Microcosm tests of the effects of temperature and microbial species number on the decomposition of *Carex aquatilis* and *Sphagnum fuscum* litter from southern boreal peatlands

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Abstract: Increased decomposition rates in boreal peatlands with global warming might increase the release of atmospheric greenhouse gases, thereby producing a positive feedback to global warming. How temperature influences microbial decomposers is unclear. We measured in vitro rates of decomposition of senesced sedge leaves and rhizomes (*Carex aquatilis*), from a fen, and peat moss (*Sphagnum fuscum*), from a bog, at 14 and 20 °C by the three most frequently isolated fungi and bacteria from these materials. Decomposition rates of the bog litter decreased (5- to 17-fold) with elevated temperatures, and decomposition of the sedge litters was either enhanced (2- to 30-fold) or remained unaffected by elevated temperatures. The increased temperature regime always favoured fungal over bacterial decomposition rates (2- to 3-fold). Different physiological characteristics of these microbes suggest that fungi using polyphenolic polymers as a carbon source cause greater mass losses of these litters. Litter quality exerted a stronger influence on decomposition at elevated temperatures, as litter rich in nutrients decomposed more quickly than litter poorer in nutrients at higher temperatures (8.0%–25.7% for the sedge litters vs. 0.2% for the bryophyte litter). We conclude that not all peatlands may provide a positive feedback to global warming. Cautious extrapolation of our data to the ecosystem level suggests that decomposition rates in fens may increase and those in bogs may decrease under a global warming scenario.

Key words: fungi, bacteria, decomposition, temperature, *Sphagnum fuscum*, *Carex aquatilis*, peatlands, climate change, microcosms.

Résumé : Des taux accélérés de décomposition dans les tourbières boréales, associés au réchauffement planétaire, pourraient augmenter la libération de gaz atmosphériques à effet de serre, entraînant ainsi une boucle d'amplification positive du réchauffement planétaire. On en sait peu sur la façon dont la température influence les décomposeurs microbiens. Nous avons mesuré les taux de décomposition de feuilles sénescentes et de rhizomes de laiche (*Carex aquatilis*) d'une tourbière basse et de mousse de tourbe (*Sphagnum fuscum*) d'une tourbière haute, à 14 et 20 °C, par les trois champignons et bactéries les plus fréquemment isolées de ces matières. Les taux de décomposition de la litière de la tourbière haute ont diminué (5 à 17 fois) avec des températures élevées, alors que la décomposition de la litière de la tourbière basse ont soit augmenté (2 à 30 fois) ou n'a pas été affectée par des températures élevées. Les différentes caractéristiques physiologiques de ces microbes indiquent que les champignons utilisant des polymères polyphénoliques en tant que source de carbone entraînent des pertes plus importantes de masse dans ces litières. La qualité de la litière a eu un impact plus important sur la décomposition à des températures élevées, puisque des litières plus riches en nutriments se sont décomposées plus rapidement que des litières pauvres en nutriments, à des températures élevées (8,0–25,7 % pour les litières de laiche *vs.* 0,2 % pour la litière de bryophyte). Nous en concluons que ce ne sont pas toutes les tourbières qui pourraient contribuer au réchauffement planétaire par une amplification positive. Une extrapolation prudente de nos données au niveau de l'écosystème indique que les taux de décomposition des tourbières basses pourraient augmenter alors que ceux des tourbières hautes pourraient diminuer dans un scénario de réchauffement planétaire.

Mots clés : champignons, bactéries, décomposition, température, *Sphagnum fuscum*, *Carex aquatilis*, tourbières, changements climatiques, microcosmes.

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Introduction

Global warming could increase atmospheric temperatures by 2050 (IPCC 2001). Climate models (including those involving greenhouse gases and sulphur dioxide) predict substantial warming (4–8 °C) in northern continental regions during spring and winter months (IPCC 2001). These regions are a mosaic of boreal forests and peatlands. Peatlands cover approximately 16% of Alberta's land surface (4.9% bogs and 11.4% fens; Vitt et al. 1996). Bogs are dominated by species of *Sphagnum* (Sphagnopsida), black spruce, and members of the Ericaceae, and fens are dominated by species of *Carex*, *Salix*, *Betula*, *Larix*, and "brown mosses" (Bryopsida, largely members of the Amblystegiaceae, such as species of *Drepanocladus*, *Campylium*, and *Scorpidium*). Species within these genera contribute significantly to the total net primary plant production (Szumigalski and Bayley 1996*a*; Thormann and Bayley 1997*a*) and the accumulation of peat in southern boreal peatlands (Kuhry and Vitt 1996; Thormann et al. 1999).

Some wetlands accumulate peat, a heterogeneous assemblage of partially decomposed plant material consisting of approximately 50% carbon (C) (Thormann et al. 1999), resulting from an imbalance between plant production and decomposition (Clymo 1965; Vitt 1990). Gorham (1991) emphasized the importance of peatlands to the global C cycle, estimating that northern peatlands store between 180 and 277 Gt C (1 Gt = 1×10^9 metric ton), which represents approximately 10%–16% of the total global terrestrial detrital C. Rates of decomposition, or C mineralization, are low in peatlands because of unfavourable hydrologic regimes, low oxygen availability, high acidity, low nutrient status, low temperature, and low litter quality (Bartsch and Moore 1985; Gorham 1991; Szumigalski and Bayley 1996*b*; Thormann and Bayley 1997*b*; Thormann et al. 2001*a*).

Fungi are the principal decomposers in wetlands and assume a more dominant role than bacteria in the upper, oxygenated soil horizon (acrotelm) (Latter et al. 1967; Williams and Crawford 1983), particularly during the initial stages of decomposition (Newell et al. 1995; Kuehn et al. 2000). However, some data are contradictory (Gilbert et al. 1998). Although fungi may be the dominant decomposers in the lacrotelm, organic matter decomposition is carried out by a consortium of different microorganisms, including fungi and bacteria (Erikson et al. 1990). The combined enzymatic activities of initial colonizers (synergism) alter the quality of the organic matter sufficiently to permit subsequent colonization by other species of fungi and bacteria. Thus, organic matter decay involves a succession of microorganisms (Thormann et al. 2003) to mineralize completely these materials.

The influence of temperature on species composition of microbial communities is not well known (Kandeler et al. 1998; Bardgett et al. 1999), although many fungi and bacteria grow across a wide temperature range (Subba Rao 1999). Also, the impact of increasing atmospheric temperatures on the abundance and type of enzyme synthesis by bacteria and fungi is unclear (Widden et al. 1989; Moorhead and Linkins 1997). While many fungi have the ability to degrade simple polymers, their ability to degrade complex polyphenolics occurs sporadically in different taxonomic groups (Domsch et al. 1980; Thormann et al. 2002), yet these compounds constitute 30%–50% of peat in some peatlands (Turetsky et al. 2000) and become more prevalent with increasing depth because they are poorly mineralized.

We initiated an in vitro study to assess the relative abilities of bacteria and fungi to decompose litter of the dominant plant species of two southern boreal peatlands at two temperature regimes. These plant litters were the moss *Sphagnum fuscum* (Schimp.) Klinggr., dominant in bogs, and the leaves and rhizomes of the sedge *Carex aquatilis* Wahlenb., native to many fens. We expected *(i)* faster mass loss rates by fungi than bacteria due to their hyphal growth habit, whereas single-celled bacteria would colonize plant litters more slowly; (*ii*) increase in mass loss rates with increasing temperatures, because the rates of growth and enzyme synthesis by fungi and bacteria are generally optimal between 20 and 35 °C; (*iii*) enhanced mass loss rates due to the synergistic relations created by the coexistence of bacteria and fungi; and (*iv*) enhanced mass loss rates resulting from the higher initial total nitrogen (TN) and total phosphorus (TP) concentrations in *Carex* than in *Sphagnum* litter.

Methods

Study area and site descriptions

The fen (54°28′N, 113°18′W) and bog (54°28′N, 113°16′W) lie within the Subhumid Low Boreal ecoclimatic region of Canada (Ecoregions Working Group 1989). The fen is dominated by *C. aquatilis*, *Carex lasiocarpa* Ehrh., and *Salix planifolia* Pursh. The bryophyte stratum is discontinuous and consists primarily of *Brachythecium mildeanum* (Schimp.) Schimp. *ex* Milde. This fen has a mean annual surface water pH of 6.9 and approximately 1 m of sedge-dominated peat. The bog is dominated by *S. fuscum*, *Picea mariana* (Mill.) BSP., and members of the Ericaceae. It has a mean annual surface water pH of 3.8 and approximately 4.5 m of *Sphagnum*-dominated peat. Vegetation composition, surface water chemistry, and physical parameters of both sites are provided in more detail elsewhere (Thormann et al. 1999, 2001*a*).

In situ decomposition study

This separate study was designed to measure in situ mass losses of the three litters over a 2-year period and to describe them using regression equations (Thormann et al. 2001*a*), which would then allow us to estimate mass loss of the three litters over the 12-week decomposition period. Briefly, we collected approximately 500 g fresh weight each of senesced attached *C. aquatilis* leaves (terminal 10 cm, yellow to pale-brown) and rhizomes (10 cm segments, soft and dark-brown as opposed to firm and light-brown rhizomes of healthy appearance) from the fen and whole *S. fuscum* plants (apical 3 cm) from the bog in early September 1997. All plant litters were collected randomly in an approximately 200 m^2 area in each site.

The plant material was oven-dried to constant mass at 60 °C, and 1.8–2.5 g randomly selected material from each litter type was placed in individual nylon mesh bags (3 cm × 6 cm, 1-mm mesh gauge). The filled bags were weighed to the nearest 0.001 g (X_i, the initial dry litter mass), sewn shut, and placed either horizontally 3–10 cm beneath (*S. fuscum*,

C. aquatilis rhizomes) or on top (*C. aquatilis* leaves) of the moss/peat surface to mimic "natural" decomposition conditions (n = 4 bags per litter type per sampling period). Decomposition bags with the individual litters were placed in the site of the origin of the litter.

Twenty-four decomposition bags per litter type, each tied to a wooden stake to avoid loss, were deployed in mid-September 1997. Four decomposition bags from each litter type were retrieved from each site after 20 and 50 days (1997), and 8, 12 (1998), 20, and 24 months (1999). Bags were cleaned immediately by removing coarse, intruding roots and other debris, such as leaves of other vascular and nonvascular plants that had grown into or through the leaf litter decomposition bags. Finer debris (soil, remaining "alien" plant parts, fungal mycelium, etc.) was removed carefully in the laboratory with forceps prior to drying to constant mass at 60 °C (X_f, the final dry litter mass after each decomposition period). Each bag was weighed again to the nearest 0.001 g, and the percent mass loss (ML) over the 2-year incubation period was determined using the following equation:

$$ML = [(X_i - X_f)/X_i] \times 100$$

Isolation of fungi and bacteria

Twenty randomly selected segments of each litter type (collected in early September 1997) were cut into smaller segments (approximately 5 mm × 5 mm in size). These were surface-sterilized for 5 min in hydrogen peroxide (10%) and washed 3× with sterile, distilled water (d-H_2O) before the isolation of fungi and bacteria.

Isolation of fungi

Five randomly selected, small segments of each litter type were placed on each of three Petri plates of potato dextrose agar (PDA) consisting of 39.0 g Difco potato dextrose agar (Difco Laboratories, Detroit, Mich.) and 1.0 L d-H_2O, final pH 5.6; PDA with rose bengal (0.03%); PDA with benomyl (0.0002%); and Mycobiotic Agar® (contains cycloheximide, 35.6 g Difco mycobiotic agar, 1.0 L d-H_2O) for the isolation of a broad spectrum of filamentous microfungi. All media were amended with oxytetracycline (0.01%) to suppress bacterial growth. Petri plates were incubated at room temperature (20 °C) in the dark, and fungi were subcultured onto malt extract agar (15.0 g Difco malt extract, 20.0 g Difco agar, 1.0 L d-H_2O, final pH 5.6) as soon as they grew from the plant material. Plates were examined regularly for 2 years to avoid isolating only heavily sporulating and fast-growing fungal taxa. For identification purposes, slide cultures 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) were prepared (Sigler 1993), stained with acid fuchsin, and mounted in polyvinyl alcohol.

Isolation of bacteria

Approximately 3 g fresh weight of each litter type was placed into 100 mL sterile phosphate buffer (P-buffer, pH = 7.25, 10 mmol L^{-1}) and homogenized in a Sorvall Omni-Mixer (Sorvall, Norwalk, Conn.) at speed setting 5 for 5 min. Serial dilutions of each homogenate in P-buffer were performed to obtain a final range of concentrations of homogenized plant material from 10^{-3} to 10^{-8} in 10 mL sterile P-buffer, before transfer of 0.2 mL of each dilution onto plate count agar (PCA: 23.5 g Difco plate count agar, 1.0 L d-H_2O, final pH 7.0). P-buffer was chosen as a suspension medium for the bacteria because it maintains live cells in an osmotic equilibrium, thereby keeping them alive for further processing. The transferred solution was spread over the agar with a bent, sterile glass rod. Triplicate plates of each litter type at each dilution were incubated at room temperature in the dark and monitored daily for the appearance of bacterial colonies. The three most frequently isolated bacteria in each litter type, as determined by morphology (shape, size, and colony colour) and physiology (ability to grow on various bacteriological growth media), were subcultured onto PCA and identified to genus (Sneath et al. 1986).

Isolation frequencies for all fungal and bacterial taxa were determined by expressing the number of isolates of each taxon as a percentage of the total number of isolates obtained from the respective plant litters. From each litter type, the three most frequently isolated fungi and bacteria were selected for the subsequent in vitro decomposition study under the assumption that they were present as decomposers during the early stages of decomposition. While our primary isolation media were designed to maximize the isolation and maintenance of a broad spectrum of fungi and bacteria, they were unavoidably biased against microbes with alternative nutritive and environmental growth conditions. A complete list of fungi and their isolation frequencies can be found in Thormann et al. (2004).

Experimental design

Preparation of microcosms

Fungi and bacteria were grown on peptone broth agar (PBA: 20.0 g Difco agar, 1.0 g Difco bacto-peptone broth, 1.0 L d-H_2O, final pH = 7.0) for 10 days at 14 °C and 20 °C in the dark before the experiment to determine the suitability of this medium. PBA was chosen because it provided some N to the growing bacteria and fungi (0.154 g L^{-1} TN; BD Diagnostic Systems, Sparks, Md.) other than the N from the plant litters. These conditions are similar to those in situ, where microbial populations have access to sources of nutrients other than the litter they mineralize, e.g., from root exudates or surficial and pore water.

Triplicate plates were inoculated with fungi indigenous to each litter type singly and in every possible combination with each other by transferring two mycelial plugs (1.0 mm × 1.0 mm × 0.5 mm) onto the respective treatment plates. For each bacterium, a suspension was prepared by transferring the bacterial colonies of 1-week-old cultures into 30 mL sterile P-buffer in sterile Pyrex culture tubes using a bent, sterilized glass rod. These suspensions were mixed with a vortex for 10 s at setting 5 before the inoculations (0.2 mL per treatment) of each litter type. As with the fungi, three bacteria indigenous to each litter type were inoculated onto the appropriate PBA plates by themselves and in every possible combination with each other. All fungal treatments also received 0.2 mL P-buffer. Triplicate Petri plates were inoculated simultaneously with all three fungi and all three bacteria (for a total of six microbial taxa per plate) indigenous to each litter type to investigate possible synergis-

tic relations during decomposition. Uninoculated plates served as controls to determine mass losses due to leaching.

Decomposition of C. aquatilis leaves and rhizomes and S. fuscum plants in vitro

The top 3 cm of four dried *S. fuscum* plants were placed in polyester mesh pouches (2.5 cm × 3.0 cm, 65-μm gauge) to minimize plant material losses during handling. The dried *C. aquatilis* litters were not placed in polyester pouches, as losses during handling were not expected on the basis of an earlier experiment. The pouches with the bryophyte litter and the sedge litters were weighed to the nearest 0.001 g (X_i) and autoclaved at 121 °C (liquid cycle) for 15 min prior to placement into the Petri plates, and then inoculated with the appropriate fungi and bacteria (see above). Petri plates were incubated in the dark, one half at 14 °C and the other half at 20 °C (room temperature). Autoclaving may have altered the carbon chemistry of the litters; however, it did not cause any discernible external ultrastructural changes in plant tissues (Tsuneda et al. 2001).

After 2, 4, 8, and 12 weeks, the litters were removed from three Petri plates of each litter type, and surficial fungal mycelium was carefully removed with forceps from the pouches or the surface of the leaves and rhizomes before drying to constant mass at 60 °C (X_f). Each bag was weighed to the nearest 0.001 g, and mass loss was determined as described. Fungi and bacteria growing in the litters could not be removed, and their biomass was not determined in this study. Therefore, our mass losses represent conservative estimates (underestimates). The 12-week decomposition period was chosen because most mass losses occur during the first 100 days of decomposition in situ (Thormann and Bayley 1997*b*; Thormann et al. 2001*a*).

Statistical analyses

Kruskal–Wallis tests (nonparametric, one-way ANOVA) were used to analyze differences in (*i*) individual litter quality variables (dependent variables: TC, TN, TC:TN, and TP; $n = 3$ per litter quality variable per litter type) among the three litter types (independent variables) and (*ii*) mass losses (dependent variables) among fungi, bacteria, and fungi in combination with bacteria (independent variables) for each temperature regime. For the second set of analyses, we combined all data on fungal mass loss and all data on bacterial mass loss ($n = 3$ for six treatments each for fungi and bacteria; hence, $n = 18$ for each treatment of fungi and bacteria). These data were then compared against the mass losses from the fungi plus bacteria treatment ($n = 3$). Kruskal–Wallis tests were followed by Tukey-type post hoc tests if there was an indication of significance in the ANOVA. Student's *t* tests were used to analyze differences in mass losses caused by fungi, bacteria, and fungi in combination with bacteria between the two temperature treatments for each litter type. Mass losses due to leaching were subtracted from all data prior to statistical analyses.

Results and discussion

Fungal vs. bacterial decomposition dynamics

The fungal dominance found at the elevated temperature may be related to morphological and physiological adaptations. Most fungi form hyphae and are able to penetrate and colonize organic materials before bacteria, which are mostly single-celled, colonial organisms. Furthermore, most bacteria require moisture to disperse, especially in soil, where they live predominantly in a thin water film covering soil particles (Erikson et al. 1990). Fungi tolerate lower water potentials, because their hyphal habit facilitates the transport of water and nutrients to the growing hyphal tip (Alexopoulos et al. 1996). Furthermore, fungi can decompose a wider range of C sources, both simple and complex (greater enzyme diversity). However, since only three of the eight fungi grew significantly more slowly at 14 than at 20 °C after 10 days (*Ph. dimorphospora*: 7 vs. 20 mm; *P. chrysogenum*: 21 vs. 36 mm; and *P. thomii*: 15 vs. 26 mm; all $p < 0.01$), we cannot explain why bacterial decomposition rates generally exceeded those of fungi at the lower temperature after 12 weeks (Table 1) on the basis of growth rates alone. We did not assess the enzymatic abilities of these fungi at 14 °C; however, it is possible that fungal enzyme synthesis was restricted at the lower temperature treatment (Widden et al. 1989; Moorhead and Linkins 1997). Since fungal decomposition did not always exceed bacterial decomposition, we rejected hypothesis 1.

Effects of temperature on fungal and bacterial decomposition dynamics

Leaching caused mean mass losses of 1.9% for *S. fuscum*, 9.1% for *C. aquatilis* rhizomes, and 15.0% for *C. aquatilis* leaves. Bacteria and fungi induced similar mass losses after 12 weeks (0.2% to 12.3%, Table 1). As temperature increased, bacteria and fungi generally caused significantly greater mass losses of the *C. aquatilis* leaf (bacteria $p < 0.01$, fungi $p < 0.01$) and rhizome litters (bacteria $p > 0.05$, fungi $p < 0.01$; Table 1). However, mass loss by bacteria and fungi of *S. fuscum* was significantly greater at the lower temperature ($p < 0.01$, Table 1). Fungi induced mass losses up to 3× greater than those caused by bacteria for each of the plant litters at 20 °C ($p < 0.05$, Table 1). At 14 °C, mass loss caused by bacteria significantly exceeded that by fungi only in the *S. fuscum* litter, and fungal mass loss at 14 °C significantly exceeded bacterial mass loss in the *C. aquatilis* leaf litter (all $p < 0.05$, Table 1).

Increasing temperature does not necessarily lead to greater decomposition rates, since the "optimal" decomposition temperatures differed for the *S. fuscum* and *C. aquatilis* litters in this study (Figs. 1–3, Table 1). However, *Ph. dimorphospora* and *M. constrictum* grew more slowly at 14 than at 20 °C after 10 days (*Ph. dimorphospora*: see previous section; *M. constrictum*: 10 vs. 13 mm, nonsignificant) and caused correspondingly significantly lower mass losses at 14 than at 20 °C after 12 weeks (*Ph. dimorphospora*: 7.7% vs. 28.4%; *M. constrictum*: 2.2% vs. 10.6%; all $p < 0.01$). Here, increasing temperatures resulted in increased decomposition rates; however, this positive relation was absent for other taxa that grew more slowly at lower temperatures (e.g., *P. thomii* and *P. chrysogenum*, data not shown).

Temperature may supercede litter quality in a hierarchy of controls on decomposition dynamics and enhance mass losses caused by fungi of some litters and not others. This is due to the differential effect of temperature on (*i*) fungal species composition and (*ii*) fungal enzyme production. For

Table 1. Mean mass losses of three litter types from two peatlands in southern boreal Alberta, Canada, after 12 weeks decomposition in vitro by bacterial, fungal, and bacterial plus fungal populations and in situ.

		Mass loss, % (±SE)			
Litter type	Temperature	Three most common bacteria	Three most common fungi	Three most common bacteria + fungi	In situ
Carex aquatilis rhizomes	14 °C	–7.3 (0.76) *a1*	–6.2 (1.21) *a1*	–24.9 (0.53) *b1*	—
	20 °C	–6.5 (0.69) *a1*	–11.4 (1.94) *b2*	–25.7 (3.01) *c1*	—
	In situ (8 °C)	—	—	—	–36.2
C. aquatilis leaves	14 °C	3.2 (1.21) *a1*	0.4 (1.86) *b1*	–1.7 (0.12) *c1*	—
	20 °C	–3.9 (0.98) *a2*	–12.3 (2.67) *b2*	–8.0 (1.30) *b2*	—
	In situ (8 °C)	—	—	—	–11.2
Sphagnum fuscum plants	14 °C	–3.2 (0.02) *a1*	–2.4 (0.06) *b1*	–3.5 (0.74) *a1*	—
	20 °C	–0.2 (0.03) *a2*	–0.5 (0.04) *b2*	–0.2 (0.01) *a2*	—
	In situ (7 °C)	—	—	—	–17.5

Note: Positive values indicate mass gains of the decomposing litters. Different italic letters indicate significant differences among the treatments for each litter type at each temperature, and different italic numbers indicate significant differences between temperature treatments for each litter type. $n = 18$ each for bacterial and fungal mass losses and $n = 3$ for bacterial plus fungal mass losses. In situ mass losses were estimated from regression equations in Thormann et al. (2001*a*). Mass losses due to leaching were subtracted from all data prior to statistical analyses.

example, the ability of *M. hiemalis* to decompose *S. fuscum* and *C. aquatilis* rhizomes depended on temperature. This zygomycete showed decreased decomposition rates with increasing temperatures in the bryophyte litter (1.8% at 14 °C, 0.0% at 20 °C) and increasing decomposition rates with increasing temperatures in the rhizome litter (5.4% mass gains at 14 °C, 4.2% mass losses at 20 °C) after 12 weeks, irrespective of the initial litter quality.

Although many fungi and bacteria can tolerate a broad temperature range (Subba Rao 1999), there are few data on the effects of increasing temperatures on microbial communities. Temperature can influence growth rates, competitive abilities, colonization patterns, enzyme syntheses, and interactions of fungi (Bissett and Parkinson 1979; Widden and Hsu 1987; Trumbore et al. 1996; Kandeler et al. 1998). For example, Kandeler et al. (1998) suggested that rising temperatures can influence decomposition kinetics of low-molecular-weight compounds, thereby influencing the ability of fungi to use such compounds as amino acids, starch, and simple sugars. Microbes have temperature optima for enzyme synthesis (Widden et al. 1989; Moorhead and Linkins 1997), and species-specific enzyme responses to rising temperatures could account for the mass loss responses observed (Widden and Hsu 1987). Since increases in temperature did not always result in increased decomposition rates by microbes, we rejected hypothesis 2.

Effects of litter quality on fungal and bacterial decomposition dynamics

TC tissue concentrations were significantly lower in *C. aquatilis* rhizomes ($p < 0.05$) than in *C. aquatilis* leaves and *S. fuscum* plants (Table 2). TN tissue concentrations were significantly higher in *C. aquatilis* leaves ($p < 0.001$) compared with *S. fuscum* and *C. aquatilis* rhizomes. The TC:TN quotient of *S. fuscum* litter was the highest ($p < 0.05$), because of low TN and high TC tissue concentrations. The TC:TN quotient of the *C. aquatilis* leaves was the lowest because of their high TN content ($p < 0.01$).

Thormann et al. (2003) found that litter quality influenced the species of microfungi recovered at various stages of decomposition of these particular tissues; however, there were no clear trends in our data. For example, *S. fuscum* had significantly lower TP tissue concentrations than the *C. aquatilis* litters (Table 2), but its mass losses were greater than those of the *Carex* leaves and less than those of the *Carex* rhizomes at 14 °C after 12 weeks. However, the TP-poor bryophyte litter decomposed more slowly than the sedge litters at 20 °C (Table 1). Moreover, although both species of *Trichoderma* showed the same enzymatic abilities (able to degrade cellulose, gelatin, pectin, and starch: Thormann 2001; Domsch et al. 1980) and growth rates (>80 mm in 10 days) in vitro (Thormann 2001), they caused significantly different mass losses of the bryophyte and sedge rhizome litters after 12 weeks (means of 0.9% and 7.2% at both temperature regimes, respectively).

The same trend in *Trichoderma* was observed for both species of *Penicillium* decomposing *S. fuscum* and *C. aquatilis* leaves (data not shown). *Phialocephala dimorphospora* and *M. constrictum* caused the largest mass losses of any single fungus at 20 °C after 12 weeks (28.4% and 10.6%, respectively; Table 3), possibly because these two fungi were the only ones able to synthesize polyphenol oxidases (PPOs) and use polyphenolics such as tannic acid and lignin (Thormann 2001). These compounds are a major component of most plant litters, often constituting up to 35% of structural polymers.

Other litter quality variables, including lignin and cellulose tissue concentrations, have a great influence on litter decomposition rates. For example, *Carex* leaves have lignin and cellulose concentrations of 25–80 mg g^{-1} and 180–240 mg g^{-1}, respectively (Bartsch and Moore 1985; Aerts and De Caluwe 1997), whereas their rhizomes have lower concentrations of both (lignin 35 mg g^{-1}, cellulose 144 mg g^{-1}; Scheffer and Aerts 2000). In contrast, *S. fuscum* has higher concentrations of "lignin-like" polymers (20–125 mg g^{-1}) and cellulose (250–400 mg g^{-1}; Bartsch and Moore 1985; Scheffer et al. 2001) than fen sedge species. We did not measure polyphenolic polymer (lignin and "lignin-like" compounds) and cellulose concentrations of the three litter types in this study. Fungi and bacteria have differing abilities to decompose these structural constituents, based largely on the degree of complexity of these molecules and the inherent

Fig. 1. Mean (%±SE) in vitro net mass changes of decomposing *Carex aquatilis* rhizomes over a 12-week period caused by its (*a*) three most frequently isolated fungi (*Mucor hiemalis*, *Phialocephala dimorphospora*, *Trichoderma harzianum*), (*b*) three most frequently isolated bacteria (*Arthrobacter* sp. 1, *Lactobacillus* sp., *Leuconostoc* sp.), and (*c*) three most frequently isolated fungi and bacteria combined. SE < 0.02% at symbols without error bars.

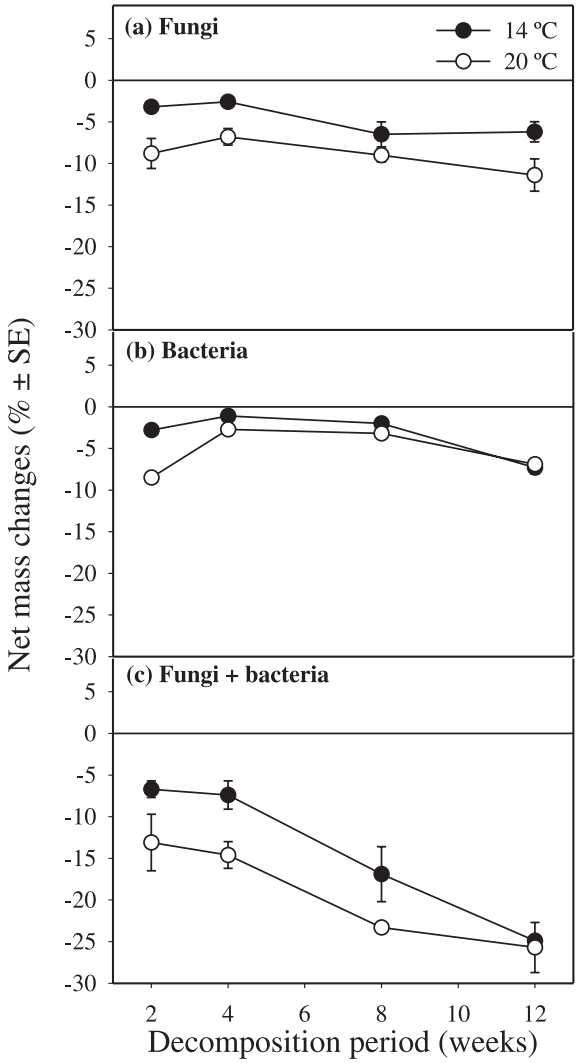
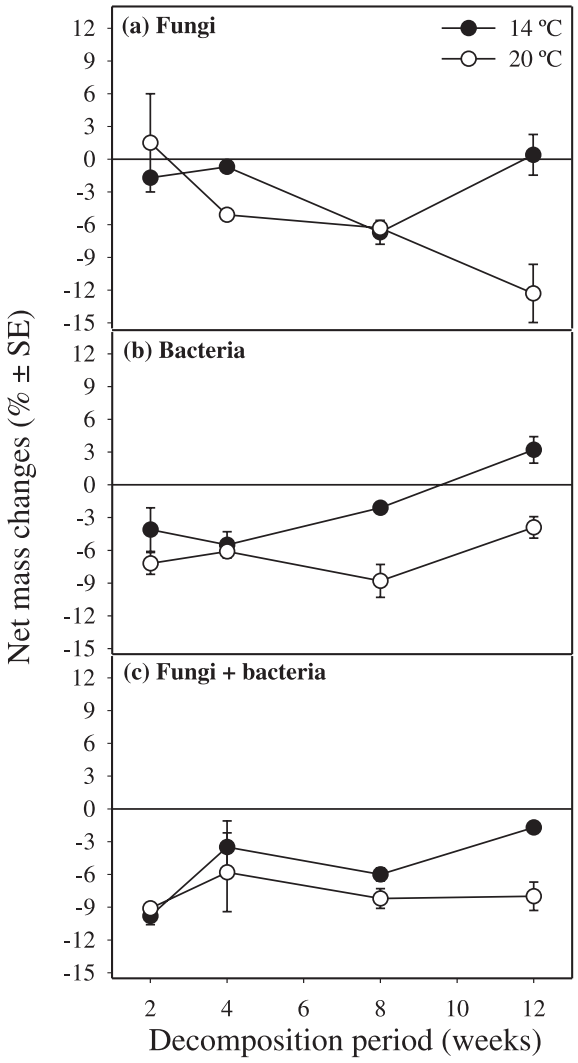


Fig. 2. Mean (%±SE) in vitro net mass changes of decomposing *Carex aquatilis* leaves over a 12-week period caused by its (*a*) three most frequently isolated fungi (basidiomycete sp. 3, *Monocillium constrictum*, *Penicillium chrysogenum*), (*b*) three most frequently isolated bacteria (*Arthrobacter* sp. 2, *Bacillus* sp. 1, *Micrococcus* sp.), and (*c*) three most frequently isolated fungi and bacteria combined. SE < 0.02% at symbols without error bars.

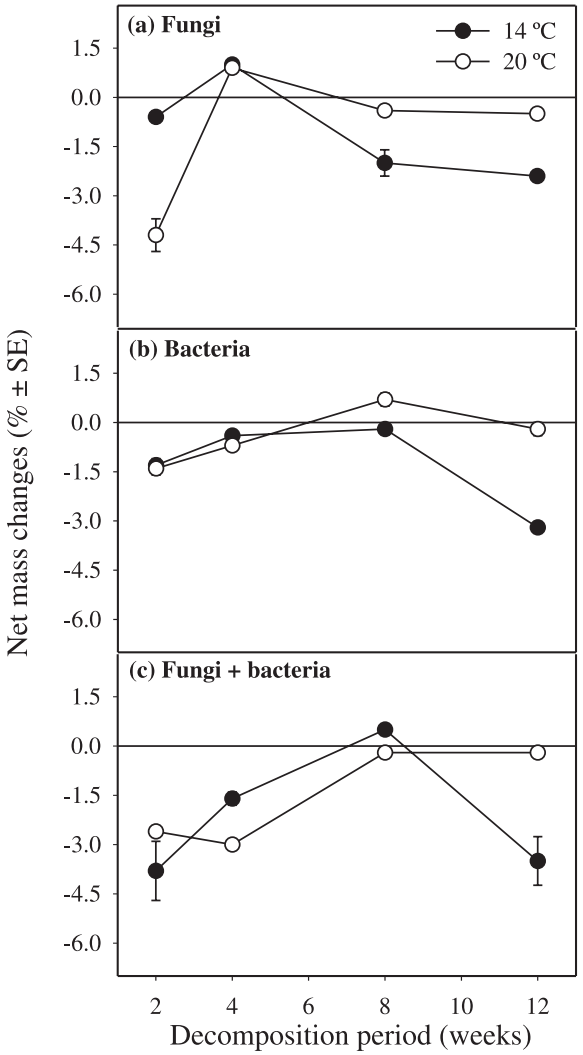


method of the enzymatic degradation of bacteria and fungi (Paul and Clark 1996).

Mass loss after 2 weeks at 20 °C was often significantly higher than that after 4 and 8 weeks and similar to or less than that at the end of the 12-week decomposition period (Figs. 1–3). This may be a result of the preferential use of compounds of low molecular weight during the initial stages of decomposition, causing rapid mass losses, and the subsequent slower degradation of the more recalcitrant structural polymers. However, microbial biomass continued to increase with continued colonization of the litter and translocation of C from the base medium, resulting in net mass gains as high as 3.2% (Figs. 2–3, Table 1). During the latter stages of decomposition, all labile compounds were likely depleted, after which the fungi and bacteria may have begun to degrade structural polymers again, resulting in net mass losses (Figs. 1–3). Alternatively, fungal biomass may have decreased because of autolysis of hyphae and the translocation of nutrients to the growing hyphal tips, thereby leading to net mass losses of the litters. However, this remains speculative, because we did not measure fungal or bacterial biomass during the 12-week decomposition process, and we did not examine the decomposed litters ultrastructurally.

Nutrient-rich litter did not necessarily decompose faster than nutrient-poor litter. Consequently, we rejected hypothesis 4. There is likely an interaction between litter quality and temperature during the process of decomposition, depending on the composition of the microbial community (a three-way interaction). Such a relation was previously proposed by Widden and Hsu (1987) using *Trichoderma* species for de-

Fig. 3. Mean (%±SE) in vitro net mass changes of decomposing *Sphagnum fuscum* plants over a 12-week period caused by its (*a*) three most frequently isolated fungi (*Mucor hiemalis*, *Penicillium thomii*, *Trichoderma viride*), (*b*) three most frequently isolated bacteria (*Arthrobacter* sp. 3, *Bacillus* sp. 2, *Brevibacterium* sp.), and (*c*) three most frequently isolated fungi and bacteria combined. SE < 0.02% at symbols without error bars.



composition studies. Our data set was too small to investigate such an interaction.

Synergism among microbial communities

When bacteria and fungi were combined, mass loss of both *C. aquatilis* litters at both temperatures generally was significantly greater than when bacteria or fungi decomposed these litters separately. This was not the case for the *S. fuscum* litter (Table 1). The greatest mass loss was that of *C. aquatilis* rhizomes (Fig. 1*c*, Table 1). While we reject hypothesis 3 (using only the three most common bacteria and fungi), microbes in situ had significantly greater decomposition rates than the selected microbes in vitro (over the 12-week decomposition period, Table 1). This is due to a much larger consortium of microbes with a much broader range of enzymatic abilities in situ than in our microcosms in vitro. For example, Thormann et al. (2001*b*) identified and (or) described over 50 microfungi from *S. fuscum*, which together had a greater impact on decomposition dynamics than the much smaller number of selected fungi in this study (17.5% in situ vs. 0.2% to 3.5% in vitro, Table 1).

Mass losses caused by individual fungi and bacteria

The most frequently isolated fungi were mostly anamorphic ascomycetes, i.e., mitosporic taxa with known affinities to ascomycetes, and zygomycetes. Species of *Penicillium* and *Trichoderma* were prevalent in all three litters. The zygomycete *Mucor hiemalis* occurred very frequently in both sedge litters (Table 4). All bacteria were Gram-positive, and most were rod-shaped. Species of *Arthrobacter* and *Bacillus* represented 50% of the most frequently isolated bacteria in these three plant litters. Species of *Arthrobacter* occurred frequently in all three litters under our isolation protocol (Table 1). Fungal decomposition rates differed among the fungi, with maximum mass losses ranging from 2.2% in *S. fuscum* (*T. viride* and *P. thomii*) to 10.6% in *C. aquatilis* leaves (*M. constrictum*) and 28.4% in *C. aquatilis* rhizomes (*Ph. dimorphospora*) after 12 weeks (Table 4). For the *Carex* litters, mass losses caused by fungi always exceeded those caused by bacteria. In contrast, mass losses of the bryophyte litter caused by bacteria and fungi were generally similar (Table 1).

Species of *Trichoderma*, *Mucor*, and *Penicillium* are common soil saprobes and have been isolated previously from peatlands and heath (McLennan and Ducker 1954; Latter et al. 1967; Thormann et al. 2001*b*). Hence, their presence in our peatland plant litters is not surprising. Conversely, species of *Monocillium* and *Phialocephala* are less frequently isolated from peatlands and, to our knowledge, our two taxa represent new records (Thormann et al. 2004). Surprisingly, these two fungi caused the greatest mass losses of any individual fungus used in this study. The taxonomic affinity of the basidiomycete remains unknown.

Distinct differences in the ability of the indigenous bacteria to decompose their respective litters were apparent, with maximum mass losses of 8.0% (*Arthrobacter* sp. 1) for an individual bacterial species to 9.8% for the combined treatment of three bacterial species decomposing *C. aquatilis* rhizomes (Fig. 1) after 12 weeks. Species of *Micrococcus*, *Bacillus*, and *Arthrobacter* represent some of the most common bacteria previously isolated from peatlands (Latter et al. 1967; Christensen and Cook 1970; Martin et al. 1982). These genera are strict aerobes or facultative anaerobes with optimal growth temperatures between 20 and 30 °C (Sneath et al. 1986). To our knowledge, *Leuconostoc*, *Lactobacillus*, and *Brevibacterium* have not been isolated from peatlands previously; however, they have growth requirements similar to the previously discussed bacteria (Sneath et al. 1986).

The use of PBA (final pH 7.0) likely biased against bacterial taxa common to *S. fuscum*, which grows in bogs under much more acidic conditions (pH 3.8 in the bog; Thormann et al. 2001*a*). For example, acidophilic bacteria and pseudomonads may be more prevalent than some of the dominant taxa of our study, which were isolated at pH 7.0.

Implications for climate change

The majority of climate models predict increases in atmospheric temperatures in most areas currently covered by

Table 2. Mean initial litter quality of *Carex aquatilis* rhizomes and leaves from a fen and *Sphagnum fuscum* from a bog in southern boreal Alberta, Canada.

	Quality component, mg g^{-1} (±SE)			
Litter type	TC	TN	TC:TN	TP
C. aquatilis rhizomes	430*a* (1.0)	7.5*a* (0.5)	57*a* (3.0)	2.1*a* (0.1)
C. aquatilis leaves	450*b* (1.0)	20.1*b* (0.1)	22*b* (0)	1.8*a* (0.2)
S. fuscum plants	460*b* (10.0)	6.8*a* (0.3)	68*c* (2.0)	0.5*b* (0)

Note: TC, total carbon; TN, total nitrogen; TP, total phosporus. Different italic letters indicate significant differences among the three plant tissues for each litter quality variable.

Table 3. Maximum in vitro mass losses of three litter types caused by fungi and bacteria by themselves or in combination after 12 weeks.

Litter type	Fungi and (or) bacteria	Mass loss, % (±SD)	Temperature, °C
Carex aquatilis rhizomes	*Phialocephala dimorphospora*	24.8 (1.3)	20
	Arthrobacter sp. 1	8.0 (3.3)	20
	Mucor hiemalis + *P. dimorphospora*	24.6 (1.3)	20
	Arthrobacter sp. 1 + *Leuconostoc* sp.	4.1 (2.1)	20
C. aquatilis leaves	*Monocillium constrictum*	10.6 (3.7)	20
	Bacillus sp. 1	8.0 (0.3)	14
	Basidiomycete sp. 3 + *M. constrictum*	24.8 (6.0)	20
	Arthrobacter sp. 2 + *Bacillus* sp. 1	10.4 (3.6)	20
Sphagnum fuscum plants	*Trichoderma viride*	2.2 (0.1)	14
	Penicillium thomii	2.2 (0.2)	14
	Brevibacterium sp.	4.5 (2.5)	14
	M. hiemalis + *T. viride*	4.4 (1.5)	14
	Bacillus sp. 2 + *Arthrobacter* sp. 3	4.4 (0.4)	14

Table 4. Most frequently isolated bacteria and fungi from *Carex aquatilis* rhizomes and leaves from a fen and *Sphagnum fuscum* from a bog in southern boreal Alberta, Canada.

Litter type	Bacteria	Fungi
C. aquatilis rhizomes	*Arthrobacter* sp. 1	*Mucor hiemalis* Wehmer
	Lactobacillus sp.	*Phialocephala dimorphospora* Kendrick
	Leuconostoc sp.	*Trichoderma harzianum* Rifai
C. aquatilis leaves	*Arthrobacter* sp. 2	Basidiomycete sp. 3
	Bacillus sp. 1	*Monocillium constrictum* (Bourchier) W. Gams
	Micrococcus sp.	*Penicillium chrysogenum* Thom
S. fuscum plants	*Arthrobacter* sp. 3	*M. hiemalis*
	Bacillus sp. 2	*Penicillium thomii* Maire
	Brevibacterium sp.	*Trichoderma viride* Pers. *ex* Gray

peatlands in Canada (IPCC 2001). Freeman et al. (2001) suggested that global warming may lead to the rapid decay of peat because of the increased activity of PPOs under aerobic conditions. PPOs are the suite of enzymes required to decompose phenolic compounds, including lignins, tannins, and lignin-like compounds. However, Freeman et al. (2001) did not address the origin of the PPOs in peat and the pH dependence of PPOs under natural conditions. The ability of fungi, other than many basidiomyceteous wood-decay fungi, and bacteria to synthesize PPOs is limited (Domsch et al. 1980; Cerniglia 1992; Paul and Clark 1996). These polymers require a suite of different enzymes, including laccases and peroxidases, to mineralize them. Thormann et al. (2001*b*) showed that less than 24% of those fungi isolated from *S. fuscum* were able to use tannic acid as a C source, whereas the same fungi used cellulose (49%) and starch (45%) to a much higher degree. What these percentages may be for the suite of fungi in situ remains unknown; however, we hypothesize that they are similar to those shown in this study. Only two of the eight fungi in this study had the ability to degrade tannic acid (*M. constrictum*, *Ph. dimorphospora*), whereas five or more were able to degrade the remaining four C sources (cellulose, gelatin, pectin, and starch; data not shown). This suggests that the natural microbial community of peatlands may have a limited ability to decompose phenolic compounds, which constitute 27%–55% of peat and become more prevalent with increasing peat depths (Turetsky et al. 2000).

Our data indicate that some of the most frequently isolated microbes from the dominant peatland plants in a bog and fen have different decomposition capabilities under an elevated temperature regime, which may be mirrored by

many other fungi and bacteria in situ. Hence, these data are useful as indicators of the potential impact of temperature on C mineralization dynamics by microbial communities in peatlands.

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