tips (Table 1, Fig. 2). These abundant MTs were identified as *Sebacina incrustans* (MT 6), *Cenococcum geophilum* (MT 14) and *Tomentella* spp. (MT 3; three OTUs; Table 1). The remaining 15 vital MTs had less than 6% abundance each (Table 1).

After the exclusion of the senescent MT 2, an overall of 18 OTUs (species) out of eight fungal genera were detected with molecular tools as mycobionts of *P. viviparum* (Table 2). Five of these fungal species were also detected as fruitbodies in the sampling area (Table 3). Sequences generated from fruitbodies helped to unambiguously identify mycobionts.

Table 1 Relative abundances of the detected morphotypes (MT) in all samples, for each sampling date (spring, summer, fall and winter) and for each plot (P1–P5); the MTs are sorted based on their abundance in all samples

MT	All samples	Spring	Summer	Fall	Winter	P1	P2	P3	P4	P5
MT 6	22.4	27.6	24.4	24.2	13.4	9.3	12.3	55.3	4.8	30.5
MT 14	20.5	27.8	19.8	15.2	19.0	29.0	5.5	6.8	38.3	22.8
MT 3	18.5	16.6	17.0	17.8	22.4	16.8	26.8	17.0	15.0	16.8
MT 2	12.6	3.8	5.6	11.0	30.0	6.5	24.3	11.8	8.3	12.3
MT 27	5.8	4.6	8.4	5.8	4.2	17.3	7.0	0.0	4.0	0.5
MT 11	4.2	4.2	9.4	1.0	2.0	2.8	1.3	2.3	7.8	6.8
MT 8	3.5	3.6	2.6	1.4	6.4	3.0	10.3	0.8	2.0	1.5
MT 39	3.0	0.0	1.4	9.0	1.6	2.5	2.3	1.0	8.0	1.3
MT 15	2.2	0.0	0.0	8.6	0.0	0.0	8.3	2.5	0.0	0.0
MT 38	1.5	0.0	5.8	0.0	0.0	0.0	0.0	0.0	7.3	0.0
MT 20	1.1	3.8	0.4	0.0	0.0	4.8	0.5	0.0	0.0	0.0
MT 21	1.1	4.2	0.0	0.0	0.0	5.3	0.0	0.0	0.0	0.0
MT 10	0.9	0.0	0.0	3.4	0.0	0.0	1.8	0.0	2.5	0.0
MT 33	0.8	0.0	3.0	0.0	0.0	0.0	0.0	0.0	1.0	2.8
MT 35	0.7	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	3.3
MT 17	0.6	2.4	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0
MT 37	0.6	0.0	2.2	0.0	0.0	0.0	0.0	2.8	0.0	0.0
MT 19	0.4	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8
MT 28	0.3	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.3	0.0
Number	19	11	12	11	9	11	11	9	12	11

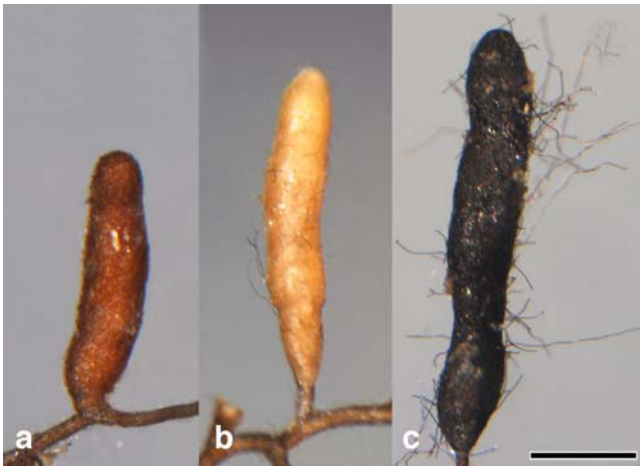

Fig. 2 Ectomycorrhizal morphotypes (MT) on *Polygonum viviparum* root tips. **a** MT 3 (*brown, with grainy surface*) was identified as *Tomentella* sp., **b** MT 6 (*yellowish, with smooth surface and with dark, presumably infectious hyphae*) as *Sebacina incrustans* and **c** MT 14 (*black, with straight thick emanating hyphae*) as *Cenococcum geophilum*. *Bar*=0.5 mm

Most OTUs (16) were basidiomycetes, and two were ascomycetes. Within the basidiomycetes, Thelephoraceae represented the highest species diversity (six OTUs of *Tomentella*, two OTUs of *Thelephora*; Table 2), followed by the Cortinariaceae (three OTUs of *Inocybe*, two OTUs of *Cortinarius*) and the Sebacinaceae (two OTUs of *Sebacina*). The Russulaceae were represented by one OTU (*Russula*). Ascomycetes were represented by one species of Helvellaceae (genus *Helvella*) and the anamorphic *C. geophilum*.

Species richness calculations based on all samples estimated a total richness of 19 OTUs (ACE), 28.6 OTUs (ICE), 19 OTUs (Chao1), 22.9 OTUs (Chao2) and 28.5 OTUs (Jack2; Table 4). Thus, actual sampling recovered at least 66% of the estimated species, when referred to the species richness estimators ICE and Jack2, which are the most sensitive to rare species and small sample size. Based on the Chao2 estimator, 83% of the mycobionts were found, but a suite of rare species remains to be detected. Based on ACE and Chao1, the total species richness of the sampling plot was detected (Fig. 3).

The composition of fungal communities associated with *P. viviparum* slightly changed within the different sampling dates, but the diversity (number of OTUs) remained comparatively constant with a mean of 11 OTUs detected per season (range=9–12 OTUs; Table 1). The highest diversity was detected in summer (12), and the lowest diversity was found in winter (9).

Based on two-way ANOVA, Shannon diversity indices of spring samples differed significantly (p=0.022) from fall and winter samples.

The dominant mycobionts (*S. incrustans* MT 6, *C. geophilum* MT 14, *Tomentella* spp. MT 3) occurred at all sampling dates with high relative abundances (range=13.4–

Table 2 Ectomycorrhizal fungal taxa (OTUs in alphabetical order) detected on root tips of *Polygonum viviparum* growing at a primary successional site in 2005

Root tip ID	Species	Genbank accession number	Sequence length	best Blast match/ voucher ID	Score	Similarity (%)
367	*Cenococcum geophilum*	EF655683	901	DQ179119	1,552	97
1444	*Cortinarius* aff. *Diasemospermus*	EF655689	677	AY748857	1,267	98
410	*C* aff. *Vernus*	EF655687	410	UDB000707	770	98
666	*Helvella* sp.	EF655691	307	UDB000177	479	Locked
415	*Inocybe rufofusca*	EF655684	736	IB20040114		100
648	*I. substraminipes*	EF655685	813	IB20050457		100
433	*Inocybe* sp. 1	EF655692	410	AB096872	353	94
615	*Russula* sp.	EF655693	202	UDB000113	400	100
603	*Sebacina incrustans*	EF655686	681	IB20060213		100
572	*Sebacina* sp.	EF655694	685	DQ974767	1,100	95
401	*Thelephora caryophyllea*	EF655687	650	IB20060087		100
519	*Thelephora* sp.	EF655695	744	AJ893305	1,201	96
595	*Tomentella atramentaria*	EF655688	581	AY702809	1,002	97
676	*Tomentella* sp. 1	EF655696	238	IB20060231		100
1149	*Tomentella* sp. 2	EF655697	738	AJ893300	1,239	96
618	*Tomentella* sp. 3	EF655698	461	AJ893327	674	93
517	*Tomentella* sp. 4	EF655699	435	U83474	453	95
495	*Tomentella* sp. 5	EF655700	464	AJ893296	912	99

Genbank Accession Number, sequence length of the rDNA ITS region, the best Blast match or Voucher ID, and Similarity (as score and in percentage) are given. Affinities of OTUs with similarities less than 97% to closely related taxa are shown with "aff." (=*affinis*) preceding the species epithet.

Table 3 Fungal fruitbody collections used in this study for identification of mycobionts (100% match with ECM root tips of *Polygonum viviparum*) with collection number, Genbank accession numbers and sequence length

Species	Voucher number (IB)	Genbank accession number	Sequence length
Inocybe rufofusca	IB20040114	EF655704	629
Inocybe substraminipes	IB20050457	EF655703	675
Sebacina incrustans	IB20060213	EF655701	942
Thelephora caryophyllea	IB20060087	EF655705	503
Tomentella sp.	IB20060231	EF655702	918

24.2%). No significant differences between seasons were found. *C. geophilum* and *S. incrustans* clearly dominated in the spring samples, but their abundances decreased in winter, whereas abundances of *Tomentella* spp. increased in winter.

Relative abundances of senescent mycorrhizal root tips (MT 2) differed significantly in winter compared to all other seasons ($p \leq 0.002$): They were comparatively rare in spring (3.8%), increased throughout the season (summer 5.6%; fall 11.0%) and were very abundant in winter (30.0%; Table 1). MT 27 (*Thelephora* spp.), MT 11 (*Sebacina* sp.) and MT 8 (*Cortinarius* spp.) were detected throughout the year, while MT 39 (*Inocybe* spp.) was found during all seasons except spring. Ten fungal species were found in one season only. However, all of them occurred with relative abundances less than 9%. All these infrequent MTs were lacking in winter. Fungal communities were comparatively evenly distributed with the most even communities in winter (Table 4).

Hierarchical cluster analyses grouped winter samples together (Fig. 4), indicating the strong influence of environmental conditions during winter on the species composition (only the linkage method 'nearest neighbour' is shown). However, a seasonal influence was not detected for the other three seasons. Rather, the dendrogram indicates spatial factors (P1 in summer and fall, P2 and P3 in spring and summer) as reasons for similarity in species composition.

Although spatial variation of fungal communities between plots seems high, Shannon diversity indices significantly differed only between plots 1 and 3 ($p=0.012$) and between plots 1 and 5 ($p=0.029$). MT 6 (*S. incrustans*), MT 14 (*C. geophilum*), MT 3 (*Tomentella* spp.) and MT 2 (senescent root tips) occurred at all sampling plots, but their abundances varied widely between plots: 4.8–55.3% for MT 6, 5.5–38.3% for MT 14 and 15–26.8% for MT 3. However, significant differences were only found as follows: relative abundances of senescent EM root tips differed significantly between plots 2 and 1 ($p=0.010$) and between plots 2 and 4 ($p=0.019$). Relative abundances of MT 14 differed significantly between plots 4 and 2 ($p=0.018$) and between plots 4 and 3 ($p=0.023$). No significant differences were found for MT 3, MT 6 and pooled abundances of the remaining MTs. *S. incrustans* clearly dominated in plot 3 but had comparatively low abundance in plot 4. Seven MTs were found in one location, and all of these occurred with relative abundances less than 8%. Omitting the most abundant MTs, shared species between plots at the same sampling date were rare. This further indicates that spatial heterogeneity of plots was high (supplementary Fig. S1).

The observed diversity was comparatively constant with a mean of 11 OTUs detected per plot (range=9 to 12 OTUs, Table 4). The highest diversity was detected in P1 and P4, and the lowest diversity was found in P3. Evenness was higher in plots than in seasons (Table 4).

DCA (supplementary Fig. S1) clustered winter samples together, but for all other seasons, fungal communities clustered because of their spatial distribution. Fungal communities of plots 1 and 4 as well as plots 3 and 5 were similar.

Table 4 Diversity indices (*S*—richness, *E*—evenness, *H*—Shannon's diversity index and *D*—Simpson's) and richness estimators (Sobs, ACE, ICE, Chao1, Chao2, Jack2) for *P. viviparum* mycorrhizae in pooled seasonal (spring, summer, fall and winter) and pooled local (P1–P5) samples

	S	*E*	*H*	D	Sobs	ACE	ICE	Chao1	Chao2	Jack2
Spring	4.00	0.788	1.100	0.580	11	11	15.3	11	13.0	16.6
summer	4.20	0.777	1.418	0.697	12	12	15.2	12	13.2	16.2
fall	4.80	0.755	1.539	0.735	11	11	13.1	11	11.6	13.8
winter	4.80	0.811	1.540	0.761	9	9	10.1	9	9.4	11.3
P1	6.00	0.903	1.614	0.775	12	12	12.4	12	12.0	11.2
P2	5.50	0.878	1.468	0.726	11	11	11.1	11	11.0	8.6
P3	4.25	0.731	1.034	0.534	9	9	9.4	9	9.0	8.5
P4	5.00	0.846	1.343	0.682	12	12	12.9	12	12.1	12.5
P5	6.00	0.787	1.537	0.749	11	11	13.1	11	11.5	13.7
all	5.30	0.844	1.399	0.693	19	19	28.6	19	22.9	28.5

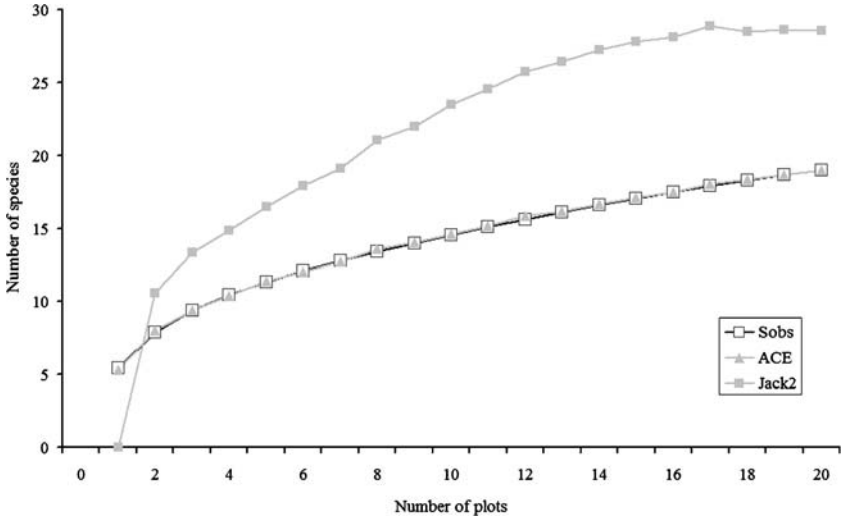

Fig. 3 Species richness estimation curves of *Polygonum viviparum* ectomycorrhizae in all plots (Sobs, ACE, Jack2)

Discussion

Diversity of *Polygonum* mycobionts

P. viviparum roots were always EM at the investigated alpine primary successional site. Nineteen EM MTs (including one MT defined for senescent EM) were found in association with *P. viviparum*, which is more than reported in previous studies (Fontana 1977; Haselwandter and Read 1980; Read and Haselwandter 1981; Treu et al. 1996). Eighteen fungal species were identified as mycobionts of *P. viviparum*. Based on current knowledge (e.g. Nara 2006; Tedersoo et al. 2006; Krpata et al. 2007), all of these are non-specific EM partners of EM plants. Species of the genera *Tomentella*, *Sebacina* and *Cenococcum* were the dominant fungal partners of *Polygonum* at this site. Fungal species belonging to these genera do not form conspicuous fruitbodies. Fruitbody data have also been an important classical tool for diversity estimation in alpine habitats (Senn-Irlet 1993, Graf 1994). Our data now demonstrate that the presence and presumably the ecological role of *Tomentella* and *Sebacina* species were especially underestimated in these habitats, as fruitbodies of these genera were never reported from alpine sites.

Species accumulation curves and species richness estimators demonstrate that the major part of the mycobiont diversity of *P. viviparum* has been captured at this site. As typical for primary successional sites, species diversity was generally low (e.g. Jumpponen et al. 2002: 13 taxa; Nara et al. 2003: 21 taxa) compared to later successional stages or climax forests (e.g. Richard et al. 2005: 140 taxa; Tedersoo et al. 2006: 172 taxa). Species richness of *Polygonum* mycobionts was probably restricted because of the limited availability of fungal partners in this environment. Such a low diversity enables a nearly complete survey of fungal communities at defined habitats. This is a clear advantage

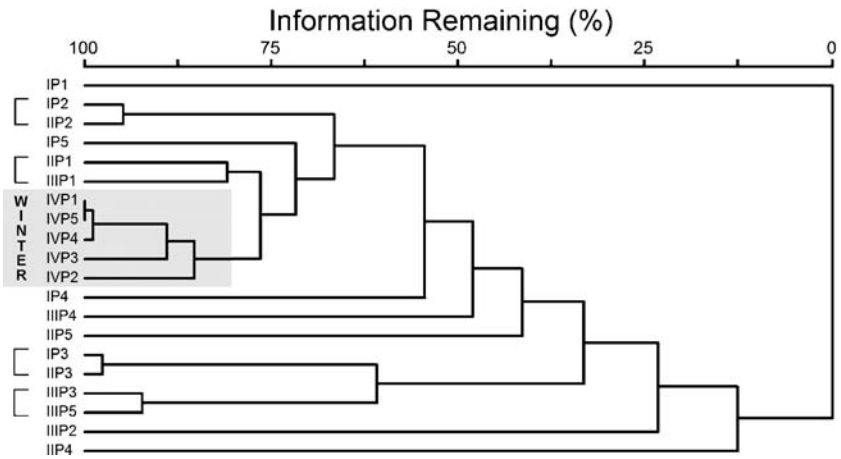

Fig. 4 Dendrogram depicting hierarchical cluster analysis of samples grouped by similarity of species composition of mycorrhizal morphotypes. *Roman numerals* indicate sampling date (*I*—spring, *II*—summer, *III*—fall, *IV*—winter). The *number preceded by P* indicates the plots (*1* to *5*). *Gray-stained area* indicates winter samples similar in species composition. The *brackets* highlight samples within the 80% limit of similar species composition

for unravelling seasonal dynamics and spatial distribution of mycobionts.

Seasonal dynamics

As early as 2 weeks after snow melt in spring, mycorrhizal root tips were well developed, vital and showed high mycobiont diversity. The relative abundance of senescent root tips differed significantly between seasons ($p<0.001$): They were lowest in spring and increased throughout the year, with a maximum in winter (frozen soil). Dominant mycobionts (*S. incrustans*, *C. geophilum* and *Tomentella* spp.) were still abundant and vital in frozen soil and did not show clear seasonal changes. The high diversity and vitality of mycobionts shortly after snow melt leads to the question of when mycorrhizae develop. Mycobiont growth and physiological activity obviously start when soil is still under snow cover. Under such conditions, soil temperature is constantly at about 0°C. Moisture conditions change early during snow melt, when melting water penetrates through the snow cover reaching the soil surface. Thus, the availability of water could be the initiating point for plant root development and the fungal colonisation of the root tips. We speculate that water and not temperature is the main limiting factor in these alpine environments with shallow soils and limited plant cover. As excellently reviewed by Nemergut et al. (2005), a number of recent studies focus on arctic and alpine soil microbial communities. Schmidt and Lipson (2004) showed that especially the transition from winter to summer is a very dynamic period, in which the dying winter community releases nitrogen that is used by plants and the microbial communities adapted to summer. Further studies focusing on the developmental dynamics of fungal communities under snow cover in alpine primary successional sites are planned.

Mycorrhizal status of *Polygonum viviparum*

P. viviparum has a unique ecology among herbaceous Polygonaceae in Europe as the only species known to form EM. Väre et al. (1992) reported that *P. viviparum* is only EM when other EM plant hosts are present. In the investigated sampling plots, *Polygonum* grew in close vicinity to either *Salix herbacea* or *K. myosuroides* (Raffl et al. 2006). We did not investigate *Polygonum* plants in sites without other EM plants. Massicotte et al. (1998) hypothesised that plant development, soil conditions or seasonal aspects could also be reasons for the contrasting data on the mycorrhizal status of *P. viviparum*. This study clearly shows that seasonal aspects and plant development do not influence the general EM status of *P. viviparum*, as plants of different ages were EM at every season. Kytöviita (2005) suggested high soil moisture to be limiting for mycorrhizal conditions. Results of this study, however, do not agree with Kytöviita (2005) because abundant and well-developed *P. viviparum* ectomycorrhizae were found in wet and water-filled depressions in spring, shortly after snow melt.

Kytöviita (2005) also postulated that for arctic regions, a general mycorrhizal symbiont shift occurs from arbuscular mycorrhizae to EM in herbaceous plants because AM may not be well adapted to nutrient uptake from cold soil. Moreover, she further assumed this shift to be more pronounced in alpine habitats because of the old age of alpine environments. Based on the high degree of ectomycorrhization in the investigated plots with only 150 years of soil development, we rather regard the presence/absence of fungal mycobionts and limiting abiotic factors of the soil environment as key factors for the symbiotic status of *P. viviparum* and other mycorrhizal host plants.

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