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Colonization of decomposing *Sphagnum* moss litter by mycorrhizal roots in two types of peatland ecosystems

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ABSTRACT

During a 35-month study on the decomposition of *Sphagnum* moss litter in poor fen and pine bog forest, an intensive colonization of litter-bags by mycorrhizal roots was observed during the decomposition process. Content of mycorrhizal roots in litter-bags, expressed as % mass of roots, was generally increasing during the decomposition in pine bog forest, and fluctuating during decomposition on poor fen, although in both cases the results were statistically insignificant. Two morphotypes of ericoid roots and two morphotypes of ectomycorrhizal roots were recorded from litter-bags on poor fen during the decomposition experiment, while in pine bog forest one morphotype of ericoid and nine morphotypes of ectomycorrhizal roots were recorded. Molecular identification of mycorrhizal roots succeeded only in the case of one ericoid and six putatively ectomycorrhizal morphotypes. Most morphotypes were recorded only once during the whole 35-month decomposition period, and only one ericoid and one ectomycorrhizal morphotypes were shared between the poor fen and pine bog forest communities.

KEY WORDS: peatlands, *Sphagnum* decomposition, roots, mycorrhizal colonization, mycorrhizae

Introduction

The mycorrhizal fungi are well known for their symbiotic relationships with plants. Their main role is assumed to increase the uptake of scarcely distributed nutrients such as various forms of nitrogen and phosphorus, which is of particular importance in ecosystems where these substances are limiting factors for plants, e.g. peatlands or boreal forests (Smith & Read 2008 Deckmyn *et al.* 2014). The mycorrhizal fungi are able to play their role owing to the great absorptive and adsorptive surface of their extraradical mycelium, and to the

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production of a wide variety of ecto- and endoenzymes, which enables them to hydrolyze and then assimilate various plant-derived organic substances, e.g. peptides, proteins, aminoacids, phospho-monoesters and phospho-diesters (Chalot & Brun 1998, Read *et al*. 2004). In addition to that, some mycorrhizal fungi were shown also to possess the capability to degrade more complicated and resistant molecules present in plant cuticle or secondary cell walls, and this occurred owing to the secretion of e.g. esterases, polygalacturonases, xylanases, cellulases, thyrosinases, peroxidases, poly- and monophenol oxidases, laccases, and even ligninases. Thus, the saprotrophic role of ectomycorrhizal fungi in ecosystems is also recently considered (Talbot *et al*. 2008, Unestam 1991, Deckmyn *et al*. 2014). There are also more and more reports on mycorrhizal fungi colonizing decomposing leaf-litter (Unestam 1991) or experimental works on the decomposition rate and changes in chemistry of decomposing litter in the presence and absence of mycorrhizal fungi (e.g. Zhu & Ehrenfeld 1996) or on the enzymatic activity in colonized plant litter (e.g. Conn & Dighton 2000). However, there are still few papers about mycorrhizal colonization of leaf litter in peatlands, while it is known that this group of fungi is also important for the functioning of these ecosystems (e.g. Thormann *et al*. 1999, Thormann 2006).

During the research on the decomposition of *Sphagnum fallax* Klinggr. litter in Puszcza Piska forest, the intensive colonization of plant debris by mycorrhizal roots was observed in litter-bags in 2011–2013. Thus, the aims of the present study were: (1) to quantify the content of mycorrhizal roots colonizing moss litter during decomposition process, and (2) to identify the mycorrhizal fungi associated with these roots during subsequent stages of leaf litter decomposition.

Material and methods

The samples were taken from two research-plots in Szeroki Bór mire complex, Pisz district, Warmian-Masurian Voivodeship: 1) poor fen with 100% of *Sphagnum* spp. cover and *Eriophorum angustifolium*, *Vaccinium oxycoccos*, *Carex limosa*, *Andromeda polifolia* in the herb layer, with moribund pine trees on the banks and pine seedlings in poor condition growing all over the fen; 2) pine bog forest with the dominance of *Sphagnum* spp. on the ground, with *Vaccinium oxycoccos*, *V. myrtillus*, *Rhododendron tomentosum* in herb layer. In late autumn 2010, litter-bags with ~10 g of air-dried *Sphagnum fallax* litter (top 10 cm of plant with removed capituli) were planted at both research plots. The bags were then collected in the spring, summer and autumn of 2011 and 2012, as well as in the spring and autumn of 2013, covering approximately 35 months of decomposition.

Three bags per collection time per sample site in 2011–2012 and two in 2013 were collected (in total, 22 bags per sample site), all ingrowing roots were extracted mechanically, and leaf-litter and roots were dried at 60^{o} C for 72 hours to constant weight and weighed to assess % ratio of roots in relation to litter mass remaining.

One additional bag per collection time was collected from both sites, for identification of mycorrhizal fungi associated with roots (in total 16 bags=samples, no replication was

possible). All bags were stored frozen until analyses. From each bag, mycorrhizal roots were isolated and divided, first into tree-roots and *Ericaceae*-roots, and then into morphotypes (according, e.g., to Aučina *et al*. 2011), using a Nikon SMZ800 stereomicroscope. Total genomic DNA was extracted from each morphotype using the GeneMATRIX Plant & Fungi DNA Purification Kit (EURx Ltd., Poland) following the manufacturer's instructions. The full ITS region, which is considered as general barcode marker for fungi (Schoch *et al*. 2012), was amplified using the primer pair ITS1f and ITS4 (White *et al*., 1990). DNA was amplified and sequenced as described by Budziszewska *et al*. (2011). The morphotypes were assigned to putative species by comparison of their ITS rDNA sequences with UNITE database (http://unite.ut.ee/) (Kõljalg *et al*. 2013), using the galaxieBLAST algorithm.

Results and discussion

It has to be noted that all tree-roots extracted from the litter-bags at all stages of decomposition were 100% mycorrhizal, although the viability of mycorrhizal tips was not quantitatively assessed, and therefore some of the samples used subsequently for molecular identification could already be dead and colonized by non-mycorrhizal fungi. As mycorrhizal associations are frequent among *Ericaceae*, all extracted roots belonging to this plant group were also considered 100% mycorrhizal. Absolute contents of roots (g) in the litter-bags during the decomposition period on the two sample sites are shown in Figs 1–2, and % contents of roots in these bags are shown in Figs. 3–4. Only in the case of the pine bog forest, a pattern of increasing content of root colonization of plant litter during decomposition could be observed. On the poor fen site, only fluctuations were recorded. However, the differences in root content between stages of decomposition for both sample sites were statistically insignificant (Kruskal-Wallis test, p=0,06476 for pine bog forest, and p=0,3288 for poor fen).

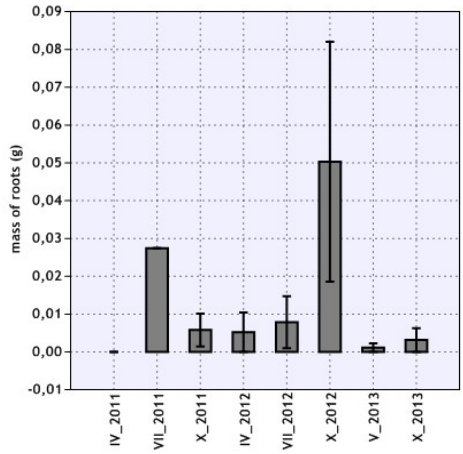

Figure 1. Mass of roots (g) colonizing *Sphagnum* litter during decomposition on poor fen. N=3 for 2011-2012, and n=2 for 2013.

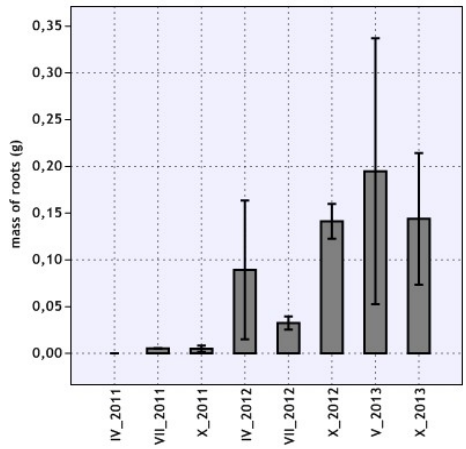

Figure 2. Mass of roots (g) colonizing *Sphagnum* litter during decomposition in pine bog forest. N=3 for 2011-2012, and n=2 for 2013.

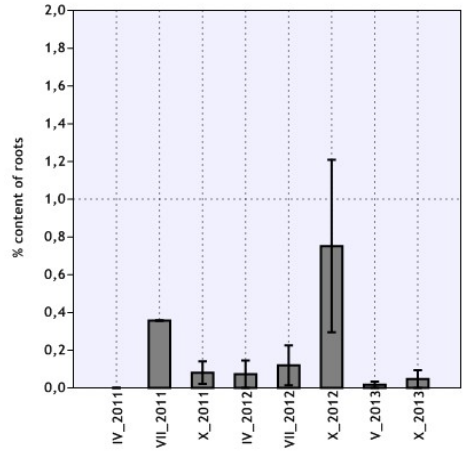

Figure 3. Content of roots (%) colonizing *Sphagnum* litter during decomposition on poor fen. N=3 for 2011-2012, and n=2 for 2013.

The results of the morphological study of mycorrhizal roots from the poor fen and from the pine bog forest are presented in Tab. 1, and Tab. 2, respectively. In total, two ericoid and 10 ectomycorrhizal morphotypes were recorded, comprising 29 samples of mycorrhizal roots (and these were subsequently used for molecular study). On the poor fen, there were two morphotypes of ericoid roots, and only two morphotypes of ectomycorrhizal roots recorded. Not surprisingly, in the pine bog forest the diversity of mycorrhizal roots was much greater, with nine ectomycorrhizal and one ericoid

morphotype recorded. Worth noting is the fact that, in total, 7 out of 10 ectomycorrhizal morphotypes were recorded only once during the whole period of decomposition, and that only one ericoid and one ectomycorrhizal morphotypes were shared between the research plots.

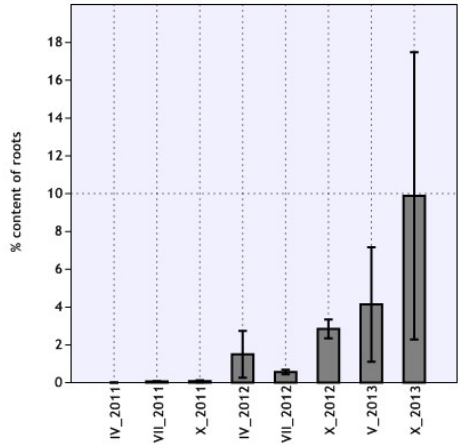

Figure 4. Content of roots (%) colonizing *Sphagnum* litter during decomposition in pine bog forest. N=3 for 2011-2012, and n=2 for 2013.

Table 1. Mycorrhizal morphotypes recorded during decomposition of *Sphagnum* litter on poor fen ("eric" – ericoid morphotypes, "m" – ectomycorrhizal morphotypes).

	IV 2011	VII 2011	X 2011	IV 2012	VII 2012	X 2012	V 2013	X 2013
ericA	-	-	+	+	+	+	+	+
ericB	-	-	+	-	-	+	+	-
mB	-	-	-	-	-	-	+	-
mC	-	-	+	-	-	-	-	-

In total, 17 out of all 29 samples of mycorrhizal roots yielded DNA of quality appropriate for molecular identification, including majority of the designated morphotypes (1 ericoid and 9 ectomycorrhizal). However, there were no simple relationships between the morphotypes and the identified sequences, i.e. where the morphotype was recorded more than once during the study, molecular identification yielded different results on different sampling seasons (data not shown). Only 7 obtained sequences could be regarded, with some caution, as mycorrhizal (Table 3). The caution is necessary in the case of wood-inhabiting saprotrophic *Ascocoryne sarcoides* and *Hypholoma*

radicosum, which could be, e.g., secondary invaders of dead or moribund roots. Some caution should also be exercised in the case of *Sebacina vermifera*, because although *Sebacinales* are well known mycorrhizal associates of Ericaceae (e.g. Weiß *et al*. 2004, Selosse *et al*. 2007), they are also known as root endophytes (Selosse *et al*. 2009, Weiß *et al*. 2011). The rest of the sequences (not shown) belonged probably to the saprotrophic secondary colonizers of roots or endophytic fungi (Geomyces sp., 7 sequences; *Cryptococcus gastricus* Reiersöl & di Menna, *Mortierella sossauensis* E. Wolf and one unidentified ascomycete; e.g. Summerbell 2005), and their invasive growth probably accounted for the variability of results of molecular identification mentioned above. Worth noting is the fact that the results of the molecular study are in accordance with field observations of macrofungal fruit-bodies (data not shown): although on the poor fen site we observed different, bryophilous species of *Hypholoma*, all species identified from pine bog forest were observed by us also as fruit-bodies.

Table 2. Mycorrhizal morphotypes recorded during decomposition of *Sphagnum* litter in pine bog forest ("eric" – ericoid morphotypes, "m" – ectomycorrhizal morphotypes).

	IV 2011	VII 2011	X 2011	IV 2012	VII 2012	X 2012	V 2013	X 2013
ericA	-	+	+	+	+	+	-	+
m1A	-	+	+	-	-	-	-	-
m2A	-	-	-	-	-	-	+	-
mC	-	-	-	-	-	-	+	-
mD	-	-	-	-	+	-	-	-
mE	-	-	-	-	+	-	-	+
mF	-	-	-	-	+	-	-	-
mG	-	-	-	-	-	+	-	-
mH	-	-	-	-	-	+	-	+
mI	-	-	-	-	-	-	-	+

Summary

Our study indicated that *Sphagnum* moss litter, decomposing in peatland ecosystems, is intensively colonized by mycorrhizal roots associated with both ericoid and ectomycorrhizal fungi. The role of these fungi in decomposition of moss litter is unknown, and was outside the scope of this study, although they certainly influence the process. In the light of the observed high variability in the colonization by mycorrhizal roots (expressed as mass or % content in

relation to remaining plant litter mass), the sample size was too small to conclude that this colonization increases with advancing stage of decomposition. The preliminary results of morphological and molecular study on mycorrhizal communities associated with roots colonizing *Sphagnum*-litter showed the presence of well-known species, occurring on the research sites also as fruit-bodies; the difference in those communities between the two sites was driven by the differences in vegetation composition. Majority of the morphotypes were recorded only once during the whole decomposition period, which could potentially indicate the existence of succession or directional changes in associated mycorrhizal communities, however the lack of replicates prevents us from giving any firm conclusions regarding this hypothesis.

Table 3. Molecular identification of putative mycorrhizal fungi recorded from roots colonizing *Sphagnum* litter during decomposition on the research sites.

IV 2011	-	-	-	-	-	-	-
VII 2011	-	-	-	+	-	-	-
X 2011	-	-	+	-	-	-	-
IV 2012	-	-	-	-	-	-	-
VII 2012	-	-	-	-	-	-	-
X 2012	-	-	-	-	-	+	+
V 2013	+	+	-	-	+	-	-
X 2013	-	-	-	-	-	-	-
species	*Sebacina vermifera* Oberw.	*Ascocoryne sarcoides* (Jacq.) J.W. Groves & D.E. Wilson	*Hypholoma radicosum* J.E. Lange	*Laccaria laccata* (Scop.) Cooke	*Thelephora terrestris* Ehrh.	*Russula emetica* (Schaeff.) Pers.(1)	*Russula emetica* (Schaeff.) Pers.(2)
site	poor fen			pine bog forest			

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Streszczenie

Podczas blisko trzyletnich badań nad rozkładem mchu torfowca na torfowisku przejściowym oraz w borze bagiennym w północno-wschodniej Polsce zaobserwowano kolonizację materiału roślinnego w woreczkach ściółkowych przez silnie zmykoryzowane korzenie. Procentowa zawartość tych korzeni, wyrażona jako stosunek ich suchej masy do suchej masy rozkładających się szczątków roślinnych, generalnie zwiększała się wraz z upływem czasu w borze bagiennym, natomiast w przypadku torfowiska przejściowego nie wykazała wyraźnych tendencji; z racji niewielkiej liczby powtórzeń zaobserwowane różnice nie były jednak istotne statystycznie. W materiale roślinnym rozkładającym się na torfowisku przejściowym odnotowano obecność dwóch morfotypów korzeni wrzosowatych oraz dwóch morfotypów korzeni ektomykoryzowych, natomiast w borze bagiennym odnotowano jeden morfotyp korzeni wrzosowatych i 9 morfotypów korzeni ektomykoryzowych. Tylko jeden morfotyp wrzosowatych i jeden ektomykoryzowy były odnotowane w obu typach siedlisk; różnice wynikały z zasadniczych różnic w składzie zbiorowisk roślinnych pomiędzy badanymi powierzchniami. Większość (7 z 10) morfotypów ektomykoryz pojawiła się tylko raz podczas całego okresu trwania eksperymentu. Badania molekularne uzyskanych morfotypów powiodły się jedynie w siedmiu przypadkach: zidentyfikowano jeden gatunek tworzący mykoryzę erikoidalną, trzy gatunki tworzące ektomykoryzy (w tym jeden tworzący dwa morfotypy) oraz dwa gatunki grzybów wielkoowocnikowych znanych jako saprotrofy, prawdopodobnie wtórnie infekujących korzenie. Sekwencje uzyskane z pozostałych badanych morfotypów należały do grzybów mikroskopijnych najprawdopodobniej kolonizujących korzenie jako saprotrofy lub endofity. Pomimo że badania niniejsze stanowią jedynie szkicowe studium, to jednoznacznie wskazują na możliwość udziału grzybów mykoryzowych w procesach rozkładu materii roślinnej w ekosystemach torfowiskowych.