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Effects of Simulated Treated Domestic Wastewater on *Sphagnum* Productivity, Decomposition and Nutrient Dynamics in a Subarctic Ladder Fen

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Abstract

Boreal and subarctic peatlands may become increasingly used for wastewater tertiary treatment. Colleagues added simulated secondarily-treated wastewater to a subarctic ladder fen over two growing seasons. We examined how carbon storage and nutrient relations changed for key *Sphagnum* species. We measured the productivity, decomposition, nutrient content, C:N and N:P ratios of *Sphagnum* species on ridges and pool edges, and the nutrient relations of a third *Sphagnum* species in pools, compared to a control fen. Closer to the point source, *S. fuscum* on ridges had a 2-fold increase in productivity, and *Sphagnum rubellum* along pond edges had a 4 to 12-fold increase in productivity, but areas beyond 50 m downgradient were not affected. The decomposition of *S. fuscum* did not change, and *S. rubellum* showed only a slight increase close to the point source. Consequently, net carbon storage increased in *Sphagnum* within <50 m of the point source. Nutrient effects were apparent in the pools and pool margins, whereas ridges were hardly affected. N:P was the most sensitive nutrient parameter, increasing within 50 m. In the short-term, subarctic peatlands exposed to simulated secondarily-treated wastewater increased their carbon storage. Ridges in string fens were most resilient to nutrient amendments.

Keywords Subarctic peatland · String fen · Aapa mire · *Sphagnum* · Carbon storage · Nutrient enrichment · Wastewater tertiary treatment · Wastewater polishing

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Introduction

Peatlands cover 3% of the Earth's land surface, but they store 15 to 30 % of global soil carbon, especially in boreal and subarctic landscapes (Limpens et al. 2008). Besides carbon sequestration, northern peatlands also provide regulating ecosystem services such as erosion protection, water quantity regulation and water filtration (Kimmel and Mander 2010). Humans commonly harness the filtering function of marshes for the tertiary treatment of domestic wastewaters (Kadlec and Wallace 2009), but we less commonly use northern peatlands, with a few exceptions (Kadlec 2009a; Ronkanen and Klove 2009).

Boreal and subarctic peatlands are dominated by ombrotrophic bogs and fens, especially string fens (Glooschenko et al. 1993). Bogs only receive water from precipitation and serve as water storage complexes, whereas fens act as conveyors of water across peatland landscapes during periods of high hydrological connectivity (Quinton et al. 2003). String fens, including ribbed fens, aapa mires and the

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narrower string fens called ladder fens, all have a have a repeating pattern of pool-ridge-pool morphology (Warner and Rubec 1997). Slow hydrological flow runs perpendicular to the raised peat ridges; it occurs during periods of high water by pool-to-pool connectivity through preferential flow paths close to the surface of the ridges (Price and Maloney 1994; Quinton et al. 2003; McCarter and Price 2017a). This gentle hydrological flow makes string fens potential candidates for the tertiary treatment of wastewater.

String fens are also acidic, nutrient-deprived ecosystems dominated by *Sphagnum* mosses on the ridges and in the pools (Sjörs 1963; Riley 2011). Their rates of productivity and decomposition depend on the local climate, the degree of waterlogging within the peatland, nutrient availability and on the dominant *Sphagnum* species present (Clymo and Hayward 1982; Turetsky et al. 2008). *Sphagnum* species, especially hummock-formers, are recalcitrant to decay (Johnson and Damman 1993; Turetsky et al. 2008; Bengtsson et al. 2016), and this makes them keystone species on the ridges, controlling hydrological relations of these fens. *Sphagnum* species also have a high cation-exchange capacity (Clymo 1963), allowing them to efficiently scavenge for nutrients in nutrient-poor environments. How would an increase in nutrient supply from secondarily-treated wastewater affect *Sphagnum* productivity and decomposition? How would it consequently affect the hydrological flow across the ridges of string fens?

Early studies reported decreased growth rates and even mortality of *Sphagnum* when it was subject to enriched atmospheric N deposition (Ferguson et al. 1984; Press et al. 1986; Woodin and Lee 1987; Verhoeven and Schmitz 1991). Aerts et al. (1992) showed that adding N to bogs that receive low atmospheric N deposition increased the growth of *Sphagnum* fourfold, while the same N addition to bogs already receiving high atmospheric N deposition did not affect their productivity, and their growth became P-limited. These results point to the need to examine multiple nutrients, especially N:P ratios, when considering the effects of nutrient enrichment of peatlands (Güsewell and Koerselman 2002). In other European experiments, high rates of simulated atmospheric N deposition decreased both *Sphagnum* production (Berendse et al. 2001; van Wijk et al. 2003) and decomposition (Bragazza et al. 2006). Bubier et al. (2007) conducted a five-year nutrient addition experiment in an ombrotrophic bog in southeastern Canada using treatments representative of elevated atmospheric N deposition (1.6 to 6.4 g m^{-2} yr^{-1}) and other treatments with added P (5 g m^{-2} yr^{-1}) and K (6.3 g m^{-2} yr^{-1}). They found that plant growth and net ecosystem CO_2 exchange (NEE) increased over the first two years, indicating an increase in carbon storage, but by the third year and onward, NEE levels decreased as the plant community shifted away from *Sphagnum* toward other mosses and vascular plants, and after four years, the treatments with

greater NPK loading had no more *Sphagnum* cover, in part because of competition for light with taller vascular plants. Larmola et al. (2013) concluded that the vegetation shifts, particularly the loss of *Sphagnum*, is why peatlands with longer-term nutrient enrichment become weaker carbon sinks.

But are these results applicable to string fens receiving nutrient enrichment from secondarily-treated wastewater? Most previous studies focus only on atmospheric N enrichment with relatively low input rates; they consider ombrotrophic bogs where the water is stagnant; and the studied peatlands have warmer climates with long-term anthropogenic influence as compared to most boreal and subarctic peatlands. Few studies report on the effects of inputs of secondarily-treated wastewater slowly flowing through peatlands. Kadlec (2009a) studied a northern temperate peatland in Michigan that received point source additions of secondarily-treated wastewater seasonally (May-October) over 40 years. The average nutrient loading rates were 4.50 g N m^{-2} yr^{-1} and 1.87 g P m^{-2} yr^{-1} within a 100 ha area, although areas closer to the discharge received higher loading. The peatland effectively retained nutrients, but within the first few years, *Typha* had displaced peatland plant assemblages (Kadlec and Bevis 2009), and peat shifted from *Sphagnum* and sedge peat to *Typha* floating mats (Kadlec 2009b). Ronkanen and Klove (2009) studied four tertiary treatment peatlands in subarctic Finland receiving secondarily-treated wastewater for 10 to 16 years with much higher loading rates of 17 to 730 g N m^{-2} yr^{-1} and 0.53 to 7.0 g P m^{-2} yr^{-1} and also found high retention of phosphorus, but do not report the effects on vegetation, peat properties or carbon sequestration. Given the increase in resource development pressures and the population growth of isolated communities in subarctic regions (e.g., (Far North Science Advisory Panel 2010) and the prevalence of string fens in this biome (Glooschenko et al. 1993), string fens may be increasingly considered for the tertiary treatment of secondarily-treated wastewater.

In a collaborative study, colleagues and ourselves studied the effects of simulated secondarily-treated domestic wastewater on a ladder fen. Our colleagues examined the hydrology (McCarter and Price 2017a), its solute transport dynamics (McCarter and Price 2017b) and its nutrient and mercury transport (McCarter et al. 2017). They showed that the ladder fen immobilized nitrate by the first ridge, ammonium by the fourth pool and phosphate by the second pool, while sulfate extended further, to the seventh pond 117 m downgradient (McCarter et al. 2017). Total mercury was also elevated three-fold in the first ridge with high methylation, and they attributed the high total mercury to increased decomposition (McCarter et al. 2017). In this study, we aimed to document the response of this point source nutrient loading on the productivity and decomposition of key *Sphagnum* species in this ladder fen, as well as the nutrient content within *Sphagnum*. We hypothesized that the addition of simulated secondarily-

treated domestic wastewater, in parallel with the nutrient transport results, will (i) increase the rate of productivity of *Sphagnum* species close to the discharge point; (ii) increase the rate of decomposition of *Sphagnum*; (iii) increase nutrient content and decrease C:N ratios in *Sphagnum*; (iv) shift the N:P ratios within the *Sphagnum*; and (v) these changes would be most evident in *Sphagnum* species growing in microtopographic positions near or below the water table rather than in ridges, which are elevated above the water table.

Methods

We conducted this study near the De Beers Canada Victor Mine, within the Attawapiskat River watershed of the Hudson Bay Lowland (HBL) in north-central Canada (52.82° N, 83.91° W; 80 m elevation; Fig. 1). The HBL is a vast peatland plain underlain by limestone bedrock and glaciomarine sediments (Martini 2006). It is the world's third largest wetland, spanning 373,700 km^2 (Abraham and Keddy 2005), with average peat depths of 1.8 m in fens to 2.2 m in bogs (Riley 2011), making it a globally significant carbon sink. Hudson Bay and James Bay heavily influence the region

and create a cool, humid, high-boreal climate, characterized by short cool summers and long cold winters (Abraham and Keddy 2005; Riley 2011). The mean annual temperature is -1.3 °C, with a mean of -22.3 °C in January, and 17.2 °C in July (Lansdowne House: 52.23 N, 87.88 W, 280 km WSW; http://climate.weather.gc.ca/climate_normals). The mean annual precipitation is 700 mm, with 291 mm falling in the growing season from June through August. The Victor Mine recorded 364 mm of precipitation during this same period in 2015, so more than normal, but only 157 mm in 2016 (De Beers Canada, unpublished data).

We studied two ladder fens: an experimental fen (EXP; 52.8547° N, 83.9431° W) and a reference fen (REF; 52.7833 °N, 83.8892° W), which are 8.5 km apart and drain into separate tributaries of the Attawapiskat River. The experimental fen had a total area of 9800 m^2 (2240 m^2 pools, 7560 m^2 ridges), a 250 m length with an elevation drop of 0.67 m, an average peat depth of 2.05 m and distinct pool-ridge morphology for about half its length (McCarter and Price 2017a). The reference fen was smaller, with an area of ~4000 m^2, 150 m in length with similar average peat depth and elevation drop to the experimental fen ridge (McCarter 2016). Their hydrology has been described in detail (McCarter and Price 2017a),

Fig. 1. Map of the study site in relation to the Hudson Bay Lowland (HBL) and the two ladder fens. Pools numbers follow the flow gradient. The arrow on pool 1 of the experimental fen shows the point of nutrient amendment. Both fens are shown at the same scale but with different orientation
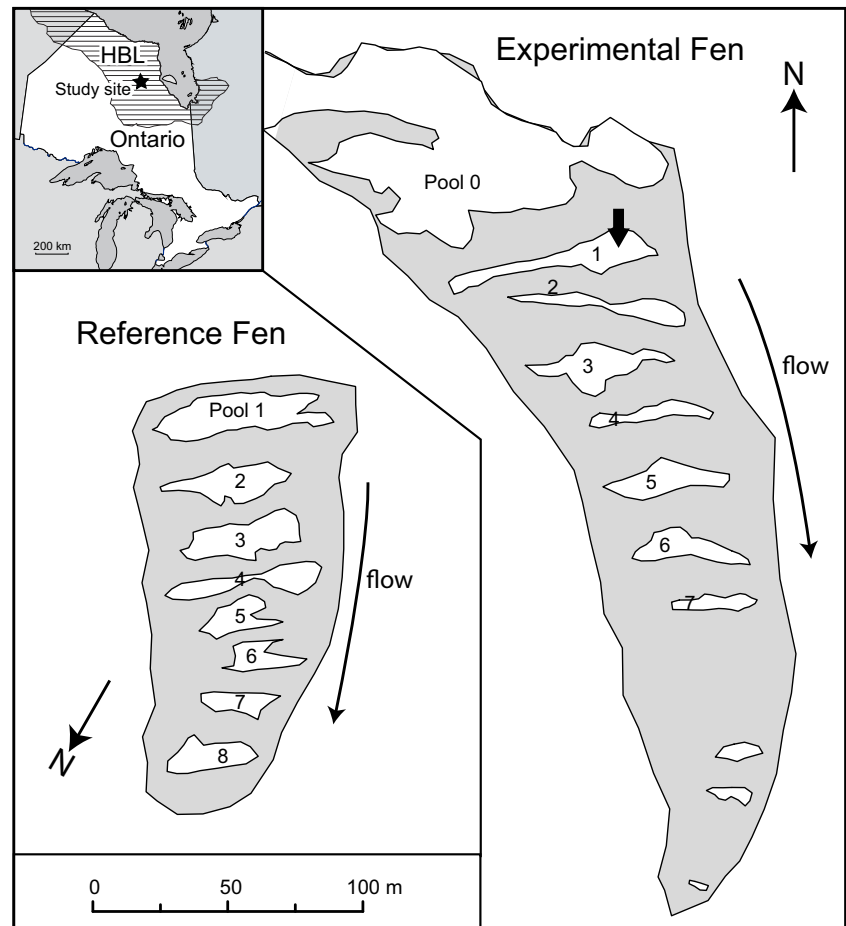
along with the solute transport dynamics (McCarter and Price 2017b) and the nutrient and mercury transport (McCarter et al. 2017). Both had pH of 4 to 6 (McCarter et al. 2017) and poor to intermediate fen vegetation with a ground cover dominated by *Sphagnum* species.

McCarter (2016) added simulated wastewater continually from a point source to the experimental fen for 51 consecutive days in the summer 2014 (July 11 - August 31) and 41 consecutive days in summer 2015 (July 4 - August 14) at an approximate rate of 38 m³ day⁻¹, but not during 2016. We used a Plant Products® custom fertilizer, derived from potassium nitrate, potassium phosphate, potassium chloride, potassium sulphate, and ammonium sulphate and diluted it so the point source contained sulfate (27.2 mg L⁻¹), nitrate (27.2 mg L⁻¹), ammonium (9.1 mg L⁻¹), phosphate (7.4 mg L⁻¹), potassium ion (24.5 mg L⁻¹) and a salt tracer with Na⁺ (25.3 mg L⁻¹) and Cl⁻ (47.2 mg L⁻¹). The rate and composition was designed to mimic domestic, secondarily-treated wastewater produced by the Victor Mine camp. The mine has a year-round population of roughly 200 persons and is required to secondarily-treat their wastewater. An electric trolling motor ensured the nutrient additions were well mixed within the first pool.

We determined differences in temperature regimes among microtopographical positions in 2016 in both fens, by placing HOBO® Pendant temperature loggers at a depth of 10 cm within the first pool and ridge and recording temperature every four hours throughout the 12-week growing season.

In May 2015, we established 72 sampling points in the experimental fen and 18 in the reference fen, using a stratified sampling design. We first selected sample zones, generally at each ridge and pool sequence in the experimental fen and at the start and end of the reference fen. Within each zone, we identified monospecific colonies of *Sphagnum fuscum* on ridge hummocks, *Sphagnum rubellum* along the edge of the pools and in low-lying preferential flow paths between pools, and *Sphagnum majus* within pools. Ridges were 29.6 ± 9.9 cm (mean ± SD, n = 24) above the water table in the experimental fen in June and July 2014, while pool edges and low-lying preferential flow paths were 7.9 ± 9.3 (n = 21) above the water table (C. McCarter, unpublished data). We randomly selected three colonies per species per zone as sampling points.

We measured the productivity of *Sphagnum fuscum* and *S. rubellum* using the crank wire method (Clymo and Reddaway 1974), with five replicate wires per sampling point, but we could not measure the productivity of the semi-aquatic *S. majus*. We measured growth from July 23 to September 8 (6.5 weeks) in 2015 and from June 6 to September 2 (12 weeks) in 2016, but we separated the 2016 season into two ~6-week periods, from June 6 to July 27 and from July 28 to September 2, the second providing a comparable time fame to the 2015 productivity measurements. We determined the dry mass per length without capitula and measured mean density of *S. fuscum* and *S. rubellum* by sampling four replicate

measurements from each sampling zone to convert length measurements into productivity per unit area.

We assessed the decomposition of all species the field by means of mass loss over time using the mesh decomposition bag technique (Johnson and Damman 1991). We harvested *Sphagnum* strands from each sample point in the experimental fen and reference fen in July 2015. We removed the top 1 cm of each strand including the capitulum and used the next 5 cm long strand segment. We oven-dried ten 5 cm strands at 30 °C for 48 hours, weighed them, placed them in 0.2 mm-mesh nylon bags and heat-sealed them closed. We returned the decomposition bags to the exact same sampling points in the field from which the *Sphagnum* strand samples originated and buried them to a depth of 10 cm, with two replicate bags per sampling point. We also dried a subsample of each group of *Sphagnum* strands at 70 °C for 48 hours to calculate a conversion factor between 30 °C to 70 °C drying temperatures. We retrieved the decomposition bags one year later in July 2016 (82% recovery), rinsed the bags with deionized water, oven-dried them at 70 °C for 48 hours, separated the remaining *Sphagnum* strands and weighed them. We calculated decomposition mass loss by correcting the initial mass at 30 °C using the calculated conversion factor. For *S. fuscum* and *S. rubellum*, we divided the mass lost by the *Sphagnum* density value from each sampling location to determine the mass lost per unit area. We did not measure the density of *S. majus*, so we could not convert its decomposition to unit area.

In August of 2016, we collected samples of *S. fuscum*, *S. rubellum*, and *S. majus* from each sampling location to determine nutrient content and stoichiometry. We again removed the capitulum and retained the next 5 cm long segment of each *Sphagnum* strand. We air-dried samples for 30 days at room temperature, ground them with a ball mill and had them analyzed at the Ontario Forestry Research Institute (OFRI) for total C and N using an elemental combustion analyzer (Vario MAX Cube CN); total Kjeldahl N using a Brann and Luebbe Technicon Traacs 800 run off a selenium dioxide digestion following industrial method 786-86T; total S using a carbon/sulfur combustion analyzer (ELTRA® CS-800); and total P, K, Ca and Mg using inductively coupled plasma (ICP; Genesis FEE ICP OES) run off the same selenium dioxide digestion as for total Kjeldahl N. Total N from combustion and the Kjeldahl technique were strongly correlated (r = 0.998), so we only report the combustion N results.

We first conducted a principal component analysis of the macronutrient data (N, S, P, K, Ca and Mg) to reduce data complexity and used the first principal component (PC1) in subsequent analyses. We first tested whether overall differences in productivity, decomposition, PC1, and C:N and N:P ratios existed among species using univariate analyses of variance, with Tukey post-hoc comparisons for the nutrient data. We also compared the growth, decomposition, and nutrient data within species using Pearson correlations. We then tested

for differences between the two fens in these variables, using distance downgradient in the fens as a covariable and the fen by distance interaction. We measured the distance downstream in the experimental fen as the distance from a central line in the first pond, since this pond was well mixed (McCarter 2016). We tested for linear regressions of these dependent variables separately within each fen against distance downgradient. In the experimental fen, we also used segmented regression to explore for breakpoints in the shape of the individual regressions downgradient from the nutrient source. We chose segmented regression models only if they were superior to the linear regression models, as determined from their type I error rate. We analyzed most data using general linear models with Statistica® version 10, but we examined for segmented or step relationships using SegReg® software (https://www.waterlog.info/segreg.htm). We verified homogeneity of variance and normality using residual plots. We applied a 5% type I error rate for statistical significance but noted borderline effects up to a 10% error rate.

Results

Temperatures at a depth of 10 cm within both fens were consistently warmer in pools than in ridges by 2.5 to 3.5 °C on average over the 2016 growing season, with median temperatures in August, the hottest month, of 16.4 °C in pools and 14.2 °C in ridges.

When we consider all sampling points, *Sphagnum fuscum* did not differ from *S. rubellum* in overall productivity at any time ($F_{1, 55} < 1.2$, $P > 0.29$). When we look at each species individually, the 6-week rates of productivity of *S. fuscum* were only weakly correlated between 2015 and 2016 ($r = 0.46$) and the overall productivity did not differ between the fens over any time period ($F_{1, 21} < 0.4$, $P > 0.56$; Table 1, Fig. 2, Online Figs. S1-S4). When we examined for effects downgradient, the productivity of *S. fuscum* did not change in the reference fen in either year ($F_{1, 4} < 1.4$, $P > 0.30$), nor in the experimental fen in 2015 ($F_{1, 17} = 2.4$, $P = 0.14$), but it showed segmented relationships in 2016 (6-weeks: $F_{3, 15} = 5.0$, $P = 0.013$; 12-weeks: $F_{3, 15} = 3.3$, $P = 0.05$), with slightly higher productivity closer to the discharge point, declining

until a common breakpoint at 48 m, after which there was no change.

For *Sphagnum rubellum*, its 6-week rates of productivity were strongly correlated between 2015 and 2016 ($r = 0.93$), and its overall productivity was greater within the experimental fen than the reference fen at all time periods (2015 6-weeks: $F_{1, 21} = 8.1$, $P = 0.009$; 2016 6-weeks: $F_{1, 22} = 8.1$, $P = 0.016$; 2016 12-weeks: $F_{1, 22} = 6.9$, $P = 0.015$; Table 1, Fig. 2 and Online Figs. S1-S4). Its productivity in the reference fen increased slightly with distance downgradient in 2015 ($F_{1, 4} = 8.8$, $P = 0.041$) but did not change in 2016 ($F_{1, 4} = 0.1$, $P > 0.76$), whereas in the experimental fen, its productivity showed strong segmented relationships ($F_{3, 16} > 27.5$, $P < 0.0001$), with much greater rates near the nutrient point source, until breakpoints of 33 to 37 m downgradient, after which there was no change, with rates similar to the reference fen.

Overall, the one-year decomposition per unit area did not differ between *S. fuscum* and *S. rubellum* ($F_{1, 53} = 2.4$, $P = 0.12$), and did not significantly correlate with any measure of productivity ($r < 0.36$). The rate of decomposition of *S. fuscum* did not change between the fens ($F_{1, 21} = 0.9$, $P = 0.35$), nor did it change with distance downgradient in either fen (REF: $F_{1, 4} = 0.4$, $P = 0.57$; EXP: $F_{1, 16} = 1.8$, $P = 0.20$; Table 1, Fig. 2b and Online Fig. S5). In contrast, the decomposition rate of *S. rubellum* was slightly faster in the experimental fen ($F_{1, 20} = 4.8$, $P = 0.040$). It did not change with distance downgradient in the reference fen ($F_{1, 4} = 4.0$, $P = 0.12$) and in the experimental fen only showed borderline faster decomposition rates near the discharge point, dropping at 31 m in a step relationship ($F_{2, 15} = 2.7$, $P = 0.099$). When we examined for decomposition effects on a percent mass lost basis, *Sphagnum majus* had greater mass lost over one year than either of the two other species (Tukey $P = 0.0001$), which were similar to each other (Tukey $P = 0.39$), and there was no significant effects with distance downgradient in either fen (Online Fig. S6).

At the end of the 2016 growing season, the first principal component (PC1) of nutrient content within the three *Sphagnum* species explained 74% of the total variation (Online Fig. S7). It was strongly correlated with N, S, P, K, and Mg content ($r > 0.90$) so reflects our simulated wastewater amendment (Online Table S1). Overall, the PC1 of *S. majus* was significantly higher than the other two species (Tukey $P > 0.0001$), indicating that it had higher overall nutrient content,

Table 1. Overall rates of productivity during the 2016 growing season and decomposition over one year (2015-2016) of *Sphagnum fuscum* and *S. rubellum* between the reference (REF) and experimental (EXP) fen

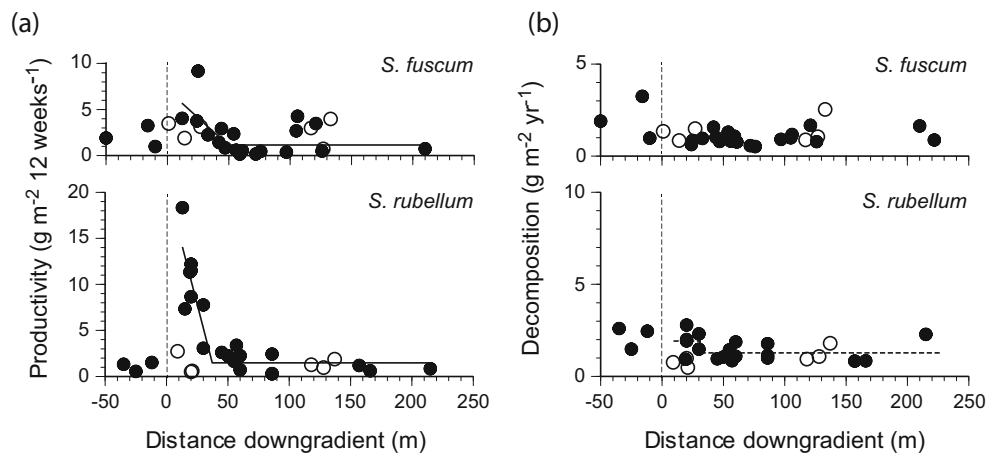| Species	Site	Productivity 2016 (g dm^{-2} 12-weeks^{-1})			Decomposition (g dm^{-2} yr^{-1})		
		n	mean	SE	*n*	mean	SE
Sphagnum fuscum	REF	6	2.69	0.49	6	1.36	0.26
	EXP	22	2.13	0.44	22	1.14	0.13
Sphagnum rubellum	REF	6	1.32	0.35	6	1.01	0.18
	EXP	23	4.44	1.03	21	1.58	0.14

(a)

(b)

Fig. 2. Scatter plots of the (**a**) productivity over 12 weeks during the growing season in 2016 and (**b**) annual decomposition as a function of distance downgradient from the nutrient input point for *Sphagnum fuscum* (top) and *Sphagnum rubellum* (bottom) in the reference fen (open circles) and the experimental fen (solid circles). We only show significant linear or segmented trend lines for the experimental fen downgradient from the discharge point (solid lines: $P < 0.05$; dashed lines: $0.05 < P < 0.10$). Note that productivity is scaled double that of decomposition. The vertical dashed line identifies the discharge point in the experimental fen

while *S. fuscum* and *S. rubellum* did not differ from each other (Tukey $P = 0.11$). PC1 of *S. fuscum* did not change between fens ($F_{1, 18} = 0.01$, $P = 0.92$), nor did it change in either fen as a function of distance downgradient (REF: $F_{1, 5} = 1.1$, $P = 0.34$; EXP: $F_{1, 13} = 0.5$, $P = 0.47$; Fig. 3a). For *S. rubellum*, PC1 was borderline higher in the experimental fen than the reference fen at the end of 2016 ($F_{1, 15} = 3.7$, $P = 0.075$), reflecting

higher nutrient content. It did not change downgradient in the reference fen ($F_{1, 4} = 0.0$, $P = 96$), but there was a borderline step decline of PC1 with distance downgradient in the experimental fen ($F_{2, 10} = 3.6$, $P = 0.065$), with a breakpoint at 51 m. For *S. majus*, PC1 was much higher in the experimental fen than the reference fen ($F_{1, 17} = 16.8$, $P = 0.0007$), reflecting higher nutrient content in the pools of the
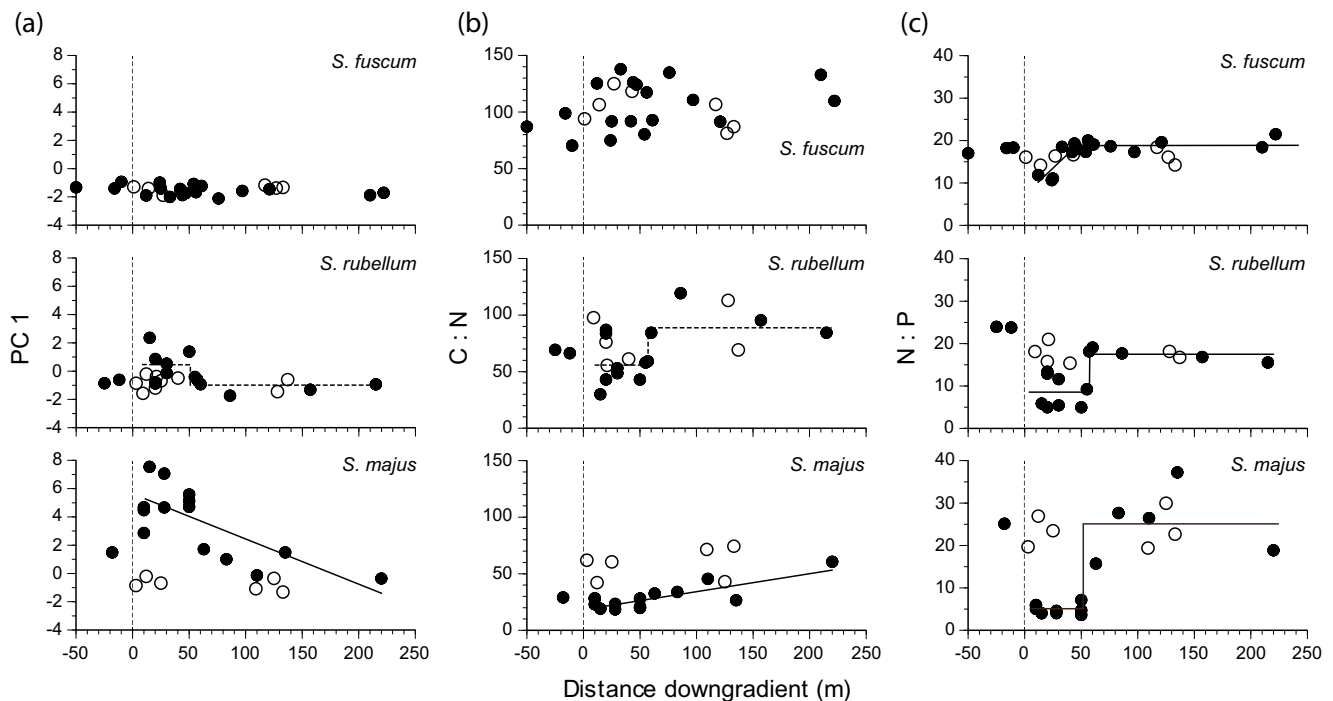
(a)   (b)   (c)

Fig. 3. Regression plots of the (**a**) first principal component (PC1), (**b**) C:N ratios and (**c**) N:P ratios of nutrient content in *Sphagnum fuscum* (top) and *S. rubellum* (middle) and *S. majus* (bottom) as a function of distance downgradient in the reference fen (open circles) and the experimental fen (solid circles). The PC1 is strongly correlated to the nutrient content ($r > 0.90$). We only show significant linear or segmented trend lines for the experimental fen downgradient from the discharge point ($P < 0.05$; dashed lines: $0.05 < P < 0.10$). The vertical dashed line identifies the discharge point in the experimental fen

experimental fen. It declined strongly and linearly in the experimental fen with distance downgradient ($F_{1, 12} = 15.7$, $P = 0.002$), but not in the reference fen ($F_{1, 4} = 0.8$, $P = 0.41$).

The C:N ratio of the three *Sphagnum* species differed at the end of the 2016 season (Tukey $P = 0.0002$ from each other), with *S. fuscum* having the highest C:N on average and *S. majus* the lowest (Fig. 3b). *S. fuscum* showed no difference in C:N between fens ($F_{1, 18} = 0.3$, $P = 0.61$) and neither was there any relationship against distance downgradient in either fen (REF: $F_{1, 5} = 2.1$, $P = 0.21$; EXP: $F_{1, 13} = 0.4$, $P = 0.54$; 104.7 ± 4.0, mean ± SE). C:N of *S. rubellum* also did not differ overall between fens ($F_{1, 15} = 0.9$, $P = 0.35$; 71.3 ± 5.1; mean ± SE), again with no effect from distance downgradient in the reference fen ($F_{1, 4} = 0.4$, $P = 0.55$), but there was a borderline step increase in the experimental fen ($F_{2, 10} = 3.5$, $P = 0.070$), with lower C:N around 56 until a step rise at a breakpoint at 57 m to C:N of 99. For *S. majus*, C:N was much lower in the experimental fen ($F_{1, 17} = 21.9$, $P = 0.0002$; REF: 59 ± 6, EXP: 29, mean ± SE) and increased linearly with distance downgradient in the experimental fen ($F_{1, 12} = 24.6$, $P = 0.0003$), from around 25 in the first pool to 60 near the end of the fen.

Overall, the *Sphagnum* species did not have different N:P ratios from each other at the end of the 2016 season ($F_{2, 64} = 0.6$, $P = 0.55$; Fig. 3c). The N:P of *S. fuscum* did not differ between fens ($F_{1, 18} = 0.1$, $P = 0.72$) and did not change with distance downgradient in the reference fen ($F_{1, 5} = 0.1$, $P = 0.76$), but it showed a significant segmented relationship in the experimental fen ($F_{1, 13} = 5.8$, $P = 0.0078$), with N:P of near 10 close to the discharge point, rising to near 20 after a breakpoint at 44 m, similar to the N:P across the reference fen. For *S. rubellum*, N:P was lower in the experimental fen ($F_{1, 15} = 7.2$, $P = 0.017$), and showed no change with distance in the reference fen ($F_{1, 13} = 0.4$, $P = 0.2$), but showed a step relationship with distance downgradient in the experimental fen ($F_{2, 10} = 12.0$, $P = 0.002$), again with lower N:P ratios around 10 closer to the discharge point, rising to just under 20 after the breakpoint at 57 m. Finally for *S. majus*, N:P was lower in the experimental fen ($F_{1, 17} = 5.6$, $P = 0.031$). There was no change with distance downgradient in the reference fen ($F_{1, 4} = 0.1$, $P = 0.76$), but the experimental fen had a strong step relationship with distance downgradient ($F_{2, 11} = 24.6$, $P = 0.0001$), with N:P near 5 until a breakpoint at 52 m, after which N:P remained around 20. This change in N:P ratio is also visible in a ternary diagram of N, P and K, and also shows that changes in K ratios do not occur (Online Fig. S8).

Discussion

A point source addition of simulated secondarily-treated wastewater, as we expected, increased *Sphagnum* productivity within the experimental ladder fen and also increased the nutrient content in *Sphagnum* (PC1), decreased the C:N ratios and decreased the N:P ratios, but we only found these effects within 50 m from the discharge point, despite two years of continuous additions of simulated wastewater during the 2014 and 2015 growing seasons. These effects extended from the first pool through to the third ridge of the experimental fen (three pools and two ridges), and beyond this point, productivity and nutrient data were comparable to the reference fen. McCarter et al. (2017) found roughly similar distances for nutrient transport, with NH_4^+ extending a bit further to the fourth pool, but PO_4^{3-} extending only to the second pool.

We also found, as we had hypothesized, that productivity and nutrient uptake were strongly linked to the microtopographical position of the *Sphagnum* species. The first few pools of the experimental fen had high hydrological connectivity, especially in 2015 when precipitation was above normal (McCarter and Price 2017a). *S. majus* in the pools was most affected, at least in terms of nutrient content, while *S. rubellum* along the edges of the pools was also strongly affected. Although we did not measure the productivity of *S. majus*, we expect that it would have followed its nutrient trends, showing a large effect. *Sphagnum* species from different microtopographical positions differ in their innate productivity. Species in the Cuspidata section of *Sphagnum*, such *S. majus* typical of pools, have higher innate growth, while those in the Acutifolia section, such as S. fuscum and *S. rubellum*, typical of hummocks and carpets, have lower innate growth rates (Bengtsson et al. 2016). Differences also exist in environmental variables between hummocks and low-lying zones along pool edges (Damman 1978; Clymo and Hayward 1982). The strong responses of *S. rubellum* for growth and nutrient relations and *S. majus* for nutrient relations must reflect their contact with the nutrient-enriched waters.

In contrast, *Sphagnum fuscum*, the species dominant in the ridges, was not affected in 2015 and was least affected in terms of productivity and nutrient content in 2016. While the innate growth of *S. fuscum* is slightly lower than *S. rubellum* (Bengtsson et al. 2016), it may have had a delayed and smaller magnitude productivity response simply because of its more than 30 cm elevation above the nutrient-enriched water table. Hummock species such as *S. fuscum* rely on passive capillary rise of water during periods of water stress (Clymo and Hayward 1982; Thompson and Waddington 2008). The above-normal precipitation in 2015 may have limited the capillary rise of nutrients and their uptake by *S. fuscum*, while the below normal precipitation in 2016 may have allowed for greater capillary rise and nutrient uptake. The ridges were also colder than pools by 2.5-3.5 °C at 10 cm depth, which may further contributed to reduced *S. fuscum* production in the ridges in contrast to the pools, although a similar temperature difference was insufficient to affect productivity in *Sphagnum balticum* (Granath et al. 2009). The temperature difference

between ridges and pools did not appear to affect decomposition.

Step shifts in N:P in all three *Sphagnum* species appears to be the most sensitive indicator of change in these nutrient-poor subarctic peatlands, more than increases in productivity and nutrient content (PC1) or decreases in C:N ratios. Koerselman and Meuleman (1996) considered plants with N:P ratios >16 to be P-limited, and those with N:P < 14 to be N-limited. All three of our *Sphagnum* species had background N:P near 20 in the reference fen and upgradient from the discharge point and downgradient beyond 50 m in the experimental fen, and consequently showed strong P-limitation under natural conditions. N:P ratios near 20 are commonly observed under natural conditions at the surface of boreal and temperate bogs and fens across Canada (Wang et al. 2015). But in the first three ponds and two ridges downgradient from the discharge point, N:P was near or below 10 for *S. fuscum* and *S. rubellum*, even below 5 for *S. majus*, indicating shifts to strong N-limitation, especially in the pools. This suggests that P transport extended further downgradient than was observed by McCarter et al. (2017), who only found elevated levels of PO_4^{3-} above 3 mg L^{-1} until the second pool. NPK nutrient enrichment experiments in southern Canada cause shifts in nutrient ratios toward K or K+N limitations (Wang et al. 2016), but our results show a shift toward N-limitation. Although this shift to N-limitation suggests that we could add even more N that would have been retained by *Sphagnum*, especially in the pools, other factors such as temperature, light or the species' maximum intrinsic growth rates may still have limited the ability of *Sphagnum* to convert these nutrients into biomass (Güsewell and Koerselman 2002).

We had also hypothesized that inputs of simulated secondarily-treated wastewater would also increase the rate of *Sphagnum* decomposition in the experimental fen closer to the discharge point, but we found no effect for *S. fuscum* and a limited, borderline effect for *S. rubellum*. *Sphagnum fuscum* has slow inherent rates of decomposition, lower than *S. rubellum* and much lower than *S. majus* (Bengtsson et al. 2016), so we expected to see differences in decomposition rates between *S. fuscum* and *S. rubellum* over the one year incubation period. *Sphagnum* decomposition also increases with decreasing C:N ratios (Limpens and Berendse 2003; Bragazza et al. 2006), and we observed lower C:N for *S. rubellum* closer to the discharge point, so we expected to have higher decomposition in this zone. Bubier et al. (2007) also did not find significant differences in the rates of decomposition of *S. capillifolium*, another Acutifolia section species similar to *S. rubellum*, after being fertilized for five years with a series of NPK mixtures; increases in rates of decomposition were due to shifts away from *Sphagnum*-dominated communities toward those dominated by vascular plants (Larmola et al. 2013). Likewise, Manninen et al. (2016) did not observe

differences in *Sphagnum* decomposition among long-term fertilization treatments in an incubation experiment. McCarter et al. (2017) suggested that elevated total mercury concentrations in the first ridge of the experimental fen were indicative of increased decomposition, but our field results do not support this suggestion, although we did find greater decomposition of *Sphagnum* closer to the discharge point in a laboratory incubation experiment (Lavallee 2017).

Sphagnum production was much greater than decomposition within 50 m downgradient from the discharge point in the experimental fen after two seasons of simulated secondarily-treated wastewater discharge, allowing for increases in carbon storage, especially around the edges of the pools and to a lesser extent in the ridges. This suggests that the structure of the ridges and hence their hydrological control over this ladder fen was not damaged and may even have been enhanced, at least over a three-year period. How long could this effect be sustained? In a temperate ombrotrophic peatlands Bubier et al. (2007) found lower carbon storage by the third year of fertilization as a result of shifts away from *Sphagnum*-dominated communities toward vascular plant communities. It is not known if and under what timeframe these subarctic ladder fens would switch in dominance toward vascular plant communities. At least through two years of nutrient amendment, *Sphagnum* species dominate and these fens remain as strong carbon sinks. If vascular plants eventually supplant *Sphagnum*, their litter may be less recalcitrant to decomposition, which would shift the carbon storage dynamics of the peatland. Longer term studies of subarctic fens receiving secondarily-treated wastewater would be needed to address this issue.

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