1 Can *Sphagnum* Leachate Chemistry Explain Differences in Anaerobic Decomposition in
2 Peatlands?
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Abstract:

Peatlands are important ecosystems in the global carbon cycle, serving as both the largest terrestrial soil carbon pool and a significant source of the greenhouse gas methane (CH₄). In *Sphagnum* moss-dominated wetlands, anaerobic decomposition, and in particular the production of CH₄, is highly variable and controlling factors are poorly understood. The main objective of this study was to determine if leachates of *Sphagnum* can explain differences in anaerobic decomposition and CH₄ production from three *Sphagnum*-dominated peatlands.

Soils from each peatland were incubated anaerobically for 40 days with *Sphagnum*-derived organic matter (S-DOM) extracted using distilled water at 25 or 60 °C in a fully-crossed experimental design. S-DOM extracted at 25 °C had a minimal effect on decomposition, but S-DOM extracted at 60 °C increased CO₂ production in all soils. The magnitude of the increased CO₂ production in response to S-DOM depended on the source site of the S-DOM. The response of CH₄ production to additions of S-DOM extracted at 60 °C was more complex. Soils from one peatland produced no CH₄ during the incubation, regardless of S-DOM source. The same S-DOM additions led to an increase in CH₄ production in a second soil, but a decrease in CH₄ production in the third soil. Stable isotopic evidence suggests that these patterns were driven by the selective inhibition or stimulation of acetoclastic methanogenesis. Taken together, these data suggest S-DOM alone does not explain differences in anaerobic decomposition in peatlands, but may play a role in regulating CO₂ and CH₄ production.

1. Introduction

Peatland soils store an estimated 462 Pg of carbon (C), serving as the largest terrestrial soil C pool (Bridgham et al., 2006 and references therein). The vast majority of this soil carbon occurs below the water table, and the fate of this C is determined, in large part, by anaerobic microbial decomposition which mineralizes stored C to carbon dioxide (CO₂) and methane (CH₄). Wetlands are the largest natural source of CH₄ to the atmosphere (Bridgham et al., 2013; Kirschke et al., 2013), with peatlands contributing a significant, but poorly constrained fraction of this CH₄ flux. Given the effectiveness of CH₄ as a greenhouse gas, understanding the controls of peatland decomposition and CH₄ dynamics is crucial for understanding feedbacks between these ecosystems and ongoing global change (Bridgham et al., 2013).

Many studies have investigated controls of anaerobic decomposition, including pH (Wang et al., 1993; Kotsyurbenko et al., 2007; Ye et al., 2012), temperature (Conrad, 2002; Glissman et al., 2004; Inglett et al., 2012), and nutrient limitation (Keller et al., 2006; Medvedeff et al., 2014) in wetland soils. Despite this work, there is still a great deal of variability in anaerobic decomposition in peatland soils, especially in *Sphagnum*-dominated ombrotrophic peatland soils, that cannot be fully explained by these controls. For example, Ye et al. (2012) demonstrated that ombrotrophic (bog) peat soils produced less CH₄ than minerotrophic (fen) peat soils, even after correcting for soil pH. This suggests that additional controls on anaerobic decomposition have yet to be elucidated.

It is likely that organic matter can play an important role in anaerobic decomposition through multiple mechanisms. While the role of organic matter as a carbon and nutrient source for microbes is well established (Reddy and DeLaune, 2008), recent evidence also suggests that organic matter (i.e., humic substances) can influence anaerobic decomposition and inhibit CH₄

production in ombrotrophic bogs through both antimicrobial properties and by serving as an organic terminal electron acceptor for microbial respiration (Blodau and Deppe, 2012; Keller and Takagi, 2013; Ye et al., unpublished data). Further, the accumulation of phenolic compounds as a result of low phenol oxidase activity under anaerobic conditions in peatlands can inhibit hydrolase enzymes reducing rates of microbial decomposition (Freeman et al., 2001).

One of the main sources of organic matter in peatlands is *Sphagnum* moss. *Sphagnum* tissue chemistry is highly variable across species (Rasmussen et al., 1995; Turetsky et al., 2008), which may affect C mineralization both through its often dominant contribution to the solid peat matrix and through its soluble components. Recent evidence demonstrates that surface DOM permeates throughout the peat profile and often accounts for a substantial fraction of C mineralization even at depth (Chanton et al., 2008; Tfaily et al., 2014). It is well established that the litter of different *Sphagnum* species decay at very different rates (e.g., Johnson and Damman, 1991; Turetsky et al., 2008) and that *Sphagnum*-derived photosynthate is a known constituent of the soil DOC pool (Fenner et al., 2004). While dissolved organic carbon originating from *Sphagnum* is highly bioavailable relative to other vegetation such as black spruce (Wickland et al., 2007), this *Sphagnum*-derived dissolved organic matter (S-DOM) may have complicated effects on anaerobic C cycling beyond its role as a source of labile C. *Sphagnum* mosses can inhibit the growth of certain microbial species and reduce decomposition rates by acidifying the environment (Stalheim et al., 2009) and releasing polyphenolic compounds, particularly sphagnum acid (*p*-hydroxy- β [carboxymethyl]-cinnamic acid) (Verhoeven and Toth, 1995). Sphagnum acid persists under anaerobic conditions (Swain and Abbott, 2013), suggesting methanogens may be in direct contact with inhibitory *Sphagnum*-derived phenolic compounds.

We tested the hypothesis that differences in rates of anaerobic decomposition and CH₄ production in *Sphagnum*-dominated peatlands are influenced by the differences in the S-DOM of the resident *Sphagnum* species. We hypothesized that (i) S-DOM from peatlands with slow rates of decomposition would inhibit anaerobic decomposition in peatland soils with naturally faster rates of decomposition, and that CH₄ production would be particularly sensitive to the S-DOM source, and (ii) the reverse would also be true in that S-DOM from peatlands with fast decomposition would stimulate anaerobic decomposition in soils with slower rates of decomposition.

2. Materials and Methods

2.1. Study Sites and Sample Collection

The three peatlands for this study are located in northern Minnesota, U.S.A. The poor fen (Bog Lake Fen, N 47°30.304'; W 93°29.339') and the weakly ombrotrophic bog (S1 Bog, N 47°30.388'; W 93°27.256') are in Marcell Experimental Forest of the U.S. Forest Service, approximately 40 km north of Grand Rapids. The strongly ombrotrophic bog (Zim Bog, N 47°10.745'; W 92°42.877') is located approximately 40 km south of the town of Virginia. All three wetlands are dominated by a near complete ground cover of *Sphagnum* moss. Bog Lake Fen is dominated by a *S. papillosum* Lindb. lawn (Olson et al., 2013), with lesser cover of *S. capillifolium* (Ehrh.) Hedw. and *S. magellanicum* Brid.. Bog Lake Fen also has extensive cover of sedges (*Carex* spp., *Eriophorum chamissonis* C.A. Mey) and occasional northern pitcher plants (*Sarracenia purpurea* L.) and ericaceous shrubs such as leather leaf (*Chamaedaphne calyculata* (L.) Moench) and bog cranberry (*Vaccinium macrocarpum* Aiton) (Kolka et al., 2011; Olson et al., 2013). The water-table depth at Bog Lake Fen averaged -7.0 cm during sampling

on July 18-20, 2013. S1 Bog contains pronounced hummock-hollow topography with hummocks dominated by *S. magellanicum* Brid. and hollows dominated by *S. angustifolium* (C.E.O. Jensen ex Russow) C.E.O. Jensen and *S. fallax* (Klinggr.) Klinggr. (D. Weston, *personal communication*). The S1 Bog overstory is comprised of black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) and tamarack (*Larix laricina* (Du Roi) K. Koch) with the understory containing ericaceous shrubs including leather leaf, Labrador tea (*Rhododendron groenlandicum* Oeder), and bog rosemary (*Andromeda polifolia* L.), forbs such as three-leaved false Solomon's seal (*Smilacina trifolia* (L.) Sloboda) and northern pitcher plant, and sedges such as three-seeded sedge (*Carex trisperma* Dewey) and cotton grass (*Eriophorum spissum* Fernald) (<http://mnspruce.ornl.gov/content/detailed-site-description>). Water-table levels at S1 Bog in July 2013 averaged -6.6 cm and -15.2 cm in hollows and hummocks, respectively. Zim Bog is dominated by a large, expansive hummock with a few sparse, but deep, hollows and was the driest of the sites with an average water-table depth of -18.8 and -33.2 cm in hollows and hummocks, respectively, in July 2013. Zim Bog has a moss layer dominated by *S. fuscum* and an overstory consisting of black spruce and similar ericaceous shrubs to S1 Bog (Bridgham et al., 1998).

Peat cores (~25 cm depth) were collected from immediately below the water table in the hollows of each site using a serrated knife (n=3 cores per site) at Bog Lake and S1 Bog and a Russian Corer at Zim Bog because of its low water table. Soil pH averaged 4.2, 4.0, and 3.8 for Bog Lake Fen, S1 Bog, and Zim Bog, respectively (Table 1). In addition to soil collection, live *Sphagnum* composite (top 0-5 cm) grab samples were collected from S1 Bog hollows in July 2013. Additional *Sphagnum* composite (top 0-5 cm) grab samples were collected from the lawn of Bog Lake Fen, Zim Bog hummocks, and S1 Bog hummocks on September 15th-17th, 2013.

We chose to use composite *Sphagnum* samples instead of sorting them by species because we had previously identified that soils from these 3 sites have very different rates of anaerobic C mineralization (unpublished data, also Table 1) and wanted to accurately represent the *Sphagnum* community at each site to determine if this could potentially explain these differences. Because of the constant depth increment used and variable growth among species, *Sphagnum* age likely varied among species across sites which could affect nutrient quantity and quality in subsequent extracts. Following collection, soil and *Sphagnum* samples were placed on ice, shipped to Chapman University, and frozen until further analysis.

2.2 Anaerobic Laboratory Incubations

To explore the effects of *Sphagnum* leachate on anaerobic decomposition, we added *Sphagnum*-derived dissolved organic matter (S-DOM) extracted from each site to the soil from each site in a fully crossed experimental design. S-DOM was prepared by extracting 10 g dry weight equivalent of *Sphagnum* from each site (Bog Lake Fen, S1 Bog hummock, S1 Bog hollow and Zim Bog hummock) with 200 mL of deionized water at either 25 °C or 60 °C for 48 hours (S-DOM₂₅ and S-DOM₆₀, respectively). To ensure that all soils received identical S-DOM additions in subsequent incubations, there was a single extraction for each *Sphagnum* community. Following extraction, samples were filter-sterilized with a 0.2 µm filtration system (Millipore, stericup), and the pH of each extract was adjusted to match the pH measured at each site following pre-incubation. Our intent was not to represent *in situ* conditions with these extraction temperatures; rather, they were utilized to extract DOM of different complexity from *Sphagnum* and explore the effect of these extracts on anaerobic decomposition in three different peat soils. We anticipated that higher extraction temperatures would yield S-DOM with higher concentrations of phenolics and proteins (Herranz et al., 2006) and elevated dissolved organic

carbon concentrations resulting from cell lyses and increased degradation of molecules of greater complexity (Gregorich et al., 2003, and references therein). It is likely that over prolonged periods of decomposition (as opposed to the 48 hour leaching period) that the compounds leached at 60 °C would become increasingly available at *in situ* temperatures.

Phenolics, dissolved organic C (DOC), protein absorbance, and specific ultra-violet absorbance (SUVA) were measured on all S-DOM extracts. Total soluble phenolics were quantified using a Folin-Ciocalteu assay (AOAC, 1950) with standard curves based on gallic acid (GA) equivalents at 760 nm wavelength on a Shimadzu UV-1700 spectrophotometer (Tokyo, Japan). DOC concentrations were analyzed following the persulfate digestion method (modified from Horwath and Paul, 1994), and CO₂ was measured on a gas chromatograph using a flame ionization detector equipped with a methanizer (SRI Instruments, Torrance, CA, USA).

Absorbance measurements were analyzed on a Shimadzu UV-1700 spectrophotometer at 254 nm for SUVA determinations and 278 nm for protein absorbance determinations (Stevens, 1992); nanopure water was used as the blank for both analyses. Samples were warmed to room temperature prior to analysis in a quartz cell cuvette with a 1.0 cm path. Specific ultraviolet absorbance (SUVA₂₅₄) was quantified as a proxy for dissolved C quality by dividing the UV absorbance_{254nm} by the DOC concentration. Values were reported as L mg⁻¹ C m⁻¹ (Weishaar et al., 2003).

Large roots and woody debris were removed from the soils, and the 3 cores from each site were homogenized. Ten grams of wet weight soil were added to 160-mL serum bottles (n=27 for each site) and pre-incubated for 22 days at 18 °C (the approximate *in situ* soil temperature in July 2013) prior to addition of S-DOM extracts to facilitate consumption of terminal electron acceptors. Additional aliquots of field-moist soil from each homogenized

sample (n=3 per site) were dried at 60 °C to a constant mass for moisture content determination. Following drying, samples were ground through a Wiley Mill fitted with a 40-mesh screen (Thomas Scientific, Swedesboro, NJ, USA) and analyzed for total C (TC) and total nitrogen (TN) with a Costech 4010 elemental analyzer (Valencia, CA). At the end of the 22 day pre-incubation, sacrificial samples (n=3 per site) were filtered through a 0.45 µm filter and analyzed for pH, phenolic concentrations, DOC, protein absorbance, and SUVA to determine initial dissolved organic matter chemistry (Table 1, 2).

In an anaerobic chamber (Coy Laboratory Products Inc., Grass Lake, MI, USA), 10 mL S-DOM extracts and 10 mL of nanopure water were added to pre-incubated soil slurries in a fully-crossed experimental design (3 soils, 4 S-DOM extracts, 2 extraction temperatures, n=3). After the addition of S-DOM, pH was measured on all samples following a 30 minute equilibration period to verify that the S-DOM did not change acidity. Serum bottles were sealed and flushed with N₂ for 15 minutes to ensure anaerobic conditions.

In addition to the S-DOM treatments, soil-only controls (10 g soil : 20 mL nanopure water) and S-DOM extract controls were analyzed concurrently (n=3 per treatment). Headspace gas was analyzed for CO₂ and CH₄ on days 1, 3, 5, 9, 12, 17, 19, 26, 33, and 40. Slurries were opened in the anaerobic chamber, soil pH was measured, and bottles were re-capped and flushed with N₂ weekly. Total CO₂ and CH₄ production were calculated after correcting for pressure, solubility and pH (Drever, 1997; Bridgham and Ye, 2013). Cumulative CO₂ and CH₄ production were calculated by summing production on each sampling date, and the total production was expressed as µmoles of gas per g dry weight soil after the 40 day incubation. After 40 days, headspace gas samples were injected into 12-mL Exetainers (LABCO Ltd.; www.labco.uk.com) and shipped to the University of California, Davis Stable Isotope Facility for δ¹³C-CH₄ analysis.

In addition, soil samples were extracted (as described previously) and analyzed for soluble phenolics, DOC, protein absorbance, and SUVA on day 40.

2.3. Statistical Analyses

To determine initial and final site differences in soil and S-DOM properties as well as CO₂ and CH₄ production, values were compared via one-way Analysis of Variance (ANOVA). Following significant ($\alpha < 0.05$) ANOVAs, post-hoc Tukey's HSD was used to test for treatment differences. Normality was determined via the Shapiro Wilk goodness-of-fit test. To achieve normality, data for phenolic concentrations, SUVA, protein absorbance, and total CO₂ production were log transformed prior to analysis. Regressions were run on S-DOM-derived gas production (CO₂ and CH₄) versus S-DOM-derived DOC and protein absorbance. S-DOM-derived effects were calculated as follows: $(X_1 - X_2)$, where X_1 =value following incubation with S-DOM and X_2 =value following incubation with no S-DOM. All statistical analyses were run in JMP v. 8.0 (SAS institute, Cary NC).

3. Results

3.1. S-DOM and Initial Soil Properties

3.1.2. Prior to incubation

To ensure uniformity of S-DOM additions, a single extraction was completed for each *Sphagnum* type at each temperature. All S-DOM additions were sources of phenolics, DOC, and protein with higher concentrations coinciding with higher extraction temperatures (Table 2).

Following the 22 day pre-incubation, soil properties were compared among Bog Lake Fen, S1 Bog, and Zim Bog (Table 1). pH was highest in Bog Lake Fen soil, followed by S1

Bog, and Zim Bog ($P<0.0001$). Soil TC was greatest in S1 Bog ($P<0.05$). TN concentrations in S1 Bog were double that of Zim Bog, with intermediate concentrations in Bog Lake Fen ($P<0.05$). Differences in TC:TN were primarily driven by differences in TN, with the highest ratio found in Zim Bog, followed by Bog Lake Fen, and the lowest ratio (approximately half that of Zim Bog) in S1 Bog soils ($P<0.05$).

CO₂ production during the pre-incubation period was greatest in Bog Lake Fen soil, followed by S1 Bog and then Zim Bog soils ($P<0.0001$, Table 1). CH₄ production from Zim Bog soil was below detection (reflecting a maximum rate of 0.0007 $\mu\text{mol g}^{-1} \text{dw soil d}^{-1}$) during both the pre-incubation and incubation. CH₄ production was approximately 4-fold greater in S1 Bog relative to Bog Lake Fen during the pre-incubation period ($P<0.05$, Table 1). No significant differences in initial dissolved phenolics, DOC, SUVA, or protein absorbance were observed between sites after the pre-incubation period and prior to the addition of S-DOM (Supplemental 1).

3.2. Response to S-DOM Amendments

Although trends between extraction temperatures were similar, the magnitude of the response of CO₂ and CH₄ production to the S-DOM₂₅ addition in each soil (Supplemental 2 and 3) was much smaller than that observed following S-DOM₆₀ addition. The addition of S-DOM₂₅ from all sources stimulated CO₂ production in Bog Lake Fen and S1 Bog soils, but only S1 Bog hollow S-DOM₂₅ stimulated CO₂ production in Zim Bog soils (Supplemental 2). Furthermore, S-DOM₂₅ from S1 Bog hummocks suppressed CH₄ production while S1 Bog hollow S-DOM₂₅ stimulated CH₄ production in Bog Lake Fen soil (Supplemental 3). Addition of Bog Lake Fen and S1 Bog hollow S-DOM₂₅ to S1 Bog soil stimulated CH₄ production. S-DOM₂₅ from Zim

Bog did not affect CH₄ production in either S1 Bog or Bog Lake Fen soil. There was no effect of S-DOM₂₅ on $\delta^{13}\text{C}$ -CH₄ in Bog Lake Fen soil; in contrast, addition of S1 Bog hollow S-DOM₂₅ enriched values when added to S1 Bog soil (Supplemental 3). Overall, we saw the largest effect of S1 Bog hollow S-DOM₂₅ on CO₂ and CH₄ production relative to all other S-DOM additions. This large response, regardless of site, was likely driven by the high DOC and protein concentrations coupled with a low SUVA value and low phenolic content in the extract (Table 2).

The addition of S-DOM₆₀ stimulated CO₂ production in soils from all sites, but the magnitude of response depended on the source of the S-DOM within soil type (Figure 1). In Bog Lake Fen soil, the greatest stimulation of CO₂ was in response to S-DOM₆₀ from S1 Bog hummocks, followed by S-DOM₆₀ from Bog Lake Fen and then S-DOM₆₀ from S1 Bog hollows and Zim Bog ($P<0.0001$, Figure 1a). Similarly, CO₂ production from S1 Bog soil was stimulated to the greatest extent by S-DOM₆₀ from the S1 Bog hummock, followed by Bog Lake Fen S-DOM₆₀, S1 Bog hollow S-DOM₆₀, and Zim Bog S-DOM₆₀ ($P<0.0001$, Figure 1b). In Zim Bog soil, CO₂ production was greatest when amended with S1 Bog hummock S-DOM₆₀, intermediate when amended with S1 Bog hollow and Bog Lake Fen S-DOM₆₀, and lowest when amended with Zim Bog S-DOM₆₀ ($P<0.0001$, Figure 1c).

S-DOM addition inhibited CH₄ production in Bog Lake Fen soil, regardless of S-DOM origin ($P=0.0038$, Figure 2a). In contrast, S-DOM₆₀ stimulated CH₄ production in S1 Bog soil ($P<0.0001$). The greatest stimulation of CH₄ production was in response to S-DOM₆₀ from S1 Bog hollow, followed by Bog Lake Fen, and then S1 Bog hummock and Zim Bog ($P<0.0001$, Figure 2b).

In Bog Lake Fen soil, S-DOM₆₀ additions yielded depleted $\delta^{13}\text{C}$ -CH₄ relative to the unamended control ($P=0.0005$, Figure 2a), although this depletion was not significant for Zim Bog S-DOM. In contrast, the addition of S-DOM₆₀ resulted in more enriched $\delta^{13}\text{C}$ -CH₄ compared to unamended controls in S1 Bog soil ($P<0.0001$, Figure 2b).

S-DOM₆₀-derived CO₂ was positively correlated to the increase of DOC in response to S-DOM₆₀ amendments in Bog Lake Fen soil ($R^2 = 0.54$, $P=0.0067$) and Zim Bog soil ($R^2=0.79$, $P=0.0003$) (Figure 3a, c); however, this relationship was not significant in S1 Bog soil ($R^2=0.24$, $P=0.104$) (Figure 3b). Similarly, increasing protein absorbance as a result of S-DOM₆₀ amendment was positively correlated with S-DOM₆₀-derived CO₂ in Bog Lake Fen and Zim Bog soils but not in S1 Bog soil (*data not shown*). CH₄ production was not correlated to S-DOM₆₀-derived DOC or protein in S1 Bog, but increasing DOC was correlated with the decreasing CH₄ production observed in Bog Lake Fen soil (Figure 3d-e).

When focusing on the S-DOM₆₀ only controls, *Sphagnum* origin had no effect on phenolic concentrations or SUVA at the end of the incubation (Supplemental 4). However, DOC and protein concentrations were highest in S-DOM₆₀ from S1 Bog hummocks and DOC concentrations were lowest in S-DOM₆₀ from Zim Bog.

The addition of S-DOM₆₀ increased soil DOC concentrations in Bog Lake Fen, Zim Bog, and S1 Bog soils, with the exception of the addition of S-DOM₆₀ from Zim Bog added to S1 Bog soil (Supplemental 5 a-c). The S-DOM₆₀ amendment had no significant effect on phenolic concentrations (Supplemental 5 d-f) or SUVA (Supplemental 6 a-c) in any soil. Protein absorbance in Bog Lake Fen soil increased when amended with S-DOM₆₀ from S1 Bog or Bog Lake Fen (Supplemental 6 d), but no significant effect was observed in the other two soils (Supplemental 6 e-f). Concentrations of DOC and phenolics were similar pre- and post-

incubation in the unamended control (Supplemental 1). Higher values of SUVA post-incubation compared to pre-incubation suggest that DOM became increasingly recalcitrant over the course of the incubation in unamended soils from S1 Bog ($P=0.0134$), although this pattern was not observed in Bog Lake Fen or Zim Bog soil (Supplemental 1). Similarly, protein absorbance increased over the incubation in S1 Bog soils without amendment ($P=0.0415$, Supplemental 1).

4. Discussion

Sphagnum is the dominant source of carbon in many ombrotrophic peatlands. There is abundant evidence that it affects C cycling through the quality of the litter and the subsequent peat that it produces in the solid form (Johnson and Damman, 1991; Verhoeven and Toth, 1995). Additionally, photosynthate from *Sphagnum* is a known constituent of the soil DOC pool (Fenner et al., 2004). This suggests DOM leached from living *Sphagnum* could stimulate anaerobic decomposition in these ecosystems by the addition of labile C (Tfaily et al., 2014) or inhibit it due high concentrations of inhibitory phenolic compounds (Verhoeven and Toth, 1995; Freeman et al., 2004; Ye et al., unpublished data), which vary widely among *Sphagnum* species (Turetsky et al., 2008). Moreover, a substantial fraction of anaerobic C mineralization can come from DOM, even at depths of up to 200 cm below the soil surface (Chanton et al., 2008; Tfaily et al., 2014) suggesting that S-DOM could potentially influence microbial processes through the peat profile.

We utilized two extraction temperatures (25 °C and 60 °C) to generate S-DOM in our experiment and explored the effects of this S-DOM on anaerobic decomposition in three peatland soils. Similar to past leachate experiments (e.g., Herranz et al., 2006), extraction at 60 °C generally resulted in higher concentrations of phenolics, DOC and protein than the extraction at 25 °C (Table 2, Supplemental 3). No short-term extraction at a single temperature will

adequately represent leachates from organic matter *in situ*, so leachates at the two temperatures more accurately represents the potential spectrum of S-DOM effects on anaerobic mineralization in the long term. While we focus on the effect of the 60 °C S-DOM extracts on anaerobic decomposition, the effects on CO₂ and CH₄ production were generally similar for 25 °C S-DOM extracts, even though the magnitude of the effects were considerably smaller. The smaller responses to S-DOM₂₅ were likely due to lower concentrations of DOC and protein with the more recalcitrant carbon (based on SUVA values) in the S-DOM₂₅ compared to the S-DOM₆₀ (with S1 Bog hollow SUVA values as the exception).

We initially hypothesized that the chemistry of S-DOM would exert a major control on anaerobic decomposition such that S-DOM from *Sphagnum* at sites with slow rates of anaerobic decomposition would inhibit decomposition in soils with faster rates of decomposition, and S-DOM from sites with fast rates of decomposition would stimulate decomposition in soils with slow rates of decomposition. Our results show a major effect of S-DOM₆₀ on anaerobic C cycling, but in a much more nuanced way than the site-specific responses of CO₂ and CH₄ production that we originally hypothesized. The relationship between the added organic C and protein contained in S-DOM and the CO₂ response in two of the soils (Figure 3 a, c) suggests the availability of labile C in S-DOM was an important factor explaining the relative responses among sources. A great deal of previous work suggests that overall anaerobic decomposition in peatland soils is limited by the availability of labile C (Segers, 1998 and references therein). Given our pre-incubation of 22 days, the majority of terminal electron acceptors (TEAs) in peat matrix were probably reduced before S-DOM addition, and any TEAs in the S-DOM would have been consumed early in the 40-day incubation period (Keller and Takagi, 2013), so the stimulated CO₂ production was likely primarily due to fermenters.

The response of CH₄ production to S-DOM amendments was more complex. Soil from Zim Bog produced no CH₄ over the course of this experiment, regardless of S-DOM addition. The reasons for this are unclear, but it is worth noting that the lack of CH₄ production continued even in the presence of microbially-accessible S-DOM which stimulated CO₂ production in this soil and was utilized by methanogens in one of the other soils in this experiment (S1 Bog soil). Slow rates of CH₄ production in anaerobic laboratory incubations are common in ombrotrophic soils (Bridgham et al., 2013 and references therein) and have been explained by a number of mechanisms. Low CH₄ production can be the result of competitive inhibition by microbial reduction of more thermodynamically favorable TEAs (Dettling et al., 2006). It is also possible that the reduction of these TEAs could be used in the process of anaerobic methane oxidation (Gupta et al., 2013). However as stated above, all TEAs were likely reduced during the long incubation (Keller and Takagi, 2013). Another possibility is that compounds in this soil, derived from *Sphagnum* or other vegetation, selectively inhibited methanogens. For example, phenolic compounds are known to have antimicrobial properties affecting certain microorganisms (Fernández et al., 1996; Mellegård et al., 2009; Stalheim et al., 2009), including some types of methanogens (Puyol et al., 2012). In addition, phenolic compounds can reduce hydrolase enzyme activity substantially (Freeman et al., 2001) ultimately affecting decompositions rates. However, soluble phenolics were not higher in Zim Bog soil compared to other soils (Supplement 1). In addition, S-DOM had no effect on the phenolic:DOC ratio (data not shown) suggesting modification of the inhibitory effect of phenolics was not affected by S-DOM addition (Freeman et al., 1990). The lower soil pH (Table 1) at Zim Bog could have also inhibited methanogens; however, factors in addition to pH may also be important (Ye et al., 2012).

S-DOM amendments led to increased CH₄ production in S1 Bog soil, suggesting that labile substrates in S-DOM were made available to methanogens in addition to CO₂-producing microbes in this soil. However, the amount of excess CH₄ produced in response to S-DOM amendments in S1 Bog soil was small compared to the amount of excess CO₂ produced in the same soil. S-DOM-derived CO₂:CH₄ ratios ranged from 12 to 477 across the S-DOM treatments. These ratios are significantly higher than the 1:1 ratio of CO₂:CH₄ expected during anaerobic decomposition under conditions where methanogenesis is not inhibited by TEA reduction (Conrad, 1999). Such high ratios are common in soil incubation experiments (Segers, 1998 and references therein), including incubations with C additions (Dettling et al., 2006), and may reflect CO₂ production from fermentation, respiratory reduction, and anaerobic methane oxidation (Bridgham et al., 2013).

The $\delta^{13}\text{C}$ -CH₄ values provide insights into the mechanism of the CH₄ responses. Methane produced through the hydrogenotrophic pathway (the reduction of CO₂ coupled to the oxidation of H₂) has $\delta^{13}\text{C}$ -CH₄ values in the range of -80‰ to -110‰. In contrast, CH₄ produced from the acetoclastic pathway (the splitting of acetate to CO₂ and CH₄) has more enriched values of $\delta^{13}\text{C}$ -CH₄ in the range of -50‰ to -60‰ (Whiticar, 1999). In the S1 Bog soil, enriched $\delta^{13}\text{C}$ -CH₄ produced in response to S-DOM amendments suggests a greater contribution of acetoclastic methanogenesis and/or a decrease in CH₄ produced by hydrogenotrophic methanogenesis. Methane oxidation also results in enriched $\delta^{13}\text{C}$ -CH₄ values (Whiticar, 1999), but it seems unlikely that CH₄ oxidation dominated S1 Bog soils amended with S-DOM due to the observed higher net CH₄ production (Figure 2b).

In contrast to the response in S1 Bog soil, S-DOM amendments inhibited CH₄ production in Bog Lake Fen soil (Figure 2a). Lower net CH₄ production could be caused by increased rates

of anaerobic CH₄ oxidation, but this seems unlikely given the depleted $\delta^{13}\text{C}$ -CH₄ signature observed in response to the S-DOM amendment in Bog Lake Fen soil (Figure 2a). Instead, the lower net CH₄ production and depleted $\delta^{13}\text{C}$ -CH₄ values suggest that S-DOM amendments selectively inhibited acetoclastic methanogenesis. This conclusion supports a previous study which found more pronounced inhibition of the acetoclastic relative to hydrogenotrophic methanogenesis following exposure to specific chlorophenolic compounds (Puyol et al., 2012). In addition to a direct inhibition of methanogenesis, S-DOM could have resulted in the competitive inhibition of CH₄ production if oxidized TEAs were introduced in the S-DOM amendments, although this should have been a transitory effect. Regardless of the specific mechanisms of inhibition, it is curious that identical S-DOM amendments caused diametrically opposite effects on CH₄ production in the two soils, apparently mediated through differential responses of the acetoclastic methanogenesis. Future work exploring microbial community shifts could provide insights into these responses.

5. Conclusions

We demonstrate that DOM leached from *Sphagnum* has large effects on anaerobic C cycling and that the effects of S-DOM on CO₂ and CH₄ are mediated by the source of the *Sphagnum* and differ between soils. The effects of S-DOM were clearer following addition of S-DOM₆₀ which contained higher concentrations of phenolics, DOC, protein, and contained a lower SUVA value relative to S-DOM₂₅ extracts. It is likely that lower concentrations of these compounds and higher SUVA values in S-DOM₂₅ extracts limited the responses compared to the S-DOM₆₀ amendments. Given new evidence that recent plant-derived DOM diffuses throughout the peat profile, this suggests that changes in *Sphagnum* communities driven by climate change

could have large effects on anaerobic C cycling within the peat profile. CO₂ production increased in response to S-DOM amendments in all soil types, suggesting that labile substrates extracted from *Sphagnum* were utilized by fermenting bacteria. The response of CH₄ production was complex, and ranged from increased CH₄ production in one soil to inhibition of CH₄ production in another soil. This response was apparently largely driven by selective inhibition or stimulation of methanogenic pathways which varied between sites. The mechanistic controls of *Sphagnum*-derived DOM on anaerobic decomposition need to be further addressed given the dramatic effects observed in this study.

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Table Captions:

Table 1. Characterization of soil prior to S-DOM addition. P-values are from a one-way ANOVA. Significant differences between initial and final values, within each soil, are designated by different letters ($P < 0.05$).

Table 2. Characterization of S-DOM extracts prior to incubation. P-values are from a one-way ANOVA. Significant differences between initial and final values, within each soil, are designated by different letters ($P < 0.05$).

Table 1. Characterization of soil prior to S-DOM addition.

	pH	TC	TN	TC:TN	CO ₂	CH ₄
<i>Site</i>	-----g kg ⁻¹ -----				--μmol g ⁻¹ dw soil --- @ 22 days	
Bog Lake Fen	4.2±0.03 ^C	432.9±1.4 ^A	6.8±0.2 ^B	63.4±1.6 ^B	220.2±11.5 ^C	2.5±0.1 ^A
S1 Bog	4.0±0.03 ^B	446.1±0.8 ^B	10.5±0.6 ^C	42.7±2.3 ^A	109.9±10.0 ^B	10.7±2.2 ^B
Zim Bog	3.8±0.03 ^A	430.4±1.4 ^A	5.0±0.3 ^A	86.6±4.1 ^C	46.3±1.3 ^A	0 ^{NA}

Table 2. Characterization of S-DOM extracts prior to incubation.

		Phenolics	DOC	Protein ₂₇₈	SUVA ₂₅₄
Site		mg GA L ⁻¹	mg C L ⁻¹	278 nm cm ⁻¹ L	mg ⁻¹ C m ⁻¹
25°C/60°C extraction					
Bog Lake Fen	Lawn	6/30	52/368	0.65/2.74	1.64/0.72
S1 Bog	Hummock	13/43	104/639	2.00/3.91	2.47/0.61
S1 Bog	Hollow	9/29	214/452	2.61/2.00	0.44/0.60
Zim Bog	Lawn	7/34	15/312	0.22/1.94	1.78/0.59

Figure Captions:

Figure 1. The effect of S-DOM extracted at 60 °C amendments on anaerobic CO₂ production in three soils following a 40 day anaerobic laboratory incubation (mean ± 1 SE, n=3). P-values are from a one-way ANOVA. Significant differences between treatments, within each soil, are designated by different letters ($P<0.05$).

Figure 2. The effect of S-DOM extracted at 60 °C amendments on CH₄ production and $\delta^{13}\text{C-CH}_4$ in soils from (a) Bog Lake Fen, and (b) S1 Bog following a 40 day anaerobic laboratory incubation (mean ± 1 SE, n=3). P-values are from a one-way ANOVA. Significant differences between treatments, within each soil, are designated by different letters ($P<0.05$). Data from Zim Bog are not shown because there was no detectable CH₄ production.

Figure 3. Relationship between S-DOM extracted at 60 °C derived DOC and S-DOM derived CO₂ and CH₄ production from Bog Lake Fen soil (a, d), S1 Bog soil (b, e), and Zim Bog soil (c) following 40 day incubation (n=3 per data point). S-DOM derived refers to the difference between S-DOM amended soils and unamended soils from the same site.

Figure 1.

Figure 2.

Figure 3.