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ORIGINAL PAPER

Correspondence of ectomycorrhizal diversity and colonisation of willows (*Salix* spp.) grown in short rotation coppice on arable sites and adjacent natural stands

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Abstract Willows (*Salix* spp.) are mycorrhizal tree species sometimes cultivated as short rotation coppice (SRC) on arable sites for energy purposes; they are also among the earliest plants colonising primary successional sites in natural stands. The objective of this study was to analyse the degree of colonisation and diversity of ectomycorrhizal (EM) communities on willows grown as SRC in arable soils and their adjacent natural or naturalized stands. Arable sites usually lack ectomycorrhizal host plants before the establishment of SRC, and adjacent natural or naturalized willow stands were hypothesized to be a leading source of ectomycorrhizal inoculum for the SRC. Three test sites including SRC stands (*Salix viminalis*, *Salix dasyclados*, and *Salix schwerinii*) and adjacent natural or naturalized (*Salix caprea*, *Salix fragilis*, and *Salix*×*mollissima*) stands in central Sweden were investigated on EM colonisation and morphotypes, and the fungal partners of 36 of the total 49 EM fungi morphotypes were identified using molecular tools. The frequency of mycorrhizas in the natural/naturalized stands was higher (two sites) or lower (one site) than in the corresponding cultivated stands. Correspondence analysis revealed that some EM taxa (e.g. Agaricales) were mostly associated with cultivated willows, while others (e.g. Thelephorales) were mostly found in natural/naturalized stands. In conclusion, we found strong effects of sites and willow genotype on EM fungi formation, but poor correspondence between the EM fungi abundance and diversity in SRC and their adjacent natural/naturalized stands. The underlying mechanism might be selective promotion of some EM fungi species by more effective spore dispersal.

Keywords Arable land · Bioenergy · Diversity · Ectomycorrhizal fungi · Willow

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Introduction

Willows (*Salix* spp.) have been cultivated for a long time for various purposes and in many regions of the world, and are today attractive to be grown as short rotation coppice (SRC) in arable soils for producing biomass for energy (Weih 2004; Kuzovkina et al. 2008). Compared to annual systems grown on arable land, the management of perennial willow or polar SRC leads to decreased mechanical disturbance of the soil and can increase the abundance and change the diversity of soil organisms (Baum et al. 2009a). Soil microbial communities,

of which mycorrhizal fungi are an integral component, are central to soil fertility and can affect both crop productivity and cropping security (Rooney et al. 2009).

Willows belong to the dual mycorrhizal plants, which form associations with ectomycorrhizal (EM) and arbuscular fungi. EM fungi seem to be the dominating mutualistic partners of willows (Püttsepp et al. 2004; Krpata et al. 2008; Hrynkiewicz et al. 2010a). They can increase the biomass production, tolerance of abiotic and biotic stress and resistance against soil-borne pathogens of their host plants (Rooney et al. 2009; Hrynkiewicz and Baum 2012).

It is known that the mycorrhizal status of willows can be affected by soil properties, plant genotype and management factors like fertilisation and rotation lengths (Baum et al. 2002; Püttsepp et al. 2004; Hrynkiewicz et al. 2010b). However, the correlation between cultivated and adjacent natural sites and potential origin of EM fungal inoculum in SRC at arable sites is unknown. It can be suspected that the autochthonous EM colonisation in arable soils is very low since host plants for EM fungi had been absent for a long time at these sites. Therefore, colonisation of EM fungi by spore transfer through air and water from adjacent EM host plants can be expected to be a main source.

The main objective of this study was to analyse the degree of colonisation and diversity of EM fungi communities on willows grown as SRC in arable soils and their adjacent natural or naturalized willow stands. We explored the hypotheses that (1) adjacent willow natural/naturalized stands are a leading source of EM fungi inoculum for SRC stands in arable soils, and (2) the mycorrhizal status of adjacent natural/naturalized willow stands will be an indicator of the mycorrhizal status of SRC stands. An alternative hypothesis was that the degree of colonisation and diversity of EM fungi communities under willows (irrespectively of the stand type) is predominately driven by physical site conditions and the host genotype.

Material and methods

Test sites and sampling procedure

The study included three test sites, with two stand types (SRC and natural/naturalized) each, all situated within a 5-km distance close to Uppsala in central Sweden (59°49′N, 17°40E). The two stand types were SRC at arable sites (SRC1, SRC2, and SRC3) and adjacent natural or naturalized willow stands (NWS1, NWS2, and NWS3). Soil properties for the study sites and the investigated willow genotypes are summarised in Table 1. The distance between the SRC and their corresponding natural or naturalized stands was less than 100 m, and the willow stands were part of rural landscape in which also other tree species were found as potential sources of EM inoculum. A variety of commercial willow clones were

Table 1 The willow genotypes or clones, and soil properties (0–20 cm depth) of the three test sites, each including a SRC stand and a corresponding natural or naturalized willow stand (NWS), located in central Sweden

	Site 1		Site 2		Site 3	
Variable	SRC	NWS	SRC	NWS	SRC	NWS
Willow genotypes/clones	*S. viminalis* L 78183 Jorunn L 78021 *S. schwerinii/viminalis* Björn Tora	*S. caprea*	*S. dasyclados* Loden Gudrun *S. schwerinii/viminalis* Björn Tordis Tora	*S. fragilis*	*S. viminalis* Jorr *S. schwerinii/viminalis* Björn Tordis Tora *S. dasyclados* Loden Gudrun	*Salix*× *mollissima*
Soil texture class (FAO)	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Clay	Clay
pH	5.6	5.2	6.2	6.0	7.5	7.3
Soil total N (mg g^{-1})	2.5	1.9	2.0	3.4	1.4	4.4
Soil total C (mg g^{-1})	34.8	23.9	26.6	51.9	14.0	51.9
C/N	13.9	12.6	13.3	15.3	10.0	11.8

grown in each SRC stand (Table 1). Stand SRC1 was divided into various monoclonal plots, c. 10×10 m in size, in which soil and root sampling for this study was performed. Stands SRC2 and SRC3 were divided into treatment plots differing in irrigation and fertilisation (Weih and Nordh 2005). The soil and root sampling for this study was done in the control treatment (without irrigation and fertilisation). The treatment plots were again divided into monoclonal sub-plots including four replicates of each clone, and sampling in SRC2 and SRC3 was carried out within the monoclonal sub-plots, which were ca. 7× 7 m in size. The SRC plantations were established in 1994 (SRC1) and 2001 (SRC2 and SRC3), and harvested in rotation cycles of 3 years. Detailed descriptions of the investigated SRC plantations are reported by Nordh and Verwijst (2004) for SRC1, and Weih and Nordh (2005) for SRC2 and SRC3. The adjacent natural or naturalized willow stands (NWS) consisted of the locally common natural species *Salix caprea* L. at NWS1, the locally less common naturalized *Salix fragilis* L. at NWS2, and the locally much less common naturalized hybrid *Salix*×*mollissima* Hoffm. Ex. Elwert at NWS3 [species descriptions according to Jonsell (2000)]. Soil and root sampling in the natural/naturalized stands was performed in plots of about 5×5 m arranged centrally in the stands. The natural/naturalized stands included trees in the same age as the SRC, but their total stand age was considerably higher than that of the SRC. The SRC and their adjacent NWS had sparse ground vegetation (few grasses and herbs) since the trees have a closed canopy during the vegetation period. The long-term mean air temperature (1961–1999) from May to September in the study area was 13.6°C and the mean annual precipitation sum was 277 mm (Alexandersson et al. 1991). The soil textural classes at the test sites were clay at site 3 and sandy loam at sites 1 and 2. The soil pH was determined in 0.01 M $CaCl_2$ at a 1:2.5 soil/solution ratio. The contents of total carbon and nitrogen (C_t, N_t) were determined with the CNS analyser (Foss Heraeus Vario EL, Hanau, Germany).

Soil and root sampling was done in June 2005. Six (sites 2 and 3) to ten (site 1) soil cores (diameter 28 mm, depth 150–200 mm) were collected in the centre of the monoclonal sub-plots (44 soil samples in total, i.e. 22 samples from SRC and 22 from NWS). The samples were collected from the uppermost 10–15 cm of soil, c. 20 cm from the stem base of willow plants and kept at 4°C until analyses. Soil cores with plant roots were soaked in tap water for 1 h to overnight in a bowl, carefully separated, and wet sieved with 2-mm, 500-μm, and 200-μm mesh. Roots were collected from the upper two sieves.

EM fungi colonisation and morphological characterisation

From each of the 44 soil samples, ten sub-samples of root fragments were randomly chosen on a grid for microscopical quantification of EM colonisation. The number of living non-colonised root tips vs. visually colonised EM root tips was counted using the method of Agerer (1991). In total, 3,957 root tips were scanned. A minimum of 20 to 496 root tips per sub-sample and 214 to 1,046 roots per site and variety were investigated. All colonised root tips collected from each sub-sample were used separately for analysis of EM fungal species diversity. In total, 1,758 EM root tips were collected. A minimum of 2 to 191 EM root tips per sub-sample and of 50 to 577 EM root tips per site (SRC1 to 3) and stand type (SRC and NWS) were investigated. The morphological–anatomical EM fungus types were distinguished by macroscopical characteristics of the fungal mantle, such as colour, surface appearance, presence of emanating hyphae and hyphal strands, as well as microscopical features such as mantle type and hyphal connections (Agerer 1987–2002). In total, 49 different EM fungi morphotypes were assigned from all sub-samples. Two to five root tips per morphotype were separately frozen in Eppendorf tubes and stored at −20°C for molecular analysis.

Molecular identification of EM fungi

The fungal taxa which formed ectomycorrhizas were identified using analysis of DNA sequences of the internal transcribed spacer (ITS) region. DNA was isolated from EM root tips using the DNAeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The ITS region within the ribosomal RNA genes was amplified using the primer pair ITS1F and ITS4 (Gardes and Bruns 1993; White et al. 1990). The PCR reactions were done according to Haug (2002) and the sequencing process according to Hrynkiewicz et al. (2008). BLAST searching with ITS sequences was performed on the GenBank (Altschul et al. 1990) and/or UNITE database (Koljalg et al. 2005). If the sequence of the EM fungus showed 98 % identities over the whole length of the sequence (about 600 to 700 bp) with a known fungus, this fungus was assumed to be the fungal partner of the mycorrhiza. DNA sequences were submitted to GenBank and accession numbers were retrieved.

Statistical analyses

Relationships between the frequency of EM fungi and the six site–stand combinations (SRC1 to SRC3, NWS1 to NWS3) were analysed by correspondence analysis (CA) to disclose the effects of different stand types (SRC and NWS) on the frequency of the most common molecularly identified EM fungi. Statistical analyses were computed using the software Statistica (Statistica for Windows software, release 5.10, 1996, StatSoft, Tulsa, OK, USA).

Results

The EM fungi colonisation of the investigated *Salix* spp. ranged from 16 to 72 % of the fine roots (Fig. 1). The highest

portions of EM roots (61 to 72 %) were observed at NWS1 and NWS2 compared to 22 to 34 % EM roots found at SRC1 and SRC2. At site 3, the level of EM fungi colonisation at the SRC was similar to the other SRC sites (27 %), but exceeded that of the adjacent NWS (16 % EM roots).

The number of different EM fungi forming taxa per site ranged from 5 to 8 in SRC stands and from 5 to 11 in NWS sites. The fungal partners of 36 different EM fungi morphotypes from all test sites were identified to family, genus, or species levels based on DNA sequences of the ITS region (Table 2). The fungi belonged to four orders of the Basidiomycetes (Agaricales, Thelephorales, Russulales, and Cantharellales) and two orders (Pezizales and Helotiales) of the Ascomycetes. Agaricales were represented by 16 EM fungi taxa. Fourteen of them were identified to species level (*Hymenogaster griseus*, *Hymenogaster vulgaris*, *Hymenogaster australis/decorus*, *Cortinarius diasemospermus*, *Cortinarius parvannulatus*, *Cortinarius atrocoeruleus*, *Hebeloma hiemale*, *Hebeloma populinum*, *Hebeloma mesophaeum*, *Hebeloma collariatum*, *Inocybe rimosa*, *Inocybe flavella*, *Inocybe squarosa*, and *Laccaria tortilis*) and two to genus level (*Hebeloma* sp. 1–2). Pezizales were represented by seven taxa of which five were identified to species level (*Tuber maculatum*/*dryophilum*, *Geopora cervina*, *G.* cf. *nicaeensis*, *Peziza depressa*, and *Genabea fragilis*) and two to genus level (*Tuber* sp. and *Geopora* sp.). Thelephorales were represented by nine taxa of which two were identified to species level (*Tomentella lapida* and *T.* cf. *coerulea*), four to genus level (*Tomentella* sp. 1–4), and three to family level (Thelephoraceae 1–3). Russulales were represented by one species (*Russula laccata*). Helotiales were represented by two taxa, which were identified to genus level (*Helotiales* sp. 1–2) and Cantharellales by one species (*Clavulina cinerea*). Thirteen morphotypes which were not identified molecularly were classified on the basis of their morphological and anatomical properties (five morphotypes at SRC1, four at NWS1, two at SRC2, and one at NWS2 and NWS3; see Table 2).

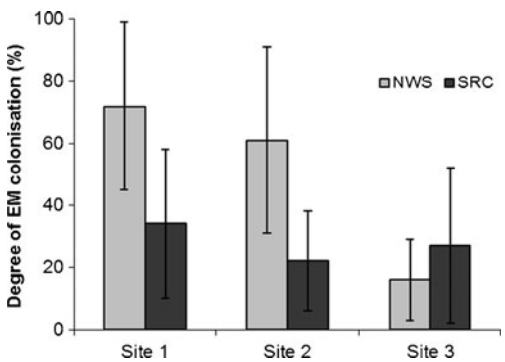

Fig. 1 Ectomycorrhizal (EM) fine root colonisation (mean value±SD) of *Salix* spp. grown in three short rotation coppice (*SRC*) and three adjacent natural/naturalized willow stands (*NWS*) in central Sweden

Members of the Agaricales and the Pezizales were observed at all test sites (Fig. 2). Agaricales belonged to the dominating EM partners at SRC2 and 3 (82 to 91 % of all EM fine roots) and also at NWS1 (60 %; Fig. 2). Pezizales were found at 4 to 8 % and at 9 to 38 % of the EM fine root tips at SRC 1–3 and at NWS 1–3, respectively. The highest portions of Thelephorales were observed at NWS2 and 3 (59 and 57 % of the EM fine roots, respectively) and at SRC1 (41 % of the EM fine roots). Fungal partners of this group were not found at SRC2 and 3. Representatives of Russulales were found only at SRC1 (46 % of the EM fine roots), Helotiales only at NWS2 (28 % of the EM fine roots), and Cantharellales only at NWS2 (0.8 % of the EM fine roots). The percentage of unidentified fungal partners constituted 12.5 % at SRC 2 and 0.2 to 2.9 % at SRC 1 and NWS 1–2.

Using CA (Fig. 3), five main orders of EM fungi taxa were correlated with the six site–stand combinations based on the frequency of EM root tips of each order. Agaricales were associated mostly with SRC (sites 2 and 3), whereas Thelephorales (sites 2 and 3) and Pezizales (sites 1 and 3) were mostly associated with NWS. An exception was NWS1, at which the willows were associated with Agaricales and Pezizales. Russulales were correlated with SRC1, where one morphotype (*R. laccata*) constituted the highest number of EM root tips. The first CA axis separated the test sites NWS2 and 3 associated with Thelephorales (negative values for associated EM colonisation frequency) from test sites SRC2 and 3 (positive values for associated EM colonisation frequency). The relative frequency of EM fungus morphotypes of Pezizales was a joint point (order) of both SRC and NWS.

Discussion

The general level of EM colonisation of *Salix* spp. in natural or naturalized stands was of a similar magnitude than the EM colonisation of *Salix repens* grown in dune ecosystems (van der Heijden et al. 1999). Since the overall levels of EM colonisation of willow fine roots can be strongly influenced by soil properties, site-specific differences are common (Hrynkiewicz et al. 2008). This means that differences in site conditions between the cultivated and natural/naturalized sites, which cannot be ruled out, have probably affected the observed fungal colonisation pattern. For example, soil water content was higher in the natural/naturalized sites (wet at NWS1 and NWS2 to almost waterlogged at NWS3) compared to the cultivated willows (moist to dry). We believe that those differences in site conditions likely affected EM colonisation pattern, but no major conclusions were made. In general, lower EM colonisation of willows in SRC than in adjacent natural/naturalized stands observed in two out of the three studied sites appeared to confirm the initially assumed low density of EM inoculum in arable soils. However,

Table 2 The fungal taxa identified from ectomycorrhizal (EM) root tips from *Salix* spp. grown at three test sites in short rotation coppice (SRC1, SRC2, and SRC3) and their adjacent natural stands (NWS1, NWS2, and NWS3) in central Sweden

Test site						Closest BLAST match acc. number and % of similarity	Classified as
SRC1	NWS1	SRC2	NWS2	SRC3	NWS3		
Pezizales							
[JQ723994] (621)	[JQ724009] (666) [JQ724010] (671) [JQ724011] (673)	[JQ724036] (518)				UDB000121[b], 99 % EU784424[a], 99 %	*Tuber maculatum/dryophilum*
	[JQ724012] (472)						
	[JQ724013] (669)						
[JQ723995] (691)			[JQ724042] (643) [JQ724043] (664)			AY748861[a], 100 %	*Tuber* sp.
				[JQ724065] (656)		FM206390[a], 98 %	*Geopora cervina*
					[JQ724068] (650)	JF908022[a], 97 %	*Geopora* cf. *nicaeensis*
			[JQ724044] (639)			FM206427[a], 99 %	*Geopora* sp.
	[JQ724014] (649)					DQ200837[a], 100 %	*Peziza depressa*
					[JQ724069] (610)	JF908763[a], 99 %	*Genabea fragilis*
Agaricales							
[JQ723996] (740) [JQ723997] (579)	[JQ724029] (710)	[JQ724039] (711)		[JQ724063] (620) [JQ724064] (712)		GU479339[a], 98 %	*Hymenogaster griseus*
	[JQ724028] (718)	[JQ724038] (704)				EU784365[a], (99 %)	*Hymenogaster vulgaris*
	[JQ724030] (670)	[JQ724037] (562) [JQ724040] (569)				DG328132[a],(99 %) GU479266[a],(100 %)	*Hymengaster australis/decorus*
	[JQ724015] (608) [JQ724018] (610)					UDB001230[b], 99 %	*Cortinarius diasemospermus*
	[JQ724020] (620)						
	[JQ724021] (604)						
	[JQ724016] (611) [JQ724017] (611)					AY669664[a], 99 %	*Cortinarius parvannulatus*
	[JQ724019] (610) [JQ724022] (611)					UDB001011[b], 99 %	*Cortinarius atrocoeruleus*
	[JQ724023] (605)						
	[JQ724024] (611)		[JQ724045] (612)			GQ869514[a], 99 %	*Hebeloma hiemale*
				[JQ724059] (609) [JQ724060] (671)		UDB000697[b] 100 %	*Hebeloma populinum*
				[JQ724062] (715)		EF451057[a], 99 %	*Hebeloma mesophaeum*
					[JQ724066] (714)	AY309962[a], 99 %	*Hebeloma collariatum*
				[JQ724061] (714)		GU817063[a], 99 %	*Hebeloma* sp. 1
					[JQ724067] (452)	UDB001188[b]. 100 % AT2003094[b], 100 %	*Hebeloma* sp. 2
[JQ723998] (736)						HQ604624[b], 98 %	*Inocybe rimosa*
	[JQ724025] (734) [JQ724026] (732)					AM882782[a], 99 %	*Inocybe flavella*
	[JQ724027] (736)		[JQ724046] (732)			FN550924[a], 99 %	*Inocybe squarrosa*
		[JQ724041] (569)				UDB001589[b], 99 %	*Laccaria tortilis*

Table 2 (continued)

Test site						Closest BLAST match acc. number and % of similarity	Classified as
SRC1	NWS1	SRC2	NWS2	SRC3	NWS3		
Thelephorales							
			[JQ724049] (687)			UDB001657[b], 98 %	*Tomentella lapida*
			[JQ724051] (685)			UBD003329[b], 97 %	*Tomentella* cf *coerulea*
[JQ724000] (602)			[JQ724052] (683)			HQ215809[a], 99 %	*Tomentella* sp. 1
			[JQ724047] (618)			HQ212232[a], 99 %	*Tomentella* sp. 2
			[JQ724048] (670)				
[JQ724001] (699)			[JQ724050] (680)			AY748883[a], 97 %	*Tomentella* sp. 3
[JQ723999] (619)					[JQ724070] (682)	AJ893328[a], 99 %	*Tomentella* sp. 4
	[JQ724031] (689)				[JQ724071] (559)	FJ554392[a], 98 %	Thelephoraceae sp. 1
	[JQ724035] (685)						
	[JQ724033] (685)						
	[JQ724034](682)					HQ667924[a], 98 %	Thelephoraceae sp. 2
	[JQ724032] (689)					AJ510271[a], 99 %	Thelephoraceae sp. 3
Russulales							
[JQ724002] (531)						UDB000915[b], 98 %	*Russula laccata*
[JQ724003] (719)							
[JQ724004] (602)							
[JQ724005] (616)							
[JQ724006] (677)							
[JQ724007] (696)							
[JQ724008] (716)							
Helotiales							
			[JQ724053] (517)			HQ625450[a], 98 %	*Helotiales* sp. 1
			[JQ724054] (520)				
			[JQ724056] (574)				
			[JQ724057] (360)				
			[JQ724055] (580)			FJ378851[a], 99 %	*Helotiales* sp. 2
Cantharellales							
			[JQ724058] (698)			EU862209[a], 100 %	*Clavulina cinerea*
Not identified							
NI-1							
NI-2							
NI-3							
NI-4							
NI-5	NI-6						
	NI-7						
	NI-8						
	NI-9	NI-10					

Table 2 (continued)

Test site						Closest BLAST match acc. number and % of similarity	Classified as
SRC1	NWS1	SRC2	NWS2	SRC3	NWS3		
		NI-11					
			NI-12		NI-13		
In total:							
8 identified taxa	11 identified taxa	5 identified taxa	10 identified taxa	5 identified taxa	5 identified taxa		
5 not identified	4 not identified	2 not identified	1 not identified		1 not identified		

Data in square brackets represent accession numbers, and values in parentheses indicate the number of base pairs

[a] Closest BLAST match in GenBank NCBI

[b] Closest BLAST match in GenBank UNITE

the pattern could also have been caused mainly by the variation in host genotypes, especially when considering that also the three NWS stands were of different willow genotypes (*S. caprea*, *S. fragilis*, *S.*×*mollissima*). Based on the limited material studied here, a dominance of site and host genotype effects on EM colonisation pattern supports our alternative hypothesis, i.e. the degree of EM colonisation of willows (irrespective of stand type) is predominately driven by the physical site conditions and host genotype. We found no indication that this conclusion would have changed substantially if we had used a greater quantity of test sites.

In the SRC of this study, the EM colonisation was in general lower than in a similar study on *S. viminalis* and *S. dasyclados* performed in Estonia (75 and 94 % colonisation, respectively) (Püttsepp et al. 2004) or on *S. viminalis* in Germany (56 to 72 %) (Hrynkiewicz et al. 2010b), but higher than on *S. viminalis* reported from another study carried out in Germany and Sweden (Baum et al. 2002). However, the frequency of EM fungi on fine roots is not necessarily correlated with the EM effect on plant functions such as biomass production (e.g. van der Heijden et al. 1999; Baum et al. 2006). For example, the identity of the fungal partners (van der Heijden and Kuyper 2003) and the host×fungus combination (Baum et al. 2009b) could have a greater impact on plant growth than the total EM colonisation.

The expected importance of site and genotype effects on EM fungus formation (alternative hypothesis) was confirmed (Fig. 1), whereas no clear correlation between the EM fungi abundance on willow fine roots under SRC and their adjacent NWS was observed in this study. This might be explained by the dominance of host genotype effects. In our study, identical and closely related willow genotypes grown in SRC formed ectomycorrhizas at a similar degree, whereas the different genotypes in the three natural stands differed considerably in EM colonisation. The observations indicate the greatest EM colonisation in the naturally and very commonly distributed *S. caprea*, lower degree of colonisation in the naturalized and less common *S. fragilis*, and the lowest colonisation in the least commonly distributed naturalized hybrid *S.*×*mollissima*. The low colonisation of *S.*×*mollissima* could also be related to the soil conditions at this site, e.g. high pH value (pH>7.0). It is interesting in this context that Pei et al. (2003) found a greater suppressive effect of this hybrid on the sporulation of leaf rust than in other willow species, possibly suggesting genetically determined anti-fungal properties.

The strongly ectomycorrhizal *S. caprea* (Fig. 1, site 1) did not promote EM fungus formation of the adjacent *Salix* clones in SRC, and the quantity of mycorrhizal colonisation in natural/naturalized willow stands seems to have only little impact on the adjacent SRC. This contradicts with the hypothesis that the mycorrhizal status of adjacent natural or naturalized willows is an indicator of the mycorrhizal status under the related SRC stands (hypothesis II). The relatively low EM colonisation

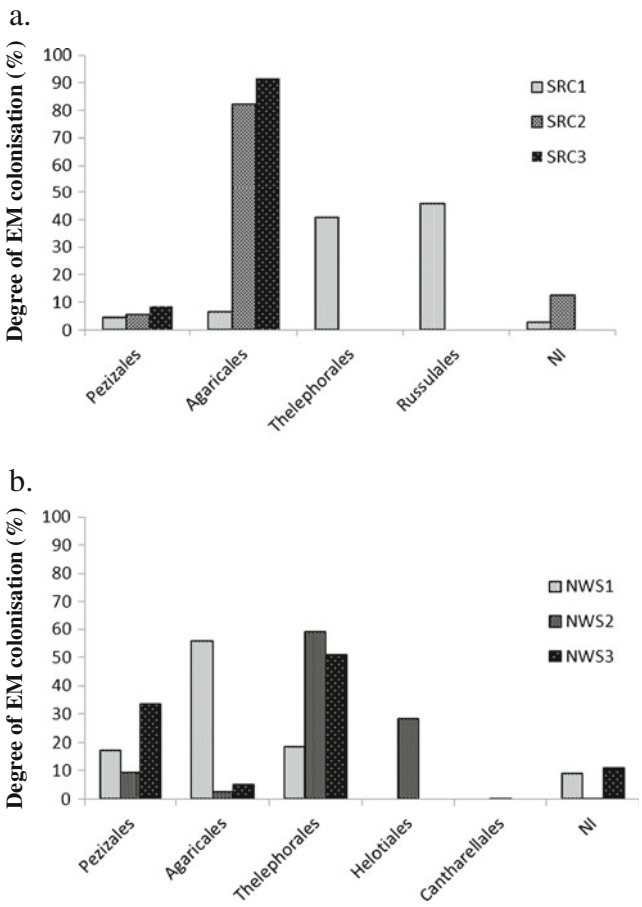

Fig. 2 Percentage of the colonisation by identified fungal partners associated with ectomycorrhizal roots of *Salix* spp. grown in three SRC (*SRC1–3*) (**a**) and three adjacent natural/naturalized willow stands (*NWS1–3*) (**b**) classified to six orders or not identified (*NI*)

under the commercial willow varieties used in the SRC plantations could also be seen in the breeding context of this material; breeding strategies predominantly focus on fast shoot growth, resistance to leaf rust and insects, and tolerance to frost (Larsson 1998), which possibly trade-offs with the abundant formation of mycorrhizas (Hrynkiewicz et al. 2010a).

The investigated *Salix* species in this study harboured 5 to 8 different EM fungus taxa per site in SRC, and 5 to 11 taxa per site in natural or naturalized stands. Those numbers of EM fungus taxa are in line with the numbers reported under other *Salix* spp., e.g. 15 EM fungus morphotypes were observed on *S. repens* at a dune site in the Netherlands (van der Heijden et al. 1999), 11 EM fungal partners were identified from *Salix* clones in SRC in Estonia (Püttsepp et al. 2004), 14 EM fungal partners of *S. caprea* were identified at heavy metal-contaminated sites (Hrynkiewicz et al. 2008), and 7 fungal taxa were identified from *S. viminalis* grown in SRC at an arable site in Germany (Hrynkiewicz et al. 2010b).

In our study, Agaricales were the most numerous group of EM symbionts, with the highest levels of EM colonisation at two SRC sites (82 and 91 % of EM fungus types observed at SRC 2 and 3, respectively). *Hymenogaster* spp. (*H. griseus*, *H. vulgaris*, and *H. australis/decorus*) dominated at SRC2 and 3 (53 and 25 % of EM fungus types identified at each site, respectively), and *Hebeloma* spp. (e.g., *H. populinum*, *H. mesophaeum*, and *H. collariatum*) dominated at SRC3 (66 % of EM fungus types at this site). Higher colonisation density of *Hebeloma* spp. in SRC might be caused partly by the earlier successional stage of these stands since *Hebeloma* spp. belong to the early stage fungi (e.g. Mason et al. 1983). In the natural stands, Agaricales were observed at all test sites but with significantly lower (2.3 to 5.5 %) level of colonisation at NWS 2 and 3. An exception was site NWS 1 where all

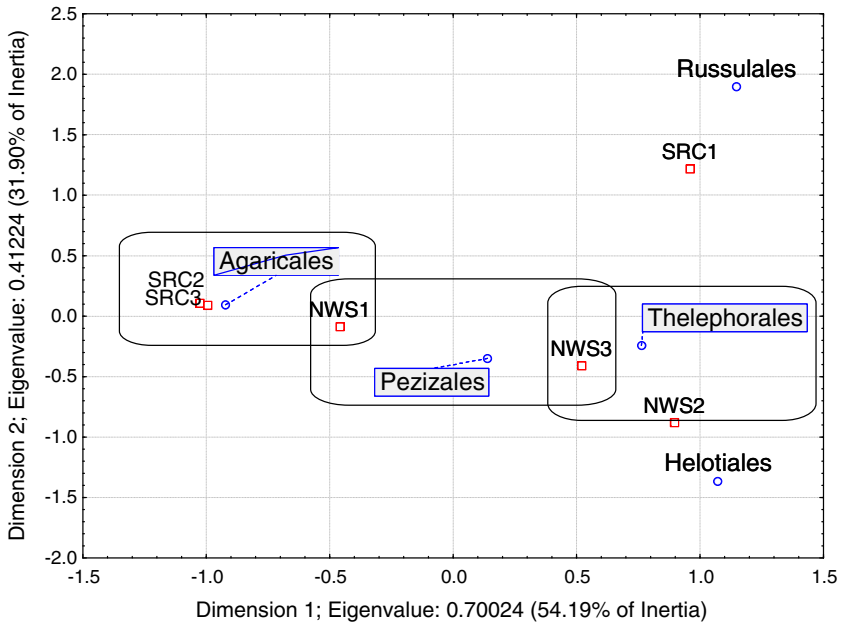

Fig. 3 Ordination of orders of ectomycorrhizal (EM) fungi (colonisation >1 % of the roots) identified on *Salix* spp., based on the colonisation densities observed in three SRC stands (*SRC1*, *SRC2*, and *SRC3*) and adjacent natural/naturalized willow stands (*NWS1*, *NWS2*, and *NWS3*) in central Sweden. The two first axes of the correspondence analysis (CA) are shown

identified *Cortinarius* spp. (*C. diasemospermus*, *C. parvannulatus*, and *C. atrocoeruleus*) were observed (51 % of all EM fungus types identified at this site). EM fungus morphotypes of *Cortinarius* spp. were identified previously by Püttsepp et al. (2004) on roots of *S. dasyclados* grown in SRC. Members of this genus are characteristic mostly for forests (Deacon and Fleming 1992) and can be negatively affected by increased N supply (Baum and Makeschin 2000; Erland and Taylor 2002). *Hymenogaster* is a genus of sequestrate basidiomycetes in the Cortinariaceae. A recent phylogenetic analysis of the Cortinariaceae suggests that *Hymenogaster* sp. is part of a monophyletic group sharing common ancestry with the epigeous mushroom genera *Hebeloma* and *Naucoria* (Peintner et al. 2001). *Hymenogaster* sp. is known as a fungal partner of *Salix* spp. on the roots of *Salix herbacea* in Svalbard and of *Salix glauca* in East Greenland (Knudsen 2006). Species of *Hebeloma* are well-known colonisers of EM plants in disturbed or primary habitats (Jumpponen and Trappe 1998; Nara et al. 2003) which can suggest that the EM fungus community is at an early successional stage with a relatively low number of fungal taxa. Relatively high colonisation with *Hebeloma* spp. was identified on *S. caprea* in heavy metal-contaminated sites in Germany (Hrynkiewicz et al. 2008) and on *Salix alba* in riparian edge forests in the Netherlands (Parádi and Baar 2006). Since EM root tips colonised by *Hebeloma* spp. were identified only at site SRC3 and NWS3 in the present study, we speculate that this genus prefers higher soil pH (pH> 7.0), like it was observed at site 3.

Thelephorales (*T. lapida*, *T.* cf. *coerulea*, *Tomentella* sp. 2 and 4, Thelephoraceae 1–3) were observed in all natural/naturalized stands (NWS1 to 3) and (at sites NWS 2 and 3) constituted the most numerous group of EM symbionts along with the highest level of EM colonisation (57–59 % of the EM fine roots). This agreed with the observed dominance of Thelephoraceae as EM fungal partners of *S. caprea* at heavy metal-contaminated sites (Hrynkiewicz et al. 2008) and of *S. alba* in riparian edge forests (Parádi and Baar 2006). In our study, tomentelloid fungi were observed only at one SRC stand (*Tomentella* spp. 1, 3, and 4). This is in line with results of Püttsepp et al. (2004), who reported also a relatively low level (<0.1 %) of Thelephoraceae on the roots of *S. dasyclados* and *S. viminalis* in SRC stands. In contrast to these results, higher levels of colonisation by Thelephoraceae (up to 20 % of the EM roots) were observed on *S. viminalis* grown in SRC at an arable site in Germany (Hrynkiewicz et al. 2010b).

Pezizales (*T. maculatum*/*dryophilum*, *Tuber* sp., *G. cervina*, *G.* cf. *nicaeensis*, *P. depressa*, *G. fragilis*, *Geopora* sp.) were observed at all investigated sites (SRC and adjacent natural stands). However, the highest colonisation of these fungal symbionts was characteristic for two of the natural sites (NWS1 and NWS3) (19 and 38 % of the EM colonisation, respectively). At all other test sites, the level of EM colonisation with this group of fungi ranged from 4 to 9 %. On the other hand, *Tuber* spp. were the only representatives of Pezizales at SRC1 and 2, *Geopora* spp. represented this group at SRC3 and at NWS 2–3. *Tuber* spp. are known to form ectomycorrhizas with *Salix* (Murat et al. 2005; Parádi and Baar 2006; Hrynkiewicz et al. 2008, 2010b). A *Geopora* sp. was identified as indigenous EM fungal species that promoted the biomass production of *S. viminalis* in fly ash (pH>8.2) (Hrynkiewicz et al. 2009). *Geopora* spp. were also the most common EM symbionts of poplar and willow clones in SRC stands (pH ~6.2) (Hrynkiewicz et al. 2010b). These observations may suggest a high preference of this genus to relatively high pH, e.g. at our site 3 (pH>7.0).

Russulales (*R. laccata*) were the dominating EM fungus morphotype at SRC1 (46 %) and were not observed at any other test site. EM fungi belonging to *Russula* spp. are characteristic for the Northern hemisphere and were observed as mycorrhizal partners of subarctic shrub willows (Clemmensen and Michelsen 2006; Hrynkiewicz et al. 2009) and seedlings in the early primary succession (Nara 2006). Their associations with fast growing tree species grown in SRC were not described in any other investigation so far.

The dominance of Agaricales and Russulales under SRC might be caused by their effective aerial spore dispersal, whereas resupinate (i.e. thin, crust-like) fungi like Thelephorales and hypogeous fungi, like *Tuber* spp. (Pezizales), have fewer opportunities for an effective aerial spore dispersal (Burnett 2003; Lilleskov and Bruns 2005). Their successful spreading in the natural stands might be bound on the activity of animal vectors (Lilleskov and Bruns 2005) and therefore be promoted on soils with lower surface disturbance caused by the lack of tillage. We cannot rule out the possibility that inoculum of multi-host EM fungi (e.g. Roy et al. 2008) was transferred not only between willows but also from other tree taxa into the willows. Such aerial spore dispersal can include long-distance transfer, which was impossible to test with the design of our study.

Helotiales in general represent putative saprobes, but were identified from EM roots of *S. fragilis* in NWS 2 in the present study. Previously, initial dominance of fungal strains belonging to this order was observed in the rhizosphere of *Andropogon gerardii* (Jumpponen 2011). Their later replacement by arbuscular mycorrhizal suggested a replacement of functional guilds comprised of saprobic fungi and mutualistic fungi (Jumpponen 2011). The EM fungus representative of the order Cantharellales (*C. cinerea*) was very rare in our material and showed weak involvement in EM fungus formation on the investigated willows. To the best of our knowledge, *Clavulina* sp. was identified as EM fungus on *Salix* in the present investigation for the first time, but other clavarioid fungi (e.g. *Multiclavula corynoides*) were previously observed on *Salix* in Estonia by Shiryaev (2009).

Conclusions

We conclude that the ectomycorrhizal colonisation of willow varieties grown on arable land in SRC is poorly correlated with the ectomycorrhizal colonisation of willows grown in adjacent natural or naturalized stands. Different EM fungal partners are characteristic for willows in plantations (SRC) and in natural/naturalized stands. Willow clones grown in SRC were associated mainly with Agaricales (*Cortinarius*, *Hymenogaster*, and *Hebeloma* spp.) and Russulales, while some Thelephorales (*Tomentella* spp.) and Pezizales (*Tuber* sp. and *Geopora* sp.) dominated the ectomycorrhiza formation of *Salix* spp. in natural or naturalized sites. Our results support our alternative hypothesis that the EM status of willows in SRC and their adjacent natural stands is predominately driven by physical site conditions and the host plant genotype. A possible impact of the taxa-specific efficiency of aerial spore dispersal was suggested and should be evaluated on its significance in subsequent investigations.

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