Soil Biology & Biochemistry 79 (2014) 91–99

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Effects of sulfate deposition on pore water dissolved organic carbon, nutrients, and microbial enzyme activities in a northern peatland

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ARTICLE INFO

Article history:
Received 22 April 2014
Received in revised form
30 July 2014
Accepted 7 September 2014
Available online 19 September 2014

Keywords:
Atmospheric deposition
Dissolved organic carbon
Peatland
Phosphorus
Microbial enzyme activity

ABSTRACT

Export of dissolved organic carbon from lakes and streams has increased throughout Europe and North America over the past several decades. One possible cause is altered deposition chemistry; specifically, decreasing sulfate inputs leading to changes in ionic strength and dissolved organic carbon solubility. To further investigate the relationship between deposition chemistry and dissolved organic carbon export in peatlands, a field experiment was conducted to compare the pore water chemistry and peat microbial enzyme activity of mesocosms receiving sulfate amendments to mesocosms receiving no additions. To consider how peatlands respond during recovery from increased inputs of sulfate, samples were also analyzed from an area of the same peatland that was previously amended with sulfate. Current additions of sulfate decreased dissolved organic carbon concentration and increased dissolved organic carbon aromaticity. Total dissolved phosphorus decreased in response to current sulfate amendments but was elevated in the area of the peatland recovering from sulfate amendment. The total dissolved phosphorus increase, which was reflected in microbial enzyme activity, may have shifted the system from P limitation to N limitation. This shift could have important consequences for ecosystem processes related to plant and microbial communities. It also suggests that the recovery from previous sulfate amendments may take longer than may be expected.

1. Introduction

In the last 25 years, dissolved organic carbon concentrations have been rising in freshwater systems across Europe and North America (Freeman et al., 2001; Evans et al., 2005; Monteith et al., 2007; Hruska et al., 2009). Dissolved organic carbon is an important source of energy in aquatic systems (Wetzel, 1992) and changes in its concentration may directly affect the dynamics of energy flow. In addition, dissolved organic carbon has been shown to influence pH (Eshleman and Hemond, 1985), metal cycling (Lawlor and Tipping, 2003), and light regime (Morris et al., 1995), which may in turn impact primary production (Urban et al., 1989).

The potential effects of increased dissolved organic carbon concentrations can have important implications for receiving waters, but there is also concern that rising export of dissolved organic carbon from the world's peatlands may indicate a loss of C sequestration in these systems (Freeman et al., 2001) If large amounts of carbon are lost from these systems in the dissolved form, the effects on receiving waters could be far-reaching. Conversely, if carbon is lost primarily in the form of carbon dioxide or methane, global climate change may be exacerbated by the large flux of greenhouse gases. The cause of increasing dissolved organic carbon export is therefore of great interest, especially if measures could be taken to slow the loss of carbon.

Over the past decade, there have been several proposed causes for rising dissolved organic carbon export based on studies of a variety of systems. Many of the potential drivers may be tied to climate change, including increased rates of microbial activity and decomposition due to rising temperature (Freeman et al., 2001; Clark et al., 2005), increasing concentrations of atmospheric carbon dioxide (Freeman et al., 2004a), water table changes that allow aerobic processes to occur (Freeman et al., 2004b), and hydrological changes, including alterations in discharge (Pastor et al., 2003), flowpath (Schindler et al., 1992), and seasonal fluctuations in precipitation, particularly drought-rewetting cycles (Mitchell and

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http://dx.doi.org/10.1016/j.soilbio.2014.09.007
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McDonald, 1992). Changes in deposition chemistry have also been investigated and linked to increased dissolved organic carbon export in several types of systems. It has been suggested that sulfate deposition, which has been declining since emissions standards were tightened in the 1970's, may be linked to trends of increasing dissolved organic carbon export (Evans et al., 2006; Monteith et al., 2007). If this change is driving the increase in dissolved organic carbon export, the response may be a sign of recovery from acid rain inputs rather than evidence of ecosystem degradation.

Few studies have examined the influence of deposition chemistry on dissolved organic carbon production specifically in peatlands. Although dissolved organic carbon has been shown to decrease in conjunction with an increase in sulfate concentration during drought years (Clark et al., 2005), the decrease in dissolved organic carbon may be due to reduced solubility under conditions of increased ionic strength, a relationship that has been documented in past research (Evans et al., 1988; Tipping and Hurley, 1988; Hruska et al., 2009).

To further investigate the relationship between atmospheric deposition and dissolved organic carbon export in peatlands, we installed mesocosms to isolate small areas of a bog and amended them with sulfate. The pore water chemistry, peat stoichiometry and microbial enzyme activities of amended mesocosms were compared with those of unamended control mesocosms. We hypothesized that sulfate amendments would decrease dissolved organic carbon concentrations, a change that could drive a response of nitrogen (N) and/or phosphorus (P). We also measured microbial enzyme allocation, which has been shown to shift in conjunction with changes in dissolved organic carbon and nutrients (Sinsabaugh and Moorhead, 1994; Allison and Vitousek, 2005; Sinsabaugh et al., 2009). This shift allows the microbial community to compensate for a discrepancy between the community's stoichiometric ratio and that of its resources. This compensation occurs as the community produces fewer enzymes to acquire a relatively abundant nutrient in order to focus more energy to produce enzymes to acquire the more limiting nutrient. For example, previous studies have shown that the activity of phosphatase, an enzyme utilized for phosphorus acquisition, increases as the availability of phosphorus decreases, indicating that the enzyme was produced to compensate for a limitation of phosphorus (Sinsabaugh, 1994).

In addition to assessing the effects of current sulfate amendments on dissolved organic carbon concentration and enzyme activity, we also sampled peat that had received sulfate amendments 3–5 years prior to this study. The goal was to gauge how long recovery from sulfate enrichment might take by comparing the recovering peat to both control peat and peat currently amended with sulfate. We expected that if the system had recovered, dissolved organic carbon and nutrient concentrations and microbial enzyme allocation would resemble control peat. However, if recovery was ongoing, the concentration of dissolved organic carbon and nutrients and pattern of enzyme allocation would presumably fall somewhere between the control and currently sulfate amended peat.

2. Materials and methods

2.1. Study site

Samples were collected from the S-6 peatland of the U.S. Forest Service's Marcell Experimental Forest (Fig. 1A) in North-central Minnesota USA (47°32′ N, 93°28′ W). The S-6 watershed (Fig. 1B) has an area of 8.9 ha with a narrow 2.0 ha peatland (Kolka et al., 2011). The upland forest of the watershed was clear cut in 1980, grazed with cattle for two years, and re-planted with white spruce and red pine in 1983. In 1987, the herbicide Garlon 4 was applied to kill willow, paper birch and hazel (Kolka et al., 2011). Additionally, a study of the effects of sulfate deposition on methylmercury production was carried out from 2001 until 2008 in which the upstream half of the peatland served as the control while the downstream half received sulfate amendments at a rate of 32 kg/ha/year via a sprinkler system (Jeremiason et al., 2006; Kolka et al., 2011).

2.2. Field sampling

Mesocosms with a diameter of approximately 48 cm and a height of approximately 66 cm were driven into hollows of the upstream half (Fig. 1B) of the peatland (which has received no prior enrichments). Specific locations were chosen to reflect dominant vegetation while avoiding close proximity to *Alnus incana* (speckled alder), a common wetland shrub known to have nitrogen-fixing bacteria associated with its roots. Treatments included sulfate and controls, with five replicates of each. Amendments were added monthly in the form of Na_2SO_4 in May 2011 through September 2011. The volume of each amendment was small (50 mL) in order to maintain a consistent pH within the mesocosms. Over the course of the field season, enrichments simulated an additional 46 kg SO_4^{2-}/ha above ambient deposition of 4.26 kg/ha (National Atmospheric Deposition Program) or a 10X treatment.

Samples were collected monthly from June 2011 through October 2011 and included both peat and pore water samples. Peat samples were taken from each mesocosm and divided into upper, middle and lower layers. The upper layer consisted of *Sphagnum* moss, which was pulled from the surface of a small area of each mesocosm prior to the collection of the middle and lower layer with a 5 cm diameter Russian peat corer. The depths of the middle and lower layer peat samples corresponded to middle and lower pore water samples, with the middle layer generally reaching a depth of approximately 40 cm and the lower layer making up the remainder of the core, typically reaching depths of 60–70 cm. Cores were spaced as evenly as possible in order to maintain a space of intact peat between each core. Holes from previous samples were maintained through the remainder of the field season and became filled with water.

Pore water was collected from each mesocosm by hand pumps and included a surface, middle, and lower sample. Upper samples were collected through tygon tubing with hand pumps. Middle and lower samples were collected by pumping through Tygon tube piezometers installed in the middle of each mesocosm to depths of 30 and 60 cm, respectively.

Peat and pore water samples were also collected from the downstream portion of the S-6 peatland (Fig. 1B; recovery area (RA) in this study), which received prior sulfate amendments as described in paragraph 2.1. The prior enrichment of the downstream portion was phased out in two stages in the direction of water flow. The first half (RA-1 in this study) received amendments for five years (2001–2006) while the remainder (RA-2 in this study) received amendments for seven years (2001–2008; Kolka et al., 2011). Due to difficulties during installation (presumably due to greater sedge cover), mesocosms in RA-2 were not installed to the same depths as those in the upstream half or RA-1. Because amendments were not being added to these mesocosms, the effect of this discrepancy is assumed to be minimal. Sampling of all mesocosms occurred on the same days using the same methods.

2.3. Laboratory methods

Each pore water sample was filtered first through an ashed GF/C pre-filter and then through a 0.45 μm membrane filter. Analyses included total dissolved nitrogen, total dissolved phosphorus, dissolved organic carbon, sulfate, and specific UV absorbance. Samples

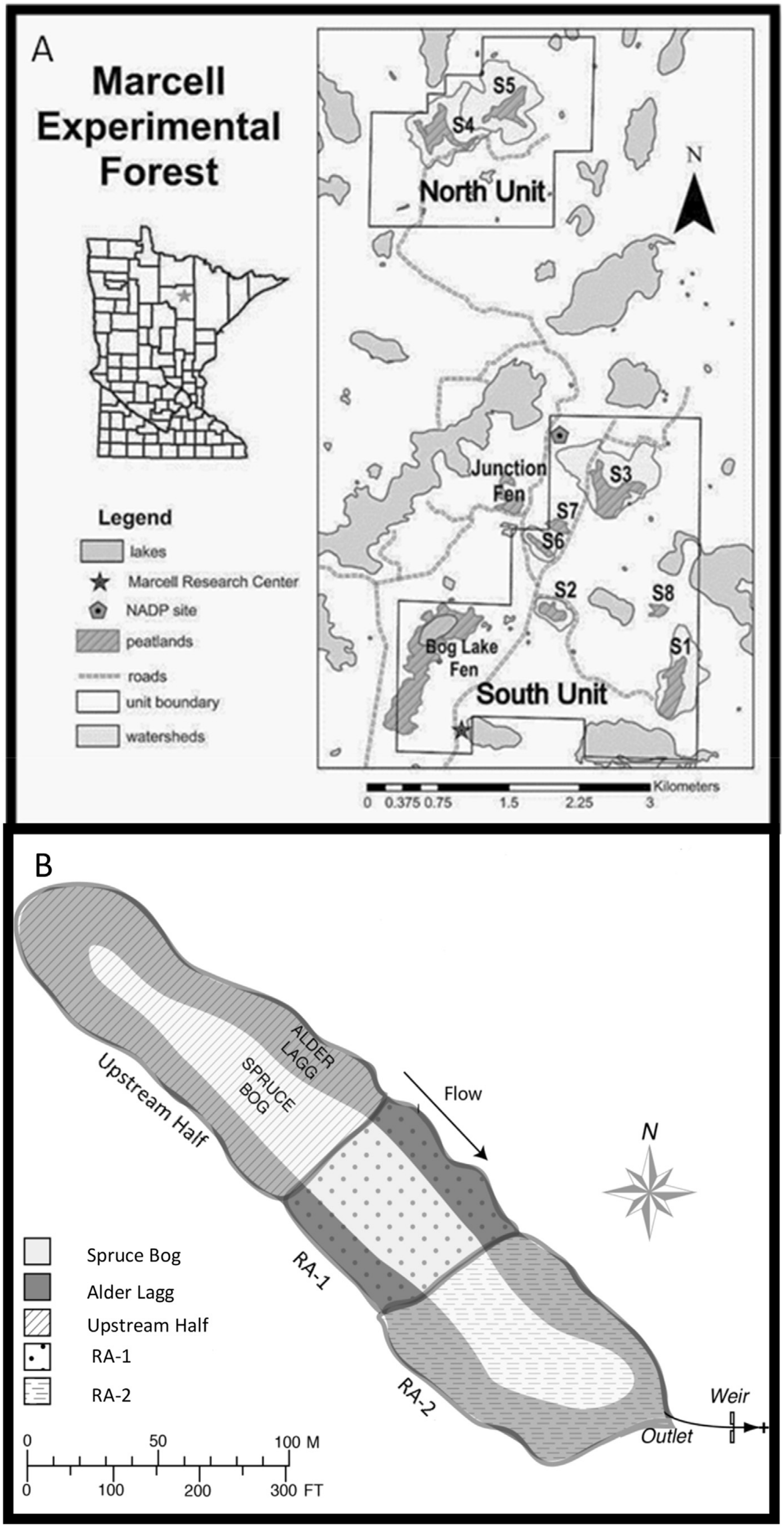

Fig. 1. Map of the Marcell Experimental Forest located in Marcell, Minnesota, USA (A) and approximate locations of sampling (B) in upstream half (1), RA-1 (2), and RA-2 (3).

for total dissolved nitrogen and total dissolved phosphorus were prepared using a persulfate digestion. This was followed by analysis with the Lachat Quikchem using the cadmium reduction method for NO_3–N, the phenolate method NH_4–N, and the molybdate-ascorbic acid method for PO_4–P (APHA, 1998). Samples were analyzed for dissolved organic carbon using the UV–persulfate method and for sulfate using ion chromatography (APHA, 1998). Filtered samples were read at 254 nm and absorbance readings were used in conjunction with dissolved organic carbon measurements to calculate specific UV absorbance ($SUVA_{254}$), which provides an estimate of the aromaticity, and therefore recalcitrance of dissolved organic carbon (EPA Method 415.3).

Peat soil samples were stored frozen in sealed plastic bags until preparation for analysis. Dried samples were ground for total C, N, and P analyses. Total C and N were measured using the combustion method on a Thermo Electron NC Soil Analyzer while total P was measured using a microwave digestion followed by analysis using the molybdate-ascorbic acid method (APHA, 1998). Thawed peat samples were analyzed for dehydrogenase activity, a measure of microbial respiration, and corrected by dry weight and carbon content as described by Hill et al. (2010). Enzyme activities were measured using methods developed by Sinsabaugh and colleagues (Sinsabaugh et al., 1997; Foreman et al., 1998; Sinsabaugh and Foreman, 2001) and included five C-acquiring glycosidases (α-D-galactosidase, β-D-galactosidase, cellobiosidase, β-D-glucosidase, β-D-xylosidase), two N-acquiring aminopeptidases (L-alanine, L-leucine aminopeptidase); and two esterases for P- and S-acquisition (phosphatase and sulfatase, respectively). Freezing is a preferred storage method for soil enzyme samples that cannot be immediately analyzed (Lee et al., 2007), and it was required in this study due to the large number of samples that were collected. Freezing has been shown to have varied effects on some enzyme activities (particularly β-N-acetylglucosaminidase and phosphatase) and minimal effects on others (DeForest, 2009). The activity of β-N-acetylglucosaminidase results in the acquisition of both C and N. For the purpose of data analysis, its activity was evenly divided between the acquisitions of the two elements. Microplates were incubated at ambient temperature, except L-alanine and L-leucine microplates, which were incubated at 30 °C. Results were adjusted for emission and extinction coefficients and corrected for quenching, incubation time, weight and carbon content.

2.4. Statistical analysis

To examine the effects of the amendments added during this study, data from the upstream half were separated from the recovery areas. Because depth was nearly always a significant factor, data were further divided so that upper, middle, and lower samples were analyzed independently from each other. All statistical analyses were performed in SAS 9.2 and included each combination of amendment and depth pooled across sampling dates. Mesocosms receiving amendments were compared to controls while mesocosms in the recovery areas were compared to control mesocosms, sulfate amended mesocosms and each other. Data were analyzed using repeated measures ANOVA. Significant results were tested further using Dunnett's test for comparisons within the upstream half and Tukey's test for comparisons between the amendment and recovery areas.

Due to clogging piezometers, lower pore water samples were difficult to collect in RA-2, with only one mesocosm reliably producing enough water for all analyses. Because data were limited for the other pore water parameters, only dissolved organic carbon and $SUVA_{254}$ are reported for lower samples in RA-2.

Dehydrogenase and enzyme activities were natural log transformed prior to analysis to normalize variances and follow the typical convention for these types of data. To explore possible shifts in enzyme allocation, total glycosidase, aminopeptidase, phosphatase, and sulfatase activities were each divided by total enzymatic activity to obtain the percentage of activity devoted to carbon, nitrogen, phosphorus and sulfur acquisition, respectively. These percentages were compared using repeated measures ANOVA.

3. Results

3.1. Sulfate amendment

Dissolved organic carbon concentration declined significantly only in middle layer pore water sample amended with S (Fig. 2, Table 1). In addition, sulfate amendments increased dissolved organic carbon aromaticity in both the middle and lower layers compared to controls ($P = 0.0001$ and 0.0011, respectively). Neither microbial enzyme allocation nor microbial respiration changed in response to sulfate amendment. Total dissolved phosphorus concentration decreased significantly in pore water in sulfate amended peat at all sample depths (Fig. 3, Table 1). Surface pore water sulfate concentrations were significantly greater in sulfate amended peat than in controls in the September and October samplings. Because there were no obvious effects of sustained increases in sulfate concentration on other parameters, data for all five months were pooled.

3.2. Recovery areas

Concentrations of dissolved organic carbon in upper pore water samples collected in the recovery areas did not differ significantly from either controls or sulfate amended mesocosms. Dissolved organic carbon concentrations in middle layer samples from RA-2 were significantly higher than those in sulfate amended samples of the same depth. In lower layer samples, there was significantly more dissolved organic carbon in both recovery areas as compared to control and sulfate amended mesocosms in the upstream half (Fig. 4; Table 3).

Dissolved organic carbon in both recovery areas was less aromatic than in sulfate amended mesocosms at all depths except lower layer samples of RA-1. RA-2 had significantly less aromatic dissolved organic carbon than RA-1 for middle and lower samples. In addition, dissolved organic carbon aromaticity in RA-2 was significantly less than controls in lower layer samples (Tables 2 and 3).

There was significantly more P present in pore water from all depths in recovering peat compared to peat currently amended

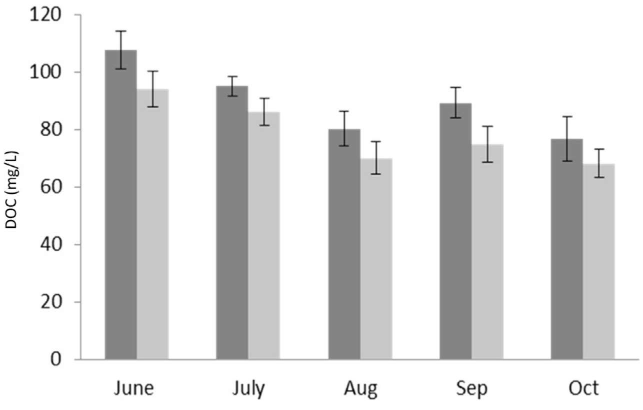

Fig. 2. Dissolved organic carbon (DOC) concentrations in middle pore water samples. Controls are represented by dark gray bars while S amendments are represented by light gray bars ($P = 0.0060$).

Table 1
Summary data averaging peat and water samples from upstream half.

	Control	+S
Upper		
Peat C:N	45.6	47.8
Peat C:P	609.9	746.2
Peat N:P	13.8	15.5
DOC:TDN	20.7	19.6
DOC:TDP	310.0	334.1
TDN:TDP	14.8	18.0
DHA (nmol INT gC^{-1} h^{-1})	12.24	12.26
%Aq$_{C}$	58.16	58.28
%Aq$_{N}$	22.08	21.84
%Aq$_{P}$	13.16	13.48
%Aq$_{S}$	6.47	6.30
TDN (ppb)	2341.0	2199.0
TDP (ppb)	301.8	173.4[a]
SO$_4$ (ppm)	0.17	1.46[a]
DOC (ppm)	47.8	42.9
SUVA$_{254}$	4.3	4.6
Middle		
Peat C:N	23.4	24.7
Peat C:P	836.1	844.5
Peat N:P	35.9	34.1
DOC:TDN	40.9	35.8
DOC:TDP	560.2	681.0
TDN:TDP	11.4	17.8
DHA (nmol INT gC^{-1} h^{-1})	12.04	12.12
%Aq$_{C}$	60.76	60.16
%Aq$_{N}$	21.76	22.24
%Aq$_{P}$	10.96	10.96
%Aq$_{S}$	6.42	6.56
TDN (ppb)	2411.0	2214.0
TDP (ppb)	415.2	158.3[a]
SO$_4$ (ppm)	0.18	0.21
DOC (ppm)	90.4	78.6[a]
SUVA$_{254}$	4.2	4.5[a]
Lower		
Peat C:N	23.4	24.4
Peat C:P	925.5	899.4
Peat N:P	39.1	36.8
DOC:TDN	31.6	30.4
DOC:TDP	372.1	754.1[a]
TDN:TDP	12.29	23.32[a]
DHA (nmol INT gC^{-1} h^{-1})	12.05	12.01
%Aq$_{C}$	65.12	62.17
%Aq$_{N}$	16.76	19.58
%Aq$_{P}$	11.52	11.46
%Aq$_{S}$	6.50	6.83
TDN (ppb)	2272.0	2359.0
TDP (ppb)	338.5	160.5[a]
SO$_4$ (ppm)	0.31	0.24
DOC (ppm)	71.8	66.6
SUVA$_{254}$	3.1	3.7[a]

[a] Result significantly different from control ($P < 0.05$) as determined by Dunnett's test performed on significant ANOVAs ($P < 0.05$).

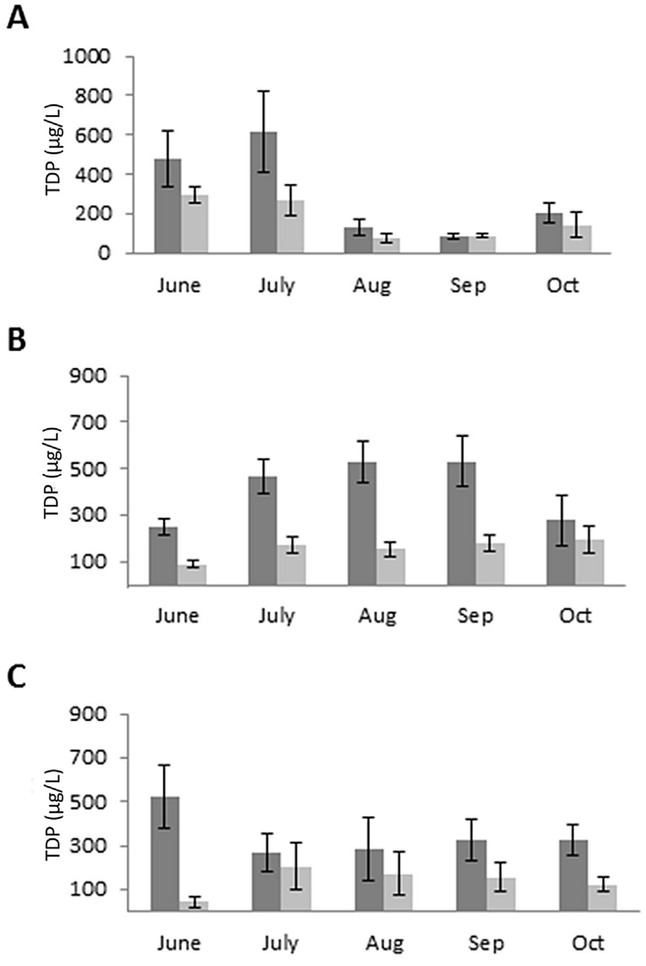

Fig. 3. Total dissolved phosphorus (TDP) concentrations in upper (A) and middle (B) and lower (C) pore water samples. Controls are represented by dark gray bars and S amendments by white bars ($P = 0.0118$, 0.0045 and 0.0048 for upper, middle and lower samples, respectively).

with sulfate. Total dissolved phosphorus concentrations in middle layer samples of RA-1 were significantly greater than controls while RA-2 had significantly greater pore water P than controls at all depths for which data were available. This was also the case for RA-1 as compared to RA-2 (Fig. 5; Tables 2 and 3).

Upper peat samples in the recovery areas had significantly smaller ratios of C:P and N:P than sulfate amended peat. N:P ratios of upper samples from RA-2 were also significantly smaller than controls. Middle layer samples from the recovery areas had significantly greater C:N ratios and significantly smaller N:P ratios than control mesocosms. These differences were also observed in middle layer samples of RA-1 compared to sulfate amended mesocosms (Tables 2 and 3).

Several shifts in enzyme allocation were observed in recovering peat as compared to control and sulfate amended peat. In the upper layer samples of RA-2, the proportion of total enzyme activity represented by P-acquiring activity was significantly smaller than in sulfate amended mesocosms. Decreased acquisition of C was accompanied by increased acquisition of N and S in middle layer samples of RA-1 compared to controls (Fig. 6; Table 3). Acquisition of P in middle layer samples was significantly decreased in RA-2 compared with control, sulfate amended and RA-1 samples. Carbon acquisition decreased in lower layer RA-1 compared to controls. C acquisition increased and N acquisition decreased in lower layer samples from RA-2 compared with sulfate amended mesocosms. These samples were similar to controls except for a significant increase in S acquisition in the RA-2 samples (Tables 2 and 3).

4. Discussion

4.1. Sulfate amendment

The hypothesis that increased sulfate inputs would decrease the concentration of dissolved organic carbon was supported in this study by a significant reduction in dissolved organic carbon concentration in middle layer pore water samples. This finding goes beyond correlative studies to show that while holding other factors constant, changes in the input of sulfate can influence the concentration, and possible export of dissolved organic carbon. Declines in dissolved organic carbon concentration were accompanied by increases in dissolved organic carbon aromaticity, indicating

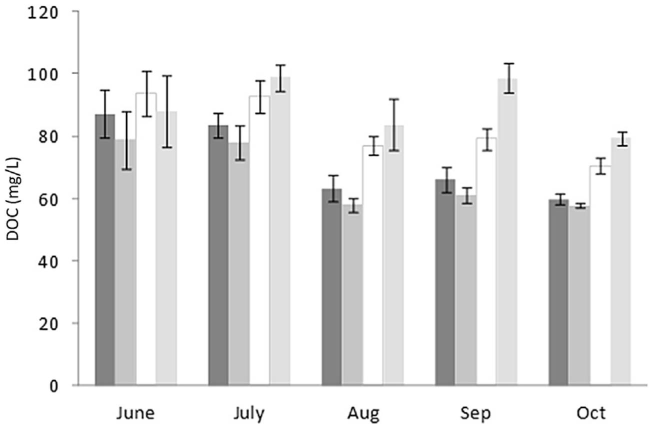

Fig. 4. Dissolved organic carbon (DOC) concentrations in lower pore water samples. Controls are represented by dark gray bars, S amendments by medium gray bars, RA-1 by white bars, and RA-2 by light gray bars.

that the dissolved organic carbon present in sulfate amended mesocosms was more recalcitrant than in control mesocosms. It is possible that the decrease in dissolved organic carbon is related to additional use of C by sulfur-reducing bacteria; additional sulfate was not detected in amended mesocosms in June through August, indicating it was either utilized by biota or escaped from the mesocosms. This could explain the decrease in dissolved organic carbon concentration and the increase in aromaticity, with the microbial community utilizing the more labile dissolved organic carbon, leaving the more recalcitrant forms in the system. However, the decrease in dissolved organic carbon observed in sulfate amended mesocosms was not associated with any change in microbial respiration or enzyme allocation compared to controls. While there is no way to be certain from the data available, it seems likely that the response of dissolved organic carbon is due to reduced solubility, as shown in past laboratory studies (Evans et al., 1988; Tipping and Hurley, 1988; Hruska et al., 2009).

Sulfate amendments resulted in a significant decrease in pore water total dissolved phosphorus concentration at all depths. This change was unexpected because typically, increased S leads to an increase in P availability (Lamers et al., 1998; Beltman et al., 2000; Lamers et al., 2002). The mechanisms driving the usual response are 1) precipitation of iron sulfide, which releases phosphate that was previously bound with iron and/or 2) competition between sulfate and phosphate for soil binding sites (Lamers et al., 1998; Beltman et al., 2000; Lamers et al., 2002). Other outcomes are possible, with one study showing no response of pore water P concentration to Sulfate amendment in a fen (Van Dijk et al., 2012) and another showing decreased labile inorganic P concentrations in a forest amended with N + S (Carreira et al., 2000). The same study also showed increased P sorption capacity and decreased P solubilization and mineralization rates in response to amendments (Carreira et al., 2000). Because iron concentrations are assumed to be low in the peatland and the observed response was a decrease in total dissolved phosphorus concentration rather than an increase, it seems reasonable to conclude that P sorption and solubility were influenced by the addition of sulfate.

4.2. Recovery areas

Despite sulfate pore water concentrations similar to those of controls, it appears that recovery is ongoing in both recovery areas. Contrary to the assumption that a recovered system would resemble controls while a recovering system would yield data between controls and sulfate amendments, several parameters showed neither response. Concentrations of total dissolved phosphorus in both recovery areas were greater than controls and sulfate amended mesocosms. At first glance it seems that with respect to P, the recovery areas responded to the sulfate amendments in the expected way with increased P availability. However because years had passed since the last amendments, it may be possible that the recovery areas initially responded in a manner similar to the upstream half with a decrease in P availability. The observed increase in P availability in this study may have only appeared as the system recovered and the chemical environment of the peat profile responded to the lack of additional sulfate, leading to solubilization of the accumulated P, and therefore higher total dissolved phosphorus concentration in the peatland pore water.

Table 2
Summary data for comparisons of recovery area averages with controls and current S amendment averages. $P < 0.05$ in Tukey's test performed in significant ANOVAs ($P < 0.05$).

	Control	+S	RA-1	RA-2
Upper				
Peat C:N	45.6	47.8	48.2	43.2
Peat C:P	609.9	746.2	562.4^{b}	444.1^{b}
Peat N:P	13.8	15.5	11.4^{b}	10.2^{a,b}
DOC:TDN	20.7	19.6	20.7	18.8
DOC:TDP	310.0	334.1	136.1^{a,b}	77.9^{a,b}
TDN:TDP	14.8	18.0	6.67^{a,b}	4.11^{a,b}
DHA (nmol INT gC^{-1} h^{-1})	12.24	12.26	12.24	12.42
%Aq$_C$	58.16	58.28	58.48	58.32
%Aq$_N$	22.08	21.84	21.84	22.52
%Aq$_P$	13.16	13.48	13.00	12.64^{b}
%Aq$_S$	6.47	6.30	6.96	6.61
TDN (ppb)	2341.0	2199.0	2177.0	2343.0
TDP (ppb)	301.8	173.4^{a}	509.7^{b}	793.7^{a,b,c}
SO$_4$ (ppm)	0.17	1.46^{a}	0.24^{b}	0.39^{b}
DOC (ppm)	47.8	42.9	43.0	42.6
SUVA$_{254}$	4.3	4.6	4.0^{b}	4.0^{b}
Middle				
Peat C:N	23.4	24.7	28.0^{a,b}	27.2^{a}
Peat C:P	836.1	844.5	772.0	782.3
Peat N:P	35.9	34.1	27.3^{a,b}	28.6^{a}
DOC:TDN	40.9	35.8	35.4	35.3
DOC:TDP	560.2	681.0	162.1^{a,b}	81.2^{a,b}
TDN:TDP	11.4	17.8	4.14^{a,b}	2.27^{a,b}
DHA (nmol INT gC^{-1} h^{-1})	12.04	12.12	12.16	12.38^{a}
%Aq$_C$	60.76	60.16	59.32^{a}	60.80^{c}
%Aq$_N$	21.76	22.24	23.08^{a}	22.36
%Aq$_P$	10.96	10.96	10.80	10.00^{a,b,c}
%Aq$_S$	6.42	6.56	6.92^{a}	6.70
TDN (ppb)	2411.0	2214.0	2377.0	2662.0
TDP (ppb)	415.2	158.3^{a}	768.5^{a,b}	1323.9^{a,b,c}
SO$_4$ (ppm)	0.18	0.21	0.22	0.16
DOC (ppm)	90.4	78.6^{a}	81.7	92.3^{b}
SUVA$_{254}$	4.2	4.5^{a}	4.2^{b}	4.0^{b}
Lower				
Peat C:N	23.4	24.4	25.4	23.8
Peat C:P	925.5	899.4	885.1	851.8
Peat N:P	39.1	36.8	34.8	36.2
DOC:TDN	31.6	30.4	35.4^{b}	NA
DOC:TDP	372.1	754.1^{a}	298.8^{b}	NA
TDN:TDP	12.29	23.32^{a}	8.50^{b}	NA
DHA (nmol INT gC^{-1} h^{-1})	12.05	12.01	11.90	11.89
%Aq$_C$	65.12	62.17^{a}	62.67	66.10^{b}
%Aq$_N$	16.76	19.58	18.88	15.24^{b}
%Aq$_P$	11.52	11.45	11.88	11.62
%Aq$_S$	6.50	6.83	6.71	7.04^{a}
TDN (ppb)	2272.0	2359.0	2402.0	NA
TDP (ppb)	338.5	160.5^{a}	314.3^{b}	NA
SO$_4$ (ppm)	0.31	0.24	0.30	NA
DOC (ppm)	71.8	66.6	81.6^{a,b}	89.1^{a,b}
SUVA$_{254}$	3.1	3.7^{a}	3.4	2.5^{a,b,c}

[a] Result differs significantly from control.
[b] Result differs significantly from S amendment.
[c] Significant difference observed between recovery areas.

Table 3
Significant *P*-values of Tukey's tests comparing controls, S amendments and recovery areas abbreviated as follows: DOC, dissolved organic carbon; TDP; total dissolved phosphorus; TDN, total dissolved nitrogen; DHA, dehydrogenase; PER, peroxidase.

	Control vs S	Control vs RA-1	Control vs RA-2	S vs RA-1	S vs RA-2	RA-1 vs RA-2
Upper						
Peat C:P				0.0340	0.0001	
Peat N:P			0.0458	0.0162	0.0010	
DOC:TDP		0.0047	<0.0001	0.0010	<0.0001	
TDN:TDP		0.0005	<0.0001	<0.0001	<0.0001	
%Aq$_P$					0.0023	
TDP			<0.0001	0.0044	<0.0001	0.0217
SO$_4$	<0.0001			<0.0001	<0.0001	
SUVA$_{254}$				0.0059	0.0146	
Middle						
Peat C:N		<0.0001	0.0013	0.0070		
Peat N:P		0.0008	0.0058	0.0120		
DOC:TDP		0.0415	0.0030	0.0023	<0.0001	
TDN:TDP	0.0024	0.0022	<0.0001	<0.0001	<0.0001	
DHA			0.0220			
PER				0.0211	0.0017	
%Aq$_C$		0.0006				0.0004
%Aq$_N$		0.0005				
%Aq$_P$			0.0028		0.0028	0.0173
%Aq$_S$		0.0036				
TDP		0.0257	<0.0001	<0.0001	<0.0001	<0.0001
DOC	0.0060				0.0095	
SUVA$_{254}$	0.0004			0.0010	<0.0001	
Lower						
DOC:TDN				0.0287		
DOC:TDP	0.0228			0.0006		
TDN:TDP	0.0041			0.0002		
PER		0.0376				
%Aq$_C$	0.0383				0.0278	
%Aq$_N$					0.0288	
TDP	0.0104			0.0104		
DOC		0.0049	<0.0001		<0.0001	
SUVA$_{254}$	<0.0001		0.0003	<0.0001	<0.0001	<0.0001

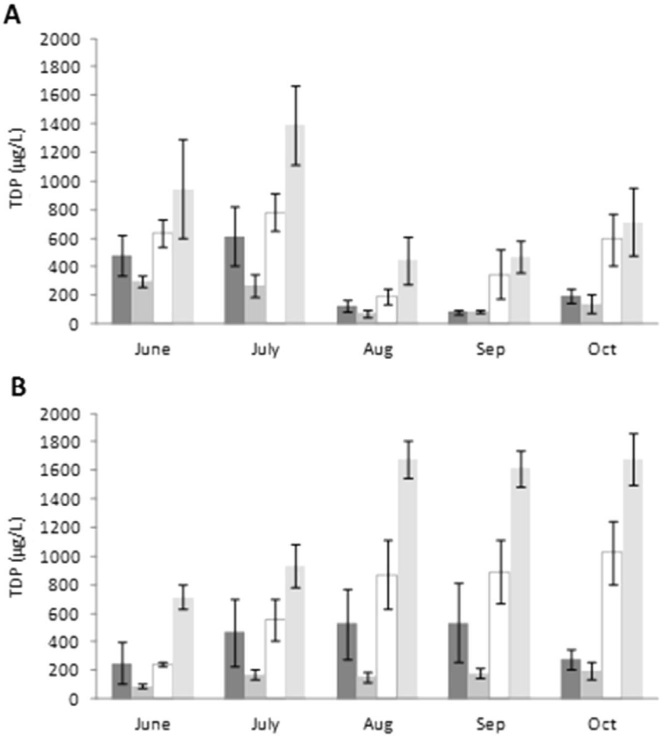

Fig. 5. Total dissolved phosphorus (TDP) concentrations in upper (A) and middle (B) pore water samples. Controls are represented by dark gray bars, S amendments by medium gray bars, RA-1 by white bars, and RA-2 by light gray bars.

The increase in total dissolved phosphorus appears to drive most of the differences observed between the recovery areas and the upstream half in upper peat and pore water samples. In upper samples, a significant decrease in N:P ratios in RA-2 compared to controls indicates that *Sphagnum* may be taking up more of the available total dissolved phosphorus which could potentially influence decomposition processes in subsequent years. Additionally, decreased total dissolved C:P and total dissolved N:P (Tables 1 and 2) in both recovery areas indicate a significant change in the stoichiometric ratios of available resources, which is reflected by the decreased effort of the microbial community to acquire P in RA-2 (Tables 2 and 3).

The concentration of total dissolved phosphorus in middle pore water samples of both recovery areas is significantly greater than control and sulfate amended mesocosms. Resulting differences in peat N:P, total dissolved C:P and total dissolved N:P are observed, with patterns similar to those seen in upper samples. The ratio of total dissolved N:P indicates that the microbial community may be limited by N in the recovery areas, with ratios averaging 4.14 in RA-1 and 2.27 in RA-2. The possibility of N limitation is supported by increased peat C:N ratios, which are driven largely by decreased peat N content in the recovery areas. *Sphagnum* N:P ratios fall below the threshold of 14, indicating that primary production is also limited by N (Koerselman and Meuleman, 1996). Additionally, there appears to be a shift in enzyme allocation in RA-1, with less effort directed towards C acquisition and greater effort at acquiring N and S. RA-2, where total dissolved phosphorus concentrations are greatest, showed a significant decrease in P acquisition, indicating that P is less limiting to the microbial community than in the upstream half.

Water chemistry data are limited for lower depths in RA-2, however RA-1 had total dissolved phosphorus concentrations and

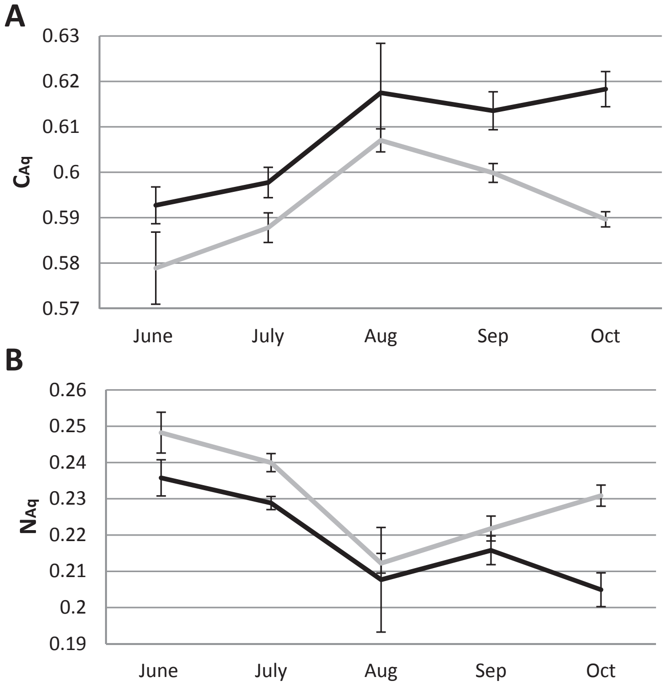

Fig. 6. Enzyme allocation (presented as proportion of total acquisition) in control (black lines) and RA-1 (gray lines) middle layer peat samples. C acquisition is displayed in Panel A while N acquisition is displayed in Panel B.

peat and pore water stoichiometric ratios that were similar to controls. The most notable difference in lower samples is the increase in dissolved organic carbon concentration in both recovery areas compared to controls and sulfate amendments. Though the difference is smaller, dissolved organic carbon seems to be responding similarly to total dissolved phosphorus, with a release of dissolved organic carbon after sulfate amendments have ended. The aromaticity of dissolved organic carbon is also significantly decreased in RA-2 as compared to control, sulfate amended, and RA-1 mesocosms, indicating that as the system begins to recover and dissolved organic carbon solubility increases, the C pool becomes larger and more labile.

4.3. Implications

While a response of total dissolved phosphorus to altered sulfate inputs was not included in the original hypotheses of this study, it was the one parameter that showed a consistent response and appears to be the driving force of many of the differences between recovering and control peat. The dramatic influence of sulfate amendments on total dissolved phosphorus concentrations could have unexpected implications, both during and following increased inputs. During long-term enrichment with sulfate, the response of total dissolved phosphorus could create or intensify P limitation. During recovery however, increased total dissolved phosphorus concentrations may result in a shift towards N limitation, which could impact plant and microbial communities, and in turn, nutrient cycling and other ecosystem processes. The response of total dissolved phosphorus and the potential impacts on nutrient limitation represent a recovery that is not transitioning between amended and pre-amended states in a predictable fashion. It is uncertain whether the recovery areas will resemble the rest of the bog in the foreseeable future. Past studies have shown that ecosystems recovering from eutrophication often follow unexpected trajectories, and due to broad environmental changes, may not return to their previous conditions (Duarte et al., 2009). Similar observations have been made of systems recovering from other types of disturbance as well. When the changes are significant enough, the system may not return to its previous condition, but rather evolve to an alternative state (Palumbi et al., 2008). If the recovery areas of the peatland do not return to their previous state, this site offers a unique opportunity for study in that there is a control half of the same peatland to serve as a reference with which to gauge the degree of recovery in the previously amended portion. This could include monitoring of chemical characteristics, but also such features as vegetation. RA-2 in particular appears to have greater sedge cover than is found in the control half of the peatland (personal observation). Because vegetation assessments were not conducted prior to amendment, there is no way to know whether this difference existed prior to amendment or was a result of it. Tracking the changes in nutrients, vegetation and microbial community function over time could provide important information regarding how recovery may proceed.

5. Conclusions

While it is likely that there are multiple causes, both local and widespread, of increasing dissolved organic carbon export, this study shows that increased inputs of sulfate can lead to decreased concentrations of dissolved organic carbon, which holding other hydrological factors constant, translates to decreased dissolved organic carbon export. After a return to ambient sulfate deposition, dissolved organic carbon may increase to levels greater than those before sulfate enrichment. We observed a consistent response of total dissolved phosphorus with perhaps the most striking response occurring in recovering peat. The increase of total dissolved phosphorus in recovering peat was reflected in microbial enzyme activities and was large enough to significantly alter peat and pore water C:N:P ratios, indicating a possible shift from P limitation to N limitation. The system does not appear to be returning to its previous condition in a linear fashion and is following an unexpected trajectory, as shown in other studies of recovering ecosystems. While this study supports previous hypotheses regarding the response of dissolved organic carbon to increased sulfate deposition, it also raises new questions about the response of P, both during and following increased sulfate inputs.

Acknowledgments

The authors would like to thank researchers at the Northern Research Station of the USDA Forest service, including Deacon Kyllander, Carrie Dorrance and Dr. Stephen Sebestyen, who provided general assistance, watershed data, and information on trends of dissolved organic matter export. Thanks to Dr. John Pastor and Dr. Josef Werne for advice on study design and previous versions of this manuscript. This research was funded through the University of Minnesota-U.S. Environmental Protection Agency Cooperative training partnership (CR83492801).

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