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Succession of microfungal assemblages in decomposing peatland plants

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Abstract

We investigated the microfungal assemblages in the decomposing tissues of dominant plant species in two peatlands in southern boreal Alberta, Canada, to determine if distinct patterns of succession of microfungi occurred throughout the first two years of decomposition. These plant species were *Sphagnum fuscum* from a bog and *Carex aquatilis* leaves and rhizomes and *Salix planifolia* leaves and roots from a riverine, sedge-dominated fen. Canonical correspondence analyses, a multivariate statistical analysis used infrequently in mycological research, revealed distinct patterns of fungal species succession in two of the five litters (*S. fuscum* and *C. aquatilis* leaves). Furthermore, our analyses showed that substantially different microfungal assemblages were associated with these litters within the first two years of decomposition. Litter quality variables, such as total nitrogen, total phosphorus, and total carbon tissue nutrient concentrations, explained most of the succession patterns and differences in the microfungal assemblages of these five litters. Our data did not reveal the classical taxonomic zygomycete – ascomycete/fungi imperfecti – basidiomycete pattern of succession during organic matter decomposition. Similarly, a succession of functional groups of microfungi, i.e., cellulose-degraders preceding lignin-degraders, generally was not apparent. Instead, microfungi with broad spectra of enzymatic abilities co-existed over the first two years of decomposition in these peatland plant litters. These microfungi have a limited ability to decompose complex phenolic polymers, such as lignin, resulting in the accumulation of peat in these ecosystems. Some microfungal taxa were not affected by changes in litter quality, environmental variables, or surface water chemistry and were present at all stages of decomposition.

Introduction

A succession of fungi during the process of decomposition has been observed in a variety of plant species in terrestrial (Frankland, 1966; Heilmann-Clausen, 2001; Kasai et al., 1995; Lumley et al., 2001; Saitô, 1966) and wetland (Apinis et al., 1972; Cabral et al., 1993; Pugh, 1958; Pugh and Mulder, 1971; Tokumasu, 1994) ecosystems. Succession generally is defined as an orderly progression of changes, in which a pioneer community colonizes a particular substrate or ecosystem and, with time, culminates in a climax community. In contrast to succession in plant communities which terminates in a climax community, succession of saprobic fungi results in the decomposition of the substrate and a climax community does not result. Fungal succession through time may be due to the process of facilitation, where species of a particular fungal community alter the substrate sufficiently to allow other species to become established and form a subsequent community (Lumley et al., 2001). For example, changes in litter quality, water potential of the litter, temperature, and pH have been shown previously to influence the fungal community of a particular substrate (Christensen and Whittingham, 1965; Dix, 1985; Lumley et al., 2001; Nilsson et al., 1992; Pugh, 1958; Pugh and Mulder, 1971).

A succession of functional groups of fungi linked to plant litter quality occurs during the process of

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decomposition. As decomposition proceeds, pools of nutrients containing nitrogen, phosphorus, and simple sugars, become scarce, while more complex structural polymers, such as lignin and lignocellulose, become comparably more dominant in the litter (Deacon, 1997). This leads to a succession of fungi in the plant litter, in which basidiomycete species may dominate over ascomycetes during the latter stages of decomposition, because they can synthesize the enzymes required to degrade complex polymers (Deacon, 1997).

Our objectives were to determine the patterns of fungal succession that occur during the process of decomposition of the dominant bog and fen vegetation in southern boreal Alberta, Canada. These plants were *Sphagnum fuscum* (Schimp.) Klinggr. from a bog and *Carex aquatilis* Wahlenb. leaves and rhizomes and *Salix planifolia* Pursh leaves and roots from a fen. These materials were selected because of their significant contribution to the total net primary production (Szumigalski and Bayley, 1997; Thormann and Bayley, 1997) and accumulation of peat in peatlands of western continental Canada (Kuhry and Vitt, 1996; Thormann et al., 1999a). We hypothesized that: (1) distinct fungal assemblages are involved in the decomposition of each of the five litters, and (2) distinct fungal succession patterns characterize the decomposition process of each of these litter types. These patterns were expected because of different total carbon (TC), total nitrogen (TN), and total phosphorus (TP) tissue concentrations and TC:TN quotients of each of these litter types (Thormann et al., 2001a).

Materials and methods

Study area and site descriptions

The riverine sedge fen (54° 28′ N, 113° 18′ W) and Perryvale bog (54° 28'N, 113° 16'W) lie within the Subhumid Low Boreal ecoclimatic region of Canada (Ecoregions Working Group, 1989). The area is characterized by mild summers and cold, snowy winters and a long-term mean annual temperature of 1.7 °C. The total mean annual precipitation is approximately 500 mm (Environment Canada, 1998).

The fen is dominated by species of *Carex* and *Equisetum fluviatile* L. The bryophyte layer is sparse and discontinuous and consists primarily of *Brachythecium mildeanum* (Schimp.) Schimp. *ex* Milde and *Tomenthypnum nitens* (Hedw.) Loeske. The bog is dominated by species of *Sphagnum*, *Picea mariana* (Mill.) BSP., and members of the Ericaceae. Vegetation composition, surface water chemistry, and physical parameters of both sites are provided in more detail in Thormann et al. (1999b, 2001a).

Sampling of living and decomposing plant material

In the bog, the top 30 mm of 20 individual *S. fuscum* plants were collected in early May, July, and September 1997. In the fen, the top 100 mm of ten *C. aquatilis* leaves, ten 100 mm segments of living *C. aquatilis* rhizomes, ten *S. planifolia* leaves, and ten 100 mm terminal root segments of *S. planifolia* were collected at the same time as the *S. fuscum* plants. These samples were used to isolate microfungi from living material of the five plant tissues.

A 2-year decomposition study using nylon mesh bags (30 × 60 mm, 1 mm gauge) was initiated in early September 1997 by collecting senesced plant samples (Thormann et al., 2001a). Briefly, between five and eight individual senesced samples of each of the above litter types were placed into each of 18 decomposition bags, i.e., 18 decomposition bags were prepared for each litter type and each bag contained 5–8 plant samples. The 90 decomposition bags were deployed in the peatlands and placed horizontally approximately 20–50 mm below the peat surface (*S. fuscum* plants, *C. aquatilis* rhizomes, and *S. planifolia* roots) or on top of the peat surface (*S. planifolia* and *C. aquatilis* leaves) to mimic natural conditions of decomposition of each type of litter. Three decomposition bags from the entire set of 18 bags for each litter type were retrieved after each of 20 and 50 days in 1997, after 250 and 365 days in May and September 1998, and after 456 and 730 days in May and September 1999 ($n = 3$ per sampling date per litter type). The 15–24 plant samples from the three decomposition bags collected at each sampling date from each litter type were combined prior to further processing (see below).

Isolation and identification of filamentous microfungi

Litterbag contents were cleaned by removing roots and other plant tissues using fine forceps and a dissecting microscope. Each of ten randomly selected decomposed samples of each litter type at each sampling date (from the total 15–24 plant segments) was cut with a sterilized scalpel into ten smaller segments (approximately 5 × 5 mm in size). These were surface-sterilized for 5 min in hydrogen peroxide (10%) and washed

with sterile, distilled water (d–H_2O) prior to being placed on primary isolation media. These were Potato Dextrose Agar (PDA, 39.0 g Difco potato dextrose agar, 1.0 L d–H_2O), PDA with rose bengal (0.03%), PDA with benomyl (0.0002%), and Mycobiotic agar® (MYC, containing cycloheximide, 35.6 g Difco mycobiotic agar, 1.0 L d–H_2O). These media were used to isolate a broad spectrum of filamentous microfungi through selection and inhibition. All media were amended with oxytetracycline (0.01%) to suppress bacterial growth.

Plates were incubated at room temperature in the dark and fungi were sub-cultured onto Malt Extract Agar (MEA, 15.0 g Difco malt extract, 20.0 g Difco agar, 1.0 L d-H_2O) as soon as they grew from the plant material. Plates were examined daily for emerging fungi for the first 2 weeks, weekly for the following 6 months, and monthly for the following 2 years of incubation. For identification purposes, slide cultures (Sigler, 1992) were prepared on mixed cereal agar (Pablum®, H. J. Heinz Company of Canada Ltd., 100.0 g mixed cereal, 15.0 g Difco agar, 1.0 L d-H_2O), stained with acidfuchsin, and mounted in polyvinyl alcohol. Only fungi that produced distinctive diagnostic colony and morphological characters were enumerated in this investigation. Non-sporulating and otherwise non-descript fungi represented less than 15% of all isolates and were excluded from this study.

The isolation frequency of each identified or described fungal taxon from each litter type was calculated by expressing the number of records of each taxon as a percentage of the total number of records of all fungi obtained at each stage of decomposition (0–730 days). The microfungus assemblage at each stage of decomposition consists of those microfungi that were isolated at that stage of decomposition (obtained from 15 to 24 plant segments per decomposition period per litter type).

Statistical analyses

Canonical Correspondence Analyses (CCA) of each litter type (*S. fuscum*, *C. aquatilis* leaves and rhizomes, and *S. planifolia* leaves and roots) individually and in combination with each other were done using CANOCO (ter Braak, 1992). This analysis ordinates communities and environmental variables, such that the relative position of the communities reflect their similarity/dissimilarity and the environmental variables are represented by vectors overlying the positions of the individual communities. The relative significance of the vectors is indicated by their length and direction from the axes origin. All ordination vectors were multiplied by 5 for a clearer representation in the figures.

Variables included in the analyses were (a) litter type, (b) length of time deployed in the field (0, 20, 50, 250, 365, 456, and 730 days), (c) surface water chemistry (nitrate, ammonium, total dissolved nitrogen, soluble reactive phosphorus, total dissolved phosphorus, total phosphorus, pH, conductivity, alkalinity, bicarbonate, dissolved organic carbon, calcium, and potassium), (d) litter quality (TC, TN, and TP tissue concentrations, and TC:TN quotients), and (e) physical variables (peat temperature, water temperature, depth of the acrotelm [oxygenated peat horizon]). Pearson's correlation coefficients among all variables and the first and second CCA axes were generated from the ordinations. Collection methods and data for (c), (d), and (e) are presented elsewhere (Thormann et al. 2001a).

Results and discussion

After 2 years decomposition, only the *S. fuscum* plant and *S. planifolia* root litters remained identifiable and had neither fragmented nor changed their colour significantly (from dark brown to pale brown). This was not surprising because bryophyte and woody plant remains form a significant portion of peat in peatlands and are readily identified in peat profiles.

Both fen leaf litters and the rhizome litter were discoloured considerably (dark brown to black). While the leaf litters were extensively fragmented, the rhizome litter had retained its structure but lost its rigidity and was very soft and flaccid. Herbaceous vascular plant materials decompose more rapidly than bryophyte and woody plant materials in peatlands and their remains are rarely identifiable in peat profiles. Thus, their loss of physical integrity and discoloration was no surprise.

The fungal assemblages of Sphagnum fuscum

The CCA of the fungal assemblages of decomposing *S. fuscum* shows a clear pattern of succession, with axis 1 separating the early and late stage microfungal assemblages (left to right, respectively) (Figure 1). The fungal assemblages of the early stages of decomposition were related to elevated TP surface water concentrations (0–50 days: 252 μg L^{-1}; 50–730 days:

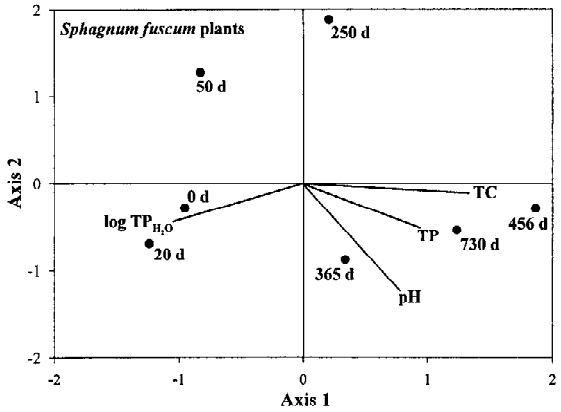

Figure 1. Canonical correspondence analyses of microfungus assemblages isolated from *Sphagnum fuscum* plants after 0, 20, 50, 250, 365, 456, and 730 days decomposition from the Perryvale bog in southern boreal Alberta, Canada. Early-stage decomposition microfungus assemblages correlated with TP_{H2O} (surface water concentration of total phosphorus), while late-stage microfungus assemblages correlated with TC (total carbon) and TP (total phosphorus) tissue concentration, and pH. Eigenvalues for axes 1 and 2 were 0.367 and 0.255, respectively.

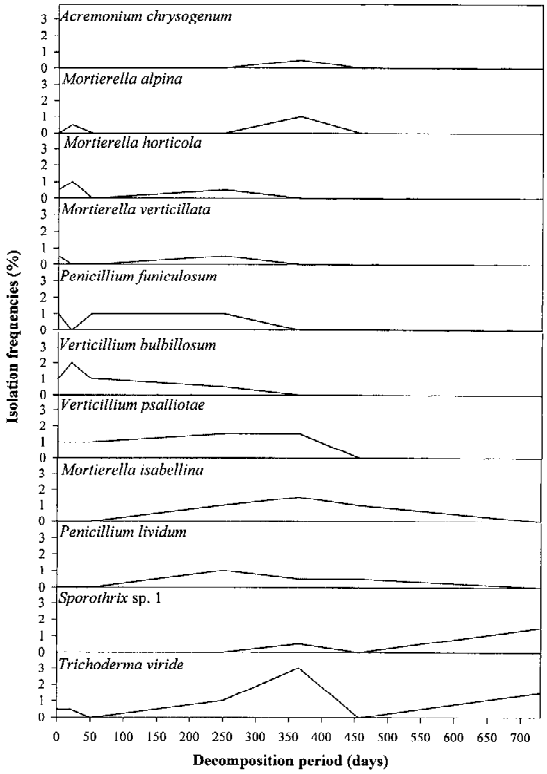

Figure 2. Isolation frequencies (%) of selected microfungi isolated during the 2-year decomposition period of *Sphagnum fuscum* in a bog in southern boreal Alberta, Canada. These fungi were selected because of their preponderance during the early to mid (0–365 days) or mid to late (365–730 days) stages of decomposition. Fungi not shown did not show clear isolation patterns during the process of decomposition.

182 μg L^{-1}) (Thormann et al., 2001a). This correlation is shown by the proximity of the surface water chemistry vector to the early stage decomposition assemblages relative to the late stage decomposition assemblages (Figure 1). TP surface water concentrations were significantly correlated with axis 1, which represents the different stages of decomposition (Table 1).

Species of *Mortierella* and *Verticillium* were among those isolated exclusively from early and mid-stage decomposing *S. fuscum* (0–365 days) (Figure 2). Therefore, we named this microfungal assemblage the '*Mortierella-Verticillium*'-dominated mycocoenosis. Conversely, *Sporothrix* sp. 1 and *Trichoderma viride* appeared predominantly in mid to late stage decomposed *S. fuscum* (365–730 days) (Figure 2), and we named this microfungal assemblage the '*Sporothrix*'-dominated mycocoenosis. In this context, 'mycocoenosis' referred to the set of fungi isolated predominantly at a particular stage of decomposition of this substrate. These mid to late stage decomposition species were affected by elevated tissue concentrations of TC (0–50 days: 462 mg g^{-1}; 50–730 days: 470 mg g^{-1}) and TP (0–50 days: 4.1 mg g^{-1}; 50–730 days: 4.4 mg g^{-1}) and lower acidity of the surface water (0–50 days: pH = 3.74; 50–730 days: pH = 3.77) (Thormann et al., 2001a), as shown by their proximity to those vectors (Figure 1). TC and TP correlated significantly with axis 1, while pH correlated significantly with axis 2 (Table 1).

Despite significant changes in the litter quality of *S. fuscum* during the 2-year decomposition period (Thormann et al., 2001a), the observed succession of microfungi was not reflected in the enzymatic profiles of the assemblages at various stages of decomposition. Microfungi with cellulolytic, lignolytic, and gelatinolytic abilities occurred throughout all stages of decomposition (Thormann et al., 2001b). Similarly, there was no classical taxonomic zygomycete – ascomycete/fungi imperfecti – basidiomycete pattern of succession during the decomposition of *S. fuscum* litter. Several fungi were unaffected by changes in litter quality, environmental variables, or surface water chemistry, e.g., several species of *Mortierella*, *Penicillium*, and *Mucor hiemalis* occurred during early, mid, and late stages of decomposition (data not shown).

Since peat consists primarily of *S. fuscum* remains, we can compare the microfungal assemblages of our study to previous studies of microfungal assemblages

Table 1. Pearson's correlation coefficients among the Canonical correspondence axes and litter quality and surface water chemistry variables of decomposing *Sphagnum fuscum* from Perryvale bog, Alberta, Canada

	Axis 1	Axis 2	TC	TP	pH	TP (H_2O)
TC	0.823 ***	−0.076	–			
TP	0.579 *	−0.343	0.233	–		
pH	0.480	−0.826 **	0.587 *	0.506 *	–	
TP (H_2O)	−0.626 *	−0.289	−0.542 *	0.132	−0.026	–

TC = Total carbon, TP = total phosphorus, TP (H_2O) = surface water concentrations of total phosphorus. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

in peat (Christensen and Whittingham, 1965; Dooley and Dickinson, 1971; Latter et al., 1967; Nilsson et al., 1992). An examination of their microfungal assemblages reveals a similar large number of species of *Penicillium*, *Trichoderma*, *Mucor*, *Mortierella*, *Acremonium*, yeasts, and *mycelia sterilia*. Comparing their species lists to ours shows an overlap of taxa between 11 and 23%. These species may be tolerant of low pH, low temperatures, and seasonal inundation. Furthermore, they may be able to colonize and decompose relatively undecomposed plant materials (Christensen and Whittingham, 1965), although an analysis of the physiological profiles of fungi from decomposing *S. fuscum* generally indicated a broad spectrum of enzymatic abilities, enabling them to utilize such carbon sources as pectin, cellulose, gelatin, and starch (Thormann et al., 2001b). Although water levels, pH, temperature, and the vegetation composition of peatlands influence the relative abundance of different fungal species (Christensen and Whittingham, 1965; Dooley and Dickinson, 1971; Latter et al., 1967; Nilsson et al., 1992), the fungal species richness of peat appears to be similar among peatlands.

Few fungi isolated from peat have been examined for their ability or mode of decomposition of the gametophytic tissues of *Sphagnum*. Thormann et al. (2002) examined mass losses of *S. fuscum* caused by nine fungi isolated from living and decomposing *S. fuscum*. Their mass losses ranged from 0.5 to 10.2% after 56 days and were considerably lower than those reported by Czastukhin (1967) (13–22% after 365 days). The mode of decomposition varies among fungi. Tsuneda et al. (2001a) examined the decomposition process of *S. fuscum* by *Oidiodendron maius* and *Acremonium cf. curvulum* and found that they degraded the cell walls of leaf tissues in different ways. Moreover, *Scleroconidioma sphagnicola* Tsuneda, Currah, and Thormann parasitizes *S. fuscum* plants by penetrating chlorophyllous cells, leading to chlorosis and finally cavitation of the cell (Tsuneda et al., 2001b). Although the *S. fuscum* cell wall itself appeared not to be degraded by this parasite, it became swollen and showed a wavy deformation following invasion (Tsuneda et al., 2001b).

The fungal assemblages of Carex aquatilis *litters*

Comparison of fungal assemblages in leaves and rhizomes

The CCA of the fungal assemblages in decomposing *C. aquatilis* leaves and rhizomes showed a clear separation of the fungal assemblages of these two litter types along axis 2, with the assemblages in the decomposing rhizomes and leaves being clustered above and below axis 1, respectively (Figure 3). The microfungal assemblages of the *C. aquatilis* leaves correlated positively with elevated tissue nutrient concentrations of TP (leaves: 1.8 mg g^{-1}; rhizomes: 1.3 mg g^{-1}) and TN (leaves: 2.2 mg g^{-1}; rhizomes: 0.7 mg g^{-1}) compared to those of the *C. aquatilis* rhizomes (Thormann et al., 2001a), especially during the late stages of decomposition (Figure 3). In contrast, the TC:TN quotient correlated most strongly with the decomposing *C. aquatilis* rhizomes (rhizomes: 64; leaves: 22) (Thormann et al. 2001a) (Figure 3) and with axis 2 (Table 2). These correlations are shown by the proximity of the litter quality vectors to the respective microfungal assemblages of the two litter types (Figure 3).

Of the fungi from decomposing *Carex* leaves and rhizomes (49 taxa), 39% occurred exclusively in leaves and 32% occurred exclusively in rhizomes, with the remainder occurring in both litters (29%). Species of *Cladosporium*, *Monocillium*, and two unidentified basidiomycetes were isolated only from decomposing *C. aquatilis* leaves, while species of *Fusarium*, *Trichoderma*, and *Phialocephala* occurred only in the decomposing *C. aquatilis* rhizomes (Figure 4). Spe-

Table 2. Pearson's correlation coefficients among the Canonical correspondence axes and litter quality variables of decomposing *Carex aquatilis* leaves and rhizomes from the riverine sedge fen, Alberta, Canada

	Axis 1	Axis 2	TC	TN	TC:TN	TP
TC	0.020	0.030	–			
TN	0.159	−0.931 ***	0.192	–		
TC:TN	−0.095	0.940 ***	0.192	−0.975 ***	–	
TP	0.644 *	−0.564 *	−0.241	0.612 *	−0.638 *	–

TC = Total carbon, TN = toal nitrogen, TC:TN = total carbon to total nitrogen quotient, TP = total phosphorus. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

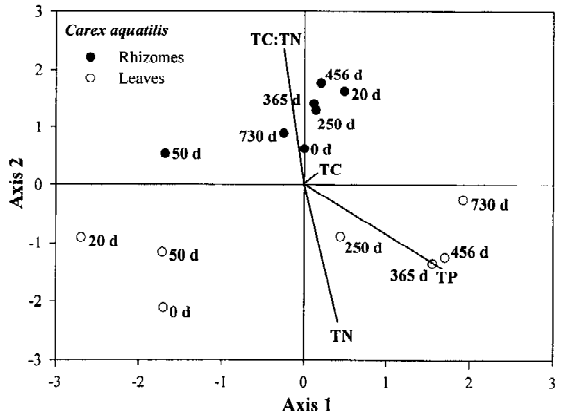

Figure 3. Canonical correspondence analysis of microfungus assemblages from *Carex aquatilis* leaves and rhizomes isolated after 0, 20, 50, 250, 365, 456, and 730 days decomposition from the riverine sedge fen in southern boreal Alberta, Canada. Axis 1 shows a succession of microfungi decomposing *C. aquatilis* leaves (absent for *C. aquatilis* rhizomes), while Axis 2 separates the two litter types. Microfungal communities of *C. aquatilis* leaves correlated with TP (total phosphorus) and TN (total nitrogen) tissue concentrations, while microfungal communities of *C. aquatilis* rhizomes correlated with TC:TN (total carbon to total nitrogen tissue concentration quotient). Eigenvalues for axes 1 and 2 were 0.619 and 0.597, respectively.

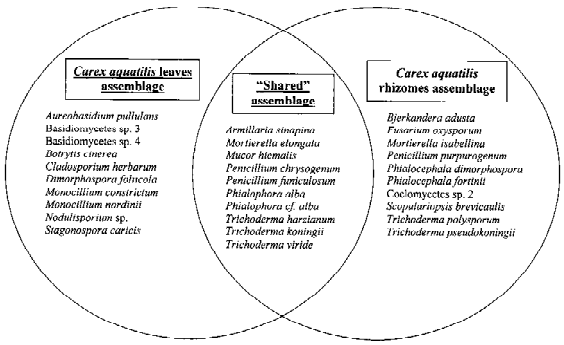

Figure 4. Microfungal assemblages (mycocoenoses) of decomposing *Carex aquatilis* leaves and rhizomes from the riverine sedge fen in southern boreal Alberta, Canada. Only the ten most frequently isolated fungal species for each assemblage are listed.

cies of *Phialophora*, *Trichoderma*, and *Penicillium* were common to both *C. aquatilis* litters (Figure 4).

Carex aquatilis leaves

A clear succession pattern was apparent for the fungal assemblages of decomposing *C. aquatilis* leaves, with axis 1 separating the fungal assemblages of different decomposition stages (Figure 3). The fungal assemblages of the early stages of decomposition (0–50 days) occurred on the left side of axis 1 and those of the late stages of decomposition (250–730 days) occurred on the right side of axis 1 (Figure 3). Elevated TP (0–50 days: 1.2 mg g^{-1}; 50–730 days: 1.8 mg g^{-1}) and TN (0–50 days: 2.1 mg g^{-1}; 50–730 days: 2.2 mg g^{-1}) (Thormann et al., 2001a) tissue nutrient concentrations correlated most strongly with the microfungal assemblages of the late stages of decomposition of the leaf litter (0–50 days) (Figure 3).

Early-stage succession species primarily belonged to *Cladosporium*, *Mortierella*, and *Mucor*, while those of the late stages of decomposition primarily belonged to *Monocillium* and *Dimorphospora* as well as basidiomycetes spp. 3 and 4 (Figure 5). Thus, we named the microfungal assemblage of the early to mid decomposition stage the '*Cladosporium-Mucor*'-dominated mycocoenosis and the microfungal assemblage of the mid to late decomposition stage the '*Monocillium*-basidiomycetes'-dominated mycocoenosis.

Although studies on the fungal assemblages of wetland plant species, such as species of *Juncus*, *Phragmites*, and *Typha*, are not uncommon (Apinis et al., 1972; Cabral et al., 1993; Tokumasu, 1994), those investigating the fungal assemblages of species of *Carex* are rare (Pugh, 1958). Similar fungal genera occur on the living and decomposing leaves of different plant species. For example, species of

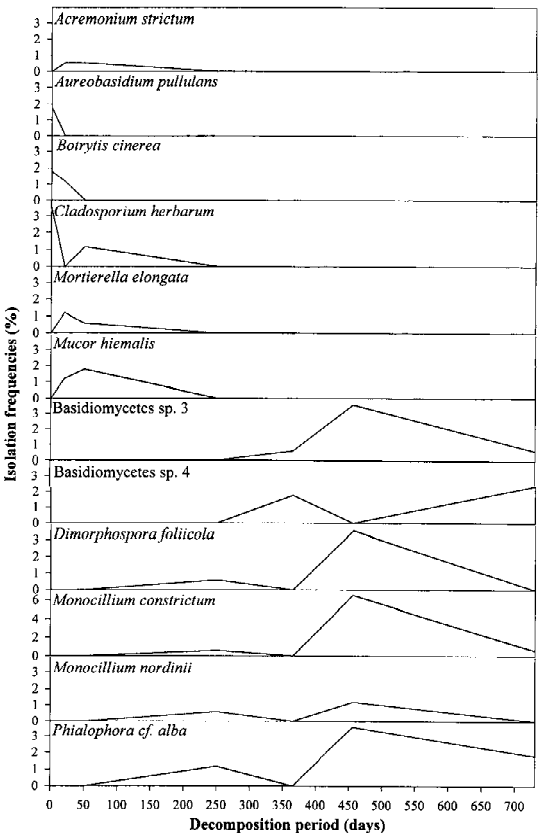

Figure 5. Isolation frequencies (%) of selected microfungi isolated during the 2-year decomposition period of *Carex aquatilis* leaves in a fen in southern boreal Alberta, Canada. Note the elevated *y*-axis scale for *Monocillium constrictum*. These fungi were selected because of their preponderance during the early to mid (0–365 days) or mid to late (365–730 days) stages of decomposition. Fungi not shown did not show clear isolation patterns during the process of decomposition.

Cladosporium, *Fusarium*, *Alternaria*, *Trichoderma*, *Penicillium*, *Mortierella*, *Acremonium*, and *Epicoccum* occur equally frequently and constitute some of the primary colonizers and saprobes of aerial plant tissues. General succession patterns of previous studies indicate that species of *Alternaria*, *Aureobasidium*, *Cladosporium*, *Epicoccum*, and *Fusarium* occur during early stages of decomposition, while species of *Mucor*, *Oidiodendron*, *Penicillium*, and *Phoma* occur during later stages of decomposition (Apinis et al., 1972; Latter and Cragg, 1967). Those patterns are supported by this study, as isolation frequencies of species of the above genera follow similar trends in decomposing *C. aquatilis* leaves in this fen (Thormann, 2001). Weakly parasitic and primary saprobic microfungi occurred during the early stages of decomposition (*A. pullulans*, *B. cinerea*), and were replaced by secondary saprobic taxa able to utilize polyphenolic compounds and cellulose (basidiomycetes spp. 3 and 4, *Monocillium constrictum*) (Thormann, 2001) during the latter stages of decomposition (Figure 5). This pattern follows the classical succession pattern of fungi and correlates with physiological profiles of fungi at various stages of decomposition (Deacon, 1997).

Carex aquatilis *rhizomes*

A clear microfungal succession pattern was absent for decomposing *C. aquatilis* rhizomes (Figure 3). However, several species of *Trichoderma* were isolated only during late stages of decomposition (365–730 days). Similarly, although species of *Phialophora* and *Phialocephala* were isolated from most stages of decomposition, their isolation frequencies increased during the late stages of decomposition (365–730 days) (Thormann, 2001).

Few studies have examined the fungal assemblages of belowground litters of wetland plant species. Pugh and Mulder (1971) examined the fungal assemblages of *Typha latifolia* L. rhizomes and roots. They found that rhizomes were almost free of colonization by fungi and that only a few dematiaceous *mycelia sterilia* were present. Roots also showed a limited range of fungal richness (*A. pullulans*, *Cladosporium herbarum*, *Helicorhoidion* sp., *Isaria* sp., and one sterile isolate) (Pugh and Mulder, 1971). Similarly, among the five litter types, the lowest number of fungi came from decomposing *C. aquatilis* rhizomes, possibly reflecting the effects of anoxia in the rooting medium within this fen. The mean acrotelm depth was 60 mm below the peat surface (Thormann et al., 2001a) and the belowground fen litters were buried between 20 and 50 mm below the peat surface to mimic natural decomposition conditions. Therefore, the rhizome litter may have been exposed to low oxygen concentrations for part of the 2-year decomposition period, thereby limiting fungal species richness.

The fungal assemblages of Carex aquatilis *rhizomes and* Salix planifolia *roots*

Comparison of their fungal assemblages

The fungal assemblages decomposing belowground litters of *C. aquatilis* and *S. planifolia* in the fen differed substantially and separated along axis 1 in the CCA (Figure 6). The fungal assemblages of the decomposing *S. planifolia* roots correlated more strongly

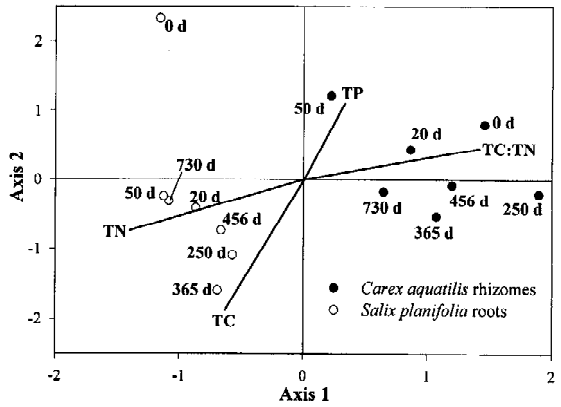

Figure 6. Canonical correspondence analyses of microfungus assemblages from *Salix planifolia* roots and *Carex aquatilis* rhizomes isolated after 0, 20, 50, 250, 365, 456, and 730 days decomposition from the riverine sedge fen in southern boreal Alberta, Canada. Axis 1 separates the litter types. Microfungal communities of *C. aquatilis* rhizomes correlated with TP and TC:TN, while microfungal communities of *S. planifolia* roots correlated with TN and TC. Abbreviations as in Figures 1 and 3. Eigenvalues for axes 1 and 2 were 0.568 and 0.385, respectively.

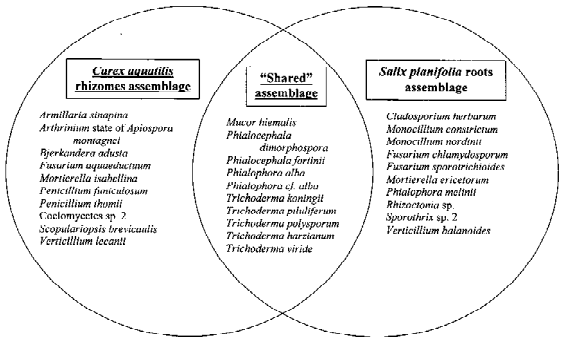

Figure 7. Microfungal assemblages (mycocoenoses) of decomposing *Carex aquatilis* rhizomes and *Salix planifolia* roots from the riverine sedge fen in southern boreal Alberta, Canada. The ten most frequently isolated fungal species for each assemblage are listed.

with TN (roots: 15 mg g^{-1}; rhizomes: 7 mg g^{-1}) and TC (roots: 485 mg g^{-1}; rhizomes: 455 mg g^{-1}) (Thormann et al., 2001a) tissue concentrations than those of the decomposing *C. aquatilis* rhizomes, as indicated by the direction of the TN and TC vectors (Figure 6). These two litter quality variables significantly correlated with axes 1 and 2, respectively (Table 3). In contrast, the fungal assemblages of decomposing *C. aquatilis* rhizomes correlated more strongly with TP tissue nutrient concentrations (rhizomes: 1.3 mg g^{-1}; roots: 1.1 mg g^{-1}) (Thormann et al., 2001a) and the TC:TN quotient (rhizomes: 64; roots: 32) (Thormann et al., 2001a) than those of the decomposing *S. planifolia* roots. This is shown by the proximity of the respective litter quality vectors to the microfungal assemblages of the two litter types (Figure 6). These litter quality variables correlated significantly with axes 2 and 1, respectively (Table 3).

Thirty-one percent of all species isolated from the rhizome and root litters (31 taxa) occurred exclusively in the rhizomes, while 38% of all fungi isolated from these litters occurred exclusively in *Salix* roots. Thirty-one percent of all species occurred in both litters. Basidiomycetes and species of *Penicillium* were isolated only from decomposing *C. aquatilis* rhizomes, while species of *Monocillium*, *Mortierella*, and *Fusarium* were isolated exclusively from decomposing *S. planifolia* roots. Species of *Phialocephala*, *Phialophora*, and *Trichoderma* occurred in both litters (Figure 7).

Salix planifolia *roots*

Species of *Acremonium*, *Cladosporium*, and *Fusarium* occurred primarily in early-stage decomposing *S. planifolia* roots, while species of *Phialophora* and *Phialocephala* were more frequently isolated from the mid- and late-stage decomposing root material. However, a clear pattern of succession did not occur (Figure 6). Studies investigating fungi associated with roots of woody plant species usually concentrate on mycorrhizal fungi, while the saprobic fungal community is investigated less commonly (Harley and Waid, 1955; Livingston and Blaschke, 1984; Summerbell, 1989). Summerbell (1989) isolated primarily *mycelia sterilia*, some belonging to the *Mycelium radicis atrovirens* (MRA) complex (*sensu* Melin, 1923), and species of *Mortierella* and *Penicillium* from *P. mariana* roots. He concluded that his rhizosphere fungi were not restricted to mycorrhizal roots of black spruce, but overlap considerably with those of roots from other plant species. We isolated two MRA species as well (*Phialocephala dimorphospora* and *Phialocephala fortinii*). Both were isolated at higher frequencies from mid- and late-stage decomposing *S. planifolia* roots. Overall, genera of microfungi isolated by Summerbell (1989) are similar to those isolated in this study, with several species of *Mortierella*, *Penicillium*, and *Trichoderma* frequently isolated from our *Salix* roots and Summerbell's *P. mariana* roots. This supports his hypothesis that microfungal assemblages of roots are similar among different plant species.

Similar isolation frequency patterns were described above for decomposing *C. aquatilis* rhizomes. It is likely that the environmental, physical, and surface water chemical variables measured in this study,

Table 3. Pearson's correlation coefficients among the Canonical correspondence axes and litter quality variables of decomposing *Carex aquatilis* rhizomes and *Salix planifolia* roots from the riverine sedge fen, Alberta, Canada

	Axis 1	Axis 2	TC	TN	TC:TN	TP
TC	−0.371	−0.918 ***	–			
TN	−0.832 **	−0.365	0.639 *	–		
TC:TN	0.844 **	0.222	−0.499 *	−0.958 ***	–	
TP	0.192	0.538 *	−0.624 *	−0.063	−0.078	–

TC = Total carbon, TN = toal nitrogen, TC:TN = total carbon to total nitrogen quotient, TP = total phosphorus. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

the similarity of the fungal assemblages of these two litter types, the relatively low number of identified species in either litter, and cultural techniques were not sufficient to show a clear succession pattern of some of these litter types throughout the decomposition process.

Succession patterns of microfungi during the decomposition of peatland plant litters

Although a succession of fungal species assemblages occurred for some of these peatland plant litters, the 'classical' pattern of fungal succession described in the literature was not observed in our study. The classical fungal succession pattern involves colonization of a particular plant substrate initially by: (1) pathogenic and weakly parasitic fungi, followed by (2) saprobic, 'sugar' fungi, (3) cellulolytic fungi, and finally (4) lignolytic fungi to complete the decomposition process (Deacon, 1997). This functional succession corresponds to a taxonomic succession. Zygomycetes, which utilize simple sugars and soluble nutrients, precede ascomycetes and fungi imperfecti, which utilize cellulose, hemicelluloses, pectin, starch, and lipids. Basidiomycetes complete the decomposition process by degrading the most decay-resistant structural plant polymers, such as lignins and related polyphenolic polymers (Deacon, 1997). These classical taxonomic and functional patterns of fungal succession have been shown in a variety of organic materials, most notably wood (Heilmann-Clausen, 2001; Lumley et al., 2001) and dung (Harper and Webster, 1964; Wicklow and Yokom, 1981). Furthermore, they most often are based on conspicuous, epigeous sporocarp surveys, which permit the efficient recording of a large number of macrofungal structures and are not hindered by culturing and identification problems and time limitations common to microfungal surveys (Heilmann-Clausen, 2001).

Our data do not show either taxonomic or functional patterns of microfungal succession. We isolated weak pathogens (*Fusarium* spp.), primary saprobes (*Mortierella* spp., *M. hiemalis*), and cellulolytic fungi (*Acremonium* spp., *Penicillium* spp., *Trichoderma* spp., *Verticillium* spp.) primarily, and these generally occurred throughout the first two years of decomposition of these plant litters (Figures 2, 5). Although lignolytic taxa (*Acremonium chrysogenum*, *M. constrictum*) were also isolated, they occurred infrequently and were not isolated predominantly from the late stages of decomposing plant litters. For example, only *A. chrysogenum* from the *S. fuscum* litter utilized tannic acid as a carbon source and it occurred during the intermediate stages of decomposition (250–456 days) (Figure 2). Those taxa that occurred predominantly during the late stages of decomposition (365–730 days; *Sporothrix* sp. 1, *T. viride*) (Figure 2) were unable to utilize this polymer as a carbon source (Thormann, 2001). Three late-stage (365–730 days) decomposition fungi from *C. aquatilis* leaves (basidiomycetes spp. 3 and 4, *M. constrictum*) were laccase positive, indicating their ability to use polyphenolic polymers as a carbon source, while others (*Monocillium nordinii*, *Phialophora cf. alba*, and *D. foliicola*) were not (Figure 5) (Thormann, 2001).

Thus, only some taxa from some of the litters fit the classical fungal succession patterns previously described from wood and dung (Harper and Webster, 1964; Heilmann-Clausen, 2001; Lumley et al., 2001; Wicklow and Yokom, 1981) and generally the classical fungal succession pattern was absent. It is possible that the decomposition period (two years) was insufficiently long to permit the isolation of 'typical' basidiomycetous lignolytic fungi, despite the use of benomyl (selective for most basidiomycetes) in one of the primary isolation media. Previous studies examining fungal succession on wood have sampled logs at low, medium, and high levels of decomposition, of-

ten spanning decades. In addition, it is possible that peatlands have a limited diversity of lignolytic fungi (Thormann et al., 2002b), despite the abundance of ectomycorrhizal fungi (basidiomycetes) associated with woody plant species in wetlands (Thormann et al., 1999b). However, Hutchison (1990) showed that most ectomycorrhizal fungi do not have the ability to use lignin and similar polyphenolic polymers as a carbon source, which has been shown to constitute up to 50% of peat (Turetsky et al., 2000).

The surface-sterilization procedure used in our study may have inadvertently eliminated some decomposer fungi. However, this was unavoidable because we wanted to assure that only endophytic fungi from the decomposing litters were isolated and opportunistic surface contaminants were eliminated prior to the litter segments being placed onto the primary isolation media. Furthermore, non-sporulating, otherwise nondescript fungi, yeasts, and chytridiomycetes either represented less than 15% of all isolates or were not isolated at all and hence were excluded from this study. However, these fungi may figure prominently in the decomposition process of organic materials *in situ* as well.

Conclusions

An investigation into the fungal assemblages of decomposing vegetation from two southern boreal peatlands in Alberta, Canada, generally revealed different fungal assemblages among different plant litters. However, the assemblages of some of the five litters were similar to each other, possibly as a result of the low number of litter type specific microfungi isolated or the similarity in the litter quality throughout the process of decomposition among others. Therefore, we rejected hypothesis 1, which stated that distinct fungal assemblages were involved in the decomposition of each of the five litter types. Furthermore, distinct succession patterns of the fungal assemblages were observed for two of the five litter types (*S. fuscum*, *C. aquatilis* leaves). However, the remaining three litter types showed no clear succession patterns, thereby rejecting hypothesis 2, which stated that distinct fungal succession patterns characterize the decomposition process of each of these litter types. The classical succession of taxonomic and functional groups of fungi during the process of decomposition, i.e., ascomycetes with cellulolytic abilities precede basidiomycetes with lignolytic abilities, was absent in all five litter types, possibly due to the short duration of the decomposition period. Instead, microfungi with broad spectra of enzymatic abilities co-existed; however, they generally had limited abilities to decompose complex phenolic polymers, such as lignin. This limitation may contribute to the accumulation of peat in these ecosystems. Litter quality variables correlated most often with the observed fungal assemblages of these litter types, indicating that these variables were more important to the individual fungal assemblages than either physical or surface water chemistry variables measured in these two peatlands.

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References

Apinis A E, Chester C G C and Taligoola H K 1972 Colonisation of *Phragmites communis* leaves by fungi. Nova Hedwig. 23, 113–124.

Cabral D, Stone J F and Carroll G C 1993 The internal mycobiota of *Juncus* spp.: microscopic and cultural observations of infection patterns. Mycol. Res. 97, 367–376.

Christensen M and Whittingham W F 1965 The soil microfungi in open bogs and conifer swamps in Wisconsin. Mycologia 57, 882–896.

Czastukhin V Y 1967 Decomposition of peat mosses by fungi. Mikol. Fitopatol. 1, 294–303.

Deacon J W 1997 Modern Mycology, 3rd edn. Blackwell Scientific Publications, Boston. 303 pp.

Dix N J 1985 Changes in relationship between water content and water potential after decay and its significance for fungal successions. Trans. Brit. Mycol. Soc. 85, 649–653.

Dooley M and Dickinson C H 1971 The ecology of fungi in peat. Ir. J. Agric. Res. 10, 195–206.

Ecoregions Working Group 1989 Ecoclimatic Regions of Canada, First Approximation. Canada Committee on Ecological Land Classification, Ecological Land Series, No. 23. Sustainable De-

velopment Branch, Canadian Wildlife Service, Conservation and Protection, Environment Canada, Ottawa. 452 pp.

Environment Canada 1998 Canadian Climate Normals, 1961–1990, Canadian Climate Program, Ottawa. http://www.cmc.ec.gc.ca/climate/normal s/e_al_ndx.htm.

Frankland J C 1966 Succession of fungi on decaying petioles of *Pteridium aquilium*. J. Ecol. 54, 41–63.

Harley J L and Waid J S 1955 A method of studying active mycelia on living roots and other surfaces in the soil. Trans. Brit. Mycol. Soc. 38, 104–118.

Harper J L and Webster J 1964 An experimental analysis of the coprophilous fungus succession. Trans. Brit. Mycol. Soc. 47, 511–530.

Heilmann-Clausen J 2001 A gradient analysis of communities of macrofungi and slime molds on decaying beech logs. Mycol. Res. 195, 575–596.

Hutchison L J 1990 Studies on the systematics of ectomycorrhizal fungi in axenic culture. II. The enzymatic degradation of selected carbon and nitrogen compounds. Can. J. Bot. 68, 1522–1530.

Kasai K, Morinaga T and Horikoshi T 1995 Fungal succession in the early decomposition process of pine cones on the floor of *Pinus densiflora* forests. Mycoscience 36, 325–334.

Kuhry P and Vitt D H 1996 Fossil carbon/nitrogen ratios as a measure of peat decomposition. Ecology 77, 271–275.

Latter P M and Cragg J B 1967 The decomposition of *Juncus squarrosus* leaves and microbiological changes in the profile of *Juncus* moor. J. Ecol. 55, 465–482.

Latter P M, Cragg J B and Heal O W 1967 Comparative studies on the microbiology of four moorland soils in the northern Pennines. J. Ecol. 55, 445–464.

Livingston W H and Blaschke H 1984 Deterioration of mycorrhizal short roots and occurrence of *Mycelium radicis atrovirens* on declining Norway spruce in Bavaria. Eur. J. For. Pathol. 14, 340–348.

Lumley T C, Gignac L D and Currah R S 2001 Microfungus communities of white spruce and trembling aspen logs at different stages of decay in disturbed and undisturbed sites in the boreal mixedwood region of Alberta. Can. J. Bot. 79, 76–92.

Melin E 1923 Experimentelle Untersuchungen über die Konstitution und Ökologie der Mycorrhizen von *Pinus sylvestris* L. und *Picea abies* (L.) Karst. *In* Mykologische Untersuchungen und Berichte. Ed. R Falck. pp. 73–334. Druck und Verlag G. Gottheilt, Kassel, Germany.

Nilsson M, Bååth E and Söderström B 1992 The microfungal communities of a mixed mire in northern Sweden. Can. J. Bot. 70, 272–276.

Pugh G J F 1958 Leaf litter fungi found on *Carex paniculata* L. Trans. Brit. Mycol. Soc. 41, 185–195.

Pugh G J F and Mulder J L 1971 Mycoflora associated with *Typha latifolia*. Trans. Brit. Mycol. Soc. 57, 273–282.

Saitô T 1966 Sequential pattern of decomposition of beech litter with special reference to microbial succession. Ecol. Rev. 16, 245–254.

Sigler L 1992 Preparing and mounting slide cultures. *In* Clinical Microbiology Procedures Handbook. Ed. H D Isenberg. pp. 6.12.1–6.12.4. American Society for Microbiology, Washington D.C. 2498 pp.

Summerbell R C 1989 Microfungi associated with the mycorrhizal mantle and adjacent microhabitats within the rhizosphere of black spruce. Can. J. Bot. 67, 1085–1095.

Szumigalski A R and Bayley S E 1997 Net aboveground primary production along a peatland gradient in central Alberta in relation to environmental factors. Écoscience 4, 385–393.

ter Braak C J F 1992 CANOCO – A FORTRAN Program for Canonical Community Ordination. Microcomputer Power, Ithaca.

Thormann M N 2001 The fungal communities of decomposing plants in southern boreal peatlands of Alberta, Canada. Ph.D. Thesis, Dept. Biol. Sci., Univ. Alta., Edmonton, AB, Canada. 256 pp.

Thormann M N and Bayley S E 1997 Aboveground plant production and nutrient content of the vegetation in six peatlands in Alberta, Canada. Plant Ecol. 131, 1–16.

Thormann M N, Szumigalski A R and Bayley S E 1999a Aboveground peat and carbon accumulation potentials along a bog-fen-marsh wetland gradient in southern boreal Alberta, Canada. Wetlands 19, 305–317.

Thormann M N, Currah R S and Bayley S E 1999b The mycorrhizal status of the dominant vegetation along a peatland gradient in southern boreal Alberta, Canada. Wetlands 19, 438–450.

Thormann M N, Bayley S E and Currah R S 2001a Comparison of decomposition of belowground and aboveground plant litters in peatlands of boreal Alberta, Canada. Can. J. Bot. 79, 9–22.

Thormann M N, Currah R S and Bayley S E 2001b Microfungi isolated from *Sphagnum fuscum* from a southern boreal bog in Alberta, Canada. Bryol. 104, 548–559.

Thormann M N, Currah R S and Bayley S E 2002 The relative ability of fungi from *Sphagnum fuscum* to decompose selected carbon substrates. Can. J. Microbiol. 48, 204–211.

Tokumasu S 1994 Trophodynamic structure of a swampy bog at the climax stage of limnological succession III. Filamentous fungi associated with the standing leaves of *Typha latifolia*. Water, Air Soil Pollut. 76, 491–499.

Tsuneda A, Thormann M N and Currah R S 2001a Modes of cell-wall degradation of *Sphagnum fuscum* by *Acremonium cf. curvulum* and *Oidiodendron maius*. Can. J. Bot. 79, 93–100.

Tsuneda A, Chen M H and Currah R S 2001b Characteristics of a disease of *Sphagnum fuscum* caused by *Scleroconidioma sphagnicola*. Can. J. Bot. 79, 1217–1224.

Turetsky M R, Wieder R K, Williams C J and Vitt D H 2000 Organic matter accumulation, peat chemistry, and permafrost melting in peatlands of boreal Alberta. Écoscience 7, 379–392.

Wicklow D T and Yokum D H 1981 Fungal species numbers and decomposition of rabbit feces. Trans. Brit. Mycol. Soc. 76, 29–32.

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