In vitro decomposition of *Sphagnum*-derived acrotelm and mesotelm peat by indigenous and alien basidiomycetous fungi

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SUMMARY

Northern peatlands have accumulated significant quantities of peat, and it has been predicted that rates of peat decomposition may increase due to climate warming. In peatlands, organic matter decomposition in the acrotelm is accomplished primarily by fungi that act differentially through time on various peat constituents. After four months of decomposition *in vitro*, I show a distinct microbiological limitation to the decomposition of *Sphagnum*-derived peat (mean mass losses of 1.1–7.1 %) by indigenous and alien basidiomycetous fungi of both acrotelm and mesotelm peat (the mesotelm is the lower part of the acrotelm *sensu lato*, in which conditions fluctuate between oxic and anoxic). Neither acrotelm nor mesotelm *Sphagnum* peat can be degraded effectively by many fungi (mean mass losses of 2.7 % and 4.3 % for acrotelm and mesotelm peat, respectively), including the ubiquitous wood decomposing basidiomycetes known to decompose some of nature's most complex polymers. Peatland basidiomycetes caused significantly greater mass losses of acrotelm and mesotelm peat than wood decay basidiomycetes (mean mass losses of 5.7 % and 1.4 %, respectively). Brown rot fungi caused significantly greater mass losses to acrotelm and mesotelm peat than white rot fungi and non-wood-decay fungi (mean mass losses of 10.1 %, 1.7 %, and 2.3 %, respectively). Rates of peat decomposition may not increase to the extent previously predicted, and peatlands may not necessarily be long-term sources of CO_2 in response to a warming climate.

KEY WORDS: carbon cycling, climate warming, peat decomposition, peatlands.

INTRODUCTION

Northern peatlands have been significant sinks of atmospheric carbon (C) for millennia, storing an estimated 455 Pg of C in peat (Gorham 1991), which represents about 10–16 % of the total global terrestrial detrital C, indicating their importance to the global C cycle. The majority of this C is stored in bogs, which are ombrotrophic peatlands that receive water and nutrients solely from precipitation. Bogs are dominated by *Sphagnum* spp.; members of the Ericaceae including *Rhododendron*, *Andromeda*, and *Vaccinium* spp.; and Pinaceae including *Picea* and *Pinus* spp. (Vitt 1994). Peat, a heterogeneous assemblage of partially decomposed organic matter, has accumulated in these peatlands since the last ice age ended, because rates of plant production exceed rates of organic matter decomposition. Chemically, peat consists of varying quantities of polysaccharides and phenolic polymers (Bland *et al.* 1968, Williams *et al.* 1998), of which the phenolic fraction, i.e. lignins and lignin-like polymers, is the most chemically complex component. Lignins are highly variable hydrophobic heteropolymers of *p*-hydroxyphenyl, guaiacyl, and syringyl units that cross-link carbohydrate polymers together, thereby providing structural integrity to cell walls of most tracheophytes (Iiyama *et al.* 1994, Lewis *et al.* 1999). Recent histochemical research has produced no conclusive evidence for the presence of lignin in bryophytes, although it supported the occurrence of unidentified phenolic compounds, lignin-like in nature, in the cell walls of bryophytes (Scheirer 1980, Ligrone *et al.* 2008) including *Sphagnum* spp. (Wilson *et al.* 1989). The complexity and abundance of the phenolic fraction of peat contributes to its slow decomposition.

Decomposition is a complex process, which includes nearly all changes in organic matter that has undergone senescence or death (Brinson *et al.* 1981). The consistently highest rates of decomposition occur in the acrotelm (oxygenated soil horizon) and result in CO_2 efflux into the atmosphere. Most litter is only partially decomposed and is buried by new litter produced in the following year(s). Litter ultimately enters the mesotelm (often anoxic, but may be periodically oxygenated, in response to fluctuating water levels; Clymo & Bryant 2008) and then the catotelm (permanently anoxic soil horizon), where decomposition is slow, and organic matter is decomposed only by anaerobic bacteria (Clymo 1984). Decomposition in peatlands is slow due to poor substrate quality, limited

nutrient supply, antimicrobial properties of the constituent plant tissues (primarily *Sphagnum* spp.), low pH, low soil temperatures, and low oxygen concentrations, particularly in the mesotelm and catotelm (e.g. Thormann & Bayley 1997, Yavitt *et al.* 1997, Aerts 2006, Stalheim *et al.* 2009).

Given the temperature sensitivity of litter decomposition and the strong positive correlation between litter decomposition rates and climatic variables (Daulat & Clymo 1998, Liski *et al.* 2003, Davidson & Janssens 2006), it has been hypothesised that climate warming may lead to increased rates of litter decomposition in peatlands (e.g. Trettin *et al.* 2006), and consequently to a long-term net flux of CO_2 into the atmosphere. It has been proposed that millennia-old peat will also decompose in response to enhanced polyphenol oxidase (PPO) activity as peat is exposed to oxygen as a result of lower water levels ("enzymic latch"; Freeman *et al.* 2001). PPOs are oxidative enzymes that hydrolyse complex carbon-carbon and ether bonds characteristic of polyphenolic polymers, including tannins, lignins, and related acid-unhydrolysable residues (Freeman *et al.* 2004). These polymers constitute a significant portion of peat and are proportionally more prevalent with increasing depth than other, less complex, structural polymers (Turetsky *et al.* 2000, Williams & Yavitt 2003), i.e. the recalcitrance of peat increases with increasing depth (Williams *et al.* 1998, Limpens *et al.* 2008). The primary types of PPOs, ligninase, peroxidase, laccase and tyrosinase are limited to certain groups of basidiomycetes, ascomycetes (Rice *et al.* 2006), bacteria (Fenner *et al.* 2005) and actinomycetes (Endo *et al.* 2003). In contrast, recent work points to a greater stability of C in peatlands than was previously thought (review in Sinsabough 2010). In addition, Laiho (2006) suggested that lowered water levels in response to climate warming may not necessarily result in increased long-term C fluxes from peatlands to the atmosphere. Rates of decomposition of mesotelm and catotelm peat and rates of decomposition of new organic matter entering the ecosystem under new environmental conditions will greatly affect whether a peatland becomes a C source, is C neutral, or remains a C sink (Laiho 2006). Moreover, Toberman *et al.* (2008) indicated a decline in not only fungal activity but also a decline in PPO activity during summer drought (lower water levels and higher temperatures) in a shallow upland heathland, and Williams *et al.* (2000) concluded that PPOs were limited more by pH than oxygen availability.

Fungi have been recognised as the principal decomposers of organic matter in the acrotelm in peatlands, with bacteria being less important overall (Thormann 2006a, 2006b). About 600 fungal taxa are known from peatlands globally (Thormann & Rice 2007). Ascomycetes and basidiomycetes are similar in terms of reported taxonomic diversity (276 and 243 species, respectively; Thormann & Rice 2007). They are the dominant fungal groups (Thormann & Rice 2007, Artz *et al.* 2007) and principally involved in the decomposition of organic matter (Thormann 2006a, 2006b, Thormann & Rice 2007, Artz *et al.* 2007). Ascomycetes have received most of the attention in peatland decomposition studies (Thormann *et al.* 2002, Rice *et al.* 2006, Thormann *et al.* 2007), which is surprising because basidiomycetes are the primary decomposers of complex polymers in terrestrial ecosystems, e.g. lignin and related polyphenolic constituents of wood. Terrestrial wood decay fungi, as well as saprobic fungi from other substrata, are widespread and have the ability to produce copious quantities of usually aerially-distributed spores. These spores have the ability to travel long distances, in some cases over 1,000 km (Hallenberg & Kueffer 2001), and are likely to be deposited in peatlands as well. These fungi may subsequently become established in peatlands, particularly as these ecosystems become more favourable environments for fungi under a warming climate, and contribute to the decomposition of peat. There are no data, however, on their ability to colonise and/or decompose peat.

Here, I examine the ability of five common upland wood decay basidiomycetes and five native peatland basidiomycetes to decompose *Sphagnum*-derived peat from the acrotelm and mesotelm of a bog. My hypotheses were that (1) wood decay and peatland fungi will decompose acrotelm peat more effectively than mesotelm peat due to the increasing recalcitrance of the latter peat, (2) wood decay basidiomycetes will be efficient decomposers of peat due their ability to decompose complex polyphenolic polymers, and (3) white rot fungi will be more efficient decomposers of peat than brown rot fungi and non-wood-decay fungi due to their greater ability to break down polyphenolic polymers, including lignin.

METHODS

Study sites

The study sites, previously described by Thormann *et al.* (2007), were in east-central Saskatchewan, Canada (two sites, one bog and one fen), and in the West Siberian Lowland, Russia (12 sites, eight bogs and four fens; Figure 1). Briefly, the Saskatchewan bog was forested and dominated by *Picea mariana* (Mill.) BSP, ericaceous shrubs and *Sphagnum* spp.

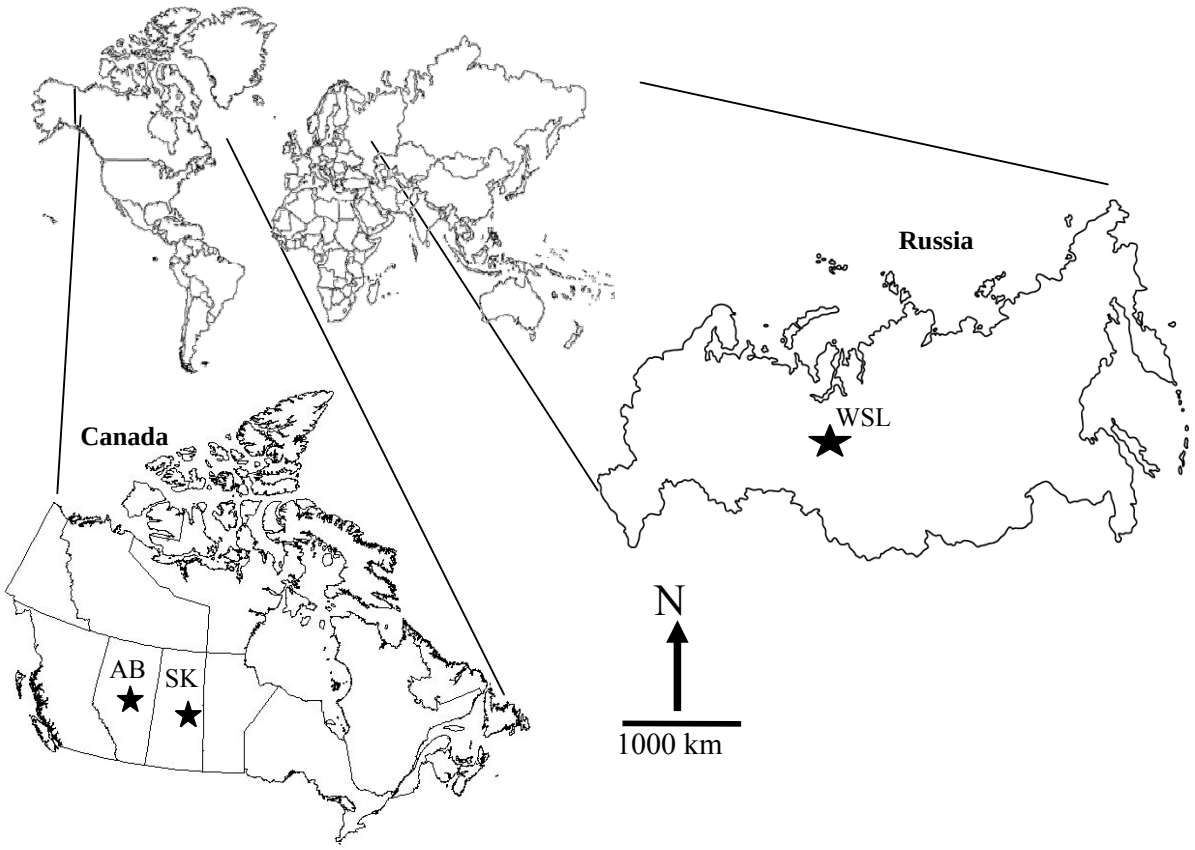

Figure 1. Locations of the study sites in Alberta (AB), Saskatchewan (SK) and the West Siberian Lowland (WSL).

typical of western continental bogs in Canada. The Saskatchewan fen was dominated by *Larix laricina* (Du Roi) K. Koch, *Betula pumila* L., *Salix* spp., *Andromeda polifolia* L., and several members of the Amblystegiaceae ("brown mosses"). The climate of central Saskatchewan is characterised by cold, snowy winters and mild summers. The average annual precipitation is 467 mm, while the mean temperatures of the three coldest and three warmest months are -16.2 ºC (December–January–February) and 14.9 ºC (June–July–August), respectively (mean annual temperature is 0.4 ºC; data from the Waskesiu Lake, SK, weather station; Environment Canada 2004).

In the West Siberian Lowland, the eight bogs were dominated by *Pinus sylvestris* L., ericaceous shrubs and *Sphagnum* spp.; and the four fens were dominated by *Betula pubescens* Ehrh., *Carex* spp., *Menyanthes trifoliata* L. and a number of bryophytes, mainly *Calliergon* spp. and *Sphagnum angustifolium* (C. Jens ex Russ.) C. Jens. The climate of West Siberia is highly continental and characterised by cold, snowy winters and mild summers similar to those in Saskatchewan. The average annual precipitation is 433–541 mm, while the mean temperatures of the three coldest and three warmest months range from -16.7 to -19.6 ºC (December–January–February) and from 15.3–16.7 ºC (June–July–August), respectively (mean annual temperature ranges from -2.1 to 0.3 ºC at the Khanty-Mansiysk, Aleksandrovskoe, Tobolsk, Kolpashev and Tara weather stations, West Siberian Lowland; Frey & Smith 2003).

Isolation of peatland basidiomycetes

Peat was collected aseptically at each sampling plot at 5 cm (acrotelm) and 25 cm (mesotelm) depths in the Saskatchewan sites and at 0–10 cm (acrotelm) and 10–20 cm (mesotelm) depths in the West Siberian sites, transferred to sterile containers, and transported in a cooler to the laboratory. The peat originated from the acrotelm and mesotelm in each site, since fungi are aerobes and no viable fungi were expected to occur in the catotelm. A sample of about 5 g (FW, randomly selected from each entire peat collection) was surface-sterilised for 5 minutes

in H_2O_2 and washed three times with sterilised distilled water (sd-H_2O) to eliminate surface contaminants before being placed onto primary isolation media. Hence, there were 14 acrotelm and 14 mesotelm samples, since there were two sites in Saskatchewan and 12 sites in the West Siberian Lowland, and acrotelm and mesotelm peat samples were extracted from all sites.

For each peat sample, nine 90 x 15 mm plastic Petri dishes were set up. All Petri dishes contained potato dextrose agar (PDA, 39.0 g Difco (Detroit, MI) potato dextrose agar, 1.0 L sd-H_2O) with oxytetracycline added to suppress bacterial growth. Three of the nine plates received no other additives and served as controls; three were amended with Benlate (50 % benomyl); and the final three plates were amended with a sterile stock solution containing Benlate, dichloran (2,6-dichloro-4-nitroaniline) and phenol in ethanol (5 mL L^{-1}). These additives have proven successful in selectively isolating generally slow-growing basidiomycetes from natural substrata (Worrall 1991) by suppressing the growth of many fungal taxa, particularly those with high growth rates and with non-basidiomycetous affinities. Five peat sub-samples (each about 2 mm^3, randomly selected from the surface-sterilised 5-g samples) were transferred aseptically to each of the nine plates.

Plates were incubated in the dark and examined daily for the first week, every other day for the following four weeks, and every five days for the following three months. Fungi emerging from the peat segments were subcultured and maintained on 1.5 % malt extract agar (MEA, 15.0 g Difco malt extract, 20.0 g Difco agar, 1.0 L sd-H_2O) and identified by extracting, amplifying, and sequencing their rDNA. Sequences were then assembled, and related sequences were found using BLAST searches (data not shown).

Collection of wood decay basidiomycetes

Fructifications of ubiquitous circumboreal polypore basidiomycetes were collected in a mixedwood stand dominated by *Populus tremuloides* Michx., *Populus balsamifera* L., *Picea glauca* (Moench) Voss, *Betula papyrifera* Marsh. and *Salix* spp. in July 2006 in central Alberta and in Edmonton, Canada. These basidiomycetes were *Cerrena unicolor* (Bull.) Murrill, *Fomes fomentarius* (L.) J.J. Kickx, *Fomitopsis pinicola* (Sw.) P. Karst., *Peniophora polygonia* (Pers.) Bourdot & Galzin and *Phellinus punctatus* (Fr.) Pilát. These wood decay basidiomycetes are common in many northern forests, including those in Canada and Russia (Gilbertson & Ryvarden 1986, 1987; Bondartseva *et al.* 2003, Kotiranta *et al.* 2005).

Fructifications were removed with a knife from the substrata (live and dead trees, logs, and stumps), placed in individual paper bags, stored in a cooler, and returned to the laboratory. Sub-samples of each fructification were flame-sterilised, placed onto PDA amended with oxytetracycline to suppress bacterial growth, and the emerging mycelium was sub-cultured onto MEA to obtain pure cultures of each fungus.

The five wood decay basidiomycetes and the five most commonly isolated peatland basidiomycetes were then used in the following experiment on the rate of peat decomposition.

***In vitro* decomposition of peat**

The experimental units were 100 × 80 mm tall glass Petri dishes, each containing about 55 mL of peptone broth agar (20.0 g Difco agar, 1.0 g Difco bacto-peptone broth, 1.0 L sd-H_2O; final pH = 6.5). Dishes were inoculated in two locations with about 3 mm^2 pieces of actively growing fungal mycelium of each of the five wood decay fungi and five peatland fungi, i.e. one fungal taxon per set of ten Petri dishes (Table 1). This medium was chosen because it provided some N to the growing fungi (0.154 g L^{-1} TN; BD Diagnostic Systems, Sparks, Md.) other than the N inherent to the peat (which was added later). Hence, these conditions are similar to those *in situ*, where microbial populations have access to sources of nutrients other than the litter they decompose, e.g. from root exudates or pore water (Thormann *et al.* 2002) or from atmospheric loading (range of N loading of 0.21–0.27 g m^{-2} yr^{-1} in continental western Canada; Munger & Eisenreich 1983).

Peat for the decomposition study was collected from a location different than those used to obtain the basidiomycete isolates in order to avoid using a decomposition substrate that might provide an advantage to fungi from the same location. The peat donor site has previously been described in Thormann *et al.* (1999). Briefly, the bog is dominated by *Sphagnum fuscum* (Schimp.) Klinggr., *S. angustifolium*, members of the Ericaceae including *Rhododendron groenlandicum* (Oeder) Kron & Judd and *Andromeda polifolia* L., and *Picea mariana* (Mill.) BSP. Thormann *et al.* (2001) previously showed that *S. fuscum* from this bog lost 17.6–20.4 % of its mass when decomposing for 2–4 months *in situ*. Peat from the acrotelm was collected at a depth of about 10 cm, and peat from the mesotelm was collected at a depth of about 35 cm. All peat samples were packed in sterile plastic bags and stored in a cooler during transport and in a refrigerator in the laboratory until processing (within four days). Polyester mesh pouches

Table 1. Peatland and wood decay basidiomycetes used in the *Sphagnum* peat decomposition study. NoF = Northern Forestry Centre Culture Collection.

Fungal taxa	Origin	Type*
Peatland taxa		
Coniophora sp. (NoF 2964)	From rich fen peat, Siberia (Russia) and bog peat, Saskatchewan (Canada)	BR
Mycena sp. (NoF 2968)	From rich fen peat, Saskatchewan (Canada)	S
Pholiota sp. (NoF 2965)	From bog peat, Siberia (Russia) and Saskatchewan (Canada)	WR
Psathyrella sp. (NoF 2967)	From bog peat, Siberia (Russia) and rich fen peat, Saskatchewan (Canada)	S
Trametes sp. (NoF 2966)	From rich fen peat, Siberia (Russia) and bog peat, Saskatchewan (Canada)	WR
Wood decay taxa		
Cerrena unicolor (Bull.) Murrill (NoF 2962)	On dead *Betula papyrifera* stump, Alberta (Canada)	WR
Fomes fomentarius (L.) J.J. Kickx (NoF 2961)	On dead *Betula papyrifera* log, Alberta (Canada)	WR
Fomitopsis pinicola (Sw.) P. Karst. (NoF 2960)	On dead *Picea glauca* log, Alberta (Canada)	BR
Peniophora polygonia (Pers.) Bourdot & Galzin (NoF 2963)	On live *Populus tremuloides* tree, Alberta (Canada)	WR
Phellinus punctatus (Fr.) Pilát (NoF 2959)	On live *Salix* sp. tree, Alberta (Canada)	WR

* BR = brown rot fungus, S = saprobe, WR = white rot fungus.

(5.5 × 5.5 cm, 65-µm gauge) were filled with about 3 g FW of either acrotelm or mesotelm peat, dried at 48 °C to constant mass, weighed to the nearest 0.01 g and autoclaved at 121 °C for 15 minutes prior to placement into the inoculated Petri dishes (one pouch per Petri dish; five pouches of acrotelm and five of mesotelm peat per fungal taxon; the peat material was moist due to the liquid cycle autoclaving procedure). Autoclaving has proven to be successful in sterilising peat samples (e.g. Thormann *et al.* 2002); however, this process may have weakened or broken down some of the bonds of the structural components of *Sphagnum* peat, thereby resulting in enhanced rates of peat decomposition. Ten Petri dishes served as controls and were not inoculated with any fungi.

After four months, pouches (n = 5 per fungus per peat depth) were removed from the Petri dishes, and surficial fungal mycelium was carefully removed with forceps. Pouches were dried at 48 °C to constant mass and weighed to the nearest 0.01 g. Mass loss, as an indicator of decomposition at the harvest time, was expressed as a percentage of the initial mass. The fungal biomass of colonised pouches was not determined in this study.

Statistical analyses

Mass losses due to leaching (mean of 3.3 % from the ten control Petri dishes) were subtracted from all mass losses prior to statistical analyses. A Kruskal-Wallis test (dependent variable: mass loss; independent variable: fungal species) was used to determine significant differences among mass losses of the ten fungal taxa after four months decomposition. This analysis was chosen because of (i) the low number of replicates for each treatment (n = 5 per fungus per peat depth) and (ii) deviations in the data from normality and homogeneity of variances (Zar 1984). Separate one-way Analyses of Variance (ANOVA) were used to compare mass losses caused by peatland and wood decay basidiomycetes (fungal groups) at each depth

(dependent variable: mass loss; independent variable: fungal groups). In addition, ANOVA was used to compare mass losses caused by the brown rot fungi, white rot fungi and non-wood-decay fungi (fungal life strategy) at each depth (dependent variable: mass loss; independent variable: fungal life strategy). *Post-hoc* Tukey's Honestly-Significant-Difference tests were used to determine where significant differences occurred among the fungal life strategy groups.

RESULTS

Mass losses of acrotelm *Sphagnum* peat ranged from 0.2–9.6 % for the peatland basidiomycetes (mean = 4.2 %, median = 3.3 %) and from 0.3–2.7 % for the wood decay basidiomycetes (mean = 1.1 %, median = 0.8 %). Mesotelm peat lost 0.3–23.4 % of its mass (mean = 7.0 %, largely driven by a mean mass loss of 23.4 % by *Coniophora* sp., median = 3.9 %) through decomposition from the peatland basidiomycetes and 0.5–4.3 % (mean = 1.6 %, median = 1.0 %) through the decomposition from wood decay basidiomycetes (Figure 2).

Overall, mass losses of peat from 10 cm and 35 cm depths did not differ significantly (χ^2 = 2.0, d.f. = 1, P = 0.159); however, mass losses at both depths differed significantly among individual basidiomycete taxa (Kruskal-Wallis test statistic = 90.5, d.f. = 19, P < 0.001). Indigenous peatland basidiomycetes always caused significantly greater mass losses of *Sphagnum* peat than wood decay basidiomycetes at both depths (10 cm depth: 4.2 % *vs*. 1.1 % for peatland basidiomycetes and wood decay basidiomycetes, respectively; F = 12.5, d.f. = 1, P = 0.001; 35 cm depth: 7.1 % *vs*. 1.6 % for peatland basidiomycetes and wood decay basidiomycetes, respectively; F = 9.9, d.f. = 1, P = 0.003; Figure 3).

Brown rot fungi (Table 1) caused significantly greater mass losses of *Sphagnum* peat at both depths compared to the white rot fungi and the non-wood-decay fungi (10 cm depth: 6.2 % *vs*. 1.6 % *vs*. 2.2 % for brown rot fungi, white rot fungi and non-wood-decay fungi, respectively; F = 8.7, d.f. = 2, P < 0.0001; 35 cm depth: 13.9 % *vs*. 1.8 % *vs*. 2.4 % for brown rot fungi, white rot fungi, and non-wood-decay fungi, respectively; F = 26.0, d.f. = 2, P < 0.0001; Figure 4).

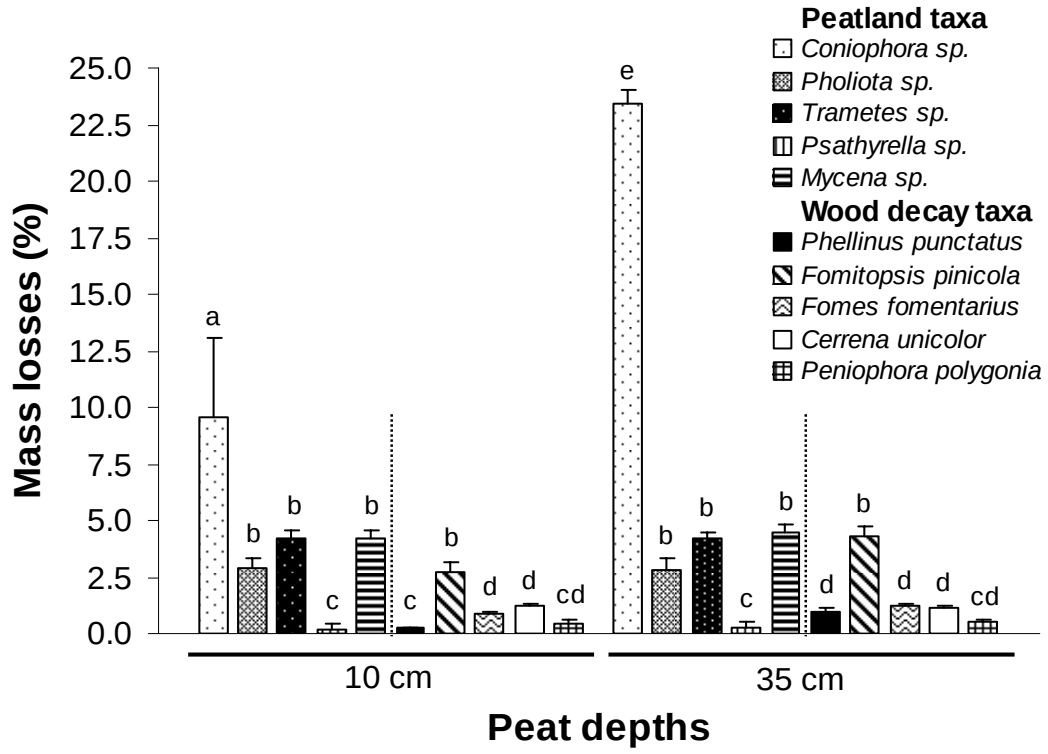

Figure 2. Mass losses (% ± SE) of *Sphagnum* peat incurred *in vitro* by peatland and wood decay basidiomycetes after four months decomposition. Letters indicate significant differences (P < 0.05) in mass losses incurred by different fungi.

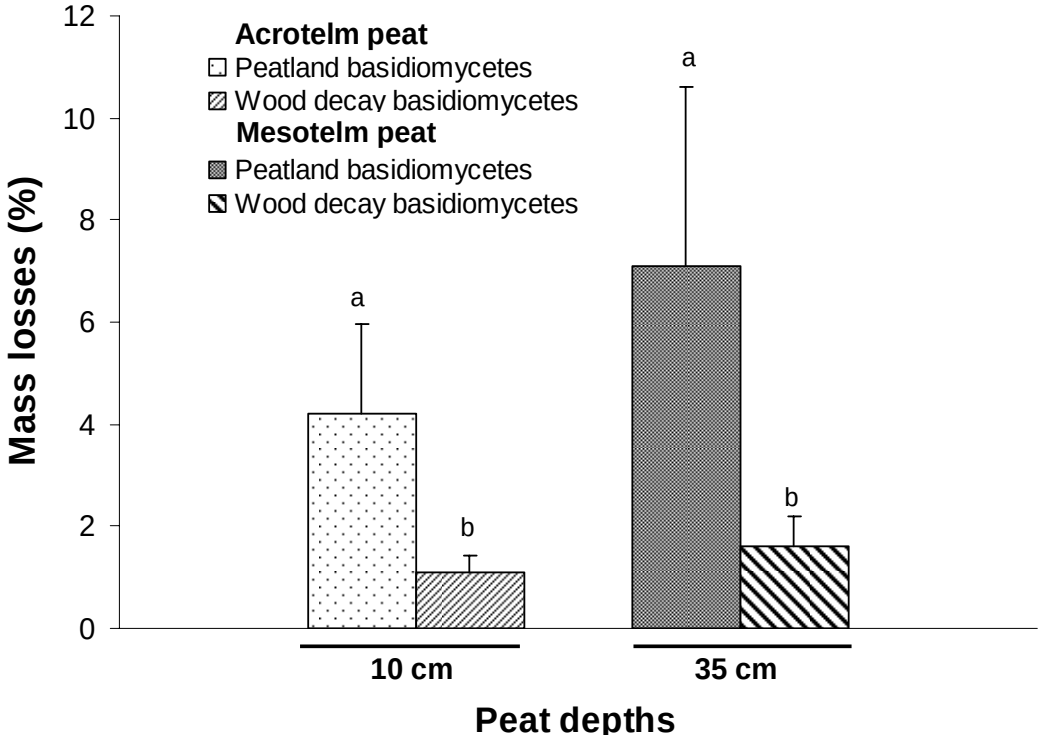

Figure 3. Mean mass losses (% ± 95 % Confidence Intervals) of acrotelm and mesotelm *Sphagnum* peat incurred *in vitro* by all peatland and wood decay basidiomycetes after four months decomposition. Letters indicate significant differences ($P < 0.05$) in mass losses at each peat depth.

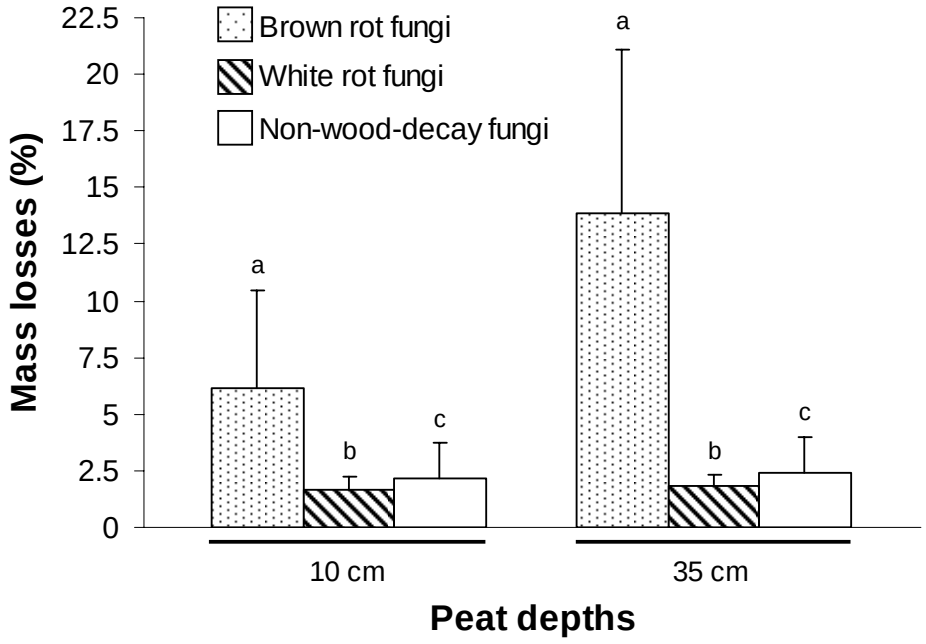

Figure 4. Mean mass losses (% ± 95 % Confidence Intervals) of acrotelm and mesotelm *Sphagnum* peat incurred *in vitro* by brown rot fungi, white rot fungi, and non-wood-decay fungi after four months decomposition. Letters indicate significant differences ($P < 0.05$) in mass losses among life strategies at each depth.

DISCUSSION

Roles of basidiomycetes and ascomycetes in the decomposition of peat

The native peatland basidiomycetes of this study were significantly more effective as peat decomposers than the alien wood decay basidiomycetes (Figure 2). Particularly *Coniophora* sp. was efficient at decomposing the *Sphagnum* peat, irrespective of depth (9.6 % and 23.4 % in the acrotelm and mesotelm, respectively). Overall, data on the ability of basidiomycetes to decompose peat are scarce (e.g. Thormann *et al.* 2002, 2007), despite representing 40.4 % of all known fungi from northern peatlands (Thormann & Rice 2007).

The enzymes responsible for degradation of the most complex polymers in peat, PPOs (e.g. ligninase, peroxidase, laccase and tyrosinase), are affected by peat temperature, oxygen availability and pH (Ruggiero & Radogna 1984, Pind *et al.* 1994, Freeman *et al.* 2001, 2004; Toberman *et al.* 2008). The effects of altered temperature and precipitation dynamics in response to climate warming on peat decomposition rates in northern peatlands remain unknown. It has been suggested that increased aeration of the peat as a result of lowered water levels might enhance the activity of PPOs (Freeman *et al.* 2001), resulting in increased peat decomposition rates. Conversely, increased aeration due to lower water levels might also decrease moisture availability and decrease the activity of PPOs, resulting in decreased peat decomposition rates, as seen in a shallow upland heathland by Toberman *et al.* (2008). Irrespective of hypothesised increased or decreased activities of PPOs in peatlands in response to altered climatic conditions, previous enzyme-based studies have generally failed to address the organisms responsible for the synthesis of these enzymes, e.g. their prevalence as part of the microbial community or their diversity. It is known that PPOs are produced by basidiomycetes, ascomycetes (Rice *et al.* 2006), bacteria (Fenner *et al.* 2005) and actinomycetes (Endo *et al.* 2003), with previous studies suggesting that fungi are the principal peat decomposers in the acrotelm (Thormann 2006a, 2006b) compared to bacteria and actinomycetes.

Within the fungi, the ability to synthesise PPOs is restricted to selected groups of basidiomycetes and ascomycetes. For example, mycorrhizal fungi are abundant in peatlands and aid plants in the acquisition of nutrients and water (Cooke & Lefor 1998, Thormann *et al.* 1999). They are also involved in the decomposition of organic matter. This is particularly true for ericoid mycorrhizal fungi (Thormann 2006b), which are common in most peatlands (Thormann *et al.* 1999) and have the ability to decompose peat but may also cause the formation of high molecular weight organic acid polymers *via* a series of complex biogeochemical pathways during the process of decomposition, thereby contributing to the accumulation of recalcitrant C polymers in peatlands (Bending & Read 1997). Similarly, ectomycorrhizal fungi also occur frequently in peatlands (Thormann *et al.* 1999); however, they appear to have limited saprobic capabilities. Other mycorrhizal fungi, e.g. vesicular-arbuscular, arbutoid and orchid mycorrhizal fungi, are uncommon in bryophyte-dominated peatlands (Thormann *et al.* 1999) and are likely to play less significant roles as organic matter decomposers.

Many ascomycetes, particularly members of the Xylariaceae, can also synthesise PPOs. Members of the Xylariaceae have a propensity to colonise lignin-rich substrata. Only one record exists, however, of a member of this family colonising peat or peat-forming plants (*Xylaria hippotrichoides* (Sowerby) Sacc. on *Sphagnum* in central Europe; Thormann & Rice 2007). Other common peatland ascomycetes can also synthesise PPOs, including species of *Acremonium*, *Aspergillus*, and *Cladosporium* (Thormann *et al.* 1999), although *in vitro* mass losses of *Sphagnum* plants were generally below 5 % after two months (Thormann *et al.* 2002), highlighting the limitations of these fungal taxa as peat decomposers.

Acrotelm *versus* mesotelm peat decomposition

Previous work has shown that *Sphagnum* peat consists of a complex mixture of polysaccharides and phenolic polymers (Bland *et al.* 1968, Williams *et al.* 1998). Comparative analyses revealed the preponderance of polysaccharides relative to phenolic polymers in surface peats, but the relative proportions approach each other with increasing depth (Williams *et al.* 1998). Consequently, peat becomes more recalcitrant with increasing depth. Moreover, PPOs are oxidases; hence, their activity decreases in the absence of oxygen, i.e. in the catotelm (Freeman *et al.* 2001, 2004). Coupled with this, smaller microbial consortia characterised by lower activity are found at increasing peat depths due to low oxygen concentrations or complete anoxia, i.e. the microbial community consists of anaerobic bacteria alone, and no fungi. This may explain the low decomposition rates of catotelm peat. Lowered water levels in conjunction with increased temperatures in some peatlands due to a warming climate (e.g. Laiho 2006, Trettin *et al.* 2006) may facilitate the expansion of aerobic decomposer communities into deeper peat deposits,

thereby potentially resulting in increased rates of decomposition of these peat deposits. This postulate has been supported (e.g. Freeman *et al.* 2001, 2004; Fenner *et al.* 2005) and refuted (e.g. Laiho 2006, Toberman *et al.* 2008) in the past and points to complex interactions between the environment and microbial communities (Sinsabough 2010).

I could not find any work that has documented the ability of fungi to decompose mesotelm or catotelm peat, with previous work focusing only on fresh peatland plant material or acrotelm peat (e.g. Thormann *et al.* 2002, 2007, Rice *et al.* 2006). My data show that neither acrotelm nor mesotelm *Sphagnum*-derived peat can be decomposed effectively by most fungi (mean mass losses of 2.7 % and 4.3 %, respectively, after four months decomposition; Figure 3), including the near-ubiquitous wood decomposing basidiomycetes known to decompose some of nature's most complex polymers. Overall, peatland basidiomycetes caused significantly greater mass losses of acrotelm and mesotelm peat than wood decay basidiomycetes (mean mass losses of 5.7 % and 1.4 %, respectively, after four months decomposition; Figure 3). Based on my data, mesotelm and catotelm peat deposits may be more stable than previously thought due to their recalcitrant nature and the restricted microbial consortia within and external to peatlands able to effectively decompose them.

Fungal life strategies and peat decomposition

In general, the white rot fungi (*Pholiota* sp., *Trametes* sp., *C. unicolor*, *F. fomentarius*, *P. polygonia*, *P. punctatus*) were poorer peat decomposers than the brown rot fungi (*Coniophora* sp., *F. pinicola*). On average after four months decomposition, mass losses by the white rot fungi averaged 1.6 % in the acrotelm and 1.8 % in the mesotelm, and mass losses by the brown rot fungi averaged 6.2 % in the acrotelm and 13.9 % in the mesotelm. Mass losses of the non-wood-decay fungi (*Mycena* sp., *Psathyrella* sp.) were intermediate to those of the brown rot and white rot fungi (mean of 2.2 % in the acrotelm and 2.4 % in the mesotelm; Figure 4).

The disparity in mass losses may be the result of the propensity of the white rot and brown rot fungi to decompose preferentially lignin and cellulosic polymers, respectively. White rot is caused primarily by basidiomycetes (Reid 1995) and some ascomycetes in the Xylariaceae (Osono & Takeda 2002), it is characterised by the removal of all cell-wall components including lignin (Blanchette 1995), a related suite of fungi is responsible for decomposing lignin in the litter (Osono & Takeda 2002, Steffen *et al.* 2002), and some white-rot fungi degrade all plant structural components simultaneously while others degrade a greater proportion of lignin (Reid 1995). In comparison, brown rot is also caused primarily by basidiomycetes, cellulose and other polysaccharides are selectively removed, and the degradation of lignin is limited (Blanchette 1995).

Thormann *et al.* (2002) examined the ability of a suite of fungi to decompose *S. fuscum* plants *in vitro*. They showed that the ascomycete *Sordaria fimicola* (Roberge *ex* Desmaz.) Ces. & De Not. caused the greatest (5.1 %) and *Mucor hiemalis* Wehmer, a zygomycete, the smallest (0.1 %) mass losses of *S. fuscum* after two months decomposition. In comparison, their only basidiomycete, an isolate similar to *Bjerkandera adusta* (Willd.:Fr.) P. Karst., caused a mass loss of 1.7 % over the same decomposition period. Their results are similar to mine and point to a generally limited ability of fungi to decompose *S. fuscum* plant tissues. Rice *et al.* (2006) determined that *Sphagnum* decomposition by selected ascomycetous and basidiomycetous fungi resembles white rot of wood (mean mass loss of 15 % after 4 months decomposition *in vitro*). They suggested that white rot basidiomycetes are proficient decomposers of *Sphagnum*-derived peat, which is contrary to my data. This discrepancy may be the result of Rice *et al.* (2006) using healthy-looking, fresh *Sphagnum* plants as opposed to peat from 10 cm and 35 cm depths, as in my study. Peat is substantially more recalcitrant than fresh plant litter and consequently would lose substantially less mass over the same decomposition period.

The skewed ratio of polysaccharides to the phenolic fraction of acrotelm peat (about 72 % to 24 %, respectively; Williams *et al.* 1998) explains the greater mass losses of the *Sphagnum* peat caused by the brown rot fungi, which preferentially degrade polysaccharide-rich polymers relative to white rot fungi. I cannot explain why mass losses by the brown rot fungi doubled with increasing depth, while those of the white rot fungi remained similar (6.2–13.9 % *vs*. 1.6–1.8 % for brown rot and white rot fungi, respectively), considering that phenolic polymers, which favour white rot fungi, become more prevalent relative to polysaccharides such as cellulose with increasing depth (Williams *et al.* 1998).

Lastly, since I did not determine the fungal biomass in the decomposed peat samples, the mass losses reported here are likely to be under-estimates of actual mass losses. Based on a mean ergosterol (fungus-specific cell wall component) concentration of 5.1 mg g^{-1} DW in soil fungi (Djajakirana *et al.* 1996) and a range in ergosterol concentration of

about 150–270 µg g^{-1} DW in peat (Potila & Sarjala 2004), the fungal biomass in peat ranges from 2.9–5.3 %, i.e. my mass losses may be under-estimating actual masses by that quantity. Nonetheless, these under-estimates are likely to be offset by over-estimates of mass losses due to my *in vitro* decomposition temperatures being higher than *in situ* decomposition temperatures and the fact that autoclaving may have altered the structural integrity of the peat samples sufficiently to enhance rates of decomposition.

CONCLUSIONS

While *in vitro* studies cannot duplicate the complexity of natural environments, they can indicate the potential roles of individual fungal species or consortia in complex biogeochemical processes. It is recommended that *in vitro* assessments of the potential roles of individual species should be coupled with surveys of taxonomic diversity and *in situ* quantification of ecological processes, such as decomposition dynamics and nutrient cycling, to better elucidate the roles of fungi in ecological processes and to better model their responses to environmental disturbances. In addition, the previously described "enzymic latch", based on the oxygen constraint of the single phenol oxidase enzyme group, is in fact a "microbiological community limitation", as fungal species capable of effectively degrading acrotelm and mesotelm *Sphagnum*-derived peat are uncommon in peatlands and even rarer in surrounding terrestrial ecosystems. As such, the abundance of fungal taxa capable of producing the enzymes needed to degrade complex polyphenolic polymers should be added to the list of factors limiting peat decomposition.

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