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ORIGINAL ARTICLE

Impact of *Heterobasidion* root-rot on fine root morphology and associated fungi in *Picea abies* stands on peat soils

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Abstract We examined differences in fine root morphology, mycorrhizal colonisation and root-inhabiting fungal communities between *Picea abies* individuals infected by *Heterobasidion* root-rot compared with healthy individuals in four stands on peat soils in Latvia. We hypothesised that decreased tree vitality and alteration in supply of photosynthates belowground due to root-rot infection might lead to changes in fungal communities of tree roots. Plots were established in places where trees were infected and in places where they were healthy. Within each stand, five replicate soil cores with roots were taken to 20 cm depth in each root-rot infected and uninfected plot. Root morphological parameters, mycorrhizal colonisation and associated fungal communities, and soil chemical properties were analysed. In three stands root morphological parameters and in all stands root mycorrhizal colonisation were similar between root-rot infected and uninfected plots. In one stand, there were significant differences in root morphological parameters between root-rot infected versus uninfected plots, but these were likely due to significant differences in soil chemical properties between the plots. Sequencing of the internal transcribed spacer of fungal nuclear rDNA from ectomycorrhizal (ECM) root morphotypes of *P. abies* revealed the presence of 42 fungal species, among which ECM basidiomycetes *Tylospora asterophora* (24.6 % of fine roots examined), *Amphinema byssoides* (14.5 %) and *Russula sapinea* (9.7 %) were most common. Within each stand, the richness of fungal species and the composition of fungal communities in root-rot infected versus uninfected plots were similar. In conclusion, *Heterobasidion* root-rot had little or no effect on fine root morphology, mycorrhizal colonisation and composition of fungal communities in fine roots of *P. abies* growing on peat soils.

Keywords Norway spruce · Root-rot · Ectomycorrhizal fungi · Pathogens · Organic soil

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Introduction

Heterobasidion spp. are among the most widespread root-rot pathogens in boreal and northern temperate forests where they cause huge economic losses (Woodward et al. 1998). *Heterobasidion* includes five species, three occurring in Europe, *H. annosum*, *H. parviporum* and *H. abietinum*, and two in North America, *H. irregulare* and *H. occidentale* (Dalman et al. 2010). Although the host species may partly overlap, among European species, *H. annosum* is mainly found on pine, *H. parviporum* on spruce and *H. abietinum* on fir (Dalman et al. 2010 and references therein). The fungi enter stands mainly through the colonisation of fresh conifer stumps; mycelia grow down to root systems and infect neighbouring healthy trees via root contacts (Rishbeth 1951). *Heterobasidion* can persist and remain active in root

systems for decades and infect trees of the next forest generation planted on infested sites (Rishbeth 1951; Stenlid 1987). *Heterobasidion* generally causes extensive heart-rot in roots and stem of Norway spruce (*Picea abies*), resulting in reduced diameter/volume growth (Bendz-Hellgren and Stenlid 1995, 1997), but it may also infect functional sapwood and cause tree mortality (Piri 2003; Piri and Korhonen 2001).

In Latvia, *P. abies* stands constitute 17 % (5374 km^2) of the total forest area. Of the *P. abies* area, 38 % (2067 km^2) is on drained peat soils (Jansons 2011). In Latvia, ca. 16 % of *P. abies* stands on peat soils are root-rot infected (Arhipova et al. 2011), which causes up to 20 % of timber loss (Gaitnieks et al. 2008). Often occurrence of *Heterobasidion* root-rot in *P. abies* stands on peat soils was suggested to be associated with development of rich vegetation and moist conditions (Stivriņa et al. 2010). Besides, it might also be promoted by alkaline and nutrient-rich groundwater, which occurs in at least 80 % of the total drained peat forest area (Zālītis 2006).

Ectomycorrhizal (ECM) fungi are commonly associated with fine roots of *P. abies* and depend on their host for nutrition (Smith and Read 1997). Some ECM fungi can protect roots against certain pathogens and promote plant growth (Sinclair et al. 1982). Several ECM fungi were also able to reduce growth of *Heterobasidion* when confronted in vitro (Napierala-Filipiak and Werner 2000). Although *Heterobasidion* is not a fine-root pathogen and it is not able to freely grow in the soil (Garbelotto and Gonthier 2013), we hypothesise that overall reduction in tree vitality as a result of root-rot infection might lead to alterations in ECM communities in tree roots. For example, Bendz-Hellgren and Stenlid (1995) suggested that observed 50 % of the growth loss could be due to allocation of resources to cope with the *Heterobasidion* infection. Furthermore, in cases where functional sapwood is infected, transport of photosynthetically derived carbohydrates to roots and ultimately to ECM symbionts might be limited, potentially leading to even more pronounced alterations in communities of associated fungi. In Latvia, fine root morphological parameters and the abundance of ECM morphotypes were compared in *Heterobasidion* root-rot infected and uninfected stands of *P. abies* growing on mineral and peat soils (Gaitnieks 2005; Gaitnieks et al. 2000). Gaitnieks (2005) observed some differences in ECM communities between root-rot infected and uninfected trees but also a significant site effect. However, in that study there was a need for more reliable identification of ECM species.

The objective of the present study was to compare fine root morphology, mycorrhizal colonisation and associated fungal communities in *Heterobasidion* root-rot infected and uninfected *P. abies* growing on peat soils. The rationale of the study was to get better understanding on whether and how *Heterobasidion* root-rot may affect indirectly morphology of fine roots and their colonisation by ECM fungi. This was expected to demonstrate that, in addition to the direct negative effect to tree health, *Heterobasidion* as other biotic stress factors (Saravesi et al. 2015; Treu et al. 2014) may also indirectly determine health and sustainability of forest ecosystems.

Material and methods

Study sites

For study, we selected four *P. abies* stands (K, O, S and M) in Latvia (Table 1). Information on the health status (defoliation) of each stand was obtained from the Latvian State Forest Research Institute "Silava" and is presented in Table 2. Defoliation was evaluated using the method described by Eichhorn et al. (2010). The presence/absence of *Heterobasidion* root-rot in each stem was determined by taking wood samples ca. 30–40 cm above the ground using an increment borer. Surface-sterilised wood samples were aseptically incubated on malt-agar media, and outgrowth of *Heterobasidion* spp. was scored after a week. Both *H. annosum* and *H. parviporum* were recorded in the samples. Based on scoring results, three or four plots, each possessing a group of three to five infected trees, and a corresponding number of closely situated plots with three to five uninfected trees were selected randomly in each site (Table 1).

Sampling

In July 2013, soil with roots was sampled from the uppermost 20-cm soil layer using a soil core 12 cm in diameter. Within each site, five replicate soil cores were taken from each of *Heterobasidion* root-rot infected and uninfected plots resulting in 140 soil cores in total (Table 1). Soil cores were placed separately in plastic bags, transported to the laboratory and stored at +4 °C until processing. *P. abies* roots were separated from soil and rinsed under tap water. Cleaned roots were cut into 1-cm segments and placed in water in Petri dishes with diameter 14 cm and a 7 × 7 mm grid on the bottom. ECM morphotypes were identified in 100-grid squares using a Leica MZ-7.5 stereomicroscope (Leica Microsystems, Wetzlar, Germany). ECM morphotypes were grouped based on colour, form and texture, and the presence and patterns of rhizomorphs or external mycelia (Agerer 1986–2006). Living and dead fine roots were identified based on their colour and elasticity of the central cylinder (Vogt and Persson 1991). Morphological parameters of fine roots including length, volume, surface area and number of root tips were determined using an Epson Perfection V750Pro scanner (Epson, Tokyo, Japan) and WinRHIZO 2005 C (Regent Instruments Inc., Canada) software. After scanning, roots were dried at 50 °C for 12 h and weighed. Soil was dried 1 week at room temperature (ca. 21 °C) and sieved (mesh size 2 × 2 mm) to separate larger fractions, which were discarded. Chemical analyses of

Table 1 Location and characteristics of four investigated forest sites, each containing *Heterobasidion* root-rot infected and uninfected plots with *Picea abies* trees

Site	Position	Plot	Health status	Soil cores sampled	Fine roots examined	ECM morphotypes detected	Forest type	Stand composition and age
K	N 56° 67.66′, E 25° 89.75′	KR1	Infected	5	675	8	*Myrtilliosa turf. mel.*	90 % *Picea abies*, 10 % *Betula pendula*. Age 41 years
		KR2		5	674	7		
		KR3		5	553	6		
		All KR		15	1902	9		
		KH1	Uninfected	5	669	8		
		KH2		5	794	7		
		KH3		5	693	8		
		All KH		15	2156	9		
All K				30	4058	12		
O	N 56° 85.42′, E 24° 79.86′	OR1	Infected	5	543	7	*Oxalidosa turf. mel.*	90 % *Picea abies*, 10 % *Betula pendula*. Age 44 years
		OR2		5	604	7		
		OR3		5	703	7		
		All OR		15	1850	8		
		OH1	Uninfected	5	689	8		
		OH2		5	802	9		
		OH3		5	497	9		
		All OH		15	1988	11		
All O				30	3838	12		
S	N 56° 70.17′, E 25° 90.55′	SR1	Infected	5	711	9	*Oxalidosa turf. mel.*	80 % *Picea abies*, 20 % *Betula pendula*. Age 67 years
		SR2		5	724	8		
		SR3		5	667	8		
		SR4		5	708	9		
		All SR		20	2810	9		
		SH1	Uninfected	5	710	9		
		SH2		5	731	9		
		SH3		5	683	8		
		SH4		5	698	8		
		All SH		20	2822	9		
All S				40	5632	9		
M	N 56° 77.47′, E 24° 13.36′	MR1	Infected	5	612	7	*Myrtilliosa turf. mel.*	60 % *Picea abies*, 40 % *Pinus sylvestris*. Age 112 years.
		MR2		5	501	9		
		MR3		5	471	8		
		MR4		5	563	10		
		All MR		20	2147	13		
		MH1	Uninfected	5	527	8		
		MH2		5	551	10		
		MH3		5	445	13		
		MH4		5	519	10		
		All MH		20	2042	16		
All M				40	4189	16		

Infected plots with trees infected by *Heterobasidion*, *Uninfected* plots with trees free of *Heterobasidion*

soil were conducted using established standard methods (International Organization for Standardization (ISO) standard and Latvian State standards (LVS)). Samples were prepared for analysis according to ISO 11464:2005. Soil pH (KCl) was potentiometrically measured in the deionised water suspension according to LVS ISO 10390. Total carbon content was determined using elemental analyser ELTRA CS530 (Eltra GmbH, Haan, Germany) according to LVS ISO 10694. The content of total N was determined using modified Kjeldahl method (ISO 11261:1995), and P was assayed by

Table 2 Defoliation of *Picea abies* in *Heterobasidion* root-rot infected and uninfected sampling plots

Site	Plots	No. of trees evaluated	Mean defoliation, %
K	Infected (KR)	12	52.9 ± 5.9*
	Uninfected (KH)	11	27.3 ± 3.6
O	Infected (OR)	22	46.4 ± 3.9**
	Uninfected (OH)	18	23.1 ± 1.7
S	Infected (SR)	27	40.4 ± 2.9**
	Uninfected (SH)	23	21.9 ± 1.1
M	Infected (MR)	12	51.0 ± 2.9**
	Uninfected (MH)	22	25.2 ± 2.0

Values show the mean ± standard error. Comparisons were done within each site

Significantly greater values are denoted by *$p < 0.01$; **$p < 0.001$

spectrophotometry using the ammonium molybdate spectrometric method in 1 M HCl extract (LVS 398). Concentration of K, Ca and Mg was determined by atomic absorption spectrophotometer with an acetylene-air flame in 1 M HCl extract.

Molecular analysis

In each site, up to five root tips of each distinct ECM morphotype were collected and used for molecular identification of fungal species. DNA extraction from individual root tips was done as described by Vainio et al. (1998). Amplification of the internal transcribed spacer (ITS) region of fungal nuclear rDNA using primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) and electrophoresis and sequencing was done as described by Klavina et al. (2013). Raw sequence data were analysed using the SeqMan version 5.07 software from DNASTAR package (DNASTAR, Madison, WI, USA) and BioEdit v. 7.0.9.0 (Hall 1999). Databases at GenBank (Altschul et al. 1997) and UNITE https://unite.ut.ee/ (Kõljalg et al. 2013) were used to determine the identity of ITS rDNA sequences. The criteria used for identification were as follows: sequence coverage >80 %; similarity to species level 97–100 %; and similarity to genus level 94–96 %. The sequences are available from GenBank under accession numbers KR019832–KR019874.

Statistical analyses

Differences in defoliation, root morphological parameters, mycorrhizal colonisation and soil chemical properties between *Heterobasidion* root-rot infected and uninfected plots of each respective study site were compared using one-way analysis of variance (ANOVA) in Minitab v.16.2.4 (Minitab, Inc., Coventry, UK). Richness of fungal species in root-rot infected and uninfected plots of each study site was compared by non-parametric chi-square test (Magurran 1988). The Shannon diversity index was used to characterise diversity of fungal communities (Shannon 1948). Fungal community structure in roots of *P. abies* and possible *Heterobasidion* root-rot effect was analysed using canonical correspondence analysis (CCA) in CANOCO 4.5 (ter Braak and Smilauer 1998). Significance of the environmental variables was tested using a generalised linear model and Gaussian distribution. MANOVA was used to evaluate degree of separation (along CCA axis 1 and 2) between the fungal communities in *Heterobasidion* root-rot infected and uninfected plots.

Results

Defoliation was significantly higher in all *Heterobasidion* root-rot infected plots than in corresponding uninfected plots (Table 2). Within O, S and M sites (Table 1), root morphological parameters were similar between *Heterobasidion* root-rot infected and uninfected plots (Table 3). In site K (Table 1), in contrast, all (except number of dead root tips) root morphological parameters in root-rot infected plots were significantly lower than those in uninfected plots (Table 3). Root mycorrhizal colonisation was high in all sites and plots (ranging between 86.4 and 100 %), and no significant differences were found between corresponding root-rot infected and uninfected plots (Table 3). Soil chemical properties varied among the sites (Table 4), but were similar between root-rot infected and uninfected plots within each site (Table 4) with some exceptions: in site K, pH and concentration of N and Ca were significantly lower in root-rot infected plots than in uninfected plots, while the C/N ratio was significantly higher in root-rot infected versus uninfected plots (Table 4).

In total, 42 fungal species were detected by ITS rDNA sequencing of analysed fine root samples of *P. abies*. Most of the species (38) were ECM (Table 5), but also saprotrophic or weakly pathogenic fungal species such as *Cryptococcus* sp., *Ilyonectria* sp. and *Trichosporon* sp. were sequenced. Within each site, a chi-square test showed that richness of fungal species did not significantly differ between root-rot infected and uninfected plots (Table 5). The Shannon diversity index was also similar between root-rot infected versus uninfected plots of each site (Table 5). The most common fungi were *Tylospora asterophora* (24.6 %), *Amphinema byssoides* (14.5 %), *Russula sapinea* (9.7 %) and *Tomentella stuposa* (7.8 %) (Table 5).

CCA ordination (Fig. 1) of fungal communities explained 27.3 % of the variation on axis 1 and 23.1 % on axis 2. CCA showed that composition of fungal communities was mainly determined by soil factor C/N ratio ($p < 0.002$); pH ($p < 0.001$) and K ($p < 0.02$) present at each site (Fig. 1). MANOVA showed that separation of fungal communities between *Heterobasidion* root-rot infected and uninfected plots was not supported significantly along axis 1 and axis 2.

Table 3 Root morphological parameters in *Picea abies* stands (K, O, S and M), each containing *Heterobasidion* root-rot infected and uninfected sampling plots (shown in parenthesis and named as in Table 1)

Morphological	K		O		S		M	
Parameter	Infected (KR)	Uninfected (KH)	Infected (OR)	Uninfected (OH)	Infected (SR)	Uninfected (SH)	Infected (MR)	Uninfected (MH)
Dry mass of fine roots, g	2.2 ± 0.2	3.4 ± 0.3**	2.9 ± 0.4	4.1 ± 0.6	3.2 ± 0.3	2.5 ± 0.2	2.8 ± 0.2	3.1 ± 0.2
Volume of fine roots, cm^3	2.3 ± 0.3	4.5 ± 0.4**	3.8 ± 0.5	5.4 ± 1.0	2.9 ± 0.3	5.3 ± 1.6	6.3 ± 1.1	6.5 ± 1.0
Length of fine roots, cm	1293 ± 123	1979 ± 163**	1273 ± 180	1810 ± 226	2254 ± 217	2760 ± 203	2568 ± 417	2664 ± 353
Surface area of fine roots, cm^2	193 ± 19.5	331 ± 28.3**	243 ± 31.3	342 ± 50.9	285 ± 28.4	381 ± 36.1*	445 ± 73.9	462 ± 64.5
Diameter of fine roots, mm	0.9 ± 0.02	1.0 ± 0.04**	1.2 ± 0.04	1.1 ± 0.04	1.6 ± 0.03	1.7 ± 0.13	1.5 ± 0.1	1.5 ± 0.09
Number of living root tips	229 ± 39.3	435 ± 47.5**	268 ± 37.3	319 ± 62.9	400 ± 43.1	352 ± 51.8	283 ± 30.4	282 ± 28.8
Number of dead root tips	247 ± 38.8	330 ± 34.4	292 ± 41.9	267 ± 38.1	491 ± 49.1	383 ± 51.3	380 ± 39.8	475 ± 36.6
Mycorrhization, %	99.7 ± 0.2	99.7 ± 0.2	99.4 ± 0.6	100 ± 0	95.2 ± 0.9	95.2 ± 1.6	89.3 ± 1.9	86.4 ± 1.7

Values show the mean ± standard error. Comparisons were done within each site

Significantly greater values are denoted by: *$p < 0.05$; **$p < 0.01$

Consequently, root-rot infected and uninfected plots of each site were more or less well grouped together on axis 1, while their position in the ordination showed relative importance of environmental variables. C/N ratio had a significant effect on fungal community structure and consequently on position of root-rot infected (MR) and uninfected (MH) plots of M site, pH on position of K and S sites, while potassium had a significant effect on position of root-rot infected (OR) and uninfected (OH) plots of O site and their separation along axis 2.

Discussion

Despite the significantly higher defoliation in root-rot infected plots, the results demonstrated that *Heterobasidion* root-rot had little or no effect on fine root morphology, root mycorrhizal colonisation (Table 3) and composition of fungal communities in fine roots of *P. abies* (Table 5, Fig. 1), thereby rejecting the hypothesis that reduction in tree vitality as a result of root-rot infection might lead to alterations in fine root morphology and mycorrhizal colonisation. In the K site, differences in root morphological parameters between root-rot infected versus uninfected plots were likely due to the significant differences in soil chemical properties between these plots (Table 4). In agreement, it was shown that pH and concentration of N in the soil may have a major effect on root morphological parameters of *P. abies* (Helmisaari et al. 2009). Previous studies suggested that mycorrhizal colonisation of Scots pine (*Pinus sylvestris*) is lower in *Heterobasidion* root-rot infected stands (Коротков 1974) and that root-rot may also affect fine root morphology of *P. abies* (Gaitnieks 2005). However, the present study did not show such effects for *P. abies* on peat soils though these effects may appear later, i.e. in older trees and after *Heterobasidion* root-rot becomes extensively established (Swedjemark and Stenlid 1993). In support, establishment and spread of *Heterobasidion* in living trees of *P. abies* is generally much slower than in non-living trees or stumps due to active defence responses and build-up

Table 4 Soil chemical characteristics of four *Picea abies* stands (K, O, S and M), each containing *Heterobasidion* root-rot infected and uninfected sampling plots (shown in parenthesis and named as in Table 1)

Chemical	K		O		S		M	
Characteristics	Infected (KR)	Uninfected (KH)	Infected (OR)	Uninfected (OH)	Infected (SR)	Uninfected (SH)	Infected (MR)	Uninfected (MH)
Moisture, %	14.3 ± 0.02	14.5 ± 0.2	8.9 ± 2.6	11.3 ± 0.1	12.9 ± 2.8	10.4 ± 2.1	13.0 ± 0.1	13.0 ± 0.1
pH (KCl)	3.7 ± 0.1	4.2 ± 0.1*	5.4 ± 0.1	4.1 ± 0.6	4.4 ± 0.1	4.5 ± 0.1	2.6 ± 0.03	2.6 ± 0.01
N total, g/kg	22.2 ± 0.5	26.0 ± 0.9*	11.3 ± 3.2	15.9 ± 0.9	14.8 ± 4.2	14.2 ± 2.3	16.6 ± 0.5	16.1 ± 0.2
C total, g/kg	471.9 ± 2.2	454.8 ± 8.6	216.6 ± 58.2	316.1 ± 37.2	273.7 ± 72.8	230.1 ± 42.2	525.7 ± 6.2	530.3 ± 3.1
C:N	21.3 ± 0.5**	17.5 ± 0.3	19.1 ± 0.5	19.8 ± 1.8	18.8 ± 1.3	19.0 ± 0.2	32.2 ± 1.1	33.6 ± 0.4
P, mg/kg	25.9 ± 7.3	46.7 ± 19.7	57.5 ± 7.7	38.8 ± 14.0	52.6 ± 7.7	83.7 ± 28.0	53.6 ± 2.9	52.7 ± 2.9
K, mg/kg	99.6 ± 16.4	129.8 ± 13.4	57.9 ± 2.3	110.4 ± 33.2	140.8 ± 30.4	135.8 ± 17.2	130.6 ± 9.6	133.0 ± 9.7
Mg, g/kg	0.7 ± 0.03	0.9 ± 0.2	1.1 ± 0.4	0.9 ± 0.3	0.5 ± 0.2	0.5 ± 0.1	0.7 ± 0.03	0.7 ± 0.02
Ca, g/kg	17.3 ± 0.8	25.0 ± 1.6*	15.4 ± 5.5	12.6 ± 3.5	9.7 ± 4.8	7.3 ± 1.8	6.1 ± 0.5	6.3 ± 0.3

Values show the mean ± standard error. Comparisons were done within each site

Significantly greater values are denoted by *$p < 0.05$; **$p < 0.01$

Table 5 Frequency of fungal taxa shown as percentage of ECM roots colonised in four *Picea abies* stands (K, O, S and M), each with *Heterobasidion* root-rot infected and uninfected sampling plots (shown in parenthesis and named as in Table 1)

Fungal species	GenBank	K		O		S		M		Total[a]
	Accession no.	Infected (KR)	Uninfected (KH)	Infected (OR)	Uninfected (OH)	Infected (SR)	Uninfected (SH)	Infected (MR)	Uninfected (MH)	
Amanita citrina	KR019832	–	1.0	–	–	–	–	–	–	0.1
Amanita porphyria	KR019833	–	–	–	–	–	–	10.1	–	1.1
Amphynema byssoides	KR019834	21.7	20.9	8.9	19.8	14.0	16.0	9.8	6.8	14.5
Amphynema sp.1	KR019835	–	–	–	–	3.4	1.5	–	–	0.9
Amphynema sp. 2	KR019836	–	–	–	–	–	–	30.5	21.7	5.6
Byssocorticium sp.	KR019837	–	1.6	–	–	–	–	–	–	0.2
Cenococcum sp.	KR019868	–	–	–	–	0.4	1.2	2.5	7.5	1.4
Clavulina sp.	KR019838	–	26.1	–	–	–	–	–	–	2.9
Cortinarius cinnamomeus	KR019840	–	–	–	–	–	–	9.5	–	1.0
Cortinarius sp.	KR019839	–	–	–	–	–	–	–	4.4	0.5
Cryptococcus sp.	KR019841	–	–	–	–	12.7	9.3	–	–	4.0
Elaphomyces muricatus	KR019869	–	–	–	–	–	–	13.1	–	1.4
Helotiales sp.	KR019871	–	–	–	–	–	–	0.1	9.5	1.0
Humaria hemisphaerica	KR019872	–	–	–	–	–	–	–	0.2	0.0
Hygrophorus olivaceoalbus	KR019843	–	–	–	–	–	–	0.6	–	0.1
Hygrophorus pustulatus	KR019842	–	5.6	–	–	–	–	–	–	0.6
Ilyonectria sp.	KR019870	–	–	–	–	3.7	2.1	–	–	1.1
Inocybe nitidiuscula	KR019844	12.8	–	–	–	7.5	14.7	–	–	4.8
Inocybe proximella	KR019845	29.6	–	–	–	–	–	–	–	1.7
Inocybe relicina	KR019846	–	–	–	–	–	–	–	11.7	1.2
Lactarius necator	KR019847	–	–	–	–	2.9	5.2	–	–	1.5
Lactarius sp.	KR019848	24.0	1.1	–	–	–	–	–	–	1.5
Lactarius tabidus	KR019849	–	–	0.4	–	–	–	1.0	0.7	0.2
Piloderma fallax	KR019851	–	–	–	–	–	–	11.9	5.5	1.9
Piloderma olivaceum	KR019852	–	–	–	–	–	–	4.0	0.3	0.5
Piloderma sp.	KR019850	–	–	–	–	–	–	–	0.4	0.0
Pseudotomentella mucidula	KR019853	–	–	–	–	–	–	3.1	1.7	0.5
Pyronemataceae sp.	KR019873	–	–	–	2.5	–	–	–	–	0.3
Russula firmula	KR019854	0.7	–	–	1.5	–	–	–	–	0.2
Russula ochroleuca	KR019855	–	–	–	–	–	–	–	0.4	0.0
Russula sapinea	KR019856	–	–	45.3	32.6	–	–	–	–	9.7
Sebacina epigaea	KR019857	–	20.6	–	–	–	–	–	–	2.3
Thelephora palmata	KR019858	–	–	–	–	3.8	3.4	–	–	1.3
Tomentella cinerascens	KR019859	11.3	–	–	–	–	–	–	–	0.7
Tomentella stuposa	KR019860	–	–	12.4	10.4	7.6	6.9	1.1	20.5	7.8
Tomentella sublilacina	KR019862	–	–	–	–	–	–	1.6	1.7	0.4
Trichosporon sp.	KR019863	–	3.3	–	–	–	–	–	–	0.4
Tylopilus felleus	KR019864	–	–	–	–	–	–	1.0	6.5	0.8

Table 5 (continued)

Fungal species	GenBank	K		O		S		M		Total[a]
	Accession no.	Infected (KR)	Uninfected (KH)	Infected (OR)	Uninfected (OH)	Infected (SR)	Uninfected (SH)	Infected (MR)	Uninfected (MH)	
Tylospora asterophora	KR019865	–	8.8	33.0	33.1	44.0	39.8	–	–	24.6
Tylospora fibrillosa	KR019866	–	4.9	–	–	–	–	–	0.5	0.6
Unidentified ascomycete	KR019874	–	–	–	0.2	–	–	–	–	0.0
Xerocomus ferrugineus	KR019867	–	6.2	–	–	–	–	–	–	0.7
All[b]		5.9	11.1	12.3	12.6	18.4	18.4	10.9	10.4	100
No. of species		6	11	5	7	10	10	15	17	42
Shannon diversity index		1.2	1.4	1.6	1.9	1.8	1.8	2.1	2.3	

[a] Relative abundance of each fungal species in all plots

[b] Relative abundance of all detected fungal species in each sampling plot

of a metabolite-rich reaction zone (Bendz-Hellgren et al. 1999; Oliva et al. 2012). Furthermore, the results suggest that in the investigated *P. abies* stands, root-rot did not restrict the flow of photosynthates belowground, as fungal communities in roots and root mycorrhizal colonisation remained largely unchanged (Fig. 1, Table 3). In contrast, tree girdling, which terminates the supply of photosynthates to roots, has been shown to have a profound effect on activity and composition of fungal communities in roots (Högberg et al. 2001). Bergemann et al. (2013) showed a significant reduction of ECM soil hyphal biomass following tree girdling while simulating the potential impact of the invasive pathogen *Phytophthora ramorum*. The latter may suggest that *Heterobasidion* root-rot may also firstly affect production and abundance of ECM hyphae in the soil while changes in abundance and composition of ECM communities in roots occur at a later stage.

Our study suggests that differences/similarities in soil properties among the different study sites were the major factors determining the abundance and composition of fungal communities in fine roots of *P. abies* (Table 4, Fig. 1). We observed large dominance of a few ECM species (Table 5), and a similar pattern was shown in a study of *Heterobasidion* root-rot infected stands in Germany (Bücking 1979). Among the dominant fungi, *T. asterophora* is one of the most constant and abundant ECM fungi on spruce roots (Eberhardt et al. 1999). *T. stuposa*, another

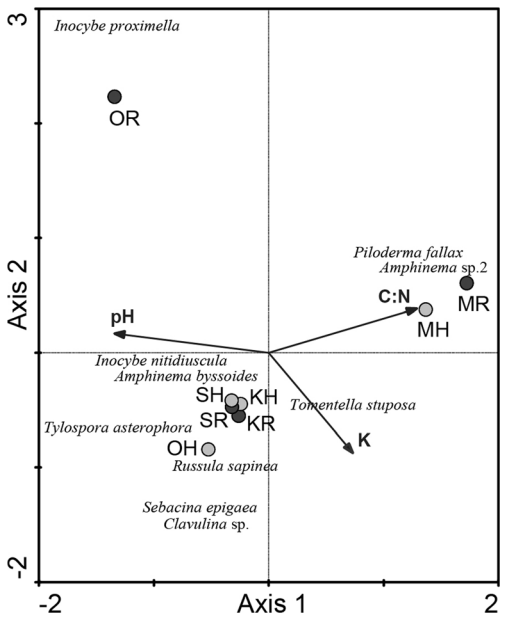

Fig. 1 Ordination diagram based on canonical correspondence analysis (CCA) of fungal communities in roots of *Picea abies* from *Heterobasidion* root-rot infected (*dark-grey circles*) and uninfected (*light-grey circles*) stands. Names of the plots (*KR*, *KH*, *OR*, *OH*, *SR*, *SH*, *MR*, *MH*) are as in Table 1. *Arrows* show environmental variables (*pH*, *C/N* and *K*) that were found to be of significant importance. *Taxonomic names* are shown in the ordination for the ten most common fungal species of a study and correspond to their position in the ordination

commonly detected ECM species, has been described as one of the most commonly encountered tomentelloid fungi in Swedish spruce stands (Kõljalg et al. 2000), and *A. byssoides* is an efficient root coloniser of *P. abies* (Menkis et al. 2011; Menkis et al. 2007; Vaario et al. 2009). Better outplanting performance of abundantly colonised seedlings (Menkis et al. 2011) and common occurrence in forest plantations (Kranabetter 2004; Menkis et al. 2007; Vaario et al. 2009) suggest that *A. byssoides* may be of particular importance to health and sustainability of *P. abies* forest stands. Thus, possibility should not be excluded that through increased supply of mineral nutrients and water to the host (Smith and Read 1997), these ECM fungi mitigated the negative effect of *Heterobasidion* root-rot, thereby contributing to similar growth parameters of *P. abies* roots in both root-rot infected and uninfected plots.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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