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Microbial activity across a boreal peatland nutrient gradient: the role of fungi and bacteria

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Abstract Microbes involved in decomposition within peatlands and the conditions that influence their activities have implications for C and greenhouse gas exchange. The objectives of this research were to characterize the role of fungal and bacterial activities in peatlands using selective antibiotic inhibition techniques across a nutrient gradient (rich to poor fens) and to search for environmental controls on the activity of each group. Bacterial activities predominated across a range of rich to poor boreal peatlands in central Ontario, Canada, although fungal activity became increasingly important in the poor sites. Linkages between soil pH and nutrient status and fungal and bacterial activities were found. However, they did not confirm our initial hypotheses that bacterial activity would be low in poor sites due to proton stress and low nutrient (particularly N) availability, whereas, fungal activity would be low in rich sites due to increased competitive ability of bacteria under near neutral pH conditions and high nutrient availability. Further work across these sites aimed at characterizing the phylogeny of the rhizosphere fungi is needed to determine if increased presence of mycorrhizae in poor sites could have explained our observed patterns. However, regardless of fungal: bacterial activity ratio differences across sites and its associated controls, microbial CO_2 production rates across fen types did not vary significantly, suggesting that the proportion of bacteria and fungi may not matter to broader carbon cycling and greenhouse gas emissions in peat soils.

Keywords Carbon dioxide · Cycloheximide · Decomposition · Fen · Soil · Streptomycin · Substrate-induced respiration

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Introduction

Mid and high latitude peatlands (mires) cover a relatively small portion of the global landscape yet have sequestered an estimated 210–450 Gt of C, equivalent to as much as one-third of all global soil C (Gorham 1991; Turunen et al. 2002). Canada contains the 2nd largest area of northern peatlands globally and the province of Ontario contains the largest peatland area nationally within Canada (Gorham 1991; McLaughlin 2004). These ecosystems have had a unique influence on Holocene-scale global cooling

due to their role in net sequestration of atmospheric CO_2 and the vast amount of C stored in peatlands makes them particularly sensitive to anthropogenic climate change- and land use-ecosystem feedbacks to the global climate (Frolking et al. 2006; Frolking and Roulet 2007). Northern peatlands accumulate C because net primary production rates surpass organic matter mineralization rates (Moore and Basiliko 2006). Net CO_2 sequestration has resulted in long-term C accumulation rates between 8 and 40 g CO_2–C m^{-2} $year^{-1}$, with a mean of 25 g C m^{-2} $year^{-1}$ in North American boreal and temperate peatlands (Gorham et al. 2003; Roulet et al. 2007). Temperature is the most dominant control on peatland C dynamics at global and regional scales (Hobbie et al. 2000), while water-table level and litter quality are dominant controls at local scales (Jaatinen et al. 2007; Laiho 2006; Moore and Basiliko 2006).

Peatlands exist across the landscape in various forms ranging from young, nutrient-rich sites (i.e., rich fens) that have a hydrologic connection with groundwater, to well-developed nutrient-poor fens and bogs that become increasingly isolated from groundwater inputs (Siegel and Glazer 1987). As a result, peat from poor fens and bogs can be drier and more acidic (McLaughlin and Webster 2010; Webster and McLaughlin 2010) and have slower rates of microbial activity, than from richer fens (Moore and Basiliko 2006).

Because of the importance of decomposition processes to peatland C sequestration and storage, microbial decomposer communities in peatlands are of particular interest as they only partially decompose plant tissues, resulting in both peat accumulation and the release of CO_2 and methane, both important global greenhouse gases (Moore and Basiliko 2006). Fungi and bacteria might have different functional roles in peat biogeochemical cycling. For example, based on a very simplified and general understanding, bacteria predominate in circumneutral pH and fertile soils and have the ability to utilize alternative electron acceptors beyond oxygen and simple organic molecules and therefore might be better adapted to compete in anoxic soil environments (Killham and Prosser 2007). In contrast, fungi typically have lower biomass N and other nutrient requirements, secrete extra-cellular enzymes, form widespread symbioses with plant roots (mycorrhizae), are key initial wood decomposers, and have chitin containing cell walls (Howarth 2007; Paul and Clark 1996). These characteristics might allow fungi to better endure ionic stress in the environment, and indeed it has been demonstrated that across upland agricultural soil pH gradients, fungi are increasingly important decomposers under more acidic conditions (Rousk et al. 2009).

Although reasonably little is known about the role of fungi in peatlands, laboratory cultivation and diversity characterization by Thormann et al. (1999) indicted that Basidiomycetes (key organisms in breaking down lignin litter in forest soils) are scant in peat soils. Thormann (2006) thus put forth the need to improve our understanding of fungi in peatland organic matter decomposition. Subsequently, Winsborough and Basiliko (2010) studied fungal and bacterial activities across three ecologically and hydrologically diverse and spatially dispersed peatlands in Canada. Using selective inhibition techniques (with antibiotics), they showed that peatland microbial activity in bogs and fens (measured as CO_2 production) was dominated by bacteria, with fungal activity greatest in a treed poor fen, although still less than bacterial activity.

The objectives of this research were to characterize the role of fungal and bacterial decomposers in peatlands using selective inhibition techniques across a nutrient gradient (rich fen to poor fen) and to search for possible environmental controls on the activities of each group of microorganisms. To meet these objectives, the following hypotheses were evaluated: first, the spectrum from rich fens to poor fens will be characterized by increasing fungal to bacterial activity ratios. We propose this results from less active fungi relative to bacteria in rich fens due to wetter conditions and competition for resources from bacteria and bacteria being less active relative to fungi in poor sites due to lower acidity tolerance and larger biomass nutrient requirements, particularly for N. Second, the spectrum of rich fens to poor fens will be characterized by decreasing rates of microbial activity. We propose this is due to more nutrient rich, bioavailable litter and peat substrate in rich fens compared to poor fens (Moore and Basiliko 2006).

Methods

In late July 2008, six fen sites located 10–20 km southwest of White River, Ontario, Canada were sampled for this study (48°21′N, 85°21′W). The sites comprised two replicates of rich, intermediate, and poor fens, and except for two sites (Intermediate Fen II

and Poor Fen II in Tables 1, 2; Fig. 2) sites were located between 2 and 5 km apart. One of each site type (Rich Fen I, Intermediate Fen I and Poor Fen I identified in Tables 1, 2; Fig. 2) were situated in long-term research watersheds maintained by the Ontario Ministry of Natural Resources' Ontario Forest Research Institute and where plant communities, hydrology, and C dynamics have been previously characterized (McLaughlin and Webster 2010; Webster and McLaughlin 2010). The rich fen sites were dominated by sweet gale (*Myrica gale*) and non-tussock forming sedges (*Eriphorum vaginatum* and *Carex* sp.) and water table depths were approximately 15 cm beneath the surface at time of sampling. The peat depth in these sites ranged from approximately 0.75 to 1.25 m and there was relatively little micro-topography. Intermediate fen sites were dominated by non-tussock forming sedges (primarily *Eriphorum vaginatum*. and *Carex* sp.) though there were some *Sphagnum* mosses and sparse ericaceous shrubs present. The water table depths were between 10 and 20 cm beneath the surface at time of sampling. In one of intermediate fen sites (Intermediate Fen II), there were distinct *Sphagnum* and shrub dominated hummocks in one area raised above the largely sedge dominated lawn areas that were sampled as one of the poor fen sites (Poor Fen II). Similar to the rich fens, there was relatively little microtopography across these two sites and peat depths ranged from 0.5 to 3 m. The poor fen sites had raised *Sphagnum* dominated hummocks with letherleaf (*Chamaedaphne calyculata*) and bog laurel (*Kalmia polifolia*) shrubs and a sparse treed overstory containing *Larix laricina* and *Picea mariniana*. The poor fen site that was embedded within the intermediate fen also had abundant pitcher plants (*Sarracenia purpurea*). The water table depths at time of sampling in the poor fen hummocks was greater than 45 cm beneath the soil surface. The peat depths from the top of the hummocks in these sites ranged between approximately 1.5 and 3.5 m. Within each site, five samples were collected along a 40-m transect (1 every 10 m), for a total of 30 samples. Samples were collected by hand with a serrated knife from the surface to 30-cm depth, sealed in freezer bags, placed on ice in coolers, and transported to the laboratory where they were stored at 4°C prior to analyses, which began shortly following sampling. Each of the 30 sample bags containing peat was homogenized by hand using a knife and scissors and mixed in a plastic bag. Any traces of living, photosynthetic plant material were removed.

Microbial activity (basal CO_2 production or basal respiration, glucose-induced microbial activity, and bacterial and fungal activity) was characterized in

Table 1 Microbial activities (measured as CO_2 production) including basal (with no substrate addition), glucose-induced, fungal:bacterial, fungal, and bacterial across all sites ($n = 6$, with five within-site replicates comprising each mean value) and peatland type ($n = 3$, with two between-site replicates comprising each mean value)

Type:	Rich fens		Intermediate fens		Poor fens	
Site:	I	II	I	II	I	II
Basal microbial activity	**1.9**^{a} (0.57)	**1.4**^{a} (1.2)	**1.1**^{a} (0.60)	**1.7**^{a} (0.90)	**1.4**^{a} (0.24)	**1.1**^{a} (0.26)
	1.7^{a} (0.34)		**1.4**^{a} (0.38)		**1.2**^{a} (0.19)	
Glucose-induced activity	**2.8**^{a} (0.86)	**3.3**^{a} (1.3)	**3.0**^{a} (1.28)	**3.8**^{a} (2.4)	**3.8**^{a} (0.40)	**3.7**^{a} (1.37)
	3.0^{a} (0.35)		**3.4**^{a} (0.55)		**3.8**^{a} (0.07)	
Fungal: bacterial activity	**0.22**^{a,b} (0.19)	**0.20**^{a} (0.23)	**0.20**^{a,b} (0.16)	**0.37**^{a,c} (0.15)	**0.72**^{c} (0.23)	**0.50**^{b,c} (0.14)
	0.21^{a} (0.02)		**0.29**^{a} (0.12)		**0.61**^{a} (0.15)	
Fungal activity	**0.30**^{a} (0.34)	**0.18**^{a} (0.11)	**0.47**^{a} (0.69)	**0.56**^{a} (0.34)	**1.6**^{b} (0.60)	**0.89**^{a,b} (0.51)
	0.24^{a} (0.08)		**0.52**^{a} (0.06)		**1.25**^{a} (0.51)	
Bacterial activity	**1.1**^{a} (0.57)	**1.2**^{a} (0.51)	**1.9**^{a} (1.3)	**1.5**^{a} (1.2)	**2.2**^{a} (0.37)	**2.0**^{a} (1.3)
	1.15^{a} (0.10)		**1.73**^{a,b} (0.26)		**2.11**^{b} (0.13)	
IAR	**1.1**	**1.2**	**1.0**	**1.2**	**1.2**	**1.3**

The three individual sites labelled with "I" have been previously studied by Webster and McLaughlin (2010). IAR is the average inhibition additivity ratio. Bold values are means and standard deviations are in parentheses. Values that are significantly different from other sites or peatland types are indicated with different superscripted letters. All units are mg CO_2 produced g^{-1}

Table 2 Peat properties (solid phase, microbial, and in solution) across all sites ($n = 6$, with five within-site replicates comprising each mean value) and peatland type ($n = 3$, with two between-site replicates comprising each mean value)

Peat type:	Rich fens		Intermediate fens		Poor fens	
Site:	I	II	I	II	I	II
Moisture, %	**747**[a] (215)	**774**[a] (220)	**914**[a] (419)	**1187**[a] (580)	**661**[a] (135)	**854**[a] (243)
	760[a] (19)		**1051**[a] (193)		**757**[a] (136)	
$OM^{s.p.}$, %	**70.2**[a] (2.7)	**77.9**[b] (5.2)	**80.2**[b] (3.2)	**88.1**[c] (3.9)	**98.1**[d] (0.31)	**96.5**[d] (2.0)
	74.0[a] (5.4)		**84.2**[a,b] (5.6)		**97.3**[b] (1.1)	
$TOC^{s.p.}$, %	**36.7**[a] (1.6)	**41.3**[b] (2.9)	**43.1**[b,c] (1.8)	**46.6**[c] (2.2)	**49.5**[c] (0.54)	**48.0**[c] (0.91)
	39.0[a] (3.2)		**44.8**[a] (2.5)		**48.7**[a] (1.1)	
$TN^{s.p.}$, %	**2.4**[a] (0.15)	**2.4**[a] (0.11)	**2.1**[a] (0.16)	**2.3**[a] (0.16)	**0.84**[b] (0.11)	**0.93**[b] (0.20)
	2.4[a] (0.04)		**2.2**[a] (0.14)		**0.89**[b] (0.06)	
$C:N^{s.p.}$	**15.3**[a] (1.34)	**17.5**[a] (1.00)	**20.4**[a] (1.07)	**20.2**[a] (2.18)	**59.4**[b] (6.79)	**53.5**[b] (12.0)
	16.4[a] (1.55)		**20.3**[a] (0.13)		**56.4**[b] (4.16)	
$Ca^{s.p.}$, mg g^{-1}	**10.4**[a] (1.51)	**10.9**[a] (2.08)	**6.73**[b] (1.32)	**6.13**[b] (2.47)	**2.28**[c] (0.56)	**3.61**[b,c] (2.09)
	10.6[a] (0.40)		**6.43**[b] (0.42)		**2.95**[c] (0.94)	
$P^{s.p.}$, mg g^{-1}	**0.85**[a] (0.14)	**0.80**[a] (0.06)	**0.85**[a] (0.15)	**0.45**[b] (0.08)	**0.43**[b] (0.03)	**0.31**[b] (0.09)
	0.82[a] (0.03)		**0.65**[a] (0.28)		**0.37**[a] (0.09)	
$S^{s.p.}$, mg g^{-1}	**0.60**[a] (0.06)	**0.56**[a] (0.05)	**0.51**[a,b] (0.04)	**0.41**[b] (0.16)	**0.09**[c] (0.01)	**0.08**[c] (0.01)
	0.58[a] (0.02)		**0.46**[a] (0.08)		**0.09**[b] (0.00)	
$Mg^{s.p.}$, mg g	**0.86**[a,b] (0.11)	**1.1**[b] (0.15)	**0.66**[a] (0.14)	**0.82**[a,b] (0.13)	**0.60**[a] (0.07)	**0.93**[a,b] (0.38)
	0.98[a] (0.17)		**0.74**[a] (0.12)		**0.77**[a] (0.24)	
$K^{s.p.}$, mg g	**0.58**[a] (0.10)	**0.69**[a] (0.09)	**0.63**[a] (0.08)	**0.91**[a] (0.36)	**0.97**[a] (0.09)	**2.1**[a] (1.0)
	0.63[a] (0.08)		**0.77**[a] (0.20)		**1.5**[a] (0.80)	
Micro. C, mg g^{-1}	**4.72**[a] (2.13)	**4.87**[a] (2.10)	**3.61**[a] (1.58)	**5.11**[a](2.79)	**3.30**[a] (0.96)	**3.87**[a] (1.30)
	4.79[a] (0.10)		**4.36**[a] (1.06)		**3.59**[a] (0.40)	
Micro. N, mg g^{-1}	**0.33**[a] (0.15)	**0.38**[a] (0.18)	**0.28**[a] (0.14)	**0.24**[a] (0.14)	**0.23**[a] (0.06)	**0.19**[a] (0.07)
	0.35[a](0.04)		**0.26**[a,b] (0.03)		**0.21**[b](0.03)	
Micro. C:N	**14.5**[a] (0.6)	**13.1**[a] (1.2)	**13.4**[a] (2.9)	**23.2**[b] (5.2)	**14.5**[a] (1.7)	**21.7**[b] (4.7)
	13.8[a] (1.0)		**18.3**[a] (7.0)		**18.1**[a] (5.1)	
DOC, mg g^{-1}	**1.52**[a] (0.46)	**1.59**[a] (0.49)	**1.36**[a] (0.47)	**2.95**[b] (0.84)	**1.44**[a] (0.39)	**1.80**[a,b] (0.99)
	1.55[a] (0.05)		**2.15**[a] (1.12)		**1.62**[a] (0.26)	
TN, mg g^{-1}	**0.63**[a,b] (0.16)	**0.63**[a,b] (0.15)	**0.72**[a,b] (0.28)	**0.94**[a] (0.39)	**0.51**[b] (0.08)	**0.65**[a,b] (0.13)
	0.63[a] (0.00)		**0.83**[a] (0.15)		**0.58**[a] (0.10)	
pH	**6.6**[a] (0.5)	**6.3**[a,b] (0.1)	**6.0**[b] (0.0)	**5.5**[c] (0.3)	**4.1**[d] (0.2)	**4.2**[d] (0.3)
	6.4[a] (0.3)		**5.7**[a] (0.4)		**4.1**[b] (0.1)	
Ca^{2+}, μg g^{-1}	**242**[a] (168)	**204**[a] (82)	**127**[a,b] (48)	**92**[a,b] (50)	**27**[b] (4)	**40**[b] (11)
	223[a] (27)		**110**[b] (25)		**34**[b] (9)	
Mg^{2+}, μg g^{-1}	**38.5**[a] (29.9)	**40.7**[a] (17.4)	**22.0**[a,b] (7.5)	**16.0**[a,b] (5.4)	**4.9**[b] (1.0)	**7.4**[b] (3.5)
	39.6[a] (1.6)		**19.0**[b] (4.2)		**6.1**[c] (1.8)	
K^{+}, μg g^{-1}	**188**[a] (203)	**46.9**[a] (21.1)	**73.1**[a] (23.5)	**103**[a] (47.9)	**49.6**[a] (14.8)	**68.8**[a] (37.4)
	117[a] (99.4)		**88.0**[a] (21.0)		**59.2**[a] (13.6)	
Na^{+}, μg g^{-1}	**152**[a] (58.5)	**138**[a] (67.3)	**139**[a] (44.1)	**222**[a] (190)	**125**[a] (36.0)	**162**[a] (68.2)
	145[a] (9.9)		**181**[a] (58.6)		**144**[a] (26.0)	
SO_4^{2-}, mg g^{-1}	**0.44**[a] (0.39)	**0.20**[a] (0.07)	**0.19**[a] (0.07)	**0.35**[a] (0.32)	**0.13**[a] (0.04)	**0.14**[a] (0.08)
	0.32[a] (0.17)		**0.27**[a] (0.11)		**0.14**[a] (0.01)	

Table 2 continued

Peat type:	Rich fens		Intermediate fens		Poor fens	
Site:	I	II	I	II	I	II
NO_3^-, μg g^{-1}	**7.6**^{a,b} (5.6)	16.6^{a} (11.3)	**8.1**^{a,b} (2.1)	**3.6**^{b} (1.86)	**2.04**^{b} (0.60)	**2.2**^{b} (1.6)
	12.1^{a} (6.4)		**5.8**^{a} (3.1)		**2.1**^{a} (0.08)	
Cl^-, μg g^{-1}	**43.2**^{a} (23.8)	**36.1**^{a} (19.8)	**40.8**^{a} (10.7)	**41.5**^{a} (11.8)	**36.1**^{a} (28.2)	**10.3**^{a} (5.3)
	39.7^{a} (5.0)		**41.2**^{a} (0.5)		**23.2**^{a} (18.2)	
SiO_2^-, μg g^{-1}	**125**^{a} (120)	**131**^{a} (64.3)	**142**^{a} (77.7)	**81.6**^{a} (55.0)	**43.7**^{a} (21.3)	**81.3**^{a} (94.6)
	128^{a} (4.8)		**112**^{a} (42.7)		**62.5**^{a} (26.6)	
Al, μg g^{-1}	**8.9**^{a,b} (4.7)	**8.4**^{a,b} (2.2)	**15.8**^{a} (7.4)	**10.1**^{ab} (4.7)	**3.7**^{b} (0.26)	**5.8**^{b} (2.5)
	8.6^{a} (0.37)		**13.0**^{a} (4.0)		**4.8**^{a} (1.5)	
Fe, μg g^{-1}	**19.0**^{a,b} (8.3)	**19.7**^{a,b} (5.1)	**26.8**^{a} (11.6)	**20.6**^{a,b} (8.3)	**9.1**^{b} (1.4)	**14.2**^{a,b} (4.8)
	19.3^{a} (0.52)		**23.7**^{a} (4.4)		**11.6**^{a} (3.6)	

The three individual sites labelled with "I" have been previously studied by Webster and McLaughlin (2010). Bold values are means and standard deviations are in parentheses. Values that are significantly different from other sites or peatland types are indicated with different superscripted letters. *s.p.* is solid peat phase

vitro as basal CO_2 production, substrate-induced CO_2 production with glucose, and selective antibiotic inhibition methods with cycloheximide plus glucose and streptomycin sulphate plus glucose following Winsborough and Basiliko (2010). Following initial tests with composite samples from each site to determine optimum glucose and antibiotic concentrations whereby adding additional glucose did not result in additional CO_2 production and additional antibiotics did not result in less CO_2 production (see Winsborough and Basiliko 2010), peat from each of the five field replicates per site was used in incubations with 2.5 g of wet peat each. Samples were weighed into airtight flasks and left slightly open to sit at room temperature for 48 h. The five replicate samples for each of the six sites received 20 ml of six different treatments (dissolved in distilled H_2O), for a total of 30 flasks per site and 180 in total. Treatments included: (i) controls with distilled H_2O, (ii) 50 mg glucose, (iii) 0.1 mg cycloheximide + 50 mg glucose, (iv) 1,000 mg streptomycin + 50 mg glucose, (v) 0.1 mg cyclohexamide + 1,000 mg streptomycin + 50 mg glucose, (vi) 0.1 mg cyclohexamide + 1,000 mg streptomycin. Gas analyses over 12 h using an infrared gas analyzer and subsequent calculations, including of the inhibition additivity ratio (IAR), which describes the degree of non-target antibiotic inhibition, were made following Winsborough and Basiliko (2010). Although this approach has been used widely in soils, it depends on stimulating microbial growth with glucose and then selectively suppressing additional respiration associated with this growth with antibiotics, meaning that the absolute rates of activity reported for fungi or bacteria are not directly comparable to basal respiration. However it does allow for comparisons across sites or with other measured soil properties (below), and if the assumption that bacteria and fungi respond equivalently to glucose additions is true, then the fungal: bacterial activity ratios are quantitatively meaningful.

Using sub-samples for each of the following analyses: moisture content was measured gravimetrically by weighing, oven drying at 90°C for 48 h, and reweighing. The pH was measured in peat and distilled water in a 1:4 ratio by weight after shaking on an oscillating shaker for 1 h at 200 rpm and using a calibrated Ag-glass electrode and meter. Total extractable- and microbial biomass C and N were measured using chloroform fumigation-K_2SO_4 extraction techniques following Basiliko et al. (2007) and using a Lachat TOC/TN IL550 analyzer (Hach Company, Loveland Colorado, USA). Total organic matter was determined by measuring loss on ignition for 5 h at 550°C (following Bengtson et al. 2007). Solid phase total C and total N concentrations were determined using an Elementar Vario Max C and N analyzer (Elementar Analysensysteme, Hanau, Germany) and total sulphur (S) using an Eltra Helios C and S analyzer (Eltra GmbH, Neuss, Germany). Total Ca, K, Mg, and P were extracted with 0.1 ml of 15.5% SeO_2 in 5 ml

concentrated sulphuric acid (H_2SO_4) digests and analyzed on a Varian Liberty Series II inductively coupled plasma spectrometer (ICP, Agilent Technologies, Santa Clara, USA). Water extractable ions Ca^{2+}, Mg^{2+}, K^+, Na^+, $SO_4{}^{2-}$, $NO_3{}^-$, $NO_2{}^-$, Cl^-, and $PO_4{}^{3-}$ were analyzer with a ICS 2000 ion chromatograph (Dionex Corp., Sunnyvale, CA) while water extractable $SiO_2{}^-$, Al, and Fe were analyzed by ICP as above (following Webster and McLaughlin 2010 and Basiliko et al. 2006).

All data were expressed per g of dry peat. Analysis of variance (ANOVA) was conducted with SYSTAT v10 (Systat Software Inc., San Jose, USA) to determine significant ($P < 0.05$) differences in all measured factors among sites. In certain cases (i.e., basal microbial activity and total extractable N) resulting residual distributions were not normal (skewness/standard error of skewness and/or kurtosis/standard error of kurtosis not near 0) and original data were log transformed and reanalyzed. For ease of interpretation however, means and standard deviations of non log-transformed data are presented. To explore linkages between measured variables, linear regression and correlation analyses were performed using SYSTAT and stepwise multiple regression analyses were performed using SigmaPlot 11.0 (Systat Software Inc., San Jose, USA). Linear regression analysis did not lead to non-normal residual distribution (see above) and therefore no data transformation was needed in calculating Pearson coefficients. Principal component analysis (PCA) was conducted with peat chemical and physical properties and biological properties using the princomp algorithm in R v.2.10.1 (R projects.com). Key factors influencing the first two principal components (explaining the majority of variance in the datasets) were reported.

Results

Basal and glucose-induced microbial activity were not significantly different among study sites or peatland type, however on average, basal microbial activity was fastest in the rich fens and slowest in the poor fens (Table 1). Fungal: bacterial activity ratios were smallest in the rich fen, and largest in the poor fens, although differences between the rich and intermediate sites were not significant (Table 1). Although both differences in fungal and bacterial activity contributed to the activity ratio differences, bacterial activity differed by less than a factor of two among the site types, while fungal activity differed by more than a factor of five (Table 1). Fungal and bacterial activities showed a strong, positive, logarithmic relationship with each other (bacterial activity $= 0.52 \times$ Ln(fungal activity) $+ 2.00$; $R^2 = 0.79$; $P = 0.02$). Inhibition additivity ratios were generally close to 1.0, indicating that non-target inhibition by cycloheximide and/or streptomycin was minor (Table 1).

Despite differences in water table positions at time of sampling, gravimetric moisture content did not differ statistically among any of the sites or by site type (Table 2). However pH, Ca^{2+} and Mg^{2+}(consistent with low concentrations of mineral carbonates in the poor fens and overall poorer nutrient status), as well as other constituents were lowest in the poor fen and highest in the rich fen (Table 2). $PO_4{}^{3-}$ was only detectable in some of the rich fen peat samples (with concentrations ranging between 3.5 and 6.4 $\mu g\ g^{-1}$) and $NO_2{}^-$ was not detectable in any samples and were not included in any further analyses. Total organic matter and C were highest, while total N lowest in the poor fens relative to the rich fens and differences were at least always significant between individual sites, if not by site type (Table 2). Microbial N was significantly smaller in the poor fens than the rich fens when compared by site type, though differences were not significant between any two of the six sites when compared individually (Table 2). Microbial biomass C and the C:N ratio were not significantly different among peatland types, however on average the poor and intermediate fens had larger biomass C:N ratios and two sites (an intermediate and poor fen) had significantly larger biomass C:N than all other sites (Table 2).

Simple linear regression analyses indicated that basal microbial activity was most strongly dependent on total microbial biomass (with significant Pearson correlation values of 0.71 and 0.65 for microbial C and N). Bacterial activity was not significantly correlated to any measured constituents based on the linear regression, but fungal activity was significantly positively correlated with peat C:N ratio and negatively correlated with pH, TN, and Ca (with Pearson values of 0.65, −0.64, −0.65, and −0.64 respectively). It was this relationship between fungal activity and measured variables that drove negative relationships between peat pH or Ca and fungal: bacterial activity ratios and a

positive relationship between peat C:N and fungal: bacterial activity ratios (Fig. 1). Statistically significant co-variation was frequent among the solid-phase peat properties and fungal activity, and therefore the reverse stepwise multiple regression analyses results might be more accurate in identifying the peat properties that controlled microbial activities. Based on reverse stepwise multiple regression analyses, fungal activity was negatively correlated with pH and total peat Mg concentrations and bacterial activities were also negatively correlated with total peat Mg concentrations, positively correlated with total K, and exhibited a parabolic response to extractable total Fe, peaking at intermediate concentrations (Table 3).

Principal component analyses illustrated differences among site types based on both peat chemical and physical factors and microbial activities and biomass (Fig. 2). Extractable K^+ was a key significant loading factor that separated sites by peatland type along the 1st component axis (PC I explained 44% of total dataset variance) while moisture content, extractable DOC, and SO_4^{2-} generally separated peat samples within peatland types along the 2nd component axis (PC II explained 15% of total dataset variance), particularly among samples from the rich or intermediate fens (Fig. 2a). Based on microbial biomass and activities, the poor and rich fens were clearly separated from each other along the 1st principal component axis (PC I explained 41% of total dataset variance) and the key significant loading factor for this component was fungal activity and fungal: bacterial activity ratios, consistent with patterns illustrated in Table 1 (Fig. 2b). Fungal: bacterial and basal microbial activity and microbial biomass C were significant factors for PC II (PC II explained 37% of total dataset variance).

Discussion

It has been well established that peat chemical characteristics, which are in large part controlled by peatland nutrient status and resulting vegetation types, play a key roles in controlling microbial activities

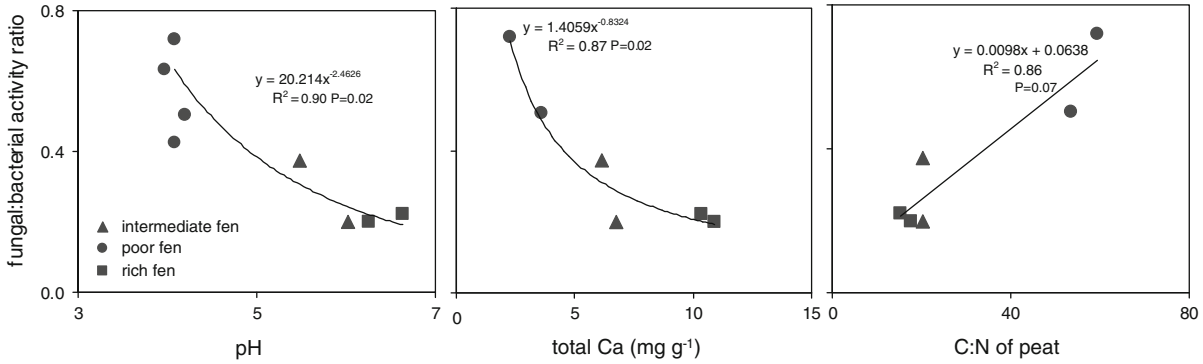

Fig. 1 Scatterplots illustrating relationships between key peat properties identified in Table 2 and fungal: bacterial activity ratios across sites ($n = 6$)

Table 3 Stepwise multiple regression results based on analysis of all sample ($n = 30$)

Microbial activity	Equation	r^2
Basal microbial activity[a]	$= 0.308 + (0.267 \times \text{microbial C}^b)$	0.48
Fungal activity[a]	$= 3.621 - (0.464 \times \text{pH}) - (0.955 \times \text{total Mg}^b)$	0.53
Bacterial activity[a]	$= 2.723 + (0.685 \times \text{total K}^b) - (0.147 \times \text{ext Fe}^c) + (0.00410 \times \text{ext Fe}^2) - (0.869 \times \text{total Mg}^{b*})$	0.62

All factors are significant ($P < 0.05$) unless indicated

ext extractable

* $P = 0.09$

[a] mg CO_2 g^{-1} peat d^{-1}, [b] mg g^{-1} peat, [c] μg g^{-1}

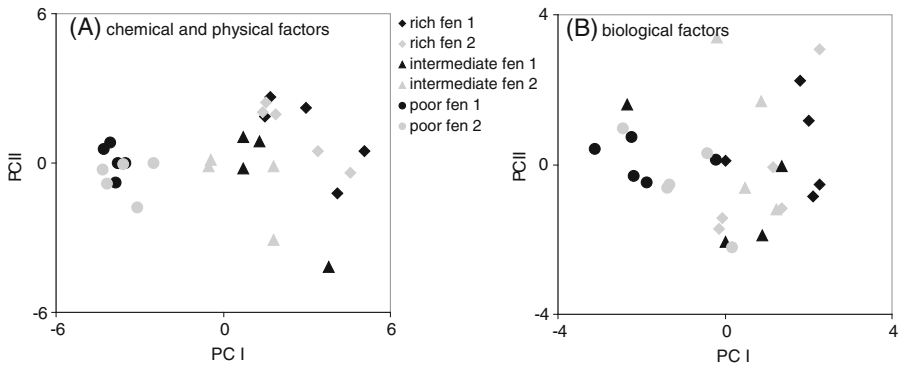

Fig. 2 Principal component analysis and scatter plots based on **a** peat chemical and physical factors in Table 2 and **b** biological factors (microbial activities and biomass) in Tables 1 and 2

(Laiho 2006; Moore and Basiliko 2006; Yavitt et al. 1997). Whether or not the microbial community structure and fungal versus bacterial activities across sites is an important control on C efflux from peatlands is less clear. Across a rich to poor peatland nutrient gradient, fungi contributed increasingly to microbial CO_2 production in peat, partially consistent with our first hypothesis.

However our proposed mechanisms to explain patterns of fungal and bacterial activity were likely incorrect: first, bacterial activity predominated across sites, and proportionally varied less across sites, indicating that bacteria were not intolerant to acidity in the poor sites as initially proposed. Second, bacterial and fungal activities were positively, not negatively, correlated across all sites, indicating that superior competitiveness of bacteria over fungi in the less acidic, nutrient rich, and more flooded sites could also not explain this pattern. Third, peat moisture content did not correlate significantly with fungal or bacterial activities or activity ratios, nor did it vary significantly across sites, potentially indicating that wetter or drier conditions in surface peat did not lead to smaller or larger fungal: bacterial activity ratios respectively as predicted, with bacteria more likely to be facultative anaerobes capable of more energetically profitable metabolism under anoxic conditions (e.g. through denitrification, generating about ½ of the energy of respiration with O_2) in contrast to fungi, which are either aerobes or typically carry out relatively unprofitable facultative fermentations generating typically two ATP molecules per glucose molecule or less than 1/18 of the energy yield of aerobic respiration (e.g. Koikei and Hattori 1975; Madigan et al. 2000). It is important to note, however, that we did not measure oxygen concentrations or redox potentials in the sites, which would have been a more appropriate means of examining the role of oxic and anoxic conditions on microbial activities. Water table levels were different between the sites, and lower in the poor fens, which might have been consistent with more oxic conditions and higher fungal activities despite similar gravimetric moisture contents. Different water table positions yet similar moisture contents in peats could have occurred because the bulk densities and physical structures of the peats across site type were markedly different (fibric in the poor fens and humified in the rich fens). Sampling at another point in time when moisture levels were different might produce different results, and specifically examining moisture, water table, and anaerobic versus aerobic controls on fungal and bacterial activities throughout peat profiles should be an objective of future studies.

Stepwise multiple regression analysis illustrated that total K concentration correlated positively with bacterial activity, perhaps consistent with relatively large requirement of this element in bacterial cells of up to 2% by dry weight (Neidhard et al. 1990). However we are at a loss to explain what possible causal mechanisms might drive negative correlations observed between extractable Mg^{2+} and both fungal and bacterial activities and the parabolic response between total Fe and bacterial activity. Both of these elements are required nutrients and toxicity issues are very rare. One speculation is that Mg^{2+} could be a long-term moisture indicator, where it increases under wet conditions or ionic strength due to less plant uptake, both of which could also inhibit microbial activity.

It is likely that nutrient availability, pH, or moisture content of the peat indirectly influenced the observed patterns of microbial activities across these sites, in particular the increase in both the rate of fungal

activity and the fungal: bacterial activity ratio (the latter which was primarily driven by differences in fungal activity). As peat aggrades in peatlands, the upper surfaces gradually become disconnected from the underlying water table causing concomitant changes in peatland vegetation and biogeochemistry (Almquist-Jacobson and Foster 1995; Siegel and Glazer 1987). The resulting oligotrophic, acidic, and lower water table conditions might facilitate mycorrhizae in the vascular plants. Indeed as well as containing the largest non-vascular plant biomass, the poor fens contain between 10 and 20 times more aboveground vascular plant biomass than the rich and intermediate fens (Webster and McLaughlin 2010), and presumably also contain substantially larger root biomass, which could support larger mycorrhizal fungal biomass and therefore microbial activity patterns seen in the present study.

Groundwater-fed North American wetlands, including fens, have been reported to contain mycorrhizae, but the occurrence and abundance of this symbiosis has been shown to be driven by soil pH and nutrient availability with lower nutrient availability leading to more frequent mycorrhizal infection (Turner et al. 2000). For example, Thormann et al. (1999) reported nutrient and trophic state of wetlands as being an important determinant of mycorrhizae presence in boreal Alberta, Canada. In wet, minerotrophic fen sites with reasonably stable water table positions, sedges also tend not to form mycorrhizae (Cornwell et al. 2001), conditions potentially similar to our pH neutral rich fens. If our speculation about the potential role of mycorrhizae across sites were true, nutrient status might be an indirect controlling factor in explaining slow fungal activity in the rich sites and faster activity in the poor sites, but only one that is mediated by the presence of mycorrhizal vascular vegetation in the latter. This explanation could still support the significant, negative correlations of fungal activity with total N content, base cation availability, and pH of peats; however the observed patterns apparently existed more as function of the plant needs facilitating fungal activity under low nutrient conditions rather than as a result of physiological differences between bacteria and fungi as initially predicted (Paul and Clark 1996; Thormann et al. 1999). An important note about this speculation that deserves discussion is that arbuscular mycorrhizal fungi associated with sedges are alleged obligate plant symbionts and should not have responded to our substrate induced respiration assay. Although approximately one-half of sedge species have been reported to be mycorrhizal, including *Eriophorum vaginatum* encountered in the rich and intermediate fens, low water tables and soil nutrient status and interrelated pH play an important role in the presence and abundance of fungi present in these symbioses (Cornwell et al. 2001; Muthukumar et al. 2004). However ericoid mycorrhizal fungi (associating with peatland shrubs) can easily grow in isolation on simple carbohydrates and ectomycorrhizal fungi associated with conifer trees/shrubs encountered in the poor sites also can assimilate non-plant derived C and could have responded to the activity assays used (Talbot et al. 2008).

Our second hypothesis predicted that basal microbial activity would have been slowest in the poor fen sites. These sites had larger fungal: bacterial activity ratios, and elsewhere fungi have been shown to support larger microbial biomass with less respiration (Basiliko et al. 2006; Sakamoto and Oba 1994). However there were no significant differences in basal rates across sites, indicating that fungal: bacterial activity ratios might not clearly link to CO_2 production rates and C release from peatlands. This contrasts Basiliko et al. (2006) who purported that increased fungal: bacterial biomass across simulated nutrient deposition plots in a bog explained decreased rates of microbial CO_2 production and Sakamoto and Oba (1994) who linked fungal versus bacterial dominance to efficiency of decomposition in upland agricultural soils. More consistent with our results, in a meta-analysis of published literature exploring fungal and bacterial dominance of soils, Strickland and Rousk (2010) found that shifts in bacterial or fungal dominance across environmental gradients often do not correlate well with changes in patterns of elemental cycling in soils and concluded that this may be due to overlap in the biogeochemical function and ecology of bacteria and fungi, or that some of the expected functional trait differences between fungi and bacteria might be incorrect. Experimental results of Rousk et al. (2009) also indicate that functional redundancy exists between bacteria and fungi in upland soils spanning a similar pH gradient as in our fen sites. Ultimately our results suggest it is the total microbial biomass, and not the proportion of fungal

and bacterial activity, that drives microbial CO_2 production rates in peat.

Winsborough and Basiliko (2010) were apparently the first to use the suppression of substrate-induced respiration with selective antibiotics in mire peat soils to partition fungal and bacterial activities. In their study, three spatially distant peatlands in central and eastern Ontario, Canada, including one of the rich fen sites in the present study, were characterized. They reported a similar finding of bacterial activity predominating over fungal activity, with fungal activity highest in a treed poor fen. The present study builds substantially on this work to include replicated peatland types with similar climatic and geologic conditions and characterization of key physicochemical and environmental variables.

In saltwater and freshwater wetlands in Florida and Georgia, USA and the Bahamas Benner et al. (1986) used a combination of radiolabeled plant substrates and selective inhibitors to characterize fungal and bacterial roles in lignin and cellulose degradation. Similarly to what was observed across northern fen sites in the present study, Brenner et al. reported predominance of bacterial activity across all sites studied. In non-peat accumulating circumneutral pH saltwater wetlands fungi did not play any significant role, whereas in two freshwater peat-accumulating swamps, fungi did play a significant role in organic matter mineralization (though still not equivalent to bacteria). Other studies of mid to high latitude peatlands have focussed on absolute and relative biomass (not activities) of fungi and bacteria in peatlands using microscopy and cell membrane lipid fingerprints. Golovchenko et al. (2007) reported general patterns for biomass that were similar to our observed patterns of activities across rich to poor peatland sites in Tver Oblast, Russia. However, they noted that in ombrotrophic sites, fungal biomass was dominant, whereas in our poor fen sites, fungal activity was significantly greater than in rich sites, but still less than bacterial. In water-table drawdown studies, Jaatinen et al. (2007) reported that relative fungal biomass (measured with phospholipid fatty acid proxies) increased in richer fen sites and decreased in poorer bog sites with drought, and that changes were mediated indirectly by changing plant communities responding to hydrological changes, consistent with our suggestion that fungal activities, in particular, might be mediated most strongly by vegetation. Increasingly studies across environmental gradients in peatlands are implicating plants as key mediators of microbial dynamics rather than direct linkages between changing conditions (temperature, nutrient availability, hydrology) and microbial activities (Basiliko et al. 2006; Berendse et al. 2001; Bragazza et al. 2006; Jaatinen et al. 2007; Tomassen et al. 2004). In some cases more than one half of annual net primary production in fens occurs below ground and both the ratios of above- to below-ground production and the depth of below ground production varies across sites and inter-annually (Chimner and Cooper 2003), and as such, exploration of plants as mediators of microbial activity in peatlands beyond simply as sources of varying quality of litter, should continue.

In summary, bacterial activities predominated across a range of rich to poor peatlands, although fungal activity became increasingly important in the poor sites. Future studies should focus on the detection and characterization of mycorrhizae across these or similar sites to help determine if linkages between fungal activities and soil pH and nutrient availability were not direct, but rather mediated indirectly via mycorrhizae in the vegetation community. Although our sites included replicate site types that are representative of many fens in central Canada in terms of hydrology and physicochemical parameters, further work addressing the influence of water table and moisture conditions on fungal and bacterial activities and associated chemical controls over time is also needed. Regardless of fungal: bacterial activity ratio differences across sites, measured microbial CO_2 production rates across peatlands did not vary significantly, and across all samples characterized, total microbial biomass, but not fungal or bacterial activity, related to bulk microbial respiration, calling into question if indeed the proportion of each group of microorganisms matters to broader C cycling and greenhouse gas emissions in peat soils.

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