Global Warming Effects on Mercury Cycling in Northern Peatlands

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Geology
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Abstract

Northern peatlands (a type of wetland with large carbon storage) are sinks for atmospheric mercury (Hg) and “hotspots” of methylmercury (MeHg) production in catchments. The effects of climate change (especially warming) are predicted to affect northern latitudes most strongly. There is concern that this warming will affect Hg cycling in northern peatlands by changing both the deposition and accumulation of inorganic Hg, as well as enhancing the production of MeHg. To better understand the influences of warming on Hg cycling in northern peatlands, I conducted field-based investigations and laboratory-based incubations on the role of vegetation, soil moisture, and soil temperature in regulating Hg cycling in two types of northern peatlands (moss-dominated fen and sedge-dominated fen). Findings of my doctoral research include plant species and leaf age affected foliar Hg uptake from the atmosphere. Foliar Hg concentrations decreased from June to July due to the dilution effect of leaf growth for all plant species (sedges and sweet gale (a shrub)). Foliar Hg concentrations then steadily increased after July until October, with a strong positive correlation with leaf age. Sedge leaves accumulated less Hg from the atmosphere over the growing season than sweet gale leaves. Leaching of Hg from litterfall differed between plant species with sedges leaching more Hg from their litterfall than sweet gale. Given the fact that climate change will increase vegetation biomass and favor sedges and shrubs abundance at the expense of Sphagnum mosses, our results provide more information to predict changes in Hg cycling in northern peatlands. Changes in soil moisture affected the cycling of Hg, sulfate (SO$_4^{2-}$), and dissolved organic matter (DOM) in northern peatlands with dry peat soils releasing more Hg, especially MeHg, and SO$_4^{2-}$, than wet and moist peat soils, and both wet and dry peat soils released more DOM than the moist peat soils during the re-wetting sampling events. There was an equilibrium between inorganic Hg concentrations in the solid phase of soils and the liquid phase of leachate. SO$_4^{2-}$ concentrations peaked in the leachate of
the initial re-wetting sampling event. As SO$_4^{2-}$ concentrations decreased, MeHg concentrations increased and peaked in the second re-wetting event. SO$_4^{2-}$ in peat soils with different soil moistures (wet, moist, and dry peat soils) depleted rapidly in the static system (no external sulfur deposition or input). These results imply that drought under global warming will increase Hg transport and affect Hg methylation by increasing the release of Hg, DOM, and SO$_4^{2-}$ during the re-wetting events in northern peatlands. Elevated soil temperature (+4.5 °C at 25 cm depth of soil) increased both inorganic Hg and MeHg concentrations in pore waters in the moss-dominated fen. However, elevated soil temperature (+3.8 °C at 25 cm depth of soil) slightly decreased inorganic Hg concentrations but significantly increased MeHg concentrations in pore waters in the sedge-dominated fen, which was due to the increase in conversion of inorganic Hg to MeHg under elevated temperature. Increases in pore water MeHg concentrations under elevated temperature were overall attributed to the enhancement in both net MeHg production and release of MeHg from soils in both fens. These findings imply that vegetation community shift and biomass increase, soil moisture decrease, and soil temperature increase under global warming can affect Hg transport and methylation in northern peatlands.

**Keywords**

Northern peatlands, mercury cycling, methylmercury, global warming, vegetation, litterfall, soil moisture, ground warming, dissolved organic matter, sulfate.
Summary for Lay Audience

Mercury (Hg) is a naturally occurring metalloid that uniquely exists as a solid, liquid, and gas at ambient temperatures, and exists everywhere on Earth. Gaseous Hg is released to the atmosphere by natural and anthropogenic processes, transported globally, and can then be assimilated by plant leaves, and finally enters soils after plant leaves senesce. Inorganic Hg can be converted into methylmercury (MeHg), a neurotoxin, by bacteria in oxygen-free soils and sediments. Northern peatlands are a place where these conditions exist, and often are “hotspots” of MeHg production. It is established that climate change is disproportionally affecting northern latitudes where most northern peatlands are found. Changes in Earth surface temperature and moisture will influence soil moisture, soil temperature, and plant species composition, which can all impact aspects of the Hg cycle. The overall goal of my thesis was to study the influence of soil warming on Hg cycling in two types of northern peatlands, a Sphagnum spp. mosses-dominated peatland and a sedge-dominated peatland. My thesis first investigated the Hg accumulation by leaves of dominant plant species, including sedges and a shrub (sweet gale), in the sedge-dominant peatland, and Hg release from these dead or senesced leaves. I found that Hg concentrations in leaves differed between plant species; shrub leaves had higher Hg concentrations than sedge leaves. Mercury concentrations in all leaves generally increased with time. Dead shrub leaves released less soluble Hg than dead sedge leaves. Future plant species composition changes under climate change will affect Hg input from plant leaves to northern peatlands. My second objective was to study how drying soils under climate change will influence Hg release from soils and net MeHg production. My results showed that drier soils released more Hg, especially MeHg during re-wetting events, such as rain, and wet soils favored MeHg production. Drier peat soils also released more sulfate (SO$_4^{2-}$) and bioaccessible dissolved organic matter (DOM), which are necessary nutrients for Hg methylators, and thus, the release of them after re-
wetting of peat soils will promote net MeHg production. My third objective was to investigate the effect of soil warming on Hg mobility and MeHg production in northern peatlands. I found that when soil temperature increased by +4.5 °C in the Sphagnum spp. mosses-dominated peatland, concentrations of inorganic Hg and MeHg in soil waters significantly increased. When soil temperature increased by +3.8 °C in the sedge-dominated peatland, inorganic Hg concentrations in soil waters slightly decreased but MeHg concentrations significantly increased. Increases in MeHg concentrations in soil waters were from both MeHg production and releases from peat soils. Overall, my study showed that changes in plant species composition and soil temperature and moisture under climate change will impact Hg input through plant leaves to northern peatlands, Hg mobility, and MeHg production in northern peatlands in the future.
Co-Authorship Statement

The three experimental chapters (Chapter 2, Chapter 3, and Chapter 4) of this thesis are planned for submission to a peer-reviewed journal. The original field experimental design, field site selection, and laboratory experimental design in chapter 2 were done by Dr. Brian Branfireun. Ting Sun conducted all experiments, including the collection of plant samples biweekly from the field site from June to October in 2018, setup and maintenance of the laboratory experiment, sample analysis, data analysis and interpretation, and initial manuscript writing. Dr. Brian Branfireun made significant contributions to data interpretation and manuscript editing. The original experimental design in chapter 3 was by Ting Sun and Dr. Brian Branfireun. Ting Sun was responsible for the conduct and maintenance of the experiment, sample collection and analysis, data analysis and interpretation, and the initial manuscript. Dr. Brian Branfireun made significant contributions to manuscript editing. For chapter 4, Dr. Brian Branfireun and Dr. Zoë Lindo designed the original experiment and selected field sites with collaboration from the Ontario Ministry of Natural Resources and Forestry. Ting Sun participated in setting up the field experimental platform and had oversight of this experiment, and was responsible for the maintenance of the experiment, pore waters samples collection, water table measurements, soil moisture and temperature measurements, sample analysis, data analysis and interpretation, and manuscript writing. Manuscripts writing and editing from this study will be credited to Ting Sun and Dr. Brian Branfireun.
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<td>Analysis of Variance</td>
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<td>BIX</td>
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<td>Carbon</td>
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Chapter 1

1 Introduction

1.1 Mercury as a global pollutant

Mercury (Hg) is a global concern due to its potential toxicity and ubiquitous presence in the environment. It is established that Hg cycles globally (Obrist et al., 2018; Selin, 2009) among the atmosphere, terrestrial ecosystems, and oceans (Figure 1.1). Mercury enters the atmosphere from both natural and anthropogenic sources (Driscoll et al., 2013). Natural sources of atmospheric Hg include volcanoes, geothermal sources, forest fires, and volatilization from mercuriferous soils, bedrock, vegetation, and oceans (Schroeder and Munthe, 1998). Mercury is also emitted to the atmosphere by anthropogenic activities, such as artisanal gold mining, coal combustion, incineration of medical waste, sewage sludge, and base metal melting (Streets et al., 2011). Although atmospheric Hg concentrations decreased by 30% with the implementation of flue gas desulfurization (FGD) and other emission controls for coal combustion between 1990 and 2010 (Zhang et al., 2016b), anthropogenic activities have increased atmospheric Hg concentrations by about 450% above natural levels since the pre-anthropogenic period (prior to 1450 AD) (Outridge et al., 2018).
The background concentrations of atmospheric gaseous elemental Hg are between 1.5 and 1.7 ng m\(^{-3}\) in the Northern Hemisphere and between 1.1 and 1.2 ng m\(^{-3}\) in the Southern Hemisphere (Lindberg et al., 2007). There are three species of Hg in the atmosphere including atmospheric gaseous elemental mercury (GEM, Hg\(^0\)), reactive gaseous mercury (RGM: HgBr\(_2\), HgCl\(_2\), Hg(CH\(_3\))Cl), and particulate-bound mercury (PBM) with GEM as the dominant species (> 95%) (Schroeder and Munthe, 1998). GEM has a long residence time in the atmosphere; generally, several months to a year (Schroeder and Munthe, 1998). After long-distance transport, GEM can be oxidized and deposited to the Earth’s surface, including in remote areas such as the Antarctic and Arctic (Lindberg et al., 2007). RGM and PBM have a shorter atmospheric residence time (hours to days) and are usually deposited at the local and regional scales (Schroeder and Munthe, 1998).

### 1.2 Mercury inputs to catchments

#### 1.2.1 Wet and dry deposition of mercury

Atmospheric Hg can be delivered to terrestrial and aquatic ecosystems by wet and dry deposition. The wet deposition of Hg involves the scavenging of gas- and aerosol-phase
RGM and PBM by precipitation (Lindberg et al., 2007; Schroeder and Munthe, 1998). GEM may also be removed from the atmosphere by precipitation after being oxidized to Hg$^{2+}$ by atmospheric oxidants, such as ozone (Wang et al., 2007), OH-radicals (Pal and Ariya, 2004), and bromine (Br) (Holmes et al., 2010). The dry deposition of Hg includes the deposition of atmospheric Hg to surfaces of vegetation, water, and soil through particle settling, and absorption of GEM by vegetation (Schroeder and Munthe, 1998). Mounting evidence suggests that dry deposition of Hg is more important than wet deposition of Hg (Demers et al., 2013; Graydon et al., 2008; Jiskra et al., 2015; Obrist et al., 2017; Risch et al., 2017; Risch et al., 2012; St. Louis et al., 2001; Zhang et al., 2016a), contributing ~84% of total Hg (wet and dry) deposition in terrestrial ecosystems (Demers et al., 2013). Vegetation plays an important role in the dry deposition of Hg (Jiskra et al., 2018; Jiskra et al., 2015; Wang et al., 2016), given the large surface area of foliage that provides large receptor sites and stomata for settling of RGM and PBM and absorption of GEM, respectively (Stamenkovic and Gustin, 2009).

1.2.2 Mercury accumulation and sequestration by vegetation

Vegetation leaves can scavenge Hg from the atmosphere (Mao et al., 2013; Obrist et al., 2017; Stamenkovic and Gustin, 2009). Vegetation is generally considered a sink for atmospheric Hg, with the majority of Hg in vegetation leaves accumulated from the atmosphere (Jiskra et al., 2018; Lindberg et al., 2007). Previous studies showed that plant roots acted as a barrier of Hg transport from soils to shoots (Wang et al., 2015), and less than 10% of Hg in roots was transported to the aboveground portion of plants (Bishop et al., 1998; Ericksen et al., 2003; Schwesig and Krebs, 2003).

Plant leaves accumulate Hg from the atmosphere through stomatal uptake and surface adsorption (Lindberg et al., 1992; Stamenkovic and Gustin, 2009). The stomatal pathway is an essential route of foliar Hg uptake from the atmosphere (Lindberg et al., 1992). Stamenkovic and Gustin (2009) suggested that the non-stomatal pathway of Hg deposition to
the leaf cuticle and subsequently retention and incorporation into leaf tissue also plays an important role in accumulating atmospheric Hg, however, the exact mechanism of the nonstomatal uptake of Hg is still unclear.

The assimilation of atmospheric Hg by leaves is affected by several factors, such as atmospheric Hg concentrations (Hanson et al., 1995), environmental factors (e.g., temperature, solar radiation, drought stress, and CO\textsubscript{2} concentrations) (Blackwell and Driscoll, 2015; Ericksen and Gustin, 2004; Zhu et al., 2016), and biological factors (e.g., leaf age, leaf placement, and plant species) (Blackwell and Driscoll, 2015; Laacouri et al., 2013). Atmosphere-vegetation Hg exchange is a bidirectional process, which depends on the atmospheric Hg concentrations and the atmospheric Hg compensation point (or compensation concentration) (Hanson et al., 1995). The Hg compensation concentration is the concentration of atmospheric Hg at which the Hg flux between leaves and the atmosphere is zero; leaves can accumulate Hg from the atmosphere when the ambient air Hg concentration is higher than this Hg compensation concentration; Hg will be released from leaves to the atmosphere when the ambient air Hg concentration is lower than this Hg compensation concentration. Although the atmospheric Hg compensation concentration being dependent on many factors (Ericksen and Gustin, 2004; Hanson et al., 1995), such as soil water, solar radiation, and plant species, gradual increases of foliar Hg concentrations over time clearly show that plants are generally a net sink of atmospheric Hg (Ericksen et al., 2003; Jiskra et al., 2018; Laacouri et al., 2013). Stomatal conductance regulates the stomatal exchange of Hg and is controlled by temperature, solar radiation, leaf surface wetness, and CO\textsubscript{2} concentrations (Lindberg et al., 1998; Millhollen et al., 2006a). Extreme temperature, high CO\textsubscript{2} concentrations, and drought stress can reduce Hg accumulation in leaves by decreasing stomatal conductance. Stomata are open in light but closed in dark. Solar radiation and wet leaf surface favor oxidation of GEM to Hg\textsuperscript{2+} on leaf cuticle and then retention of Hg\textsuperscript{2+} in leaves. Foliar Hg assimilation rate reaches a peak at the mid-growing
season and subsequently decreases due to the decrease in photosynthetic activity (or stomatal activity) of leaves at the end of the growing season (Laacouri et al., 2013; Lee et al., 2000). The outer tree leaves can receive more solar radiation and have a lower specific leaf area, a measure of leaf area per unit mass (cm² g⁻¹), than the interior leaves, indicating that they have more biomass per unit leaf area, which may store more Hg (Laacouri et al., 2013). Moreover, foliar Hg accumulation is different among plant species (Bushey et al., 2008; Siwik et al., 2009). For instance, conifers accumulate more atmospheric Hg per leaf unit area than deciduous species likely because conifers have more leaf hairs and cuticle materials (e.g., more lipids) that provide more favorable surface area and chemistry for atmospheric Hg accumulation (Obrist et al., 2012).

Mercury in leaves is predominantly bound in the leaf tissues (epidermal, stomatal cell walls, mesophyll, and vascular tissues) (Beauford et al., 1977; Laacouri et al., 2013; Stamenkovic and Gustin, 2009), however, the mechanism is unknown. A small amount of Hg (PBM and RGM) is adsorbed to leaf surfaces (Laacouri et al., 2013). Foliar Hg in leaf tissues is immobilized (Laacouri et al., 2013; Lodenius et al., 2003; Stamenkovic and Gustin, 2009), but Hg on leaf surfaces can be removed by precipitation (Rea et al., 2001; Rea et al., 2000). Sunlight and/or atmospheric oxidants can also transform surface Hg²⁺ to Hg⁰, which is then emitted back to the atmosphere (Millhollen et al., 2006b; Zhang and Lindberg, 1999).

1.2.3 Importance of plant litterfall in catchment mercury mass balance

Litterfall is a critical Hg input to terrestrial ecosystems (Risch et al., 2012; Wang et al., 2016). Wang et al. (2016) reported that ~75% of Hg dry deposition can be attributed to litterfall. Risch et al. (2012) and St. Louis et al. (2001) also pointed out that more than 50% of annual Hg deposition in forests was from Hg dry deposition via litterfall.

Mercury in litterfall is transported to soils via wash-off of Hg in aerosols and particles on litterfall surfaces and decomposition of litterfall that incorporates foliar Hg into soil organic
matter (SOM). Litterfall decomposition is the dominant process in delivering litterfall Hg to soils, given that the majority of foliar Hg is bound in leaf tissues (Laacouri et al., 2013). Litterfall decomposition includes two simultaneous processes: (1) leaching of soluble compounds; and (2) the concomitant mineralization and humification of lignin, cellulose, and other compounds by a series of microorganisms, such as bacteria and fungi (Couteaux et al., 1995). Leaching is the rapid loss of soluble leaf compounds (sugars, phenolics, hydrocarbons, and glycerides) shortly after immersion of leaves, leading to up to 30% of mass loss within 24 h (Gessner et al., 1999). Humification is the formation of amorphous substances (humus) from decomposing organic matter. Mineralization is the degradation of humus to inorganic nutrients (e.g., CO$_2$ and inorganic nitrogen and phosphorus).

Litterfall decomposition is controlled by environmental conditions (temperature, soil moisture, precipitation), litterfall quality, and soil organisms (macrofauna, fungi, and bacteria) (Couteaux et al., 1995; Krishna and Mohan, 2017). Microbial metabolism normally increases with ambient temperature but rapidly decreases when the temperature is above an optimum value (Kirschbaum, 1995). Low soil moisture limits microbial metabolism (De Santo et al., 1993), as soil moisture content increases, microbial metabolism rises until reaching an optimum peak. Higher soil moisture contents do not limit microbial metabolism until anaerobic conditions suppress the oxygen consumption of microbial metabolism (Couteaux et al., 1995). Drier soils damage the litterfall structure, resulting in the rapid leaching of soluble compounds during precipitation events (Gessner et al., 1999).

Decomposition rates of litterfall are higher in species with higher nitrogen (N) contents and lower carbon to nitrogen (C:N) ratios (Aerts, 1997; Manzoni et al., 2010; Singh and Gupta, 1977) because N is often considered as a limiting nutrient for decomposers (Aerts, 1997). Taken together, factors that affect litterfall decomposition can also influence Hg input from litterfall to soils.
1.3 Mercury methylation in catchments

1.3.1 Mercury methylation and controlling factors

Methylmercury (MeHg), a potent neurotoxin, is the product of Hg methylation (Gilmour et al., 1992). Hg methylation is facilitated by anaerobic microorganisms, such as sulfate-reducing bacteria (SRB) (Gilmour et al., 1992), iron-reducing bacteria (FeRB) (Fleming et al., 2006; Kerin et al., 2006), and methanogenic archaea (Hamelin et al., 2011) with the SRB as the main methylators (Compeau and Bartha, 1985; Gilmour et al., 1992) in anaerobic freshwater environments (e.g., lake sediments and wetlands). Recent studies have identified gene clusters (HgcA and HgcB) in these microorganisms that are reliable predictors of the capability of microbes to methylate Hg (Gilmour et al., 2013; Parks et al., 2013). The gene HgcA encodes a corrinoid protein that acts as a methyl carrier, and HgcB encodes a ferredoxin that acts as an electron donor required for bacterial Hg methylation (Parks et al., 2013). By screening for the specific gene clusters (HgcAB), Gilmour et al. (2013) found more methylators in environments other than SRB and FeRB, including methanogens, syntrophic, acetogenic, and fermentative Firmicutes.

Mercury methylation by bacteria is an intracellular reaction (Gilmour et al., 2011; Hsu-Kim et al., 2013; Parks et al., 2013; Schaefer et al., 2011). Inorganic Hg in bacteria’s extracellular surroundings passes the outer and inner cell membranes and reaches into the cell cytosol (Gilmour et al., 2011; Hsu-Kim et al., 2013). Hg methylation is carried out by enzymes, such as methyltransferase and acetyl-coenzyme A (CoA), in the cell cytosol (Siciliano and Lean, 2002). The methyl groups are transferred from methyl-tetrahydrofolate (methyl-THF) to Hg with enzymes acting as methyl groups carriers (Siciliano and Lean, 2002). MeHg has little affinity for the cell surface possibly because of the selectivity of cell surface receptors and transporters for Hg-ligand complexes rather than MeHg-ligand complexes (Graham et al.,
MeHg is finally exported from the cell to the ambient to avoid the buildup of Hg inside the cells by diffusion and to reduce MeHg toxicity to bacteria (Schaefer et al., 2011).

Pathways for Hg uptake by bacteria generally include passive diffusion of small neutrally-charged Hg complexes, such as HgCl$_2$ and HgS$^0$ complexes (Barkay et al., 1997; Benoit et al., 1999), and active uptake of specific Hg-complexes with low-molecular-weight dissolved organic matter (DOM) such as Hg-cysteine complexes via an energy-dependent transmembrane protein pump (Golding et al., 2002; Schaefer and Morel, 2009; Schaefer et al., 2011). Here, DOM is the organic matter dissolved in soil solution that can pass a 0.45 µm filter, and low-molecular-weight DOM is that their molecular weight (or size) is lower than 1000 daltons (or < 1 nm) (Benner et al., 1992) such as organic acids, sugars, amino acids (Kalbitz et al., 2000). Previous studies also showed that nanoparticulate metacinnabar (β-HgS(s), a black mineral-HgS) instead of HgS$^0$ is the primary small neutrally-charged Hg species utilized by Hg methylators (Gerbig et al., 2011; Graham et al., 2012; Zhang et al., 2012).

Mercury methylation is affected by a wide variety of factors that regulate microbial activity and the bioavailability of inorganic Hg (Paranjape and Hall, 2017; Ullrich et al., 2001).

1.3.1.1 Microbial activity

Sulfate-reducing bacteria is a group of anaerobes that utilizes sulfate (SO$_4^{2-}$) as their terminal electron acceptor for energy generation (Muyzer and Stams, 2008), and is often considered as the dominant Hg methylator in both freshwater and estuarine sediments (Compeau and Bartha, 1985; Gilmour et al., 1992). Evidence supporting this is that the addition of SO$_4^{2-}$ to sulfur-limited catchments significantly increased MeHg production (Branfireun et al., 2001; Branfireun et al., 1999; Gilmour et al., 1992; Mitchell et al., 2008a), and the addition of molybdate, a metabolic inhibitor of SRB, significantly inhibited Hg methylation (Compeau and Bartha, 1985; Gilmour et al., 1992; King et al., 1999). Additionally, previous studies
showed that Hg methylation is correlated with sulfate-reduction rates (Gilmour et al., 1992; King et al., 2000; King et al., 1999). However, not all SRB can methylate Hg (Gilmour and Henry, 1991; King et al., 2000). SRB species, such as Desulfovibrio gigas and Desulfovibrio desulfuricans aestuarii can not methylate Hg but Desulfovibrio desulfuricans ND132 can in the natural environment (Gilmour and Henry, 1991). Macalady et al. (2000) found that Desulfobacter groups are more abundant than Desulfovibrio groups and are important methylators in lake sediments. King et al. (2000) also noted that the Desulfobacteriaceae group is more effective in Hg methylation than the Desulfovibrio group.

Activities of SRB are affected by concentrations of SO$_4^{2-}$ and bioaccessible organic matter (OM) that are important nutrients for SRB. Gilmour and Henry (1991) proposed that the optimal SO$_4^{2-}$ concentration for Hg methylation by SRB ranges from 19 to 48 mg L$^{-1}$; below that range, methylation and the sulfate-reduction process is limited; whereas, above that range, methylation is inhibited due to the increase of sulfide (one byproduct generated from the sulfate-reduction process) that can decrease the bioavailability of Hg by forming large HgS complexes. Orem et al. (2011) found that the MeHg production was inhibited in sulfate-enriched areas (> 20 mg L$^{-1}$) due to the buildup of sulfide, and the Hg methylation was limited in sulfate-deficient areas (< 1 mg L$^{-1}$). Jeremiason et al. (2006) and Corrales et al. (2011) also demonstrated that SO$_4^{2-}$ concentrations below 1 mg L$^{-1}$ did not favor MeHg production.

Bioaccessible organic matter as an energy source is required for all bacterial metabolism, including that of the SRB. The addition of bioaccessible OM to bioaccessible OM-limited sediments can stimulate Hg methylation (Ullrich et al., 2001). A previous study has shown that the combined addition of bioaccessible OM and SO$_4^{2-}$ to peatlands significantly stimulated the net Hg methylation (Mitchell et al., 2008a). Windham-Myers et al. (2009) also observed that the removal of vegetation in a wetland decreased net Hg methylation due to the reduction in the supply of bioaccessible OM from root exudates to SRB.
All microbial growth and metabolism is also controlled by temperature. It has been observed that Hg methylation activity peaks in summer because temperature increase directly elevated overall microbial growth and metabolism (Canario et al., 2007; Hintelmann and Wilken, 1995). However, Sagemann et al. (1998) and Tsukamoto et al. (2004) found that temperature decrease from room temperature to 6 °C did not cause a decline in SRB activity, and pointed out that SRB growth and metabolism were more likely controlled by nutrients than by temperature.

1.3.1.2 Bioavailability of Hg

Bioavailable Hg includes neutral and dissolved Hg complexes, such as HgCl$_2$, HgS$^0$, and β-HgS(s) that can passively pass through cell membranes and are subsequently methylated by Hg methylators (Barkay et al., 1997; Benoit et al., 1999; Graham et al., 2012). Complexes of Hg and low-molecular-weight DOM (e.g., Hg-cysteine) are also bioaccessible, which can be actively taken up by bacteria (Schaefer and Morel, 2009; Schaefer et al., 2011). Hg bioavailability is affected by chloride and sulfide concentrations, DOM quantity and characteristics, and pH (Ullrich et al., 2001). Chloride can combine with Hg forming chloride-Hg complexes, such as HgCl$_2$, HgCl$_3$$^-$, and HgCl$_4$$^{2-}$ and, among them, the neutral form HgCl$_2$ is bioaccessible for Hg methylators (Barkay et al., 1997; Ullrich et al., 2001). In sulfidic sediments, sulfide out-competes other ligands for combining with Hg due to the high formation constants of Hg-S complexes, which leads to HgS$^0$ as the dominant neutral dissolved Hg complex (Benoit et al., 1999). However, sulfide accumulation in aquatic ecosystems limits MeHg production by enhancing the precipitation of HgS(s) (Benoit et al., 1999; Ullrich et al., 2001; Winfrey and Rudd, 1990). Previous studies suggested that Hg methylation was positively related to HgS$^0$ concentrations only at low sulfide concentrations ($<10^{-6}$ mg L$^{-1}$); as sulfide concentration increase, MeHg production decreased because of the formation of solid species of HgS(s) (Benoit et al., 2001; Benoit et al., 1999).
Mercury bioavailability is also affected by DOM. Many pieces of evidence suggested that DOM enhances the photolytic reduction of Hg by donating electrons to Hg$^{2+}$ (Alberts et al., 1974; Gu et al., 2011; Jiang et al., 2015; Jiskra et al., 2015; Zheng and Hintelmann, 2010), which will reduce Hg bioavailability due to the evasion of gaseous Hg$^0$. The bioavailability of Hg is affected by forming complexes with DOM, given the strong affinity between Hg and sulfur ligands in DOM (Xia et al., 1999; Xia et al., 1998). Hg can be actively taken up by Hg methylators by forming Hg-ligand complexes, such as Hg-cysteine (Schaefer and Morel, 2009; Schaefer et al., 2011). Leclerc et al. (2015) confirmed that Hg complexation with low-molecular-weight DOM (e.g., cysteine) promoted the bioavailability of Hg. However, the high-molecular-weight DOM (molecular weight $\geq$ 1000 daltons, or size $\geq$ 1 nm; i.e., humic substances) reduces Hg bioavailability in the absence of sulfide because the Hg-DOM complexes are too large to pass through bacteria cell membranes (Barkay et al., 1997; Gilmour et al., 2011; Ravichandran, 2004). In the presence of sulfide, the high-molecular-weight DOM increases Hg bioavailability by coating the β-HgS(s) and then increasing electrostatic repulsion and preventing aggregation and precipitation of β-HgS(s) (Deonarine and Hsu-Kim, 2009; Gerbig et al., 2011; Miller et al., 2007; Ravichandran et al., 1999; Slowey, 2010). The formation of DOM-Hg complexes is affected by pH (Barkay et al., 1997; Ravichandran, 2004). At low pH, DOM is less negatively charged, which decreases its potential to combine with Hg and consequently makes Hg more available to Hg methylators (Barkay et al., 1997).

1.3.2 Mercury sequestration and methylation in northern peatlands

Peatlands are one type of wetland that stores abundant partially decomposed organic matter due to their low temperatures, waterlogged and acidic conditions that limit decomposition rates (Rydin and Jeglum, 2013). Peatlands are found in the tropics, temperate regions, and the boreal (Figure 1.2). Peatlands that locate at the boreal are named northern peatlands or boreal peatlands. The largest peatlands are in Canada and are particularly located at northern
latitudes in Québec and Ontario. Northern peatlands cover approximately 3% of the global land area but store about 30% of the global terrestrial carbon (Gorham, 1991). Northern peatlands are broadly placed in two classifications: bogs and fens (Rydin and Jeglum, 2013). True ombrotrophic (nutrient-poor) bogs are isolated from catchment runoff with precipitation as their only primary source of nutrients and water. Fens are hydrologically connected to surface water and/or groundwater runoff and as such have higher pH and dissolved mineral and nutrient concentrations. Fens are further classified along a nutrient gradient from nutrient-poor to rich, although in all cases they are more productive than true bogs. True bogs are acidic with approximate pH ranges from 3.5 to 4.2; *Sphagnum* mosses are their dominant vegetation. As for fens, pH ranges from 4 to 8 along the nutrient gradient with nutrient-poor fens having the lowest pH (4–5.5) and nutrient-rich fens having the highest pH (6.8–8); nutrient-poor fens are dominated by *Sphagnum* mosses, vascular plants (ericaceous shrubs), and trees; nutrient-intermediate fens are dominated by vascular plants (graminoid plants and deciduous shrubs); nutrient-rich fens are dominated by vascular plants (graminoid plants).

Figure 1.2 The distribution of peatlands. (Downloaded and modified from the website of wcscanada.org)
Peatlands are important sinks of atmospherically-deposited Hg (Liu et al., 2018; Perez-Rodriguez et al., 2018; St. Louis et al., 1994; Wallschläger et al., 2000). Hg sequestration and methylation in peat is different between sites and within the same sites, which is related to vegetation type, plant species composition, and peat decomposition (Rydberg et al., 2010a). For instance, Hg concentrations are significantly higher in Sphagnum mosses than in vascular plants (Moore et al., 1995; Rydberg et al., 2010a). However, vascular plant-dominated fens have higher peat Hg concentrations than the moss-dominated fens (Rydberg et al., 2010a), likely because those peat soils in moss-dominated peatlands have a lower shading compared to peat soils in vascular plant-dominated fens, which favors the photo-reduction of Hg$^{2+}$ to Hg$^0$ in moss-dominated peatlands (Gustin et al., 2006b). Peat Hg concentrations are highest between 25 and 50 cm peat depth followed by a decline towards the peat depth and surface (Rydberg et al., 2010a), because there is a higher degree of peat decomposition closer to the peat surface at the above groundwater discharge sites (Franzen et al., 2004) where are aerobic zones in the peat profile and can receive nutrients from the groundwater ecosystems. The highest pore water MeHg concentrations have been found in the near-surface peat at the groundwater discharge sites where is the transition between the anaerobic and aerobic zones in peat soils (Branfireun et al., 1996). The plausible explanation is that this transition can provide both anaerobic conditions and more SO$_4^{2-}$ that from groundwater ecosystem and oxidation release from peat soils to SRB. Therefore, any changes in vegetation type, species composition, and decomposition rates can affect Hg cycling in peat soils.

Northern peatlands are often considered as sources of MeHg to downstream ecosystems, given their anaerobic conditions (Branfireun et al., 1999; Mitchell et al., 2008b; St. Louis et al., 1994). St. Louis et al. (1994) found that catchments with some peatlands had 4–15 times greater MeHg yields than those with no peatlands. Branfireun et al. (1996; 1998) also found that peatlands are a large MeHg source. Moreover, the addition of SO$_4^{2-}$ to peatlands
significantly increased MeHg concentrations in pore waters (Branfireun et al., 1999; Mitchell et al., 2008a; Coleman-Wasik et al., 2015), which suggests that sulfate-reduction is the dominant mechanism of MeHg production in peatlands.

1.4 Global warming implications for mercury cycling

The Earth’s climate is changing due to the increase in atmospheric greenhouse gasses (e.g., CO₂ and methane) driven mainly by anthropogenic fossil fuel combustion (IPCC, 2018). The mean global temperature is expected to be 1.5 °C above pre-industrial levels between 2030 and 2052 with more extreme temperature variations at higher latitudes (IPCC, 2018). Climate change (especially global warming) can affect the global cycle of Hg (Krabbenhoft and Sunderland, 2013; Obrist et al., 2018). An increase in precipitation intensity and extreme storms under global warming may increase the wet deposition of atmospheric Hg (Krabbenhoft and Sunderland, 2013). As temperatures rise, less gaseous and aerosol halogens (e.g., Br and Cl) are released to the atmosphere from the ocean with uncertain mechanisms (Stern et al., 2012), which will result in the decrease of the oxidation of GEM to RGM and then Hg wet deposition (Holmes et al., 2010). More gaseous Hg species in oceans will be released back to the atmosphere under higher temperatures (Krabbenhoft and Sunderland, 2013; Macdonald et al., 2005). Moreover, forest wildfire activity will increase under enhanced air temperature (Westerling et al., 2006), leading to a re-emission of volatilized Hg to the atmosphere (Kumar et al., 2018; Turetsky et al., 2006). Also, warming will increase permafrost melting that will further release more Hg from organic-rich soils (Rydberg et al., 2010b).

1.4.1 Global warming impacts on mercury mobility in northern peatlands

It is anticipated that temperatures at higher latitudes will reach up to 6 °C above pre-industrial levels between 2030 and 2052 (IPCC, 2018). Global warming may affect Hg
cycling in northern peatlands by decreasing water tables and increasing soil temperature, vegetation biomass and community composition shifts, and microbial metabolism (Figure 1.3). Global warming is generally accompanied by decreases in water table levels and corresponding surface soil moisture (Tarnocai, 2009). Warmer and drier conditions in northern peatlands are expected to impact Hg mobility (Carpi and Lindberg, 1998; Ericksen et al., 2006; Gustin and Stamenkovic, 2005; Haynes et al., 2017; Coleman-Wasik et al., 2015), microbial community activity and structure (Nunes et al., 2015; Peltoniemi et al., 2015), decomposition rates of litter (Couteaux et al., 1995; Krishna and Mohan, 2017) and SOM (Davidson and Janssens, 2006), DOM concentrations in pore waters (Kalbitz et al., 2000), and plant abundance and community composition (Dieleman et al., 2015; Haynes et al., 2017; Mäkiranta et al., 2018). The PEATcosm mesocosm experiment has observed that treatment mesocosms with a lower water table and more vegetation have a stronger Hg depositional trend than treatment mesocosms with a higher water table and more vegetation; shrubs have greater sorption of Hg onto foliar surfaces and stomatal uptake of atmospheric Hg into leaf tissue compared to sedges; soil warming increase Hg emission fluxes (Haynes et al., 2017). However, leaves in the PEATcosm mesocosm experiment were collected in July, Hg concentrations in these leaves cannot reflect the actual input of litter Hg to soils, given that foliar Hg concentrations are positively related to time (Laacouri et al., 2013). In addition, effects of decreasing water table and increasing soil temperatures on concentrations of dissolved IHg and MeHg in pore water are unclear.
Figure 1.3 Environmental controls on Hg cycling in northern peatlands.

Global warming is decreasing water table levels and soil moisture content in northern peatlands due to the less frequent precipitation events and high evapotranspiration (Tarnocai, 2009; Winter, 2000). Changes in water table levels can increase the oxidative release of Hg from peat soils to pore waters due to the exposure of previously saturated soils to air (Gustin et al., 2006a; Coleman-Wasik et al., 2015). Soil moisture shows a positive correlation with soil Hg re-emission when soil moisture is below saturation (Carpi and Lindberg, 1998; Ericksen et al., 2006; Gillis and Miller, 2000). The mechanisms for this are that gaseous Hg\(^0\) in the soil pore spaces is displaced by the infiltrated water, causing an increase in the Hg\(^0\) emission; and thicker water film around the soil particles provides a medium for Hg transformation to Hg\(^0\) and thus increasing the Hg emission rate (Gillis and Miller, 2000). Changes in biochemical and physical soil characteristics under drier conditions (Kaiser et al., 2015) can also increase the leaching of Hg from peat soils (Lodenius et al., 1987).

Microbial community activity and structure can also be significantly influenced by changes in temperature and hydrological conditions (Nunes et al., 2015; Peltoniemi et al., 2015), which may affect decomposition rates of litterfall and soils and subsequently Hg mobility in northern peatlands. However, some researchers pointed out that microbial populations
(bacterial, fungal, and archaeal) may adapt to changes in hydrological conditions in the long-term (Peltoniemi et al., 2009; Strickland and Rousk, 2010). Haynes et al. (2015) reported that microbial communities can rapidly adapt to the changes in plant community composition and subsequent changes in bioaccessible DOM substrate under higher global warming. As a result, global warming increases microbial metabolism and decomposition rates (Davidson and Janssens, 2006; Krishna and Mohan, 2017) in the short-term, leading to increases in releases of DOM and Hg from peat soils. After microbial community activity and structure stabilize in the long-term, the effects of global warming on Hg mobility may lessen.

Plant biomass and community composition are predicted to change with global warming (Dieleman et al., 2015; Mäkiranta et al., 2018). A field experiment showed that warming and drought can reduce plant aboveground biomass in northern peatlands (Mäkiranta et al., 2018). Plant community components are changing from Sphagnum mosses to vascular plants in many peatland types under global warming (Dieleman et al., 2015; Fenner et al., 2007). Changes in plant biomass and community composition can further affect Hg input from litterfall to peat soils, given that atmospheric Hg accumulation by leaves is different among plant species (Moore et al., 1995; Siwik et al., 2009).

The influences of changes in soil temperature and moisture under global warming on Hg release from peat soils to pore waters are unclear. Although Moore et al. (1995) reported that Hg concentrations in wetland plants follow the sequence: grassland herbs < trees and shrubs < aquatic macrophytes < Sphagnum spp. mosses < lichens < fungi, little information is available about atmospheric Hg accumulation by peatland plants. Litterfall Hg input to peat soils through leaching is also unknown.

1.4.2 Global warming impacts on mercury methylation in northern peatlands

Global warming is expected to impact the process of MeHg production in peatlands by affecting microbial metabolism as well as concentrations of bioaccessible inorganic Hg and
OM and $\text{SO}_4^{2-}$ in pore waters. Metabolism of all microbes, including Hg methylators, normally increases with temperature up to the optimum temperature (Ullrich et al., 2001). Low moisture limits microbial metabolism (De Santo et al., 1993); as soil moisture increases, microbial metabolism increases up to an optimum plateau (Couteaux et al., 1995); when anaerobic conditions arise, the aerobes’ metabolism is suppressed but anaerobes’ metabolism is enhanced. Various studies have shown that decomposition rates in wetlands are increased under more aerobic conditions (Clark et al., 2009; Dieleman et al., 2016; Worrall et al., 2006), leading to the increase of DOM (Dieleman et al., 2016) that will further affect Hg bioavailability and then net MeHg production.

Changes in vegetation community composition with decreases in moss and increases in vascular plant abundance under global warming (Dieleman et al., 2015) can affect DOM quantity and characteristics because vascular plant litter contains high concentrations of limiting nutrients (e.g., nitrogen and phosphorous) and is more bioaccessible compared to Sphagnum moss litter (Mastný et al., 2018). Additionally, previous studies have reported that elevated temperature increased aboveground productivity and belowground production of vascular plants (Breeuwer et al., 2010; Kane et al., 2014; Mäkiranta et al., 2018), which will subsequently increase DOM concentrations in pore waters.

Water table fluctuation can increase the oxidation of reduced sulfur and release of $\text{SO}_4^{2-}$, thus increasing net MeHg production (Feng et al., 2014; Coleman-Wasik et al., 2015). Elevated temperatures, however, can increase the gaseous evasion of S compounds (e.g., hydrogen sulfide and carbonyl sulfides) from northern peatlands and then decrease the net MeHg production (Åkerblom et al., 2013). So far, it is unclear that the direct effects of varying soil moisture contents and rising soil temperature on net MeHg production in northern peatlands, given uncertainties in changes, such as DOM quantity and characteristics and $\text{SO}_4^{2-}$ concentrations in pore waters under different soil moisture contents and increased soil temperature.
1.5 Objectives of my doctoral research

Because both Hg cycling and global warming are linked to vegetation biomass and community, hydrology, microbial activity, DOM quantity and characteristics, SO$_4^{2-}$ concentrations, and pH in northern peatlands, it is important to understand how global warming will impact Hg cycling in northern peatlands, given northern peatlands as “hotspots” of MeHg production. I anticipated that global warming will increase Hg input and net MeHg production likely by increasing vascular plant biomass, the oxidative release of Hg, MeHg, DOM, and SO$_4^{2-}$ due to the decreasing soil moisture content, and microbial metabolism under warmer conditions in northern peatlands. So far, the accumulation of Hg by leaves as well as the patterns of Hg leaching from litterfall into peatland soils remains largely unknown. It is also unclear that how different soil moisture contents and soil warming will affect Hg mobility and MeHg production in northern peatlands, given uncertainties in changes, such as DOM quantity and characteristics and SO$_4^{2-}$ concentrations in pore waters under different soil moisture contents and increased soil temperature. In this thesis (Figure 1.4), my overall goal is to better understand how global warming will affect Hg cycling in two types of northern peatlands (i.e., moss-dominated fen and sedge-dominated fen). The specific goals are to:

1) Assess Hg accumulation by dominant plants in sedge-dominated fen and Hg leaching from litterfall to peatland soils;

2) Determine the influence of different soil moisture contents on net MeHg production and inorganic Hg concentrations in pore waters;

3) Quantify the effects of increased soil temperature on Hg cycling in northern peatlands.
Figure 1.4 The location of research sites and pictures of research peatland types. Research sites are located at A) 817 ha sub-watershed of the Lake Superior basin near White River Ontario, Canada (48°21’ N, 85°21’ W; modified from Henry and Smith, (2001), in B) a moss-dominated fen and a sedge-dominated fen (modified from Mack, (2017). Pictures of C) moss-dominated fen and D) sedge-dominated fen.

In my second chapter, I conducted a field-based investigation to examine Hg accumulation in dominant plant species, few-seeded sedge [Carex oligosperma Michx.], wire sedge [Carex lasiocarpa Ehrh], tussock sedge [Carex stricta Lamb.], and sweet gale [Myrica gale L.] over one growing season. I also conducted a laboratory-based incubation experiment to examine Hg leaching from these plant species litters to peat soils. In my third chapter, I conducted a laboratory-based incubation experiment to examine the effects of different soil moisture contents on net MeHg production and inorganic Hg concentrations in pore waters. In my fourth chapter, I analyzed the influences of soil warming on total Hg and MeHg
concentrations in the moss-dominated fen and the sedge-dominated fen on a large-scale field warming experiment over three years.
1.6 References


Millhollen, A. G., Gustin, M. S., Obrist, D. (2006b). Foliar mercury accumulation and
exchange for three tree species. Environmental Science and Technology 40, 6001-6006.


Journal 15, 85-100.
Resources Journal 34, 453-466.
Society of America Journal 62, 1240-1246.
Chapter 2

2 Foliar mercury accumulation and mercury leaching from litter in a northern sedge peatland

2.1 Introduction

Mercury (Hg), especially methylmercury (MeHg), is a global concern due to its potential toxicity and ubiquitous presence in the environment (Morel et al., 1998). Hg is emitted to the atmosphere from both natural (e.g., volcanoes, wildfires, and geothermal activity) and anthropogenic activities (e.g., coal combustion, artisanal gold mining, and incineration of medical waste) (Schroeder and Munthe, 1998; Streets et al., 2011). Atmospheric Hg exists as gaseous elemental mercury (GEM, Hg\(^0\)), reactive gaseous mercury (RGM, Hg\(^{2+}\)), and particulate-bound mercury (PBM, Hg\(_p\)) with GEM as the dominant species (> 95%) (Schroeder and Munthe, 1998). RGM and PBM have a short atmospheric residence time ranging from hours to days, whereas GEM has a longer atmospheric residence time of several months to a year and thus is transported globally (Schroeder and Munthe, 1998). These atmospheric Hg species are eventually deposited into aquatic and terrestrial ecosystems via wet deposition (precipitation, such as rain, snow, and fog) and dry deposition (particle settling or direct partitioning to vegetation, water, and soil surface, or direct absorption by vegetation foliage) (Lindberg et al., 2007). Mounting evidence suggests that Hg dry deposition is more important than wet deposition, contributing 70%~85% of total Hg deposition (dry and wet deposition) in terrestrial ecosystems (Graydon et al., 2008; Risch et al., 2017; Risch et al., 2012; St. Louis et al., 2001; Wang et al., 2016; Zhang et al., 2016), and more than 70% of Hg dry deposition is by vegetation litterfall/incorporation into soil organic matter (SOM) (Obrist et al., 2017; Wang et al., 2016).
Vegetation leaves accumulate Hg from the atmosphere through stomatal uptake (Lindberg et al., 1992) and a non-stomatal pathway of Hg deposition to the leaf cuticle and possibly subsequent retention and incorporation into leaf tissue (Stamenkovic and Gustin, 2009). Vegetation-atmosphere Hg exchange is a bidirectional process (Ericksen and Gustin, 2004; Zhu et al., 2016), depending on several factors such as the ambient air Hg concentration (Ericksen et al., 2003; Ericksen and Gustin, 2004; Fay and Gustin, 2007), the Hg compensation point (or compensation concentration) (Hanson et al., 1995), environmental conditions (e.g., temperature, solar radiation, drought stress, and CO₂ concentrations) (Blackwell and Driscoll, 2015; Ericksen and Gustin, 2004; Zhu et al., 2016), and biological factors (e.g., leaf age, leaf placement, and plant species) (Blackwell and Driscoll, 2015; Laacouri et al., 2013). Hg concentrations in leaves are generally positively correlated with the levels of ambient air GEM (Ericksen et al., 2003; Ericksen and Gustin, 2004; Fay and Gustin, 2007). The Hg compensation concentration is the concentration of atmospheric Hg at which the Hg flux between leaves and the atmosphere was zero. Vegetation assimilates Hg from the atmosphere when ambient air Hg concentration is above the Hg compensation point. Foliar Hg will be released to the atmosphere when ambient air Hg concentration is below the Hg compensation point. However, the hypothesis of compensation point cannot explain the accumulation of Hg by vegetation. Many studies found that vegetation is a net sink of atmospheric Hg (Ericksen et al., 2003; Fleck et al., 1999; Frescholtz et al., 2003; Frescholtz and Gustin 2004; Jiskra et al., 2018; Laacouri et al., 2013; Millhollen et al., 2007a; Obrist et al., 2017; Rea et al., 2002; Rutter et al., 2001a), despite the fact that the leaf compensation point concentrations (0.5–33 ng m⁻³; Wright and Zhang, 2015) are generally above the background Hg concentrations in air (1-2 ng m⁻³) (Lindberg et al., 2007).

Environmental conditions regulate plant stomatal activity and then the stomatal exchange of atmospheric Hg (Lindberg et al., 1998; Millhollen et al., 2006a). For instance, extreme temperature and drought reduced stomatal activity leading to a decrease in Hg assimilation.
by leaves (Lindberg et al., 1998). Biological conditions are related to leaf roughness, leaf area, stomatal numbers and activity, and cuticular thickness, and thus control leaves accumulating Hg from the atmosphere (Obrist et al., 2012; Zhang et al., 2009).

The majority of Hg in leaves is bound in leaf tissues (i.e., epidermis, stomatal cell walls, mesophyll, and vascular tissues) (Laacouri et al., 2013). The mechanism is unknown, but Hg$^0$ in leaves must be oxidized to Hg$^{2+}$ and incorporated into leaf tissues. Less than 10% of total foliar Hg (i.e., RGM and PBM) is adsorbed to the surface of leaves (Amado Filho et al., 2002; Laacouri et al., 2013; Stamenkovic and Gustin, 2009). Hg in leaf tissues is immobilized (Laacouri et al., 2013; Lodenius et al., 2003; Stamenkovic and Gustin, 2009), whereas surface Hg can be reduced to Hg$^0$ by oxidants and then re-emitted to the atmosphere (Zhang and Lindberg, 1999) and can also be removed by precipitation (Rea et al., 2001; Rea et al., 2000). Retained Hg in leaves eventually enters soils with litterfall.

Field investigations (Risch et al., 2012; St. Louis et al., 2001), laboratory incubation (Ericksen et al., 2003), and stable Hg isotope studies (Hintelmann et al., 2002; Obrist et al., 2017) showed that Hg deposition via litterfall is a vital input of Hg to soils and contributes 2–5 times greater atmospheric Hg to soils relative to non-vegetated landscapes. A statistical model (Monte Carlo simulation) estimated that the input of global Hg deposition via litterfall is 2–6 times higher than Hg emission from the forest floor, making global forest ecosystems an atmospheric Hg sink (Wang et al., 2016). Vegetation thus plays an essential role in the cycling of Hg, given that ~80% of terrestrial surfaces globally are vegetated (Rea et al., 2002; St. Louis et al., 2001).

Forest ecosystems are important sinks of atmospheric Hg and have received widespread attention of researchers (Risch et al., 2012; St. Louis et al., 2001; Wang et al., 2016; Zhang et al., 2009); however, studies about foliar Hg accumulation in other plant types or other ecosystems such as northern peatlands are few (Moore et al., 1995) despite their critical role
in the carbon cycle (Gorham, 1991) and Hg cycles (St. Louis et al., 1994). Northern peatlands store 500 ± 100 Gt of carbon and accumulate > 40 cm depth of peat (partially decomposed vegetation matter) due to slow decomposition rates in their anaerobic and acidic conditions and low temperatures (Rydin and Jeglum, 2013). Northern peatlands are generally classified as either bog or fen ecosystems according to their moisture-aeration, pH-base richness, dominated vegetation, and hydrological conditions (Rydin and Jeglum, 2013). Specifically, bogs are acidic (pH < 4.5) nutrient-poor peatlands, which are hydrologically isolated from groundwater systems and dominated by Sphagnum spp. mosses. Unlike bogs, fens are along a gradient of nutrient status from nutrient-poor to nutrient-rich, have a range of pH values from 4.5 to greater than 6.5, and receive nutrients and water from precipitation, groundwater and/or surface runoff. Nutrient-rich fens are dominated by vascular plants-graminoid plants, nutrient-intermediate fens are dominated by vascular plants-graminoid plants and deciduous shrubs, and nutrient-poor fens are dominated by Sphagnum spp. mosses, vascular plants-shrubs, and trees (Rydin and Jeglum, 2013; Webster and McLaughlin, 2010).

Most studies on Hg pollution in peatlands have focused on MeHg because peatlands are often considered as significant MeHg sources to downstream ecosystems (Branfireun et al., 1996; Mitchell et al., 2008; St. Louis et al., 1994). Peatlands have anaerobic conditions, non-limiting amounts of inorganic Hg (St. Louis et al., 1994) and carbon (Gorham, 1991), and available but limiting sulfate (Blodau et al., 2007; Schmalenberger et al., 2007) that support the methylation of Hg by sulfate-reducing bacteria (SRB), the dominant methylating microbes in these systems. Little information is available about the inputs of atmospheric Hg by leaves in peatlands. Moore et al. (1995) reported that Hg levels in nonvascular plants (fungi, lichens, and mosses) are almost an order of magnitude higher than those in vascular plants in wetlands, and the Hg concentrations follow the sequence: grassland herbs < trees and shrubs < aquatic macrophytes < Sphagnum spp. mosses < lichens < fungi. More studies
are needed to fill in the knowledge gap of atmospheric Hg accumulation by vegetation leaves in northern peatlands.

Vascular plant-dominated fens are an important type of peatland in the northern hemisphere (Rydin and Jeglum, 2013). In these fen systems, vascular plants, not bryophytes, are the dominant peat-forming plants (Frolking et al., 2001; Thormann and Bayley, 1997). Vascular plants play an important role in the Hg cycle in northern peatlands. Previous studies showed that the annual input of aboveground vegetation biomass to peatland soils is higher for vascular plants than bryophytes (i.e., *Sphagnum* spp. mosses) (Frolking et al., 2001; Thormann and Bayley, 1997). Moreover, compared to *Sphagnum* spp. mosses, vascular plants contain a lower ratio of carbon (C): nitrogen (N) and are more bioaccessible (Hobbie, 1996; Lyons and Lindo, 2019), given that N is a limiting nutrient to decomposers in peatland ecosystems (Manzoni et al., 2010; Moore et al., 2006; Parton et al., 2007). Thus, Hg assimilation by vascular plants and subsequent deposition with litterfall and incorporation into SOM after decomposition plays an important role in the peatland Hg mass balance.

Northern peatlands are experiencing rising temperatures (IPCC, 2018), which are expected to change plant abundance and community composition and then Hg input through litterfall. Weltzin et al. (2000) found that the productivity of vascular plants in fens increased under higher temperatures. Increased vegetation biomass is expected to increase Hg$^0$ deposition flux by up to 20% in north mid-latitudes by 2050 (Zhang et al., 2016). However, Mäkiranta et al. (2018) observed that the temperature increase had no significant effect on aboveground plant biomass, and global warming-induced drought reduced plant aboveground biomass in two sedge-dominant fens. Several studies have shown that warming in northern peatlands will alter plant community composition with a decrease in *Sphagnum* spp. mosses abundance alongside an increase in vascular plants (Buttler et al., 2015; Dileman et al., 2015; Weltzin et al., 2000). Moore et al. (1995) found that *Sphagnum* spp. mosses accumulated higher
levels of Hg from the atmosphere than vascular plants, but Hg input via the litterfall of *Sphagnum* spp. mosses to peat soils is less than that via vascular plant litterfall, given the antimicrobial properties of *Sphagnum* spp. mosses that inhibit decomposition. So far, little information is available for Hg accumulation by vascular plants in northern peatlands. More relevant data are needed to better understand the role of vascular plants in the peatland Hg mass balance, especially under the conditions of global warming.

The input of Hg in vascular plant litterfall to peatland soils follows the sequence: (1) wash-off of aerosols, particles, and gases from leaf surfaces, (2) leaching of water-soluble components, and (3) incorporation into SOM after the microbial decomposition of litterfall. Leaching is the initial phase of litterfall breakdown in aquatic environments and leads to up to 30% of mass loss within 24 h after immersion of litterfall (Gessner *et al.*, 1999). The matter rapidly leached from litterfall is generally soluble organic matter (OM), such as sugars, phenolics, hydrocarbons, and glycerides (Gessner *et al.*, 1999), that provides energy for microbial metabolism, including Hg methylators. The importance of leaching of soluble Hg from litterfall remains unknown, but it is important to quantify the amount of soluble Hg leached from litterfall, given that rapid released Hg is readily methylated by bacteria compared to “old” Hg in peat soils (Branfireun *et al.*, 2005; Feng *et al.*, 2014; Hintelmann *et al.*, 2002).

Previous studies showed that Hg mass in live leaf leachate is insignificant compared to that on leaf surfaces and that in SOM (Rea *et al.*, 2001; Rea *et al.*, 2000). Compared to live leaves, litterfall generally lacks structural integrity and likely leaches more Hg. Moreover, it has been established that the quantity and characteristics of dissolved organic matter (DOM) are closely related to Hg mobility in terrestrial and aquatic ecosystems, given the strong affinity between Hg and thiol ligands in DOM (Haitzer *et al.*, 2002; Ravichandran, 2004).
The rapid and abundant leaching of DOM from litterfall may also lead to large amounts of Hg leaching.

The quantity and characteristics of DOM affect Hg methylation. The bioaccessible DOM can provide energy for SRB and then influence Hg methylation. Complexes of Hg with low-molecular-weight DOM (molecular weight < 1000 daltons, or size < 1 nm), such as cysteine, can be actively taken up by Hg methylators (Schaefer and Morel, 2009; Schaefer et al., 2011). High-molecular-weight DOM (molecular weight ≥ 1000 daltons, or size ≥ 1 nm; namely, humic substance) reduce Hg bioavailability in the absence of sulfide because these Hg-DOM complexes are too large to pass SRB cell membranes (Ravichandran, 2004). In sulfidic conditions, high-molecular-weight DOM prevents the growth of HgS(s) into large clusters leading to a higher Hg bioavailability (Gerbig et al., 2011; Miller et al., 2007; Ravichandran et al., 1999). Several studies also showed that DOM that with high-molecular-weight and high aromaticity (aromatic molecule content) hindered HgS(s) growth and then increased net MeHg production (Graham et al., 2013; Hall et al., 2008; Mitchell and Gilmour, 2008). Therefore, it is necessary to quantify the leaching of Hg and DOM from vascular plant litterfall to better understand the peatland Hg mass balance and Hg methylation in northern peatlands.

The overall objective of this study is to more mechanistically link the vascular plant community (i.e., sedges and shrubs) to the peatland Hg cycle in a vascular plant-dominated fen. We use “sedge-dominated fen” instead of “vascular plant-dominated fen” hereafter, given that sedges are the primarily dominant plants in this study site (Webster and McLaughlin, 2010). The specific objectives of this study are to:

(1) quantify the accumulation of Hg in leaves of dominant sedges and shrub species in a sedge-dominated fen over a growing season;
(2) experimentally estimate the Hg input to surficial peat pore waters from litterfall through leaching at the end of the growing season;

(3) quantify and characterized dissolved organic matter associated with the leaching process.

2.2 Materials and methods

2.2.1 Study site

Samples were collected from a sedge-dominated fen (10.2 ha) located in an 817 ha sub-watershed of the Lake Superior basin near White River Ontario, Canada (48°21’ N, 85°21’ W). The weather data (air temperature and precipitation) was provided by the Ontario Ministry of Natural Resources and Forestry, who installed a weather station and monitored the environmental conditions in this study site. The mean annual air temperature and precipitation from 2012 to 2018 were 1.7 °C and 721 mm, respectively. For the sample collecting year (2018), the mean annual air temperature and precipitation were 1.2 °C and 730 mm, respectively. The sedge-dominated fen is surrounded by a mixed-wood deciduous and coniferous forest with two small streams running along the northern and southwestern edges. The growing season is from May 1st to August 31st. The sedge-dominated fen is mostly open and dominated by three sedge species: few-seeded sedge [*Carex oligosperma* Michx.], wire sedge [*Carex lasiocarpa* Ehrh], tussock sedge [*Carex stricta* Lamb.] (Lyons and Lindo, 2019). Sweet gale [*Myrica gale* L.] is the only primary shrub (Lyons and Lindo, 2019; Palozzi and Lindo, 2017). The characteristics of these plants are described in *Wetland Plants of Ontario* by Newmaster, Newmaster *et al.* (1997). In brief, the few-seeded sedge is 40–100 cm tall; their leaves are 1–3 mm wide, stiff, smooth, edges rolled in toward midrib and rounded in cross-section, and red-tinged at the base. The wire sedge is 30–100 cm tall; leaves are arching, narrow, 1–2 mm wide, wire-like, folded along the midrib, and angular. Tussock sedge is 40–140 cm tall; leaves are 3–6 mm wide, lowest leaves reduced to
bladeless sheaths. Sweet gale is a deciduous shrub, up to 1.5 m tall; leaves are alternate, 3–6 cm long, toothed at tip; sweet gale is a nitrogen-fixer and its root nodules contain nitrogen-fixing bacteria that are symbiotic. In this study, few-seeded sedges and wire sedges were mixed during plant sample collection as they are indistinguishable in size and form from one another when not in flower/seed.

2.2.2 Sample collection and analysis

Five locations several hundred meters apart were selected in the sedge-dominated fen to serve as within-site replicates to account for potential local-scale variability. Leaves of few-seeded sedge/wire sedge, tussock sedge, and sweet gale were collected using a clean blade in June, July, August, and during senescence in October 2018 in each plot, totaling 60 samples. During the October sampling event, part of the sedge leaves was still green and still standing, and although senesced, shrub leaves were sampled from the branch to ensure that there was no mixing with previous years' fallen leaves. Disposable nitrile gloves were worn during the sample collection. All samples were double bagged with two polyethylene bags and transported to the lab using a clean cooler. Leaves of each species that were collected from each plot in October 2018 were divided for foliar total Hg (THg) analyses and a foliar Hg leaching experiment. Leaves were stored frozen until they were returned to the university laboratory.

Foliar total mercury. At the Western university, leaves were rinsed three times with deionized water (18.2 MΩ cm) and then freeze-dried for 48 h. Freeze-dried leaf samples were subsequently ground and homogenized with a stainless-steel blade grinder. All powdered samples were stored in polyethylene bags for further chemical analysis. Precautions were performed to avoid any cross-contamination during the process. Disposable nitrile gloves were worn all the time. The electric grinder was thoroughly cleaned with deionized water (18.2 MΩ cm) after each sample grinding and completely dried with Kimwipes® (Kimtech
Science™). THg in leaves was analyzed by thermal decomposition, amalgamation, and atomic absorption spectrometry using a Milestone™ DMA-80 (EPA method 7473) with the National Research Council Canada, DORM-4 (fish, lobster, squid) as the Certified Reference Material (CRM). Each analytical run for THg included 10% method blanks, 10% duplicates, and 20% matrix spikes. The detection limit for THg was 0.05 ng g\(^{-1}\). All method blanks were below the detection limits. The relative standard deviation (RSD) was < 10% for duplicate samples. Recoveries of THg for matrix spikes and CRM (DORM-4) was 101.08 ± 3.08%. All recoveries of CRM were comparable well with the certified values: 0.41 ± 0.04 mg kg\(^{-1}\).

Leaf C content (%C; w/w), N content (%N; w/w), and the ratio of leaf C content and N content (C:N) before and after the foliar Hg leaching experiment was analyzed using a CNS analyzer (Vario Isotope Cube; Elementar). Birch leaf Organic Analytical Standard (Betula papyrifera Marsh.) was the CRM. Each analytical run for C and N included 10% method blanks and 10% duplicates (no matrix spikes for C and N). The detection limits for C and N were 0.26 mg g\(^{-1}\) and 0.02 mg g\(^{-1}\), respectively. All method blanks were below the detection limits. The relative standard deviation (RSD) was < 10% for duplicate samples. Recoveries of CRM for C and N were 99.16 ± 0.30%, and 101.62 ± 0.88% of the certified values, respectively.

**Foliar mercury leaching experiment.** For the Hg leaching experiment, leaves of sedges and sweet gale that were collected in October 2018 were rinsed twice with 100 mL of deionized water (18.2 MΩ cm) to quantify particulate or loosely-bound Hg and DOM that can be easily removed/leached from the leaf surface. This water was reserved for subsequent analysis. After rinsing, the leaves were gently oven-dried at 40 °C for 48 h, and then leaves of each species from each plot were relatively evenly separated into three groups and weighted, totaling 45 groups. The foliar leaching experimental procedure followed the design of Rea *et al.* (2000) and Del Giudice and Lindo (2017). These oven-dried litter samples were leached
in 150 mL of deionized water in clean 250 mL PETG bottles. All PETG bottles were capped, double bagged, and incubated in the dark at room temperature (~21 °C) for 48 h. Litter was gently swirled at the beginning and end of the leaching experiment to ensure complete wetting. Following the leaching, the leachate was vacuum filtered through a 0.45 μm glass fiber filter into clean 250 mL PETG bottles. Leachate from each sample was split into two aliquots. One was preserved by acidifying to 0.5% (vol/vol) with high-purity HCl for dissolved total Hg (THg\textsubscript{aq}) analysis and stored in 250 mL PETG bottles; the other was stored in the clean 60 mL Amber glass bottles and analyzed within 2 d for the quantity and characteristics of DOM. All samples were stored in the dark at 4 °C for further analysis. Method blanks of the leaching experiment were performed at the same time following the same procedure.

Previous studies showed that mass loss was related to plant type and was primarily due to losses of soluble and bioaccessible DOM (Del Giudice and Lindo, 2017; Gessner \textit{et al.}, 1999). Thus, litter was taken out of each PETG bottle, oven-dried at 40 °C for 48 h, and re-weighed after leaching. The dry leaf weight before and after the leaching process was used to calculate the mass loss. These re-dried litter samples after leaching were ground and homogenized prior to the measurement for %C, %N, and C:N as described above.

The dissolved total Hg (THg\textsubscript{aq}) concentrations in the rinse water and leachate were analyzed using Environmental Protection Agency (EPA) method 1631. Samples were oxidized for 12 h with BrCl oxidation, neutralized using hydroxylamine, reduced to Hg\textsuperscript{0} by SnCl\textsubscript{2} reduction, purged onto gold traps, thermally desorbed in argon, and finally analyzed by cold vapor atomic fluorescence spectroscopy (CVAFS, Tekran 2600, Tekran Inc., Canada) (Bloom and Fitzgerald, 1988). The detection limit for THg\textsubscript{aq} was 0.072 ng L\textsuperscript{-1}. The instrument, Tekran 2600, was calibrated daily. Each analytical run included 10% method blanks (deionized water), 10% sample duplicates, and 20% matrix spikes. Check standards (made from 1000
ppm stock standard) were included in every ten samples. All method blanks were below the detection limit. The RSD was < 10% for all duplicate samples. Recoveries of matrix spikes and check standards were 104.52 ± 8.23%.

Dissolved organic matter is quantified as dissolved organic carbon (DOC), given that DOC comprises the vast majority of DOM (Leenheer and Croue, 2003). DOC concentrations in rinse water and leachate were measured using an iTOC Aurora 1030 (OI Analytical, College Station, TX, USA) using the persulfate wet oxidation method. Dissolved organic carbon in the liquid was oxidized to CO₂ gas by the persulfate wet oxidation and the amount of CO₂ was subsequently determined by measuring the infrared absorbance of CO₂ gas.

Concentrations of THgₐq and DOM in both rinse waters and leachate are presented as the mass of solute per mass of dry material. DOC concentrations in blanks were less than 1 mg L⁻¹. Each run included 10% deionized water blanks, 10% sample duplicates, 10% matrix spikes, and check standards. Deionized water blanks were generally less than 1 mg L⁻¹. The RSD was < 10% for sample duplicates. Recoveries of matrix spikes and check standards were about 104.60 ± 5.36%.

DOM in leachate was characterized as specific ultraviolet absorbance at a wavelength of 254 nm (SUVA₂₅₄), an indicator of the molecular weight (or size) and aromaticity (the content of aromatic molecules) of DOM (Weishaar et al., 2003). Higher SUVA₂₅₄ values suggest that DOM contains more high-molecular-weight and aromatic DOM contents (Weishaar et al., 2003). Sample absorbance was measured at λ = 254 nm using a Horiba Aqualog® fluorescence spectrofluorometer with a xenon lamp. SUVA₂₅₄ values were determined by dividing the absorbance at 254 nm by the DOM concentration of the same sample and multiplied by 100 and are reported in the unit of L mg C⁻¹ m⁻¹ (Weishaar et al., 2003).

Fluorescence excitation-emission matrices (EEMs) were also collected for calculating informative optical indices that reflect differences in DOM characteristics in leachate using a
Horiba Aqualog® fluorescence spectrofluorometer with a xenon lamp. The ultrapure closed water blank was used to correct the inner-effects of Horiba Aqualog® fluorescence spectrofluorometer on EEMs. Aqualog® directly reported the fluorescence intensity as arbitrary units (A.U.). The reported EEMs were then converted to optical indices using R Software (R Core Team 2012). Three common indices were chosen in this study: the fluorescence index (FI), the humification index (HIXEM), and the biological index or ‘freshness’ index (BIX). FI reflects DOM sources and characteristics with lower FI values (< 1.2) indicating that DOM is terrestrially derived (resulting from decomposition and leaching of plant and soil organic matter) and has higher aromaticity, while higher FI values (> 1.8) indicting that DOM is microbially derived (originating from processes as extracellular release and leachate of algae and bacteria) and has lower aromaticity (Fellman et al., 2010; McKnight et al., 2001). HIXEM is an indicator of humic substance content or the extent of humification that converts lower-molecular weight organic matter derived from animal and plant products to more condensed and higher-molecular-weight organic matters by microbes. High HIXEM (> 1.0) values reflect the high humification of DOM and DOM is composed of more highly condensed and higher molecular weight molecules (Fellman et al., 2010; Hansen et al., 2016; Huguet et al., 2009; Ohno, 2002). BIX reflects the contribution of autochthonous (or microbially derived) DOM with higher BIX values (> 1.0) reflecting that more low-molecular-weight DOM was recently produced by microbes (Fellman et al., 2010; Huguet et al., 2009). For DOM quality (i.e., SUVA254, FI, HIXEM, and BIX), there were no reference materials to assess method performance, but 10% of samples were run in duplicates with their RSDs < 10%.

2.3 Statistical analysis

Results were analyzed using IBM SPSS statistics software (IBM SPSS Inc. 24.0). The repeated-measures ANOVA was performed to compare the difference in foliar THg
concentrations among different plant species over the growing season and to analyze the effect of leaf age on foliar Hg concentrations. Linear regressions were analyzed to examine the relationship between foliar THg accumulation and leaf age. Differences in the foliage quality (%C, %N, and C:N) were analyzed using a multivariate ANOVA. One-way ANOVA was used to determine the effects of plant species on concentrations of THg_{aq} and DOM quantity and characteristics in leachate. The repeated-measures ANOVA, multivariate ANOVA and one-way ANOVA were followed by a post hoc test (Bonferroni’s significant difference; honestly significant difference at the 95% confidence interval). Linear regressions were used to examine the nature of the relationship between THg_{aq} concentrations and SUVA_{254} in leachate. Data are presented as the mean ± standard deviation (SD). Coefficient of determination (R²) and significance p-values (p) are presented for linear regression fits, and p < 0.05 was considered significant.

2.4 Results and discussion

2.4.1 Foliar mercury accumulation in peatland plants

Foliar THg concentrations were related to time/leaf age (F_{(1,73,24,26)} = 42.75, p < 0.001) and plant species (F_{(1,23,23,38)} = 29.38, p < 0.001) (Figure 2.1). Based on post hoc tests, foliar THg concentrations were significantly different between plant species and between the sampling months, except that there was no significant difference in foliar THg concentrations between June and August. The mean foliar THg concentrations (n = 5) in June follow the sequence: few-seeded sedge/wire sedge < tussock sedge < sweet gale. In July these means approximately decreased by 30% (few-seeded sedge/wire sedge), 40% (tussock sedge), and 47% (sweet gale), respectively. The decrease of THg concentrations is likely because of the dilution of leaf growth, although specific changes in leaf biomass were not quantified as part of this study. Foliar THg concentrations were positively related to time after July (few-seeded sedge/wire sedge: F_{(1,13)} = 185.79, p < 0.001, R² = 0.93; tussock sedge: F_{(1,13)} =
200.87, $p < 0.001$, $R^2=0.94$; sweet gale: $F_{(1,13)} = 70.72$, $p < 0.001$, $R^2=0.84$). The mean foliar THg concentrations in October few-seeded sedge/wire sedge, tussock sedge, and sweet gale were 1.7, 1.3, and 2.0 times higher than the initial concentrations in June, showing a clear pattern of continuous THg accumulation from the atmosphere over time as has been shown for forests (Laacouri et al., 2013; Millhollen et al., 2006b; Rea et al., 2002), given that plant roots act as a barrier of Hg transport from soils to shoots (Wang et al., 2015).

Mercury accumulation in leaves is affected by many factors, such as atmospheric Hg concentration, environmental conditions (e.g., solar radiation and temperature), and biological factors (e.g., leaf age, plant species, leaf area, and leaf placement) (Blackwell and Driscoll, 2015; Ericksen et al., 2003; Ericksen and Gustin, 2004; Laacouri et al., 2013; Millhollen et al., 2006a). Since all samples were collected in the same location, factors such as atmospheric Hg concentration and environmental conditions were deemed the same, leaving only biological factors as an explanation for differences.
Figure 2.1 The intraseasonal trend in foliar total mercury (THg) concentrations (ng g\(^{-1}\)) of few-seeded sedge/wire sedge, tussock sedge, and sweet gale (ng g\(^{-1}\)). Vertical bars are mean ± SD (n = 5).

**Leaf age.** Leaf age is an important biological factor in controlling foliar concentrations (Ericksen *et al.*, 2003; Laacouri *et al.*, 2013). The positive relationship between foliar THg concentrations and time after July suggests that leaves of all species here continued to assimilate atmospheric Hg over the growing season right up to senescence. Some studies have found that the rate of foliar Hg uptake decreased toward the end of the growing season (Ericksen *et al.*, 2003; Laacouri *et al.*, 2013; Poissant *et al.*, 2008), which appears to be because of the decrease of photosynthetic activity at the end of the growing season (Koike *et al.*, 2004). Despite the decline of foliar Hg uptake at the late growing season, foliar Hg concentrations continue to increase right up to senescence because of the immobilization of the majority of foliar Hg (Laacouri *et al.*, 2013; Lodenius *et al.*, 2003; Stamenkovic and Gustin, 2009).
The foliar Hg concentrations for species in this study increased between 1.3 and 2.0 times over the growing season, which was smaller than that for trees (Laacouri et al., 2013; Poissant et al., 2008; Rea et al., 2002). Poissant et al. (2008) observed that Hg concentrations in maple foliage increased by three times during the growing season. Rea et al. (2002) found that foliar Hg concentrations increased ten-fold from spring bud break to autumn litterfall in northern mixed-hardwood forests. Laacouri et al. (2013) also reported that foliar Hg concentrations in trees (i.e., ginkgo, horse chestnut, red oak, sugar maple, American elm, and tamarack) increased by more than eleven-fold over the growing season.

The difference in foliar Hg concentrations between trees and sweet gale and sedges in this study (few-seeded sedge/wire sedge, tussock sedge, sweet gale) is not surprising given that foliar Hg concentrations differ significantly among vegetation types (Demers et al., 2007; Moore et al., 1995; Obrist et al., 2012; Richardson and Friedland, 2015). A likely reason is there are different leaf surface areas, leaf surface: weight ratios, life span (Juillerat et al., 2012), leaf morphology (Obrist et al., 2012), and canopy structure among vegetation types (Blackwell and Driscoll, 2015; Demers et al., 2007; Obrist et al., 2012), although all of these measures were not made for this study.

**Plant species.** Plant photosynthesis, transpiration, growth rates, and leaf area are typically different among plant species (Antúnez et al., 2001; Kloeppel et al., 2000; Laacouri et al., 2013; Millhollen et al., 2006b; Walters and Reich, 1999). The mean foliar THg concentrations in tussock sedge were 1.2 times higher than that in few-seeded sedge/wire sedge. A plausible explanation is that tussock sedge has a larger leaf area than few-seeded sedge/wire sedge (Newmaster et al., 1997). A larger leaf area reflects more stomates and thus more leaf accumulation of atmospheric Hg (Laacouri et al., 2013), given that stomates play an important role in accumulate Hg\(^0\) from the atmosphere (Millhollen et al., 2006; Stamenkovic and Gustin, 2009). Although sweet gale has a larger leaf area than few-seeded
sedge/wire sedge and tussock sedge (Newmaster et al., 1997), the mean foliar THg concentrations in sweet gale were 1.7 and 1.4 times higher than that in few-seeded sedge/wire sedge and tussock sedge over time, respectively. A likely reason is that leaf morphology is very different between sweet gale and sedges. Few-seeded sedge/wire sedge is 30–100 cm tall and 1–3 mm wide; tussock sedge is 40–140 cm tall and 3–6 mm wide; sedge leaves are vertical and generally not facing the atmosphere; sweet gale is up to 1.5 m tall and their leaves are 3–6 cm long, 2 cm wide, and adequately spread out in the air (Newmaster et al., 1997). Kozlowski and Pallardy (1997) reported that leaves near the top of the canopy are generally saturated at higher light intensities and have higher rates of photosynthesis and stomatal conductance than those near the bottom of the canopy. Sweet gale, in this study, are taller than sedges, and their leaves are adequately spread out in the air, suggesting that their leaves are generally saturated at higher light intensities and have higher stomatal conductance compared to sedge leaves, which may consequently increase the stomatal uptake of Hg from the atmosphere.

**Leaf quality.** Measures of leaf quality (%C, %N, and C:N) were significantly different among plant species ($F_{(6,104)} = 59.64, p < 0.001$) over the growing season ($F_{(9,124)} = 45.42, p < 0.001$) (Figure 2.2). Based on post hoc tests, foliar %C, %N, and C:N had significant differences between sweet gale and sedges (few-seeded sedge/wire sedge and tussock sedge) but not between few-seeded sedge/wire sedge and tussock sedge; there was no significant difference in foliar %C between sampling months; foliar %N and C:N showed a significant difference between sampling months, except between July and August. Foliar %C, %N, and C:N of few-seeded sedge/wire sedge and tussock sedge were similar to each other. Foliar %C and %N were much lower in these sedges than sweet gale, and it was the opposite for foliar C:N. Leaf N is an element of proteins of photosynthetic machinery that is responsible for photosynthetic capacity (Evans, 1989; Wright et al., 2004), thus, a higher foliar %N is usually together with a higher %C given higher net photosynthesis. Deciduous shrubs (i.e.,
sweet gale) generally have a higher foliar %C and %N than grasses (Wright et al., 2004), because sweet gale root nodules contain symbiotic nitrogen-fixing bacteria that increase the fixation of nitrogen in sweet gale (Newmaster et al., 1997; Vitousek et al., 2002).
Figure 2.2 Peatland plants traits: (A) carbon content (%C), (B) nitrogen content (%N), and (C) the ratio of carbon content to nitrogen content (C:N) over 2018 growing season. Vertical bars are mean ± SD (n = 5).
There were no significant increases in foliar %C ($F_{(1,55)} = 7.92, p > 0.05$) but strong decreases of foliar %N ($F_{(1,55)} = 58.32, p < 0.001$) over the growing season (Figures 2.2A and 2.2B). The strong decreases in foliar %N with leaf life-span can be attributed to the re-translocation of N to new leaves (Reich et al., 1992). The values of foliar C:N were increasing with time ($F_{(1,55)} = 128.71, p < 0.001$) (Figure 2.2C), which is a function of the decreases of foliar %N rather than the changes in %C, given the strong decrease of foliar %N and the slight increase of foliar %C.

**Relationships between litterfall quality and THg concentrations.** Litterfall (i.e., leaves collected from October) with higher foliar %C and %N had higher foliar THg concentrations based on the positive relationships between foliar %C and THg concentrations ($F_{(1,13)} = 191.09, p < 0.05, y = 0.78x – 28.20, R^2 = 0.94$) and between foliar %N and THg concentrations ($F_{(1,13)} = 82.38, p < 0.05, y = 7.16x – 1.96, R^2 = 0.93$) (Figures 2.3 A and 2.3 B), suggesting that litterfall with higher %C and %N have a higher input of atmospheric Hg to soils. It is not surprising that THg concentrations were negatively related to foliar C:N during senescence ($F_{(1,13)} = 175.10, p < 0.05, y = 0.18x – 18.33, R^2 = 0.86$; Figure 2.3 C), given that foliar C:N was negatively related to foliar %C and %N. It is established that Hg is positively related to organic matter content (Ravichandran, 2004; Yin et al., 1996), given the affinity of Hg to reduced sulfur groups in organic matter (Obrist et al., 2009; Ravichandran, 2004; Skyllberg et al., 2000; Xia et al., 1999). Higher foliar C and N content reflects higher plant productivity and more organic matter (Wright et al., 2004). Thus, higher C and N content in litterfall indirectly indicates a higher input of Hg via litterfall to soils.
Figure 2.3 Correlations between (A) THg concentrations and C contents, (B) THg concentrations and N contents, and (C) THg concentrations and ratios of C content and N content (C:N) in litter. The circle represents few-seeded sedge/wire sedge, the square represents tussock sedge, and the triangle presents sweet gale. All linear correlations are statistically significant (p < 0.05).
2.4.2 Mercury leaching from litterfall

**Surficial mercury.** The mean concentration of Hg from the twice surface rinse of litter (expressed per gram of dry litter) was 0.02 ± 0.01 ng g⁻¹ and 0.01± 0.00 ng g⁻¹, respectively, indicating that mass of Hg that was loosely bound on the leaf surface was small relative to the total litter Hg concentration (8.83 ± 2.38 ng g⁻¹), with only 0.4% Hg (tussock sedge: 0.6%; few-seeded sedge/wire sedge: 0.3%; sweet gale: 0.3%) contribution to the total THg mass in litters.

**Mercury leached from litter.** The mean concentration of THg_{aq} from 48 hrs leaching of litter (expressed per gram of dry litter) is shown in Figure 2.4. The mean concentrations of THg_{aq} had significant differences between plant species ($F_{(2,41)} = 11.55, p < 0.001$). Based on post hoc tests, there were significant differences in THg_{aq} concentrations between sweet gale and sedges (few-seeded sedge/wire sedge and tussock sedge) but not between few-seeded sedge/wire sedge and tussock sedge. Sweet gale litter leached the least Hg among these plant species, which is likely due to their hydrophobic waxy cuticle limiting the leaching of Hg from litters. In addition, Obrist *et al.* (2009) observed a positive relationship between Hg and N in both soils and litter and speculated that N groups in OM play an important role in the retention of Hg in terrestrial ecosystems. In this study, sweet gale leaves have a higher %N than tussock sedge and few-seeded sedge/wire sedge, which can also partially explain why less Hg was leached from sweet gale litter than sedges.
Figure 2.4 Concentrations of soluble total mercury (THg\textsubscript{aq}) from leaching of litter. Boxplot displays median (50\textsuperscript{th} percentile; the inside line of the box), first quartile (25\textsuperscript{th} percentile; lower bound of the box), third quartile (75\textsuperscript{th} percentile; upper bound of the box), whiskers (all measures between 5\textsuperscript{th} percentile and 25\textsuperscript{th} percentile and between 75\textsuperscript{th} percentile and 95\textsuperscript{th} percentile; the straight line below and above the box), and outliers (individual points outside of the percentile of 5\textsuperscript{th} and 95\textsuperscript{th}). n = 15.

Only 3.0\%, 2.9\%, and 0.3\% of the THg present in tussock sedge, few-seeded sedge/wire sedge, and sweet gale litters were leached from leaves, respectively. The percentages of Hg that leached from tussock sedge, few-seeded sedge/wire sedge leaves were 5.5 and 10.6 times higher than that from rinses, while the percentage of Hg that leached from sweet gale was similar to that from rinse water (0.3\%). However, Rea \textit{et al.} (2000) showed that surface washoff of loosely bound and particulate Hg was a rapid and larger source of Hg in forest throughfall compared to continuously foliar Hg leaching from live leaves. It is likely because these target plants in Rea \textit{et al.}'s (2000) study were trees that have a larger leaf surface than sedges and shrubs, and larger leaf surfaces provide more efficient collectors for atmospheric reactive gaseous Hg (Zhang \textit{et al.}, 2009). Additionally, dry leaves (litter) lack structural
integrity compared to the live leaves, leading to rapid leaching of soluble constituents (Gessner et al., 1999), including Hg.

2.4.3 Quantity and characteristics of leachate dissolved organic matter

The quantity and characteristics of DOM in leachate. The mean concentrations of DOM from 48 hrs leaching of litter (expressed per gram of dry litter and represented by DOC concentrations), which has been shown in Figure 2.5A. The mean DOM concentrations in leachate were significantly different between plant species ($F_{(2,42)} = 34.95$, $p < 0.001$). Del Giudice and Lindo (2017) reported that the loss of soluble DOM accounted for the majority of the mass loss during litter leaching. In this study, there was also a significant difference in plant species for mass loss during litter leaching ($F_{(2,42)} = 11.62$, $p < 0.05$; Figure 2.5B).

Based on post hoc tests, mass loss was significantly different between sweet gale and sedges (few-seeded sedge/wire sedge and tussock sedge) but not between few-seeded sedge/wire sedge and tussock sedge. The mass loss of different plant species during litter leaching followed the sequence: few-seeded sedge/wire sedge (8.1%) < tussock sedge (11.5%) < sweet gale (17.7%).

Percentage changes in foliar %C, %N, and C:N between before and after leaching showed that N was more easily released from sedges than C while it was the opposite for sweet gale (Table 2.1). Given the majority of C content (44.9–52.1%) and the small amount of N (0.6–1.5%) in all leaves, soluble DOM in leachate is more available as soluble C, although sometimes N was more easily leached from litters than C. Leachings of soluble C can provide energy to microbes (Del Giudice and Lindo, 2017; Jung et al., 2014; Uselman et al., 2012), and will further increase microbial decomposition.
Figure 2.5 Concentrations of dissolved organic carbon (DOC) from leaching of litter. Boxplot displays median (50th percentile; the inside line of the box), first quartile (25th percentile; lower bound of the box), third quartile (75th percentile; upper bound of the box), whiskers (all measures between 5th percentile and 25th percentile and between 75th percentile and 95th percentile; the straight line below and above the box), and outliers (individual points outside of the percentile of 5th and 95th). n = 15.

Table 2.1 Loss of foliar carbon (C) and nitrogen (N) content and change in the ratio of carbon content to nitrogen content (C:N) during leaching of litterfall. n = 15

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Loss of foliar C during leaching (%)</th>
<th>Loss of foliar N during leaching (%)</th>
<th>Change in C:N during leaching</th>
</tr>
</thead>
<tbody>
<tr>
<td>sweet gale</td>
<td>−1.0</td>
<td>+8.1</td>
<td>−8.7</td>
</tr>
<tr>
<td>tussock sedge</td>
<td>−2.0</td>
<td>−10.3</td>
<td>+10.7</td>
</tr>
<tr>
<td>few-seeded sedge/wire sedge</td>
<td>−3.1</td>
<td>−12.3</td>
<td>+10.7</td>
</tr>
</tbody>
</table>
Characteristics of DOM also varied among plant species (Figure 2.6 and Table 2.2). Significant differences were found in SUVA\textsubscript{254} (F\((2,42) = 24.02, p < 0.001\)), HIX\textsubscript{EM} (F\((2,42) = 3.82, p < 0.05\)), FI (F\((2,42) = 11.24, p < 0.001\)), and BIX (F\((2,42) = 125.48, p < 0.001\)) among plant species. Based on post hoc tests, there were significant differences in SUVA\textsubscript{254} between sweet gale and sedges (few-seeded sedge/wire sedge) only and BIX between all plant species; there were no significant differences in HIX\textsubscript{EM} between plant species. The mean value of SUVA\textsubscript{254} in leachate followed the sequence: tussock sedge > few-seeded sedge/wire sedge > sweet gale leaves, respectively (Figure 2.6), indicating that DOM that leached from tussock sedge and few-seeded sedge/wire sedge leaves had higher aromaticity and less bioaccessibility than that from the sweet gale leaves. These results are supported by indexes of FI and HIX\textsubscript{EM} (Table 2.2). DOM in the leachate of tussock sedge and few-seeded sedge/wire sedge litter had lower values of FI and HIX\textsubscript{EM} than that of sweet gale leaves, indicative of the presence of less bioaccessible and more aromatic DOM contents in sedges than in sweet gale. All BIX values (0.26–0.73) measured in this study were lower than 1.0, reflecting that DOM is mainly terrestrially derived (leaching from litterfall) in this study. Although DOM leached from different litters has different characteristics, leaching of litters is a substantial source of DOM to surrounding ecosystems (Davis III et al., 2003; Davis et al., 2006; Del Giudice and Lindo, 2017). Importantly, the leaching of soluble DOM (e.g., organic acids, sugars; amino acids) can provide energy and nutrients for microbes (Davis III et al., 2003), which will subsequently stimulate biological degradation and Hg methylation.
Figure 2.6 Dissolved organic matter characteristics as measured by specific ultraviolet absorbance at the wavelength 254 nm (SUVA<sub>254</sub>), n = 15. Boxplot displays median (50<sup>th</sup> percentile; the inside line of the box), first quartile (25<sup>th</sup> percentile; lower bound of the box), third quartile (75<sup>th</sup> percentile; upper bound of the box), whiskers (all measures between 5<sup>th</sup> percentile and 25<sup>th</sup> percentile and between 75<sup>th</sup> percentile and 95<sup>th</sup> percentile; the straight line below and above the box), and outliers (individual points outside of the percentile of 5<sup>th</sup> and 95<sup>th</sup>).

Table 2.2 The mean fluorescence indices of dissolved organic matter characteristics<sup>a</sup>

<table>
<thead>
<tr>
<th>Index</th>
<th>Tussock sedge</th>
<th>Few-seeded sedge/wire sedge</th>
<th>Sweet gale</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI</td>
<td>1.19 ± 0.10</td>
<td>1.31 ± 0.09</td>
<td>1.49 ± 0.27</td>
</tr>
<tr>
<td>HIX&lt;sub&gt;EM&lt;/sub&gt;</td>
<td>0.16 ± 0.03</td>
<td>0.16 ± 0.02</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>BIX</td>
<td>0.53 ± 0.05</td>
<td>0.63 ± 0.06</td>
<td>0.35 ± 0.04</td>
</tr>
</tbody>
</table>

<sup>a</sup>Lower values of the FI (< 1.2) suggest dissolved organic matter (DOM) has higher aromaticity and is primarily composed of high-molecular-weight DOM, while high FI values (> 1.8) indicate that DOM has lower aromaticity and is mainly composed of low-molecular-weight DOM. DOM with high HIX<sub>EM</sub> (> 1) values is composed of more highly condensed and higher molecular weight molecules. In contrast, higher BIX (> 1.0) values reflect that more low-molecular-weight DOM is recently produced, generally, by microbes. All indices are unitless, n = 15.
Correlation between THg_{aq} concentrations and SUVA_{254} values in leachate. Correlations between the concentrations of soluble THg_{aq} and SUVA_{254} values in leachate are shown in Figure 2.7. The concentrations of soluble THg_{aq} were not significantly related to SUVA_{254} values (F(1,41) = 39.51, p < 0.001, y = 0.14x – 0.18, R^2 = 0.49) based on statistic analysis. Hg is tightly and readily bound to reduced sulfur groups (i.e., thiols) in DOM (Ravichandran, 2004; Xia et al., 1999), especially those with higher aromaticity that have more reduced sulfur groups (Dittman et al., 2009). Mercury weakly binds to carboxyl and phenol functional groups in DOM after all thiol groups are occupied at relatively high Hg concentrations (Drexel et al., 2002; Graham et al., 2012), which is atypical in most natural environments in which Hg concentrations are relatively low. Although Hg was not significantly related to SUVA_{254}, an indicator of the aromaticity of DOM (Weishaar et al., 2003), DOM with higher aromaticity still plays an important role in controlling Hg mobility, given that the amount of reduced sulfur groups far exceed the amount of Hg in natural environments (Ravichandran, 2004).

![Graph](image)

Figure 2.7 Correlations between the concentrations of total mercury (THg_{aq}) and the specific ultraviolet absorbance at the wavelength 254 nm (SUVA_{254}) in leachate. The circle represents few-seeded sedge/wire sedge, the square represents tussock sedge, and the triangle presents sweet gale.
2.5 Conclusions

This study shows that the widely-observed pattern of foliage accumulation of Hg from the atmosphere and changes in foliar Hg concentrations over time are the same in peatland vascular plants as they are for forest trees and that the patterns are related to time/leaf age and plant species. Although foliar THg concentrations in litterfall in my study (6–12 ng g⁻¹) are relatively lower than that in the forest litterfall (17–238 ng g⁻¹) (Wang et al., 2016), Hg input through litterfall to peatland soils cannot be neglected, given that peatlands are “hotspots” of MeHg production (St. Louis et al., 1994). Foliar leaching of lower molecular weight DOM from peatland shrubs such as sweet gale provides substrate for bacteria and can contribute to enhanced microbial metabolism, including sulfate reduction and Hg methylation. The dissolved Hg that is associated with higher molecular weight DOM leaching from ubiquitous sedge litter may deliver atmospherically-derived Hg in a bioaccessible form relatively more quickly than the much slower release of tissue-associated Hg through the decomposition of plant tissues and incorporation into SOM. Thus, there are both fast and slow pathways for the supply of inorganic Hg to sites of methylation in peatlands that have the potential to shift under climate change, given that peatland plant species composition and biomass will certainly change under climate change (Dieleman et al., 2015). This study is one of the few to quantify Hg accumulation by the predominant plant species in northern peatlands, demonstrating that sweet gale has higher foliar Hg levels than sedges over one growing season. This study is also one of the first to demonstrate that Hg leached from litterfall is less than 5% of foliar Hg. However, future studies of the annual amount of litterfall produced by different plant species should be done to have a better understanding and prediction of climate change effects on the mass of Hg input from litterfall to peat soils.
2.6 References


Kloepfel, B., Gower, S., Vogel, J., Reich, P. (2000). Leaf-level resource use for evergreen and deciduous conifers along a resource availability gradient. Functional Ecology 14,
281-292.


Chapter 3

3 Moisture content and wetting and drying cycles regulate peat pore water mercury, methylmercury and related chemistry

3.1 Introduction

Methylmercury (MeHg), a potent neurotoxin, is the most toxic species of mercury (Hg) in the environment (Clarkson and Magos, 2006). Given its lipophilic properties, MeHg readily bioaccumulates and biomagnifies in the food web, threatening humans and wildlife health (Zahir et al., 2005). MeHg is primarily produced in oxygen-free environments (e.g., lake sediments and wetlands) by anaerobic microbes, such as sulfate-reducing bacteria (SRB) (Gilmour et al., 1992), iron-reducing bacteria (FeRB) (Fleming et al., 2006), methanogenic archaea (Hamelin et al., 2011), and fermentative Firmicutes (Gilmour et al., 2013) with SRB as the widely accepted primary Hg methylators (Compeau and Bartha, 1985; Ullrich et al., 2001).

In addition to the supply of bioavailable inorganic Hg (IHg), MeHg production is related to the controls that regulate the activity of SRB and the bioavailability of inorganic Hg (IHg), such as anaerobic conditions, concentrations and characteristics of dissolved organic matter (DOM), and sulfate (SO$_4^{2-}$) concentrations (Ullrich et al., 2001). Bioaccessible DOM and SO$_4^{2-}$ are nutrients for SRB (Muyzer and Stams, 2008). Changes in concentrations of bioaccessible DOM and SO$_4^{2-}$ can influence SRB metabolism and then net MeHg production. The concentrations of bioaccessible IHg also control MeHg production (St. Louis et al., 1994; Ullrich et al., 2001). Bioaccessible IHg includes dissolved neutral-Hg complexes, such as HgCl$_2$ and HgS$_0$ that can passively cross SRB cell membranes (Barkay et al., 1997; Benoit
Mounting studies have suggested that nanoparticles of metacinnabar (ß-HgS(s)) instead of HgCl₂ and HgS⁰ are the primary neutrally-charged bioaccessible IHg species for SRB (Gerbig et al., 2011; Graham et al., 2012; Zhang et al., 2012). Moreover, DOM plays an important role in controlling Hg bioavailability, given that Hg tightly combines with the reduced sulfur groups in DOM forming complexes of DOM-Hg (Ravichandran, 2004; Xia et al., 1999). Complexes of Hg with high-molecular-weight DOM (molecular weight ≥ 1000 daltons, or size ≥ 1 nm; sometimes referred to as humic substances) are too large to be metabolized directly by SRB (Barkay et al., 1997; Ravichandran, 2004), but complexes of Hg with low-molecular-weight DOM (molecular weight < 1000, or size < 1 nm) are bioaccessible to SRB (Bravo et al., 2017; Leclerc et al., 2015; Schaefer and Morel, 2009; Schaefer et al., 2011). Previous studies also found that DOM with higher molecular weight and more complex aromatic fractions are more effective in increasing Hg bioavailability by preventing the growth of nanoparticulate ß-HgS(s) into large clusters in the presence of sulfide (Deonarine and Hsu-Kim, 2009; Graham et al., 2012; Slowey, 2010; Zhang et al., 2012).

Wetlands support environmental conditions that favor net MeHg production and are usually considered as important sources of MeHg to downstream lakes and streams (Branfireun et al., 1996; Branfireun et al., 1998; Compeau and Bartha, 1985; St. Louis et al., 1994; St. Louis et al., 1996). Northern wetlands, particularly northern peatlands (peat (partially decomposed plant matter)-accumulated wetlands), are sensitive to altered hydrology driven by climate change (IPCC, 2018). Forecasted climate change is expected to decrease the groundwater level and corresponding soil moisture in non-permafrost northern wetlands by increasing evapotranspiration rates and shifting precipitation patterns (e.g., longer periods of drought) (Tarnocai, 2009). Changes in soil moisture will affect Hg budget in soils, given that the annual Hg budget in soils is generally balanced by inputs of Hg via throughfall and litterfall and outputs of Hg via volatilization, soil sequestration, and streamflow fluxes.
Many studies have shown that soil moisture is an important factor in affecting Hg emissions from soils to the atmosphere (Carpi and Lindberg, 1998; Ericksen et al., 2006; Gabriel and Williamson, 2004; Gillis and Miller, 2000; Gustin and Stamenkovic, 2005; Lindberg et al., 1999; Zhu et al., 2016). For instance, the addition of water to dry soils increases Hg\(^0\) emission because of the replacement of Hg\(^0\) from soil binding sites by water molecules (Lindberg et al., 1999). Hg emission is suppressed in saturated soil due to the filling of water into the soil pore space (Gustin and Stamenkovic, 2005). However, only a few studies have investigated the direct impacts of soil moisture status on concentrations of MeHg in wetland soils (Holloway et al., 2009; Tanner et al., 2018), especially the concentrations of dissolved MeHg that can be transported to downstream ecosystems. Holloway et al. (2009) found that soil MeHg concentrations correlated well with soil moisture in wetlands and suggested that higher soil moisture contents facilitated the formation of more anaerobic environments required by Hg methylators. Tanner et al. (2018) observed that both soil MeHg and pore water MeHg concentrations in wetlands decreased during the drought season because the exposure of saturated soils to air limited the activity of Hg methylators, given the oxygen toxicity for anaerobes including Hg methylators (Jenney et al., 1999; Minz et al., 1999; Muyzer and Stams, 2008; Wang et al., 2014).

Dissolved MeHg in soils is either from net MeHg production or desorption/leaching of MeHg from the solid phase and microbial decomposition of soil organic matter (SOM). Previous studies in wetlands found a greater decomposition rate of SOM in dry periods due to the exposure of previously saturated soils to air (Dieleman et al., 2016; Fenner and Freeman, 2011; Kwon et al., 2013; Worrall et al., 2006), and correspondingly an increase in mobilization of MeHg from dry soils to pore waters during re-wetting events (e.g., precipitation and runoff) (Coleman-Wasik et al., 2015). The majority of MeHg (> 99%) is bound to SOM with little in soil solutions as complexes of DOM-MeHg and MeHg-SH (the combination of MeHg and HS\(^-\)) in different types of northern wetland soils (Liem-Nguyen et al. etc.

(Grigal, 2002).
al., 2017), and thus either increased desorption/leaching of MeHg or decomposition rates of SOM can promote the mobility of MeHg from soils to pore waters. Moreover, dry conditions disrupt biochemical and physical soil characteristics, such as decreased soil aggregation, and re-wetting events will subsequently increase desorption/leaching of soil matter (Kaiser et al., 2015), including MeHg. However, Tóth et al. (2017) observed that decomposition rates of SOM decreased by 93.7% in soils after a six month severely dry period than in the wetter soils in a grassland, because severe drought caused the death of large proportions (up to 69%) of soil microbes, such as bacteria and archaea, that having less drought resistance and adaptation (Haynes, 2000; Kaiser et al., 2015; Kwon et al., 2013; Sparling and Ross, 1988; Srivastava, 1997). Previous studies also found that desorption/leaching of soil matter and microbial decomposition rates are positively correlated to the severity and duration of drought (Kaiser et al., 2015; Tóth et al., 2017). Thus, soil moisture status and drought duration will further affect dissolved MeHg concentrations in soils through direct effects on net MeHg production or indirect influence on MeHg release from soils.

Soils with lower soil moisture content have a further and greater effect on net MeHg production after re-wetting and/or flooding. Previous studies showed that periodic drying and re-wetting of soils enhanced Hg methylation due to the release of SO₄²⁻ that stimulated the activity of SRB (Feng et al., 2014; Coleman-Wasik et al., 2015). Drought exposes previously anaerobic peat to air, resulting in the oxidation of reduced S compounds to SO₄²⁻ (Eimers et al., 2007; Jokic et al., 2003). Re-wetting events promote the release of SO₄²⁻ from soils to pore waters and then net MeHg production (Coleman-Wasik et al., 2015). Furthermore, drought is expected to increase the release of IHg from soils. Although the store of IHg in pore waters of some wetlands was sufficient to provide IHg for MeHg production in situ (Moore et al., 1995), and the “new” IHg that is from wet and dry deposition and runoff was more readily methylated than “old” IHg that from soil release (Branfireun et al., 2005; Feng
et al., 2014; Hintelmann et al., 2002), IHg released from soils is still important in net MeHg production in Hg-limit wetlands.

Drought also changes DOM concentrations and characteristics (Dieleman et al., 2016; Kaiser et al., 2015), which will have further effects on SRB metabolism and Hg bioavailability and then net MeHg production. A greater decomposition rate in soils with lower water tables can increase concentrations of both bioaccessible DOM and high-molecular-weight DOM because of the increase of both microbial decomposition of organic matter (OM) and release of extracellular enzymes under more aerobic conditions (Dieleman et al., 2016). Soil structures are changed (or soil aggregation is decreased) under drought conditions, making the previous sequestered organic matter more available as bioaccessible DOM (Kaiser et al., 2015; Lundquist et al., 1999).

Drought and re-wetting cycles have been shown to increase the release of IHg, MeHg, SO$_4^{2-}$, and DOM (Feng et al., 2014; Gilmour et al., 2004; Coleman-Wasik et al., 2015). These previous studies, however, did not consider the effects of drought on changes in DOM characteristics that are important in controlling net MeHg production (Mitchell et al., 2008; Paranjape and Hall, 2017; Ullrich et al., 2001). However, Mitchell et al. (2008) reported that the combined addition of SO$_4^{2-}$ and bioaccessible DOM to peat soils significantly increased net MeHg production compared to the addition of SO$_4^{2-}$ alone. Additionally, the combined effects of varying soil moisture contents and drought duration on dissolved MeHg concentrations in wetland soils are unknown so far. There is a need to determine the effects of soil moisture status and drought duration on MeHg concentrations in pore waters to better understand the contributions of MeHg from gradually drying peat soils to downstream ecosystems under climate change in wetlands. It is also important to elucidate the influences of soil moisture content and drought and re-wetting cycles on concentrations of pore water IHg, SO$_4^{2-}$, and DOM and characteristics of DOM to have a better understanding of changes
in net MeHg production after re-wetting of peat soils. The overall objective of this study was to experimentally quantify the effects of varying soil moisture conditions and wetting and drying cycles on concentrations of dissolved MeHg, IHg, DOM, and SO$_4^{2-}$ and DOM characteristics in soils. Specifically, I sought to:

A) determine the direct impact of different peat soil moisture status and drought duration on dissolved MeHg concentrations in peat soils;

B) quantify concentrations of SO$_4^{2-}$ and IHg and DOM concentrations and characteristics in the leachate of different peat soil moisture status during the re-wetting events to provide more information for a better understanding of the importance of different peat soil moisture status in controlling net MeHg production;

C) link SO$_4^{2-}$ and DOM concentrations and characteristics to MeHg concentrations in leachate from peat soils with different soil moisture contents.

### 3.2 Materials and methods

#### 3.2.1 Experimental design

The experimental peat soil sampling site is a *Sphagnum*-dominated fen near White River, Ontario, Canada (48°21’ N, 85°21’ W), which is a part of a long-term experimental site maintained by the Ontario Ministry of Natural Resources and Forestry. Approximate 64 L of wet peat soil was sampled from between 20 cm and 60 cm below the ground surface using a 40 cm clean narrow-blade saw in the middle of September of 2019. Samples were double bagged with two clean plastic bags that were then sealed to keep samples saturated. Disposable nitrile gloves were worn during the sampling. Samples were then transferred to the laboratory in the University of Western Ontario in a cooler and then stored at 4 °C in the dark until further processing. The saturated peat monolith was put in a clean plastic
container. Large intact living plant material was removed from the peat soils, which were further homogenized manually. Disposable nitrile gloves were worn all the time to avoid contamination. The homogenized and saturated peat was spread evenly on the bottom of a clean plastic tray and then air-dried at room temperature for three weeks. Peat in the clean plastic tray was homogenized manually every day during the air-drying period to ensure the peat dried evenly.

Five grams of air-dried and homogenized peat was placed in an acid-washed 250 mL glass jar. There were 21 replicates in total that were further divided into three groups with seven jars in each group. Three treatments were: dry (5.0 g + no added water, × 7 replicates) treatment, moist (5.0 g + 12.5 g deionized water, × 7 replicates) treatment, and wet (5.0 g + 25 g deionized water, × 7 replicates) treatment. One method blank treatment (one empty jar) was implemented simultaneously. The total weight of each jar with peat and water (W₀) for each treatment was recorded. Jars for the moist treatment and the wet treatment were capped to avoid water evaporation. Jars for the dry treatment were uncapped leaving peat soils continually air-drying. All treatment jars were placed on a lab bench at room temperature (~21 °C).

For the re-wetting treatment of all samples, an additional 100 mL of deionized water was added to each jar after a period of incubation. The peat was gently swirled at 90 rpm to ensure complete wetting. After 18 h, leachate in each jar was poured through a clean nylon mesh (1 mm; allowing leachate but not peat through) into a clean 125 mL PETG bottle. Leachate was then filtered through a 0.45 µm glass fibre filter into a clean 125 mL PETG bottle. The absolute volume of each leachate sample was recorded. Each leachate sample was split into two aliquots. About 60 mL leachate was for the analysis of dissolved total Hg (THg) and MeHg and preserved by acidifying to 0.5% (vol/vol) with high-purity HCl and stored in a clean 125 mL PETG bottle; the another was for the analysis of dissolved SO₄²⁻.
and DOM and stored in a clean 60 ml Amber glass bottle. All leachate was stored in the dark at 4 °C until analysis.

Peat in the moist treatment and the wet treatment jars were re-wetted by adding deionized water to the original weight (W₀) on the same day after sampling, and then jars were capped for both moist and wet treatments leaving the peat in the dry treatment air-drying. Re-wetting sampling events were repeated five times. The intervals between each re-wetting sampling event were two weeks for the first four re-wetting sampling events and 24 weeks between the last two re-wetting sampling events. The total duration of this incubation was thirty-two weeks. One-hundred and five samples were collected in total with twenty-two subsamples for dissolved THg and MeHg and twenty-two subsamples for DOM and SO₄²⁻ for each sampling event.

3.2.2 Leachate analysis

Dissolved total Hg (THg) concentrations in leachate were analyzed according to Environmental Protection Agency (EPA) method 1631 using the Tekran 2600 Automated Total Mercury Analyzer. All species of Hg in leachate were firstly oxidized to Hg²⁺ by bromine monochloride. Hg²⁺ was further converted to Hg⁰ with stannous chloride reduction after samples being neutralized with hydroxylamine. Hg⁰ in sample solutions was purged, trapped on the gold traps, thermally desorbed in argon, and finally analyzed by cold vapor atomic fluorescence spectroscopy (CVAFS, Tekran 2600, Tekran Inc., Canada) (Bloom and Fitzgerald, 1988). Each analytical run included 10% method blank (deionized water), 10% sample duplicates, 10% matrix spike, 10% matrix spike duplicate, and 10% check standard (made from 1000 ppm stock standard). The instrument was calibrated daily and its detection limit was 0.072 ng L⁻¹. All coefficient of determinations (R²) of daily calibration curves were > 0.9975. The relative percentage differences (RPD) of sample duplicates and matrix
spike duplicates were < 10%. The recoveries of matrix spikes and check standards were 99.45 ± 5.02%. All method blanks were below the method detection limit.

Dissolved MeHg was determined according to methods described in Bloom (1989) and Liang et al. (1994). Samples were distilled with ammonium pyrrolidine dithiocarbamate (APDC) in an acid-cleaned Teflon vessel to remove organic matter. Following distillation, all Hg species in leachate were aqueous-phase ethylated with sodium tetraethyl borate, purged using nitrogen, trapped on Tenax traps, thermally desorbed in helium, and separated by a chromatographic column. Separated Hg species were further successively converted to Hg⁰ and finally analyzed by cold vapor atomic fluorescence spectroscopy (CVAFS, Tekran 2700, Tekran Inc., Canada). Each analytical run included 10% method blank (deionized water), 10% sample duplicates, 10% matrix spike, 10% matrix spike duplicate, and 10% check standard (made from 1000 ppm stock standard). The instrument was calibrated daily and its detection limit was 0.002 ng L⁻¹. All R² of daily calibration curves were > 0.9975. The RPD of sample duplicates and matrix spike duplicates were < 10%. The recoveries of matrix spikes and check standards were 96.46 ± 3.74%. All method blanks were below the method detection limit. Concentrations of dissolved IHg were calculated as THg minus MeHg concentrations.

Sulfate in leachate was determined by ion chromatography on a Dionex ICS-1600. Each analytical run included 10% method blanks, 10% sample duplicates, 10% matrix spikes, 10% matrix spike duplicates, 10% check standards (made from 1000 ppm stock standard). The detection limits were 0.02 mg L⁻¹. The RSD was < 10%. Method blanks were below the detection limit. The recoveries of matrix spikes, matrix spike duplicates, and check standards were 104.50 ± 9.35%.

Concentrations of DOM in leachate were analyzed using the persulfate wet oxidation method on the instrument iTOC Aurora 1030 (OI Analytical, College Station, TX, USA). The carbon
of DOM in leachate was oxidized firstly to CO$_2$ with the persulfate wet oxidation and the amount of CO$_2$ was then determined by the infrared absorbance of CO$_2$ on a iTOC Aurora 1030. The stability of the instrument was daily checked with standard solutions. Each run included 10% method blanks (deionized water), 10% sample duplicates, 5% matrix spikes, and check standards. Equipment blanks of DOM were less than 1 mg L$^{-1}$. Method blanks were less than 1 mg L$^{-1}$. The RSD was < 10% for sample duplicates. Recoveries of matrix spikes and check standards were 106.35 ± 5.75%. All concentrations of THg, MeHg, IHg, SO$_4^{2-}$, and DOM were present as the mass of THg, MeHg, IHg, SO$_4^{2-}$, and DOM per liter of leachate.

The aromaticity of DOM in leachate was characterized by the specific ultraviolet absorbance at a wavelength of 254 nm (SUVA$_{254}$) (Weishaar et al., 2003), using a Horiba Aqualog® fluorescence spectrofluorometer with a xenon lamp. Higher values of SUVA$_{254}$ means DOM has higher molecular weight and more aromatic molecule contents, while lower SUVA$_{254}$ values indicate that DOM compounds are more bioaccessible (Weishaar et al., 2003). SUVA$_{254}$ was calculated by dividing the sample absorbance at $\lambda = 254$ nm by the DOM concentration of the same sample and multiplying by 100. SUVA$_{254}$ was reported in the unit of L mg C$^{-1}$ m$^{-1}$. Each analytical run included 10% sample duplicates with their RSD < 10%.

The characteristics of DOM were also characterized by fluorescence optical indices, including fluorescence index (FI), humification index (HIX$_{EM}$), and the biological index or ‘freshness’ index (BIX) that were determined using R Software (R Core Team 2012) based on fluorescence excitation-emission matrices (EEMs). EEMs were measured using a Horiba Aqualog® fluorescence spectrofluorometer with a xenon lamp. An ultrapure closed water blank was used to eliminate inner-effects on EEMs. The EEMs are directly reported as arbitrary units (A.U.) from Aqualog® and then converted to optical indices (FI, HIX$_{EM}$, and BIX) using R Software. Higher values of FI (> 1.8) indicate that DOM compounds originate
from processes as leachate of algae and bacteria and extracellular release and are more bioaccessible, while lower FI values (< 1.2) indicate that DOM is from decomposition and desorption/leaching of plant and SOM and has higher aromaticity (Fellman et al., 2010). HIX\textsubscript{EM} is an indicator of the extent of humification or humic substance content with higher values indicate a higher degree of humification and more humic substance (Fellman et al., 2010). BIX shows the freshness of DOM that higher values of BIX (> 1.0) suggesting more low-molecular-weight DOM was produced recently, generally by microbes (Fellman et al., 2010). There were 10% sample duplicates in each analytical run with their RSD < 10%.

### 3.2.3 Statistical analysis

The repeated measures ANOVA (Statistica 13.3, StatSoft. Inc. 2017) was used to compare the differences in concentrations of IHg, MeHg, DOM, and SO\textsubscript{4}\textsuperscript{2-}; and DOM characteristics (SUVA\textsubscript{254}, FI, HIX\textsubscript{EM}, and BIX) in the three soil treatments (dry, moist, and wet). The repeated-measures ANOVA was followed by a post hoc test (Tukey’s significant difference; honestly significant difference at the 95% confidence interval). Additional one-way ANOVAs were performed to determine significant differences among treatments in each sampling event. Linear regressions were performed to examine the relationship between concentrations of Hg (i.e., IHg and MeHg) and DOM concentrations and characteristics and between concentrations of MeHg and SO\textsubscript{4}\textsuperscript{2-} in leachate. Data are presented in the text as the mean ± standard deviation (SD). Coefficient of determination (R\textsuperscript{2}) and significance p-values (p) are presented for linear regression fits, with a level of significance set at p < 0.05.
3.3 Results

3.3.1 Effects of soil moisture contents and wetting and drying cycles on pore water mercury concentrations

The concentrations of IHg and MeHg and the proportion of THg as MeHg (%MeHg) in leachate are shown in Figure 3.1. Leachate from the first re-wetting sampling event had higher concentrations of IHg and MeHg in the wet and moist treatments than that in the dry treatment, but this trend did not persist over subsequent sampling events. A plausible explanation is the addition of deionized water to homogenized soils in the wet-peat and moist-peat treatments at the beginning of the experiment resulted in an initially greater release of Hg (IHg and MeHg) from peat due to initial prolonged leaching. Hg data from the initial sampling event in all treatments were discounted in the further statistic analysis.
Figure 3.1 Mercury (Hg) in leachate from each re-wetting sampling event after short-terms (2 weeks) drying and a long-term (24 weeks) drying under wet, moist, and dry soils conditions: A) inorganic mercury (IHg) concentrations (ng L\(^{-1}\)), B) methylmercury (MeHg) concentrations (ng L\(^{-1}\)), and, C) the proportion of total mercury (THg) as MeHg (%MeHg). Vertical bars are mean ± SD (N = 5). The same letters above bars of each rewetting sampling event denote that values (i.e., IHg concentrations, MeHg concentrations, and %MeHg) are not significantly different at the 0.05 levels.

**IHg concentrations.** Concentrations of IHg in leachate showed significant differences among the three treatments of peat soil moisture status over time \((F_{(1.72,44.77)} = 28.76, p < 0.001;\) Figure 3.1A). Based on post hoc tests, IHg concentrations in the dry treatment were significantly higher (~1.3-times) than that in both the moist and wet treatments overall. There was no significant difference in IHg concentrations between the moist treatment and the wet treatment \((p > 0.05)\). These results suggested that re-wetting events significantly increased
the dissolved IHg concentrations in the dried peat soils than in the moist and wet peat soils that had similar IHg concentrations.

Time of sampling had a significant effect on concentrations of dissolved IHg in peat soils only in the dry treatment (the wet treatment: $F_{(3,18)} = 2.31$, $p = 0.11$; the moist treatment: $F_{(3,15)} = 0.38$, $p = 0.77$; the dry treatment: $F_{(3,18)} = 12.57$, $p < 0.001$). Based on post hoc tests, in the dry treatment, the mean IHg concentrations were significantly higher in leachate from the last re-wetting sampling event than that from other re-wetting sampling events; there were no significant differences in the mean IHg concentrations over the rest of the sampling events. Concentrations of IHg in leachate were stable for moist and wet treatments over time. These experimental time patterns for all treatments suggest that there was generally an equilibrium in IHg concentrations between the pore water and peat soil, but long-term (twenty-four weeks) severe drought conditions significantly increased the concentrations of dissolved IHg in pore waters.

**MeHg concentrations.** MeHg concentrations in leachate were significantly affected by peat soil moisture status over time ($F_{(2,46)} = 31.06$, $p < 0.001$; Figure 3.1B). Based on post hoc tests, there were significant differences in MeHg concentrations between treatments overall. The mean MeHg concentrations in the dry treatment were 1.7 and 1.4-times higher than that in the wet treatment and the moist treatment. The amount of pore water MeHg released from dried peat soils far exceeds the amount of pore water MeHg that was produced and released from the wet and moist peat soils, although the net MeHg production was not quantified as part of this study.

Concentrations of MeHg in all treatments were significantly affected by the time of sampling (the wet treatment: $F_{(3,18)} = 17.12$, $p < 0.001$; the moist treatment: $F_{(3,12)} = 78.71$, $p < 0.001$; dry treatments: $F_{(3,9)} = 6.56$, $p < 0.05$). MeHg concentrations in leachate were generally decreasing over the course of the experiment. Based on post hoc tests, the mean MeHg
concentrations in the second re-wetting sampling event were the highest over the experiment but only significantly higher than the last re-wetting sampling event in the dry treatment; in both the moist and wet treatments, the mean MeHg concentrations were significantly higher in leachate from the second re-wetting sampling event than that from the subsequent sampling events, and there were no significant differences among the rest of the sampling events.

%MeHg. The proportion of THg as MeHg (%MeHg) in sediment is generally used as an assessment of net MeHg production, but %MeHg alone in waters cannot reflect the net Hg methylation because MeHg in waters is controlled by both net MeHg production and direct release from soils due to the decoupling of MeHg between overlying waters and submerged soils (Liu et al., 2020; Skyllberg, 2008). The values of %MeHg in this study were used to compare the effects of drying and re-wetting events on the concentrations of IHg and MeHg in leachate. For instance, if %MeHg values are higher in the dry treatment than in the wet treatment, the dry treatment readily increases the concentrations of MeHg relative to IHg concentrations in pore waters compared to the wet treatment.

There was a significant difference in %MeHg over the experiment affected by peat soil moisture status ($F_{(2,46)} = 4.62, p < 0.05$) and time of sampling in all treatments (the wet treatment: $F_{(3,18)} = 15.44, p < 0.001$; the moist treatment: $F_{(3,12)} = 29.39, p < 0.001$; dry treatments: $F_{(3,12)} = 9.86, p < 0.05; Figure 3.1C$). The mean values of %MeHg were highest in the dry treatment among these three treatments. Based on post hoc tests, the mean %MeHg in the wet treatment was significantly lower (~ 0.8-times) than that in both the moist and dry treatment overall; there was no significant difference in the mean %MeHg values between the moist and the dry treatment. These results suggested that MeHg concentrations in leachate are more strongly affected by the peat soil moisture status than IHg concentrations.
Relatively more MeHg than IHg was released from extremely dried soils compared to that from moist and wet peat soils.

The experimental time patterns of %MeHg had a similar trend as that of MeHg concentrations in leachate. The mean %MeHg values were highest in leachate from the second re-wetting sampling event, and then gradually decreasing down to the lowest in leachate from the last sampling event, which can be attributed to the gradual decrease of MeHg concentrations in leachate and the relative stability or even an increase of IHg concentrations in leachate over experimental time. Based on post hoc tests, in the wet treatment, there were significant differences in %MeHg between re-wetting sampling events with two exceptions. There were no significant differences between the fourth re-wetting event and the second and third re-wetting events; in the moist treatment, %MeHg showed significant differences between re-wetting sampling events (except the fourth re-wetting sampling event); in the dry treatment, there were significant differences in %MeHg between the second re-wetting event and the last re-wetting sampling event and between the third re-wetting sampling event and the fourth re-wetting sampling event. These results suggested that MeHg was more readily released from peat soils and depleted in the static system compared to IHg.

3.3.2 Effects of soil moisture contents and wetting and drying cycles on pore water sulfate concentrations

Sulfate concentrations in leachate from the initial re-wetting sampling event were significantly higher than that from the rest of re-wetting sampling events for all treatments (the wet treatment: \( F_{(4,24)} = 107.00, p < 0.001 \); the moist treatment: \( F_{(4,20)} = 49.86, p < 0.001 \); the dry treatment: \( F_{(4,20)} = 258.43, p < 0.001 \); Figure 3.2). Based on post hoc tests, in the dry treatment, the concentrations of \( \text{SO}_4^{2-} \) were significantly higher in the initial re-wetting sampling event than in the subsequent sampling events, and were significantly higher in the
second re-wetting sampling event than in the third and fourth re-wetting sampling events; in the moist and wet treatments, \( \text{SO}_4^{2-} \) concentrations were significantly higher in the initial re-wetting sampling event than in the rest re-wetting sampling events, and there were no significant differences in \( \text{SO}_4^{2-} \) concentrations between the rest re-wetting sampling events. The likely reasons for the highest \( \text{SO}_4^{2-} \) concentrations in all treatments in the initial re-wetting sampling event over time are the oxidation of reduced S compounds to \( \text{SO}_4^{2-} \) due to the pre-drying treatment of peat soils before the incubation experiment. Concentrations of \( \text{SO}_4^{2-} \) in leachate dramatically decreased after the initial re-wetting sampling event, which demonstrated the rapid oxidation of reduced S compounds to \( \text{SO}_4^{2-} \) after exposing the previously saturated peat soils to air and rapid depletion of \( \text{SO}_4^{2-} \) during the re-wetting sampling events.

![Figure 3.2 Results of sulfate (SO_4^{2-}) in leachate from each rewetting sampling event after short-terms (2 weeks) drying and long-term (24 weeks) drying under wet, moist, and dry treatment conditions of soils. Vertical bars are mean ± SD (N = 5). The same letters above bars of each rewetting sampling event denote that SO_4^{2-} concentrations are not significantly different at the 0.05 levels.](image-url)
Given the interference from the pre-treatment of peat soils for the initial samples, one-way ANOVA and the repeated measures of ANOVA was used to analyze the differences in SO$_4^{2-}$ concentrations among peat soils with different peat soil moisture contents for the initial re-wetting sampling event and the rest of re-wetting sampling events, separately. In the initial re-wetting sampling event ($F_{(2,17)} = 112.55$, $p < 0.001$), the mean SO$_4^{2-}$ concentrations followed the sequence: the dry treatment < the wet treatment < the moist treatment; there were significant differences between treatments based on post hoc tests. In the subsequent re-wetting sampling events ($F_{(1.11,35.40)} = 14.89$, $p < 0.001$), the mean SO$_4^{2-}$ concentrations were significantly higher in the dry treatment than that in the moist and wet treatments; no significant difference was found between the moist treatment and the wet treatment based on post hoc tests.

### 3.3.3 Effects of soil moisture contents and wetting and drying cycles on concentrations and characteristics of pore water dissolved organic matter

**DOM concentrations.** The mean DOM concentrations followed the sequence: the wet treatment < the moist treatment < the dry treatment, but this sequence was not persisted over subsequent sampling events. A likely reason is decomposer metabolism declined because the addition of deionized water to peat soils in the wet-peat and moist-peat treatments at the beginning of the experiment decreased aerobic conditions; over the subsequent experimental time, anaerobic decomposers gradually thrived in the anaerobic conditions in the wet and moist peat soils. The initial sample data of DOM concentrations and characteristics for all treatments were not included in the further statistic analysis over the rest of the experimental time (Figure 3.3).

There was a significant difference in DOM concentrations over the experiment influenced by the peat soil moisture status ($F_{(1.37,35.60)} = 13.55$, $p < 0.001$; Figure 3.3A). Based on post hoc tests, the mean DOM concentrations in the moist treatment were significantly lower than that
in the wet and dry treatments; no significant differences were found between the wet treatment and the dry treatment, suggesting that both wet and dry peat soils increased the DOM concentrations in leachate.

Time of sampling also had a significant effect on concentrations of DOM in all treatments (the wet treatment: \( F_{(3,18)} = 361.20, p < 0.001 \); the moist treatment: \( F_{(3,15)} = 179.06, p < 0.001 \); the dry treatment: \( F_{(3,18)} = 335.70, p < 0.001 \)). For all treatments, DOM concentrations were gradually decreasing with the time of re-wetting sampling events after each short-term (two weeks) drying event but sharply increased by 2.6, 2.0, 2.7 times after a long-term drying (twenty-four weeks) for the wet treatment, moist treatment, and the dry treatment, respectively. Based on post hoc tests, in the wet treatment, the mean DOM concentrations were significantly lower in the fourth re-wetting sampling event than in the rest of the re-wetting sampling events; the mean DOM concentrations were significantly higher in the last re-wetting sampling event than in the rest of re-wetting sampling events; there were no significant differences in the mean DOM concentrations between the second and the third re-wetting sampling event. There were significant differences in DOM concentrations between re-wetting sampling events except between the third and the fourth re-wetting sampling event in both the moist and the dry treatment.
Figure 3.3 Changes in (A) dissolved organic carbon (DOC) concentrations, and (B) the specific ultraviolet absorbance at the wavelength 254 nm (SUVA\textsubscript{254}) values for all treatments over the experiment. Vertical bars are mean ± SD (N = 5). The same letters above bars of each rewetting sampling event denote that DOM concentrations or SUVA\textsubscript{254} values are not significantly different at the 0.05 levels.

**SUVA\textsubscript{254}.** SUVA\textsubscript{254} in leachate was significantly affected by the peat soil moisture status over time ($F_{(2,52)} = 19.62$, $p < 0.001$; Figure 3.3B). Based on post hoc tests, the mean SUVA\textsubscript{254} values were significantly lower in the dry treatment than in the moist and wet treatments; no significant differences were found between the moist treatment and the wet treatment, implying that DOM in the dry treatment had lower aromaticity than that in the moist and wet treatments.
The mean SUVA$_{254}$ were significantly affected by the time of sampling for all treatments (the wet treatment: $F_{(3,18)} = 11.32$, $p < 0.001$; the moist treatment: $F_{(3,15)} = 7.94$, $p < 0.05$; dry treatments: $F_{(1.50,9.02)} = 9.08$, $p < 0.05$). Based on post hoc tests, in the wet treatment, the mean SUVA$_{254}$ was significantly lower in the last re-wetting sampling event than in the second and the third re-wetting sampling events, and no significant differences were found between other re-wetting sampling events; in the moist treatment, a significant difference in SUVA$_{254}$ was found between the second and the last re-wetting sampling event; in the dry treatment, the mean SUVA$_{254}$ was significantly lower in the last re-wetting sampling event than in the rest of re-wetting sampling events and relatively stable in the prior sampling events. These results suggested that both long-term (twenty-four weeks) air-drying of peat soils in the dry treatment and long-term (twenty-four weeks) maintaining of wet and moist conditions in the wet and moist treatments increased the accumulation of DOM with lower aromaticity.

**FI.** There were significant differences in FI affected by peat soil moisture status ($F_{(2,48)} = 18.32$, $p < 0.001$; Figure 3.4A). The dry treatment had significantly higher FI (~ 1.1-times) than both the moist and wet treatments. There were no significant differences in FI between the moist treatment and the wet treatment. These results suggested that microbial activity contributed more bioaccessible DOM in the dry treatment than in the moist and wet treatments.
Figure 3.4 Changes in fluorescence indices indicative of dissolved organic matter (DOM) characteristics over time in three-peat soils moisture status. (A) fluorescence index (FI), (B) humification index (HIXEM), and (C) biological index (BIX). Vertical bars are mean ± SD (N = 5). The same letters above bars of each rewetting sampling event denote that fluorescence indices are not significantly different at the 0.05 levels.

FI values were also influenced by time of sampling in the moist treatments but not in the wet and dry treatments (the wet treatment: $F_{(3,12)} = 4.77$, $p = 0.05$; the moist treatment: $F_{(3,12)} =$
10.79, p < 0.05; the dry treatment: $F_{(3,18)} = 1.97$, $p = 0.15$). Based on post hoc tests, in the wet treatment, there were no significant differences in FI between re-wetting sampling events. In the moist treatment, FI values in leachate from the last re-wetting sampling event were significantly higher than that from the second and the third re-wetting sampling events, and no significant differences were found between other re-wetting sampling events. In the dry treatment, FI values were generally stable over time but significantly higher in the last re-wetting sampling event than in the fourth re-wetting sampling event. The mean FI in all treatments was lower than 1.2, indicating that DOM was primarily from decomposition and desorption/leaching of plant and SOM, although processes such as microbial activity can also produce some DOM.

**HIX\textsubscript{EM}**. There was a significant difference in HIX\textsubscript{EM} over time affected by peat soil moisture status ($F_{(1.50,35.94)} = 18.15$, $p < 0.001$; Figure 3.4B). Based on post hoc tests, the mean HIX\textsubscript{EM} values were significantly lower in the dry treatment than in the moist and wet treatments; there were no significant differences between the moist treatment and wet treatment. These results suggested that dried peat soils had a higher degree of humification or released more humic substance during the re-wetting sampling events than the moist and wet peat soils.

Time of sampling significantly influenced the HIX\textsubscript{EM} in the dry treatment but not in the moist and wet treatments (the wet treatment: $F_{(3,12)} = 2.06$, $p = 0.16$; the moist treatment: $F_{(1.21,4.83)} = 1.01$, $p = 0.42$; the dry treatment: $F_{(3,18)} = 160.47$, $p < 0.001$). Based on post hoc tests, the mean HIX\textsubscript{EM} values were significantly lower in the last re-wetting sampling event than the rest of the sampling events, and the mean HIX\textsubscript{EM} values in the third re-wetting sampling event were highest but only significantly higher than the second and last re-wetting sampling events in the dry treatment. These results suggested that drought duration had no clear effects on humic substance content in the moist and wet treatments but significantly decreased the humic substance content after a long-term drying (twenty-four weeks).
**BIX.** The BIX in all treatments were lower than 0.5 reflecting that DOM in peat soils had greater aromaticity, which was consistent with the above results of FI and HIXEM. There were no significant differences in BIX among all treatments ($F_{(2,48)} = 0.51, p = 0.61$; Figure 3.4C), indicating that peat soil moisture status did not significantly affect the contributions of microbially derived DOM. Time of sampling had no significant influences on BIX in all treatments (the wet treatment: $F_{(3,12)} = 3.36, p = 0.05$; the moist treatment: $F_{(3,12)} = 2.71, p = 0.91$; the dry treatment: $F_{(3,18)} = 1.03, p = 0.40$).

### 3.3.4 Relationships between mercury concentrations and SUVA$_{254}$ and between concentrations of methylmercury and sulfate

There was no relationships between IHg concentrations and SUVA$_{254}$ in all treatments (wet treatment: $F_{(1,33)} = 0.072, p = 0.79, R^2=0.01$; moist treatment: $F_{(1,30)} = 8.83, p < 0.05, R^2 = 0.23$; dry treatment: $F_{(1,33)} = 7.27, p < 0.05, R^2 = 0.18$) and between concentrations of MeHg and SUVA$_{254}$ (wet treatment: $F_{(1,33)} = 12.38, p < 0.05, y = 0.01x – 0.003, R^2 = 0.27$; moist treatment: $F_{(1,31)} = 0.68, p = 0.41, R^2 = 0.02$; a dry treatment: $F_{(1,33)} = 0.01, p = 0.91, R^2 = 0.02$). There were no significant correlations between concentrations of MeHg and SO$_4^{2-}$ in all treatments (wet treatment: $F_{(1,33)} = 12.38, p < 0.05, y = 28.80x + 3.75, R^2 = 0.27$; moist treatment: $F_{(1,31)} = 0.68, p = 0.41, R^2 = 0.02$; dry treatments: $F_{(1,33)} = 0.01, p = 0.91, R^2 = 0.02$).

### 3.4. Discussion

#### 3.4.1 Effects of peat soil moisture status on concentrations of inorganic mercury, methylmercury, and sulfate and dissolved organic matter concentrations and characteristics

The concentrations of IHg, MeHg, and SO$_4^{2-}$ measured in leachates from the dry treatment were generally significantly higher than that from the moist and wet treatments, which is consistent with the finding of Coleman-Wasik *et al.* (2015) that drought and re-wetting
events increased the release of total Hg, MeHg, and SO$_4^{2-}$ from peat soils. The exposure of previously anaerobic peat soils to oxygen is associated with an increase in the decomposition of SOM (Evans et al., 2005; Fenner and Freeman, 2011; Kaiser et al., 2015) and oxidation of reduced S compounds to SO$_4^{2-}$ (Eimers et al., 2003; Eimers et al., 2007; Jokic et al., 2003). Given the majority of Hg (IHg and MeHg) is binding with the reduced sulfur groups in SOM (Liem-Nguyen et al., 2017), an increase in the decomposition of SOM will lead to the release of Hg from soils. Moreover, drought disrupts soil biochemical and physical structures, such as decreases soil aggregation (Kaiser et al., 2015), promoting the release of potential IHg, MeHg, and SO$_4^{2-}$ from peat soils during re-wetting events.

There were generally no significant differences in the concentrations of IHg, MeHg, and SO$_4^{2-}$ between the moist and wet treatments, which was contrary to a previous study that found more oxygen filtration to previously saturated soils increased the oxidation of SOM and reduced S compounds (Coleman-Wasik et al., 2015). In this study, the moist peat soils had more aerobic conditions than the wet peat soils, but both of them were in oxygen deficit conditions in most of the experimental time because the mason jars for these peat soils were capped in most of the experimental time to avoid the evaporation of soil waters. Thus, it is not surprising for the similar concentrations of IHg, MeHg, and SO$_4^{2-}$ in the moist and wet treatments. Furthermore, the nonsignificant differences in MeHg concentrations in the moist and wet treatments can also be explained by the similar net MeHg production because of the nonsignificant different SO$_4^{2-}$ concentrations in the moist and wet treatments.

The %MeHg was significantly affected by peat soil moisture status with the %MeHg values significantly higher in the dry treatment than in the wet treatment, suggesting that the dry treatment readily increased the concentrations of dissolved MeHg relative to IHg concentrations compared to the wet treatment. The high %MeHg values in the dry treatment can not attribute to the net MeHg production, given that Hg methylation requires the
anaerobic environments (Ullrich et al., 2001). There are two possible reasons for the higher %MeHg values in the dry treatment: (1) IHg has a stronger binding strength than MeHg to the reduced sulfur groups (Liem-Nguyen et al., 2017), with the average partitioning coefficient for total Hg and MeHg to be 4.0 ± 0.4 and 3.2 ± 0.4, respectively (Liu et al., 2020); (2) saturated conditions decreases MeHg diffusion from soils to waters (Marvin-DiPasquale et al., 2009).

The peat soil moisture status affected DOM concentrations. Both the dry and wet treatments significantly increased pore water DOM concentrations than the moist treatment, which agrees well with previous studies that both wet and dry conditions increase the release of DOM from soils (Kalbitz et al., 2000). Increased aerobic conditions as water table drops can enhance decomposition of SOM (Alm et al., 1999; Christensen et al., 1998; Dieleman et al., 2016; Fenner and Freeman, 2011; Kwon et al., 2013), leading to an increase in DOM concentrations in pore waters (Banas and Gos, 2004; Frank et al., 2014; Freeman et al., 2001a; Glatzel et al., 2003; Moore and Clarkson, 2007; Wallage et al., 2006; Worrall et al., 2006). Severe drought increases fungi biomass but causes the death of large proportions (up to 69%) of bacteria and archaea that with less drought resistance and adaptation (Haynes, 2000; Kaiser et al., 2015; Kwon et al., 2013; Sparling and Ross, 1988; Srivastava, 1997), increasing microbial cell lysis and intracellular solutes release (Kalbitz et al., 2000). Severe drought also disrupts soil biochemical and physical structure, such as breaking apart soil aggregates, exposing and release physically protected DOM (Kaiser et al., 2015; Lundquist et al., 1999). However, several studies found that anaerobic conditions had higher DOM concentrations than aerobic conditions because of the slower degradation and less efficient decomposition of organic matter under anaerobic conditions (Du and Li, 2017; Kalbitz et al., 2000). Freeman et al. (2004) also observed that drought significantly decreased peat DOM concentrations because the aerobic conditions increase the release of carbon dioxide rather than DOM as the major end product of decomposition. These studies support our results that
the wet treatment had higher DOM concentrations than the moist treatment. More specific studies are needed to clarify the mechanisms of soil moisture effects on DOM concentrations in peat soils.

The dry treatment had the lowest SUVA$_{254}$ and HIX$_{EM}$ but highest FI among the three treatments of peat soil moisture status, suggesting that DOM from the dry treatment had less aromaticity and lower molecular weight than that from the moist and wet treatments. The values of BIX were lower than 1.0 and not affected by peat soil moisture status, which can be attributed to the large terrestrial-derived DOM (resulting from decomposition and leaching of soil organic matter) might overwhelm contributions from microbial-derived DOM (originating from processes as extracellular release and leachate of bacteria). These results agree well with previous studies that soils under dry conditions released more DOM with low aromaticity than the soils under wetter conditions (Kaiser et al., 2015; Kwon et al., 2013; Van Gaelen et al., 2014; Zsolnay, 2003). It has been established that microbial-derived organic compounds (intracellular solutes, cell wall, and cell lysis) and DOM that leached from soils, such as organic acids and sugars (Kaiser et al., 2015) are generally more bioaccessible for microbes (Kalbitz et al., 2003a; Kalbitz et al., 2003b), while DOM from the decomposition of SOM generally had higher aromaticity and molecular weights (Kaiser et al., 2015).

### 3.4.2 Effects of drought duration on concentrations of inorganic mercury, methylmercury, and sulfate and dissolved organic matter concentrations

Based on the statistic analysis, not including the data from the initial re-wetting sampling event, concentrations of IHg in leachate were stable over time in the moist and wet treatments; in the dry treatment, IHg concentrations in leachate were stable during the short term drying (two weeks) and re-wetting events but significantly increased after long-term drying (twenty-four weeks). A plausible explanation is there is an equilibrium of IHg
concentrations between peat soils and pore waters (Miretzky et al., 2005), but a long-term severe drought of peat soils may break the equilibrium by increasing decomposition rates and disturbing soil structures, and consequently increases the release of IHg from peat soils to pore waters.

Concentrations of MeHg and %MeHg values were highest in the second re-wetting sampling event and lowest in the last re-wetting sampling events in the dry treatment, suggesting that abundant of MeHg was released from peat soils in the second re-wetting sampling, and lesser MeHg was released over the rest of experimental time. These results confirm that MeHg is more readily released from peat soils than IHg (Liem-Nguyen et al., 2017; Liu et al., 2020). In the moist and wet treatments, MeHg concentrations were highest in the second re-wetting sampling events. No significant differences were found over the subsequent re-wetting sampling events. One possible reason is more MeHg was physically released from peat soils during the second re-wetting sampling event, given that MeHg is readily released from soils compared to IHg (Liem-Nguyen et al., 2017; Liu et al., 2020). Another reasonable explanation is the new inputs of SO$_4^{2-}$ due to the oxidation of sulfur enhanced the SRB activity and then net MeHg production, but as the depletion of SO$_4^{2-}$ in this static system, it has lesser effects on net MeHg production. However, the highest SO$_4^{2-}$ concentrations were found in the initial re-wetting sampling event but the highest MeHg concentrations were in the second re-wetting event in the moist and wet treatments, which can be attributed to the lag effects of SO$_4^{2-}$ on MeHg concentrations. This result agrees well with the previous study that drought and re-wetting increased the new input of SO$_4^{2-}$ to pore water and as the SO$_4^{2-}$ concentrations decline, MeHg concentrations and %MeHg values increased further (Coleman-Wasik et al., 2015).

Concentrations of SO$_4^{2-}$ were significantly higher in the initial re-wetting sampling events than in the rest of re-wetting sampling events in all treatments. A plausible explanation is the
pre-treatment (air-drying of peat) before the experiment led to the rapid oxidation of reduced S compounds to SO$_4^{2-}$ due to the exposure of saturated peat soils to oxygen. In the dry treatment, reduced sulfur compounds were continued oxidized to SO$_4^{2-}$ over the rest of the experimental time but depleted quickly in this static system. These results are consistent with previous studies in wetlands and stream systems that SO$_4^{2-}$ concentrations in pore waters increased after dry periods (Eimers et al., 2003; Eimers et al., 2007; Coleman-Wasik et al., 2015), and successive droughts eventually result in smaller SO$_4^{2-}$ export as sulfur pools is rapidly depleted in the low sulfur deposition zone (Eimers et al., 2007; Coleman-Wasik et al., 2015). In the moist and wet treatments, there were no significant differences in SO$_4^{2-}$ concentrations over the experiment (except the initial re-wetting sampling event), supporting the previous observations that under saturated and anoxic conditions, SO$_4^{2-}$ was retained in peat soils through microbial reduction of SO$_4^{2-}$ (Eimers et al., 2003). Although SO$_4^{2-}$ concentrations in this study were low ($\leq 2.259 \pm 0.292$ mg L$^{-1}$), similar to previously reported in other peatlands (Mitchell et al., 2008b; Coleman-Wasik et al., 2015), it still has significant effects on Hg methylation in wetlands given the fact that SO$_4^{2-}$ concentrations ranging from 1 to 20 mg L$^{-1}$ leads to the maximum MeHg production in anaerobic environments (Orem et al., 2011).

Concentrations of DOM in leachate were highest in the last re-wetting sampling event in all treatments. Increased oxygenation under longer drought conditions has been shown to stimulate the activity of phenol oxidase enzymes leading to the degradation of phenolic compounds (hydrolase or microbial metabolic inhibitors) and then enhancing peat decomposition (Fenner and Freeman, 2011; Freeman et al., 2001b). Long term drought also breaks soil biochemical and physical structure (e.g., changing microbial community structure by decreasing bacteria and archaea biomass and increasing fungi biomass, and breaking apart soil aggregates), resulting in the release of more bioaccessible dead microbial biomass, lysis, and potential DOM from peat soils (Kaiser et al., 2015). In the moist and wet treatments, the
highest DOM concentrations in the last re-wetting sampling event can be attributed to the accumulation of DOM in twenty-four weeks.

3.4.3 Relationships between mercury concentrations and dissolved organic matter concentrations and characteristics

There were no correlations between concentrations of Hg (IHg and MeHg) and SUVA$_{254}$. Many studies observed the correlations between dissolved Hg concentrations and DOM concentrations and between particulate Hg concentrations and particulate organic matter concentrations (Brigham et al., 2009; Dennis et al., 2005; Grigal, 2002; Riscassi and Scanlon, 2011; Yin and Balogh, 2002). Some studies, however, showed that the relative aromaticity of DOM was more strongly correlated to dissolved Hg concentrations than DOM concentrations (Burns et al., 2013; Dittman et al., 2009; Dittman et al., 2010; Jiang et al., 2018; Lavoie et al., 2019; Riscassi and Scanlon, 2011; Shanley et al., 2008) because aromatic fractions of DOM contain the majority of reduced sulfur groups (strong Hg binding sites) (Ravichandran, 2004). DOM with higher aromaticity also plays a larger role in stabilizing nanoparticles $\beta$-HgS(s) or preventing the growth of nanoparticles $\beta$-HgS(s) into large clusters (Gerbig et al., 2011; Ravichandran et al., 1999), leading to increases in the bioavailability of IHg and then net MeHg production (Hall et al., 2008), which may explain the positive relationship between MeHg concentrations and SUVA$_{254}$.

There are five likely reasons for the results that there were not always relationships between Hg and SUVA$_{254}$: (1) the amount of reduced sulfur groups far exceeds the amount of Hg in natural environments (Ravichandran, 2004). (2) dissolved Hg concentrations in aquatic environments can be affected by protein-like compounds that contain thiol ligands but not link to SUVA$_{254}$ (Schaefer and Morel, 2009); (3) Hg is likely bound to sulfide species such as cinnabar (Barnett et al., 1995; Miller et al., 2013) or suspended matter (Brigham et al., 2009; Grigal, 2002; Mason et al., 1999) rather than to DOM. There were apparently
suspended particles in the moist and wet treatments. (4) there is an equilibrium of IHg in the soil-pore water system with the majority of IHg adsorbed on solid surfaces (Miretzky et al., 2005), while DOM concentrations can easily be affected by many factors, such as microbial activity, soil moisture, precipitation, and water fluxes (Kalbitz et al., 2000). (5) some studies found that MeHg has a weaker binding strength than IHg to the reduced sulfur groups in DOM (Liem-Nguyen et al., 2017; Liu et al., 2020; Burns et al., 2012; Hall et al., 2008; Lescord et al., 2018). Thus, it is not surprising to find that there were no significant relationships between MeHg concentrations and SUVA_{254} in the moist and dry treatments, given that there were no positive relationships between IHg concentrations and SUVA_{254}.

3.4.4 Relationships between concentrations of methylmercury and sulfate

Sulfate is an important nutrient for SRB (Muyzer and Stams, 2008). In natural anaerobic conditions, the addition of SO_4^{2-} can increase the net MeHg production (Branfireun et al., 1999; Mitchell et al., 2008a). However, there were no significant correlations between concentrations of SO_4^{2-} and MeHg in this study, which does not mean SO_4^{2-} did not affect MeHg production because a clear higher MeHg concentration and %MeHg in the second re-wetting sampling event were found right after the highest SO_4^{2-} concentrations in the initial re-wetting sampling event. Moreover, Except SO_4^{2-}, Hg methylation is also affected by Hg bioavailability, DOM concentrations and characteristics, and other environmental factors (i.e., anaerobic conditions and hydrologic fluctuation) (Ullrich et al., 2001). Thus, the results confirmed that new inputs of SO_4^{2-} increased MeHg concentrations but as the SO_4^{2-} depletion, other factors can continually affect net MeHg production.

3.5 Conclusions and implications

This study demonstrated that concentrations of dissolved IHg, MeHg, DOM and SO_4^{2-} and characteristics of DOM were affected by peat soil moisture status and the duration of
drought. Dry peat soils released more dissolved IHg, MeHg, and SO$_4^{2-}$ than the moist and wet peat soils during re-wetting events. Both dry and wet peat soils had higher pore water DOM concentrations than the moist peat soils. DOM in the dry peat soils had lower aromaticity than the moist and wet peat soils. These results indicate that drought under global warming in the northern peatland can increase the potential threat of MeHg to humans and wildlife in the downstream ecosystems, given that long-term severe drought can directly increase the release of MeHg from peat soils and the potential net MeHg production due to the enhanced release of IHg, bioaccessible DOM, and SO$_4^{2-}$ during drying and re-wetting events. Although positive correlations were not always observed between concentrations of MeHg and the aromaticity of DOM and between concentrations of MeHg and SO$_4^{2-}$ in this study, the increased oxidative release of bioaccessible DOM and SO$_4^{2-}$ still plays an important role in stimulating SRB metabolism, given that both bioaccessible DOM and SO$_4^{2-}$ are important nutrients to SRB (Mitchell et al., 2008; Muyzer and Stams, 2008). Together, these findings contribute new insight into how peat soil moisture content and drying duration affect concentrations of dissolved IHg, MeHg, DOM, and SO$_4^{2-}$ and characteristics of DOM in wetlands. These findings also provide useful information for predicting subsequent net MeHg production in peat soils after re-wetting of dried peat soils.
3.6 References


Bloom, N. (1989). Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. Canadian Journal of Fisheries and Aquatic Sciences 46, 1131-1140.


organic carbon: observations, possible causes and environmental impacts.

Environmental Pollution 137, 55-71.


microbial mercury methylation under sulfidic conditions. Environmental Science and Technology 46, 2715-2723.


organic matter (DOM) from the topsoil. Zeitschrift fuer Pflanzenernaehrung und Bodenkunde 160, 475-483.
environment. Environmental Science and Technology 43, 8548-8553.
Sparling, G. and Ross, D. (1988). Microbial contributions to the increased nitrogen


Chapter 4

4 Effects of increased ground temperature on mercury cycling in northern peatlands

4.1 Introduction

Mercury (Hg), a toxic element, is considered a global pollutant due to its ubiquitous presence in the environment (Driscoll et al., 2013). Atmospheric Hg originates from both natural and anthropogenic sources (Driscoll et al., 2013). Natural sources of atmospheric Hg include volcanoes, forest fires, geothermal sources, and volatilization from mercuriferous soils, vegetation, and oceans (Schroeder and Munthe, 1998). Anthropogenic sources of atmospheric Hg include coal combustion, artisanal gold mining, incineration of medical waste, sewage sludge, and base metal melting (Streets et al., 2011). The dominant Hg species (atmospheric gaseous elemental mercury (GEM); > 95%) in the atmosphere has a long atmospheric residence time of several months to a year (Pirrone et al., 2010; Schroeder and Munthe, 1998; Selin, 2009), and therefore is widely dispersed and deposited to even remote areas (Enrico et al., 2016; Martinez-Cortizas et al., 1999), making Hg a global pollutant.

Mercury can be converted into methylmercury (MeHg) in anaerobic environments (e.g., lake sediments and wetlands) by anaerobic microorganisms, such as sulfate-reducing bacteria (SRB) (Gilmour et al., 1992), iron-reducing bacteria (FeRB) (Fleming et al., 2006; Kerin et al., 2006), methanogenic archaea (Hamelin et al., 2011), and syntrophic and acetogenic bacteria (Gilmour et al., 2013) with SRB as the dominant Hg methylators, particularly in sediments and young peat soils (< 1000 years) (Compeau and Bartha, 1985; Gilmour et al., 1992; Hu et al., 2020). MeHg is a potent neurotoxin and the most toxic form of Hg (Clarkson and Magos, 2006). Owing to its lipophilic and protein-binding properties, MeHg
bioaccumulates and biomagnifies, posing a threat to upper trophic level wildlife and humans (Mergler et al., 2007).

Net MeHg production is affected by factors that regulate microbial activity and the bioavailability of inorganic Hg (IHg), such as sulfate (SO\textsubscript{4}\textsuperscript{2−}), dissolved organic matter (DOM), and temperature (Ullrich et al., 2001). Low SO\textsubscript{4}\textsuperscript{2−} concentrations (< 1 mg L\textsuperscript{−1}) limit SRB metabolism and thus Hg methylation (Corrales et al., 2011; Jeremiason et al., 2006; Orem et al., 2011). At SO\textsubscript{4}\textsuperscript{2−} concentrations higher than 20 mg L\textsuperscript{−1}, the buildup of byproduct sulfide during SRB metabolism may result in the precipitation of HgS(s) that can decrease the bioavailability of Hg (Orem et al., 2011).

Dissolved organic matter provides energy for SRB growth and metabolism (Ullrich et al., 2001). DOM also serves as a Hg carrier facilitating Hg transport and acts as a strong chelating agent for Hg (Ravichandran, 2004; Ullrich et al., 2001), given the strong affinity between Hg and reduced sulfur ligands in DOM (Xia et al., 1999). Complexes of Hg with low-molecular-weight DOM (molecular weight (or size) < 1000 daltons (or 1 nm)), such as cysteine, can be taken up by SRB (Leclerc et al., 2015; Schaefer and Morel, 2009; Schaefer et al., 2011). High-molecular-weight DOM (molecular weight (or size) ≥ 1000 daltons (or ≥ 1 nm)), sometimes referred to as humic substances, decreases Hg bioavailability in the absence of sulfide because the complexes of DOM-Hg are too large to pass through bacteria cell membranes (Barkay et al., 1997; Gilmour et al., 2011). In the presence of sulfide, high-molecular-weight DOM can increase Hg bioavailability by coating the bioaccessible nanoparticulate metacinnabar (ß-HgS(s)) and then increasing electrostatic repulsion and preventing aggregation and precipitation of ß-HgS(s) (Deonarine and Hsu-Kim, 2009; Gerbig et al., 2011; Miller et al., 2007; Slowey, 2010). The formation of DOM-Hg complexes is affected by pH. DOM is less negatively charged under lower pH, which subsequently
decreases the binding between DOM and Hg, making Hg more available for Hg methylators (Barkay et al., 1997; Ravichandran, 2004).

Temperature is also important in controlling net MeHg production. All microbial growth and metabolism including SRB are highly temperature-dependent with higher microbial growth and metabolism under moderately higher temperatures (Comeau, 2008). This is supported by previous studies that found Hg methylation peaked in summer (Canario et al., 2007; Hintelmann and Wilken, 1995). Warmer conditions elevate decomposition rates (Archer, 2004; Dieleman et al., 2016; Kirschbaum, 1995) and subsequently DOM concentrations (Dieleman et al., 2016; Du and Li, 2017; Evans et al., 2005; Freeman et al., 2001; Kalbitz et al., 2000; Kane et al., 2014) that are expected to affect the mobility of Hg in aquatic ecosystems (Ravichandran, 2004). Higher temperatures are also suggested to increase the release of bioaccessible DOM from root exudates and microbial activity (Dieleman et al., 2016; Kane et al., 2014). DOM from root exudates and microbial activity normally has lower molecular weight and is more bioaccessible (Jones et al., 2009) than that from the decomposition of soil organic matter (SOM) (Nebbioso and Piccolo, 2013), which further increases microbial metabolism and affects Hg bioavailability (Ullrich et al., 2001).

Northern peatlands are water-saturated for a long period and have accumulated > 40 cm depth of peat (partially decomposed plants) due to slow decomposition rate and low temperatures associated with the relatively northern latitudes (Rydin and Jeglum, 2013), which support environmental conditions that favor net MeHg production (Branfireun et al., 1996; Branfireun et al., 1998). Northern peatlands are usually classified as either bog or fen ecosystems according to their hydrological conditions, nutrient status, dominated vegetation, and pH-base richness (Rydin and Jeglum, 2013). Bogs are acidic (pH < 4.5), nutrient-poor, and Sphagnum spp. mosses-dominated peatlands and are hydrologically isolated from groundwater systems. Fens have a higher pH ranging from 4.5 to greater than 6.5. Fens are
along a gradient of nutrient status from nutrient-poor to nutrient-rich, and receive nutrients and water from groundwater and/or surface runoff and precipitation. Nutrient-poor fens are dominated by *Sphagnum* spp. mosses, vascular plants, such as shrubs and trees. Nutrient-intermediate fens are dominated by vascular plants (e.g., graminoid plants and deciduous shrubs).

Northern peatlands are already experiencing temperature increases due to climate change (IPCC, 2018). The IPCC 2018 special report states that the mean global temperature is likely to reach 1.5 °C above pre-industrial levels between 2030 and 2052 with the extreme temperature up to 6 °C in high latitudes (IPCC, 2018). Several studies and the IPCC 2018 special report also have shown that global warming is altering hydrology with gradually drier conditions in northern peatlands (Dai, 2011; Helbig *et al.*, 2020; Tarnocai, 2009).

As northern peatlands are considered to be sinks of atmospheric Hg and SO$_4^{2-}$ and, importantly, “hotspots” of MeHg production (Branfireun *et al.*, 1996; Branfireun *et al.*, 1998; Branfireun *et al.*, 1999; Mitchell *et al.*, 2008b), and these processes are strongly governed by temperature and hydrology, the overall objective was to experimentally quantify the influences of ground warming on pore water Hg concentrations in two northern peatlands: a moss-dominated, poor nutrient fen and a sedge-dominated, intermediate nutrient fen.

Specifically, my objectives were to:

(1) determine the direct impact of ground warming on IHg and MeHg concentrations in pore waters of two types of northern peatlands (a moss-dominated fen and a sedge-dominated fen);

(2) quantify changes in centers around MeHg production, such as concentrations of SO$_4^{2-}$ and DOM concentrations and characteristics under elevated ground temperature.

(3) compare the differences between the two fens in ground warming effects on concentrations
of IHg, MeHg, SO$_4^{2-}$, DOM concentrations and characteristics.

4.2 Methods

4.2.1 Study sites

The experiment was conducted in a sedge-dominated fen (10.2 ha) and a moss-dominated fen (4.5 ha) located in an 817 ha sub-watershed of the Lake Superior basin near White River, Ontario, Canada (48°21’ N, 84°20’ W). These two sites are part of a long-term White River Experimental Watershed Study site monitored by the Ontario Ministry of Natural Resources and Forest Research Institute, who also provided the weather data, including air temperature and precipitation. The field-based study was performed from June to October of 2017, 2018, and 2019. The mean air temperature from June to October of each year was 12.3 °C, 8.7 °C, and 12.9 °C for 2017, 2018, and 2019, respectively. The mean precipitation from June to October of each year was 299.05 mm, 277.15 mm, and 219.40 mm for 2017, 2018, and 2019, respectively.

The two fens are ~2 km apart with catchments of mixed-wood coniferous and deciduous forests. The sedge-dominated fen vegetation community is dominated by Carex spp., with a low shrub overstorey of mainly sweet gale [Myrica gale L.] and leatherleaf [Chamaedaphne calyculata L. Moench]. The moss-dominated fen groundcover is dominated by Sphagnum spp. mosses, with a low shrub cover of mainly leatherleaf and Labrador tea [Rhododendron groenlandicum], and a sparse overstorey of coniferous trees including tamarack [Larix laricina] and black spruce [Picea mariana]. The sedge-dominated fen is relatively more nutrient-rich because of groundwater and surface water connections, reflected in the vegetation community, higher pore water pH, and a generally wetter condition than the moss-dominated fen (Rydin and Jeglum, 2013).
4.2.2 Field experiment design

Sixteen experimental plots (eight control and eight warmed) along with two reference plots were established at each of the two study peatlands (Figure 4.1 and 4.2). Reference plots were several hundred meters (sedge-dominated fen) or dozens of meters (moss-dominated fen) away from these experimental plots and were rarely disturbed artificially over the experiment. Reference plots were established to elucidate the effects of artificial disturbance on samples and experimental results. The plots were arranged in a block design (2 control, 2 warmed in each block) to address small-scale spatial factors (e.g., microtopography, vegetation biomass and composition, moisture variability, and light intensity) in the two sites. Cylindrical PVC collars (1 m diameter; 40 cm deep) were inserted into the peat of each plot (10 cm aboveground; 30 cm belowground).
Figure 4.1 Research site and experimental design. Research sites A) the moss-dominated fen, and B) the sedge-dominated fen is located at 817 ha sub-watershed of the Lake Superior basin near White River Ontario, Canada (48°21’ N, 85°21’ W). Ground warming was simulated using C) the open-top chambers (OTCs) and D) six soil heating robs. Target increased soil temperature was controlled by E) a custom control system.
Figure 4.2 Experimental design schematic diagram: A) moss-dominated fen and B) sedge-dominated fen.
**Chamber warming:** Experimental ground warming was implemented in stages beginning with passive warming in 2017. Passive warming was accomplished by resting clear, 1.2 m tall, open-topped chambers (OTCs) on the collars at each warmed plot. Passive warming was implemented from June to October in 2017 and 2018 and June and October in 2019. The mean surface peat (5 cm) temperatures (measured by Lyons *et al.* at the same experiment) were consistently raised by 0.38 °C and 0.27 °C under passive warming for the moss-dominated fen and sedge-dominated fen, respectively (*Lyons et al.*, 2020). In late June 2019, active warming was added to the warmed plots to raise peat temperatures to a target of 4 °C above ambient peat temperatures to a depth of 50 cm. Active warming was performed circumferentially by installing six 50 cm heating rods (60 W Watlow FireRod® immersion heaters) and the heating of the peat to the target set-point temperature was automatically controlled by a custom control system designed by Zesta Engineering (Mississauga ON). Two thermocouples were installed inside and outside of each OTCs, respectively, monitoring the 25 cm depth of soil temperature. The heating system automatically controlled and adjusted the heating rods to maintain the designed temperature difference between the inside and outside peat of OTCs based on the measured temperatures from the two thermocouples. The active warming was from the end of June (June 28) to September 20th, 2019 in both sites. Most of the heating plots in two peatland sites were generally maintained at the constant offset of +4 °C. The mean increased soil temperatures under active warming were +4.5 ± 0.9 °C and +3.8 ± 0.8 °C in the moss-dominated fen and sedge-dominated fen, respectively. However, the system failed to consistently warm several plots at both sites over the entire season (3 of 8 plots in the moss-dominated fen and 2 of 8 plots in the sedge-dominated fen) because of the failed working of heating rods in these plots; data from these warmed plots are thus not included as part of the actively warmed data for this analyses. Clear OTCs were left on the warmed plots during active heating, also contributing to the passive warming effects consistent with previous years. Manual measurements of soil temperature were performed
weekly during chamber sampling by measuring temperatures at soil depths 25 cm at three random locations in each plot via Fisherband™ Long-Stem Digital Thermometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Surface soil moisture integrated over the top 10 cm was measured during chamber sampling within each OTC at three different locations using an HH2 Moisture Meter (Delta-T Devices, Burwell, Cambridge, UK). Water table elevation (under the peat surface, cm) was provided by the Ontario Ministry of Natural Resources and Forest Research Institute for 2017 from a single well at weather stations located immediately adjacent to the experimental plots. In June 2018, TD-Diver™ and Baro-Diver® data loggers were deployed in 4 locations across both sets of experimental plots to measure water table levels every 15 minutes during the rest of the experimental period.

4.2.3 Sampling and analysis

**Sampling.** An integrated groundwater sampling well was permanently installed in each experimental and reference plot. Sampling wells were custom-fabricated from Teflon® (5 cm O.D., 4 cm I.D., 50 cm long; slotted along entire length), and capped at both ends to prevent contamination. An acid-washed (10% v/v) 6.35 mm (0.25 cm O.D.) Teflon sampling line was installed in each well secured with a Teflon compression fitting in the vented top cap, and passed through the sidewall of the chamber to allow for water sampling without having to remove the OTCs or otherwise disturb the plot. Sample lines were kept capped and clean between sampling.

Wells were pre-purged using a Geotech® GeoPump (Geotech Ltd., North Aurora, ON, Canada) and acid-washed Masterflex® C-FlexUltra tubing (Cole-Palmer Instrument Co.). Pore waters were collected weekly (2017 and 2019) or bi-weekly (2018) into pre-acid washed 500 mL PETG (polyethylene terephthalate glycol-modified; Thermo Scientific™ Nalgene™). A second smaller volume was withdrawn and used for pH measurements.
(Mettler Toledo Seven2GO™Pro) in the field immediately after the water sample was taken. All pore water samples were kept cool and dark in the field in a clean cooler and then transported to the local field laboratory for filtering and preservation. All samples were then filtered with 0.5 μm glass-fiber filters in an enclosed vacuum filter apparatus with Teflon wetted surfaces, and transferred to acid-cleaned 250 mL PETG bottles (total Hg (THg); MeHg), 60 mL HDPE (high-density polyethylene; Ions) bottles, and 60 mL amber glass (total DOM and optical characterization) bottles for further analysis. Filtered pore waters for Hg analysis were preserved by acidifying to 0.5% (vol/vol) with OmniTrace® hydrochloric acid. All filtered samples were stored at 4 °C until they were returned to the university analytical lab for analysis.

Field, equipment, and filter blanks and sample duplicates were collected for each sampling date. Field blanks were performed by directly pouring the deionized water (18.2 MΩ cm) into the 250 mL PETG bottles. Sample duplicates were performed by randomly choosing one plot and then simultaneously collecting two pore water samples. Equipment blanks were performed by directly pumping deionized water (18.2 MΩ cm) into the 500 mL PETG bottles using the acid-cleaned Masterflex C-FlexUltra tube. Filter blanks were performed by filtering the deionized water (18.2 MΩ cm) into 250 mL PETG bottles, 60 mL HDPE bottles, and 60 mL amber glass bottles. All field blanks, filter blanks, equipment blanks, and sample duplicates were stored and handled in the same way as field samples.

Standard ultraclean sampling protocols were used throughout sample collection (US EPA 1996). Before sample collection, all sampling equipment and sample containers for Hg and DOM analysis were cleaned using soapy water for 1 h followed by a MilliQ water rinse and soaked in 10% (v:v) HNO₃ overnight followed by a MilliQ water rinse in the university laboratory. Sample containers for ions analysis were cleaned using soap for 1 h followed by a MilliQ water rinse. After cleaning, sampling equipment and sample containers were
individually double-bagged and shipped to the sampling site. Clean hands/dirty hands techniques were used during sample collection. There were two people in a sampling team with one person as “dirty hands” and another as “clean hands”. All operations including preparation of sampling, operation of any machinery, and all other activities that do not directly contact with samples and the inner sample bottles were handled by “dirty hands”. “Clean hands” was responsible for all operations involving contact with sample bottles and the transfer of samples from the sampling equipment to the sample bottles. Clean disposable nitrile gloves were worn all the time to avoid contamination.

**THg analysis.** Environmental Protection Agency (EPA) method 1631 was used for THg analysis in pore waters (EPA, 2002). Specifically, all samples were oxidized 12 h with BrCl oxidation, neutralized by hydroxylamine, reduced to Hg\(^0\) using SnCl\(_2\) reduction, purged onto gold traps, thermally desorbed in argon gas, and finally analyzed by cold vapor atomic fluorescence spectroscopy (CVAFS, Tekran 2600, Tekran Inc., Canada).

**MeHg analysis.** The dissolved MeHg was determined by the methods introduced in Bloom (1989) and Liang et al. (1994). Pore water samples were distilled in a pre-acid-washed Teflon vessel to remove the humus. All Hg species in pore waters were further aqueous-phase ethylated with sodium tetraethyl borate, purged onto Tenax traps, thermally desorbed in helium gas, separated by a chromatographic column, and finally converted to Hg\(^0\) and analyzed by CVAFS (Tekran 2700, Tekran Inc., Canada).

Instruments for THg and MeHg were calibrated daily. All coefficients of determination (R\(^2\)) of daily calibration curves were > 0.9975. Each analytical run included 10% method blanks, 10% sample duplicates, 20% matrix spikes, and 10% check standards (made from 1000 ppm stock standard). The detection limit for THg and MeHg was 0.072 ng L\(^{-1}\) and 0.002 ng L\(^{-1}\), respectively. All method blanks were below the detection limit. The relative percentage differences (RPD) were < 10% for all duplicate samples and matrix spikes. Recoveries of
matrix spikes and check standards were 107.53 ± 6.26% and 96 ± 2.76% for THg and MeHg, respectively.

**DOM quantity and characteristics.** DOM concentrations in pore waters were determined by the persulfate wet oxidation method. DOM in pore waters was firstly oxidized to CO$_2$ with the persulfate wet oxidation, and then the amount of CO$_2$ was analyzed by the infrared absorbance of CO$_2$ on the iTOC Aurora 1030 (OI Analytical, College Station, TX, USA). Three check standards were run at the beginning of each run to ensure the stability of the instrument. Each run included 10% method blanks, 10% sample duplicates, 5% matrix spikes, and 10% check standards. Blanks of equipment and method were less than 1 mg L$^{-1}$. All relative standard deviation (RSD) of sample duplicates was < 10%. Recoveries of matrix spikes and check standards were about 105 ± 4.33%.

The characteristics of DOM in pore waters were determined by the specific UV absorbance at 254 nm (SUVA$_{254}$) and optical indices including fluorescence index (FI), humification index (HIX$_{EM}$), and biological index or freshness index (BIX). The SUVA$_{254}$ is an indicator of DOM aromaticity (aromatic molecules contents) (Weishaar et al., 2003). Higher SUVA$_{254}$ indicates higher DOM aromaticity. FI reflects DOM sources with higher FI values (> 1.8) suggesting DOM is microbially derived (originating from processes such as extracellular release and leachate of algae and bacteria) and has lower aromaticity; lower FI values (< 1.2) indicating DOM is terrestrially derived (originating from decomposition and leaching of plant and soil organic matter) and has higher aromaticity (Fellman et al., 2010). HIX$_{EM}$ indicates the humic substance content or the extent of humification that converts simple organic matter derived from animals and plants to more condensed and higher molecular weights organic matters by microbes (Fellman et al., 2010; Hansen et al., 2016; Huguet et al., 2009). High HIX$_{EM}$ values (> 1.0) demonstrate the high humification of DOM that contains more highly condensed and complex molecules. BIX denotes the freshness of DOM
that was generally produced by microorganisms (Fellman et al., 2010). Higher BIX values (> 1.0) mean DOM is more bioaccessible and is predominantly produced by microbes.

The values of SUVA$_{254}$ were measured and calculated using EPA methods (415.3). The absorbance at $\lambda = 254$ nm was analyzed using a Horiba Aqualog® fluorescence spectrofluorometer with a xenon lamp. SUVA$_{254}$ values were determined by dividing the absorbance at 254 nm by the samples' DOM concentration and then multiplying by 100. The reported SUVA$_{254}$ was in the unit of L mg C$^{-1}$ mL$^{-1}$. Fluorescence excitation-emission matrices (EEMs) were measured using a Horiba Aqualog® fluorescence spectrofluorometer with a xenon lamp for calculating informative optical indices (FI, HIX$_{EM}$, and BIX). An ultrapure closed water blank was used to correct the inner-effects during the measurement of EEMs. The reported EEMs from Aqualog® were calculated to optical indices (i.e., BIX and FI) by R Software. There were 10% sample duplicates in each analytical run with their RSD less than 10%.

**Sulfate analysis.** SO$_4^{2-}$ in pore waters was analyzed by ion chromatography on a Dionex ICS-1600 (Thermo Scientific™ Dionex™, Canada). The detection limit of this instrument was 0.02 mg L$^{-1}$. There were 10% method blanks, 10% sample duplicates, 10% matrix spikes, 10% matrix spike duplicates, 10% check standards (made from 1000 ppm stock standard) in each analytical run. Method blanks were below the instrument detection limit. The recoveries of check standards and matrix spikes and duplicates were 105.50 ± 8.25%. All RSDs were < 10%.

### 4.2.4 Statistical analyses

All statistical analyses were performed using Statistica 13.3 (StatSoft. Inc., 2017). The effects of soil temperatures on concentrations of pore water IHg, MeHg, SO$_4^{2-}$, and DOM and characteristics of DOM (SUVA$_{254}$, FI, HIX$_{EM}$, and BIX) were analyzed using a repeated-measures ANOVA for each site separately. Linear regression was used to test the
relationships between concentrations of MeHg and SO$_4^{2-}$ and between the concentrations of Hg (i.e., IHg and MeHg) and DOM. Origin 9.3 (Microcal Software Inc., MA) software was used to visualize data. Data are shown as the mean ± standard deviation (SD). Coefficient of determination ($R^2$) and significance probabilities (p) are presented for linear regression fits, with a level of significance set at $p < 0.05$.

4.3 Results and discussion

4.3.1 Effects of ground warming on physical environmental conditions

**Water table.** Water table levels (below peat soil surface) were approximately 10 cm lower in the moss-dominated fen than in the sedge-dominated fen (Figure 4.3). The mean water table levels during the time from June to August in both fens were generally higher in 2017 than in 2018 and 2019 (the moss-dominated fen: $-14.5 \pm 2.9$ cm (2017), $-16.8 \pm 2.6$ cm (2018), and $-20.3 \pm 5.3$ cm (2019); the sedge-dominated fen: $-0.5 \pm 4.0$ cm (2017), $-5.6 \pm 4.6$ cm (2018), and $-7.0 \pm 3.9$ cm (2019)). The total annual precipitation was approximately 598 mm, 554 mm, and 439 mm in 2017, 2018, and 2019, respectively.
Figure 4.3 Water table levels and daily precipitation in the moss-dominated fen and the sedge-dominated fen over the experiment (2017-2019).

Soil moisture. Warming decreased soil moisture in both fen peatlands (Table 4.1). At the moss-dominated fen, passive warming had no significant effects on soil moisture (p > 0.05); soil moisture decreased approximately 1.5% (% v/v) under active warming ($F_{(1,63)} = 5.51$, $p < 0.05$). At the sedge-dominated fen, soil moistures decreased about 3.0% (% v/v) and 4.1% (% v/v) under passive warming in 2017 and 2018, respectively, (2017: $F_{(1,57)} = 5.00$, $p < 0.05$; 2018: $F_{(1,71)} = 10.37$, $p < 0.05$); passively warmed plots in 2019 had no significant effects on soil moistures ($F_{(1,46)} = 4.01$, $p = 0.05$); soil moisture decreased approximately 6.1% (% v/v) under active warming in 2019 ($F_{(1,58)} = 15.36$, $p < 0.001$).
Table 4.1 Soil moisture (± standard deviation; % v/v) in the control and warmed plots in the moss-dominated fen and the sedge-dominated fen over the experiment.

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Moss-dominated Fen</th>
<th>Sedge-dominated Fen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control plots</td>
<td>Warmed plots</td>
</tr>
<tr>
<td>2017</td>
<td>June</td>
<td>41.8 ± 7.5</td>
<td>41.0 ± 10.5</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>25.4 ± 9.3</td>
<td>23.7 ± 8.2</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>34.4 ± 9.2</td>
<td>32.5 ± 10.4</td>
</tr>
<tr>
<td>2018</td>
<td>June</td>
<td>21.6 ± 9.5</td>
<td>21.0 ± 9.7</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>20.2 ± 9.1</td>
<td>19.2 ± 8.1</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>20.8 ± 9.2</td>
<td>19.8 ± 6.7</td>
</tr>
<tr>
<td>2019</td>
<td>June</td>
<td>16.2 ± 8.8</td>
<td>16.3 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>July</td>
<td>17.5 ± 6.8</td>
<td>15.3 ± 7.1</td>
</tr>
<tr>
<td></td>
<td>August</td>
<td>16.9 ± 6.6</td>
<td>14.9 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>September</td>
<td>15.5 ± 8.4</td>
<td>15.1 ± 8.0</td>
</tr>
<tr>
<td></td>
<td>October</td>
<td>18.2 ± 6.3</td>
<td>13.3 ± 6.1</td>
</tr>
</tbody>
</table>

4.3.2 Response of pore water mercury concentrations to ground warming

Inorganic mercury. At the moss-dominated fen (Figure 4.4a), both passive warming (+0.38 °C; (Lyons et al., 2020)) and active warming (+4.5 °C) significantly increased pore water IHg concentrations over the experiment (passive warming (2017: $F_{(1,87)} = 15.62$, $p < 0.001$; 2018: $F_{(1,55)} = 5.97$, $p < 0.05$; 2019: $F_{(1,31)} = 6.48$, $p < 0.05$); active warming (2019: $F_{(1,39)} = 9.03$, $p < 0.05$). At the sedge-dominated fen (Figure 4.4b), there was no significant effect of passive warming (+0.27 °C; (Lyons et al., 2020)) on pore water IHg concentrations in either 2017 or 2018 (2017: $F_{(1,87)} = 1.79$, $p = 0.18$; 2018: $F_{(1,55)} = 3.01$, $p = 0.09$). Passive
warming in 2019 significantly increased pore water IHg concentrations ($F_{(1,31)} = 5.00, p < 0.05$). There was no significant impact of active warming (+3.8 °C) in 2019 on pore water IHg concentrations ($F_{(1,47)} = 0.53, p = 0.47$). Indeed under active warming, IHg concentrations in pore waters were slightly lower in the actively warmed plots (mean = 11.41 ± 0.88 ng L$^{-1}$) than in the control plots (mean = 12.52 ± 1.62 ng L$^{-1}$).
Figure 4.4 Concentrations of inorganic mercury (IHg) and methylmercury (MeHg) and the proportion of total Hg (THg) as MeHg (%MeHg) in pore waters in the control and warmed plots in the moss-dominated fen and the sedge-dominated fen over the experiment. Pore water IHg concentrations in A) the moss-dominated fen and B) the sedge-dominated fen; pore water MeHg concentrations in C) the moss-dominated fen and D) the sedge-dominated fen; %MeHg changes in pore waters in E) the moss-dominated fen and F) the sedge-dominated fen. Colors represent the following sources: black for the control plots; orange for the passively warmed plots; red for the actively warmed plots. Each value represents the mean ± standard deviation.
These results indicate that soil temperature acted as a significant control of pore water IHg concentrations in the moss-dominated fen. Previous studies showed that rising temperatures increased decomposition rates of soil organic matter and then DOM concentrations (Dieleman et al., 2016; Kalbitz et al., 2000), which subsequently can promote the release of IHg from peat soils, given that DOM is a Hg carrier facilitating Hg mobility (Ravichandran, 2004; Xia et al., 1999). This is supported by the results that pore water DOM concentrations were significantly higher under elevated ground temperatures in the moss-dominated fen (see section 4.3.3). At the sedge-dominated fen, warming was also expected to increase the decomposition rate and then IHg release from peat soils; however, there were generally no significant effects of warming on pore water IHg concentrations over the experiment. High water table levels in 2017 corresponding with more anaerobic conditions might counteract the enhancing impact of passively elevated temperatures on metabolisms of the dominant decomposers-aerobes. In 2018, although higher pore water DOM concentrations in the passively warmed plots than in the control plots (see section 4.3.3) reflected a higher decomposition rate, there was no increase in pore water IHg concentrations under warmer conditions. One likely reason is that warmer conditions increased Hg methylation, which was denied by the below result that pore water MeHg concentrations were significantly lower in the passively warmed plots than in the control plots (Figure 4.4d). Another plausible explanation is that increased DOM under warmer conditions may enhance photochemical reduction of IHg (Ravichandran, 2004). More studies are needed to elucidate this mechanism. In 2019, the slight decreases in pore water IHg concentrations under the active warming can be partially attributed to having more IHg being converted to MeHg under active warming, given that there were higher MeHg concentrations in the actively warmed plots than in the control plots (Figure 4.4d).
A decrease in soil moisture due to ground warming in both fen peatlands was expected to increase the oxidative release of IHg from peat soils to pore waters (Coleman-Wasik *et al.*, 2015). However, decreases in soil moisture under warming generally did not correspond with a compensatory increase but instead a decrease in pore water IHg concentrations in experimental months. A plausible explanation is that the effects of soil moisture on pore water IHg concentrations were very little compared to ground warming in this study.

Pore water IHg concentrations were different between the experimental plots (the control and warmed plots) and the reference plots, among years, and between sites (Table 4.2). At both fen peatlands, the mean IHg concentrations in pore waters of each year were approximately 2-fold higher in the experimental plots than in the reference plots. One plausible explanation is more IHg was released from peat soils to pore waters together with DOM in the experimental plots, given that the mean DOM concentrations were significantly higher in the experimental plots than in the reference plots over the experiment in both fen peatlands (see section 4.3.3). Another likely reason is more IHg was converted to MeHg in the reference plots, based on the observation that MeHg concentrations were higher in the reference plots than in the experimental plots over the experiment in both fen peatlands, except in 2019 in the sedge-dominated fen that active warming in 2019 significantly increased both concentrations of IHg and MeHg in pore waters.
Table 3.2 The mean concentrations of inorganic mercury (IHg) and methylmercury (MeHg) and the percentage of total Hg (THg) as MeHg concentrations (%MeHg) over the experiment of each year in the moss-dominated fen and the sedge-dominated fen. Values are presented as mean ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Moss-dominated fen</th>
<th>Sedge-dominated fen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reference plots</td>
<td>Treatment plots</td>
</tr>
<tr>
<td>IHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2017</td>
<td>2.11 ± 0.57</td>
<td>3.71 ± 2.15</td>
</tr>
<tr>
<td>2018</td>
<td>2.26 ± 0.87</td>
<td>4.46 ± 3.47</td>
</tr>
<tr>
<td>2019</td>
<td>2.27 ± 0.80</td>
<td>4.13 ± 3.34</td>
</tr>
<tr>
<td>MeHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2017</td>
<td>0.65 ± 0.27</td>
<td>0.51 ± 0.29</td>
</tr>
<tr>
<td>2018</td>
<td>0.53 ± 0.24</td>
<td>0.41 ± 0.25</td>
</tr>
<tr>
<td>2019</td>
<td>0.55 ± 0.18</td>
<td>0.47 ± 0.29</td>
</tr>
<tr>
<td>%MeHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2017</td>
<td>25.44 ± 12.07</td>
<td>14.43 ± 8.42</td>
</tr>
<tr>
<td>2018</td>
<td>21.03 ± 11.44</td>
<td>11.47 ± 8.41</td>
</tr>
<tr>
<td>2019</td>
<td>20.65 ± 8.21</td>
<td>12.25 ± 7.77</td>
</tr>
</tbody>
</table>

The mean pore water IHg concentrations in the experimental plots were highest in 2018 and 1.20 and 1.11 times higher in 2018 and 2019 than in 2017, respectively, in the moss-dominated fen. 2019 was 1.38 and 1.32 fold higher in pore water IHg concentrations in the experimental plots than in 2017 and 2018, respectively, in the sedge-dominated fen. These results are likely because of the interactive effects of temperatures and drought conditions on pore water IHg concentrations. The wetter conditions provide more anaerobic environments for Hg methylation at the expense of IHg (Ullrich et al., 2001). Moderately higher temperatures increase Hg methylation by stimulating SRB activity (Ullrich et al., 2001). Drought and re-wetting events can increase the oxidative release of Hg from peat soils (Coleman-Wasik et al., 2015). Drought can also disrupt soil biochemical and physical
structures (e.g., soil aggregates) (Kaiser et al., 2015) and then increase the release of potential IHg from peat soils during re-wetting events. At the moss-dominated fen, lower pore water IHg concentrations in 2017 and 2019 than in 2018 can be attributed to higher Hg methylation under wetter environments in 2017 and under warmer conditions in 2019, respectively, which was supported by the results of pore water MeHg concentrations that were higher in 2017 and 2019 than in 2018. Higher pore water IHg concentrations in 2018 and 2019 than in 2017 were likely due to increased oxidative release of IHg from drier peat soils in 2018 and 2019. At the sedge-dominated fen, pore water IHg concentrations were higher in 2019 than in 2018, which might be attributed to more oxidative releases of IHg from the drier peat soils in 2019. There were very high pore water IHg concentrations in several individual sampling events (moss-dominated fen: October 4, 2017, October 7, 2018; sedge-dominated fen: August 13, 2017, August 27, 2017, July 11, 2018, September 16, 2019) after heavy precipitation events, confirming that drought conditions and re-wetting events played an important role in IHg release from peat soils. Not all sampling events that after re-wetting events had very high pore water IHg concentrations due to the dilution of IHg.

The sedge-dominated fen was overall higher in pore water IHg concentrations than the moss-dominated fen. The mean IHg concentrations in pore waters were 1.98, 1.58, and 2.42-fold higher in the sedge-dominated fen than those in the moss-dominated fen in 2017, 2018, and 2019, respectively. There are several plausible reasons: 1) sedge-dominated fen received more IHg from groundwater ecosystem and upland runoff, given its higher water table levels and connection to surface water whereas moss-dominated fen had lower water table levels and was disconnected to surface waters; 2) moss-dominated fen had a lower shading compared to the vascular plant-dominated fen, which may increase the photo-reduction of Hg$^{2+}$ to Hg$^0$ (Gustin et al., 2006b); 3) there was more IHg input from litterfall to peat soils in the sedge-dominated fen than in the moss-dominated fen, given that litterfall is an important Hg input to soils (Ericksen et al., 2003; Hintelmann et al., 2002; Risch et al., 2012; St. Louis
et al., 2001) and the annual input of aboveground vegetation biomass to peat soils is higher for sedges than mosses (Frolking et al., 2001; Thormann and Bayley, 1997); 4) more IHg was transformed to MeHg in the moss-dominated fen, based on the higher pore water MeHg concentrations than in the sedge-dominated fen (Figure 4.4c and 4.4d).

**Methylmercury.** There was no significant effect of passive warming on pore water MeHg concentrations in the moss-dominated fen (2017: $F_{(1,87)} = 1.24$, $p = 0.27$; 2018: $F_{(1,55)} = 2.62$, $p = 0.11$; 2019: $F_{(1,31)} = 0.78$, $p = 0.38$; Figure 4.4c). Active warming significantly increased pore water MeHg concentrations ($F_{(1,39)} = 9.50$, $p < 0.05$). At the sedge-dominated fen, pore water MeHg concentrations were significantly higher in the control plots than in the passively warmed plots in both 2017 ($F_{(1,87)} = 8.65$, $p < 0.05$) and 2018 ($F_{(1,54)} = 14.09$, $p < 0.001$; Figure 4.3d). There was no significant effect of passive warming on pore water MeHg concentrations in 2019 ($F_{(1,31)} = 0.69$, $p = 0.41$). Active warming in 2019 significantly increased pore water MeHg concentrations ($F_{(1,46)} = 18.93$, $p < 0.001$).

It has been established that MeHg concentrations in sediments are controlled by both Hg methylation and demethylation (Martín-Doimeadios et al., 2004; Pak and Bartha, 1998; Ullrich et al., 2001). Previous studies found that MeHg demethylation rates peaked early in the summer and then decreased rapidly, whereas Hg methylation rates peaked in the late summer and decreased in fall and thus pointed out that both Hg methylation rates and demethylation rates increased in summer but relatively higher temperatures more favored Hg methylation whereas relatively lower temperatures more favored MeHg demethylation (Korthals and Winfrey, 1987; Ramlal et al., 1993). Field investigations (Canario et al., 2007; Hintelmann and Wilken, 1995; Korthals and Winfrey, 1987) and stable isotope tracer experiments (Hudelson et al., 2020; Mauro et al., 1999; St Pierre et al., 2014) confirmed that Hg methylation rates increased under higher temperatures, likely because higher temperatures increase the metabolism of all microbes, including Hg methylators (Bisogni Jr
and Lawrence, 1975; Ullrich et al., 2001; Yang et al., 2016). Some studies found that Hg methylation potentials increased under elevated temperatures (≥ + 4 °C) but demethylation potentials did not show a compensatory increase with warming (Hudelson et al., 2020; St Pierre et al., 2014), supporting the result that the active warming in this study had greater effects on increasing net Hg methylation than promoting MeHg demethylation, although MeHg demethylation rates were not part of this study. Passively elevated temperatures did not increase pore water MeHg concentrations in the moss-dominated fen and even decreased pore water MeHg concentrations in the sedge-dominated fen, supporting that a slight increase in temperature is more favorable to MeHg demethylation than Hg methylation (Korthals and Winfrey, 1987; Ramlal et al., 1993).

Except for net MeHg production, pore water MeHg in actively warmed plots may also be released from soil organic matter. Warmer conditions can stimulate microbial metabolism and subsequently increase decomposition rates and then DOM concentrations (Kalbitz et al., 2000), which consequently increases the release of MeHg from peat soils to pore water, given the strong combination between Hg and the reduced sulfur groups in DOM (Xia et al., 1999). This is supported by the result that both pore water DOM and MeHg concentrations were significantly increased under actively elevated temperatures (see section 4.3.3). The increased net MeHg production under active warming in the sedge-dominated fen can also be particularly attributed to the significantly increased pore water SO₄²⁻ concentrations under active warming (Figure 4.5), given that SO₄²⁻ is generally a limiting nutrient for SRB in northern peatlands and the addition of SO₄²⁻ significantly increased the MeHg production (Branfireun et al., 1999; Mitchell et al., 2008a). There were no significant increases in pore water SO₄²⁻ concentrations in the passively warmed plots in both fens (p > 0.05). Active warming had no significant effects on pore water SO₄²⁻ concentrations in the moss-dominated fen (p > 0.05). Active warming significantly increased pore water SO₄²⁻ concentrations in the
sedge-dominated fen (F(1,47) = 24.31; p < 0.001). It has been established that rising
temperature stimulates SRB metabolism and then SO$_4^{2-}$ reduction (Holmer and Storkholm, 2001). The drier conditions, however, increase the oxidation of reduced sulfur compounds (Jokic et al., 2003; Mandernack et al., 2000). The increase in pore water SO$_4^{2-}$ concentrations in the actively warmed plots in the sedge-dominated fen peatlands can be attributed to the significantly decreased soil moistures under active warming. There was a significant increase in soil moisture but not in pore water SO$_4^{2-}$ concentrations in the passively warmed plots in 2017 and 2018 in the sedge-dominated fen and the actively warmed plots in 2019 in the moss-dominated fen than in the control plots. A plausible explanation is the enhanced effect of drier peat soils on the oxidation of reduced sulfur compounds (Jokic et al., 2003; Mandernack et al., 2000) might be counteracted by higher temperature effects on increasing SO$_4^{2-}$ reduction (Holmer and Storkholm, 2001) and gaseous S evasion (Åkerblom et al., 2013).

There were no significant relationships between concentrations of MeHg and SO$_4^{2-}$ in all treatments (control, passive warming, active warming) in the moss-dominated fen and the control and active warming treatments in the sedge-dominated fen. A significantly negative relationship was found between concentrations of MeHg and SO$_4^{2-}$ in the passive warming treatment in the sedge-dominated fen (F(1,140) = 4.42, p < 0.05). Significant relationships between concentrations of MeHg and SO$_4^{2-}$ do not always exist, given that SO$_4^{2-}$ is not the only factor controlling net MeHg production (Ullrich et al., 2001). The negative relationship between concentrations of MeHg and SO$_4^{2-}$ in the passive warming treatment in the sedge-dominated fen was consistent with the previous study that net MeHg production continued to increase with declining SO$_4^{2-}$ concentrations (Coleman-Wasik et al., 2015).
Figure 4.5 Changes in sulfate (SO$_4^{2-}$) concentrations (± standard deviation) under elevated temperature conditions in A) the moss-dominated fen and B) the sedge-dominated fen.

Pore water MeHg concentrations differed between the experimental plots and the reference plots, among years, and between sites (Table 4.2). The mean MeHg concentrations of each year were 1.27, 1.29, and 1.17-fold higher in the reference plots than in the experimental plots in 2017, 2018, and 2019, respectively, in the moss-dominated fen. The mean MeHg concentrations in the reference plots were 2.08 and 1.54-fold higher than that in the treatment plots in 2017 and 2018, respectively, but were 0.65 times that in the experimental plots in
2019 in the sedge-dominated fen. At the moss-dominated fen, higher pore water MeHg concentrations in the reference plots appeared to be predominantly determined by other factors, such as IHg bioavailability, rather than by SO$_4^{2-}$, given that pore water SO$_4^{2-}$ concentrations were higher in the reference plots than in the experimental plots only in 2019 (Table 4.3). At the sedge-dominated fen, the reference plots were in the upland-peatland interface zones, where can receive nutrients, such as SO$_4^{2-}$, from upland runoff making these zones “MeHg hot spots”. This was supported by higher SO$_4^{2-}$ concentrations in the reference plots than in the experimental plots in 2017 and 2018. The concentrations of MeHg and SO$_4^{2-}$ in 2019 were lower in the reference plots than in the experimental plots, which is likely due to the increased net MeHg production under active warming and the increased oxidative releases of SO$_4^{2-}$ and MeHg under drier conditions in the experimental plots.

Table 4.3 The mean concentrations of sulfate (SO$_4^{2-};$ mg L$^{-1}$) over the experiment in the moss-dominated fen and the sedge-dominated fen. Values are presented as mean ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Moss-dominated fen</th>
<th>Sedge-dominated fen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reference plots</td>
<td>Treatment plots</td>
</tr>
<tr>
<td>2017</td>
<td>0.14 ± 0.07</td>
<td>0.17 ± 0.10</td>
</tr>
<tr>
<td>2018</td>
<td>0.31 ± 0.23</td>
<td>0.34 ± 0.23</td>
</tr>
<tr>
<td>2019</td>
<td>0.45 ± 0.59</td>
<td>0.33 ± 0.54</td>
</tr>
</tbody>
</table>

Pore water MeHg concentrations in the experimental plots were overall lowest in 2018 in both fen peatlands, which can be attributed to the interactive effects of water table decrease and temperature increase. Wetter environments in 2017 provided more anaerobic conditions for net MeHg production. Actively elevated temperatures in 2019 significantly increased net
MeHg production. Therefore, higher pore water MeHg concentrations in 2017 and 2019 than in 2018 were because more MeHg was produced under wetter conditions in 2017 and under warmer peat soils in 2019.

The moss-dominated fen generally had 1.38, 1.71, and 1.17-fold higher pore water MeHg concentrations in the experimental plots than the sedge-dominated fen in 2017, 2018, and 2019, respectively. Water table levels were lower in the moss-dominated fen than in the sedge-dominated fen, resulting in more oxidative releases of MeHg from peat soils. There were also higher net MeHg productions in the moss-dominated fen than in the sedge-dominated fen, based on that moss-dominated fen had lower pore water IHg concentrations but higher pore water MeHg concentrations than the sedge-dominated fen.

**Percentage of total Hg as MeHg.** Percentage of total Hg as MeHg concentrations (%MeHg) is often used as an indication of net MeHg production in sediment (Benoit et al., 2003; Paranjape and Hall, 2017a; Sunderland et al., 2004). %MeHg alone in waters or pore waters cannot entirely reflect net MeHg production because MeHg in pore waters is affected not only by net MeHg production but also by the decoupling of MeHg between submerged soils and overlying waters (Liu et al., 2020; Skyllberg, 2008). %MeHg values in this study were used only to support the explanation of the results of MeHg concentrations in pore waters over the experiment.

At the moss-dominated fen, there were no significant differences in the mean %MeHg between the passively warmed plots (2017: 13.85 ± 8.36%; 2018: 11.36 ± 8.73%; 2019: 6.77 ± 3.30%) and the control plots (2017: 15.02 ± 8.49%; 2018: 11.59 ± 8.15%; 2019: 9.31 ± 4.86%); the mean %MeHg was slightly higher in the actively warmed plots than in the control plots in 2019 (actively warmed plots: 16.99 ± 9.60%; control plots: 13.50 ± 7.12%) (Figure 4.4e). For the sedge-dominated fen, there were no significant differences in %MeHg between the passively warmed plots (2017: 5.30 ± 2.13%; 2018: 3.18 ± 1.30%; 2019: 3.12 ±
1.53%) and the control plots (2017: 5.55 ± 2.24%; 2018: 4.15 ± 2.09%; 2019: 3.28 ± 1.43%); the mean %MeHg was slightly higher in the actively warmed plots (5.26 ± 4.21%) than in the control plots (3.79 ± 2.68%; Figure 4.4f). These results are consistent with the suggestion that active warming increased Hg methylation and/or release of MeHg from peat soils to pore waters.

At both fen peatlands, the mean %MeHg of each year was lower in the experimental plots than in the reference plots, supporting that net MeHg production was higher in the reference plots. %MeHg was overall higher in 2017 than in 2018 and 2019 and lowest in 2018 in both fen peatlands, confirming that there were highest net MeHg production rates in 2017 with the highest water table among years, and there was higher net MeHg production in 2019 than in 2018 due to the active warming. The mean %MeHg was higher in the moss-dominated fen than the sedge-dominated fen, supporting that net MeHg production was higher in the moss-dominated fen.

4.3.3 Response of dissolved organic matter concentrations and characteristics to ground warming

**Dissolved organic matter concentrations.** At the moss-dominated fen, both passive warming and active warming significantly increased DOM concentrations in pore waters (2017: $F_{(1,86)} = 19.32$, $p < 0.001$; 2018: $F_{(1,55)} = 12.27$, $p < 0.001$; 2019 (passive warming: $F_{(1,28)} = 14.58$, $p < 0.001$; active warming: $F_{(1,39)} = 7.25$, $p < 0.05$); Figure 4.6a). At the sedge-dominated fen, passive warming had no significant effects on pore water DOM concentrations in 2017 ($F_{(1,87)} = 0.68$, $p = 0.41$); passive warming significantly increased pore water DOM concentrations in 2018 ($F_{(1,54)} = 4.39$, $p < 0.05$) and in 2019 ($F_{(1,31)} = 8.48$, $p < 0.05$); active warming significantly increased DOM concentrations in pore waters in 2019 ($F_{(1,47)} = 4.89$, $p < 0.05$; Figure 4.6b). These results revealed that elevated temperature generally increased pore water DOM concentrations, which is likely due to higher
decomposition rates under higher temperatures (Dieleman et al., 2016; O'Donnell et al., 2016).

![Figure 4.6 Dissolved organic matter (DOM) concentrations (± standard deviation) in A) the moss-dominated fen and B) the sedge-dominated fen.](image)

Pore water DOM concentrations differed between the experimental plots and the reference plots, among years, and between sites (Table 4.4). At both fen peatlands, the mean DOC concentrations were higher in the experimental plots than in the reference plots over the experiment, confirming that warming increased pore water DOM concentrations. Pore water DOM concentrations in the experimental plots were decreasing annually in both fens, which is contrary to previous studies in which DOM concentrations increased with the decreasing
water tables (Dieleman et al., 2016; Höll et al., 2009; Hribljan et al., 2014; Worrall et al., 2006) and then exposing previously saturated soils to air and consequently leading to an increase in decomposition rates of SOM (Dieleman et al., 2016; Fenner and Freeman, 2011; Kwon et al., 2013; Worrall et al., 2006). A plausible explanation is the aerobic conditions under drier peat soils may also increase the mineralization or efficient decomposition of DOM (Kalbitz et al., 2000).

Pore water DOM concentrations were 1.54, 1.47, and 1.23 fold higher in the moss-dominated fen than in the sedge-dominated fen in 2017, 2018, and 2019, respectively. Peat soils are primarily comprised of partially decomposed plants (Rydin and Jeglum, 2013), and thus the dominant plants play an essential role in controlling DOM characteristics and concentrations. It is established that sedges contain a lower ratio of carbon: nitrogen and are more bioaccessible than mosses (Hobbie, 1996; Lyons and Lindo, 2019), given that nitrogen is a limiting nutrient to decomposers (Manzoni et al., 2010; Moore and Basiliko, 2006; Parton et al., 2007). DOM from root exudates of sedges is also more bioaccessible than that from Sphagnum spp. mosses root exudates (Bragazza et al., 2013; Dieleman et al., 2017). More bioaccessible DOM, therefore, can be consumed by microbes in the sedge-dominated fen compared to the DOM with higher aromaticity and lower bioaccessibility in the moss-dominated fen. Water table levels were more than 10 cm lower in the moss-dominated fen than in the sedge-dominated fen, which may further lead to the high pore water DOM concentrations in the moss-dominated fen because of the exposure of more peat soils to air promoting decomposition rates of SOM (Dieleman et al., 2016).
Table 4.4 The mean concentrations of dissolved organic matter (DOM; mg L\(^{-1}\)) over the experiment in the moss-dominated fen and the sedge-dominated fen. Values are presented as mean ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Moss-dominated fen</th>
<th>Sedge-dominated fen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference plots</td>
<td>Treatment plots</td>
<td>Reference plots</td>
</tr>
<tr>
<td>2017</td>
<td>38.85 ± 9.20</td>
<td>43.68 ± 10.35</td>
</tr>
<tr>
<td>2018</td>
<td>38.28 ± 6.44</td>
<td>41.55 ± 9.31</td>
</tr>
<tr>
<td>2019</td>
<td>33.86 ± 9.00</td>
<td>34.41 ± 10.57</td>
</tr>
</tbody>
</table>

Dissolved organic matter characteristics. Values of SUVA\(_{254}\), FI, HIX\(_{EM}\), and BIX in both fens are shown in Table 4.5. At the moss-dominated fen, SUVA\(_{254}\) was significantly higher in the passively warmed plots than in the control plots in both 2017 (F\(_{(1,86)}\) = 12.47, p < 0.001) and 2018 (F\(_{(1,55)}\) = 9.00, p < 0.05) but not in 2019 (F\(_{(1,30)}\) = 2.27, p = 0.14). There was no significant impact of passive warming on FI in pore waters over the experiment (2017: F\(_{(1,87)}\) = 2.01, p = 0.16; 2018: F\(_{(1,55)}\) = 0.59, p = 0.44; 2019: F\(_{(1,30)}\) = 0.0009, p = 0.97). Values of HIX\(_{EM}\) were significantly higher in the passively warmed plots than in the control plots in 2017 (F\(_{(1,87)}\) = 4.50, p < 0.05) and 2019 (F\(_{(1,30)}\) = 8.66, p < 0.05) but not in 2018 (F\(_{(1,55)}\) = 0.004, p = 0.95). Passively warmed plots had significantly higher BIX than the control plots only in 2019 (2017: F\(_{(1,87)}\) = 3.82, p = 0.05; 2018: F\(_{(1,55)}\) = 3.34, p = 0.07; 2019: F\(_{(1,30)}\) = 6.11, p < 0.05). There was no significant effect of active warming on SUVA\(_{254}\) (F\(_{(1,39)}\) = 2.66, p = 0.11), FI (2019: F\(_{(1,39)}\) = 2.06, p = 0.16), HIX\(_{EM}\) (F\(_{(1,39)}\) = 0.73, p = 0.40), and BIX (F\(_{(1,39)}\) = 0.04, p = 0.85). These results suggested that DOM had higher aromaticity in the passively warmed plots than in the control plots in both 2017 and 2018, and there was no significant difference in the aromaticity of DOM between the warmed plots and the control plots in
Table 4.5 The mean observations and standard deviation of specific ultraviolet absorbance at 254 nm (SUVA$_{254}$, L mg C$^{-1}$ mL$^{-1}$) and fluorescence indices (FI, HIX$_{EM}$, and BIX) in pore waters under the ground warming treatment at both sites. Passive warming was from June to October in 2017 and 2018 and in June and October of 2019; active warming was from July to September in 2019. Values are presented as mean ± standard deviation.

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Control plots</th>
<th>Warmed plots</th>
<th>Control plots</th>
<th>Warmed plots</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUVA$_{254}$</td>
<td>2017 June-October</td>
<td>4.204 ± 0.475</td>
<td>4.321 ± 0.579</td>
<td>3.998 ± 0.452</td>
<td>3.965 ± 0.456</td>
</tr>
<tr>
<td></td>
<td>2018 June-October</td>
<td>4.601 ± 0.372</td>
<td>4.840 ± 0.509</td>
<td>4.629 ± 0.334</td>
<td>4.614 ± 0.377</td>
</tr>
<tr>
<td></td>
<td>2019 June and October</td>
<td>4.571 ± 0.451</td>
<td>4.668 ± 0.591</td>
<td>4.729 ± 0.444</td>
<td>4.674 ± 0.511</td>
</tr>
<tr>
<td></td>
<td>July-September</td>
<td>5.300 ± 0.471</td>
<td>5.165 ± 0.451</td>
<td>5.550 ± 0.446</td>
<td>5.312 ± 0.503</td>
</tr>
<tr>
<td>FI</td>
<td>2017 June-October</td>
<td>1.328 ± 0.083</td>
<td>1.341 ± 0.087</td>
<td>1.526 ± 0.112</td>
<td>1.523 ± 0.114</td>
</tr>
<tr>
<td></td>
<td>2018 June-October</td>
<td>1.306 ± 0.037</td>
<td>1.302 ± 0.037</td>
<td>1.506 ± 0.065</td>
<td>1.512 ± 0.062</td>
</tr>
<tr>
<td></td>
<td>2019 June and October</td>
<td>1.326 ± 0.036</td>
<td>1.325 ± 0.041</td>
<td>1.487 ± 0.028</td>
<td>1.491 ± 0.039</td>
</tr>
<tr>
<td></td>
<td>July-September</td>
<td>1.323 ± 0.143</td>
<td>1.314 ± 0.049</td>
<td>1.504 ± 0.043</td>
<td>1.530 ± 0.041</td>
</tr>
<tr>
<td>HIX$_{EM}$</td>
<td>2017 June-October</td>
<td>0.943 ± 0.042</td>
<td>0.951 ± 0.051</td>
<td>0.951 ± 0.019</td>
<td>0.952 ± 0.019</td>
</tr>
<tr>
<td></td>
<td>2018 June-October</td>
<td>0.460 ± 0.010</td>
<td>0.460 ± 0.011</td>
<td>0.454 ± 0.012</td>
<td>0.452 ± 0.015</td>
</tr>
<tr>
<td></td>
<td>2019 June and October</td>
<td>0.771 ± 0.181</td>
<td>0.784 ± 0.182</td>
<td>0.816 ± 0.206</td>
<td>0.807 ± 0.214</td>
</tr>
<tr>
<td></td>
<td>July-September</td>
<td>0.467 ± 0.012</td>
<td>0.471 ± 0.013</td>
<td>0.462 ± 0.010</td>
<td>0.458 ± 0.010</td>
</tr>
<tr>
<td>BIX</td>
<td>2017 June-October</td>
<td>0.379 ± 0.018</td>
<td>0.375 ± 0.017</td>
<td>0.477 ± 0.026</td>
<td>0.479 ± 0.026</td>
</tr>
<tr>
<td></td>
<td>2018 June-October</td>
<td>0.427 ± 0.010</td>
<td>0.425 ± 0.010</td>
<td>0.531 ± 0.044</td>
<td>0.563 ± 0.103</td>
</tr>
<tr>
<td></td>
<td>2019 June and October</td>
<td>0.437 ± 0.014</td>
<td>0.431 ± 0.014</td>
<td>0.519 ± 0.014</td>
<td>0.526 ± 0.018</td>
</tr>
<tr>
<td></td>
<td>July-September</td>
<td>0.433 ± 0.017</td>
<td>0.435 ± 0.011</td>
<td>0.534 ± 0.029</td>
<td>0.560 ± 0.026</td>
</tr>
</tbody>
</table>
2019; passive warming significantly increased concentrations of more condensed DOM in 2017 and 2019 and more bioaccessible DOM in 2019 in the moss-dominated fen.

At the sedge-dominated fen, passive warming had no significant effects on SUVA\textsubscript{254} (p > 0.05), FI (p > 0.05), HIX\textsubscript{EM} (p > 0.05), and BIX (p > 0.05). Active warming significantly decreased SUVA\textsubscript{254} (F\textsubscript{(1,47)} = 12.50, p < 0.001) but significantly increased FI (F\textsubscript{(1,47)} = 17.25, p < 0.001), HIX\textsubscript{EM} (F\textsubscript{(1,55)} = 19.59, p < 0.001), and BIX (F\textsubscript{(1,47)} = 56.01; p < 0.001). These results indicated that passive warming had no significant effects on DOM characteristics, but active warming significantly increased the concentrations of more bioaccessible DOM.

O'Donnell \textit{et al.} (2016) observed that warming increased the concentrations of DOM with higher aromaticity by enhancing the decomposition rates of SOM. Warming can also increase concentrations of bioaccessible DOM by stimulating microbial metabolism and increasing plant root exudates (Dieleman \textit{et al.}, 2016; Kane \textit{et al.}, 2014). DOM characteristics in the moss-dominated fen and sedge-dominated fen reflected that warming increased concentrations of both aromatic and bioaccessible DOM by enhancing both decomposition rates of SOM and microbial metabolisms and plant root exudates, and with the increase of soil temperature, relatively more bioaccessible DOM was produced.

### 4.3.4 Relationships between pore water concentrations of mercury and dissolved organic matter under ground warming

Relationships between concentrations of Hg (IHg and MeHg) and DOM are shown in Figure 4.7. Based on linear regression analysis, relationships between concentrations of IHg and DOM were not significant in both fens. Previous studies have reported correlations between Hg and DOM concentrations (Brigham \textit{et al.}, 2009; Dennis \textit{et al.}, 2005; Grigal, 2002; Riscassi and Scanlon, 2011; Yin and Balogh, 2002). However, the positive correlation between Hg and DOM concentrations is not always found in natural environments (Hurley \textit{et al.}, 1998). Although only a small fraction of reduced sulfur groups in organic matter is
available for binding with Hg (Haitzer et al., 2002; Ravichandran, 2004), the reduced sulfur groups in the organic matter still far exceed Hg amount in the natural aquatic environments (Ravichandran, 2004). The noncorrelated concentrations of IHg and DOM did not mean that DOM was not important for Hg mobility, given the strong affinity between Hg and reduced sulfur groups in DOM (Xia et al., 1999).

pH can influence the binding of Hg to DOM (Haitzer et al., 2003; Ravichandran, 2004), with lower pH inhibiting the complexation of Hg and DOM (Barkay et al., 1997). Although the warming treatment had no significant effects on pH in both sites (p > 0.05), the mean pH was higher in the sedge-dominated fen (5.49) than that in the moss-dominated fen (4.19) over the experiment, which may have led to weaker relationships between Hg and DOM in the moss-dominated fen than in the sedge-dominated fen.

There were no significantly linear relationships between MeHg concentrations and DOM concentrations in all treatments in both fen peatlands. It has been established that net MeHg production is controlled by many factors such as temperature, SO$_4^{2-}$ concentrations, DOM concentrations and characteristics (Ullrich et al., 2001), not just by DOM concentrations (Mitchell et al., 2008a). In this study, the increased temperature corresponding with increases in SO$_4^{2-}$ and DOM concentrations may interact and then promote the net MeHg production. In addition, pore water MeHg concentrations are not only controlled by net MeHg production but also by the release of MeHg from peat soils, which can explain the lacking correlations between concentrations of MeHg and DOM in these treatments.
Figure 4.7 Correlations A) between concentrations of inorganic mercury (IHg) and dissolved organic matter (DOM) and B) between concentrations of methylmercury (MeHg) and DOM in the moss-dominated fen; correlations C) between concentrations of IHg and DOM and D) between concentrations of MeHg and DOM in three treatments in the sedge-dominated fen. Each point denotes the one-time point at one plot.
4.4 Conclusions

Global warming may affect Hg cycling in northern peatlands, therefore, we studied the influences of elevated temperatures on Hg cycling in two types of northern peatlands: moss-dominated fen and sedge-dominated fen. At the moss-dominated fen, both passive and active warming significantly increased pore water IHg concentrations. There were no significant effects of passive warming on pore water MeHg concentrations, but active warming significantly increased MeHg concentrations in pore waters. At the sedge-dominated fen, both passive and active warming generally did not affect pore water IHg concentrations. Indeed under active warming, IHg concentrations in pore waters were slightly decreased. Passive warming significantly decreased pore water MeHg concentrations, whereas active warming significantly increased MeHg concentrations in pore waters. Although these results showed that higher soil temperatures can significantly increase pore water MeHg concentrations at least in a short term (several years), the amounts of MeHg that will be exported from both types of northern peatlands to downstream ecosystems remains unknown. Future studies of quantification of the amounts of runoff should be done to have a better prediction of the amounts of MeHg that will be exported to downstream ecosystems. Temperature increasing is expected to alter vegetation community composition (Dieleman et al., 2015; Turetsky et al., 2012; Weltzin et al., 2000) that plays an important role in controlling Hg input via litterfall (Risch et al., 2012; Wang et al., 2016), DOM concentrations and characteristics in pore waters (Bragazza et al., 2013; Dieleman et al., 2017; Hobbie, 1996; Lyons and Lindo, 2019), all of which are controls on MeHg production in northern peatlands. In addition, microbial metabolism will adapt to the warming soil conditions with time (Bradford, 2013; Melillo et al., 2002). A long-term field experiment, therefore, is needed to better understand the effects of global warming on Hg cycling in northern peatlands.
4.5 References


Bloom, N. (1989). Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. Canadian Journal of Fisheries and Aquatic Sciences 46, 1131-1140.


Science and Technology 36, 3564-3570.
mercury methylation across a peatland chronosequence: from sulfate reduction to

methylation and demethylation potentials in Arctic lake sediments. Chemosphere
248, 126001.

Properties of fluorescent dissolved organic matter in the Gironde Estuary. Organic
Geochemistry 40, 706-719.


Jeremiason, J. D., Engstrom, D. R., Swain, E. B., Nater, E. A., Johnson, B. M.,
methylmercury production in an experimental wetland. Environmental Science and
Technology 40, 3800-3806.


Kane, E. S., Mazzoleni, L. R., Kratz, C. J., Hribljan, J. A., Johnson, C. P., Pypker, T. G.,
Chimner, R. (2014). Peat porewater dissolved organic carbon concentration and
lability increase with warming: a field temperature manipulation experiment in a


decomposition, and the effect of global warming on soil organic C storage. Soil
Biology and Biochemistry 27, 753-760.


changes in decomposition and microbial properties in tropical peat soils exposed to
the short-term drought. Soil Biology and Biochemistry 60, 33-44.

Leclerc, M., Planas, D., Amyot, M. (2015). Relationship between extracellular low-
molecular-weight thiols and mercury species in natural lake periphytic biofilms.
Environmental Science and Technology 49, 7709-7716.


Chapter 5

5 General discussion

The inherent complexity of ecosystems and the interacting factors, such as vegetation biomass and community composition, hydrologic fluctuation and changes in soil moisture contents, microbial metabolisms, and concentrations of nutrients, complicate the exact prediction of the effects of global warming on MeHg production in northern peatlands. To address some of this uncertainty, my doctoral research was designed to better understand Hg input through vegetation into peatland soils (chapter 2), effects of peat soil moisture content and drought durations on pore water inorganic Hg (IHg), MeHg, $\text{SO}_4^{2-}$, and DOM concentrations and DOM characteristics (chapter 3), and influences of elevated ground temperature on Hg cycling in northern peatlands (chapter 4).

5.1 The role of organic matter in controlling mercury mobility in northern peatlands

As the fate of Hg in northern peatland is linked to reduced sulfur groups in organic matter (Xia et al., 1999), the controls that govern organic matter quantity and characteristics also regulate Hg cycling. Vegetation plays an important role in accumulating Hg from the atmosphere and transporting atmospheric Hg to soils through litterfall (Risch et al., 2017), because that the majority of Hg combines with the reduced sulfur groups in organic compounds in leaf tissues and consequently is incorporated in leaves (Laacouri et al., 2013). Dissolved organic matter that is produced from the decomposition of soil organic matter (SOM) or litterfall further transfers Hg from soils to pore waters (Ravichandran, 2004). In this thesis, I demonstrate that plant species, leaf age, and leaf quality (chapter 2), and environmental conditions, including soil moisture (chapter 3) and soil temperature (chapter 4) regulate Hg cycling in northern peatlands by controlling foliar Hg accumulation over a growing season and Hg release from litterfall into peat soils and from peat soils to the pore water.
Northern peatlands are important sinks of carbon (Gorham, 1991) and accumulate > 40 cm depth of peat (partially decomposed vegetation matter) because production rates exceed decomposition rates in the anaerobic and acidic conditions and the low temperatures associated with the high latitude (Rydin and Jeglum, 2013). Northern peatlands, however, are understudied regarding Hg cycling relative to other vegetation types, such as forest (Risch et al., 2012; St. Louis et al., 2001; Wang et al., 2016). It is necessary to elucidate the processes and mechanisms of Hg cycling within it, given that northern peatlands are important “hotspots” of MeHg production (Branfireun et al., 1996; Mitchell et al., 2008). Additionally, northern peatlands are experiencing more pronounced effects of climate change, such as higher temperature increase and longer periods of drought compared to other areas (IPCC, 2018) and vegetation community shift (Dieleman et al., 2016); thus, understanding the role of plant species, leaf age, and leaf quality, and environmental conditions, including soil moisture and soil temperature on Hg cycling, is critical to forecasting changes of global Hg cycling under climate change.

Plant species differ in leaf biomass and quality from leaf age to the decomposition of litter with different photosynthetic capacities, leaf longevities, leaf quality (foliar carbon and nitrogen content), and decomposition rates (Tuomi et al., 2009). Accumulation of atmospheric Hg by vegetation is affected by many factors, such as biological factors (e.g., leaf age and plant species) (Blackwell and Driscoll, 2015; Laacouri et al., 2013; Moore et al., 1995) and environmental conditions (e.g., temperature and drought stress) (Blackwell and Driscoll, 2015; Ericksen and Gustin, 2004; Zhu et al., 2016). I observed that foliar Hg concentrations were positively related to leaf age; foliar Hg concentrations varied among plant species with higher foliar Hg levels in sweet gale than in sedges over the growing season; less than 5% of foliar Hg was leached from litterfall; Hg release was positively related to the release of DOM with high aromaticity during leaching and was related to plant species with lower Hg being released from sweet gale litterfall than from sedge litterfall.

Decomposition rates of SOM are closely related to hydrology and soil temperature (Dieleman et al., 2016). Exposure of previously saturated peat soils to air can increase
microbial decomposition of SOM (Fenner and Freeman, 2011; Kown et al., 2013; Worrall et al., 2006). Warmer conditions also lead to a greater decomposition rate of SOM by stimulating microbial metabolism (Comeau, 2008). Higher decomposition rates in northern peatlands are generally corresponding with higher concentrations of DOM (Dieleman et al., 2016; Du and Li, 2017; Freeman et al., 2001), especially aromatic DOM (Kaiser et al., 2015), that plays an important role in transport Hg (Ravichandran, 2004), given the strong affinity between Hg and the reduced sulfur groups in DOM (Xia et al., 1999). I found that drier conditions increased the mobility of Hg from peat soils to pore waters (chapter 3), and elevated peat soil temperatures also increased concentrations of pore water Hg (chapter 4). Although the positive relationships between Hg concentrations and the concentrations or aromaticity of DOM did not always exist in this study, DOM still plays an important role in controlling Hg mobility, given that the reduced sulfur groups in the organic matter far exceed the amount of Hg in natural aquatic systems (Ravichandran, 2004).

Previous studies have shown that global warming will decline Sphagnum mosses biomass but increase vascular plant biomass in northern peatlands (Dieleman et al., 2015; Lyons et al., 2020; Turetsky et al., 2012), which is expected to increase atmospheric Hg input through litterfall to peat soils, given that the annual input of aboveground vegetation biomass is higher for vascular plants than mosses (Frolking et al., 2001; Thormann and Bayley, 1997). Drought and higher soil temperatures in the future global warming will also increase the oxidative release of Hg from peat soils to pore waters due to the increase in decomposition rates of SOM. Overall, future global warming may increase Hg input to peat soils and pore waters.

5.2 Importance of dissolved organic matter concentrations and characteristics and sulfate concentrations in controlling methylmercury production in northern peatlands

Net MeHg production in northern peatlands is controlled by factors that regulate Hg bioavailability and sulfate-reducing bacteria (SRB) activity, such as DOM concentrations and characteristics and sulfate (SO$_4^{2-}$) concentrations (Ullrich et al., 2001). The increase
of bioaccessible DOM and $SO_4^{2-}$ in pore waters can further increase net MeHg production in anaerobic conditions, given that both bioaccessible DOM and $SO_4^{2-}$ are nutrients for SRB (Ullrich et al., 2001). As the initial phase of litterfall breakdown, leaching leads to releases of an abundance of bioaccessible DOM from litterfall (Del Giudice and Lindo, 2017; Gessner et al., 1999), which can stimulate microbial metabolism, including SRB. Drying and re-wetting of peat soils can increase net MeHg production by enhancing the oxidative release of $SO_4^{2-}$ (Feng et al., 2014; Coleman-Wasik et al., 2015). Higher temperatures can also increase both bioaccessible and aromatic DOM concentrations (Dieleman et al., 2016).

I observed that mass loss (8%-18%) during litter leaching was primarily due to the loss of soluble DOM (chapter 2), which can provide energy for microbes (Davis III et al., 2003), including SRB, given that leached soluble DOM from litter contains abundant bioaccessible DOM (Del Giudice and Lindo, 2017; Jung et al., 2014). Re-wetting events can increase more release of bioaccessible DOM and $SO_4^{2-}$ from dried peat soils compared to from wet and moist peat soils (chapter 3). Soil temperature increase (~ +4 °C) can also increase the production of more bioaccessible DOM in pore waters in northern peatlands (chapter 4). The decline of Sphagnum mosses biomass and increase of vascular plant biomass in northern peatlands (Dieleman et al., 2015; Lyons et al., 2020; Turetsky et al., 2012) will increase the input of bioaccessible DOM to peat soils, given that the annual input of aboveground vegetation biomass to peat soils is higher for vascular plants than mosses (Frolking et al., 2001; Thormann and Bayley, 1997). A previous study, however, showed that higher temperatures increase $SO_4^{2-}$ reduction (Holmer and Storkholm, 2001) and gaseous sulfur evasion (Åkerblom et al., 2013). In addition, microbial metabolism will adapt to the warming soil conditions with time (Bradford, 2013; Melillo et al., 2002). My study suggests that vascular biomass increase, drought, and soil temperature increase due to global warming can stimulate net MeHg production by increasing the concentrations of bioaccessible DOM and $SO_4^{2-}$, but with the gaseous sulfur evasion and the adaption of microbial metabolism to the higher temperatures (Åkerblom et al., 2013), net MeHg production may be inhibited, given that $SO_4^{2-}$ is a limit nutrient in northern peatlands (Branfireun et al., 1999).
5.4 Study limitations and future efforts

As much of my research was conducted in only two types of northern peatlands (i.e., moss-dominated fen and sedge-dominated fen), there are limitations in understanding of global warming impacts on Hg cycling in northern peatlands, given that northern peatlands have many types, such as ombrotrophic bogs, moss-dominated fens, sedge-dominated fens (Rydin and Jeglum, 2013). Peatland types may affect Hg cycling because the vegetation biomass and community composition, pore water chemistry (e.g., DOM concentrations and characteristics, pH, and SO$_4^{2-}$ concentrations), and water tables are different within peatland types (Rydin and Jeglum, 2013). More northern peatland types may be considered in future research to have a better understanding of vegetation role in Hg input into peat soils and impacts of soil moisture changes and soil temperature increases on Hg cycling (Hg mobility and methylation) in northern peatlands.

One major limit in studying Hg accumulation by foliage in chapter 2 is that I could not determine the relative contributions of original atmospheric Hg, re-emitted gaseous Hg from soils, and soil Hg to foliar Hg, although it is established that more than 90% of foliar Hg is from the atmosphere (Ericksen et al., 2003; Mao et al., 2013; Schwesig and Krebs, 2003). Atmospheric Hg comes from primary Hg emission and secondary Hg emission (or re-emission of Hg) (Driscoll et al., 2013). Primary Hg emission is that Hg in long-lived lithospheric reservoirs is transferred to the atmosphere by natural and anthropogenic activities; secondary Hg emission is that deposited Hg is re-emitted to the atmosphere from soil, vegetation, lakes, and oceans. However, concentrations of atmospheric Hg that are re-emitted from soils were difficult to measure in the field without the assistance of the use of natural stable isotope tracers. Soil Hg contributions to foliar Hg were also not included in my study. However, to fully understand foliar Hg sources and “new” Hg input from plant leaves to peat soils, the stable isotope tracer technique and an isotope mixing model can unravel these connections in future studies.

My research about the effects of varying soil moisture contents and drought duration on Hg leaching from peat soils in chapter 3 was conducted under controlled laboratory conditions; there are limitations to the interpretation of my work. For example,
pretreatment, including removing large intact living plant material from peat soils and homogenizing and drying peat soils, directly altered soil structure. Given that plants can provide bioaccessible DOM through root exudates (Jones et al., 2009) to microbes that can affect decomposition rates, the removal of plants might affect microbial biomass, community, and metabolisms. The removal of plants, homogenization, and dry treatment of peat soils may increase Hg leaching due to changes in soil porosity.

Laboratory conditions in chapter 3 did not reflect the natural field conditions. The laboratory incubation was at room temperature (~21 °C), while air temperatures in the field are not consistent and change with time and weather. Higher temperatures increase decomposition rates and leaching of DOM from peat soils (Dieleman et al., 2016) and subsequently affect Hg mobility and methylation. Deionized water releases Hg compounds with relatively high water solubility and with weak adsorptions only, whereas a more ionically balanced solution in natural precipitation may increase Hg release due to ion exchange (Issaro et al., 2009). Thus, the conclusions and results presented in chapter 3 are context-specific and are not directly applicable to field conditions.

The main limitation of chapter 4 is the ground warming treatment. The ground warming temperature in my study was approximately 4 °C above the ambient temperature at 50 cm depth of soil. These warming conditions were implemented over a relatively short term, whereas equivalent warming even under anthropogenic climate change will take decades to manifest. The impact of this transient heating disturbance versus a more gradual change may be assessed through modeling in the future.

The warming experiment in chapter 4 was sampled only from June to October of each year because these study sites were flooded or covered by snow during other times, making it difficult to collect samples. The warming experiment was conducted only across three years (two years for passive warming treatment and one year for active warming treatment). A long-term active warming treatment may have elicited a stronger influence on Hg cycling due to structure and function changes in northern peatlands under global warming. That being said, Dieleman et al. (2015) found that a significant vegetation community shift from Sphagnum spp. mosses to vascular plants occurred after
four months under elevated temperatures (+4 °C and +8 °C). Lyons et al. (2020) also found that climate warming increased plant community heterogeneity in northern peatlands over the course of the same experimental study presented here, suggesting that the ecosystem-level response to warming is relatively fast. Over a longer-term manipulation, it will be more clear if the ecosystem responses are transient or represent a shift in function.

The field experiment in chapter 4 cannot elucidate the relative contributions of soil moisture content and soil temperature to net MeHg production and oxidative release of MeHg from peat soils. Global warming is decreasing water tables and soil moisture contents and increasing soil temperatures (IPCC, 2018). The increased oxidative release of SO$_4^{2-}$ during drought and re-wetting events (Coleman-Wasik et al., 2015) and increased soil temperature can have integrated effects on enhancing net MeHg production by stimulating SRB metabolism (Coleman-Wasik et al., 2015; Ullrich et al., 2001). Drought and re-wetting events (Feng et al., 2014; Coleman-Wasik et al., 2015) and warmer soil conditions (Dieleman et al., 2016) can increase decomposition rates of SOM and subsequently oxidative release of MeHg. A factorial experiment and stable isotope tracer technique are suggested to unravel relative contributions of soil moisture content and soil temperature to net MeHg production and oxidative release of MeHg from peat soils in future studies.

Future research should also examine the integrated effects of increased CO$_2$, elevated temperature, and lowered water tables on Hg cycling in northern peatlands. It has been a consensus that anthropogenic activities are elevating both global temperature and atmospheric CO$_2$ concentrations and decreasing water table levels in northern peatlands (IPCC, 2018). These changes are anticipated to significantly decrease the resilience and stability of peatland ecosystems (Dieleman et al., 2015; Weltzin et al., 2000). Thus, changes in peatland ecosystems may further affect Hg cycling in northern peatlands. A better understanding of the global warming impacts on Hg cycling in northern peatlands will arise if both elevated temperature and CO$_2$ concentrations are considered in future researches.
Finally, the mechanism by which ground warming affects net Hg methylation in northern peatlands is not resolved by my data (chapter 4). MeHg concentrations and %MeHg in pore waters in chapter 4 can not reflect net Hg methylation rates because MeHg in pore waters can be from net Hg methylation and demethylation and the release from soils through desorption (Liu et al., 2020). It is not possible to determine the contributions of net MeHg production (net Hg methylation and demethylation rates) and the release of MeHg from peat soils to pore waters just based on MeHg concentrations and %MeHg values. The methylation of Hg is affected by a wide variety of factors, such as microbial activity, the concentration of bioaccessible Hg, DOM concentrations and characteristics, and concentration of electron acceptors (i.e., sulfate), all of which are affected by redox-potential, temperature, and pH (Paranjape and Hall, 2017; Ullrich et al., 2001). The contributions of each factor to net Hg methylation rate changes under soil warming are unclear. Thus, more research is needed to clarify the effects of warming and corresponding changes in factors, such as microbial metabolism and bioavailability of Hg, on net MeHg production and MeHg release from soils.

5.5 Conclusions and significance

Overall, my doctoral research provides insights into the effects of global warming on Hg cycling in northern peatlands. Specifically, the field-based study of Hg accumulation by the dominant plant leaves and the laboratory-based incubation of Hg leaching from litters contribute to better predictions of Hg inputs via plant leaves to peat soils in the future. This is important because there remains a large knowledge gap around Hg inputs by litterfall in northern peatlands, and there is an expectation that global warming will lead to vegetation community composition shifts with an increase in vascular plant abundance at the expense of Sphagnum mosses in northern peatlands (Buttl er et al., 2015; Dieleman et al., 2015; Weltzin et al., 2000). Vascular plants have a higher annual input of vegetation biomass to peatland soils than Sphagnum mosses (Frolking et al., 2001; Thormann and Bayley, 1997). Once deposited to the ground, vascular plant litter will release Hg more rapidly from litter to peat soils than Sphagnum mosses, given that vascular plants are more degradable (Hobbie, 1996; Lyons and Lindo, 2019).
Methylmercury mobility and production under varying soil moistures and different drought duration remain largely unknown. This research will contribute to a better prediction of MeHg transport and risk to downstream ecosystems of northern peatlands. Drier peat soil conditions have a higher potential to release more MeHg, and re-wetting of peat soils will increase net MeHg production due to the oxidative release of $\text{SO}_4^{2-}$, IHg, and bioaccessible DOM.

Projected global temperature is increasing, especially in northern peatlands (IPCC, 2018), while there is a large knowledge gap around net MeHg production changes under global warming in northern peatlands. This research provides direct evidence that ground warming will significantly increase net MeHg production in the future. Further, ground warming will increase the release of DOM, which will also promote the MeHg input from northern peatlands to downstream ecosystems, given the strong combination between Hg and reduced sulfur grounds in DOM (Xia et al., 1999).
5.6 References


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