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MOLECULAR BIOGEOCHEMISTRY OF A POOR FEN

By

Zhimin Song

A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

In Environmental Engineering

MICHIGAN TECHNOLOGICAL UNIVERSITY

2018

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This dissertation has been approved in partial fulfillment of the requirements for the
Degree of DOCTOR OF PHILOSOPHY in Environmental Engineering.

School of Forest Resources and Environmental Science

Dissertation Advisor: *Paul V. Doskey*

Committee Member: *Erik A. Lilleskov*

Committee Member: *Evan S. Kane*

Committee Member: *Daisuke Minakata*

School Dean: *Andrew J. Storer*

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Preface

This preface serves as an introduction to the contents of the three main chapters in this dissertation. The three chapters covers the projects which I was working under the direction of Dr. Paul Doskey in the school of Forest Resources and Environmental Science at Michigan Tech from Fall 2016 to Summer 2018. These projects would not have been completed without the unconditional support from Dr. Erik Lilleskov and Dr. Evan Kane. The second chapter covers the effect of PFGs and seasonal effects on the dissolved organic matter in the porewater; the third chapter covers the variation of organic/inorganic nitrogen in the porewater with respect to season and PFGs; the fourth chapter summarizes the effect of PFGs and peat sampling depth on the variation of organic matters in the peat samples. All the chapters were written by Ms. Zhimin Song and proofed by Dr. Paul Doskey. The main results were intended to submit to Biogeochemistry.

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Zhimin Song

Michigan Technological University

Abstract

Peatlands store 30% of the world's soil carbon. Climate change may transform peatlands from net carbon sinks to carbon sources, which could result in a positive feedback to global warming. Variations in climate may rapidly change the carbon balance of peatlands and increase greenhouse gas emissions through accelerated decomposition of peat and dissolved organic matter (DOM). The rate of decomposition of natural organic matter follows the general order: carbohydrates > proteins > lipids > lignin $\approx$ tannin. As result, the quality and quantity of organic matter in peat and DOM in porewater are important to evaluate peatland stability. In this study, porewater samples were collected monthly from May to October in 2017 to study the effects of plant functional groups (PFGs) on DOM, dissolved inorganic and organic nitrogen through the season in a poor fen with replicate plots dominated by different PFGs in Nestoria, Michigan, USA (46.34°N 88.16°W). Three peat cores samples were collected in fall 2016 to understand how the composition of OM varied in different depths and PFGs.

The composition of the peat porewater showed strong statistical significance with plot type and season. In terms of PFG effects, the unmanipulated plots showed the highest decomposition rate in the porewater was possible because of the synergistic effect between the sedge and Ericaceae. Ericaceae-only plots showed low enzymatic activities could be the anoxic condition and low decomposition rate. Sedge-only plots exhibited the highest concentrations of total dissolved organic carbon and proteinaceous species throughout the field experiment might due to the fast decomposition rates of the labile

compounds with O₂ present. Sedge plots also showed lower concentration of free amino acids and have higher impact on N-containing DOM (NDOM) composition than ericaceous plots. Lower total phenolics, higher polysaccharides, and higher carbonyl/lignin, polysaccharides/lignin, E2/E3 values were observed in sedge plot than the unmanipulated and ericaceous plot. In terms of season effects, the lowest percentage of degraded materials was observed in the early season, while the highest was observed in the late season. The composition of the DOM was possible regulated on the seasonal time scale by fresh inputs from sedges and Ericaceae, microbial decomposition of labile OM, and accumulation of refractory organic compounds. The early season showed high concentration of arginine, while low concentration of ammonium was observed in fall. The maximum concentration of protein was found in June or July with a concentration less than 0.15 mg N/L. The molecular diversity of NDOM increased with season, and the NDOM changed dramatically with season by reduction, oxidation, hydration, and condensation reactions. The effect of peat sampling depth on the OM composition was also investigated. Highest DOC, TDN and protein and lowest total phenolics and C/N were observed at 20-40 cm; the concentrations of ammonium and TFAA decreased while the DOC and TDN increased with sampling depth. The observed PFG and season impact on the composition of DOM will offer great guidance for the prediction of the DOM decomposition in peatland with respect to climate change.

Chapter 1 Introduction

Peatlands are wetland ecosystems where the rate of production of organic matter exceeds the rate of decomposition. These carbon-rich ecosystems encompass about 3% of the land surface and contain ~30% of the global soil carbon pool (Batjes 2014). . Climate change may transform peatlands from net carbon sinks to carbon sources. Variations in climate may rapidly change the C balance of peatlands and increase emissions of greenhouse gases like methane and carbon dioxide through accelerated decomposition of peat and dissolved organic matter (DOM) (Bridgham et al. 2008; Moore et al. 1998). The rate of OM respiration is strongly related to the molecular composition of organic matter (OM) in peatlands. Each class of OM has a unique decay rate. Thus, to advance understanding of the mineralization rate of OM requires identification of the various classes of OM in peatlands.

Poor fens are a class of peatlands that is acidic, nutrient poor, saturated, groundwater-fed and typically dominated by sedges, ericaceous shrubs, and *Sphagnum* mosses (Andersen et al. 2013). Peatland vegetation influences leaf and root litter quality, O₂ availability (and thus, redox potential), composition of root exudates, root symbiosis, and the nutrient composition of porewater through competition with microbes for nutrients (Romanowicz et al. 2015). Peatland-adapted sedges are perennial graminoids with a deep root structure, which allows the plants to extract water and nutrients from deep zones of the peat that is unavailable to other plant groups. Specialized structures (aerenchyma) in sedges transport O₂ via diffusion or mass flow from the atmosphere deep into the anoxic peat, which

influences the composition of microbial populations and enzyme activities (Flessa and Fischer 1992). Respiration of OM is more energetically favorable when O₂ is used as the electron acceptor, and thus, decomposition of OM in the vicinity of sedge roots is expected to be more rapid than in the rhizosphere of peatland plants that lack aerenchyma or in non-rhizosphere peat. Free-living aerobic heterotrophic fungi and prokaryotes live near sedge roots and process the OM, which releases nutrients for uptake by plants and the microbial population. Sedge root exudates can also stimulate microbial activity and perhaps prime decomposition. Ericaceous shrubs are shallow-rooted and lack aerenchyma. Ericoid mycorrhizal fungi associated with the roots of Ericaceae produce a complex mixture of extracellular enzymes (EEs), including proteases, phosphatases, phenol oxidases, chitinases and cellulases (Cairney and Burke 1998). The EEs process the complex mixture of OM in peat and release nutrients for uptake by plants and the microbial population (Read et al. 2004). *Sphagnum* mosses access water through precipitation and capillarity action (Rydin et al. 2013). Decay of *Sphagnum* is slow compared to production (Johnson and Antoni 1991). *Sphagnum* mosses lack roots and are vulnerable to fluctuations in the depth of the water table (Gignac et al. 2000).

Availability of O₂ in the rhizosphere regulates enzyme activities. Oxidative enzymes like phenol oxidase require O₂ to process phenolic compounds. Low O₂ availability in peat will lead to accumulation of polyphenols, which will further suppress the activities of hydrolytic enzymes like β -1,4-glucosidase, β -1,4-N-acetyl-glucosaminidase, and acid-phosphatase. Oxygen availability in the rhizosphere is regulated by photosynthesis, plant type, microbial activity, water column O₂, and ambient water temperature (Greve et al.

2003). Plants can obtain O₂ via internal photosynthetic production during daylight and passive diffusion from the water column into leaves during darkness (Borum et al. 2005). The ambient water temperature influences plant and sediment respiration, photosynthesis rates, plant growth (Raun and Borum 2013), and the O₂ diffusion rate (Elgetti Brodersen et al. 2016).

Dissolved organic carbon (DOC) is the most active portion of soil organic matter due to its abiotic and biotic reactivity (Khadka et al. 2016). Recalcitrant humic substances and labile compounds, such as carbohydrates, peptides, amino acids, carboxylic acids, and alcohols are included in the broad classification of DOC (Hribljan et al. 2014; Sachse et al. 2005). The origin of DOC in peatlands includes substances from peat and plant tissue decomposition, litter leachate, plant root exudates, and microbial biomass (Kalbitz et al. 2000). Microbial biomass and root exudates, which are composed of simple proteins, amino acids and sugars, and short-chain carboxylic acids, are more easily processed than plant-derived material, which is composed of cellulose, lignin, and tannins.

Nitrogen is a plant and microbial macronutrient and plays a substantial role in the cycling of organic matter in peatlands and is often the limiting nutrient for peatland plants. The availability of nitrogen influences microbial, fungal and plant productivities. The dominant dissolved inorganic nitrogen (DIN) in peatlands is ammonium because the water saturated condition inhibit nitrification. The dissolved organic N (DON) species include amino acids, amino sugars, proteinaceous compounds, and a complex mixture of non-hydrolysable organic N species (Kielland 1995). Microbial decomposition of proteinaceous material and uptake of amino acids and inorganic nitrogen by plants are

influenced by environmental factors, which produce seasonal variations in the DIN and DON pool. To predict peatland responses to climate change, it is important to understand the dynamics of DIN and DON.

In this study, porewater samples were collected monthly from May to October in 2017 at a peatland in Nestoria, Michigan. The vegetation in the sampling plots was manipulated in a replicated design to produce sedge- and shrub-only plots. These were used to study the seasonal variation of porewater DOM, DIN and DON in the poor fen dominated by different plant functional groups (PFG). Additionally, three peat cores samples were collected in fall 2016 to understand how the composition of OM varied in different depths and PFG. Ultra-high resolution mass spectrometry (UHR-MS) was used to analyze the molecule-level characteristics of OM of the trajectories of the biotic transformations of peat-derived OM and the elemental composition was further based on the elemental ratio of carbon, oxygen and hydrogens for visualization.

Reference

- Andersen R, Chapman SJ, Artz RRE (2013) Microbial communities in natural and disturbed peatlands: A review *Soil Biology and Biochemistry* 57:979-994 doi:<http://dx.doi.org/10.1016/j.soilbio.2012.10.003>
- Batjes NH (2014) Total carbon and nitrogen in the soils of the world *European Journal of Soil Science* 65:10-21 doi:10.1111/ejss.12114_2
- Borum J, Pedersen O, Greve TM, Frankovich TA, Zieman JC, Fourqurean JW, Madden CJ (2005) The potential role of plant oxygen and sulphide dynamics in die-off events of the tropical seagrass, *Thalassia testudinum* *Journal of Ecology* 93:148-158 doi:10.1111/j.1365-2745.2004.00943.x
- Bridgham SD, Pastor J, Dewey B, Weltzin JF, Updegraff K (2008) Rapid carbon response of peatlands to climate change *Ecology* 89:3041-3048
- Cairney J, Burke R (1998) Extracellular enzyme activities of the ericoid mycorrhizal endophyte *Hymenoscyphus ericae* (Read) Korf & Kernan: their likely roles in decomposition of dead plant tissue in soil *Plant and Soil* 205:181-192
- Elgetti Brodersen K, Koren K, Lichtenberg M, Kuhl M (2016) Nanoparticle-based measurements of pH and O₂ dynamics in the rhizosphere of *Zostera marina* L.: effects of temperature elevation and light-dark transitions *Plant Cell Environ* 39:1619-1630 doi:10.1111/pce.12740
- Flessa H, Fischer W (1992) Plant-induced changes in the redox potentials of rice rhizospheres *Plant and soil* 143:55-60
- Gignac LD, Halsey LA, Vitt DH (2000) A bioclimatic model for the distribution of Sphagnum-dominated peatlands in North America under present climatic conditions *Journal of Biogeography* 27:1139-1151 doi:10.1046/j.1365-2699.2000.00458.x
- Greve TM, Borum J, Pedersen O (2003) Meristematic oxygen variability in eelgrass (*Zostera marina*) *Limnology and Oceanography* 48:210-216

- Hribljan JA, Kane ES, Pypker TG, Chimner RA (2014) The effect of long-term water table manipulations on dissolved organic carbon dynamics in a poor fen peatland *Journal of Geophysical Research-Biogeosciences* 119:577-595 doi:10.1002/2013jg002527
- Johnson LC, Antoni WHD (1991) Species-Controlled Sphagnum Decay on a South Swedish Raised Bog *Oikos* 61:234-242 doi:10.2307/3545341
- Kalbitz K, Solinger S, Park J-H, Michalzik B, Matzner E (2000) Controls on the dynamics of dissolved organic matter in soils: a review *Soil science* 165:277-304
- Khadka B, Munir TM, Strack M (2016) Dissolved organic carbon in a constructed and natural fens in the Athabasca oil sands region, Alberta, Canada *Sci Total Environ* 557-558:579-589 doi:10.1016/j.scitotenv.2016.03.081
- Kielland K (1995) Landscape patterns of free amino acids in arctic tundra soils *Biogeochemistry* 31:85-98
- Moore TR, Roulet NT, Waddington JM (1998) Uncertainty in Predicting the Effect of Climatic Change on the Carbon Cycling of Canadian Peatlands *Climatic Change* 40:229-245 doi:10.1023/a:1005408719297
- Raun AL, Borum J (2013) Combined impact of water column oxygen and temperature on internal oxygen status and growth of *Zostera marina* seedlings and adult shoots *Journal of Experimental Marine Biology and Ecology* 441:16-22 doi:<http://dx.doi.org/10.1016/j.jembe.2013.01.014>
- Read DJ, Leake JR, Perez-Moreno J (2004) Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes *Canadian Journal of Botany* 82:1243-1263
- Romanowicz KJ, Kane ES, Potvin LR, Daniels AL, Kolka RK, Lilleskov EA (2015) Understanding drivers of peatland extracellular enzyme activity in the PEATcosm experiment: mixed evidence for enzymic latch hypothesis *Plant and Soil* 397:371-386 doi:10.1007/s11104-015-2746-4
- Rydin H, Jeglum JK, Jeglum JK (2013) *The biology of peatlands*, 2e. Oxford university press,

Sachse A, Henrion R, Gelbrecht J, Steinberg CEW (2005) Classification of dissolved organic carbon (DOC) in river systems: Influence of catchment characteristics and autochthonous processes *Organic Geochemistry* 36:923-935
doi:<http://dx.doi.org/10.1016/j.orggeochem.2004.12.008>

Chapter 2 Molecular biogeochemistry of a poor fen. I.

Dissolved organic matter in porewater

Zhimin Song¹, Maryam Khaksari², Erik A. Lilleskov^{3,4}, Evan S. Kane⁴, and Paul V. Doskey^{4,5}

¹Department of Civil and Environmental Engineering, Michigan Technological University, Houghton, Michigan 49931, USA, ²Department of Chemistry, Michigan Technological University, Houghton, Michigan 49931, USA, ³USDA Northern Research Station, Houghton, MI 49931, USA, ⁴School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, USA, ⁵Atmospheric Sciences Program, Michigan Technological University, Houghton, Michigan 49931, USA

Abstract Climate change may transform peatlands from net carbon sinks to carbon sources, which could result in a positive feedback impact on global warming. Recent studies have shown the seasonal variation of dissolved organic matter (DOM) in a different ecosystem. However, our understanding of the seasonal diagenesis of DOM in different plant functional group (PFG) dominated site is limited in peatland. In this study, the effects of PFG on peat porewater composition through the season was thoroughly investigated in a poor fen dominated by different PFG in Nestoria, Michigan, USA (46.34°N 88.16°W). The composition of the peat porewater showed strong statistical significance with PFG and season. The lowest percentage of degraded materials was observed in the early season, while the highest was observed in the late season. The unmanipulated plots showed the highest decomposition rate due to the synergistic effect

between the sedge and Ericaceae. Low enzymatic activities were observed in ericaceous-only plots due to the anoxic condition and low decomposition rate. Sedge-only plots exhibited the highest concentration of DOC and protein due to fast decomposition rates of the labile compounds with O₂ presents. The observed PFG impact on the seasonal composition of DOM will offer great guidance for the prediction of the DOM decomposition in peatlands with respect to climate change.

Keywords Porewater · sedge · Ericaceae · Dissolved organic matter

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Introduction

Peatlands are wetland ecosystems in which the rate of production of organic matter exceeds the rate of decomposition. These carbon (C)-rich ecosystems comprise 3-5% of the Earth's land surface and contain approximately 30% of global soil C, which is equivalent to about 455 Pg of C (Blodau 2002; Dahl and Zoltai 1997; Gorham 1991; Meingast et al. 2014; Trettin et al. 2006). A large amount of C stored in peatlands is sensitive to variations in the climate system (Bridgman et al. 2008), which regulates the global C balance (Tarnocai et al. 2009; Trettin et al. 2006). Increases in temperature and lowering of the water table in peatlands that are attributed to climate change may enhance organic matter (OM) decomposition rates, increase C losses as carbon dioxide (CO₂), methane (CH₄), and dissolved organic carbon (DOC) (Dieleman et al. 2016; Rouse et al. 1997), and release inorganic nitrogen (N) and amino acids (AAs) (Scarpelli 2016). Decomposition of OM is regulated by temperature, moisture and aeration that affect redox potential, pH, enzyme activity (Limpens et al. 2008; Schneckner et al. 2014), and OM availability. Biodegradability of OM in peatlands is important to the internal cycling of C, N, and phosphorus (P) (Tfaily et al. 2013) and is related to the composition of OM inputs. The molecular composition of OM in peatlands influences microbial activity and regulates pH, mineral complexation, and the mobility of heavy metals (Kane et al. 2014).

Variations in climate can drive changes in the relative abundance of plant functional groups (PFGs) in fens, which in turn affect OM decomposition. Sedges, ericaceous

shrubs, and *Sphagnum* mosses are the dominant PFGs in poor fens. Shrubs typically increase in abundance as water tables fall (Strack et al. 2015) and *Sphagnum* growth is slowed as temperatures increase (Walker et al. 2015). Sedges are favored by wetter microplots in peatlands, and thus, abundance of sedges might increase in peatlands as water tables rise. Litter quality, rhizosphere molecular oxygen (O₂) availability, nutrient acquisition pathways, and root symbiotic relationships are regulated by plant functional type (Romanowicz et al. 2015). The seasonal cycle of peatland vegetation also alters the composition of the microbial community and leads to distinct seasonal patterns of enzyme activities that regulate the decomposition of OM (Kaiser et al. 2010).

Plant litter, microbial biomass, and root exudates are typical sources of dissolved OM (DOM) (Kalbitz et al. 2000). DOM is an important C source for microorganisms and contains organically bound nutrients such as N, P, and sulfur (S) (Marschner and Kalbitz 2003). Nutrient and substrate availability are affected by biotic and abiotic transformations of DOM. The concentration and molecular composition of DOM in peatlands vary seasonally and are controlled by the production, consumption, and transport of DOM (Singh et al. 2014). Seasonal variations in the levels and composition of DOM have been observed in a variety of ecosystems (Brooks et al. 2007; Fellman et al. 2009; O'Donnell et al. 2010; Porcal et al. 2013; Wilson et al. 2013). Fellman et al. (2009) reported a distinct seasonal variation in the quality and lability of DOM in wetland and bog watersheds in Alaska. The most labile, protein-rich, and biodegradable DOC (BDOC) was observed during spring runoff. Lowest levels of BDOC were found in the summer months. Concentrations of DOM with high aromaticity and a high degree of

humification were greatest in a catchment during autumn (Catalán et al. 2013). High levels of DOM in autumn were attributed to leaf fall that supplied a fresh input of labile DOM. Processes regulating the concentration of DOM include increasing OM decomposition during the warm spring and summer months, autumn leaf fall, microbial production (Buckeridge et al. 2013) and turnover (Schmidt et al. 2007), and microbial mineralization of OM. Kaiser et al. (2001) observed seasonal variations in the composition of DOM in forest soils from several plots. Lignin-derived phenols, carbohydrates, and amino sugars were highest in summer and autumn and neutral carbohydrates and amino sugars were dominant in winter and spring. However, seasonal variations in the molecular composition of DOM in peatland plots dominated by various PFGs have not been investigated.

Seasonal diagenesis of DOM in a peatland dominated by various PFGs was investigated in a poor fen using locations that were manipulated to contain sedge- and Ericaceae-only plots. The following hypotheses were examined: (1) The DOM of sedge rhizospheres will be characterized by labile organic compounds and a low abundance of phenolic species, (2) Dissolved OM with high aromaticity and low O/C and N/C atomic ratios will be observed in Ericaceae-only plots, and (3) Increases in O₂ availability provided by sedge roots will enhance decomposition of woody biomass in unmanipulated plots. Porewater was collected monthly immediately after spring snowmelt into the fall season. Hypotheses were tested through bulk organic, compound class, and molecular analysis of the dissolved phase of porewater using a combination of ultra high

performance liquid chromatography (UHPLC), spectral indices, and electrospray ionization, ultra-high resolution mass spectrometry (ESI-UHR-MS).

Material and methods

Site description

The study was conducted in an extensive poor fen in Nestoria, Michigan, USA (46.34°N 88.16°W) (Meingast et al. 2014). Vegetation in the peatland included sedge (*Carex* spp.), ericoid shrubs (*K. polifolia*, *C. calyculata*, *R. groenlandicum*, *A. polifolia* var. *glaucophylla*, *Vaccinium* spp.) and *Sphagnum* moss (*S. magellanicum*, *S. angustifolium*, and *S. fuscum*). The study plots included 3 blocks with each block containing 3 plots. Plant communities were manipulated to create the following 3 types of plots: (1) Unmanipulated (U) - sedges and Ericaceae were left intact, (2) Sedge-only (S) – sedges were left intact and Ericaceae were removed, and (3) Ericaceae-only (E) – Ericaceae were left intact and sedges were removed. *Sphagnum* moss was left intact in all plots.

Sample collection

Level loggers in wells constructed of perforated polyvinylchloride (PVC) pipe were used to measure the depth of the water table (Kane et al. 2014). Samples of porewater were collected from piezometers constructed from PVC pipe. The piezometers had 10-cm-slotted sections, which were located at depths of 20 cm and 40 cm (Romanowicz et al. 2015). Nitex nylon mesh was used to form a water-permeable barrier between each section and the adjacent peat.

Porewater samples were collected each month in 2017 during the snow-free season from May to October. Samples for analysis of oxidation-reduction potential (ORP) and pH; total soluble iron (Fe) and manganese (Mn); and total DOC (TDOC), total dissolved N (TDN) and dissolved inorganic nitrogen [nitrate (NO_3^-) and ammonium (NH_4^+)], and organic molecular species were stored in 60-mL amber vials (VWR, Radnor, PA), 15-mL polypropylene (PP) conical centrifuge tubes (VWR international, Batavia, IL), and 40-mL amber volatile organic analysis (VOA) vials (Thermo Fisher Scientific, Waltham, MI), respectively. The amber vials and PP centrifuge tubes were cleaned with soap water and rinse with distilled water in preparation for sample collection. The VOA vials were burned in furnace at 550° C, cleaned with distilled water, dried in oven, flushed with high-purity compressed N_2 (Superior Water and Welding, Chassel, MI), and evacuated in preparation for sampling. Glass fiber filters (0.45- μm ; VWR international, Batavia, IL) were cleaned before use by flushing with 10 mL of MilliQ water and autoclaving. All

samples were filtered in the field with the exception of 2, 20-mL aliquots, which were used for analysis of ORP and pH. Samples were packed into a cooler with ice packs and transported to the laboratory, where they were immediately stored at 4° C.

Sample analysis

The ORP was determined in the field by injecting a 20-mL aliquot into a sealed flow-through cell containing a Hach ORP probe (IntelliCALMTC301; Hach Company, Loveland, CO) (Romanowicz et al. 2015). The probe was calibrated with an ampoule of Light's solution. All electrode potential (E_h) values were normalized to pH 7 (E_h 7) according to pH- E_h relationships for Quinhydrone (Bier 2009). The pH was measured in the laboratory using an accumet™ Model 25 pH meter (Fisher Scientific). For the measurements of TDOC and TDN, 20-mL aliquots were acidified to ~pH 2 with concentrated HCl and analyzed on a TOCV analyzer with TDN module (Shimadzu Scientific Instruments, Columbia, MD). Nitrate and ammonium were determined on ICS-2100 and ICS-1100 ion chromatographic systems (Thermo Scientific, Location), respectively, which were run in parallel. Total soluble Fe and Mn were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES; PerkinElmer Optima 7000DV, PerkinElmer Corporation, Waltham, Ma).

Total phenolics (tannin and lignin; TPHs) were analyzed by adapting a Hach method to a microplate technique (Romanowicz et al. 2015). In each microplate well, 83 μL of sample and 166 μL of MilliQ water were added to achieve a 1:3 dilution. To eliminate interference from Fe(II), 10 μL of TanniVer reagent and 20 μL of 0.1 g mL^{-1} sodium pyrophosphate solution were added to each microplate well. The plates were shaken for 30 s after adding 50 μL sodium carbonate solution (Hach Company, Loveland, CO), incubated for 25 min, and the absorbance at 700 nm was read on a SpectraMax M2 plate reader (Molecular Devices, Sunnyvale, California). The TPHs were quantified with aqueous standards of tannic acid (J.T.Baker, Inc., Philipsburg, NJ).

Monosaccharides (MCHOs) and polysaccharides (PCHOs) were measured by the 2,4,6-tripyridyl-s-triazine (TPTZ) spectrophotometric method (Myklestad et al. 1997). For measurement of MCHOs, 0.1 mL of sample, 0.9 mL of MilliQ water, and 1 mL of potassium ferricyanide solution (0.7 mM) were mixed in a pre-combusted, screw cap test tube (Fisher Scientific International Inc., Pittsburgh, PA) using a Vortex mixer (Fisher Scientific International Inc., Pittsburgh, PA). The mixture was heated at 100 °C for 10 min in an incubator (Hach Company, Loveland, CO) and 1 mL of ferric chloride solution (2 mM) and 2 mL of TPTZ solution (2.5 mM) were added immediately. After 30 min, the sample was transferred to a 10 mm disposable cuvette and the absorbance at 595 nm was measured with a UV-Visible spectrophotometer (Hach DR 5000, Hach). The MCHOs were quantified with aqueous standards of D-glucose. Total dissolved saccharides (TDCHOs) were determined by the TPTZ spectrophotometric method following hydrolysis of a 4 mL sample with 4 mL of 0.1 M HCl in a 5 mL flame-sealed, amber

glass ampoule (VWR international, Batavia, IL) at 100 °C for 20 h. The concentration of PCHOs was determined by subtracting the concentration of MCHOs in the sample from the TDCHOs measurement. The MCHO and PCHO concentrations were corrected for interference by Fe(II).

Proteinaceous species were determined following hydrolysis to AAs [33]. Equal volumes (0.5 mL) of sample and 12 N HCl (Fisher Scientific International Inc., Pittsburgh, PA) were transferred to a 2-mL amber ampoule (VWR international, Batavia, IL), which was sealed under vacuum and heated at 110 °C for 20 h. The hydrolysate was dried under vacuum in a desiccator to remove the acid, redissolved in 1 mL of MilliQ water, and filtered with a 0.2- μ m cellulose acetate membrane filter (VWR international, Batavia, IL) in preparation for analysis. The AAs in the hydrolysate and the filtered porewater sample [i.e., free amino acids (FAAs)] were determined by an on-line derivatization technique using an UHPLC with a fluorescence detector (Dionex Ultimate 3000; Dionex, Sunnyvale, CA). The derivatization reagent was a mixture of 1 mL of *o*-phthalaldehyde (OPA) reagent (Sigma-Aldrich, St. Louis, MO) and 2 μ L of 3-mercaptopropionic acid (MPA) reagent (Sigma-Aldrich, St. Louis, MO). Standards and samples were mixed on-line with the derivatizing reagent prior to injection into the UHPLC. The AAs were separated by gradient elution at 2 mL min⁻¹ through a C-18 analytical column (Zorbax Eclipse AAA, 3.5 μ m, 4.6 $\times$ 150 mm; Agilent Technologies, Santa Clara, CA) using two eluents, i.e., eluent A (borate buffer, pH 7.8) and eluent B (45:45:10 by volume of acetonitrile, methanol, and water (Dorresteyn et al. 1996; Kutlan et al. 2002). The AAs were quantified with an aqueous mixed standard containing

aspartate, glutamate, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, valine, methionine, phenylalanine, isoleucine, leucine, lysine, proline, and cysteine. The level of FAAs in the filtered porewater sample was subtracted from the amount of AAs in the hydrolysate to obtain the concentration of proteinaceous species in the sample. Dissolved organic nitrogen species other than proteinaceous species and amino acids were estimated by subtracting levels of the proteinaceous species, amino acids, and inorganic nitrogen ($\text{NO}_3^- + \text{NH}_4^+$) from the TDN concentration.

Molecular formulas for individual organic species composing several classes of organic compounds were determined by ESI-UHR-MS (Orbitrap Elite Hybrid Ion Trap Mass Spectrometer, Thermo Scientific). Samples were acidified to pH 2.0 with HCl and desalted by passing 10 mL through 1-mL Oasis HLB solid-phase extraction cartridges containing 30 mg of sorbent (Waters Corporation, Milford, MA). The cartridges were pre-conditioned with 1 mL of HPLC-grade methanol and 1 mL of MilliQ water. Cartridges containing sorbed analytes were rinsed with MilliQ water and dried, the species were eluted with 1 mL of methanol/water 90/10 (v/v), and the eluents were stored at 4 °C prior to analysis. Desalted samples were introduced by direct infusion at 5 $\mu\text{L min}^{-1}$ to the ESI-UHR-MS and analyzed in negative ion mode. The heater and capillary temperatures were 80 °C and 265 °C, respectively, and the sheath and auxiliary gas flow rates were 10 arb and 5 arb, respectively. The spray voltage was set to 2.4 kV and broadband mass spectra were collected at 100-800 m/z at a resolution of 240,000. An average of 100 full scans were obtained for each mass spectrum. The instrument was externally calibrated in negative ion mode with the standard negative Thermo calibration

mixture Ultramark 1621 (0.001%), sodium dodecyl sulfate (29ug), sodium taurocholate (54ug) in a solution of 50% acetonitrile/50% methanol/24% water/1% acetic acid per 10mL of solution (Waltham, MA, USA). Mass accuracy was greater than 3 ppm.

Sierra Analytics Composer64 Software (Sierra Analytics, Modesto, CA, USA) was used to calculate all possible molecular formulas having the empirical formula $C_{2-200}H_{4-400}O_{1-40}N_{0-3}S_{0-1}$. The software calculator used the Kendrick mass analysis to differentiate homologous ion series based on the difference in CH_2 increments. The *de novo* cutoff was set to 500 Da with a minimum abundance of 0.05%. Data from the ESI-UHR-MS analysis was visualized using the van Krevelen diagram (Grannas et al. 2006; Hedges 1990; Ohno et al. 2014). The y - and x -axes of a 2-dimensional van Krevelen diagram represent the H/C and O/C atomic ratios in the molecular formulas, respectively. Due to the similarities of molecular formulas of various classes of organic species, signals for the molecular formulas are found in clusters in specific regions of the van Krevelen diagram. The clusters represent several classes of organic compounds (i.e., unsaturated hydrocarbons, condensed aromatics, lipids, carbohydrates, amino sugars, proteins, tannins, and lignin) (Guigue et al. 2016; Kim et al. 2003). The nominal oxidation state of carbon (NOSC) and aromaticity index (AI) were calculated from Eqns. 1 (Lv et al. 2016) and 2 (Guigue et al. 2016), respectively as follows:

$$NOSC = 4 - \frac{4C+H-3N-2O-2S}{c} \quad (1)$$

$$AI = \frac{1+C-O-S-0.5H}{C-O-S-N} \quad (2)$$

Fluorescence and absorbance spectra of the samples were also used to characterize the DOM (Veverica *et al.*, 2016; Coble *et al.*, 2016a, b). Spectra were collected simultaneously in a 1-cm quartz cuvette using an AqualogVR (Horiba Instruments, City, NJ). Data was normalized to the Raman area of a MilliQ water blank. Specific UV absorptivity at 254 nm (SUVA₂₅₄), which is an indicator of the degree of aromaticity (Teixeira *et al.* 2011), was calculated by dividing the absorbance at 254 nm by the DOC concentration (mg C L⁻¹). The index, E2/E3, which is used to gauge molecular size, is the ratio between the absorbance at 254 nm and the absorbance at 365 nm. The E2/E3 index is inversely related to the average molecular size. The humification index (HIX), which is an indicator of the degree of DOM humification, is operationally defined as the area under the emission spectra from 435–480 nm divided by the area under the fluorescence spectra (excitation λ = 254 nm) from 300–345 nm and 435–480 nm (Hansen *et al.* 2016). The index, A/T, is used as an indicator of the recalcitrant/labile nature of the DOM and is operationally defined as the ratio of the peak of the emission at 450 nm from the excitation at 260 nm (A) to the peak of the emission at 304 nm from the excitation at 275 nm (T). The fraction of degraded material increases with increasing values of A/T (Hansen *et al.* 2016).

Statistical analysis

Porewater properties with the exception of depth of the water table and ORP were Box-Cox transformed to normalize distributions. A value of 0.01 was added to concentrations of PCHOs to eliminate zero values. To account for repeated measures, a mixed model using JMP pro 13.2.1 (SAS Institute Inc., Cary, NC) was run with the following response variables: pH, ORP, temperature, depth of the water table, Mn, Fe, TDOC, TDN, PCHOs, MCHOs, TPHs, proteinaceous species, SUVA₂₅₄, E2/E3, HIX, and A/T. The mixed model included the following fixed factors: plot type (S, E, U), sampling depth (20 cm, 40 cm), date of the year (DOY) of the sample collection (130, 160, 188, 216, 260, 292), all two and three-way interactions, and sampling block. Individual plot nested within the plot type was included as a random effect. Probability (p) values less than 0.05 were considered statistically significant.

Matrices of DOM formulas derived from mass spectra and relative abundances using distance-based permutation matrices' analysis of variance (PERMANOVA) statistical models visualized with a non-metric multidimensional scaling (nMDS) technique with Bray-Cutis similarity were used to evaluate relationships between DOM molecular composition and fixed factors. Matrices were fourth-root transformed prior to application of PERMANOVA statistical models and the nMDS technique. The PERMANOVA models included plot type, sampling depth, DOY, all two and three-way interactions, and plot nested with plot type and depth. Type II sums of squares was used for the

PERMANOVA statistical models. The PERMANOVA statistical models and nMDS technique were conducted in Primer 6.1.15 (PRIMER-E, Plymouth, UK).

Molecular formulas that produced the strongest patterns in the datasets were identified. A Monte Carlo method was used to determine the significance of observed maximum indicator molecular formulas that elicited responses. Three indicator species analyses were performed: (1) plot type indicators for all the DOY, $p < 0.01$; (2) DOY indicators for all the plot types, $p < 0.001$; and (3) plot type indicators for specific DOY, $p < 0.01$. Analyses were run in PC-ORD 6.20 (Gleneden Beach, Oregon).

Results

Physical and chemical properties

The depth of the water table varied considerably during the field experiment and was lowest in August on DOY 216 (Fig. 1a) when the highest mean temperature for all plot types and depths was also observed (Fig. 1b). Temperature variations between different depth and plot types that were observed on the same DOY were generally higher from May to August than those observed in September and October. Porewater temperatures

were unaffected by the plot type ($p>0.05$). Mean temperatures with depth during the field experiment for all plot types were higher at 20 cm than at 40 cm (Fig. 1c).

Porewater pH varied significantly with plot type and DOY; however, there were no individual effects ($p>0.05$) of the plot type on pH (Fig. 1d). The highest pH values were observed in June (DOY 160) when pH levels for the 3 plot types were similar. Sedge-only plots exhibited the lowest pH values during the field experiment. The pH values for unmanipulated plots were lower or similar to the Ericaceae-only plots with the exception of DOY 130 when pH levels for the unmanipulated plots were higher. The mean pH values of porewater with depth during the field experiment for all plot types was greater at 20 cm than at 40 cm ($p<0.0001$) (Fig. 1e.). The ORP values of the porewater varied significantly with DOY and plot type (Fig. 1f). The Ericaceae-only and unmanipulated plots exhibited the lowest and highest ORP values, respectively, with the exception of DOY 216 when ORP values for sedge-only plots were greater than the unmanipulated plots.

Concentrations of total Fe in porewater were higher in the Ericaceae-only plots than levels in the sedge-only and the unmanipulated plots with the exception of DOY 188 (Fig. 2a). Levels of total Fe were generally lowest in the unmanipulated plots during the field experiment. Plot type did not exhibit an individual effect on total Fe ($p>0.05$); however, there were interactions between plot type and DOY ($p<0.05$) and between plot type and depth ($p<0.002$). Total Fe concentrations were highest at a depth of 40 cm for all plot types (Fig. 2b).

Porewater concentrations of total Mn varied significantly during the field experiment ($p < 0.0001$) with the lowest levels being observed on DOY188 in July (Fig. 2c). Levels of total Mn in the Ericaceae-only plots were higher than concentrations in sedge-only plots. Concentrations of total Mn were highest in unmanipulated and sedge-only plots at depths of 20 cm and 40 cm, respectively (Fig. 2d). Unlike the sedge-only plots, unmanipulated and Ericaceae-only plots exhibited higher total Mn concentration at depths of 20 cm than the levels at depths of 40 cm. An interaction was observed between plot type and depth ($p < 0.0001$).

Dissolved Organic Matter composition by compound class and spectral characteristics

Concentrations of TDOC in porewater generally increased from approximately 30 mg C L⁻¹ in May to about 45 mg C L⁻¹ in October with the highest levels being observed in September (Fig. 3a). Unmanipulated plots exhibited the lowest TDOC concentrations during the field experiment (Fig. 3a). Levels of TDOC were highest at a depth of 40 cm (Fig. 3b). Concentrations of TDOC in sedge-only plots were higher than the levels in Ericaceae-only plots at all depths (Fig. 3b). Partitioning of the TDOC into various classes of organic compounds is shown in Fig. 3c. The unidentified classes typically represented

the largest fraction of the TCDOC. The MCHOs represented the largest fraction of identified organic compound classes.

Significant interactions ($p > 0.05$) were observed between depth and DOY and the concentrations of MCHOs, between DOY and the level of PCHOs, and between plot type, depth, and DOY for the concentrations of the TPHs. Levels of the MCHOs and TPHs were highest on DOY 216 (Figs. 4a and 5a). Concentrations of the PCHOs were highest on DOY 188 and lowest on DOY 216 (Fig. 4c). The concentration of MCHOs and TPHs increased with sampling depth (Figs. 4b and 5b). Levels of TPHs were highest for the sedge plots (Fig. 5c).

Levels of TDN in porewater increased during the growing season, attaining a maximum in DOY 292 (October) (Fig. 6a). Similar to the trends in TDOC, sedge-only plots exhibit slightly higher TDN concentrations than the Ericaceae-only plots and levels were lowest in unmanipulated plots. Concentrations of TDN increased with depth in the porewater (Fig. 6b). The largest fraction of TDN was unidentified species with the exception Ericaceae-only plots on DOY 130 and the unmanipulated plot at a depth of 20 cm on DOY 130 (Fig. 6c). Significant interactions ($p > 0.05$) were observed between plot type and the level of proteinaceous species (Table 1). Sedge-only plots exhibited slightly higher levels of proteinaceous species than the other plot types (Fig. 6d).

Variations of $SUVA_{254}$ indices with PFG ($p = 0.0006$) and DOY ($p < 0.0001$) were distinct. Higher values of $SUVA_{254}$ were observed at unmanipulated plots than Ericaceae-only plots (Fig. 7a). Values of $SUVA_{254}$ diminished with season through September (Fig. 7b). Mean values of E2/E3 indices were highest at a depth of 40 cm ($p = 0.0005$; Fig.

7c). Seasonal variations of E2/E3 were similar to the trends observed with SUVA₂₅₄ (Fig. 7d). Values of E2/E3 for the unmanipulated plots were higher than the values for other plot types with the exception of DOY 188 (Fig. 7d). Ericaceae-only plots exhibited lower values of E2/E3 than the other plot types except on DOY 260. The HIX indices for all plot types were lowest on DOY 130 (Fig. 7e). Lower values of HIX for unmanipulated plots than sedge and ericaceous plots were observed from DOY 130 to DOY 188 and higher HIX indices were observed for unmanipulated plots from DOY 216 to DOY 292. The A/T index exhibited significant seasonal variations ($p < 0.0001$). The lowest and highest values of A/T were observed on DOY 130 and on DOY 260, respectively (Fig. 7f).

Molecular composition of dissolved organic matter

The O/C and H/C atomic ratios of various classes of naturally occurring organic compounds are presented in Table 2. Seasonal variation in the classes of compounds containing only C, H, and O atoms (e.g., C₈H₁₂O) were negligible; however, classes of compounds containing S and N (e.g., C₁₀H₁₀N₂O₅S) varied significantly. The molecular composition of the DOM was significantly influenced by plot type and DOY ($p < 0.05$) and the interaction between plot types and DOY. There were also significant interactions between plot types, DOY, and depth.

Statistical analyses of the molecular data were used to identify the indicators for all DOY, the DOY indicators for all plot types, and the plot type indicators for all DOY. Results of the nMDS analysis indicate that small values on axis 1 represent the late season and large values on axis 2 represents the early season (Fig. 8). Proteinaceous species, SUVA₂₅₄ and E2/E3 indices, and temperature are positively correlated with the early season and total Fe, TDOC, TPHs, TDN, MCHOs, water table, and the HIX indices are positively correlated with the late season.

An analysis of plot-type indicators for all DOY indicated that the number of indicators were similar for the Ericaceae- and sedge-only plots (Fig. 9a). Unmanipulated plots exhibited the fewest number of condensed aromatic- and lignin-like compounds (Figs. 9a, 9b). Lipid-like compounds were only observed in the sedge-only plots and more tannin-like indicators were found in Ericaceae-only plots. Contributions by the proteinaceous species were greatest for the Ericaceae-only plots. The O/C ratios of lignin-like compounds in sedge-only plots were lower than the ratios in Ericaceae-only plots. The lowest average molecular weight (MW) and the highest AI of the plot-type indicators was found for the sedge-only plots (Fig. 9c). The unmanipulated plots exhibited the highest values of the N/C atomic ratio and the NOSC (Fig. 9c).

An analysis of DOY indicators for all plot types indicated that there were more indicators on DOY 160, 260, and 292 (Fig. 10a). Approximately 75% of the indicators that were observed on DOY 292 were tannin- and lignin-like species. The contribution of lignin-like compounds to the DOM changed significantly through the season (Figs. 10a, 10b). The lowest and highest contribution of lignin-like compounds occurred on DOY 188

and DOY 260, respectively. The highest contribution by the lignin-like compounds on DOY 260 was consistent with highest average MW of the DOY indicators (Fig. 10c). Seasonal trends in the AI and the NOSC were similar. The highest average values of the AI occurred on DOY 160 when contributions by the condensed aromatics were highest. Values of the AI were negative on DOY 130, 188 and 216. Average values of the N/C atomic ratio were highest on DOY 130.

Comparing DOM molecular formulas in the various plot types indicated there were significant differences between plot type by DOY, with the exception of DOY 292 (Table 4). On DOY 188, there was also a significant difference between the two sampling depths (Table 3). Plot type indicators were identified for DOY 130, 160, 216, and 260, and for each depth for DOY 188. More unique compounds with low O/C were observed on DOY 130 in the sedge-only and unmanipulated plots (Figs. 11a, 12a). A single tannin-like indicator was found in the Ericaceae-only plots. Values of NOSC, AI, N/C were lower in the sedge-only plots on DOY 130; however, the average MW of the indicators were slightly higher (Fig. 13a). Contributions of condensed aromatics, unsaturated hydrocarbons, and lignin were greater in the sedge-only plots than in the unmanipulated plots on DOY 130. Fewer plot-type indicators were observed on DOY 160 (Figs. 11b, 12b). More tannin-like species were found in Ericaceae-only plots than the sedge-only and unmanipulated plots. The largest contributions by condensed aromatics and lignin-like compounds were observed in the sedge-only plots. Sedge-only plots also exhibited the highest AI values on DOY 160 (Fig. 13b). All 3 plot-type indicators for the unmanipulated plots were unsaturated hydrocarbons. Values of the average MW and the

NOSC in the unmanipulated plots were lower than the values in the sedge- and Ericaceae-only plots. On DOY 188, the sedge-only and unmanipulated plots exhibited unique plot-type indicators at the sampling depth of 20 cm (Fig. 11c, 12c); however, at the 40 cm, the unique indicator compounds were only found in Ericaceae-only plots (Fig. 11d, 12d). Smaller values of the average MW and AI of the plot-type indicators and higher values of the NOSC and N/C atomic ratios were found at 20 cm in the unmanipulated plots (Fig. 13c). Approximately 1600 plot-type indicators were observed in the Ericaceae-only plots on DOY 216 (Fig. 11e). Contributions were greatest for the lignin- and tannin-like species in the Ericaceae-only plots (Figs. 11e, 12e). The average MW of the plot-type indicators was high on DOY 216 and N/C atomic ratios and the NOSC was low (Fig. 13e). Contributions of tannin- and lignin-like species were greatest for the Ericaceae- and sedge-only plots, respectively, on DOY 260 (Figs. 11f and 12f). Plot-type indicators in the unmanipulated plots had lower O/C atomic ratios and average values of MW and NOSC than indicators in Ericaceae- and sedge-only plots (Fig. 13f). Highest values of the NOSC, AI, MW, and N/C were observed for plot-type indicators in sedge-only plots.

Discussion

Seasonal effects

Concentrations of TDOC and TDN increased from DOY 130 to DOY 292 (Figs. 3a, 6a). Values of the N/C atomic ratio of the DOM were highest late in the season (Figs. 9c, 10c). Levels of TDOC in snowmelt water are typically lower than the concentrations in porewater of peatlands in spring (Anneli et al. 2008), which likely explains the low levels of TDOC observed on DOY 130. Plants require high concentrations of mineral nutrients and carbohydrates to grow stems and leaf sheaths early in the growing season (Silvan et al. 2004), which may explain the low concentrations of polysaccharides and monosaccharides that were observed on DOY 130 and DOY 160 (Figs. 4a, 4c). Microbial decomposition of DOM is accelerated during the summer as temperature increases with concomitant increases in enzyme activities. Peroxidases, which catalyze decomposition of lignin, use Mn. Concentrations of MCHOs (Fig. 4a) and TPHs (Fig. 5a) increased from DOY 188 to DOY 216, levels of PCHOs decreased (Fig. 4c), and concentrations of total dissolved Mn increased. Dehydration of PCHOs to form TPHs and subsequent hydrolysis to MCHOs might explain the trends. Decomposition of macromolecular lignin by manganese peroxidase that produces phenolic monomers might contribute to the low levels of total dissolved Mn and high concentration of TPHs on DOY 188. Exudation of DOM from plant roots likely increases as plant roots grow during the summer, which might also increase TDOC inputs to porewater late in the growing season. Fresh inputs of

fallen leaves or a reduction in the consumption of plant nutrient might also contribute to rising TDOC and TDN levels and increases in the N/C atomic ratio late in the season (Olsrud and Christensen 2004).

The SUVA₂₅₄ and E2/E3 indices diminished during the growing season (Figs. 7b, 7d, respectively), which implies a decrease in aromaticity accompanied by an increase in DOM molecular size. The trend suggests that aromatic compounds with small molecular sizes like monomeric phenolic species are abundant early in the growing season and polymeric species with more alicyclic character are abundant late in the growing season. Aromatic DOM with a large molecular size has been observed in a bog (Broder et al. 2017), which is contrary to observations from the subject study. Trends in the MW of the DOY indicators (Fig. 10c) were similar to trends in the E2/E3 indices (Fig. 7d). Low values of the HIX index, which represents the degree of humification, and the MW of the DOY indicators on DOY 130 are indicative of the presence of low molecular weight, monomeric species. However, seasonal trends in AIs of the DOY indicators were different than the trends in SUVA₂₅₄. The AIs increased sharply after DOY 130 to a maximum value, diminished sharply after DOY 160 and then increased throughout the rest of the season (Fig. 10c). The SUVA₂₅₄ represents the average aromatic content of the bulk DOM; however, the AI only represents the aromaticity of the DOY indicators, i.e., the small fraction of DOM amenable to analysis by ESI-UHR-MS in the negative ion mode. The UHR-MS analysis is restricted to organic substances with masses less than 500 Da and proton donating species, i.e., compounds having carboxylic acid and alcohol functionalities. Results from the molecular analysis are contrary to the spectral analysis

of bulk DOM and suggests that some polymeric OM with aromatic character might be forming in porewater during the growing season. Seasonal variations in the $SUVA_{254}$ indices were directly opposite to trends in concentrations of the TPHs, which include hydroxylated aromatic compounds like tannin, lignin, phenols, and cresols. However, some of the phenolic species might be multifunctional with carboxylic acid moieties that are not quantified by the TPH analytical procedure.

The smallest values of the A/T indices, which is indicative of a small proportion of degraded material, most likely high molecular weight, polymeric species in the bulk DOM, were found on DOY 130 and the largest values of the A/T indices were observed on DOY 260, which is indicative of a large proportion of degraded material, most likely low molecular weight, monomeric species (Fig. 7f). The trends in A/T indices are directly opposite the variations in MW of the DOY indicators, which indicate an increase in MW throughout the growing season. However, the low values of the HIX indices and MW values on DOY 130 are in agreement. The melting snowpack in spring likely flushes low molecular weight, water-soluble compounds into porewater, and thus, low values of HIX indices and the MWs of DOY indicator species are expected. Values of HIX indices after DOY 130 are relatively constant, which indicates a constant degree of humification after spring. Large values of A/T indices late in the season might indicate that root exudates, which are composed of low molecular weight compounds, contribute to the DOM. However, seasonal trends in the A/T indices do not agree with variations in the HIX indices, which are more similar to the trends in the MW of DOY indicator species.

The discrepancies are likely due to spectral analysis of the bulk DOM versus molecular analysis of a small fraction of the DOM by ESI-UHR-MS.

PFG effects

Concentrations of TDOC in Ericaceae-only and unmanipulated plots were similar and were highest in sedge-only plots, with the exception of DOY 130 and DOY 160 when TDOC levels were lower in the unmanipulated plots (Fig. 3a). Aerenchyma tissues in sedges are conduits between the atmosphere and the anoxic rhizosphere. Bacterial decomposition of OM is accelerated in the O₂-rich microenvironment that surrounds sedge roots. Ericoid mycorrhizal (ERM) fungi assist the Ericaceae with nutrient acquisition via the decomposition of OM. Seasonal trends in TDOC might indicate a larger contribution of root exudates to the DOM in sedge-only plots and more fungal decomposition of DOM than bacterial decomposition in the peatland rhizosphere. Highest values of the SUVA₂₅₄ indices were observed in the unmanipulated plots (Fig. 7a), which might indicate more extensive decomposition of macromolecular aromatic species like lignin and accumulation of low molecular weight, water-soluble aromatic products (Hribljan et al. 2014). Values of the E2/E3 indices were generally larger for the unmanipulated plots than the sedge- and Ericaceae-only plots (Fig. 7d), which indicates a larger contribution by low molecular weight species in the unmanipulated plots. Low

molecular weight, fungal decomposition products of lignin and tannin and root exudates from sedge might be responsible for the abundance of low molecular weight species like the TPHs in unmanipulated plots (Fig. 5c). Concentrations of proteinaceous species were lower in the unmanipulated plots (Fig. 6d). Many proteinaceous species are enzymes and thus the low levels might indicate high rates of DOM decomposition.

Sedge-only plots exhibited the highest concentrations of TDOC throughout the season (Fig. 3a). Woody shrub litter is composed of more recalcitrant, lignin- and tannin-like OM than sedge litter. Extracellular enzymes (EEs) like various lignin-modifying enzymes (LMEs) contain Fe, Mn, or copper and are released by ERM fungi and decompose the lignin-like OM. Higher EE activity in Ericaceae-only plots may have contributed to the low levels of TDOC. The high concentrations of proteinaceous species and the presence of lipid-like compounds in sedge-only plots (Figs. 6d and 9a, respectively) are likely attributed to elevated levels of bacterial biomass in the O₂-rich microenvironment surrounding sedge roots. High levels of TPHs in sedge-only plots (Fig. 5c) and large contributions of lignin-like and condensed aromatic species to the plot-type indicators (Fig. 11f) with a high AI (Fig. 9c) indicate that lignin-like species accumulated in the sedge-only plots. However, average MWs of the molecular species in sedge-only plots were smaller than the average values in Ericaceae-only plots, which might indicate larger contributions of polymeric degradation products of the lignin-like species with high aromatic character in sedge-only plots. The low O/C atomic ratios of molecular species in sedge-only plots relative to Ericaceae-only plots (Fig. 12) shows that hydroxy, methoxy, and carboxylic acid functionalities are likely being removed from the lignin-like species

via enzymatic activity, which produces low molecular weight, condensed aromatic species.

Concentrations of the TPHs (Fig. 5c) and values of the SUVA₂₅₄ indices (Fig. 7a) and AIs (Fig. 13), were lower and average MWs were generally higher for Ericaceae-only than the sedge-only plots. Tannin- and lignin-like species contributions to the plot-type indicators were large, which is contrary to trends in the TPHs, SUVA₂₅₄ indices, and AIs. However, the tannin- and lignin-like indicators were nearly all observed on DOY 216, which might make the spectral and other chemical data misleading. The anoxic conditions of the Ericaceae rhizosphere likely suppressed fungal decomposition of the DOM and the free-living saprotroph activity, which allowed the tannin- and lignin-like species to accumulate. Concentrations of total dissolved Fe at Ericaceae-only plots were high (Fig. 2a), which might explain the low enzymatic activities at the Ericaceae-only plots relative to the sedge-only and unmanipulated plots.

DOM composition and interactions between plot type and day of the year

There were significant interactions between plot type and DOY on the E2/E3 indices and HIX indices. The interaction was most pronounced on DOY 130, when the unmanipulated plots exhibited high E2/E3 indices and low HIX indices and the Ericaceae-only plots exhibited the opposite trend (Figs. 7d and 7e, respectively). The

average MW of the plot type indicators for the unmanipulated plots was smaller and the NOSC was higher on DOY 130 (Fig. 13a). Many of the plot type indicators were lignin-like and condensed aromatics with low O/C atomic ratios; however, some species with high O/C atomic ratios were observed (Fig. 12a). The trend in the unmanipulated plots indicates the DOM had a small molecular size and a low degree of humification, which is consistent with a melting snowpack in spring that flushes low molecular weight, water-soluble compounds into porewater. The opposite trend in the Ericaceae-only plots on DOY 130 indicates high molecular weight DOM species with a high degree of humification. The HIX indices and E2/E3 indices exhibited the same trends between plot type and DOY on DOY 160 and DOY 130. The plot type indicators for Ericaceae-only plots on DOY 160 had large contributions of tannin-like compounds (Fig. 11b) with high molecular weight (Fig. 13b). PFG indicators for unmanipulated plots on DOY 160 exhibited unsaturated hydrocarbons with low molecular weight, which are likely degradation products of plant and microbial lipids.

The HIX indices for unmanipulated plots were higher than the HIX indices for Ericaceae-only plots on DOY 216 and 292, which is opposite the trends on DOY 130 and 160. The indices indicate a lower degree of humification in the Ericaceae-only plots; however, the average MW of the plot-type indicators on DOY 216 were high and lignin- and tannin-like species made large contributions to the indicators. The molecular data indicates a high degree of humification, which is inconsistent with the HIX indices of the bulk DOM that indicate a low degree of humification in Ericaceae-only plots. Evergreen shrubs form all or most of their leaves in August unlike sedges, which form their leaves

in spring (Leppälä et al. 2008). Thus, high photosynthetic efficiency of the Ericaceae in August will lead to higher production of labile carbohydrates that will be transported to ERM fungi. Elevated levels of ERM fungi activity may suppress saprotroph activity with a concomitant decrease in DOM decomposition. The ERM fungi lack the class II peroxidases, which are capable of degrading lignin and complex humic substances. Thus, more lignin would accumulate in Ericaceae-only plots, which is consistent with the molecular data.

The HIX indices for unmanipulated and Ericaceae-only plots on DOY 260 were similar and greater than the HIX indices for sedge-only plots (Fig. 7e). The N/C atomic ratios, AIs, average molecular weight and NOSC of the plot-type indicators for sedge-only plots were greater than the values for the unmanipulated and Ericaceae-only plots (Fig. 13f). Contributions of the lignin-like and amino sugars to the plot-type indicators were larger than the contributions in the unmanipulated and Ericaceae-only plots (Fig. 11f). Reaction of amino sugars, polypeptides, and amino acids with the phenolic moieties of lignin produces humic substances. The HIX indices of the bulk DOM in sedge-only plots indicate a low degree of humification; however, the molecular data indicates precursors to humic substances like amino sugars and lignin-like species were present. Levels of proteinaceous species and the contribution of tannin-like species to the plot-type indicators in Ericaceae-only plots were greater than the concentrations and contributions in unmanipulated plots (Figs. 6d and 11f, respectively). Tannin-like compounds complex with proteinaceous species to produce macromolecular species during humification, which is consistent with the molecular data and HIX indices.

Depth effects

Concentrations of TDOC, MCHOs, TPHs, and TDN in porewater at a sampling depth of 40 cm were higher (Figs. 3b, 4b, 5b, and 6b, respectively) and the E2/E3 indices were greater (Fig. 7c) than the levels and indices at 20 cm. Warmer temperatures at 20 cm likely accelerated decomposition of macromolecular organic species, which produced smaller molecules like monosaccharides. Additionally, root and microbial activity is expected to be high in shallow depths of the fen where O₂ availability is high, which might be responsible for lower levels of TDN. The TDOC and TDN appeared to accumulate in deeper zones of the fen where O₂ availability is low.

There were significant interactions between PFG and depth on TDOC concentrations (Fig. 3b). Differences in TDOC levels with depth were greatest for the unmanipulated plots. Concentrations of total dissolved Fe were lower at depths of 20 cm than that at 40 cm (Fig. 2b), which could be due to sequestration of Fe by LMEs. Unlike the trends exhibited by Fe, total dissolved Mn concentrations in Ericaceae-only and unmanipulated plots were higher at 20 cm than 40 cm (Fig. 2d). Manganese is also present in LMEs, and thus, like Fe low levels of Mn at 20 cm were expected. Ericaceae roots typically reach depths of about 20 cm, which could be modifying the redox chemistry of the rhizosphere. The O₂ could consume faster with ericaceous roots at 20 cm because the microbes and roots compete for O₂. The reducing condition results in more Mn dissolved. The depth of the water table and ORP might also regulate total dissolved Mn levels. When the water

table is high and ORP is low, which indicates reducing conditions, Mn would be present in the dissolved phase. As the water table is lowered, conditions might become more oxidizing, which would diminish the solubility of Mn.

Ericaceae-only plots exhibit plot-type indicators at 40 cm on DOY 188 that are unique from the plot-type indicators in sedge-only and unmanipulated plots (Fig. 12d). Sedge roots extend down to depths of below 40 cm, transporting O₂ deep into the rhizosphere of the sedge-only and unmanipulated plots, which alters microbial decomposition of the DOM. Plot-type indicators at a depth of 20 cm in unmanipulated plots have lower MW and AI and higher NOSC and N/C atomic ratios than the sedge-only plots (Fig. 13c). The ERM fungi associated with Ericaceae roots at a depth of 20 cm and increased O₂ availability provided by sedge roots likely enhance enzyme activities and microbial decomposition of the DOM.

Conclusions

Strong seasonal variations of DOM composition and interactions between DOY and plot type were observed. Composition of the DOM may be regulated on the seasonal time scale by fresh inputs from sedge and Ericaceae, microbial decomposition of labile OM, and accumulation of refractory organic compounds. There appeared to be some evidence of the early stages of humification on the seasonal time scale, i.e., reaction of phenol

moieties with amino sugars and complexation of proteinaceous substances by tannin-like species. Concentrations of TDOC were a minimum in May when melting snow flushed DOM characterized by a low average MW and high aromaticity through the fen. Late in the season unique molecular species could be contributed by root exudates and leaf fall that produced DOM with little aromaticity. Contributions of tannin- and lignin-like compounds to the plot-type indicators in the Ericaceae-only plots were high that is possible due to inputs of the woody structure of Ericaceae. The refractory compounds persisted in Ericaceae rhizosphere may arise from a combination of anoxia and suppression of free-living saprotroph activity by the ERM fungi. Sedge-only plots exhibited the highest concentrations of TDOC and proteinaceous species throughout the field experiment. The TPHs were abundant may due to rapid decomposition of labile compounds in the O₂-rich microenvironment surrounding sedge roots that allowed more refractory compounds like lignin and tannin to accumulate.

Variations in the temperature and water table of peatlands with concomitant changes in various plant functional groups are expected in a warming climate. Observing impacts of plant functional groups on seasonal variations of the composition of DOM will be useful in predicting the accumulation of OM in peatlands as the climate changes. Some evidence of humification, which acts to sequester C in peatlands, was apparent on a seasonal time scale. However, year-to-year observations of DOM behavior will be required to understand effects of climate change on long- and short-term storage of C in peatlands.

References

- Anneli Å, Ishi B, Martin B, Kevin B, Mats J, Hjalmar L (2008) Dissolved organic carbon characteristics in boreal streams in a forest-wetland gradient during the transition between winter and summer *Journal of Geophysical Research: Biogeosciences* 113 doi:doi:10.1029/2007JG000674
- Bier AW (2009) Introduction to oxidation reduction potential measurement Hach Company, Lit
- Blodau C (2002) Carbon cycling in peatlands — A review of processes and controls *Environmental Reviews* 10:111-134 doi:10.1139/a02-004
- Bridgham SD, Pastor J, Dewey B, Weltzin JF, Updegraff K (2008) Rapid carbon response of peatlands to climate change *Ecology* 89:3041-3048
- Broder T, Knorr K-H, Biester H (2017) Changes in dissolved organic matter quality in a peatland and forest headwater stream as a function of seasonality and hydrologic conditions *Hydrology and Earth System Sciences* 21:2035
- Brooks PD, Haas PA, Huth AK (2007) Seasonal variability in the concentration and flux of organic matter and inorganic nitrogen in a semiarid catchment, San Pedro River, Arizona *Journal of Geophysical Research: Biogeosciences* 112:n/a-n/a doi:10.1029/2006JG000275
- Buckeridge KM, Banerjee S, Siciliano SD, Grogan P (2013) The seasonal pattern of soil microbial community structure in mesic low arctic tundra *Soil Biology & Biochemistry* 65:338-347 doi:10.1016/j.soilbio.2013.06.012
- Catalán N, Obrador B, Alomar C, Pretus JL (2013) Seasonality and landscape factors drive dissolved organic matter properties in Mediterranean ephemeral washes *Biogeochemistry* 112:261-274 doi:10.1007/s10533-012-9723-2

- Dahl TE, Zoltai SC (1997) Forested northern wetlands of North America. Lewis Publishers, Boca Raton, FL, USA,
- Dieleman CM, Lindo Z, McLaughlin JW, Craig AE, Branfireun BA (2016) Climate change effects on peatland decomposition and porewater dissolved organic carbon biogeochemistry *Biogeochemistry* 128:385-396 doi:10.1007/s10533-016-0214-8
- Dorresteyn RC, Berwald LG, Zomer G, de Gooijer CD, Wieten G, Beuvery EC (1996) Determination of amino acids using o-phthalaldehyde-2-mercaptoethanol derivatization effect of reaction conditions *Journal of Chromatography A* 724:159-167 doi:[http://dx.doi.org/10.1016/0021-9673\(95\)00927-2](http://dx.doi.org/10.1016/0021-9673(95)00927-2)
- Fellman JB, Hood E, D'Amore DV, Edwards RT, White D (2009) Seasonal changes in the chemical quality and biodegradability of dissolved organic matter exported from soils to streams in coastal temperate rainforest watersheds *Biogeochemistry* 95:277-293 doi:10.1007/s10533-009-9336-6
- Gorham E (1991) Northern Peatlands: Role in the Carbon Cycle and Probable Responses to Climatic Warming *Ecological Applications* 1:182-195 doi:10.2307/1941811
- Grannas AM, Hockaday WC, Hatcher PG, Thompson LG, Mosley-Thompson E (2006) New revelations on the nature of organic matter in ice cores *Journal of Geophysical Research: Atmospheres* 111:n/a-n/a doi:10.1029/2005JD006251
- Guigue J, Harir M, Mathieu O, Lucio M, Ranjard L, Lévêque J, Schmitt-Kopplin P (2016) Ultrahigh-resolution FT-ICR mass spectrometry for molecular characterisation of pressurised hot water-extractable organic matter in soils *Biogeochemistry* 128:307-326 doi:10.1007/s10533-016-0209-5
- Hansen AM, Kraus TEC, Pellerin BA, Fleck JA, Downing BD, Bergamaschi BA (2016) Optical properties of dissolved organic matter (DOM): Effects of biological and photolytic degradation *Limnology and Oceanography* 61:1015-1032 doi:10.1002/lno.10270

- Hedges J (1990) Compositional indicators of organic acid sources and reactions in natural environments *Organic acids in aquatic ecosystems* Wiley:43-63
- Hribljan JA, Kane ES, Pypker TG, Chimner RA (2014) The effect of long-term water table manipulations on dissolved organic carbon dynamics in a poor fen peatland *Journal of Geophysical Research-Biogeosciences* 119:577-595
doi:10.1002/2013jg002527
- Kaiser C et al. (2010) Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil *New Phytologist* 187:843-858 doi:10.1111/j.1469-8137.2010.03321.x
- Kaiser K, Guggenberger G, Haumaier L, Zech W (2001) Seasonal variations in the chemical composition of dissolved organic matter in organic forest floor layer leachates of old-growth Scots pine (*Pinus sylvestris* L.) and European beech (*Fagus sylvatica* L.) stands in northeastern Bavaria, Germany *Biogeochemistry* 55:103-143 doi:10.1023/a:1010694032121
- Kalbitz K, Solinger S, Park J-H, Michalzik B, Matzner E (2000) Controls on the dynamics of dissolved organic matter in soils: a review *Soil science* 165:277-304
- Kane ES, Mazzoleni LR, Kratz CJ, Hribljan JA, Johnson CP, Pypker TG, Chimner R (2014) Peat porewater dissolved organic carbon concentration and lability increase with warming: a field temperature manipulation experiment in a poor-fen *Biogeochemistry* 119:161-178 doi:10.1007/s10533-014-9955-4
- Kim S, Kramer RW, Hatcher PG (2003) Graphical method for analysis of ultrahigh-resolution broadband mass spectra of natural organic matter, the van Krevelen diagram *Analytical Chemistry* 75:5336-5344
- Kutlán D, Presits P, Molnár-Perl I (2002) Behavior and characteristics of amine derivatives obtained with o-phthaldialdehyde/3-mercaptopropionic acid and with o-phthaldialdehyde/N-acetyl-L-cysteine reagents *Journal of Chromatography A* 949:235-248

- Leppälä M, Kukko-Oja K, Laine J, Tuittila E-S (2008) Seasonal dynamics of CO₂ exchange during primary succession of boreal mires as controlled by phenology of plants *Ecoscience* 15:460-471 doi:10.2980/15-4-3142
- Limpens J et al. (2008) Peatlands and the carbon cycle: from local processes to global implications – a synthesis *Biogeosciences* 5:1475-1491 doi:10.5194/bg-5-1475-2008
- Lv JT, Zhang SZ, Luo L, Cao D (2016) Solid-phase extraction-stepwise elution (SPE-SE) procedure for isolation of dissolved organic matter prior to ESI-FT-ICR-MS analysis *Analytica Chimica Acta* 948:55-61 doi:10.1016/j.aca.2016.10.038
- Marschner B, Kalbitz K (2003) Controls of bioavailability and biodegradability of dissolved organic matter in soils *Geoderma* 113:211-235
doi:[http://dx.doi.org/10.1016/S0016-7061\(02\)00362-2](http://dx.doi.org/10.1016/S0016-7061(02)00362-2)
- Meingast KM et al. (2014) Spectral detection of near-surface moisture content and water-table position in northern peatland ecosystems *Remote Sensing of Environment* 152:536-546 doi:10.1016/j.rse.2014.07.014
- Myklestad SM, Skånøy E, Hestmann S (1997) A sensitive and rapid method for analysis of dissolved mono- and polysaccharides in seawater *Marine Chemistry* 56:279-286 doi:[https://doi.org/10.1016/S0304-4203\(96\)00074-6](https://doi.org/10.1016/S0304-4203(96)00074-6)
- O'Donnell JA, Aiken GR, Kane ES, Jones JB (2010) Source water controls on the character and origin of dissolved organic matter in streams of the Yukon River basin, Alaska *Journal of Geophysical Research: Biogeosciences* 115:n/a-n/a doi:10.1029/2009JG001153
- Ohno T, Parr TB, Gruselle MC, Fernandez IJ, Sleighter RL, Hatcher PG (2014) Molecular composition and biodegradability of soil organic matter: a case study comparing two new England forest types *Environ Sci Technol* 48:7229-7236 doi:10.1021/es405570c

Olsrud M, Christensen TR (2004) Carbon cycling in subarctic tundra; seasonal variation in ecosystem partitioning based on in situ ¹⁴C pulse-labelling Soil Biology and Biochemistry 36:245-253 doi:<https://doi.org/10.1016/j.soilbio.2003.08.026>

Porcal P, Dillon PJ, Molot LA (2013) Seasonal changes in photochemical properties of dissolved organic matter in small boreal streams Biogeosciences 10:5533-5543 doi:10.5194/bg-10-5533-2013

Romanowicz KJ, Kane ES, Potvin LR, Daniels AL, Kolka RK, Lilleskov EA (2015) Understanding drivers of peatland extracellular enzyme activity in the PEATcosm experiment: mixed evidence for enzymic latch hypothesis Plant and Soil 397:371-386 doi:10.1007/s11104-015-2746-4

Rouse WR et al. (1997) Effects of climate change on the freshwaters of arctic and subarctic north America Hydrological Processes 11:873-902 doi:10.1002/(SICI)1099-1085(19970630)11:8<873::AID-HYP510>3.0.CO;2-6

Scarpelli T (2016) The role of amino acids in the nitrogen cycle of peatlands Open Access Master's Thesis, Michigan Technological University

Schmidt S et al. (2007) Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil Ecology 88:1379-1385

Schnecker J et al. (2014) Effects of soil organic matter properties and microbial community composition on enzyme activities in cryoturbated arctic soils PLoS ONE 9:e94076 doi:10.1371/journal.pone.0094076

Silvan N, Tuittila ES, Vasander H, Laine J (2004) Eriophorum vaginatum plays a major role in nutrient immobilisation in boreal peatlands Annales Botanici Fennici 41:189-199

Singh S, Inamdar S, Mitchell M, McHale P (2014) Seasonal pattern of dissolved organic matter (DOM) in watershed sources: influence of hydrologic flow paths and autumn leaf fall Biogeochemistry 118:321-337 doi:10.1007/s10533-013-9934-1

- Strack M, Kaing E, Perkins M, Munir TM (2015) Carbon dioxide flux and net primary production of a boreal treed bog: Responses to warming and water-table-lowering simulations of climate change vol 12. *Biogeosciences*,
- Tarnocai C, Canadell JG, Schuur EAG, Kuhry P, Mazhitova G, Zimov S (2009) Soil organic carbon pools in the northern circumpolar permafrost region *Global Biogeochemical Cycles* 23:GB2023 doi:10.1029/2008GB003327
- Teixeira MC, Azevedo JCRd, Pagioro TA (2011) Spatial and seasonal distribution of chromophoric dissolved organic matter in the Upper Paraná River floodplain environments (Brazil) *Acta Limnologica Brasiliensia* 23:333-343
- Tfaily MM, Hamdan R, Corbett JE, Chanton JP, Glaser PH, Cooper WT (2013) Investigating dissolved organic matter decomposition in northern peatlands using complimentary analytical techniques *Geochimica et Cosmochimica Acta* 112:116-129 doi:<http://dx.doi.org/10.1016/j.gca.2013.03.002>
- Trettin C, Laiho R, Minkinen K, Laine J (2006) Influence of climate change factors on carbon dynamics in northern forested peatlands *Canadian Journal of Soil Science* 86:269-280
- Walker TN, Ward SE, Ostle NJ, Bardgett RD (2015) Contrasting growth responses of dominant peatland plants to warming and vegetation composition *Oecologia* 178:141-151 doi:10.1007/s00442-015-3254-1
- Wilson HF, Saiers JE, Raymond PA, Sobczak WV (2013) Hydrologic Drivers and Seasonality of Dissolved Organic Carbon Concentration, Nitrogen Content, Bioavailability, and Export in a Forested New England Stream Ecosystems 16:604-616 doi:10.1007/s10021-013-9635-6

Table 1 Mixed-effects model of predictors of Monosaccharides, total phenolics, protein, and polysaccharides.

Source	Monosaccharides	Polysaccharide	Total Phenolics	Protein
Plot type	0.2219	0.2199	0.0325	0.0293
Depth	<.0001	0.0749	<.0001	.
Plot type*Depth	0.8306	0.4494	0.4017	.
DOY	<.0001	0.0016	<.0001	.
Plot type*DOY	0.8586	0.3038	0.7721	.
Depth*DOY	0.9097	0.5057	0.8908	.
Plot type *Depth *DOY	0.858	0.5319	0.9218	.
Block	0.1304	0.2247	0.0107	0.0117

Table 2 The classification of the organic compounds.

Compound class	Abbreviation	O/C range	H/C range
Lipid-like	Lipid	0-0.29	1.6-2
Protein-like	Protein	0.29-0.6	1.5-2
Amino sugar	AS	0.6-0.67	1.5-2
Carbohydrate-like	Carb	0.67-1	1.5-2
Unsaturated hydrocarbons	UH	0-0.29	1-1.6
Condensed aromatics	CA	0-0.4	0-0.7
Lignin-like	Lignin	0.29-0.65	0.7-1.5
Tannin-like	Tannin	0.65-1	0.5-1.5

Table 3 Summary of results from PEMANOVA analysis for all plant functional group (PFG) at depth 20 and 40 cm on all day of the year (DOY). (Main test)

Source	df	SS	MS	Pseudo-F	<i>P</i> <i>n</i> (perm)	Unique perms
PFG	2	1868	934.01	2.6642	0.002	998
Depth	1	479.33	479.33	1.3672	0.165	998
DOY	5	23168	4633.5	15.205	0.001	998
PFG*Depth	2	891.94	445.97	1.2721	0.164	996
PFG*DOY	10	7944.5	794.45	2.6069	0.001	996
Depth*DOY	5	1815.7	363.14	1.1916	0.138	997
PFG*Depth*DOY	10	3815.8	381.58	1.2522	0.04	998
Plot(PFG*Depth)	12	4206.9	350.58	1.1504	0.107	998
Residuals	60	18285	304.74			
Total	107	62474				

*Note: Bolded values are significant at $p < 0.05$.

Table 4 Summary of results from PEMANOVA for each DOY.

Source	df	<i>p</i> (perm)					
		DOY130	DOY16 0	DOY18 8	DOY21 6	DOY26 0	DOY29 2
PFG	2	0.015	0.024	0.012	0.003	0.007	0.108
Depth	1	0.473	0.277	0.047	0.211	0.195	0.332
PFG*Depth	2	0.768	0.245	0.01	0.11	0.22	0.731

*Note: Bolded values are significant at $p < 0.05$.

Figure Captions

Fig. 1 Variation of the environmental factors (physical properties) in the peatland with plot types, DOY or Depth: **(a)** Water table in the peatland; **(b)** DOY impacts on temperature; **(c)** Effect of sampling depth on temperature; **(d)** pH of the porewater with plot types and DOY interaction; **(e)** Effect of sampling depth on pH; **(f)** Oxidation reduction potential (ORP) of the porewater with plot types and DOY interaction

Fig. 2 Variation of different minerals in the porewater: **(a)** Seasonal variation of Total Fe with different plot types; **(b)** Depth impacts on Total Fe with different plot types; **(c)** Seasonal variation of Total Mn; **(d)** Depth impact on Total Mn with different plot types

Fig. 3 **(a)** Seasonal variation of TDOC with different plot types; **(b)** Depth and plot types impacts on TDOC; **(c)** Partition of TDOC changed with season

Fig. 4 **(a)** Seasonal variation of monosaccharides; **(b)** The effect of sampling depth on monosaccharides; **(c)** Seasonal variation of polysaccharides

Fig. 5 (a) Seasonal variation of total phenolic; (b) The effect of plot types on total phenolics; (c) Plot type impacts on total phenolics

Fig. 6 (a) Seasonal variation of TDN with different plot types; (b) Depth impacts on TDN; (c) Partition of DON changed with season; (d) The effect of plot types on proteinaceous species

Fig. 7 Pore water DOC ultraviolet-visible (UV-Vis) and fluorescence spectroscopic parameters. (a) Effect of plot types on $SUVA_{254}$; (b) Seasonal variation of $SUVA_{254}$; (c) Effect of sampling depth on the ratio of E2/E3; (d) Seasonal variation of E2/E3 with different plot types; (e) Seasonal variation of HIX with different plot types; (f) Seasonal variation of A/T.

Fig. 8 Non-Metric multidimensional scale ordination for the molecular composition of DOM for 108 porewater samples. The arrow in the ordination diagram represents environmental variables fitted to the ordination ($r > 0.2$). Length of the arrow represents the correlation.

Fig. 9 Plot types indicator for 20 cm and 40 cm on all DOY. **(a)** Percentage of Class group; **(b)** Van Krenvlen diagram; **(c)** Average NOSC, N/C, AI, MW (molecular weight)

Fig. 10 DOY indicator for all plot types at both sampling depths of 20 cm and 40 cm. **(a)** Percentage of Class group; **(b)** Van Krenvlen diagram; **(c)** Average NOSC, N/C, AI, MW (molecular weight)

Fig. 11 Percentage of Class group **(a)** DOY 130, **(b)** DOY 160, **(c)** DOY 188 at 20 cm, **(d)** DOY 188 at 40 cm. **(e)** DOY 216, **(f)** DOY 260

Fig. 12 Van Krevlen diagram **(a)** DOY 130, **(b)** DOY 160, **(c)** DOY 188 at 20 cm, **(d)** DOY 188 at 40 cm, **(e)** DOY 216, **(f)** DOY 260

Fig. 13 Average NOSC, N/C, AI, MW (molecular weight) **(a)** DOY 130, **(b)** DOY 160, **(c)** DOY 188 at 20 cm, **(d)** DOY 188 at 40 cm, **(e)** DOY 216, **(f)** DOY 260

Fig. 1

Fig. 2

Fig. 3

Fig. 4

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Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 12

Fig. 13

Chapter 3 Molecular biogeochemistry of a poor fen.

II. Dissolved nitrogen species in porewater

Zhimin Song¹, Maryam Khaksari², Erik A. Lilleskov^{3,4}, Evan S. Kane⁴, and Paul V. Doskey^{4,5}

¹Department of Civil and Environmental Engineering, Michigan Technological University, Houghton, Michigan 49931, USA, ²Department of Chemistry, Michigan Technological University, Houghton, Michigan 49931, USA, ³USDA Northern Research Station, Houghton, MI 49931, USA, ⁴School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, USA, ⁵Atmospheric Sciences Program, Michigan Technological University, Houghton, Michigan 49931, USA

Abstract The growth of vegetation, fungi and bacteria in poor fens is often limited by the availability of inorganic and organic nitrogen in porewater. The composition of organic nitrogen, concentration of amino acids and inorganic nitrogen of pore water from a poor fen dominated by different plant function types were studied during six months. Low concentration of ammonium was observed in fall. The concentration of arginine was high in early season and the total free amino acid concentration was below 0.004 mg N/L. The season variations in protein concentration were showed with the maximum concentration in June or July and less than 0.15 mg N/L. Plant functional group and season significantly influenced the concentration of free amino acids and nitrogen containing dissolved organic matter (NDOM) composition. Sedges play higher impact on

NODM composition than ericaceous shrubs. The molecular diversity of NDOM was increasing with season. The NDOM changed dramatically with season by reduction, oxidation, hydration and condensation reaction.

Keywords Amino acids · organic · nitrogen · peatland

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Introduction

Poor fens are acidic, nutrient-poor, peat-saturated, groundwater-fed, and typically dominated by sedges, ericaceous shrubs, and *Sphagnum* mosses (Andersen et al. 2013). Nitrogen (N) is a plant and microbial macronutrient and plays a substantial role in the cycling of organic matter (OM) in peatlands. Many of the northern peatlands receive very small external inputs of nutrients like inorganic N (IN) species. The availability of N influences microbial and plant productivities. The N pool of peatlands is composed of IN and organic N (ON). The dominant IN species are ammonium (NH_4^+), which readily sorbs to negatively charged particles that are abundant in soils and water, and nitrate (NO_3^-), which is mobile in aquatic systems. Levels of NO_3^- in peatlands are very low due

to the water-saturated conditions and uptake by microbes and plants (Aestrom and Aaltonen 2005; Tiemeyer and Kahle 2014). Thus, internal cycling of ON species in peatlands is an important source of N.

Dissolved ON (DON) species include amino acids (AAs), amino sugars, proteinaceous compounds, and a complex mixture of non-hydrolysable ON species (Kielland 1995). Many of the AAs are water soluble and are readily available for uptake by plants and microbes (Lipson et al. 2001). The typical amino acids observed in peat include glycine, serine, aspartate, alanine, threonine, glutamate, valine, and leucine (Swain et al. 1959). Microbial communities and plants, including sedge and *Sphagnum* moss, assimilate AAs directly from soil matrices (Jones and Darrah 1993; Kielland 1994; Leadley et al. 1997; Jones et al. 2005; Krab et al. 2008). Soil microbes prefer specific AAs, particularly the smaller molecules like glycine, and readily compete with plants for the nutrient source (Lipson et al. 1999). Some sedge species like *Kobresia myosuroides* assimilate glycine more rapidly than glutamate.

Sedges, Ericaceae and *Sphagnum* mosses have distinct nutrient acquisition pathways, litter quality, and root structures (Romanowicz et al. 2015), which are expected to uniquely affect AA concentrations in peat porewater. Microbial decay rates of proteinaceous substances in the oxygenated rhizosphere of sedge are likely enhanced due to the energetically favorable use of molecular oxygen (O₂) as an electron acceptor in the respiration of OM (Flessa and Fischer 1992). Weintraub and Schimel (2005) observed differences in total free AA (TFAA) concentrations in ericaceous shrub and sedge-dominated ecosystems. Serine, glycine, alanine, arginine, and threonine were the most

abundant AAs in soil dominated by sedge, and glycine, cysteine, alanine, arginine, and valine were prevalent in soil dominated by ericaceous shrubs.

Microbial decomposition of proteinaceous material and uptake of AAs by plants and microbes are influenced by environmental factors that produce seasonal variations in the FAA pool. Elevated levels of AAs following snow melt and spring thaw have been observed in many studies and attributed to root turnover, litter leachates, and lysis of microbial cells (Mack 1963; Kielland 1995; Lipson et al. 2001). Kielland (1995) observed peak concentrations of TFAA in Alaskan tussock and shrub tundra plots in June (after thawing) and August (before freezing) and minimum TFAA concentrations in July during the growing season. Weintraub and Schimel (2005) made similar observations in Alaskan tussock, shrub, and wet sedge ecosystems and found levels of TFAA and NH_4^+ were near the detection limit in the middle of July. Low AA concentrations in summer were attributed to peak rates of microbial decomposition of proteinaceous material and competition for assimilation of available AAs by plants and microbes.

Internal cycling of inorganic and organic forms of N in peatlands is poorly understood. As northern peatlands are transformed by a warming climate, variations in the distribution of plant functional groups (PFGs) are expected. For example, ericaceous shrubs may replace sedges as temperatures rise and water tables fall. Thus, it is important to understand the influence of various PFGs on plant-microbe interactions and the transformations of N.

The study was conducted in a peatland where the vegetation in several locations had been manipulated to contain sedge- and Ericaceae-only plots. The following hypotheses

were examined: (1) Amino acids will be abundant in porewater immediately following snowmelt due to a flush of dead microbial biomass and (2) Amino acids will diminish in concentration through spring and summer as root exudates from a mature plant population decrease. Porewater was collected once a month from May to October. Hypotheses were tested through bulk N, compound class, and molecular analysis using a combination of ion chromatography (IC), ultra-high performance liquid chromatography (UHPLC), spectral indices, and electrospray ionization, ultra-high resolution mass spectrometry (ESI-UHR-MS).

Material and methods

Site description, experimental design, and sample collection

Meingast et al. (2104) and Song et al. (2018) describe in detail the site of the investigation and experimental design, respectively. Briefly, an extensive poor fen in Nestoria, Michigan, USA (46.34°N 88.16°W) was selected for the study. Locations in the peatland were manipulated to contain sedge- and Ericaceae-only plots. Monthly samples of porewater were collected at depths of 20 cm and 40 cm from slotted sections of piezometers from May through October 2017. Samples were filtered in the field through 0.45- μ m glass fiber filters (Supplier, Location) into amber, 40-mL volatile organic

analysis vials (Thermo Fisher Scientific, Waltham, MI) that were flushed with N₂ and evacuated in the laboratory in preparation for sample collection. Porewater samples were transported on ice to the laboratory within 2 h where they were immediately stored at 4° C.

Sample analysis

Aliquots of porewater (20 mL) were acidified to ~ pH 2 with concentrated HCl, and analyzed for total dissolved N (TDN) on a TOCV analyzer with a TDN module (Shimadzu Scientific Instruments, Columbia, MD, USA) (Hribljan et al. 2014). Dual IC systems (ICS-2100, ICS-1100; Thermo Scientific, Waltham, MA) that were run in parallel were used to measure NH₄⁺ and NO₃⁻. The IC systems were equipped with a 25-μL injection loop and DS6 heated conductivity detectors. The IC system for separating NH₄⁺ from the mixture of cations included an AG-11 guard column, an AS-11 HC analytical column, and an AERS-500 4-mm self-regenerating suppressor. A CS12A analytical column with CG12A guard column and a CERS-500 4-mm self-regenerating suppressor was used on the IC system to separate NO₃⁻ from the mixture of anions. The detection limit for both NH₄⁺ and NO₃⁻ was 0.05 μM. The DON was determined as the difference between TDN and the total DIN (i.e., Σ NO₃⁻ + NH₄⁺).

Analysis of compound classes of organic species in porewater is described in detail by Song et al. 2018). Briefly, proteinaceous species were determined as AAs following hydrolysis with 6 N HCl (Supplier, Location) (Keil and Kirchman 1991). A Dionex Ultimate 3000 UHPLC (Dionex, Sunnyvale, CA) with fluorescence detector was used to separate and detect individual AAs in the filtered porewater (i.e., free AAs) and in the hydrolysates. The AAs were determined as o-phthaldialdehyde derivatives by an on-line derivatization technique (Dorresteyn et al. 1996; Kutlan et al. 2002). A gradient elution through a C-18 analytical column was used to separate the amino acids. An aqueous mixed standard containing aspartate (ASP), glutamate (GLU), serine (SER), histidine (HIS), glycine (GLY), threonine (THR), arginine (ARG), alanine (ALA), tyrosine (TYR), valine (VAL), methionine (MET), phenylalanine (PHE), isoleucine (ILE), leucine (LEU), lysine (LYS), proline (PRO), and cystine (CYS) was used for quantitation.

Concentrations of proteinaceous species were obtained by the difference between the concentrations of AAs in the hydrolysate and the FAAs. Levels of proteinaceous species, FAAs, and DIN were subtracted from the TDN concentrations to estimate the concentrations of DON species other than proteinaceous species and FAAs.

Molecular formulas for organic species were determined by ESI-UHR-MS (Orbitrap Elite Hybrid Ion Trap Mass Spectrometer, Thermo Scientific) and are described in detail by Song et al. (2018). Briefly, samples were acidified and desalted by solid-phase extraction in preparation for analysis [5]. All possible molecular formulas having the empirical formula $C_{2-200}H_{4-400}O_{1-40}N_{0-3}S_{0-1}$ were calculated using Composer64 Software (Supplier, Location). Van Krevelen diagrams were constructed to visualize the data for compounds

containing nitrogen (Grannas et al. 2006; Hedges 1990; Ohno et al. 2014). Signals for the molecular formulas of various classes of organic matter are found in clusters in specific regions of the van Krevelen diagram (Kim et al. 2003; Guigue et al. 2016) (Fig. 1).

Linear trajectories of the organic molecular species in the 3-dimensional space of the diagrams will be used to evaluate seasonal variations in biotic and abiotic processing of DOM. For example, carboxylation (+ COOH) of phenolic functionalities in lignin will move points representing the species upward and to the left in the lignin cluster of Fig. 1.

Eqns. 1 and 2 were used to calculate the nominal oxidation state of carbon (NOSC; Lv et al. 2016) and aromaticity index (AI; Guigue et al. 2016) as follows:

$$\text{NOSC} = 4 - \frac{4\text{C} + \text{H} - 3\text{N} - 2\text{O} - 2\text{S}}{\text{C}} \quad (1)$$

$$\text{AI} = \frac{1 + \text{C} - \text{O} - \text{S} - 0.5\text{H}}{\text{C} - \text{O} - \text{S} - \text{N}} \quad (2)$$

Statistical analysis

Matrices of the molecular formulas of N-containing DOM (NDOM) derived from relative abundances determined by ESI-UHR-MS and concentrations of amino acids were analyzed using distance-based permutation matrices' analysis of variance (PERMANOVA) statistical models and a non-metric multidimensional scaling (nMDS) with Bray-Cutis similarity. Matrices were fourth root transformed prior to application of

the statistical methods. Plot type, sampling depth, DOY, all two and three-way interactions, and plot nested with plot type and sampling depth are all included in the PERMANOVA models, which used Type II sums of squares. An analysis of the molecular formulas of indicator species was performed to understand the molecular species and amino acids that were responsible for the strongest patterns in the datasets. The observed maximum indicator values for a response were determined by a Monte Carlo method. Analyses of four indicator species (only for NDOM) were performed: (1) plot-type indicators for all the DOY; (2) DOY indicators for all the plot types; (3) plot-type indicators for specific DOY; and (4) DOY indicators for each plot type. Primer 6.1.15 (PRIMER-E, Plymouth, UK) was used to conduct the PERMANOVA statistical tests and the nMDS technique. Analyses of indicator species were run in PC-ORD 6.20 (Glenneden Beach, Oregon).

Results

Dissolved inorganic nitrogen, free amino acids, and proteinaceous species in porewater

Concentrations of FAAs were significantly influenced by the DOY ($p < 0.05$) and the interaction between PFG and DOY (Table 1). The amino acids data were further analyzed

to find the DOY indicator for all the PFG (Table 2). The most significant DOY indicators for DOY 130, 160, 188, and 216 are HIS, ARG, THRE and LYS, and LEU, respectively. Seasonal variations of four of the DOY indicators and GLY, which was one of the most abundant FAA, proteinaceous species, TFAAs, and NH_4^+ at the Ericaceae-only, sedge-only, and the unmanipulated plots at two sampling depths (20 cm and 40cm) are shown in Figs. 2, 3, and 4, respectively. The only DIN species that was present at concentrations greater than the detection limit was NH_4^+ .

The five individual AAs followed similar trends and were typically present at $< 15 \text{ nmol L}^{-1}$ at a depth of 20 cm (Fig. 2a) and at $\sim 20 \text{ nmol L}^{-1}$ at a depth of 40 cm in the Ericaceae-only plots (Fig. 2b). Concentrations of the FAAs at the depth of 40 cm were relatively higher than the levels at 20 cm. Concentrations of ARG and GLY were higher than the other AAs during the early growing season. Levels of ARG gradually diminished following a maximum at DOY 160 and concentrations of GLY were higher than the other AAs after DOY 216. The concentration of HIS reached a maximum in June (DOY 160) and then levels gradually diminished. Concentrations of THR and LEU increased after DOY 160 and then gradually returned after DOY 216 to levels observed in early spring. Concentrations of proteinaceous species and NH_4^+ were similar at both depths and decreased from July (DOY 188) to October (DOY 292) (Figs. 2c, 2d). Levels of the TFAAs at 20 cm increased before DOY 188 and then maintained a constant concentration late in the season. Concentrations of TFAAs at 40 cm were greater than the levels at 20 cm and decreased after reaching peak levels in July (DOY 188).

Concentrations of individual AAs at the sedge-only plots were $< 20 \text{ nmol L}^{-1}$ at both depths (Figs. 3a, 3b). Trends in levels of the individual AAs were similar with the exception of GLY, which exhibited greater fluctuations in concentration compared to the other AAs. Concentrations of proteinaceous species were nearly constant ($\sim 0.1 \text{ mg N L}^{-1}$) at both depths (Figs. 3c, 3d). Levels of NH_4^+ attained a maximum in May (DOY 130). Concentrations of NH_4^+ and TFAAs at 20 cm decreased from DOY 130 to DOY 160. After DOY 160, levels of NH_4^+ were nearly constant and the concentrations of TFAAs decreased. Similar trends in NH_4^+ concentrations were observed at a depth of 40 cm (Fig. 3d). Levels of TFAAs diminished slightly through the season with maxima at DOY 188 and DOY 260.

Larger fluctuations in the levels of GLY than the other AAs were observed at the unmanipulated plots, which is similar to trends observed at the Ericaceae- and sedge-only plots (Figs. 4a, 4b). Concentrations of the other AAs were similar and $< 10 \text{ nmol L}^{-1}$. Peak levels of the AAs were observed on DOY 160 and DOY 216 at depths of 20 cm and 40 cm, respectively. Concentrations of proteinaceous species were similar at the two sampling depths and nearly constant ($\sim 0.1 \text{ mg N L}^{-1}$) (Figs. 4c, 4d). Levels of NH_4^+ and TFAAs at 20 cm increased slightly before DOY 160 and then quickly diminished to constant levels. Concentrations of NH_4^+ at a depth of 40 cm were nearly constant. Levels of TFAAs diminished slightly through the season with the exception of a peak in concentrations at DOY 216.

A comparison between FAAs for the plot types on the same DOY pointed to a significant difference between plot types on DOY 130, DOY 160 and DOY 188 and an

interaction between plot type and depth on DOY 260 (Table 3). There was also a significant difference between the two depths on DOY 166 (Table 3). Plot-type, depth, and plot-type indicators at both sampling depths were identified for DOY 130, DOY 160, and DOY 188, for DOY 160, and for DOY 260, respectively (Table 4). All of the plot-type indicators on DOY 130 are for sedge-only plots; however, none of the indicators are significant. On DOY 160, GLY, THRE, and LYS were identified as significant plot-type indicators for the unmanipulated plots. More plot-type indicators were identified for 20 cm than 40 cm on DOY 160; however, only VAL and LYS were found to be marginally significant (p close to 0.05). Plot-type indicators for the Ericaceae-only plots on DOY 188 included GLU, ARG, ALA, and PHE. The plot-type indicators for sedge- and Ericaceae-only plots at 20 cm on DOY 260 were ASP and ALA and ILE, respectively. Six significant plot-type indicators (i.e., ASP, GLU, THRE, ARG, MET, and PHE) were identified for Ericaceae-only plots at a depth of 40 cm.

Composition of nitrogen-containing, dissolved organic matter in porewater

The number of unique molecular formulas for N-containing dissolved OM (NDOM) increased through the season with slight variations in the number for the plot types (Fig. 5a). Trends in the average relative abundance of the NDOM species and the number of unique molecular formulas for the NDOM were similar (Fig. 5b). Concentrations of DON for all plot types increased throughout the season for all plot types (Fig. 5c).

The NDOM composition was significantly influenced by plot type, DOY and depth ($p < 0.05$) and by interactions between plot type and DOY, depth and DOY, plot type and depth, and plot-type, depth, and DOY (Table 5). Analysis of nMDS showed that the small number of nMDS axis 1 represented the late season and the large number of nMDS axis 2 represented the early season (Fig. 6). The FAAs that were positively correlated with the early season were ARG, HIS, and TYR, with ARG exhibiting the strongest correlation with the early season.

Seasonal trends for species at the molecular level were examined by identifying unique molecular formulae for every DOY and for all plot types (Fig. 7) and for each plot type (Figs. 8, 9 and 10). A single indicator was observed in May (DOY 130) and July (DOY 188) (Fig. 1a). Contributions of labile compounds like unsaturated hydrocarbons, lipids, and carbohydrates and lowest average molecular weight (MW) were found in Aug. (DOY 216) (Fig. 1b). Similar numbers of indicators were observed in June (DOY 160) and September (DOY 260): however, the O/C atomic ratio of NDOM in September was higher than the O/C atomic ratio in June (Figs. 7c, 7d), which is inconsistent with values of NOSC that were higher than the values in September. Contributions by lignin- and tannin-like and condensed aromatics and the average MW were greater in September than June. The N/C atomic ratio and AI increased and the average MW decreased from September to October (DOY 292).

Results from PERMANOVA for each plot type indicated that impacts of DOY for each plot type were significant (Table 6). The Ericaceae-only and unmanipulated plot types exhibited impacts related to depth and interactions between depth and DOY, and thus,

DOY indicators were identified for each plot type at each depth. At E20, Contributions of condensed aromatics and values of AI change significantly through the season at the Ericaceae-only plots at a depth of 20 cm (Fig. 8). Principal components of the NDOM were lignin- and tannin-like and condensed aromatic species (Fig. a). Values of the NOSC decreased and increased from DOY 130 to DOY 260 and from DOY 260 to DOY 292, respectively (Fig. 8b). The N/C atomic ratio was high early in the season (DOY 130 and 160) and late in the season (DOY 292) and relatively constant midway through the season. Highest and lowest values of the average MW ($\sim 500 \text{ g mol}^{-1}$) occurred on DOY 260 and DOY 160, respectively. More DOY indicators were identified throughout the season, with the exception of DOY 130 and DOY 292, at a depth of 40 cm (Figs. 8d). Contributions of the condensed aromatics were highest on DOY 160, which is consistent with the highest values of AI (Fig. 8e). Similar contributions of tannin-like species were observed on DOY 260 and DOY 292. Ericaceae-only plots at a depth of 40 cm on DOY 216 had higher N/C atomic ratios and NOSC values than the Ericaceae-only plots at a depth of 20 cm. As for E40, Trends in the average MW of species at Ericaceae-only plots at a depth of 40 cm were similar to the trends at a depth of 20 cm; however, average MWs were lower (Fig. 8e). Tannin-like compounds on DOY 292 had higher O/C atomic ratios than the tannin-like species observed on DOY 260 (Fig. 8f).

The greatest number of DOY indicators at a depth of 20 cm occurred on DOY 160 and DOY 292 (Fig. 9a). Contributions of the condensed aromatics and lignin-like species were greatest on DOY 160. Tannin-like species were most abundant plot-type indicators on DOY 260 and DOY 292. Values of the AI were $>$ and < 0.67 on DOY 160, and DOY

260 and 292, respectively (Fig. 9b). Values of the NOSC and N/C atomic ratio increased throughout the season. The average MW of the species was approximately 350 g mol⁻¹ on DOY 160, increased to 470 g mol⁻¹ on DOY 260, and then decreased to 425 g mol⁻¹ on DOY 292. Lignin-like species with low and high O/C atomic ratios were observed on DOY 160, and DOY 260 and DOY 292, respectively (Fig. 9c). The DOY indicators at a depth of 40 cm were greatest on DOY 260 and DOY 292 (Fig. 9d). Contributions of lignin- and tannin-like species to the DOY indicators were the greatest on DOY 216 and DOY 260, respectively. Trends in the AIs and average MWs were similar at depths of 40 cm and 20 cm (Figs. 9 b, 9e); however, the average MW was lower at a depth of 40 cm for most DOY. Values of the NOSC and N/C atomic ratios were higher at a depth 40 cm than the values at a depth of 20 cm on DOY 160, DOY 260, and DOY 292. The O/C atomic ratios of lignin-like species increased from DOY 160 to DOY 260 and to DOY 292 (Fig. 9c).

The number of plot-type indicators at the unmanipulated plots at a depth of 20 cm was greatest on DOY 260 and DOY 292 (Fig. 10a). Contributions of lignin-like compounds decreased from DOY 188 to DOY 292. About 45% of NDOM on DOY 260 was condensed aromatics. Contributions of tannin-like species and condensed aromatics were slightly higher and slightly lower, respectively, on DOY 292 than the abundances on DOY 188. Values of the average MW at a depth of 20 cm varied from 300-400 g mol⁻¹ throughout the season (Fig. 9b). The average MW exhibited a decreasing trend for DOY 188, 260, and 292; however, values of the NOSC and N/C atomic ratios showed an increasing trend. Tannin-like compounds on DOY 192 exhibited smaller O/C atomic

ratios than the ratios on DOY 188 (Fig. 10c). For the unmanipulated plots at a depth of 40 cm, the number of plot-type indicators was greatest on DOY 260 and DOY 292 (Fig. 10d). Approximately 70% of the DOY indicators on DOY 216 were condensed aromatics and lignin-like species. Similar contributions of condensed aromatics were found at each depth on DOY 260. Unlike the other plot types, values of the NOSC and average MW for the unmanipulated plots increased from DOY 216 to 292 and average MWs and values of NOSC were lower and higher, respectively, on DOY 292 than on DOY 260 (Fig. 8e, 9e, 10e). Condensed aromatics with high O/C atomic ratios appeared at a depth of 40 cm on DOY 260 that were not present at a depth of 20 cm (Fig. 10c, 10f). There were more tannin-like compounds with higher O/C ratios on DOY 292 at depth of 40 cm than the species at a depth of 20 cm.

Impact of plant functional group on the nitrogen-containing, dissolved organic matter composition

Contributions of lignin-like species and condensed aromatics to the NDOM plot-type indicators were greater than 60% (Fig. 11a). Unmanipulated plots exhibited the smallest contribution of condensed aromatics, lowest values of the AI, and greatest contribution of lignin-like compounds (Figs. 11a, 11b). An $AI > 0.5$ for the sedge- and Ericaceae-only plots indicates that aromatic molecules were ubiquitous in porewater of those plot types. Proteinaceous species and amino sugars were only observed in sedge-only plots. The

NDOM in the sedge-only plots exhibited the lowest average MWs and highest values of the NOSC. Tannin-like compounds were abundant in the Ericaceae-only plots.

The PERMANOVA statistical model of NDOM from different plot types on the same DOY, indicated significant PFG effects with the exception of DOY 292 (Table 7). There were also impacts related to depth on DOY 160 and DOY 188. Sedge-only and unmanipulated plots had more unique compounds on DOY 130 (Fig. 12a). Values of the NOSC, the AI, the N/C atomic ratio were lower and the average MWs were higher in sedge-only plots than the values in unmanipulated (Fig. 12b). The NDOM in unmanipulated plots had higher N/C atomic ratios (Fig. 12c).

Sedge-only plots exhibited the highest number of plot-type indicators on DOY 160 at a depth of 20 cm (Fig. 13a). Like the trends observed on DOY 130, contributions of the tannin- and lignin-like species and condensed aromatics were approximately 80% in sedge-only plots. Values of the AI and NOSC were highest in the sedge-only plots; however, the average MWs were lower than the MWs observed in the Ericaceae-only plots (Fig. 13b). The greatest number of plot-type indicators at a depth of 40 cm was observed on DOY 160 (Fig 14a). The average MW of the NDOM and the AI at the Ericaceae-only plots were about 400 g mol^{-1} and > 0.67 , respectively (Fig. 14b). More tannin-like species with O/C atomic ratios > 0.8 were observed in the sedge-only plots than the other plot types (Fig. 14c).

Unmanipulated plots at a depth of 20 cm exhibited the greatest number of unique compounds on DOY 188 (Fig. 15a). The NDOM in unmanipulated plots had a slightly higher average MW ($\sim 400 \text{ g mol}^{-1}$) and lower value of NOSC on DOY 188 (Fig. 15b)

compared to the average MW and NOSC on DOY 130 (Fig. 13b). Molecular species in the lignin-like region of the van Krevelen diagram for the unmanipulated plots moved upward (higher H/C atomic ratio) and to the right (higher O/C atomic ratio), and downward (lower H/C atomic ratio) and to the left (lower O/C atomic ratio) in the region of condensed aromatics (Fig. 15c). At a depth of 40 cm, the greatest number of unique compounds was observed in the Ericaceae-only plots (Figs. 16a). Species in the lignin- and tannin-like regions moved to regions of lower O/C atomic ratios from DOY 160 to DOY 188 (Figs. 14c and 16c, respectively).

The greatest number of unique compounds on DOY 216 was observed in Ericaceae-only plots (Fig. 17a). Sedge-only plots exhibited smaller contributions of condensed aromatics than the Ericaceae-only and unmanipulated plots. Contributions of the lignin-like species on DOY 216 were less than the contributions on DOY 188 for all plot types (Figs. 17a and 15a, respectively). Sedge-only plots had higher values of the NOSC and N/C atomic ratio and a lower average MW than the Ericaceae-only plots (Fig. 17b). Values of AI were similar for the plot types. The O/C atomic ratios for lignin-like species in the Ericaceae-only plots on DOY 216 were higher than the O/C atomic ratios on DOY 188 (Fig. 17c and 16c, respectively).

A similar number of plot-type indicators were identified for the unmanipulated and sedge-only plots on DOY 260 (Fig. 18a). Contributions of tannin-like and condensed aromatic and lignin-like compounds in the sedge-only plots were 95% of the NDOM and were higher and lower, respectively than the contributions in the unmanipulated plots (Fig. 18a). The value of the AI for NDOM in the unmanipulated plots was > 0.67 , which

is consistent with the large contribution of condensed aromatics to the plot-type indicators. Sedge-only plots exhibited higher values of the NOSC than the other plot types, which was the same trend observed on other DOY.

Discussion

Seasonal trends of amino acids and dissolved inorganic nitrogen species

Arginase activity is high during plant germination (Witte 2011), and thus, a significant correlation of ARG with the early season (Table 2, Fig. 6) was expected. Of the 17 AAs that were measured, ARG has the lowest C/O atomic ratio and is used for N storage in many plants (Hildebrandt et al. 2015). The abundance of LYS is low in plant tissue; however, LYS was identified as a DOY indicator for DOY 188 when porewater temperatures were high. Lysine has been identified as a product of microbial decomposition of proteinaceous species (Henry and Jefferies 2002), which might be responsible for the significant correlation of LYS with high porewater temperatures.

Concentrations of FAAs in the sedge-only plots were consistently lower than the levels in the Ericaceae-only plots and trends in the concentrations were stronger (Fig. 2a, 2b, 3a, 3b). Levels of TFAAs in the Ericaceae-only plots diminished from DOY 188 (July) and then increased from DOY 260 to DOY 292 at each depth (Fig 2c, 2d). Similar trends

have been observed in shrub tundra (Kielland 1995). Concentrations of TFAA at the sedge-only plots also decreased from DOY 160 to DOY 188; however, the levels increased from DOY 216 to DOY 260 and then diminished to the end of the season. Trends in the levels of proteinaceous species in the Ericaceae- and sedge-only plots were similar to seasonal patterns in TFAA concentrations. Mineralization of DON species early in the season might be responsible for elevated concentrations of TFAAs in June. Maxima in the concentrations of TFAAs on DOY 292 in Ericaceae- and sedge-only plots and on DOY 292 and DOY 260, respectively, might arise from proteolysis in senescing leaves and transport of AAs to roots. Sedges senesce earlier than ericaceous shrubs, which is probably responsible for the earlier peak in TFAA levels in the sedge-only plots. Maxima in concentrations of NH_4^+ at Ericaceae- and sedge-only plots were typically observed on DOY 130 and diminished throughout the rest of the season. Weintraub and Schimel (2005) observed a decline in NH_4^+ levels in wet sedge and shrub environments beginning in early July. Sampling depth had a minimal effect on variations of NH_4^+ and protein in the sedge-only plots; however, the correlation with depth was significant for the Ericaceae-only plots. Trends in the concentrations of TFAAs for different sampling depths at the Ericaceae-only plots were different; however, variations of TFAA levels at different sampling depths at sedge-only plots were similar. Unlike the roots of Ericaceae, sedge roots can grow deeper than 40 cm. Thus, sedge roots can influence concentrations of DN species at both 20 cm and 40 cm, which apparently results in similar trends in levels of species deep into the porewater.

Concentrations of AAs in the unmanipulated plots—particularly LEU and THRE—showed relatively similar variations throughout the season. Variations in concentrations of proteinaceous species throughout the season for all plot types were similar, which indicates a lack of sensitivity of the levels of proteinaceous species to plot type, sampling depth, and season, and steady state conditions for proteinaceous species. Levels of NH_4^+ in the unmanipulated plots were nearly constant with the exception of early spring. Trends in NH_4^+ concentrations at a depth of 20 cm were similar to the patterns observed at sedge-only plots. However, Ericaceae-only plots exhibited a diminishing trend in NH_4^+ concentrations at both sampling depths, which demonstrates that sedges may have a stronger influence on DIN than Ericaceae.

Seasonal patterns of nitrogen-containing dissolved organic matter

The number of NDOM formulae and DON concentration increased throughout the season; however, the trends were dissimilar (Figs. 4a and 4b, respectively), which indicates a direct correlation between the number of NDOM formulae and the concentration of DON. The results indicate the molecular diversity of NDOM increased throughout the season; however, Roth et al. (2015) observed a decline of molecular diversity of DOM species in forest soils from March to May and during November. Differences in soil types are likely responsible for the behavior of the DON species; however, the study might have missed changes that occurred between May and

November. The character of the NDOM, which was significantly influenced by plot type, DOY, depth, plot type and DOY interaction, depth and DOY interaction, and DOY, plot type, and depth interaction, was more dynamic than the composition of the DOM (Song et al., 2018). Plants and microbes are actively involved in transforming DOM to acquire N, which is an essential macronutrient. Thus, both strong correlations of NDOM concentrations with the various plot characteristics and dynamic variations in NDOM levels were expected.

The large contribution of labile compounds with low average MW on DOY 216 (Figs. 7c, 7d) might be the result of accelerated transformations of DOM associated with the elevated temperatures of the porewater (Song et al. 2018). The H/C and O/C atomic ratios of molecular formulae in regions of the condensed aromatics and lignin-like species increase from DOY 160 to DOY 260, which is indicative of hydration reactions that add water (H₂O) to the molecule (Reemtsma 2009). The average MW on DOY 260 was higher than the MW on DOY 160 and N/C atomic ratio was lower on DOY 260 than the ratio on DOY 160. The likely explanation for trends in MW and the N/C atomic ratio is microbial decomposition of labile DON species, which lowers MW, decreases the N/C ratio, and recovers N to meet nutritional needs. The largest contributions of tannin-like species were observed on DOY 292 (Song et al. 2018). Accumulation of DOM occurs when there is a scarcity of nutrients like N (Smolander et al. 2012) that occurs when levels of NH₄⁺ and TFAs are low and the growing season is short (Thoss et al. 2004). Formation of tannin-protein complexes might explain large contributions of tannin-like

species to the DOY indicators on DOY 292 and the relatively low levels of proteinaceous species.

The N/C atomic ratio in the Ericaceae-only plots at a depth of 20 cm began decreasing at DOY 130 and continued to decrease until DOY 188, which might have marked the beginning of the active growth cycle for ericaceous shrubs (Fig. 7c). The high N/C atomic ratio on DOY 292 might indicate the decrease in plant consumption of nutrients during the fall season. The dramatic decrease in the contribution of condensed aromatics from DOY 260 to 292 may indicate the condensed aromatics are an active pool of NDOM late in the growing season. The NOSC can be used as an indicator of the lability of DOM, with high values of NOSC representing the most rapidly decomposing species (Fred et al. 2016). Values of NOSC increased dramatically from DOY 260 to 292, which might indicate an increase in microbial decomposition from DOY 260 to DOY 292 and/or increase in labile N-containing root exudates like the amino sugars that appeared on DOY 292 (Fig. 8a). The average MW for NDOM in the Ericaceae-only plots was lower at 40 cm than 20 cm depth, which is the depth to which roots of ericaceous shrubs typically extend. Ericoid mycorrhizal fungi associated with the shrub roots were likely responsible for decomposing NDOM to acquire N. The labile and low MW NDOM were decomposed most rapidly leaving a large pool of high MW compounds at a depth of 20 cm. The H/C and O/C atomic ratios for molecular formulae in the tannin region of the van Krevelen diagram increased from DOY 260 to DOY 292 (Fig. 7c), which indicated hydration of tannin-like species or production of more hydrated compounds late in the growing season.

The NDOM in sedge-only plots late in the growing season is apparently more labile than NDOM that is observed early in the season. Values of the O/C atomic ratio of lignin-like compounds were higher and the NOSC and N/C atomic ratio increased during the season. Refractory NDOM decomposing throughout the growing season to the more labile NDOM or fresh inputs of labile NDOM from fall leaf litter and root exudates might have been responsible for increasingly labile NDOM late in the growing season. In addition, root growth late in the season might increase the rate of microbial N immobilization. The O/C atomic ratio of molecular formulae in the lignin region of sedge-only plots increased from DOY 160 to DOY 260 (Fig. 9c, 9f), which indicates oxidation of lignin-like species. Large contributions of tannin-like species to the DOY indicators for NDOM in Ericaceae- and sedge-only and unmanipulated plots on DOY 188 might explain low concentrations of NH_4^+ observed on the same DOY. Fixation of N by high-molecular-weight condensed tannins decreases N mineralization (Smolander et al. 2012).

Effects of plot type on the composition of nitrogen-containing dissolved organic matter

Proteinaceous species and amino sugars were only observed in the sedge-only plots (Fig. 11a), which is consistent with a high rate of decomposition of N-containing lignin- and tannin-like species in the O₂-rich rhizosphere of sedges. Contributions of N-containing condensed aromatics to the plot-type indicators were greater in sedge- and Ericaceae-only plots and contributions of lignin-like species dominated the indicators in the unmanipulated plots. The low average MW and high NOSC of NDOM in the sedge-only plots were consistent with an active microbial community in an O₂-rich rhizosphere (Fig. 11b). The number of plot-type indicators for the sedge-only plots were greater than the number of indicators for the Ericaceae-only plots on 4 of the 6 sampling days (Figs. 12a-18a). Sedge likely has a greater influence on NDOM composition due to increased availability of O₂ in the rhizosphere. Changes in the O/C and H/C atomic ratios of lignin-like species of the NDOM and condensed aromatics in the unmanipulated plots, respectively, indicated that the lignin-like and condensed aromatic species from DOY 130 to DOY 188 were apparently hydrated and reduced, respectively (Figs. 10c, 10f). van Krevelen diagrams for the Ericaceae-only plots indicate lignin- and tannin-like species were reduced, which is consistent with the reducing conditions of an Ericaceae rhizosphere with low O₂ availability.

Conclusion

Concentrations of TFAA for the three plot types were $< 4 \mu\text{g N L}^{-1}$. A maximum in the levels of the TFAA at the Ericaceae- and sedge-only plots was observed on DOY 188. The DOY and plot type exerted the greatest influence on the concentration and composition of the TFAA pool. Trends in the levels of GLY and THRE were similar throughout the season. The most abundant FAA during parts of the growing season was GLY, which is important in the photorespiration process that occurs in C3 plants like sedge and Ericaceae. Arginine is an important N storage compound, which correlated with the period of germination early in the growing season. There were lower concentration of free amino acids in sedges plots than ericaceous plots. Concentrations of proteinaceous species in all plot types were $< 150 \mu\text{g N L}^{-1}$. Maximum levels were observed in June or July. Inhibition of N mineralization of N-containing, tannin-like species (e.g., tannin-protein complexes) was likely responsible for the low concentrations of NH_4^+ that were observed late in the growing season. Fresh input from fall leaves and root exudates, and an accelerated rate of microbial N immobilization, produced a higher molecular diversity of NDOM compounds with high NOSC late in the growing season.

The abundance of NDOM species indicated that there was considerable fixation of N into organic forms throughout the season. The NDOM composition changed dramatically throughout the season and various reactions, like oxidation, reduction, hydration, and condensation that are typical of the humification process were observed with the aid of the van Krevelen diagrams. Effects of sedge-only plots on trends of NH_4^+ and NDOM molecular composition were stronger than the patterns observed for Ericaceae-only plots. Seasonal variations in the composition of the FAAs and NDOM for sedge- and

Ericaceae-only plots indicate that environmental conditions and plant functional group influence NDOM bioavailability. Thus, more accurate predictions of the dynamics of N cycling in peatlands in a changing climate will require comprehensive, molecular analysis of DIN and DON species like the strategy proposed here.

References

- Aestrom M, Aaltonen E (2005) Changes in leaching patterns of nitrogen and phosphorus after artificial drainage of a boreal forest- a paired catchment study in Lappajaervi, western Finland *Boreal environment research* 10:67-78
- Andersen R, Chapman SJ, Artz RRE (2013) Microbial communities in natural and disturbed peatlands: A review *Soil Biology and Biochemistry* 57:979-994
doi:<http://dx.doi.org/10.1016/j.soilbio.2012.10.003>
- Dorresteyn RC, Berwald LG, Zomer G, de Gooijer CD, Wieten G, Beuvery EC (1996) Determination of amino acids using o-phthalaldehyde-2-mercaptoethanol derivatization effect of reaction conditions *Journal of Chromatography A* 724:159-167 doi:[http://dx.doi.org/10.1016/0021-9673\(95\)00927-2](http://dx.doi.org/10.1016/0021-9673(95)00927-2)

- Flessa H, Fischer W (1992) Plant-induced changes in the redox potentials of rice rhizospheres *Plant and soil* 143:55-60
- Fred W, D. CG, S. MC, P. BT, Rob R (2016) The effective oxidation state of a peatland *Journal of Geophysical Research: Biogeosciences* 121:145-158
doi:doi:10.1002/2015JG003182
- Grannas AM, Hockaday WC, Hatcher PG, Thompson LG, Mosley-Thompson E (2006) New revelations on the nature of organic matter in ice cores *Journal of Geophysical Research: Atmospheres* 111:n/a-n/a doi:10.1029/2005JD006251
- Guigue J, Harir M, Mathieu O, Lucio M, Ranjard L, Lévêque J, Schmitt-Kopplin P (2016) Ultrahigh-resolution FT-ICR mass spectrometry for molecular characterisation of pressurised hot water-extractable organic matter in soils *Biogeochemistry* 128:307-326 doi:10.1007/s10533-016-0209-5
- Hedges J (1990) Compositional indicators of organic acid sources and reactions in natural environments *Organic acids in aquatic ecosystems* Wiley:43-63
- Henry HAL, Jefferies RL (2002) Free amino acid, ammonium and nitrate concentrations in soil solutions of a grazed coastal marsh in relation to plant growth *Plant Cell and Environment* 25:665-675 doi:10.1046/j.1365-3040.2002.00849.x

Hildebrandt TM, Nesi AN, Araújo WL, Braun H-P (2015) Amino acid catabolism in plants *Molecular plant* 8:1563-1579

Hribljan JA, Kane ES, Pypker TG, Chimner RA (2014) The effect of long-term water table manipulations on dissolved organic carbon dynamics in a poor fen peatland *Journal of Geophysical Research-Biogeosciences* 119:577-595
doi:10.1002/2013jg002527

Jones D, Darrah P (1993) Influx and efflux of amino acids from *Zea mays* L. roots and their implications for N nutrition and the rhizosphere *Plant and Soil* 155:87-90

Jones DL, Shannon D, Junvee-Fortune T, Farrar JF (2005) Plant capture of free amino acids is maximized under high soil amino acid concentrations *Soil Biology and Biochemistry* 37:179-181

Keil RG, Kirchman DL (1991) Dissolved combined amino acids in marine waters as determined by a vapor-phase hydrolysis method *Marine Chemistry* 33:243-259
doi:[http://dx.doi.org/10.1016/0304-4203\(91\)90070-D](http://dx.doi.org/10.1016/0304-4203(91)90070-D)

Kielland K (1994) Amino acid absorption by arctic plants: implications for plant nutrition and nitrogen cycling *Ecology* 75:2373-2383

- Kielland K (1995) Landscape patterns of free amino acids in arctic tundra soils
Biogeochemistry 31:85-98
- Kim S, Kramer RW, Hatcher PG (2003) Graphical method for analysis of ultrahigh-resolution broadband mass spectra of natural organic matter, the van Krevelen diagram Analytical Chemistry 75:5336-5344
- Krab EJ, Cornelissen JH, Lang SI, van Logtestijn RS (2008) Amino acid uptake among wide-ranging moss species may contribute to their strong position in higher-latitude ecosystems Plant and Soil 304:199-208
- Kutlán D, Presits P, Molnár-Perl I (2002) Behavior and characteristics of amine derivatives obtained with o-phthaldialdehyde/3-mercaptopropionic acid and with o-phthaldialdehyde/N-acetyl-L-cysteine reagents Journal of Chromatography A 949:235-248
- Leadley PW, Reynolds JF, Chapin FS (1997) A model of nitrogen uptake by *Eriophorum vaginatum* roots in the field: Ecological implications Ecological Monographs 67:1-22

- Lipson DA, Raab TK, Schmidt SK, Monson RK (1999) Variation in competitive abilities of plants and microbes for specific amino acids *Biology and Fertility of Soils* 29:257-261
- Lipson DA, Raab TK, Schmidt SK, Monson RK (2001) An empirical model of amino acid transformations in an alpine soil *Soil Biology and Biochemistry* 33:189-198
- Lv JT, Zhang SZ, Luo L, Cao D (2016) Solid-phase extraction-stepwise elution (SPE-SE) procedure for isolation of dissolved organic matter prior to ESI-FT-ICR-MS analysis *Analytica Chimica Acta* 948:55-61 doi:10.1016/j.aca.2016.10.038
- Mack A (1963) Biological activity and mineralization of nitrogen in three soils as induced by freezing and drying *Canadian Journal of Soil Science* 43:316-324
- Meingast KM et al. (2014) Spectral detection of near-surface moisture content and water-table position in northern peatland ecosystems *Remote Sensing of Environment* 152:536-546 doi:10.1016/j.rse.2014.07.014
- Ohno T, Parr TB, Gruselle MC, Fernandez IJ, Sleighter RL, Hatcher PG (2014) Molecular composition and biodegradability of soil organic matter: a case study comparing two new England forest types *Environ Sci Technol* 48:7229-7236 doi:10.1021/es405570c

- Reemtsma T (2009) Determination of molecular formulas of natural organic matter molecules by (ultra-) high-resolution mass spectrometry: Status and needs *Journal of Chromatography A* 1216:3687-3701
doi:<http://dx.doi.org/10.1016/j.chroma.2009.02.033>
- Romanowicz KJ, Kane ES, Potvin LR, Daniels AL, Kolka RK, Lilleskov EA (2015) Understanding drivers of peatland extracellular enzyme activity in the PEATcosm experiment: mixed evidence for enzymic latch hypothesis *Plant and Soil* 397:371-386
doi:[10.1007/s11104-015-2746-4](https://doi.org/10.1007/s11104-015-2746-4)
- Roth V-N, Dittmar T, Gaupp R, Gleixner G (2015) The Molecular Composition of Dissolved Organic Matter in Forest Soils as a Function of pH and Temperature *PLoS ONE* 10:e0119188 doi:[10.1371/journal.pone.0119188](https://doi.org/10.1371/journal.pone.0119188)
- Smolander A, Kanerva S, Adamczyk B, Kitunen V (2012) Nitrogen transformations in boreal forest soils—does composition of plant secondary compounds give any explanations? *Plant and Soil* 350:1-26 doi:[10.1007/s11104-011-0895-7](https://doi.org/10.1007/s11104-011-0895-7)
- Swain F, Blumentals A, Millers R (1959) Stratigraphic Distribution of Amino Acids in Peats from Cedar Creek Bog, Minnesota, and Dismal Swamp, Virginia’
LIMNOLOGY

- Thoss V, Shevtsova A, Nilsson M-C (2004) Environmental manipulation treatment effects on the reactivity of water-soluble phenolics in a subalpine tundra ecosystem Plant and Soil 259:355-365 doi:10.1023/b:plso.0000020984.17403.82
- Tiemeyer B, Kahle P (2014) Nitrogen and dissolved organic carbon (DOC) losses from an artificially drained grassland on organic soils Biogeosciences 11:4123-4137
- Weintraub MN, Schimel JP (2005) The seasonal dynamics of amino acids and other nutrients in Alaskan Arctic tundra soils Biogeochemistry 73:359-380 doi:10.1007/s10533-004-0363-z
- Witte C-P (2011) Urea metabolism in plants Plant Science 180:431-438 doi:https://doi.org/10.1016/j.plantsci.2010.11.010

Table 1 Summary of results from PERMANOVA analysis for free amino acids (FAA) in all plot types at depth 20 and 40 cm on all day of the year (DOY). (Main test)

Source	df	SS	MS	Pseudo-F	<i>P</i> <i>n</i> (perm)	Unique perms
Plot type	2	3283.8	1641.9	1.8224	0.088	997
Block	2	2194.6	1097.3	1.2179	0.267	999
Depth	1	1544.6	1544.6	1.7144	0.141	999
DOY	5	49208	9841.6	10.923	0.001	997
Plot type x Depth	2	1261.7	630.87	0.70022	0.688	998
Plot type x DOY	10	19341	1934.1	2.1467	0.001	998
Depth x DOY	5	4735.2	947.03	1.0511	0.379	998
Plot type x Depth x DOY	10	6221.3	622.13	0.69051	0.915	998

*Note: Bolded values are significant at $p < 0.05$.

Table 2 Amino acids indicator at different DOY.

	DOY	P
Glutamate	130	0.1346
Serine	130	0.3391
Histidine	130	0.0002
Glycine	130	0.1378
Alanine	130	0.7712
Arginine	160	0.0002
Threonine	188	0.0396
Isoleucine	188	0.0784
Lysine	188	0.0028
Tyrosine	216	0.1656
Valine	216	0.4151
Phenylalanine	216	0.215
Leucine	216	0.0002
Asparate	260	0.5383
Methionine	260	0.2573

*Note: Bolded values are significant at $p < 0.05$.

Table 3 Summary of results from PERMANOVA for each DOY

Source	df	<i>p</i> (perm)					
		DOY 130	DOY 160	DOY188	DOY216	DOY260	DOY 292
Plot type	2	0.691	0.012	0.006	0.095	0.199	0.159
Depth	1	0.738	0.007	0.077	0.361	0.096	0.295
Plot type x Depth	2	0.198	0.818	0.53	0.504	0.03	0.451

*Note: Bolded values are significant at $p < 0.05$.

Table 4 Amino acids PLOT TYPE and depth indicators

	Plot type indicator on DOY 130		Plot type indicator on DOY 160		Depth indicator on DOY 160		Plot type indicator on DOY 188		Plot type indicator on DOY 260 at 20cm		Plot type indicator on DOY 260 at 40cm	
	Plot type	P	Plot type	P	Depth	P	Plot type	P	Plot type	P	Plot type	P
ASP	S	0.2468	U	0.3393	20	0.6857	E	0.3393	S	0.0392	E	0.0438
GLU	S	0.4147	U	0.6757	20	0.3317	E	0.001	E	0.7185	E	0.032
SER	S	0.3101	U	0.2146	20	0.8254	S	0.4359	S	0.221	E	0.5243
HIS	S	0.0934	E	0.0614	20	0.2116	E	0.2863	S	0.2212	E	0.1594
GLY	S	0.3955	U	0.0216	20	0.2883	E	0.5243	S	0.2853	E	0.5403
THR	S	0.1718	U	0.04	20	0.4853	S	0.2653	S	0.1014	E	0.0338
ARG	S	0.2655	E	0.2164	20	0.4103	E	0.0052	E	1	E	0.0338
ALA	S	0.2494	U	0.1092	20	0.5187	E	0.0246	E	0.0362	E	0.1058
TYR	S	0.3191	U	0.1326	40	0.9486	E	0.0964	S	0.7207	E	0.066
VAL	S	0.6293	U	0.1246	20	0.0546	E	0.1398			E	0.066
MET	S	1	S	0.2997	20	1	E	0.0738	S	0.7049	E	0.0338
PHE	S	0.4599	U	0.0612	20	0.4339	E	0.0058	E	0.2903	E	0.0236
ISO	S	0.063	U	0.2851	20	0.2314	E	0.0586	E	0.0362	E	0.097
LEU	S	1	U	0.0512	20	0.1308	S	0.1332	E	1	E	1
LYS	S	0.2236	U	0.0204	20	0.0668	S	0.2148	S	1	U	0.5071

*Note: Bolded values are significant at $p < 0.05$.

Table 5 Summary of results from PERMANOVA analysis of nitrogenous dissolved organic matter (NDOM) for all plot types at depth 20 and 40 cm on all day of the year (DOY). (Main test)

Source	df	SS	MS	Pseudo-F	<i>P</i> <i>n</i> (perm)	Unique perms
DOY	5	67131	13426	10.839	0.001	999
Plot type	2	8342	4171	3.3673	0.001	998
Depth	1	2026.1	2026.1	1.6357	0.015	998
Block	2	3161.6	1580.8	1.2762	0.048	998
Plot type x DOY	10	32584	3258.4	2.6305	0.001	995
Depth x DOY	5	8139.6	1627.9	1.3142	0.004	996
Plot type x Depth	2	3658.4	1829.2	1.4767	0.008	998
Plot type x Depth x DOY	10	14714	1471.4	1.1879	0.006	994
Residuals	70	86708	1238.7			
Total	107	226470				

*Note: Bolded values are significant at $p < 0.05$.

Table 6 Summary of results from PEMANOVA for each plot type

Source	df	<i>p</i> (perm)		
		E	S	U
DOY	5	0.001	0.001	0.001
Depth	1	0.003	0.312	0.01
Block	2	0.369	0.729	0.075
Depth x DOY	5	0.01	0.496	0.002

Table 7 Summary of results from PEMANOVA for each DOY

Source	df	$p(\text{perm})$					
		DOY 130	DOY 160	DOY18 8	DOY216	DOY260	DOY 292
Depth	1	0.197	0.076	0.005	0.481	0.103	0.526
Block		0.161	0.717	0.195	0.704	0.748	0.537
Plot type	2	0.001	0.05	0.015	0.005	0.002	0.058
Plot type x Depth	2	0.283	0.047	0.016	0.35	0.183	0.537

Figure Captions

Fig. 1 The van Krevelen plot illustrating regions for the major classes of DOM and the displacements for expected different types of reactions. Adapted from (Grannas, Hockaday et al. 2006). The lines are noted as (1) Carboxylation or decarboxylation; (2) methylation, demethylation or alkyl chain elongation; (3) oxidation or reduction; (4) hydration or condensation; and (5) hydrogenation or dehydrogenation.

Fig. 2 Seasonal dynamics of five individual amino acids in porewater **(a)** in E20, **(b)** in E40; TFAA, ammonium, and protein in porewater in **(c)** in E20, and **(d)** in E40. Note E20 is porewater sample extracting from 20 cm at ericaceous plots. E40 is from 40 cm.

Fig. 3 Seasonal dynamics of five individual amino acids **(a)** in S20, **(b)** in S40; TFAA, ammonium, and protein in porewater in **(c)** in S20, and **(d)** in S40. Note S20 is porewater sample extracting from 20 cm at sedge plots. S40 is from 40 cm.

Fig. 4 Seasonal dynamics of five individual amino acids **(a)** in U20, **(b)** in U40; TFAA, ammonium, and protein in porewater in **(c)** in U20, and **(d)** in U40. Note U20 is porewater sample extracting from 20 cm at unmanipulated plots. U40 is from 40 cm.

Fig. 5 Overview of the abundance of molecular formulae and DON concentration at different PLOT TYPE through the season. **(a)** The number of molecular formulae; **(b)** The average relative abundance (RA) of nitrogen-containing compounds for different PLOT TYPE through the season. Error bar indicates standard deviation between different sampling blocks; and **(c)** DON concentration

Fig. 6 Non-metric multidimensional scaling (nMDS) ordination for the molecular composition of NDOM for 108 porewater samples. The arrow in the ordination diagram represents environmental variables fitted to the ordination ($r > 0.2$). Length of the arrow represents the correlation.

Fig. 7 DOY indicator for all PLOT TYPE at both sampling depths of 20 cm and 40 cm. **(a)** Percentage of Class group; **(b)** Average NOSC, N/C, AI, MW (molecular weight); **(c)** Van Krevelen diagram for DOY 130 to DOY 160; and **(d)** Van Krevelen diagram for DOY 292

Fig. 8 DOY indicators of NDOM for E20 and E40. Percentage of Class group **(a)** E20, **(d)** E40; Average NOSC, N/C, AI, MW (molecular weight) **(b)** E20, **(e)** E40; Van Krevelen diagram **(c)** E20, **(f)** E40

Fig. 9 DOY indicators of NDOM for S20 and S40. Percentage of Class group **(a)** E20, **(d)** E40; Average NOSC, N/C, AI, MW (molecular weight) **(b)** E20, **(e)** E40; Van Krenvlen diagram **(c)** E20, **(f)** E40

Fig. 10 DOY indicators of NDOM for U20 and U40. Percentage of Class group **(a)** E20, **(d)** E40; Average NOSC, N/C, AI, MW (molecular weight) **(b)** E20, **(e)** E40; Van Krenvlen diagram **(c)** E20, **(f)** E40

Fig. 11 PLOT TYPE indicator of NDOM for all DOY **(a)** Percentage of Class group; **(b)** Average NOSC, N/C, AI, MW; **(c)** Van Krenvlen diagram

Fig. 12 PLOT TYPE indicator of NDOM for DOY 130 **(a)** Percentage of Class group; **(b)** Average NOSC, N/C, AI, MW; **(c)** Van Krenvlen diagram

Fig. 13 PLOT TYPE indicator of NDOM for DOY 160 at 20 cm **(a)** Percentage of Class group; **(b)** Average NOSC, N/C, AI, MW; **(c)** Van Krenvlen diagram

Fig. 14 PLOT TYPE indicator of NDOM for DOY 160 at 40 cm **(a)** Percentage of Class group; **(b)** Average NOSC, N/C, AI, MW; **(c)** Van Krenvlen diagram

Fig. 15 PLOT TYPE indicator of NDOM for DOY 188 at 20 cm **(a)** Percentage of Class group; **(b)** Average NOSC, N/C, AI, MW; **(c)** Van Krenvlen diagram

Fig. 16 PLOT TYPE indicator of NDOM for DOY 188 at 40 cm **(a)** Percentage of Class group; **(b)** Average NOSC, N/C, AI, MW; **(c)** Van Krenvlen diagram

Fig. 17 PLOT TYPE indicator of NDOM for DOY 216 **(a)** Percentage of Class group; **(b)** Average NOSC, N/C, AI, MW; **(c)** Van Krenvlen diagram

Fig. 18 PLOT TYPE indicator of NDOM for DOY 260 **(a)** Percentage of Class group; **(b)** Average NOSC, N/C, AI, MW; **(c)** Van Krenvlen diagram

Fig. 1

Fig. 2

Fig. 3

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Fig. 5

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Fig. 18

Chapter 4 Molecular biogeochemistry of a poor fen.

III. Organic matter transformation in peat

Zhimin Song¹, Maryam Khaksari², Erik A. Lilleskov^{3,4}, Evan S. Kane⁴, and Paul V. Doskey^{4,5}

¹Department of Civil and Environmental Engineering, Michigan Technological University, Houghton, Michigan 49931, USA, ²Department of Chemistry, Michigan Technological University, Houghton, Michigan 49931, USA, ³USDA Northern Research Station, Houghton, MI 49931, USA, ⁴School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, USA, ⁵Atmospheric Sciences Program, Michigan Technological University, Houghton, Michigan 49931, USA

Abstract Peat diagenesis at the Nestoria, Michigan, USA was characterized using Fourier-transformed infrared (FTIR) spectroscopy and carbon and nitrogen content, and ultra-high-resolution mass spectrometry (UHR-MS) for the water-extractable organic matter (WEOM) of the peat. We found organic matter (OM) molecular compositions were different in sedge-only, Ericaceae-only, and unmanipulated (sedge + Ericaceae) plots, as well as at different sampling depths. In particular, the sedge plots showed lower total phenolics, higher polysaccharides, and higher carbonyl/lignin, polysaccharides/lignin, E2/E3 values than the unmanipulated and Ericaceae plots. The unmanipulated plots inferred high microbial activities which increased the nitrogen mineralization. Moreover, the %C and %N of the solid peat increased with depth and the

highest C/N ratio was found in surface peat samples because of less decomposed fresh vegetation. The sampling depths 20-30 and 30-40 cm are the most active with highest DOC, TDN and protein and lowest total phenolics and C/N. The highest humification degree in different sampling depth varied in sedge, ericaceous and unmanipulated plots.

Keywords Peat · water-extractable organic matter · sedge · Ericaceae

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Introduction

Peatlands are acidic, nutrient-poor, and carbon (C)-rich terrestrial ecosystems that encompass about 3% of the Earth's land surface and contain ~30% of the global soil C (Batjes 2014). Climate change may rapidly change the carbon balance of peatlands and increase greenhouse gas emissions through accelerated decomposition of organic matter (OM) in the peat matrix (Moore et al. 1998; Bridgham et al. 2008). The rate of microbial decomposition of natural OM follows the general order: carbohydrates > proteins > lipids > lignin $\approx$ tannin. Moreover, temperature, nutrient availability, and redox potential affect decomposition rate of OM. Nutrient availability and redox potential of the peat matrix is influenced by plant functional groups (PFG), and thus, the rate of decomposition of OM in the peat matrix is expected to differ in peat dominated by various PFGs. Thus, a molecular analysis of the quality and quantity of the OM is required to evaluate the stability of OM in peat.

Peat is an organic soil with high moisture content that consists mainly of partially decomposed plant residues. The layers of peat classified by depth include the acrotelm (0 to ~30 cm), mesotelm (30 to ~75 cm), and catotelm (below 75 cm). The acrotelm layer is characterized by a fluctuating water table and oxic conditions and is dominated by living plants and their roots. The mesotelm layer is typically anoxic; however, oxic conditions are occasionally created by fluctuations in the water table. The anoxic conditions of the

water-saturated catotelm layer that slows the rate of OM decomposition and leads to storage of most of the peatland C.

Water-extractable OM (WEOM) is the most dynamic and labile fraction of OM in peat. The WEOM is characterized by easily decomposed organic compounds like carbohydrates, lignin monomers, phenolic species, and organic nitrogen (N) compounds (Landgraf et al. 2006). The molecular composition and abundance of WEOM in peat are sensitive to climate warming. Delarue et al. (2011) observed a higher consumption of water extractable organic carbon (WEOC) in a *Sphagnum*-dominated poor fen as air temperature increased. Consumption of WEOC resulted in the accumulation of aliphatic and/or aromatic compounds at a depth of 0-5 cm. The carbohydrate content of the WEOC of the deep layer (7.5-10 cm) likely increased due to leaching of phenolic compounds by plant roots. Thus, quantifying the molecular composition of WEOM can be used to evaluate the potential C loss from peatlands.

(Tfaily et al. 2014) used Fourier Transform Infrared (FTIR) Spectroscopy and OM decomposition proxies like C/N atomic ratio, bulk density, and water content to examine diagenesis of OM in peat and observed that C cycling at depth is related to processes at the surface. The OM of the acrotelm layer of peat covered by *Sphagnum* mosses exhibited a high C/N atomic ratio and an abundance of carbohydrates, which was characteristic of newly formed and poorly decomposed plant litter. Humification and decomposition was observed in the mesotelm layer of the peat. The OM of the mesotelm layer exhibited utilization of oxygenated compounds, like carboxyl - and oxygen-

containing-alkyl-C (COOH-alkyl-C and O-alkyl-C, respectively) and accumulation of N- and alkyl C-containing compounds. The O-alkyl-C was identified as a sensitive indicator of peat decomposition. The OM of the catotelm layer was stable and contained aromatic compounds and lignin-like species. Coccozza et al. (2003) found OM of the acrotelm layer to be highly dynamic and characterized by oxidative processes that unevenly distributed the OM at different stages of decomposition. Conditions of the mesotelm layer varied between aerobic and anaerobic, humification was induced by microbial activity, and there was evidence of the decomposition of polysaccharides and lignin-derived residues. The catotelm layer exhibited a constant and homogeneous distribution of OM, which was due to the anoxic conditions that slowed decomposition of OM. Artz et al. (2006) used FTIR spectroscopy and observed that the concentration of polysaccharide markers diminished with depth in peat; however, levels of lignin-like species, alkene, aromatic, and aliphatic C, and COOH-alkyl-C increased with depth. A similar trend in polysaccharides was reported by Leifeld et al. (2012). An analysis of lignin-like species and lipid biomarkers with depth in permafrost peat indicated the concentration of lignin monomers increased with depth and contributions of the lipids followed the trend: n-alkanols > sterols $\geq$ n-alkanes $\geq$ triterpenols (Routh et al. 2014). The acrotelm layer of the peat was dominated by organic species with various O-containing functionalities that decreased in a logarithmic manner with an increase of n-alkanes and lignin.

The subject investigation examined the molecular composition of solid peat and the WEOM of the peat. Peat core samples were collected from sedge- and Ericaceae-only and unmanipulated plots located in a poor fen (Song et al., 2018a and b) to study the

composition effects of plant functional group (PFG) on variations of the molecular composition and OM with depth in peat. The following hypotheses were tested: 1) Contributions of labile compounds like saccharides to the WEOM will be greatest in surface layers of the peat; 2) Contributions of aromatic species to the OM of peat from Ericaceae-only plots will be greater than the contributions in sedge-only and unmanipulated plots; (3) The abundance of total phenolics (TPHs) in WEOM from Ericaceae-only plots will be greater than the abundances in sedge-only and unmanipulated plots; and 4) The C/N atomic ratio of the surface layers of peat will be higher than the ratios observed in deep layers of the peat. The composition of solid peat was examined using FTIR spectroscopy and elemental analysis to determine C/N atomic ratios. The WE matter (WEM) was studied using a combination of ion chromatography (IC), ultra-high performance liquid chromatography (UHPLC), spectral indices, and electrospray ionization, ultra-high resolution mass spectrometry (ESI-UHR-MS).

Material and methods

Site description, experimental design, and sample collection

The site of the investigation, a poor fen Nestoria, Michigan, USA (46.34°N 88.16°W) has been previously described in detail (Meingast et al. 2014; Song et al. 2018a and b). Core samples of peat (10 cm diameter × 50 cm depth) were collected following the procedure

of Chimner et al. (2014) in September 2016 from sedge- and Ericaceae-only and unmanipulated plots (Hribljan et al. 2014). The cores were sectioned with a knife into 10 cm layers in field and stored in polyethylene bags. The peat core sections were transported on ice to the laboratory for processing and analysis. The sections were freeze dried and homogenized by grinding to a fine powder ($< 250 \mu\text{m}$) using a ball mill grinder (SPEX CertiPrep 8000 series; SPEX SamplePrep, Metuchen, NJ) (Chambers et al. 2011; Ott 2013). Freeze-dried peat was stored in glass jars in at -18°C prior to analysis.

Chemical analysis of solid peat

Subsamples of the sections were analyzed for total carbon (TC), total nitrogen (TN) using the method of (Keller 2006) with a Costech 4010 Elemental Combustion system (Costech Analytical Technologies, Inc., Valencia, CA). Peat FTIR spectroscopy was carried out to study the structure of the peat organic matter. Subsamples for FTIR spectroscopic analysis were mixed and homogenized with potassium bromide (KBr) spectrograde powder (International Crystal Labs, Garfield, NJ, USA) in a sample-to-KBr ratio of 1:10 (w:w). Following homogenization, the mixture was dried at 60°C , poured into a sample cup, and leveled to the rim of the cup. The FTIR spectra were recorded by diffuse reflectance using a Nicolet iS5 spectrometer with an iD Foundation – Diffuse accessory (Thermo Scientific, Waltham, MA). Sixty-four scans were averaged across the $4000\text{--}400 \text{ cm}^{-1}$

range of wavenumbers at a resolution of 4 cm^{-1} . The spectrum of pure KBr was used for background subtraction and a baseline correction was applied to remove baseline distortions. Minimal peak heights were added to all the wavenumbers to normalize peaks with negative absorbances. Wavenumber ratios representing the polysaccharides/lignin, carbonyl/lignin, and lipids/polysaccharides compositions were $1060\text{ cm}^{-1}/1620\text{ cm}^{-1}$, $1725\text{ cm}^{-1}/1620\text{ cm}^{-1}$, and $2920\text{ cm}^{-1}/1060\text{ cm}^{-1}$, respectively (Hribljan et al. 2017).

Chemical Analysis of water-extractable matter

The WEM was extracted from peat by the hot-water extraction method (Heller and Weiss 2015). Briefly, freeze-dried and homogenized subsamples of peat (0.06 g) were added to 48 mL of MilliQ water ($18.2\text{ M}\Omega\cdot\text{cm}$) in glass bottles (Fisher Scientific International Inc., Pittsburgh, PA) to obtain soil/water ratios of 1/800 (w/v). The samples were mixed, septum lined caps were affixed to the bottles, and the mixture was heated in an oven at $100\text{ }^{\circ}\text{C}$ for 1 h. The mixtures were cooled to $25\text{ }^{\circ}\text{C}$ following extraction and centrifuged for 10 min at $2600\times g$. The supernatants were decanted and filtered through $0.45\text{ }\mu\text{m}$ pre-rinsed glass fiber filters (VWR international, Batavia, IL).

Song et al. (2018a and b) describe in detail the techniques for the analysis of WEM. Briefly dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured on a TOCV analyzer with TDN module (Shimadzu Scientific Instruments,

Columbia, MD, USA) (Hribljan et al. 2014). Ammonium (NH_4^+) and nitrate (NO_3^-) were quantified using dual IC systems (ICS-2100, ICS-1100; Thermo Scientific, Waltham, MA) that were run in parallel. The difference between the TDN and the total DIN (i.e., $\Sigma \text{NO}_3^- + \text{NH}_4^+$) represented the dissolved organic nitrogen (DON).

Analytical methods to measure compound classes of the WEOM are described in detail by Song et al. (2018a and b). Proteinaceous species were determined as amino acids (AAs) following acid hydrolysis (Keil and Kirchman 1991). The AAs were separated and quantified by UHPLC (Dionex Ultimate 3000; Dionex, Sunnyvale, CA). The difference between concentrations of AAs in the hydrolysate and the free AAs (FAAs) in the WEOM represented the level of proteinaceous species in the WEOM. Total phenolics (TPHs) were quantified as tannin and lignin by a Hach method (Loveland, CO, USA) that was adapted to a microplate technique (Romanowicz et al. 2015). The 2,4,6-tripyridyl-s-triazine (TPTZ) spectrophotometric method was used to measure free monosaccharides (MCHOs) and total dissolved saccharides (TDCHOs) following acid hydrolysis (Myklestad et al. 1997). The concentration of PCHOs was determined by difference from the free MCHO and TDCHO measurements.

Song et al. (2018a and b) describe in detail the determination of molecular formulae for organic species by ESI-UHR-MS. Briefly, all possible molecular formulae in samples treated by solid-phase extraction (Oasis HLB, Water Corporation, MA, USA) and having the empirical formula $\text{C}_{2-200}\text{H}_{4-400}\text{O}_{1-40}\text{N}_{0-3}\text{S}_{0-1}$ were calculated using Composer64 Software (Sierra Analytics, Modesto, CA, USA). Visualization analyses of the ESI-UHR-

MS data were performed using van Krevelen diagrams of the WEOM (Hedges 1990; Grannas et al. 2006; Ohno et al. 2014). The nominal oxidation state of carbon (NOSC) and aromaticity index (AI) were calculated for structural interpretation using Eqns. 1 (Lv et al. 2016) and 2 (Guigue et al. 2016), respectively as follows:

$$\text{NOSC} = 4 - \frac{4\text{C} + \text{H} - 3\text{N} - 2\text{O} - 2\text{S}}{\text{C}} \quad (1)$$

$$\text{AI} = \frac{1 + \text{C} - \text{O} - \text{S} - 0.5\text{H}}{\text{C} - \text{O} - \text{S} - \text{N}} \quad (2)$$

Fluorescence and absorbance spectra of the WEOM were acquired by UV/VIS spectroscopy on an AqualogVR (Horiba Instruments, New Jersey) and various spectral characteristics were used to determine spectral indices (Veverica *et al.*, 2016; Coble *et al.*, 2016a, b). Song et al. (2018a and b) describe the various spectral indices in detail. Briefly, the Specific UV Absorptivity at 254 (SUVA₂₅₄), the E2/E3 index, the humification index, and the A/T index are used as indicators of the degree of aromaticity, molecular size, the degree of humification, and the recalcitrant/labile nature, respectively, of the bulk OM.

Statistical analysis

The statistical analysis in this study is limited to inference given that there is no true replication, only the pseudoreplication derived from multiple depth. The Data from the FTIR spectroscopic and ESI-UHR-MS analyses were used to identify indicator species for the various plot types. Monte Carlo experiments were used to determine the significance of observed maximum indicator species for a response. p-values less than 0.05 were considered significant. The FTIR spectra of solid peat were used to identify plot-type indicators, with depths as the replicates. Plot-type indicators for all depths and depth indicators for all plot types were derived from the molecular data acquired from the ESI-UHR-MS analyses of WEOM. Analyses of indicator species was run in PC-ORD 6.20 (Gleneden Beach, Oregon).

Results

Chemical characterization of solid peat

The C and N content of the solid peat increased with depth (Fig. 1). The Ericaceae-only plot exhibited the most dramatic decrease of carbon content from the surface to the deepest layer of the peat (Fig. 1a). The average C content for the deepest peat layers of three plot types was about 49%. The surface layer of the unmanipulated plot exhibited a greater C content (~47.4%) than the surface layers of the Ericaceae- and sedge-only plots. The C content of the various layer of the sedge-only plots were greater than the C contents of the other plots with the exception of the surface layer (0-10 cm) where the C content of the unmanipulated plot was greater. The N content for various layers of peat from the three plot types was 0.6% - 1.2%. Fluctuations of the C and N contents with depth in the unmanipulated plot were small compared to fluctuations in the Ericaceae- and sedge-only plots (Fig. 1). The Ericaceae-only plot exhibited lower N contents in most layers of the peat than the N contents throughout the cores from the unmanipulated and sedge-only plots. The lowest C/N ratios were observed at depths of 20-30 cm for the sedge- and Ericaceae-only plots and at 30-40 cm for the unmanipulated plot.

The FTIR spectra obtained from five different depths of the three plot types were averaged and are compared in Fig. 2. Wavenumbers for indicators of the various plot types are marked by a yellow box. The polysaccharides region of the spectra [1030-1080 cm^{-1} (Grube et al. 2006)] was identified as an indicator of the sedge-only plot ($p < 0.05$). Absorbances attributed to polysaccharides were highest in the sedge-only plot and lowest in unmanipulated plot. The Ericaceae-only plot exhibited a marginally significant aromatic signature [1600-1650 cm^{-1} (Niemeyer et al. 1992)]. An aliphatic signature [2850 cm^{-1} and 2920 cm^{-1} (Niemeyer et al. 1992)] was characteristic of the

unmanipulated plot ($p < 0.05$). The FTIR spectra from five layers in each plot type are shown in Fig. 3. Unmanipulated and sedge-only plots exhibited larger variations with depth than the Ericaceae-only plots. Polysaccharides, aromatic compounds, and aliphatic species were more abundant in the surface layer of peat from the unmanipulated and sedge-only plots than levels in deepest layers.

Ratios of absorbances for regions of the FTIR spectra that are characteristic of various chemical species and compound classes were calculated as a function of depth for the plot types (Fig. 4). Larger variations of the lipids/polysaccharides (Lip/Pol) ratio as a function of depth in the three plot types were observed than variations in the carbonyl/lignin (Car/Lig) and polysaccharides/lignin (Pol/Lig) ratios. The Lip/Pol ratio in the Ericaceae-only plot was highest in the 20-30 cm layer (Fig. 4a). Variations in the Car/Lig and Pol/Lig ratios with depth in the Ericaceae-only plot were small (1.2-1.5). As for the sedge plots, The Car/Lig and Pol/Lig ratios for the sedge-only plot were all higher than the ratios in the same layers of the Ericaceae-only plot (Fig. 4b). The Lip/Pol ratio for the sedge-only plot increased with depth. Fluctuations in the three ratios with depth in the unmanipulated plot were small (Fig. 4c). Values of the Car/Lig and Pol/Lig ratios were 1.2-1.5; however, there was a slight increase in the Lip/Pol ratio with depth.

Chemical characteristics of water-extractable organic matter

Values of the spectral indices of the WEOM from five layers of peat for the three plot types are shown in Fig. 5. Values of the E2/E3 index for all layers of peat from the sedge-only plot were higher than values of the index for the Ericaceae-only and unmanipulated plots. Values of the E2/E3 index were highest in the 0-10 cm and 10-20 cm layers of the peat from the sedge-only and unmanipulated plots, respectively. However, the maximum value of the E2/E3 index was observed in the 40-45 cm layer from the Ericaceae-only plot. Slight increases in the HIX and A/T indices with depth were observed for the three plot types; however, values of the SUVA₂₅₄ index decreased with depth in the three plot types.

Concentrations (normalized to the mass of extracted peat) of DOC and various compound classes in WEOM from five layers of peat from the three plot types are shown in Fig. 6.

Levels of DOC were 15-70 mg C g⁻¹ and reached a maximum in the 20-30 cm layer.

Variations in DOC concentrations with depth in the Ericaceae-only plot were higher than variations in the DOC levels with depth in the sedge-only and unmanipulated plots. The DOC concentrations of peat layers from the unmanipulated plot were < 26 mg C g⁻¹ and relatively constant with depth. The concentrations of TPHs were lowest in the 20-30 cm layer of peat from the three plots. Levels of TPHs in layers of peat from the sedge-only plot were relatively lower than concentrations of TPHs in layers of peat from the other plot types. The level of TPHs in the surface layer (0-20 cm) of the Ericaceae-only plot was higher than concentrations of TPHs in surface layers of the other plot types.

Concentrations of MCHOs and PCHOs in peat from all plot types decreased with depth from 15-20 to ~ 8 mg C g⁻¹. Oxalate and formate levels for layers of peat from the three

plot types were 0.01-0.35 mg C g⁻¹ and decreased with depth. Concentrations of acetate decreased with depth in the unmanipulated and sedge-only plots. The maximum and minimum concentrations of acetate in the Ericaceae-only plot (1.65 mg C g⁻¹ and 0.06 mg C g⁻¹, respectively) were observed in the 40-45 cm and 20-30 cm layers, respectively. Levels of propionate were low in all layers of peat from the three plot types.

Concentrations (normalized to the mass of extracted peat) of N species in WEOM from five layers of the peat from the three plot types are shown in Fig. 7. Levels of TDN, DON, and proteinaceous species were higher (300-900 µg N g⁻¹) than the levels of TFAAs (< 150 µg N g⁻¹) and NH₄⁺ (< 90 µg N g⁻¹). Concentrations of NH₄⁺ in layers of peat from the unmanipulated plot were higher than the levels in the other plot types. Concentrations of NO₃⁻ in layers of peat from the three plot types were below the detection limit. The DON was the main fraction of TDN in layers of peat from the three plot types. Layers of peat from the Ericaceae-only plots exhibited larger variation in TDN and DON concentrations than the other two plot types.

Levels of proteinaceous species in peat from the three plot types were relatively constant with depth; however, there was a maximum in concentrations in the 20-30 cm layer (Fig. 7). The lowest levels of proteinaceous species were 100, 120, and 190 µg N g⁻¹ in the 40-45 cm layer from the Ericaceae- and sedge-only and unmanipulated plots, respectively. Concentrations of proteinaceous species with depth in peat from the unmanipulated plot were higher than the levels in the other plots.

The TFAA concentrations in peat from the three plot types were $< 130 \mu\text{g N g}^{-1}$ and decreased with increasing depth (Fig. 7). Levels of TFAAs were highest in peat from the unmanipulated plots. Concentrations (nmol L^{-1}) of individual AAs in the water extracts of layers of peat from the three plot types are shown in Fig. 8. The most abundant AAs in WEOM from the Ericaceae-only plot were GLU, ASP, and ALA (Fig. 8a). Levels decreased with increasing depth in peat in the Ericaceae-only plot. A similar trend was observed in sedge-only plot; however, the decrease in concentrations of the three AAs with depth was more dramatic (Fig. 8b). Abundances of GLU, ASP, and ALA were also high in peat from the unmanipulated plot; however, unlike the other plots, GLY and SER were abundant (Fig. 8c).

Approximately 4500 unique molecular formulae were identified in the WEOM of the peat. Less than 20 depth indicators were found for each layer of the peat from the three plots and no indicator was identified for the 10-20 cm layer (Fig. 9a). Lignin-like species were present in every layer of the peat (Fig. 7 b) and unsaturated hydrocarbons (UHs) and lignin-like species were abundant in the 0-10 cm layer. Contributions of proteinaceous species were highest in the 20-30 cm and 40-45 cm layers, which is consistent with the higher N/C atomic ratios of those layers compared to other layers of the peat (Fig. 9c). All AI values were < 0.5 . The average molecular weights (MWs) of the depth indicators were $180\text{-}500 \text{ g mol}^{-1}$ and varied greatly with depth. Values of the average MW and NOSC of the depth indicators were highest in the 30-40 cm layer.

Most of the plot-type indicators were found in the Ericaceae-only and unmanipulated plots (Fig. 9a). Lipids, UHs, and proteinaceous species were abundant in the unmanipulated plots. About 75% of the plot-type indicators in Ericaceae plots were attributed to lignin- and tannin-like species (Figs. 9a, 9b). The N/C atomic ratio for the unmanipulated plot was higher and values of the average MW and NOSC were smaller than the ratios and values for plot-type indicators of the Ericaceae plot. The values of AI for the three plot types were < 0.5 .

The nMDS technique was applied to visualize differences between the molecular composition of the WEOM and DOM in porewater from a previous study (Song et al. 2018). The molecular composition of the WEOM of peat is distinct from the DOM of peat porewater (Fig. 11). Molecular composition of the WEOM of the sedge-only, Ericaceae-only, and unmanipulated plots vary along axis 1, along axis 2, and at the intersection of axes 1 and 2, respectively.

Discussion

Effects of plot type on molecular composition of peat and water-extractable organic matter

Levels of TPHs in WEOM of sedge-only plots were lower than the TPH concentrations in the other plots (Fig. 6). Sedge has much less woody biomass than Ericaceae, and thus, there will be very little input of lignin via litter to sedge-only plots. The abundance of PCHOs in peat and WEOM of peat from sedge-only plots was higher than the amount in peat from the other plot types (Fig. 2a). Polysaccharides are a major constituent of plant cell walls. Peat from the sedge-only plot also had a higher Pol/Lig ratio than peat from the Ericaceae-only plot (Fig. 4). The trends indicate the polysaccharide content is much higher in sedge litter than Ericaceae litter. Peat from the sedge-only plot had a higher Car/Lig ratio (Fig. 4) and the value of the E2/E3 index (Fig. 5) of the WEOM was higher than the ratio and index in peat and WEOM from the Ericaceae-only plot. High values of the E2/E3 index indicate an abundance of low MW species, which are produced during microbial decomposition of the peat. Functionalities of lignin-like species are oxidized to carbonyl containing moieties during the early stages of decomposition. Microbial decomposition in sedge-only plots is facilitated by the ability of sedge to transfer molecular oxygen (O₂) to roots through aerenchyma deep into the sedge rhizosphere, which accelerates the rate of OM decomposition by microbes.

The higher concentrations of TPHs were found in the surface peat of the Ericaceae-only plot than the levels in surface peat of the other plot types (Figs. 6a, 6b), which is

consistent with the woody structure of ericaceous shrubs. The high absorbance attributed to aromatics in FTIR spectra of peat from the Ericaceae-only plot (Fig. 2) is consistent with the high concentration of TPHs in WEOM from the same plot (Fig. 6). Values of the SUVA₂₅₄ index of the WEOM were higher above 20 cm than below 20 cm in the Ericaceae-only plot (Fig. 5), which is the extent of root penetration of ericaceous shrubs into the rhizosphere. Lignin-like species in the Ericaceae litter is the likely source of the aromatic. Most of the plot-type indicators in WEOM from peat of the Ericaceae-only plot were tannin- and lignin-like species (Fig. 9a). Ericoid mycorrhizal fungi associated with roots of ericaceous shrubs, which extend to depths of ~20 cm into the rhizosphere, may suppress free-living saprotrophs, potentially limiting C mineralization with a concomitant accumulation of lignin- and tannin-like species. Ericaceae do not have aerenchyma, which limits O₂ availability and creates reducing conditions that slow the rate of decomposition.

The FTIR spectra of peat in the unmanipulated plot exhibited absorbance characteristic of the CH₂ functionality of lipids and waxes (Fig. 2). Contributions of lipids to the plot-type indicators of WEOM of peat from the unmanipulated plot were greater than the contributions from the other plot types (Fig. 9a). Concentrations of proteinaceous species and NH₄⁺ concentrations in WEOM of peat from the unmanipulated plot were higher than the levels of WEOM from the other plot types (Fig. 7). The DOM of porewater from unmanipulated plots at the same site was more enriched in proteinaceous species and lipids than sedge-only and unmanipulated plots (Song et al. 2018). The major organic constituents of bacteria are proteins and lipids, and thus, abundances of these compound

classes in peat and WEOM of peat from the unmanipulated plot are indicative of an active microbial population. Microbial decomposition of N-containing OM might also explain higher concentration of NH_4^+ in unmanipulated plots than the other plot types.

Effects of depth on molecular composition of peat and water-extractable organic matter

The DOC concentrations were highest in WEOM of peat in the 20-30 cm and 30-40 cm layers (Fig. 6), which is contrary to the results of Zaccone et al. (2009) who found that DOC levels in WEOM of peat from a bog decreased with depth. The peat and WEOM from the surface layers exhibited higher abundances of PCHOs (Figs. 2 and 6, respectively), which indicates inputs from plant litter. Concentrations of NH_4^+ and TFAAs in the WEM of peat decreased and the DON increased with depth (Fig 7). The observations indicate rapid microbial and plant consumption of readily available nitrogen species like NH_4^+ and FAAs and accumulation of more recalcitrant forms of ON in deeper layers of the peat.

Values of the A/T index for WEOM were low in surface layers of all plot types, which indicates a low abundance of degraded substances and is consistent with the higher concentrations of MCHOs, PCHOs, and carboxylic acids in WEOM in the surface layers (Fig. 5 and Fig. 6, respectively). High values of the E2/E3 index of WEOM, which indicate an abundance of low-molecular-weight species, were found for the surface layers

of sedge-only and unmanipulated plots and in deep layers of the Ericaceae-only plot. High values of the E2/E3 index and low values of the A/T index of WEOM from surface layers of the peat in sedge-only and unmanipulated plots indicated small contributions of degraded substances with low molecular weights, which is consistent with high concentrations of CHOs and carboxylic acids (Fig. 5 and Fig. 6, respectively). Values of the A/T and E2/E3 indices for WEOM from surface layers of the Ericaceae-only plots indicate the substances are poorly degraded and have high molecular weights, which is consistent with large contributions of lignin- and tannin-like species to the plot-type indicators (Fig. 9a).

The C and N contents of peat from all the plots increased with depth (Fig. 1), which is similar to the trends observed by Tfaily et al. (2014). During humification, N is fixed into refractory humic substances in soil (Jackson et al. 1989), which might explain increases of N with depth in peat. Song et al. (2018b) observed fixation of N into organic forms like lignin- and tannin-like species in porewater at the site of the subject investigation. Retention of N in microbial biomass (Damman 1988) or plant litter might also explain increases of N content with depth in peat. However, the proteinaceous species and nucleic acids of microbes is more labile than the tannin- and lignin-like species of plant litter to which N becomes fixed during the early stages of humification. Contributions of proteinaceous species and lipids to the WEOM of peat in the deepest layer of the peat were larger than contributions to the WEOM from other layers of the peat (Figs. 9a, 9b), and might represent contributions of refractory tannin-protein complexes and lipids incorporated into the refractory, wax matrix of plant litter.

The high C/N ratio in the surface layer of peat is likely due to fresh inputs of high C-containing plant litter. Plant cell walls are low in N and instead contain high C-containing compounds like cellulose, hemicellulose, lignin, and tannin. The C/N ratio of surface peat samples from the unmanipulated plot was lower than the ratios observed in the other plots. The low C/N ratio might reflect a more advanced stage of decomposition. Also, shrubs and sedges might diminish light penetration to the surface of the unmanipulated plot and decrease the abundance of *Sphagnum* moss (Potvin et al. 2015). The N content of *Sphagnum* mosses is low; however, the decay of *Sphagnum* moss litter is slow compared to the decay of vascular plant litter (Asada et al. 2005). The humification process reduces the C/N ratio of natural organic matter (Waksman and Tenney 1927). The degree of humification is inversely related to the CHO concentration and proportional to values of the HIX index. The Ericaceae- and sedge-only plots exhibited highest values of the HIX index and lowest concentrations of CHOs in WEOM of the 20-30 cm layer and values of the HIX index were a maximum and levels of CHOs were a minimum in the 30-40 cm layer of the unmanipulated plot (Figs. 5 and 6, respectively). The results indicate high degrees of humification in the 20-30 cm layer of Ericaceae- and sedge-only plots and the 30-40 cm layer of the unmanipulated plot.

The Lip/Pol ratio increases and the Car/Lig and Pol/Lig ratios decrease during the humification process (Kalbitz et al. 1999; Tfaily et al. 2014b). Plant lipids are protected from microbial decomposition by a wax matrix and the PCHOs in plants are more labile than the lignin of the plant cell wall. The Lip/Pol ratios of peat vary inversely with Car/Lig and Pol/Lig ratios as expected. The highest Lip/Pol ratios were observed in 20-30

cm, 40-45 cm, and 30-40 cm layers in Ericaceae- and sedge-only, and unmanipulated plots, respectively, which is consistent with high values of HIX indices for WEOM from those layers (Fig. 5). The C/N ratios, concentrations of PCHOs, and values of the HIX index were similar in the 20-30, 30-40 and 40-45 cm layers of peat from the sedge-only plot. The Pol/Lig and Car/Lig ratios were also similar in the 30-40 and 40-45 cm layers; however, the Lip/Pol ratio was clearly a maximum in the 40-45 cm layer. The results indicate the greatest degree of humification in the sedge-only plot occurs in the 40-45 cm layer. Very few depth indicators were identified in the WEOM for the various plot types (Fig. 9a); however, plot-type indicators were abundant for Ericaceae-only and unmanipulated plots (Fig. 10a). The WEOM of peat from the Ericaceae- and sedge-only plots have litter inputs with distinctly different molecular compositions. The degree of humification in the sedge- and Ericaceae-only plots are distinctly different due to availability of O₂ in the sedge rhizosphere.

Conclusion

The sedge-only plot exhibited lower TPHs, higher PCHOs, higher Car/Lig and Pol/Lig ratios, and higher values of the E2/E3 index than the unmanipulated and Ericaceae-only plots. The trends may be due to the aerenchyma and roots of sedge that deliver O₂ deep into the rhizosphere, which accelerates microbial decomposition of the OM. The shallow

roots of Ericaceae, which lack aerenchyma to facilitate transfer of O₂ into the rhizosphere, and the ericoid mycorrhizal fungi limited C mineralization in the rhizosphere of ericaceous shrubs. High microbial activity may increased the N mineralization in the unmanipulated plot. Concentrations of DOC, TDN, and proteinaceous species in WEOM of peat were highest and the C/N ratio of peat and levels of TPHs in WEOM of peat were lowest in 20-40 cm layer of peat, which indicates intermediate layers of the peat are the most active plots of humification. Levels of NH₄⁺ and TFAAs decreased with depth and concentrations of DOC and TDN increased. Values of the E2/E3 and A/T indices of WEOM from surface layers of the sedge-only and unmanipulated plots were high and low, respectively. The greatest degrees of humification were observed in WEOM from the 20-30 cm, 30-40 cm, and 40-45 cm layers in the Ericaceae-only, unmanipulated, and sedge-only plots, respectively.

References

Artz R, Chapman S, Campbell C (2006) Substrate utilisation profiles of microbial communities in peat are depth dependent and correlate with whole soil FTIR profiles *Soil Biology and Biochemistry* 38:2958-2962 doi:10.1016/j.soilbio.2006.04.017

Asada T, Warner B, Aravena R (2005) Effects of the early stage of decomposition on change in carbon and nitrogen isotopes in *Sphagnum* litter *Journal of Plant Interactions* 1:229-237 doi:10.1080/17429140601056766

Batjes NH (2014) Total carbon and nitrogen in the soils of the world *European Journal of Soil Science* 65:10-21 doi:10.1111/ejss.12114_2

Bridgham SD, Pastor J, Dewey B, Weltzin JF, Updegraff K (2008) Rapid carbon response of peatlands to climate change *Ecology* 89:3041-3048

Chambers FM, Beilman D, Yu Z (2011) Methods for determining peat humification and for quantifying peat bulk density, organic matter and carbon content for palaeostudies of climate and peatland carbon dynamics *Mires and Peat* 7:1-10

Cocozza C, D'Orazio V, Miano TM, Shotyk W (2003) Characterization of solid and aqueous phases of a peat bog profile using molecular fluorescence spectroscopy, ESR and FT-IR, and comparison with physical properties *Organic Geochemistry* 34:49-60
doi:[https://doi.org/10.1016/S0146-6380\(02\)00208-5](https://doi.org/10.1016/S0146-6380(02)00208-5)

Damman AWH (1988) REGULATION OF NITROGEN REMOVAL AND RETENTION IN SPHAGNUM BOGS AND OTHER PEATLANDS *Oikos* 51:291-305
doi:10.2307/3565310

Delarue F, Laggoun-Défarge F, Buttler A, Gogo S, Jassey VEJ, Disnar J-R (2011) Effects of short-term ecosystem experimental warming on water-extractable organic matter in an ombrotrophic *Sphagnum* peatland (Le Forbonnet, France) *Organic Geochemistry* 42:1016-1024 doi:<https://doi.org/10.1016/j.orggeochem.2011.07.005>

Grannas AM, Hockaday WC, Hatcher PG, Thompson LG, Mosley-Thompson E (2006)
New revelations on the nature of organic matter in ice cores *Journal of Geophysical
Research: Atmospheres* 111:n/a-n/a doi:10.1029/2005JD006251

Grube M, Lin J, Lee P, Kokorevicha S (2006) Evaluation of sewage sludge-based
compost by FT-IR spectroscopy *Geoderma* 130:324-333

Guigue J, Harir M, Mathieu O, Lucio M, Ranjard L, Lévêque J, Schmitt-Kopplin P
(2016) Ultrahigh-resolution FT-ICR mass spectrometry for molecular characterisation of
pressurised hot water-extractable organic matter in soils *Biogeochemistry* 128:307-326
doi:10.1007/s10533-016-0209-5

Hansen AM, Kraus TEC, Pellerin BA, Fleck JA, Downing BD, Bergamaschi BA (2016)
Optical properties of dissolved organic matter (DOM): Effects of biological and
photolytic degradation *Limnology and Oceanography* 61:1015-1032
doi:10.1002/lno.10270

Hedges J (1990) Compositional indicators of organic acid sources and reactions in natural
environments *Organic acids in aquatic ecosystems* Wiley:43-63

Heller C, Weiss K (2015) Approaching a Standardized Method for the Hot-Water Extraction of Peat Material to Determine Labile SOM in Organic Soils Communications in Soil Science and Plant Analysis 46:1044-1060 doi:10.1080/00103624.2015.1019082

Hribljan JA, Kane ES, Chimner RA (2017) Implications of Altered Hydrology for Substrate Quality and Trace Gas Production in a Poor Fen Peatland Soil Science Society of America Journal 81:633-646 doi:10.2136/sssaj2016.10.0322

Hribljan JA, Kane ES, Pypker TG, Chimner RA (2014) The effect of long-term water table manipulations on dissolved organic carbon dynamics in a poor fen peatland Journal of Geophysical Research-Biogeosciences 119:577-595 doi:10.1002/2013jg002527

Jackson LE, Schimel JP, Firestone MK (1989) SHORT-TERM PARTITIONING OF AMMONIUM AND NITRATE BETWEEN PLANTS AND MICROBES IN AN ANNUAL GRASSLAND Soil Biology & Biochemistry 21:409-415 doi:10.1016/0038-0717(89)90152-1

Kalbitz K, Geyer W, Geyer S (1999) Spectroscopic properties of dissolved humic substances - a reflection of land use history in a fen area *Biogeochemistry* 47:219-238
doi:10.1007/bf00994924

Keil RG, Kirchman DL (1991) Dissolved combined amino acids in marine waters as determined by a vapor-phase hydrolysis method *Marine Chemistry* 33:243-259
doi:[http://dx.doi.org/10.1016/0304-4203\(91\)90070-D](http://dx.doi.org/10.1016/0304-4203(91)90070-D)

Keller JK (2006) Controls of Microbial Carbon Cycling in Northern Peatlands. ProQuest,

Landgraf D, Leinweber P, Makeschin F (2006) Cold and hot water-extractable organic matter as indicators of litter decomposition in forest soils *Journal of Plant Nutrition and Soil Science* 169:76-82 doi:10.1002/jpln.200521711

Leifeld J, Steffens M, Galego-Sala A (2012) Sensitivity of peatland carbon loss to organic matter quality *Geophysical Research Letters* 39:L14704
doi:10.1029/2012gl051856

Lou T, Xie H (2006) Photochemical alteration of the molecular weight of dissolved organic matter *Chemosphere* 65:2333-2342 doi:10.1016/j.chemosphere.2006.05.001

Lv JT, Zhang SZ, Luo L, Cao D (2016) Solid-phase extraction-stepwise elution (SPE-SE) procedure for isolation of dissolved organic matter prior to ESI-FT-ICR-MS analysis *Analytica Chimica Acta* 948:55-61 doi:10.1016/j.aca.2016.10.038

Meingast KM et al. (2014) Spectral detection of near-surface moisture content and water-table position in northern peatland ecosystems *Remote Sensing of Environment* 152:536-546 doi:10.1016/j.rse.2014.07.014

Moore TR, Roulet NT, Waddington JM (1998) Uncertainty in Predicting the Effect of Climatic Change on the Carbon Cycling of Canadian Peatlands *Climatic Change* 40:229-245 doi:10.1023/a:1005408719297

Myklestad SM, Skånøy E, Hestmann S (1997) A sensitive and rapid method for analysis of dissolved mono- and polysaccharides in seawater *Marine Chemistry* 56:279-286 doi:[https://doi.org/10.1016/S0304-4203\(96\)00074-6](https://doi.org/10.1016/S0304-4203(96)00074-6)

Niemeyer J, Chen Y, Bollag J-M (1992) Characterization of humic acids, composts, and peat by diffuse reflectance Fourier-transform infrared spectroscopy Soil Science Society of America Journal 56:135-140

Ohno T, Parr TB, Gruselle MC, Fernandez IJ, Sleighter RL, Hatcher PG (2014) Molecular composition and biodegradability of soil organic matter: a case study comparing two new England forest types Environ Sci Technol 48:7229-7236
doi:10.1021/es405570c

Ott CA (2013) Carbon in the Peatlands in the Great Lakes Region

Potvin LR, Kane ES, Chimner RA, Kolka RK, Lilleskov EA (2015) Effects of water table position and plant functional group on plant community, aboveground production, and peat properties in a peatland mesocosm experiment (PEATcosm) Plant and Soil 387:277-294 doi:10.1007/s11104-014-2301-8

Romanowicz KJ, Kane ES, Potvin LR, Daniels AL, Kolka RK, Lilleskov EA (2015) Understanding drivers of peatland extracellular enzyme activity in the PEATcosm experiment: mixed evidence for enzymic latch hypothesis Plant and Soil 397:371-386 doi:10.1007/s11104-015-2746-4

Routh J, Hugelius G, Kuhry P, Filley T, Tillman PK, Becher M, Crill P (2014) Multi-proxy study of soil organic matter dynamics in permafrost peat deposits reveal vulnerability to climate change in the European Russian Arctic Chemical Geology 368:104-117 doi:<https://doi.org/10.1016/j.chemgeo.2013.12.022>

Tfaily MM et al. (2014a) Organic matter transformation in the peat column at Marcell Experimental Forest: Humification and vertical stratification Journal of Geophysical Research-Biogeosciences 119:661-675 doi:10.1002/2013jg002492

Tfaily MM et al. (2014b) Organic matter transformation in the peat column at Marcell Experimental Forest: Humification and vertical stratification Journal of Geophysical Research: Biogeosciences 119:661-675 doi:doi:10.1002/2013JG002492

WAKSMAN SA, TENNEY FG (1927) THE COMPOSITION OF NATURAL ORGANIC MATERIALS AND THEIR DECOMPOSITION IN THE SOIL: II. INFLUENCE OF AGE OF PLANT UPON THE RAPIDITY AND NATURE OF ITS DECOMPOSITION—RYE PLANTS *Soil Science* 24:317-334

Zaccone C, D'Orazio V, Shotyk W, Miano TM (2009) Chemical and spectroscopic investigation of porewater and aqueous extracts of corresponding peat samples throughout a bog core (Jura Mountains, Switzerland) *Journal of Soils and Sediments* 9:443-456

Figure Captions

Fig. 1 (a) Percentage of carbon; (b) Percentage of nitrogen of solid peat from different plots at each depth; (c) The C/N ratio at different depth in each site

Fig. 2 Mean Fourier-transform infrared spectra of peat harvested across different plots (Unmanipulated, Sedge, Ericaceae). Note: the color bar A represents the significance,

blue = low P values, Red = P values. The color bar B represents the indicator from different plots, Blue= unmanipulated, Green = Sedge, Red= Ericaceae.

Fig. 3 Fourier-transform infrared spectra of peat harvested from (a) unmanipulated site; (b) sedge site; (c) ericaceous site

Fig. 4 Ratios of Fourier-transform infrared peak heights for (a) Ericaceous plots, (b) Sedges plots, (c) Unmanipulated plots at 5 depths

Fig. 5 Optical properties of peat extract for each site at (a) 0-10 cm, (b) 10-20 cm, (c) 20-30 cm, (d) 30-40 cm, (e) 40-45 cm.

Fig. 6 Composition of dissolved organic carbon of peat extract for each plots at (a) 0-10 cm, (b) 10-20 cm, (c) 20-30 cm, (d) 30-40 cm, (e) 40-45 cm.

Fig. 7 Dissolved nitrogen, dissolved organic nitrogen and inorganic nitrogen of peat extract for each plots at (a) 0-10 cm, (b) 10-20 cm, (c) 20-30 cm, (d) 30-40 cm, (e) 40-45 cm.

Fig. 8 The concentration of each amino acids at different depths in (a) Ericaceous plots; (b) Sedge plots; (c) Unmanipulated plots.

Fig. 9 Depth indicator for all PFG. (a) Percentage of Class group; (b) Van Krenvlen diagram; (c) Average NOSC, N/C, AI, MW (molecular weight). Note: AS is amino sugar, UH is unsaturated hydrocarbons, Carb is carbohydrate, CA is condensed aromatics

Fig. 10 PFG indicator of DOM for all Depth. (a) Percentage of Class group; (b) Van Krenvlen diagram; (c) Average NOSC, N/C, AI, MW (molecular weight)

Fig. 11 Non-Metric multidimensional scale ordination for the molecular composition of DOM for 108 porewater samples and water extractable organic matter of peat

Note: E1,S1 and U1 are the water extractable samples, and other samples are the porewater.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig.11

Chapter 5 Conclusion

In summary, the effect of PFGs and season on the quantity and quality of organic matter in peat and DOM in porewater were thoroughly investigated to evaluate the stability of the peatland.

In terms of the PFG effect on the composition of peat porewater, the unmanipulated plots showed highest decomposition rate compared with the sedge-only and Ericaceae-only plots, which is possible related to the synergistic effect between the two plant functional groups. The Ericaceae-only plots inferred lower enzymatic activities compared with other two plots; the sedges-only plots showed highest concentration of DOC and protein could due to fast decomposition rates of the labile compounds. In terms of seasonal variations of the composition of peat porewater, the late season showed highest percentage of degraded materials while the early season showed the lowest, which may be contributed by root exudates and leaf fall that produced DOM with little aromaticity.

In terms of PFGs effect on the inorganic and organic nitrogen in the porewater, sedges plots showed lower concentration of free amino acids and play higher impact on NDOM composition than Ericaceae plots. The early season showed high concentration of arginine, while low concentration of ammonium was observed in fall. The maximum concentration of protein was found in June or July with a concentration less than 0.15 mg N L^{-1} . NDOM compounds with high NOSC were found in the growing season due to fresh input from fall leaves and root exudates; dramatic change in NDOM composition was observed because of oxidation, reduction, hydration, and condensation reactions.

In terms of PFGs and sampling depth effect on the OM composition in the peat, FTIR, chemical analysis, and UHR-MS analysis were performed. For PFGs effect, the sedge plots showed lower total phenolics, higher polysaccharides, and higher Car/Lig, Pol/Lig, E2/E3 values than the unmanipulated and ericaceous plots. The unmanipulated plots may exhibit high microbial activities, which could increase the nitrogen mineralization. For the effect of peat depth, 20~40 cm was the most active layer with highest DOC, TDN and protein and lowest total phenolics and C/N. The concentrations of ammonium and TFAA were decreasing while the DOC and TDN were increasing with the increase of sampling depth. Higher humification degree was observed at 20~30 cm in the ericaceous plots, at 30~40 cm in the unmanipulated plots and at 40 ~45 cm in the sedges plots.

Chapter 6 Future work

Further understanding of the rate of decomposition of OM in peatlands response to climate change requires quantitation of (1) the various compound classes and intermediate decomposition products that comprise DOM in porewater, (2) the different classes of OM and WEOM in peat from mesotlem layer, and (3) the seasonal activity of enzymes, which are induced by the various classes of OM, in peatland dominated by different plant functional types.

The rate of decomposition of organic matter (OM) in the peat matrix can be estimated by the following rate expression:

$$\frac{d[OM]}{dt} = -k_{bio} \{enzyme\} \{OM\} - k_{chem} \{OM\} - k_{photochem} \{OM\}$$

where: $[OM]$ is the concentration of organic matter, k_{bio} , k_{chem} , and $k_{photochem}$ are the rate constants for biotransformation, chemical transformation and photochemical transformation, $\{OM\}$ is the activity of the organic matter, and $\{enzyme\}$ is the enzyme activity.

- (1) In this study, we were able to classify the OM and quantify some of the class of the OM in porewater and peat dominated by various plant functional types. However, predicting the responses of the C, N, and P cycles of peatlands to climate change requires quantification of all the classes of DOM in porewater.

- (2) The mesotelm layer (30-75 cm) is most active zone with redox oscillation associated with fluctuation water table, as a result, increasing in temperature and lowering of the water table in peatlands would impact the mesotelm layer most. The quantification of OM and WOEM in this layer, therefore, would be of great importance.
- (3) Quantification of the seasonal variation of enzyme activities is directly related to the decomposition rate of the OM. Enzymes are induced by the presence of molecules with molecular structure (i.e., linear, branched, cyclic) and the functionality (e.g., -OH, -NH₂, -CHO, -COOH) of the organic molecule. Various classes of natural OM, i.e., lipids, carbohydrates, lignins, proteins, amino sugars, and tannins have characteristic structures and functionalities. Thus, decomposition of the various classes of OM is enzyme-specific. Classes of enzymes like cellulases, ligninases, amylases, proteases, and lipases are specific to decomposition of cellulose, lignin, starch, protein, and fatty acid esters. Enzyme activities are related to soil temperature, water table level, redox potential, nutrients, and substrates. Plant seasonal cycles affect the composition of root exudates and carbon and nitrogen availability for the soil microbial community. Alteration of the microbial community composition affects the degradation of soil OM and the accessibility of nutrients for uptake by plants (Kaiser et al. 2010). Thus, cycles of plant and enzyme activities are intertwined and exhibit strong seasonal patterns (Wallenstein et al. 2009).

Reference

- Kaiser C et al. (2010) Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil *New Phytologist* 187:843-858 doi:10.1111/j.1469-8137.2010.03321.x
- Wallenstein MD, McMahon SK, Schimel JP (2009) Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils *Global Change Biology* 15:1631-1639