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# The effects of legacy sulphur deposition on methylmercury production in northern peatlands; geochemical and biological considerations

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## **Abstract**

Mercury is a ubiquitous element with a complex geochemical cycle. Aquatic ecosystems such as wetland soils convert inorganic mercury to organic, neurotoxic methylmercury through the activity of sulphate-reducing bacteria (SRB). Sulphate stimulates the activity of SRB, and the production of methylmercury in these environments. My aim was to investigate the effect that legacy sulphate has on Hg methylation in northern peatlands through a laboratory sulphate addition experiment with differentially sulphate-exposed peats and a field study of peatlands subjected to different levels of sulphate. Results from the laboratory study indicate that peatlands in regions of higher atmospheric sulphate deposition show enhanced Hg methylation responses compared to pristine peatlands, while field results indicate that sulphate deposition increases Hg methylation dependence on other nutrients as opposed to sulphate supply. Management for peatlands impacted by industrial sulphate sources will have to consider legacy sulphate deposition within peatland geochemical context to mitigate potential Hg methylation.

## **Keywords**

Methylmercury, sulphate, peatlands, sulphate-reducing bacteria, sulphide, bioavailability, biogeochemistry

## Summary for Lay Audience

Mercury (Hg) is an element that occurs everywhere in the natural environment. Mercury in nature rarely reaches levels that would be harmful to the health of wildlife or humans. However, in wetland soils, Hg transforms into methylmercury (MeHg) which is the form of Hg that can easily build up in living tissue. Methylmercury is produced by small organisms known as sulphate-reducing bacteria (SRB) that live in wetland soils. Because SRB use sulphate ( $\text{SO}_4^{2-}$ ) to live and grow, giving SRB more  $\text{SO}_4^{2-}$  has the potential to increase the amount of MeHg they produce. Human activities such as fossil fuel burning and mining can be a source of  $\text{SO}_4^{2-}$  to wetlands such as peatlands, increasing MeHg production. Peatlands are wetlands that are covered with large amounts of built up dead and decaying plant matter known as peat. The first goal of my thesis was to link  $\text{SO}_4^{2-}$  in peatlands from human sources to MeHg production in peatlands. My second goal was to determine if past  $\text{SO}_4^{2-}$  release to peatlands affects the ability of peatlands to produce MeHg in the future. In my laboratory study, I found that when peat taken from an area that had higher levels of atmospheric  $\text{SO}_4^{2-}$  deposition is given more  $\text{SO}_4^{2-}$ , these peats are able to produce more MeHg compared to peats from areas with lower atmospheric  $\text{SO}_4^{2-}$  deposition. In my field study, I found that in peatlands that have high levels of  $\text{SO}_4^{2-}$  additions such as those surrounding a mine,  $\text{SO}_4^{2-}$  does not have a large effect on MeHg production because the SRB are not limited by  $\text{SO}_4^{2-}$ . The supply of other nutrients such as carbon that the bacteria need for growth become more important for MeHg production instead. These studies show that MeHg production in peatlands is not simply linked to the amount of sulphate in the environment but is also influenced by other factors that control the growth of SRB. Recovery plans need to consider not only the level of  $\text{SO}_4^{2-}$  that has been added to these wetlands, but the balance of other nutrients as well and what this means for MeHg production in the future.

## List of Abbreviations

C/N	Carbon/nitrogen
CRM	Certified reference material
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
Fe <sup>2+</sup>	Ferrous iron
IHg	Inorganic mercury
Fe	Iron
Hg	Mercury
HgS <sub>(s)</sub>	Mercury (II) sulphide (cinnabar)
MeHg	Methylmercury
THg	Total mercury
SO <sub>4</sub> <sup>2-</sup>	Sulphate
SRB	Sulphate-reducing bacteria
S <sup>2-</sup>	Sulphide
UNEP	United Nations Environment Programme
U.S. EPA	United States Environmental Protection Agency
WEIS	Wood Environment & Infrastructure Solutions

## **Co-Authorship Statement**

I hereby declare that I am the sole author of this thesis, except where noted (below). I understand that my thesis may be made electronically available to the public. Study design and execution for both Chapters 2 and 3 was developed by myself and Dr. Brian Branfireun. Experimental set-up for the laboratory experiments was based off that developed by Lauren Twible and Dr. Brian Branfireun. All sample collection and analysis was performed by myself, and Biotron analytical technicians Jeff Warner and Dr. Erin Mann.

Exception to sole authorship:

For all chapters Dr. Brian Branfireun acted as an editor of all written work and advisor on data presentation and interpretation, and as such, will be listed as co-author on all subsequent publications based on this work.

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# Chapter 1

## 1 Introduction

This chapter presents a general introduction of mercury (Hg) and sulphur biogeochemistry in aquatic systems, with an emphasis on wetlands. The biological and geochemical factors that influence the production of methylmercury (MeHg) in these environments will be discussed, and overall thesis objectives and significance are presented.

### 1.1 Mercury in the environment

Mercury (Hg) is a globally distributed, naturally occurring element with a complex biogeochemical cycle. Its three oxidation states Hg(0), Hg(I) and Hg(II) allow it to undergo a variety of geochemical transformations (Liu *et al.*, 2012), with Hg(II) being the dominant form of Hg in sediments, soils, and natural waters, and Hg(0) being the dominant form found in the atmosphere (Ullrich *et al.*, 2001). Hg(0) in the atmosphere is easily transported long distances because of its relatively long atmospheric lifetime (Selin, 2009). The lower solubility of Hg(0) in water means that it is retained in the atmosphere instead of deposited (Selin, 2009), which makes the atmosphere a key transport route for Hg. Deposition of Hg to aquatic and terrestrial systems occurs via dry deposition of particulate Hg and wet deposition of dissolved Hg(II) species (Shroeder & Munthe, 1998) following Hg(0) oxidation, a process that increases Hg(II) solubility, and therefore removal, from the atmosphere (Liu *et al.*, 2012). Hg(0) is also directly removed from the atmosphere via foliar uptake by vegetation (Lindberg *et al.*, 1992; Gustin & Lindberg, 2005; Obrist *et al.*, 2017).

Mercury can be released to the atmosphere through several natural processes including volatilization from soil (Schluter, 2000), emission from catchments due to fire (Wiedinmyer & Friedli, 2007), re-emission from oceans (Shroeder & Munthe, 1998), and volcanic activity (Ferrara *et al.*, 2000). It is important to keep in mind, however, that a

significant fraction of Hg released from natural sources actually constitutes a re-emission of previously deposited natural and anthropogenic Hg, especially in environments where background Hg concentrations are low (Gustin *et al.*, 2000; Liu *et al.*, 2012). This re-emission is of particular concern as Hg emissions from anthropogenic sources have increased the burden of atmospheric Hg. Presently, anthropogenic emissions of Hg account for as much as 30% of the total amount of Hg entering the atmosphere each year (UNEP, 2013). Anthropogenic sources of Hg are predominantly from small-scale gold mining and the burning of coal, but lesser sources include cement production and non-ferrous metal production (UNEP, 2013).

In addition to atmospheric inputs of Hg, aquatic systems also receive significant inputs of Hg(II) from terrestrial runoff (Selin, 2009). The transport of this Hg to aquatic systems is largely facilitated by organic matter, which binds Hg and delivers it to aquatic systems during high flow events (Driscoll *et al.*, 1995; Watras, Morrison, & Host, 1995; Eklöf *et al.*, 2012). The ability of organic matter to bind Hg is attributed to reduced sulphur groups such as sulphonic acids and thiols that have a high affinity for Hg species (Haitzer *et al.*, 2003; Skyllberg *et al.*, 2000). The efficiency with which Hg binds to these groups is influenced by both the ratio of Hg to organic matter (Haitzer, Aiken, & Ryan, 2002), as well as the ratio of Hg to sulphur groups (Hesterberg *et al.*, 2001). Ultimately, the flux of Hg bound to organic matter is regulated by hydrologic flow paths and hydrologic connectivity between terrestrial and aquatic systems (Scherbatskoy *et al.*, 1998; Schuster *et al.*, 2008; Demers, Driscoll, & Shanley, 2010).

## 1.2 Methylmercury production in aquatic systems

Despite the anthropogenic enrichment of the atmospheric Hg pool, ambient concentrations of Hg in the natural environment rarely exceed thresholds that pose a health risk (Clarkson *et al.*, 2003). Of greater concern with respect to environmental and human health is the organic form of Hg, methylmercury (MeHg). Because it is an organic compound, the excretion of MeHg by living organisms is much slower compared to inorganic Hg, and can therefore accumulate in living tissue (Kidd, Clayden, & Jardine, 2012). As a result, MeHg constitutes the majority of Hg found in organic tissue (Bloom,



1992). In addition only MeHg not inorganic Hg is able to biomagnify in food webs, a process by which a chemical becomes increasingly more concentrated in higher trophic levels (Swackhammer, 2003). Through biomagnification, MeHg becomes increasingly more concentrated in higher trophic level consumers and as a potent neurotoxin, poses a health risk to predatory birds, mammals, and fish (Mergler *et al.*, 2007; Scheuhammer *et al.*, 2007) as well as humans. However, outside of organic tissue, MeHg generally constitutes a relatively small fraction of the total Hg (THg) in the natural environment (Krabbenhoft, Branfireun, & Heyes, 2005).

That being said, aquatic environments such as lake sediments (Winfrey and Rudd, 1990; Matilainen *et al.*, 1991; Krabbenhoft *et al.*, 1998), and wetlands (Branfireun *et al.*, 1996; Gilmour *et al.*, 1998; Mitchell, Branfireun, & Kolka, 2008) can constitute significant sources of MeHg to the surrounding environment, which is attributed to active Hg methylation within these systems (St. Louis *et al.*, 1994). Mercury methylation within these systems is mediated by the activity of a variety of anaerobic bacteria that are able to thrive in the anoxic conditions inherent to sediments and wetland soils, which include members of the iron-reducing bacteria (Fleming *et al.*, 2006; Kerin *et al.*, 2006), methanogens (Wood *et al.*, 1968; Hamelin *et al.*, 2011), and sulphate-reducing bacteria (Gilmour *et al.*, 1992; King *et al.*, 2001).

The ability of these bacterial groups to methylate Hg has recently been linked to the presence of the *hgcA/B* gene cluster (Parks *et al.*, 2013). This gene cluster is distributed across two phyla of bacteria (*Proteobacteria* and *Firmicutes*) and one phylum of Archaea (*Euryarchaeota*; Parks *et al.*, 2013). The *Deltaproteobacteria* clade contains the highest number of known methylators (Christensen *et al.*, 2016). Members of this clade are also the strongest methylators; that is, these species methylate Hg at higher rates, and include the iron and sulphate-reducers (Kerin *et al.*, 2006; King *et al.*, 2000; Gilmour *et al.*, 2013). Methylation of Hg is an enzymatic process, proposed to occur by way of the acetyl-coenzyme A pathway (Choi, Chase Jr., & Bartha, 1994). Indeed, the *hgcA/B* gene cluster determined by Parks *et al.* (2013) encodes two essential components of the acetyl-coenzyme A pathway; a putative corrinoid protein likely responsible for transfer of the methyl group to Hg, and a ferredoxin-like protein likely responsible for corrinoid

reduction. The physiological purpose of Hg uptake is still unknown but may be due to accidental uptake during non-discriminatory metal transport by bacterial cells (Drott *et al.*, 2007; Gilmour *et al.*, 2011).

It is important to note, however, that MeHg produced in these environments reflects a balance between Hg methylation, and MeHg demethylation. Methylmercury demethylation is thought to largely be facilitated by the activity of microbes and has been linked to the bacterial detoxification pathway encoded by the *mer* operon (Robinson & Tuovinen, 1984). However, other studies indicate that in natural systems with lower concentrations of Hg, an oxidative demethylation pathway used by methanogens and sulphate-reducers dominates demethylation reactions (Marvin-Dipasquale *et al.*, 2003). These biotic MeHg demethylation processes seems to be more extensive in aerobic conditions (Oremland, Culbertson, & Winfey, 1991). Demethylation in aquatic systems can also occur abiotically through MeHg degradation from UV light (Rudd *et al.*, 1996), although this process is thought to be less relevant for systems secluded from UV light such as deep sediments where biotic demethylation is more significant (e.g. Ramlal, Rudd, & Hecky, 1986).

### 1.3 The sulphate-reducing bacteria

The sulphate-reducing bacteria (SRB) in particular have long been established as the principle methylators of Hg in natural environments (Compeau & Bartha, 1985). Indeed, Hg methylation has been found to be significantly correlated with sulphate reduction in anoxic environments (Choi & Bartha, 1994; King *et al.*, 1999). Sulphate-reducing bacteria use sulphate ( $\text{SO}_4^{2-}$ ) as their terminal electron acceptor during anaerobic respiration, producing sulphide ( $\text{S}^{2-}$ ) as a metabolic end product and MeHg as a metabolic by-product. The methylation of Hg by SRB is dependent on multiple environmental factors that include pH, temperature, supply of nutrients, and availability of inorganic Hg (Ullrich *et al.*, 2001). Generally, higher temperatures favour Hg methylation largely because of the positive effect on overall bacterial activity (Bisogni & Lawrence, 1975). Although SRB can show decreased activity in the acidic pH range (Connell & Patrick,

1968), a lower pH also increases the desorption of Hg from soil organic matter (Yin *et al.*, 1997; Skjellberg *et al.*, 2000), potentially increasing its availability for methylation.

The supply of organic matter for decomposition by SRB constitutes another key control on Hg methylation by SRB. Dissolved organic matter (DOM) can both promote Hg methylation by acting as an electron donor in SRB metabolism (Schartup *et al.*, 2013; Hsu-Kim *et al.*, 2013), and inhibit methylation due to its ability to bind inorganic Hg and make it less available for methylation (Miller *et al.*, 2007; Hammerschmidt *et al.*, 2008). The ability of DOM to complex Hg is dependent upon its composition, as Hg binds to acidic functional groups of DOM which include carboxylic acids, phenols, alcohols, and thiols, with a preference for thiols in particular (Ravichandran, 2004).

Sulphur geochemistry constitutes an important control not only on SRB activity, but also on Hg bioavailability and partitioning. It is well established that the activity of SRB is related to the abundance of  $\text{SO}_4^{2-}$  as an electron acceptor (Choi & Bartha, 1994; Gilmour *et al.*, 1992; King *et al.*, 1999). However, increases in SRB activity in response to increased  $\text{SO}_4^{2-}$  abundance also results in an increase in  $\text{S}^{2-}$  as the metabolic end product. Sulphide has a high binding affinity for Hg, and precipitated HgS complexes are unavailable for uptake by the SRB (Compeau and Bartha, 1985; Benoit *et al.*, 1998; Benoit *et al.*, 1999; Gilmour *et al.*, 1998). At lower  $\text{S}^{2-}$  concentrations, these solid, charged complexes are less likely to precipitate (Benoit *et al.*, 1999). The decrease in available inorganic Hg can cause a subsequent decrease in Hg methylation.

Sulphur geochemistry also has an important role in determining Hg partitioning between aqueous and solid phases. As previously mentioned, Hg species have high binding affinities for reduced sulphur compounds of organic matter (Skjellberg *et al.*, 2000; Hesterberg *et al.*, 2001). However, when  $\text{S}^{2-}$  concentrations are high, there exists competition between reduced sulphur binding sites in solid phase organic matter, and dissolved  $\text{S}^{2-}$  such that a greater proportion of Hg exists in dissolved or precipitated HgS complexes in the aqueous phase (Skjellberg, 2008). This balance between Hg in the solid and aqueous phase can have significant implications for the availability of Hg for bacterial uptake. In addition, the partitioning of Hg in anoxic environments can be

impacted by the presence of dissolved ferrous iron ( $\text{Fe}^{2+}$ ), which can complex dissolved  $\text{S}^{2-}$ , thus preventing the formation of insoluble HgS complexes (Howarth & Jørgensen, 1984; Bailey *et al.*, 2017). It is important to note, however, that since dissolved neutral HgS complexes are the ones preferentially taken up by SRB (Benoit *et al.*, 1999), a large decrease in free  $\text{S}^{2-}$  can limit bacterial Hg uptake and therefore Hg methylation (Liu, Valsaraj, & Delaune, 2009; Ulrich & Sedlak, 2010). The availability of Hg for bacterial uptake thus reflects a complex balance between solid and aqueous phase partitioning, and between dissolved and precipitated Hg complexes.

## 1.4 Peatlands as methylmercury production hotspots

Peatlands are wetlands with vegetation often dominated by *Sphagnum* mosses, and by definition have at least 40 cm of peat (organic soil) accumulation (Clymo *et al.*, 1998; Limpens *et al.*, 2008). Peatlands are known regions of MeHg production (e.g. Branfireun, Heyes, & Roulet, 1996; Mitchell, Branfireun, & Kolka, 2008), due to conditions that support sulphate reduction (Urban, Eisenreich, & Grigal, 1989; Spratt, Morgan, & Good, 1987). The factors that govern the degree of SRB activity in peatland soils and the production of MeHg are similar to other environments of MeHg production. However, peatlands are relatively nutrient limited systems, and so the availability of  $\text{SO}_4^{2-}$  is often a limiting factor. Sulphate inputs to peatlands can come from a variety of natural sources, which include groundwater (Branfireun & Roulet, 2002), and upland runoff (Mitchell, Branfireun, & Kolka, 2008; Demers, *et al.*, 2013). The amount of  $\text{SO}_4^{2-}$  that is delivered to these environments is dependent on factors such as local hydrology and topography. For example, during high flow events, the increase in hydrologic connectivity facilitates transport of nutrients from uplands to wetlands (Demers, Driscoll, & Shanley, 2010), and uplands that are more concave can increase the delivery of these nutrients (Mitchell, Branfireun, & Kolka, 2009).

In addition to these natural sources of  $\text{SO}_4^{2-}$ , anthropogenic activities have accelerated the deposition of  $\text{SO}_4^{2-}$  to peatlands, including remote northern peatlands. Although emissions of sulphur dioxide ( $\text{SO}_2$ ) from activities such as coal-burning and metal smelting have been decreasing since the 1980s (Canada-United States Air Quality

Committee, 2012), regions that have been heavily industrialized still have higher wet deposition of sulphur compared to more pristine regions (Vet *et al.*, 2014). The acidification of aquatic environments is not only detrimental to the health of wildlife (Wright & Schindler, 1995), but can also stimulate the production of MeHg (Gilmour & Henry, 1991; Gilmour *et al.*, 1992). In more pristine environments, mining operations in regions rich in sulphide minerals can increase the release of  $\text{SO}_4^{2-}$  to surrounding wetlands as these minerals become oxidized upon exposure to air (Al, Martin, & Blowes, 2000; Berndt & Bavin, 2012). This also potentially increases the production of MeHg from these systems.

Not only does  $\text{SO}_4^{2-}$  deposition from anthropogenic sources have the potential to directly stimulate the activity of SRB, but it also has the potential to change SRB community structure (Strickman *et al.*, 2016). As not all SRB methylate Hg, or methylate Hg at the same rate (King *et al.*, 2000), re-structuring of these communities could have significant implications for MeHg production from peatland environments. In northern peatlands where climate effects are projected to increase water table draw-down events (Sheffield & Wood, 2008), previously reduced sulphur can be re-oxidized (Coleman Wasik *et al.*, 2015), resulting in further  $\text{SO}_4^{2-}$  legacy effects with potential consequences for MeHg production.

## 1.5 Thesis objective and significance

The objective of this thesis was to further investigate the link between sulphur deposition, and MeHg production in northern peatlands across a range of atmospheric and point source depositions of  $\text{SO}_4^{2-}$ . While several studies have investigated the link between  $\text{SO}_4^{2-}$  addition and MeHg production in wetlands (Harmon *et al.*, 2004; Jeremiason *et al.*, 2006; Mitchell, Branfireun, & Kolka, 2008; Bergman *et al.*, 2012), there are no studies to my knowledge that take a comparative approach across wetlands that have been differentially impacted by  $\text{SO}_4^{2-}$  loads. In addition, studies on  $\text{SO}_4^{2-}$  loading have focused on the immediate response of peatlands to additional  $\text{SO}_4^{2-}$ , and less is known about how peatlands that have already been  $\text{SO}_4^{2-}$  exposed will respond to further  $\text{SO}_4^{2-}$  inputs. The specific objectives of this thesis were therefore to:

- 1) Determine if legacy exposure to atmospheric sulphur deposition increases Hg methylation of northern peats in response to further inputs of  $\text{SO}_4^{2-}$ , and
- 2) Determine if the relationship between sulphur and net MeHg production and accumulation in northern peatlands is affected by the magnitude and source of  $\text{SO}_4^{2-}$  inputs.

The first objective was investigated in Chapter 2, that reports on a laboratory study in which  $\text{SO}_4^{2-}$  was added continuously to peat cores from peatlands that fall along a latitudinal gradient of  $\text{SO}_4^{2-}$  deposition in Ontario. The Hg methylation response of these cores to  $\text{SO}_4^{2-}$  addition was evaluated along with other geochemical variables. The second objective was investigated in Chapter 3, which reports on a field study of natural, elevated atmospheric, and elevated point source  $\text{SO}_4^{2-}$  gradients in Ontario peatlands. The relationship between sulphur and MeHg accumulation in peat samples was assessed from a sulphur availability, and geochemical perspective. Collectively, this research will provide insight into Hg methylation responses of northern peatlands to various inputs of  $\text{SO}_4^{2-}$ , and the geochemical influences that can help predict this response. The results of this study will be particularly pertinent for peatlands that are impacted by industrial activities that increase the supply of  $\text{SO}_4^{2-}$  to these systems, as management plans will need to consider that these environments could constitute long-term sources of MeHg to the surrounding watershed.

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## Chapter 2

### 2 Evaluating Mercury Methylation Along a Latitudinal Gradient of Sulphate Deposition

This chapter reports on a series of laboratory experiments investigating the effects of sulphate ( $\text{SO}_4^{2-}$ ) addition on methylmercury (MeHg) production in peats. By using peats from regions that have different histories of atmospheric  $\text{SO}_4^{2-}$  deposition, I attempt to reveal the effects of long-term  $\text{SO}_4^{2-}$  loading on MeHg production in peatlands and discuss potential explanations and implications of these effects.

#### 2.1 Introduction

Mercury (Hg) is a naturally occurring trace element with a complex global geochemical cycle. This complexity is in part due to the fact that it can exist under natural conditions in gaseous, liquid, and solid forms (Krabbenhoft *et al.*, 2005). The global transport of Hg is largely facilitated by the atmosphere, which constitutes a significant pool of Hg (Swain *et al.*, 1992). Although release of Hg to the atmosphere can come from natural sources such as volcanism, forest fires, and geothermal activity (Pirrone *et al.*, 2010; Liu *et al.*, 2012), anthropogenic activities such as coal burning and cement production account for as much as 30% of all global Hg emissions to the atmosphere (UNEP, 2013).

While all Hg species are toxic to some degree, of particular concern is the organic species of Hg, methylmercury (MeHg). Methylmercury readily bioaccumulates in organic tissue, biomagnifies in food chains (Bloom, 1992), and is a potent neurotoxin (Clarkson, Magos, & Myers, 2006). However, MeHg generally makes up a small fraction of the total Hg present in the environment with the exception of specific aquatic environments, namely lake sediments (Winfrey and Rudd, 1990; Krabbenhoft *et al.*, 1998), and wetland soils (Branfireun *et al.*, 1996; Gilmour *et al.*, 1998; Mitchell *et al.*, 2008), which can contribute a significant amount of MeHg to the surrounding environment.

The key to understanding the high MeHg output from wetland systems is the very specific environmental conditions that are present there. Because wetland soils are often waterlogged and anoxic, they are conducive to the anaerobic biogeochemical processes



required for the metabolism of known groups of Hg-methylating bacteria. These include members of the iron-reducing bacteria (Fleming *et al.*, 2006; Kerin *et al.*, 2006), methanogens (Kennedy, Rosen, & Wood, 1968; Hamelin *et al.*, 2011), and the sulphate-reducing bacteria (Compeau and Bartha, 1985; Gilmour *et al.*, 1992; King *et al.*, 2001).

The sulphate-reducing bacteria (SRB) in particular are the principle methylators of inorganic Hg in anoxic environments (Compeau and Bartha, 1985). Sulphate-reducing bacteria use sulphate ( $\text{SO}_4^{2-}$ ) as their terminal electron acceptor during the decomposition of organic matter, producing sulphide ( $\text{S}^{2-}$ ) as an end product. During this process, SRB uptake Hg from the environment through proposed active (Schaefer *et al.*, 2011) or passive (Benoit *et al.*, 1999; Drott *et al.*, 2007) transport mechanisms and convert it into MeHg as a by-product of their metabolism.

As a key metabolite for SRB,  $\text{SO}_4^{2-}$  constitutes a key control on MeHg methylation by SRB. It is well established that the addition of  $\text{SO}_4^{2-}$  to nutrient poor systems such as wetlands stimulates the activity of the SRB community and subsequently the output of MeHg from the wetland (Gilmour *et al.*, 1992; Branfireun *et al.*, 1999; Harmon *et al.*, 2004; Jeremiason *et al.*, 2006). However, increased  $\text{SO}_4^{2-}$  reduction often leads to the build-up of dissolved  $\text{S}^{2-}$ , the end product of  $\text{SO}_4^{2-}$  reduction. Sulphide has a high affinity for Hg and in anoxic environments, the formation of charged  $\text{HgS}_{(s)}$  complexes can result in Hg methylation inhibition due to the inability of SRB to uptake these complexes (Benoit *et al.*, 1999; Skyllberg, 2008). As a result, there exists a trade-off between high rates of  $\text{SO}_4^{2-}$  reduction when  $\text{SO}_4^{2-}$  is supplied, and subsequent inhibition of Hg methylation due to the build-up of dissolved  $\text{S}^{2-}$ . Some researchers have called this intermediate level of  $\text{SO}_4^{2-}$  that stimulates high rates of MeHg production the ‘Goldilocks Zone’ of Hg methylation (Johnson *et al.*, 2016). This ‘Goldilocks Zone’ is also consistent with the fact that dissolved neutral  $\text{HgS}$  complexes are the dominant Hg species taken up by methylating bacteria in pure cultures (Benoit *et al.*, 1999; Drott *et al.*, 2007). An intermediate level of  $\text{SO}_4^{2-}$  reduction in wetland soils should therefore ensure that there is enough  $\text{S}^{2-}$  produced to facilitate uptake of Hg, but not so much as to form high levels of charged, insoluble  $\text{HgS}_{(s)}$  complexes.

However, there are several other factors other than the supply of  $\text{SO}_4^{2-}$  and the build-up of dissolved  $\text{S}^{2-}$  that impact the ability of wetland soils to produce MeHg, among them carbon content, iron content, and SRB community composition. Soils with both a higher organic carbon content (Groffman *et al.*, 1996; Sutton-Grier, Ho, & Richardson, 2009), and higher carbon quality (Yavitt, Lang, & Wieder, 1987; Bridgham & Richardson, 1992) have higher levels of microbial decomposition, and therefore higher activity of SRB. Dissolved organic carbon (DOC) can also impact Hg methylation in wetland soil pore waters due to its ability to bind inorganic Hg and make it less bioavailable for methylation (Miller *et al.*, 2007; Hammerschmidt *et al.*, 2008).

Iron (Fe) content in wetland soils can also have an impact on Hg methylation potential. Dissolved ferrous iron ( $\text{Fe}^{2+}$ ) has the ability to complex dissolved  $\text{S}^{2-}$ , forming insoluble FeS (Howarth & Jørgensen, 1984). In this way, soils with higher Fe contents have a higher buffering capacity for the build-up of dissolved  $\text{S}^{2-}$  as the product of sulphate reduction (Heijs *et al.*, 1999). With less free  $\text{S}^{2-}$ , there is less formation of  $\text{HgS}_{(s)}$  complexes, meaning there is potentially more Hg available for methylation (Bailey *et al.*, 2017). However, if  $\text{Fe}^{2+}$  completely depletes free dissolved  $\text{S}^{2-}$ , this can potentially inhibit methylation as there are no neutral, dissolved HgS complexes available for bacterial uptake (Liu, Valsaraj, & Delaune, 2009; Ulrich & Sedlak, 2010).

Lastly, the SRB community composition of wetland soils also has the ability to impact MeHg production potential. Not all SRB methylate Hg (Ekstrom, Morel, & Benoit, 2003), and those that can do not always methylate Hg at the same rate (King *et al.*, 2000; Ranchou-Peyruse *et al.*, 2009). Some researches attribute this ability to biochemical pathways specific to certain types of SRB (Choi, Chase, & Bartha, 1994). Therefore, changes to the relative abundance, and types of SRB in wetland soils could have significant implications for MeHg production.

Interestingly, long-term  $\text{SO}_4^{2-}$  addition to wetland soils can shift the composition of the SRB community. Strickman *et al.* (2016) found that the overall SRB community structure, as well as the community structure of *Deltaproteobacteria* (which include numerous potent Hg-methylating bacteria) shifted in experimentally  $\text{SO}_4^{2-}$ -amended

wetland plots compared to control plots. In addition, the bacterial species diversity was lower at these  $\text{SO}_4^{2-}$ -amended plots compared to control plots, and changes in % MeHg in wetland soils were significantly correlated with changes in the *Deltaproteobacteria* community (Strickman *et al.*, 2016).

This suggests that changes in only a subset of the SRB community in response to  $\text{SO}_4^{2-}$  addition can have a significant impact on Hg methylation. Indeed, Hausmann *et al.* (2016) found that low abundance groups of SRB with less than 0.1% relative genome abundance in soil samples respond significantly to inputs of  $\text{SO}_4^{2-}$  compared to relatively higher abundance groups. This change in bacterial community composition could be part of a so called  $\text{SO}_4^{2-}$  ‘priming’ effect of wetland bacterial communities, in which past exposure to  $\text{SO}_4^{2-}$  allows the SRB community to sustain elevated Hg methylation even after  $\text{SO}_4^{2-}$  addition has declined (Coleman Wasik *et al.*, 2015). Addition of a nutrient required for growth or metabolism can cause a priming effect in bacterial communities through several potential mechanisms. These include activation of dormant microbes that respond specifically to the nutrient, biomass increases in faster-growing competitive microbes, or overall increases in microbial activity that increases soil organic matter decomposition (Kuzyakov, Friedel, & Stahr, 2000; Blagodatskaya and Kuzyakov, 2008).

The aim of the current study was to investigate the potential  $\text{SO}_4^{2-}$  priming effect that long-term  $\text{SO}_4^{2-}$  loading has on northern peatlands. Peatlands are wetlands with highly organic soils (Clymo, Turunen, & Tolonen, 1998; Limpens *et al.*, 2008), and are known hotspots of Hg methylation (e.g. Branfireun *et al.*, 1996; Mitchell *et al.*, 2008) due to conditions that support  $\text{SO}_4^{2-}$  reduction (Spratt, Morgan, & Good, 1987; Urban *et al.*, 1989). More specifically, this study aims to determine: 1) if legacy  $\text{SO}_4^{2-}$  deposition to peatlands affects MeHg production in response to further  $\text{SO}_4^{2-}$  inputs, and 2) if the Hg methylation response of differently  $\text{SO}_4^{2-}$ -exposed peat to further  $\text{SO}_4^{2-}$  inputs is similar across a range of  $\text{SO}_4^{2-}$  addition concentrations.

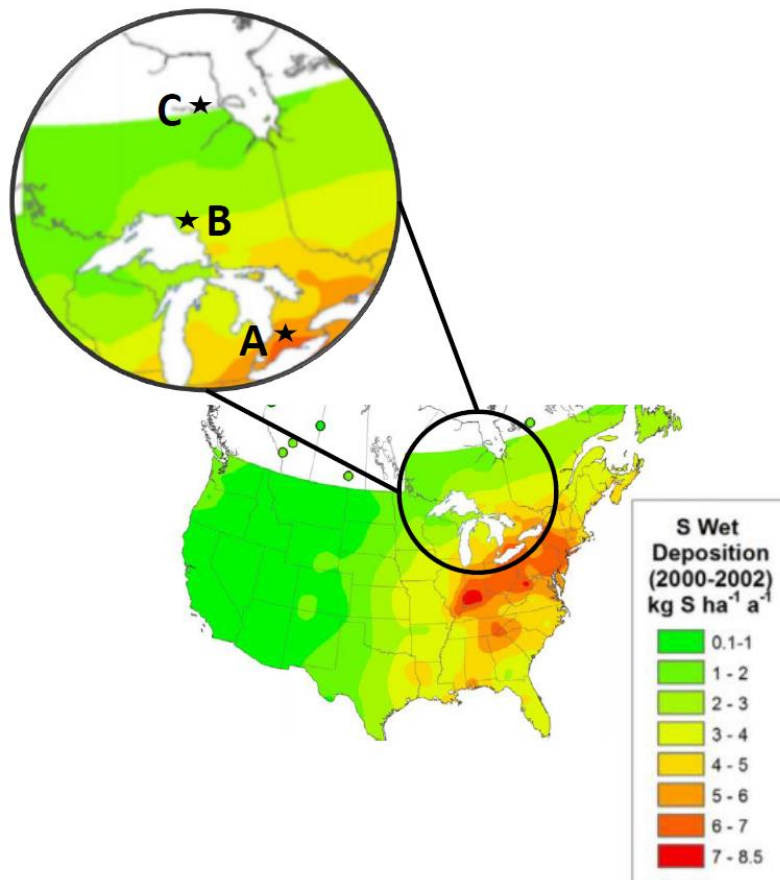
To answer these research questions, a series of controlled laboratory experiments were performed in which distilled water, and a range of concentrations of  $\text{SO}_4^{2-}$  were applied to peat cores in a flow-through system. The response of peat cores to  $\text{SO}_4^{2-}$  addition was

assessed in terms of Hg and sulphur biogeochemistry. The peat cores were taken from three sites across a historical atmospheric  $\text{SO}_4^{2-}$  deposition gradient. I hypothesized that peats with a history of high  $\text{SO}_4^{2-}$  deposition have an enhanced Hg methylation response to further  $\text{SO}_4^{2-}$  inputs compared to peats with a history of low  $\text{SO}_4^{2-}$  deposition due to a  $\text{SO}_4^{2-}$  priming effect. I predicted that when peats with a history of high  $\text{SO}_4^{2-}$  deposition are supplied with further  $\text{SO}_4^{2-}$ , the SRB will more readily respond to these added nutrients by producing more MeHg compared to sites with a history of low  $\text{SO}_4^{2-}$  deposition. I expected that this response will become more enhanced with larger additions of  $\text{SO}_4^{2-}$ . Understanding how legacy  $\text{SO}_4^{2-}$  loading alters MeHg production is needed to inform management strategies for peatland-dominated watersheds impacted by long-term  $\text{SO}_4^{2-}$  deposition from industrial sources. Northern ecosystems and communities in the Canadian boreal and subarctic, where over 90% of Canadian peatlands are located (Warner and Asada, 2006), will be particularly vulnerable to land-use changes that increase  $\text{SO}_4^{2-}$  release to the surrounding environment, and increase MeHg production as a result.

## 2.2 Methods

### 2.2.1 Field sites and sample collection

The three regions chosen for this study fall along a latitudinal transect in Ontario, which represents a gradient of historic atmospheric sulphur deposition. In Ontario, sulphur deposition has historically been highest at southern latitudes due to heavy industrialization, as can be seen in Figure 2.1 (adapted from Vet *et al.*, 2014), which also shows the specific sites chosen for this study.



**Figure 2.1:** Mean annual wet deposition of sulphur in Kg S/ha/year in North America in the years 2005–2007, adapted from Vet *et al.* (2014).

Locations of the three study sites along a latitudinal transect in Ontario are as follows:

A) The Sifton Bog in London, Ontario

B) White River, Ontario

C) DeBeers Victor Mine James Bay, Ontario

The most southerly site is the Sifton Bog located in London, Ontario (42°58'17.5"N 81°19'30.8"W). Sifton Bog is an acidic peat bog located within the city of London. It is a kettle lake wetland consisting of a shallow open pond of ~2 m depth at its centre with a maximum peat depth of ~10 m (Judd, 1957; City of London, 2009). The pond is surrounded by a floating *Sphagnum* moss mat that transitions into a closed spruce-tamarack swamp forest with a peat depth of ~2 m (Judd, 1957; City of London, 2009). The upland slopes surrounding the wetland consist of young to mature deciduous forest and shrub thickets (Judd, 1957; City of London, 2009). The sampling location for the current study was located within the *Sphagnum* moss mat. This site is hereafter referred to as the southern Ontario site.

The mid-latitude site is a poor nutrient fen located in White River, Ontario (48°21'13.3"N 85°20'17.6"W). This site is part of a long-term monitoring project maintained by the Ministry of Natural Resources and Forestry (MNRF) as part of the White River Experimental Watershed Study. The site is characterized by *Sphagnum* moss, shrubs, and

trees (black spruce and tamarack) with a peat depth of 0.5–3m overlying sandy deposits in the wetland portion of the fen, and 0.05–1 m in the upland portion of the fen (Webster & McLaughlin, 2010). A further description of the experimental area can also be found in Myers *et al.* (2012). This site is hereafter referred to as the low boreal site.

The most northerly site is located in the second largest peatland complex in the world; the James Bay Lowlands surrounding the DeBeers Victor Mine, which is ~90 km west of Attawapiskat. The peatland complex is characterized by 1.5–2.5 m of peat overlying mineral sediments (McCarter & Price, 2017). The region is covered by a range of peatland types from carbonate-rich fens to mineral poor bogs (Corson & Campbell, 2013; Riley, 2011). Similar to the other sites, the fens in this region are dominated by *Sphagnum* mosses, as well as a significant abundance of *Carex* sedges and cotton grass (Leclair, Whittington, & Price, 2015; Riley, 2011). The specific sampling location chosen is a reference fen (52°49'34.8"N 83°54'07.9"W) that is far enough removed from the mine that it is not affected by aquifer drawdown caused by mining activities (McCarter & Price, 2017). This site is hereafter referred to as the subarctic site.

A single peat block of ~30 x 30 x 30 cm was collected from each site using a handsaw and shovel to extract the peat after the top 10 cm of vegetation was removed. Peat from the southern Ontario site was collected in June 2018, from the low boreal site in July 2018, and from the subarctic site in August 2018. The peat was kept sealed, and saturated in a black plastic bag to exclude light, and was kept at +4 °C during transport from the field sites and during storage. Storage period pre-experiment differed between sites, as experiments were initiated in November 2018 for the southern Ontario site, in February 2019 for the low boreal site, and May 2019 for the subarctic site. Surface peat bulk density was measured at all sites by cutting a 5 x 5 x 5 cm sub section of each peat core using a hand saw after removing the top 10 cm of vegetation. After sampling, the peat cube was preserved at +4 °C in a sealed plastic bag, uncompressed, until analysis in the laboratory. Each sample was then weighed on an aluminum tin with an analytical balance, the weight was recorded, and the sample was placed in an oven at 60 °C for ~96 h. To validate that all water was evaporated from the peat, peat samples were removed

from the oven after 96 h, weighed, placed in the oven for an additional hour, and weighed again to determine if there was any further water loss.

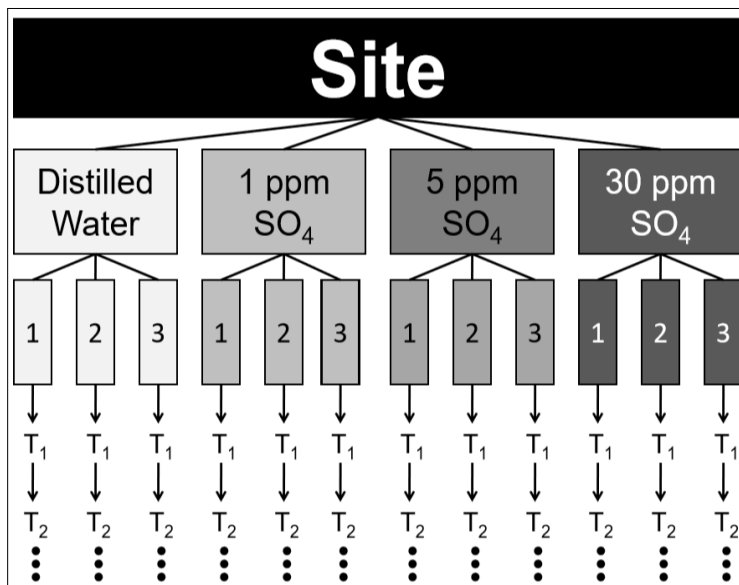
Once peat samples were completely dry, they were weighed again, and the mass was recorded. Bulk density in  $\text{g/cm}^3$  was then calculated by dividing the dry weight of peat by the volume of the peat sample ( $125 \text{ cm}^3$ ). The moisture content of the peat was calculated as the ratio of water to dry peat for each site. The field bulk densities and moisture contents of the three samples from the field sites are listed in Table 2.1.

**Table 2.1:** Bulk density ( $\text{g/cm}^3$ ) and moisture content measurements for peat samples collected from the three study sites used in the column experiments.

Study Site	Southern Ontario	Low Boreal	Subarctic
Bulk Density ( $\text{g/cm}^3$ )	0.057	0.052	0.054
Water:Peat Ratio	14.9	13.7	13.2

### 2.2.2 Experimental design and setup

The study design involved the application of 1, 5, and 30 ppm  $\text{SO}_4^{2-}$  solutions and a distilled water control to peat cores from the three study sites in a flow-through system over a period of ~13 days. A separate experiment was run for each study site, in which the  $\text{SO}_4^{2-}$  solutions and distilled water control were pumped through twelve columns of peat, with three columns used per  $\text{SO}_4^{2-}$  solution. The study used a mixed design, with peat column (the subjects) crossed with time (within subject factor) and nested within sulphate treatment and site (between subject factors). Figure 2.2 presents an overview of the experimental design.



**Figure 2.2:** Overview of experimental design for column experiments. For each of the three sites, a separate experiment is run applying a distilled water control, and three SO<sub>4</sub><sup>2-</sup> treatments to the columns. Each treatment has three replicate columns (represented here with the numbers 1–3), and each column is sampled periodically at pre-determined time intervals (represented here with T<sub>1</sub>, T<sub>2</sub>, etc.).

The experimental set-up used in the laboratory experiments is parallel to that developed by Twible (2017). The concentrations of SO<sub>4</sub><sup>2-</sup> to be added to the columns were the same used by Twible (2017) based off of the range of observed SO<sub>4</sub><sup>2-</sup> concentrations in the subarctic peatland study location when exposed to elevated SO<sub>4</sub><sup>2-</sup> loading. For more details on the study, see McCarter, Branfireun, & Price (2017). This range of SO<sub>4</sub><sup>2-</sup> concentrations ensured that there would be an observable Hg methylation response.

To further validate the use of these specific concentrations, I performed a calculation of the SO<sub>4</sub><sup>2-</sup> solution concentration needed for the flow-through system to reach atmospheric deposition rates similar to those for southern Ontario (the region with the highest historic SO<sub>4</sub><sup>2-</sup> deposition). This calculation was based on the data from Vet *et al.* (2014) and was adjusted for the time period of the experiment, surface area of peat inside the column, and column flow rate. For full details on the calculation, see Figure A1 in Appendix A. The calculated SO<sub>4</sub><sup>2-</sup> concentration was reasonably close to 1 ppm SO<sub>4</sub><sup>2-</sup>. The waste water additions at the subarctic peatland in McCarter, Branfireun, & Price (2017) were approximately 30 ppm SO<sub>4</sub><sup>2-</sup>, and so the SO<sub>4</sub><sup>2-</sup> additions chosen for this study represent elevated atmospheric deposition (1 ppm SO<sub>4</sub><sup>2-</sup>), elevated point source SO<sub>4</sub><sup>2-</sup> addition (30 ppm SO<sub>4</sub><sup>2-</sup>), and an intermediate SO<sub>4</sub><sup>2-</sup> addition (5 ppm SO<sub>4</sub><sup>2-</sup>).



Flow rates for the experiment were also similar to those used by Twible (2017) but were slightly higher for the current study to ensure consistent flow and to minimize blockage issues. Flow rates were set to 15–20 mL/h which falls within the range of flow volumes in peatland systems under natural hydraulic gradients reported previously (Rezanezhad *et al.*, 2016; Branham *et al.*, 2014). Volume of samples at each time point were recorded to ensure flow rates were consistent, and flow rates for individual columns were adjusted accordingly.

Peat cores from the study sites were packed into twelve 30 cm long x 4.8 cm wide (543 mL) Kimble® Kontes® Chromaflex® glass chromatography columns to a consistent bulk density of 0.07 g/cm<sup>3</sup>. A bulk density of 0.07 g/cm<sup>3</sup> was selected as the target bulk density of peat for packing the columns because it is similar to field bulk densities reported in the literature for northern, *Sphagnum* peatlands (Yu, 2012; Loisel *et al.*, 2014) and is slightly higher than the average bulk density determined for the sites used in the present study. Using a bulk density that was slightly higher than field bulk density for the sampled sites also ensured that gaps in the peat were minimal and the columns stayed saturated and well mixed to promote SO<sub>4</sub><sup>2-</sup> reduction.

The peat cores were first homogenized by tearing the peat core into ~1 cm<sup>3</sup> pieces in a glove bag under nitrogen, ensuring an anaerobic environment was maintained. Once the peat was thoroughly homogenized and mixed, subsamples were taken for bulk density trials to determine the wet mass of homogenized peat that yielded a bulk density of 0.07 g/cm<sup>3</sup> when packed into a 125 cm<sup>3</sup> cube. This mass was then extrapolated for the volume of the glass columns used in the experiments. For a full description of the calculation, see Figure A2 in Appendix A.

The glass columns were packed with peat using a glass rod attached to a round Polyethylene Terephthalate Glycol (PETG) bottle cap to ensure even compaction of peat material throughout the column. Columns were packed in a glove bag under nitrogen to maintain an anaerobic environment. Columns were closed with PTFE fittings with 20 µm porosity bed supports at both ends and were held upright in a metal frame with attached clamps. PTFE tubing of 1/16" diameter was connected to the column bed supports via

CTFE threaded adaptors, and CTFE flangeless fittings at both column inlet and outlets. Luer locks were used to connect the 1/16" inner diameter tubing at column inlets to Masterflex® two-stop silicon platinum-cured tubing for use with a twelve-cassette Carter Manostat peristaltic pump. Luer locks were used at the pump inlet to connect the Masterflex® tubing with PTFE 1/16" inner diameter tubing which was fed into the  $\text{SO}_4^{2-}$  solutions and distilled water. Figure B1 in Appendix B shows a picture of the experimental set-up.

The three  $\text{SO}_4^{2-}$  solutions and distilled water control were prepared in 20 L acid-cleaned Nalgene® carboys. A 1000 ppm  $\text{SO}_4^{2-}$  solution was first prepared by dissolving 1.81 g of  $\text{K}_2\text{SO}_4$  in 1 L of MilliQ deionized water (18.2 M $\Omega$ ) using a volumetric flask. A volume of 20, 100, and 600 mL of this solution was diluted with distilled water to a final volume of 20 L to prepare the 1, 5, and 30 ppm  $\text{SO}_4^{2-}$  solutions respectively. The solutions and distilled water control were pH-adjusted to 5 ( $\pm 0.2$ ) to more accurately mimic the pH typical of peatland pore waters (Rydin, 2013) using 150-170  $\mu\text{L}$  of OmniTrace hydrochloric acid. Sulphate concentrations of the solutions were then validated on a Dionex ICS-1600 ion chromatography system, run isocratically with an AS-14 anion column.

### 2.3.3 Outflow collection and analysis

Column outflow was sampled at 24 h intervals for the first three sampling periods, in 48 h increments for the following three to four sampling periods, and 72 h increments for the final three sampling periods. Column outflow was collected during sampling in acid-washed, bagged, 125 mL Nalgene® PETG bottles over a period of 6 h. Samples were kept in coolers with ice packs during sampling to maximize preservation during collection. After sampling, samples were vacuum filtered through 0.5  $\mu\text{m}$  glass fibre filter papers, and aliquots were taken for  $\text{S}^{2-}$  and DOC analysis. Filter blanks were prepared every other sampling period. Samples were then preserved for Hg analysis through acidification to 1% v/v with OmniTrace® hydrochloric acid. Samples were kept refrigerated and in the dark until analysis.

Dissolved organic carbon samples were analyzed using an OI Analytical Aurora 1030W Combustion TOC Analyzer. Approximately 10 mL of sample was transferred to 50 mL glass sample vials for use on the 1088 autosampler. High turbidity samples were diluted by a factor of 0.25–2 with deionized water to ensure that sample concentrations fell within the calibrated DOC range of 1–100 ppm. Analytical duplicates were run every 10 samples, as well as low (5 ppm), medium (50 ppm), and high (100 ppm) QC standards that bracketed the range of sample concentrations. All QC standards and duplicate recoveries were required to fall within 20% of expected values. Both reagent, and analytical blanks were run per batch to ensure lack of contamination in phosphoric acid and sodium persulphate reagents used in the wet oxidation method. Fresh reagents were made every 2 weeks.

Sulphide analysis was performed according to the methylene blue spectrophotometric method outlined in Cline (1969) adjusted for smaller sample sizes and adapted for use with the Horiba Aqualog® spectrofluorometer. Sixteen calibration standards from 0.03–32.06 ppm  $S^{2-}$  were prepared from  $Na_2S \cdot 9H_2O$  and deaerated reagent water (prepared by bubbling  $N_2$  through MilliQ deionized water). Standards were mixed on a magnetic stir plate in 125 mL filter flasks while the flasks were continuously purged with  $N_2$  to prevent oxidation. Five mL aliquots of each standard were injected into BD Vacutainer® tubes using a 12 mL syringe and 20G 1 inch BD PrecisionGlide® needle. Four mixed diamine reagents were prepared to the concentrations outlined in Cline (1969). Each reagent has a different diamine concentration that is compatible for use with a specified range of  $S^{2-}$  standards outlined in Cline (1969). The diamine reagent contains N,N-dimethyl-*p*-phenylenediamine sulphate, the chemical responsible for producing the blue colour central to the spectrophotometric method.

Diamine reagent was injected into each standard tube in 0.4 mL aliquots using a 1 mL syringe. The standards were mixed by inverting the tubes several times. The reagent and standard were allowed to react for 20 minutes before a ~4 mL aliquot was poured into 4 mL quartz cuvettes for analysis on the Horiba Aqualog®. Single absorbance measurements for each standard were taken at 670 nm with an integration time of 0.1 s and recorded. For each specified range of standards requiring a different concentration of

diamine reagent, three absorbance trials were run, and the average absorbance value for each standard was used to generate a calibration curve. In each trial, a cuvette containing MilliQ deionized water and the appropriate addition of diamine reagent was run as an instrument blank. Each calibration curve had an associated  $R^2$  value of at least 0.99. A summary of the calibration data is shown in Figure A3 in Appendix A.

Samples for  $S^{2-}$  analysis were collected using 12 mL syringes immediately after the bulk water sample was collected. The syringes were attached to the column outflow Teflon tubing using Luer lock adaptors, and approximately 6 mL of sample was drawn into the syringe. The Luer lock connection was then broken, and a 20G 1 inch BD PrecisionGlide® needle was fitted to the syringe using the Luer lock fittings. Five mL aliquots of the samples were immediately injected into 7 mL plastic BD Vacutainer® tubes to prevent oxidation of the sample. The appropriate diamine reagent was then injected in a 0.4 mL aliquot using a 1 mL syringe and 20G 1 inch BD PrecisionGlide® needle. The sample was mixed by inverting each tube several times, and samples were left to react for 20 minutes.

The samples were then run on the Horiba Aqualog® using the same procedure outlined for running  $S^{2-}$  calibration standards. Collection and analysis of the samples was staggered to standardize reaction time of the sample and diamine reagent before analysis. Blank correction was performed for each sample by analyzing oxidized column outflow samples that had been exposed to oxygen for at least 24 h and subtracting their absorbance from the absorbance of the corresponding  $S^{2-}$  sample. This ensured that any ambient absorbance at 670 nm from the sample matrix itself was accounted for in  $S^{2-}$  concentration calculations. The final concentration for each sample was calculated following blank correction using the linear equations derived from the calibration curves for each range of  $S^{2-}$  concentrations.

All elemental analysis was performed in the Biotron Analytical Services Laboratory (Western University, London, ON). Methylmercury and total Hg (THg) water analysis was performed following the cold vapour atomic fluorescence spectrometry (CVAFS) procedures outlined in EPA method 1630 (U.S. EPA, 1998) and 1631 (U.S. EPA, 2002)

respectively. Briefly, samples for MeHg analysis were diluted in 40 mL Teflon™ vials and distilled for ~3 h after the addition of ammonium pyrrolidine dithiocarbamate to volatilize MeHg present in the sample. The distillation vials were placed in a 125 °C heating block and glass receiver vials in a cooling block at 4 °C captured distillate. Distillate transfer took place through polyfluorinated plastic tubing using ultra purity N<sub>2</sub> as the carrier gas. All samples, as well as method blanks consisting of MilliQ deionized water, and 1.2 ppt quality control (QC) standards were standardized to an acidification of 0.5% using OmniTrace® hydrochloric acid before distillation.

A 30 mL aliquot of distillate was then transferred to glass instrument vials. Ascorbic acid was added to samples and vials were shaken to dissipate free halogens. The pH of all samples, blanks, and QC standards were standardized to ~4.5 by adding a 2 M acetate buffer. Lastly, tetraethyl borate was added to instrument vials to ethylate MeHg in the samples for detection on the Tekran® 2700 automated methylmercury analysis system. Method performance was monitored by method blanks, QC standards, 1.2 ppt sample matrix spikes, and duplicates run every 10 samples, and instrument performance was monitored by 0.5 ppt on-going precision recovery standards run every 10 samples. The instrument was calibrated for every new run, in the MeHg range of 0.02–9.0 ppt. Matrix spike and QC standard recoveries were required to fall within 33% of expected values, while on-going precision recovery standards were required to fall within 15% of their expected values.

Samples for THg analysis were diluted in 30 mL glass instrument vials, and samples were digested for ~24 h with the addition of a bromine monochloride solution. A hydroxylamine hydrochloride solution was then added, and vials were shaken to dissipate free halogens. Finally, stannous chloride was added to all samples to convert all elemental Hg in the samples to gaseous elemental Hg for analysis on the Tekran® 2600 automated mercury analysis system. All method blanks, 0.125 ng QC standards, and 0.125 ng matrix spikes were analyzed following the same CVAFS procedure outlined above. Instrument performance was monitored using 0.125 ng on-going precision recovery standards. The instrument was calibrated for every new run, in the THg range of 0.02–1.0 ng. Matrix spike and QC standard recoveries were required to fall within 33%

of expected values, while on-going precision recovery standards were required to fall within 15% of their expected values. Inorganic Hg (IHg) values were derived from THg values by subtracting MeHg concentrations from THg concentrations. Inorganic Hg measured and calculated in this way represents all Hg species in the sample that are not in the methylated form.

### 2.3.4 Peat soil analysis

Subsamples of peat for elemental analysis were taken both prior to, and after each experiment. Pre-experiment subsamples were taken from homogenized bulk peat from each site, while post-experiment subsamples from each column were taken after manual re-homogenization of peat cores. All peat subsamples were stored in a -80 °C freezer until lyophilization. Samples were lyophilized for ~96 h or until all water was sublimated from the peat, then thoroughly homogenized by pulse grinding in a stainless steel grinder ensuring sample integrity was maintained by cleaning with acetone in between samples. Samples were then analyzed for THg, MeHg, % sulphur, and carbon/nitrogen (C/N) ratios, with additional analysis of total Fe on pre-experiment subsamples. The results of these analyses for the pre-experiment subsamples are shown in Table 2.2. Further information on elemental concentrations of Hg, Fe, and sulphur as well as C/N ratio at these sites can also be found in the results section of Chapter 3.

**Table 2.2:** Initial values of THg, MeHg, % sulphur, C/N Ratio, and total Fe in homogenized peat from the three field sites used in the column experiments. Concentrations are based on dry weights of lyophilized peat samples.

Site	Total Mercury (ng/g)	Methylmercury (ng/g)	Sulphur (%)	C/N Ratio	Iron (mg/g)
<b>S. Ontario</b>	79.11	3.55	0.311	30.3	1.75
<b>Low Boreal</b>	119.27	8.91	0.302	22.4	2.64
<b>Subarctic</b>	82.66	1.83	0.121	34.0	1.21

Total Hg analysis on solid peat samples was carried out using a Milestone Direct Mercury Analyzer (DMA)-80 following EPA method 7473 (U.S. EPA, 2007). Analytical duplicates, blanks, and 50 ng matrix spikes were run every 10 samples. The certified reference material (CRM) MESS-3 ( $0.091 \pm 0.009$  mg/kg Hg) was also run every 10

samples to validate instrument performance throughout the run. All matrix spike, duplicate, and CRM recoveries were required to fall within 20% of their expected value. All blanks were required to fall below the method reporting limit (0.24 ng). Methylmercury analysis on solid peat samples was performed by first digesting ~100 mg dry peat samples with 4.0 M HNO<sub>3</sub> followed by a microwave digestion for 4 h at 82 °C. Digestate was diluted with MilliQ deionized water before analysis on the Tekran® 2700, once again following the EPA 1630 (U.S. EPA, 1998) protocol. As with water samples, IHg concentration in solid peat samples was calculated as the difference between THg and MeHg concentration.

Analysis for % sulphur C/N ratios was performed using a CHNS purge and trap chromatography system (Elementar vario ISOTOPE cube). Calibration of the instrument was validated each run with daily factor sample recoveries of sulfanilamide (41.85 % C, 18.62 % S, 16.27% N) which were required to fall within 10% of their targets. Consistent performance of the instrument was validated by including the CRM B2166 (48.09% C, 2.12% N, 0.17 % S) every 10 samples. Certified reference material recovery was required to fall within 15% of expected values. Analytical duplicates and blanks were run every 10 samples. Blanks consisted of empty tin boats used for sample packaging and were required to fall below the method reporting limit (MRL) which was 0.077 mg C, 0.006 mg N, and 0.017 mg S. Duplicate recoveries were required to fall within 20% of one other. Approximately 30 mg of dry sample was used, and a 5:1 ratio of sample to tungsten trioxide was used to ensure complete sulphur oxidation during analysis. Analysis of total iron in solid peat samples was performed by digesting samples with an acid microwave digestion, and then running them on an Agilent 7700 inductively coupled plasma mass spectrometry (ICP-MS) system according to EPA method 200.8 (U.S. EPA, 1994) following filtration.

### 2.2.5 Statistical analysis

All statistical analyses were performed using the statistical computing software R version 3.6.1 (R Core Team, 2019). The nlme linear and nonlinear mixed effects models package (Pinheiro *et al.*, 2019) in R was used for analysis of all water sample time series data.

Mixed linear models were assigned for each dependent variable with main effects of time,  $\text{SO}_4^{2-}$  treatment, and site and with column (1–12) as the random effect. Backwards selection was used to select the model of best fit for the data based on AIC values, and all models were fit using restricted maximum likelihood. The Anova function, part of the car package in R (Fox & Weisberg, 2019), was used to summarize significance of main effects and interactions via type III Wald Chi-Squared tests. Additional variance structures were specified for main factors where their inclusion resulted in better model fit. The assumptions of normality and homoscedasticity were validated through residual histograms, QQ-plots, and standardized residual plots. In most cases, data required  $\log_{10}$  transformation to homogenize residuals.

Changes in solid peat Hg and sulphur concentrations in the experimental columns were analyzed using two-way ANOVAs with site and treatment as main factor effects. Assumptions were validated through histograms of residuals, QQ-plots, Levene's test for equality of variances in the car package (Fox & Weisberg, 2019), and Cook's distance for identification of influential data points. Tukey's (HSD) tests were run on the ANOVA models to identify group differences. All figures were created using the R package ggplot2 (Whickham, 2016).

## 2.3 Results

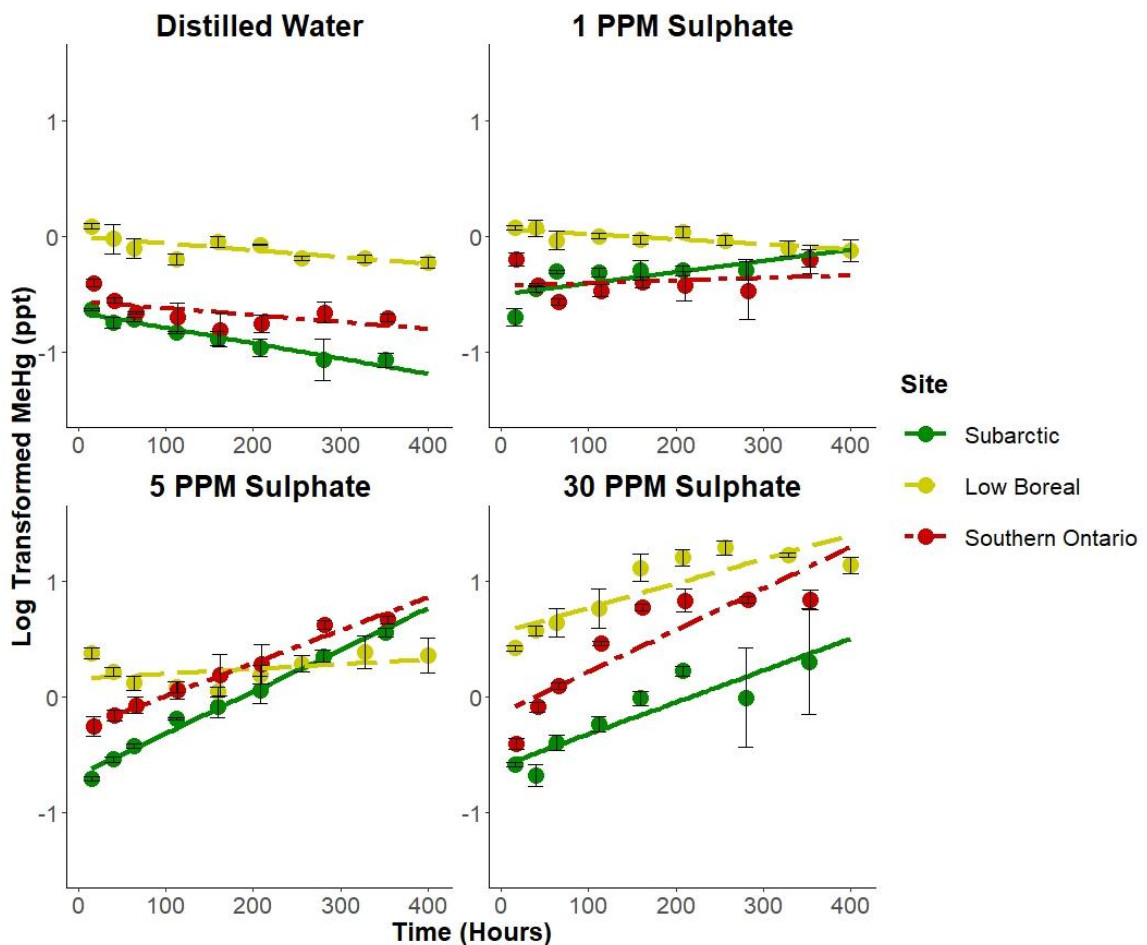
### 2.3.1 Column outflow biogeochemistry

**Methylmercury:** The results from the type III Wald Chi-Squared test show that there is a significant three-way interaction between  $\text{SO}_4^{2-}$  treatment, site, and time ( $\chi^2(6) = 37.86$ ,  $p < 0.05$ ). This suggests that the effect that site and  $\text{SO}_4^{2-}$  treatment have on MeHg concentration over time was highly dependent on the interaction between these two factors. This can be seen visually in Figure 2.3; the linear regression lines for the majority of  $\text{SO}_4^{2-}$  treatment and site combinations have different slopes for MeHg concentration measured over time.

Slopes of MeHg concentration over time were negative for all sites in the control treatment, but generally increased when moving to higher  $\text{SO}_4^{2-}$  treatments. The subarctic



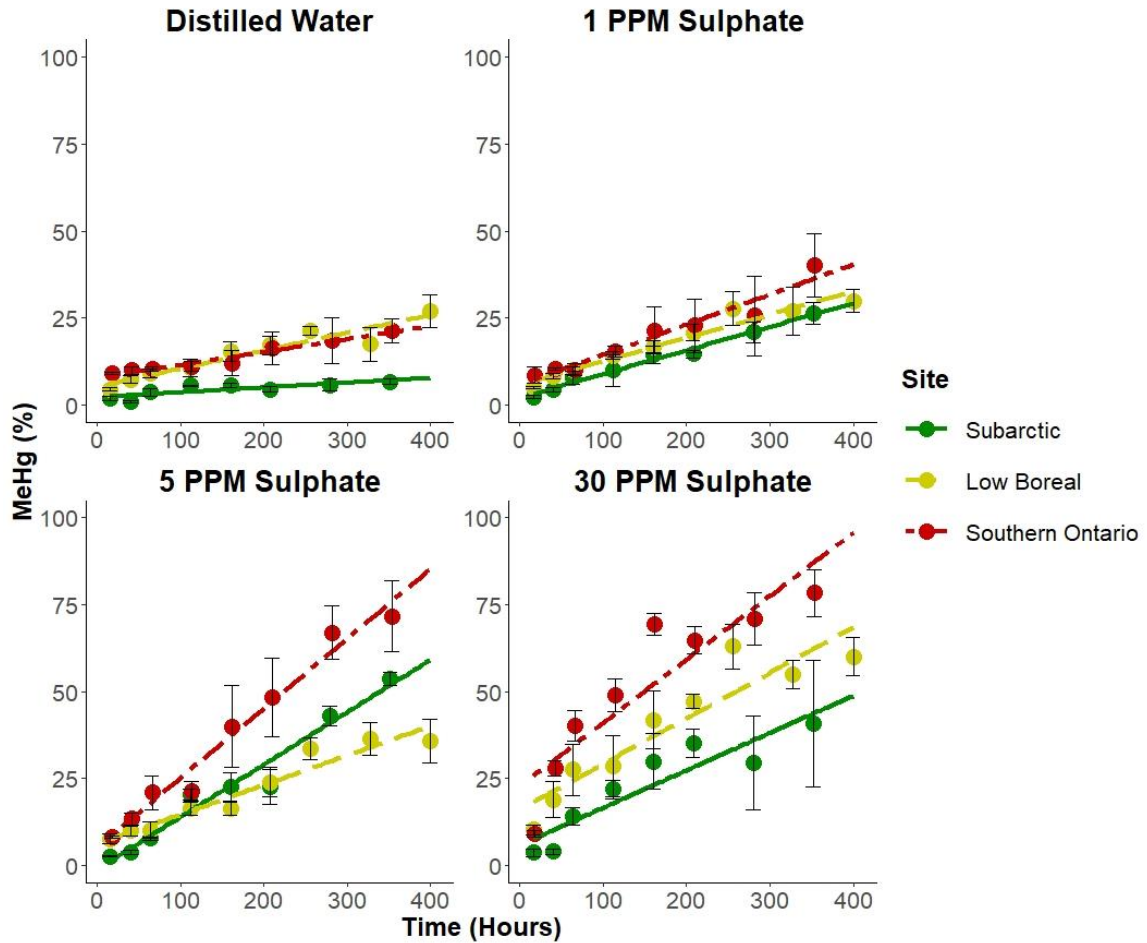
site displayed significantly higher slopes for MeHg increase over time within the 1 ppm and 5 ppm  $\text{SO}_4^{2-}$  treatment compared to the other two sites ( $p < 0.05$ ) with the low boreal site having the lowest slopes in these treatments. However, in the 30 ppm treatment, the slopes of MeHg increase over time were not significantly different between the subarctic site and the low boreal ( $p = 0.12$ ) and southern Ontario ( $p = 0.89$ ) sites. The y-intercepts for MeHg concentration over time were highest for the low boreal site in all  $\text{SO}_4^{2-}$  treatments and were significantly different from those of the subarctic site in both the distilled water control and 30 ppm  $\text{SO}_4^{2-}$  treatment ( $p < 0.05$ ). The low boreal site also had the highest overall concentration of MeHg in outlet waters compared to all other treatment and site combinations (maximum of  $19.66 \pm 2.68$  ppt).



**Figure 2.3:** Log transformed MeHg concentration (ppt) in column outlet waters measured over 400 hours. Points represent the average of three replicate samples with standard error bars. Linear regression lines are drawn through the points for each site (represented by different colours) within each  $\text{SO}_4^{2-}$  treatment.

**Percent Methylmercury:** The results for % MeHg change over time are shown in Figure 2.4. Percent MeHg values are generated by dividing MeHg concentrations by THg in column outflow waters and multiplying by 100. Percent MeHg values more accurately reflect active Hg methylation in peat cores because they account for changes in THg partitioning between aqueous and solid phases. That is, absolute MeHg concentrations indicate the quantity of MeHg present in a given phase, while % MeHg values indicate the proportion of THg in that phase that is in the methylated form. Although none of the y-intercepts of % MeHg change over time were significantly different between treatments or sites, the slopes of these lines were impacted by site and treatment, as is evident from the significant interactions between time and treatment ( $\chi^2(3) = 108.35$ ,  $p < 0.05$ ), as well as time and site ( $\chi^2(2) = 15.70$ ,  $p < 0.05$ ). Much like the absolute MeHg concentration, the slope of % MeHg over time generally increased with larger  $\text{SO}_4^{2-}$  additions. The southern Ontario site reached the highest % MeHg in column outflow waters, with a maximum value of  $78.36 \pm 6.72$  % occurring at 354 hours in the 30 ppm  $\text{SO}_4^{2-}$  treatment.

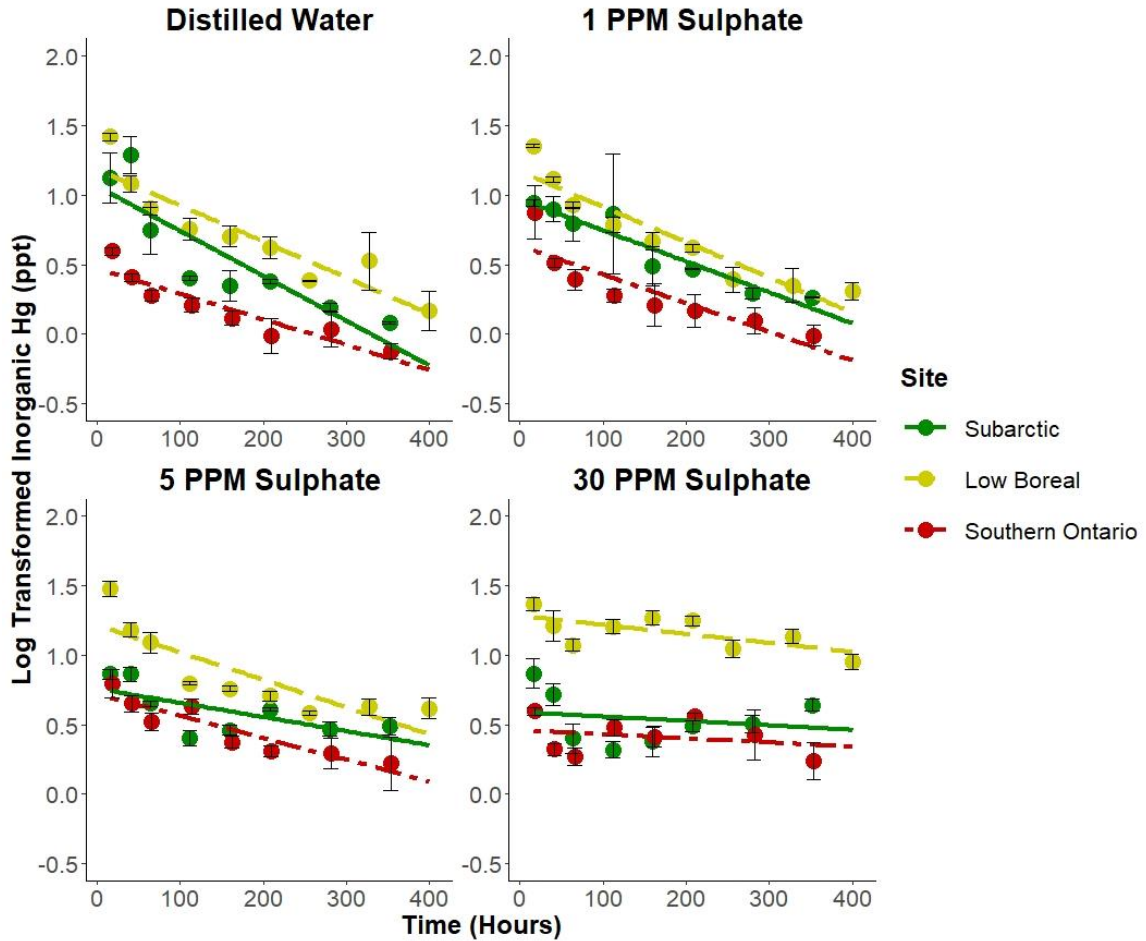
Slopes for all three sites were similar in the 30 ppm and 1 ppm  $\text{SO}_4^{2-}$  treatments, but differences between the sites were apparent in the distilled water control and 5 ppm  $\text{SO}_4^{2-}$  treatments. In the distilled water control, the low boreal site and southern Ontario site had higher slopes compared to the subarctic site by  $0.034 \pm 0.0087$  %/h ( $p < 0.05$ ) and  $0.022 \pm 0.009$  %/h ( $p < 0.05$ ) respectively. In the 5 ppm  $\text{SO}_4^{2-}$  treatment, the subarctic and southern Ontario sites had similar slopes for % MeHg increase over time, but the low boreal site had a significantly lower slope by approximately  $0.01 \pm 0.018$  %/h ( $p < 0.05$ ). Regardless, all sites showed an increase in % MeHg over time for all treatments, including the distilled water control.



**Figure 2.4:** MeHg (%) in column outflow water measured over 400 hours. Points represent the average of three replicate samples with standard error bars. Linear regression lines are drawn through the points for each site (represented by different colours) within each  $\text{SO}_4^{2-}$  treatment.

**Inorganic mercury:** The type III Wald Chi-Squared test for IHg concentration in column outflow indicates that there is a significant two way interaction between treatment and time ( $\chi^2(3) = 62.14, p < 0.05$ ), but not between site and time ( $\chi^2(2) = 5.03, p = 0.08$ ). These results suggest that  $\text{SO}_4^{2-}$  treatment has a significant effect on the slope of IHg concentration over time but site does not, as can be seen visually from Figure 2.5. With subsequent  $\text{SO}_4^{2-}$  treatments, the slope of IHg concentration over time increased but remained similar between sites within each treatment. However, all slopes remained negative for all treatments, indicating a net loss of IHg over time. All y-intercepts of IHg concentration over time were similar for sites within the same treatment, with the exception of the low boreal site which had a significantly higher y-intercept value in the

30 ppm  $\text{SO}_4^{2-}$  treatment ( $p < 0.05$ ). Because of this high y-intercept and shallow slope, IHg concentrations for the low boreal site remained elevated in the 30 ppm treatment for the duration of the experiment at  $9.07 \pm 1.05 - 23.62 \pm 2.35$  ppt.

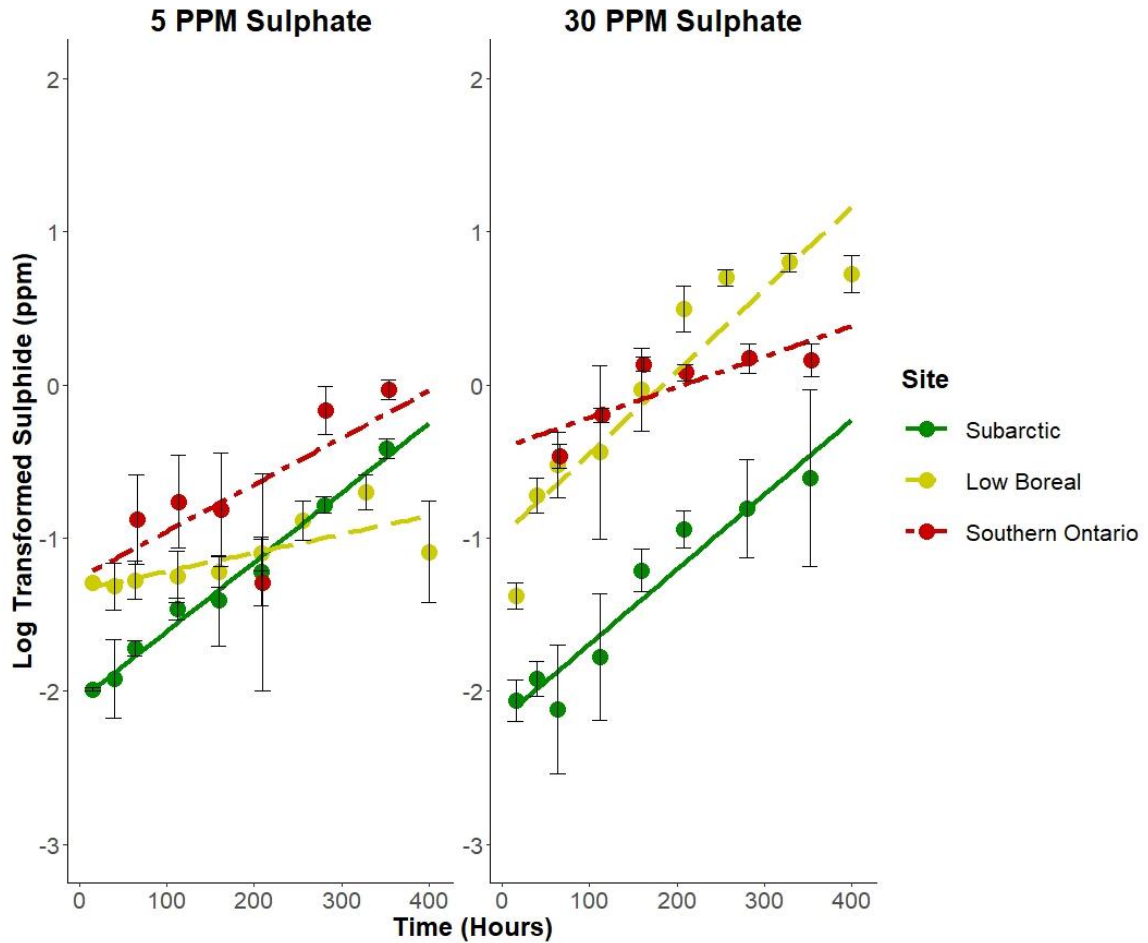


**Figure 2.5:** Log transformed inorganic Hg (ppt) in column outflow water measured over 400 hours. Points represent the average of three replicate samples with standard error bars. Linear regression lines are drawn through the points for each site (represented by different colours) within each  $\text{SO}_4^{2-}$  treatment.

**Sulphide:** The results for dissolved  $\text{S}^{2-}$  data are visualized in Figure 2.6. Note that only the 5 and 30 ppm  $\text{SO}_4^{2-}$  treatments are shown due to the prevalence of values below detection limit for the distilled water control and 1 ppm  $\text{SO}_4^{2-}$  treatment. The results from the type III Wald Chi-Squared test show that there is a significant interaction between time,  $\text{SO}_4^{2-}$  treatment, and site ( $\chi^2(2) = 21.45$ ,  $p < 0.05$ ). However, this interaction is mainly driven by the low boreal site, which showed a significantly different slope of  $\text{S}^{2-}$  concentration over time compared to the other two sites within both  $\text{SO}_4^{2-}$  treatments

( $p < 0.05$ ), as well as a significantly different slope between  $\text{SO}_4^{2-}$  treatments ( $p < 0.05$ ). Within the 5 ppm  $\text{SO}_4^{2-}$  treatment, the low boreal site had the lowest slope of  $\text{S}^{2-}$  concentration over time compared to the other sites, while in the 30 ppm  $\text{SO}_4^{2-}$  treatment, this site showed the highest slope. Both the subarctic and southern Ontario sites did not have significantly different slopes for  $\text{S}^{2-}$  concentration over time within both  $\text{SO}_4^{2-}$  treatments, and slopes for both sites did not change significantly between  $\text{SO}_4^{2-}$  treatments.

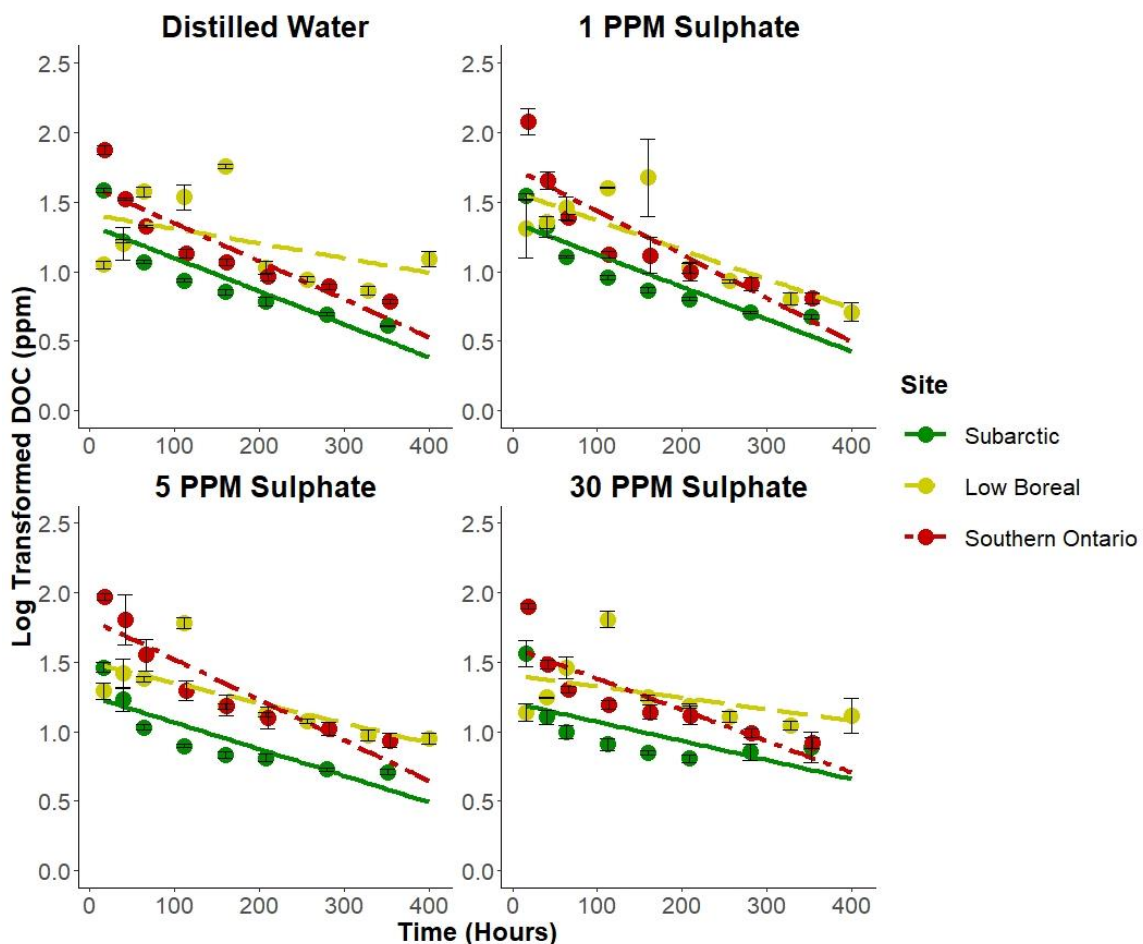
The y-intercepts of  $\text{S}^{2-}$  concentration over time in the 5 ppm  $\text{SO}_4^{2-}$  treatment were significantly higher than the subarctic site for the low boreal ( $p = 0.017$ ) and southern Ontario ( $p = 0.02$ ) sites, with the southern Ontario site having the highest y-intercept. The y-intercepts for the subarctic and low boreal sites did not change significantly between the 5 and 30 ppm  $\text{SO}_4^{2-}$  treatments, but the y-intercept of the southern Ontario site was significantly higher in the 30 ppm  $\text{SO}_4^{2-}$  treatment compared to the 5 ppm  $\text{SO}_4^{2-}$  treatment ( $p = 0.046$ ). Overall  $\text{S}^{2-}$  production was highest in the 5 ppm  $\text{SO}_4^{2-}$  treatment for the southern Ontario site, reaching a maximum of  $0.95 \pm 0.13$  ppm at 354h, and was highest in the 30 ppm  $\text{SO}_4^{2-}$  treatment for the low boreal site, reaching a maximum of  $6.40 \pm 0.84$  ppm at 328h.



**Figure 2.6:** Log transformed dissolved  $S^{2-}$  (ppm) in column outflow water measured over 400 hours. Points represent the average of three replicate samples with standard error bars. Linear regression lines are drawn through the points for each site (represented by different colours) within each  $SO_4^{2-}$  treatment.

**Dissolved organic carbon:** The results for DOC in column outflow are shown in Figure 2.7. While  $SO_4^{2-}$  treatment did not have a significant effect on DOC concentration over time, both time and site did, as well as the interaction between these two factors ( $\chi^2(2) = 28.29$ ,  $p < 0.05$ ). Time had a negative effect on DOC concentration, with all slopes for DOC concentration over time being negative. Across all  $SO_4^{2-}$  treatments, the low boreal site showed a more positive slope than the subarctic site ( $p = 0.007$ ) while the southern Ontario site showed a more negative slope than the subarctic site ( $p = 0.001$ ). Y-intercepts of DOC concentration over time were lower across all  $SO_4^{2-}$  treatments for the low boreal site ( $p = 0.002$ ), and higher across all  $SO_4^{2-}$  treatments for the southern Ontario site ( $p < 0.05$ ). This is consistent with the higher average values of DOC concentration over

time for the southern Ontario site across  $\text{SO}_4^{2-}$  treatments, which varied from  $22.34 \pm 4.76$  to  $34.55$  ppm compared to the low boreal ( $21.83 \pm 3.57$  to  $25.92 \pm 5.90$  ppm) and subarctic ( $11.16 \pm 1.69$  to  $12.76 \pm 2.10$  ppm) sites.



**Figure 2.7:** Log transformed DOC (ppm) in column outflow water measured over 400 hours. Points represent the average of three replicate samples with standard error bars. Linear regression lines are drawn through the points for each site (represented by different colours) within each  $\text{SO}_4^{2-}$  treatment.

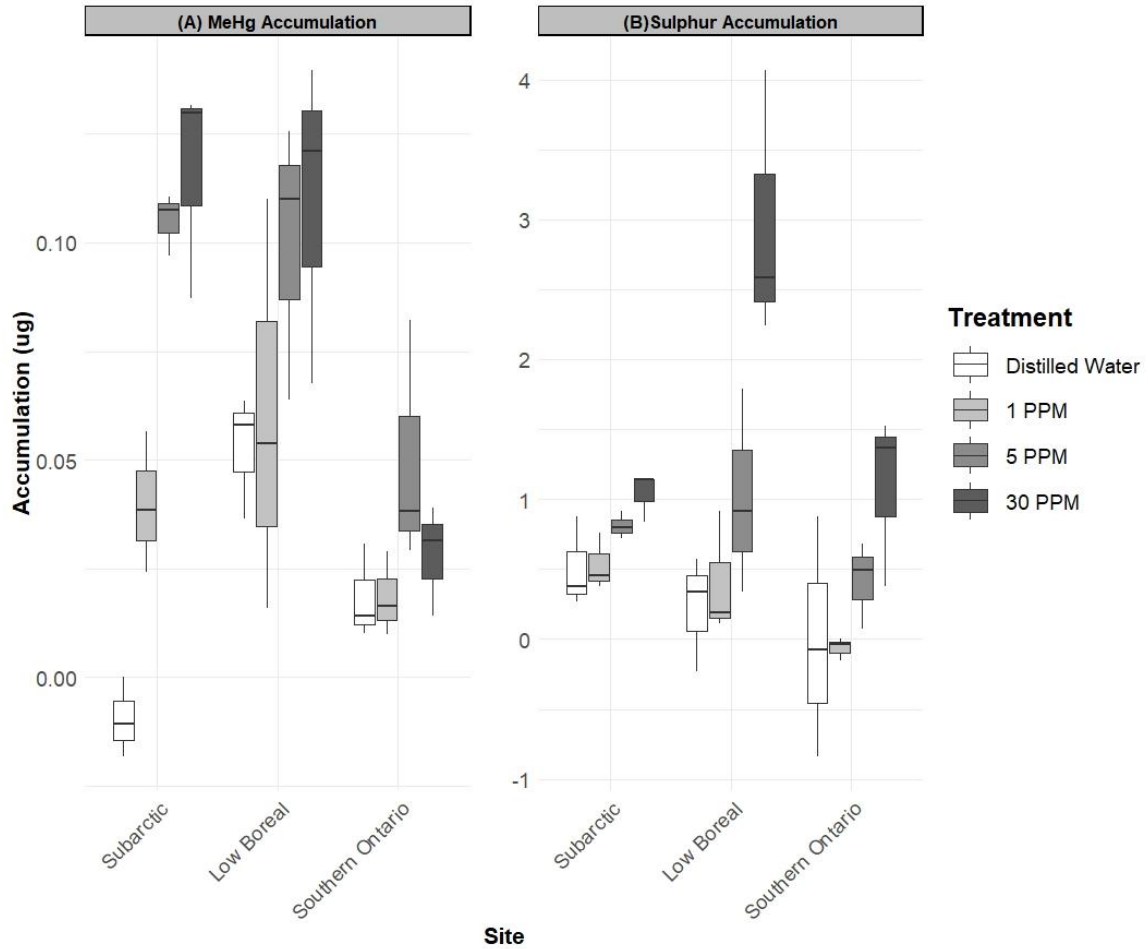
### 2.3.2 Peat core chemistry

**Methylmercury and sulphate:** Mercury and sulphur accumulation were calculated based on the difference between initial and final concentrations of sulphur, IHg, and MeHg in dry samples from peat cores. Accumulation values are based on the dry weight of peat cores (38.01 g) calculated by multiplying the target dry bulk density used for packing columns ( $0.07 \text{ g/cm}^3$ ) by the volume of the columns ( $543 \text{ cm}^3$ ). The results of the two-way ANOVA for MeHg accumulation in the experimental peat cores show that both

site ( $F(2,33)=14.07$ ,  $p<0.05$ ) and treatment ( $F(3, 32)=16.15$ ,  $p<0.05$ ) had a significant effect on MeHg accumulation, and that there is also an interaction between these two factors ( $F(11,24) =3.32$ ,  $p=0.016$ ). The southern Ontario site showed significantly lower MeHg accumulation in its peat cores compared to the low boreal ( $p<0.05$ ) and subarctic ( $p=0.006$ ) sites. On average the low boreal site had higher MeHg accumulation by  $0.05 \pm 0.02 \mu\text{g}$ , and the subarctic site had higher MeHg accumulation by  $0.03 \pm 0.02 \mu\text{g}$  compared to the southern Ontario site. For all sites, both the 30 ppm and 5 ppm  $\text{SO}_4^{2-}$  treatments increased MeHg accumulation on average by  $0.06 \pm 0.03 \mu\text{g}$  compared to the distilled water control. The distilled water control and the 1 ppm  $\text{SO}_4^{2-}$  treatment were not significantly different for MeHg accumulation ( $p=0.37$ ), and neither were the 5 ppm and 30 ppm  $\text{SO}_4^{2-}$  treatments ( $p=0.99$ ). These results are shown in Figure 2.8A.

The results from the two-way ANOVA for sulphur accumulation show that both site ( $F(2,33)=7.04$ ,  $p=0.004$ ) and treatment ( $F(3,32)=15.20$ ,  $p<0.05$ ) had a significant effect on accumulation in peat cores, as well as their interaction ( $F(11,24)=3.07$ ,  $p=0.02$ ). Overall sulphur accumulation was highest in the low boreal site, although the low boreal site was only significantly different for sulphur accumulation when compared to the southern Ontario site ( $p=0.003$ ) which had the lowest overall sulphur accumulation. This trend, however, appears to be in part driven by the high sulphur accumulation in the 30 ppm  $\text{SO}_4^{2-}$  treatment for the low boreal site. Although from Figure 2.8B it is apparent that sulphur accumulation generally increased with higher  $\text{SO}_4^{2-}$  additions, only the 30 ppm  $\text{SO}_4^{2-}$  treatment was significantly different from the other treatments. The 30 ppm  $\text{SO}_4^{2-}$  treatment increased sulphur accumulation on average by  $0.95 \pm 0.68 \mu\text{g}$  compared to the 5 ppm  $\text{SO}_4^{2-}$  treatment ( $p=0.004$ ), by  $1.41 \pm 0.68 \mu\text{g}$  compared to the 1 ppm  $\text{SO}_4^{2-}$  treatment ( $p<0.05$ ), and by  $1.46 \pm 0.68 \mu\text{g}$  compared to the distilled water control ( $p<0.05$ ).



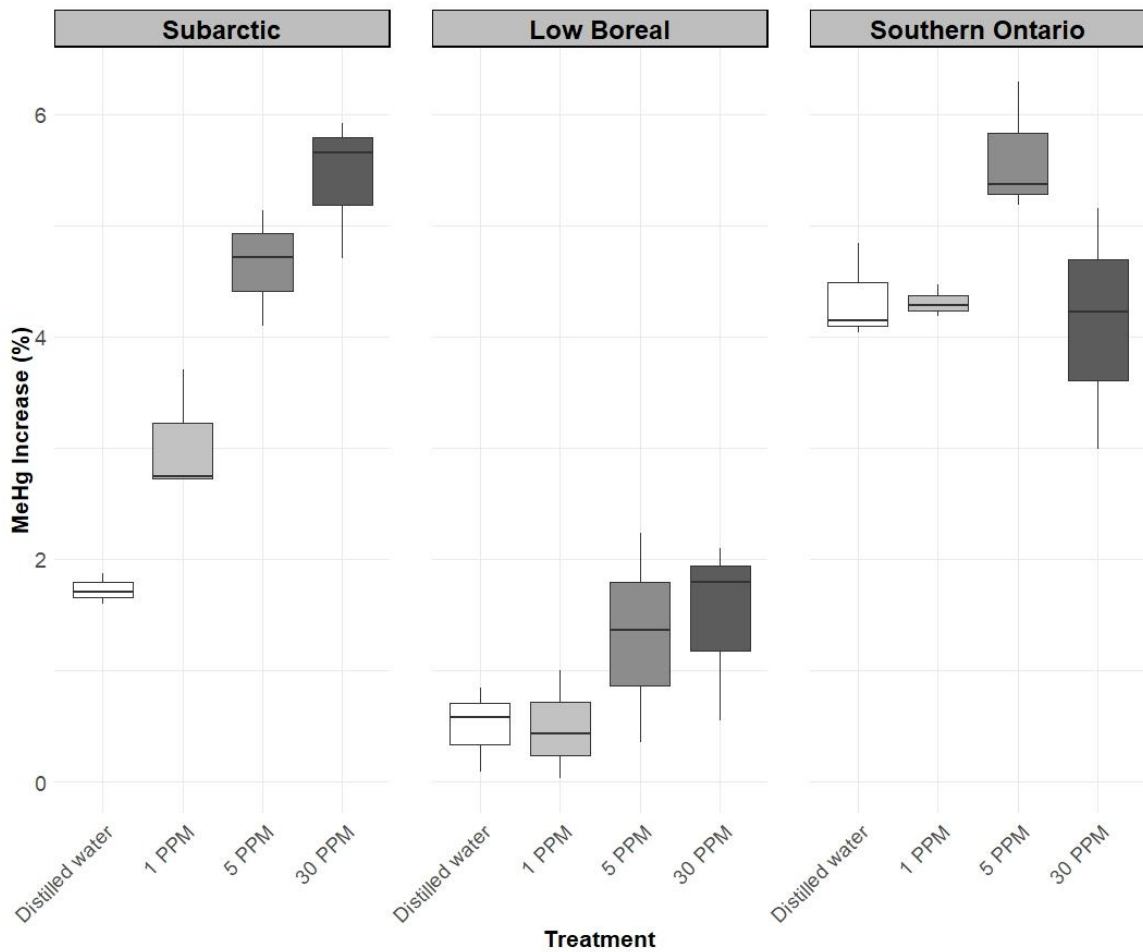


**Figure 2.8:** Accumulation ( $\mu\text{g}$ ) of (A) MeHg and (B) Sulphur in peat cores post-column experiment. Boxes represent the interquartile range of three column replicates for each site and  $\text{SO}_4^{2-}$  treatment combination. Treatments are listed in the legend by colour, and sites are listed along the x-axis. Horizontal lines inside boxes represent median values, and vertical lines above, and below boxes represent maximum and minimum values respectively.

Percent MeHg increase was calculated by taking the difference in % MeHg in peat cores between the start and end of the experiment. Similar results to those for absolute MeHg accumulation are seen in % MeHg increase in peat cores; significant site ( $F(2, 33)=111.31, p<0.05$ ), treatment ( $F(3,32)=15.14, p<0.05$ ), and site by treatment interaction ( $F(11,24)=5.78, p<0.05$ ) effects are produced by the two-way ANOVA. These results are shown in Figure 2.9.

The southern Ontario site showed the highest overall increase in % MeHg in peat cores compared to the subarctic ( $p<0.05$ ) and low boreal ( $p<0.05$ ) sites. On average, the

subarctic site showed  $0.88 \pm 0.64$  less % MeHg increase compared to the southern Ontario site, and the low boreal site showed  $3.65 \pm 0.64$  less % MeHg increase compared to the southern Ontario site. Once again, the 5 and 30 ppm  $\text{SO}_4^{2-}$  treatments were not significantly different in terms of % MeHg increase in peat cores ( $p=0.92$ ) and neither were the distilled water control and 1 ppm  $\text{SO}_4^{2-}$  treatments ( $p=0.48$ ). However, the 30 ppm  $\text{SO}_4^{2-}$  treatment did increase the % MeHg in peat cores compared to the distilled water control by  $1.49 \pm 0.81$  % ( $p<0.05$ ) on average, while the 5 ppm  $\text{SO}_4^{2-}$  treatment increased % MeHg compared to the distilled water control by  $1.67 \pm 0.81$  % ( $p<0.05$ ) on average.



**Figure 2.9:** MeHg increase (%) in peat cores post-column experiment. Boxes represent the interquartile range of three column replicates for each site and  $\text{SO}_4^{2-}$  treatment combination. Results are separated by site, and treatments are listed along the x-axis. Horizontal lines inside boxes represent median values, and vertical lines above, and below boxes represent maximum and minimum values respectively.

**Inorganic mercury and carbon/nitrogen ratios:** Based on the two-way ANOVA for IHg accumulation in peat cores, there was no significant effect of site ( $F(2,33)=0.88$ ,  $p=0.43$ ), treatment ( $F(3,32)=0.28$ ,  $p=0.84$ ), or their interaction ( $F(11,24)=1.06$ ,  $p=0.41$ ) on IHg accumulation. While  $\text{SO}_4^{2-}$  treatment did not significantly affect C/N ratios in the peat cores, site did have a significant effect ( $F(2,33)=274.0$ ,  $p<0.05$ ). The low boreal site was the only site that differed significantly from the other two sites, on average having C/N ratios that were  $9.19 \pm 1.18$  lower than the southern Ontario site, and  $9.98 \pm 1.16$  lower than the subarctic site. Both the subarctic site and the southern Ontario site had similar C/N ratios, of  $32.59 \pm 0.27$  for the subarctic site, and  $31.80 \pm 0.49$  for the southern Ontario site.

## 2.4 Discussion

### 2.4.1 Evidence of a sulphate priming effect

As initially predicted, the site with a history of the highest  $\text{SO}_4^{2-}$  deposition (the southern Ontario site) showed the largest overall Hg methylation response. Higher legacy  $\text{SO}_4^{2-}$  deposition at the lower latitude sites is confirmed by the initial % sulphur values in the cores, which are highest for the low boreal and southern Ontario sites. The enhanced response of the southern Ontario site to  $\text{SO}_4^{2-}$  inputs is most apparent in the % MeHg data, for which the southern Ontario site not only shows the largest cumulative increase in % MeHg in column outflow waters, but also shows the highest rate of increase of % MeHg over the course of the experiment. This data would seem to support the  $\text{SO}_4^{2-}$  priming hypothesis; the southern Ontario site, being previously exposed to higher levels of  $\text{SO}_4^{2-}$ , shows an increased rate of MeHg production due to a difference in the bacterial community itself or the geochemical environment (Coleman-Wasik *et al.*, 2015).

The fact that both the low boreal site and southern Ontario site showed a greater increase in % MeHg over time compared to the pristine subarctic site in the distilled water treatment supports the idea that the ability of these peats to methylate MeHg even in the absence of significant  $\text{SO}_4^{2-}$  inputs is enhanced because of past  $\text{SO}_4^{2-}$  exposure. There also appears to be higher levels of SRB activity at the onset of the experiment for both the low boreal and southern Ontario sites as evidenced by the higher y-intercepts of  $\text{S}^{2-}$

concentration over time compared to the subarctic site. Again, this supports the theory that past exposure to  $\text{SO}_4^{2-}$  has primed the SRB at the low boreal and southern Ontario sites such that they respond more readily to further inputs of  $\text{SO}_4^{2-}$ . Although the southern Ontario site does not accumulate as much MeHg by mass as the other two sites, it does show the highest % MeHg increase, and since % MeHg in soils and sediments is often used as a proxy for long-term Hg methylation potential (Drott *et al.*, 2008; Bailey *et al.*, 2017), this result is strong evidence for the enhanced Hg methylation potential of the southern Ontario peat compared to the other two sites.

#### 2.4.2 The 'Goldilocks Zone' of sulphate addition

With higher additions of  $\text{SO}_4^{2-}$ , the slope of the change in % MeHg over time also increased, and this response was consistent across sites. This result is expected as an increase in Hg methylation following the addition of  $\text{SO}_4^{2-}$  to wetland soils has long been established (e.g. Branfireun *et al.*, 2001; Jeremiason *et al.*, 2006; Bergman *et al.*, 2012). However, as can be seen from Figure 2.9, the solid phase % MeHg increase for both the southern Ontario and low boreal sites was lower than would be expected for the 30 ppm  $\text{SO}_4^{2-}$  addition. The southern Ontario site in particular showed the highest level of % MeHg increase in the solid phase not for the 30 ppm  $\text{SO}_4^{2-}$  addition, but for the intermediate level of  $\text{SO}_4^{2-}$  addition (5 ppm).

This result would seem to support the 'Goldilocks Zone' of MeHg production proposed by Johnson *et al.* (2016); MeHg production is highest when there is a sufficient level of  $\text{SO}_4^{2-}$  to stimulate SRB activity, but not so much as to cause the production of high levels of  $\text{S}^{2-}$  that can inhibit Hg methylation through insoluble  $\text{HgS}_{(s)}$  formation (Gilmour *et al.*, 1998; Benoit *et al.*, 1999). For example, Bailey *et al.* (2017) found that at dissolved  $\text{S}^{2-}$  concentrations of more than 0.65 ppm, Hg methylation potential decreased in sediments due to  $\text{S}^{2-}$  inhibition. In the current experiment, dissolved  $\text{S}^{2-}$  concentrations in the 30 ppm  $\text{SO}_4^{2-}$  treatment reached levels as high as 3 and 6 ppm for the southern Ontario and low boreal sites respectively, which may explain the lower % MeHg increases in peat cores in the 30 ppm  $\text{SO}_4^{2-}$  treatment. Comparatively, the column outflow from the subarctic site had concentrations of  $\text{S}^{2-}$  less than 1 ppm for the duration of the experiment.

However, this decrease in MeHg production at the highest levels of  $\text{SO}_4^{2-}$  addition is not observed in column outflow waters for any of the sites. It is possible that the flow rate of this experiment was sufficiently high enough to flush newly produced  $\text{S}^{2-}$  from the peat and therefore circumvent  $\text{S}^{2-}$  inhibition, or at the very least, prolong the time period before  $\text{S}^{2-}$  in the system built to inhibitory levels. Indeed, similar column experiments have shown that the accumulation of metabolic end products can slow microbial activity and hence decomposition in peat soils, and that removal of these end products can free the system from end-product inhibition (Bonaiuti, Blodau, & Knorr, 2017).

If this is indeed the case, then the smaller values of % MeHg increase in peat cores for the low boreal and southern Ontario sites in the 30 ppm  $\text{SO}_4^{2-}$  treatment could have less to do with decreasing rates of MeHg production with higher  $\text{SO}_4^{2-}$  additions, and more to do with MeHg binding capacity of the peat cores themselves. Higher levels of  $\text{S}^{2-}$  can compete with binding sites within the peat cores, keeping MeHg in the aqueous phase (Skylberg, 2008). However, if there is a larger sulphur/MeHg ratio in the peat itself due to higher sulphur sequestration following  $\text{SO}_4^{2-}$  reduction by SRB, this means there are more available binding sites for MeHg since Hg species have a high affinity for reduced sulphur compounds (Skylberg *et al.*, 2000; Hesterberg *et al.*, 2001). There is likely a trade-off between high sulphur accumulation in the peat that can bind MeHg, and high dissolved  $\text{S}^{2-}$  production that can also bind MeHg in the aqueous phase for these two sites. For the subarctic site, higher % MeHg increase in the 30 ppm  $\text{SO}_4^{2-}$  treatment compared to the other two sites could be explained by the lower concentration of  $\text{S}^{2-}$  in column outflow that is available to bind MeHg. As a result, more MeHg is retained in the solid phase. Therefore, it appears that the optimal level of  $\text{SO}_4^{2-}$  addition that will increase % MeHg in peat cores differs between sites based on the level of  $\text{SO}_4^{2-}$  reduction and resulting  $\text{S}^{2-}$  production that occurs.

### 2.4.3 Mercury partitioning versus methylmercury production

If only considering the absolute concentration of MeHg in outflow, the low boreal site produced the highest concentration of MeHg. Although the absolute concentration of MeHg in outflow for the low boreal site is elevated throughout the experiment compared

to the other two sites regardless of  $\text{SO}_4^{2-}$  treatment, it does not necessarily show the largest increase over time. That is, the y-intercepts of MeHg concentration over time are higher for the low boreal site across treatments, but the slopes are not necessarily steeper compared to the other two sites. This suggests that rather than higher levels of active MeHg production, there is simply a higher concentration of ambient MeHg present in the low boreal peat that was mobilized from solid peat to column outflow via leaching or decomposition of peat organic matter (Drexel *et al.*, 2002; Regnell & Hammar, 2004). The fact that the slopes and y-intercepts of absolute MeHg concentration over time for the low boreal site remained relatively constant between the distilled water control and the 1 and 5 ppm  $\text{SO}_4^{2-}$  treatments does seem to suggest that MeHg re-partitioning from the solid to the aqueous phase is influencing MeHg concentration in outflow at lower  $\text{SO}_4^{2-}$  additions, rather than active Hg methylation. The mass of MeHg in initial peat cores pre-experiment was indeed higher for the low boreal site, which had a MeHg mass approximately 2× higher than the southern Ontario site and 4× higher than the subarctic site.

Despite these high MeHg concentrations in outlet waters, the low boreal site showed only an intermediate % MeHg increase compared to the other two sites. The reason for this likely lies in the IHg data. For the low boreal site, IHg concentrations were slightly higher within each  $\text{SO}_4^{2-}$  treatment compared to the other two sites and were significantly higher than the other two sites in the 30 ppm  $\text{SO}_4^{2-}$  treatment. What this suggests is that because IHg concentrations in outflow were also elevated, the elevated concentrations of MeHg for the low boreal site were essentially ‘diluted’, leading to lower values of % MeHg. Since IHg concentration can constitute an important control on Hg methylation rates (Hsu-Kim *et al.*, 2013; Ma, Du, & Wang, 2019) this increase in IHg concentration has the potential to increase Hg methylation rates. However, as can be inferred from the % MeHg values, this excess IHg was not converted into MeHg, and so the southern Ontario site despite having less absolute MeHg in outflow, methylates more of the available IHg compared to the low boreal site. The low values of % MeHg increase in solid peat over the course of the experiment for the low boreal site provides further support for this conclusion. The elevated IHg in outflow for the low boreal site

specifically is likely due to ambient levels of IHg being higher in the low boreal peat, as can be confirmed from Table 2.2.

What is also apparent from the IHg data is that the concentration of IHg in column outflow differs not only by site as discussed above, but also by  $\text{SO}_4^{2-}$  treatment. The slope of IHg concentration in outflow over time increases with higher  $\text{SO}_4^{2-}$  additions, indicating that more IHg is being partitioned into the aqueous phase from the solid phase with higher  $\text{SO}_4^{2-}$  additions. The reason for this result could be a combination of two factors. First, increased microbial activity in response to higher  $\text{SO}_4^{2-}$  and subsequent peat decomposition could be releasing IHg from the peat (Regnell & Hammar, 2004). Second, increased partitioning of IHg in peat to the aqueous phase could be occurring due to higher  $\text{S}^{2-}$  levels as the result of higher  $\text{SO}_4^{2-}$  reduction. Since dissolved  $\text{S}^{2-}$  has a strong affinity for IHg (Hammersmidt *et al.*, 2008), high levels of  $\text{S}^{2-}$  could outcompete reduced sulphur binding sites in peat organic matter and repartition solid IHg to aqueous HgS complexes (Reimers & Krenkel, 1974; Drexel *et al.*, 2002). As is evident from the  $\text{S}^{2-}$  results, the higher  $\text{SO}_4^{2-}$  additions (5 and 30 ppm) do in fact produce higher levels of dissolved  $\text{S}^{2-}$  in column outflow.

The increase in IHg with increasing  $\text{SO}_4^{2-}$  addition is most evident for the low boreal site, particularly in the 30 ppm  $\text{SO}_4^{2-}$  treatment. In the 30 ppm  $\text{SO}_4^{2-}$  treatment, the low boreal peat produced significantly more dissolved  $\text{S}^{2-}$  than the other two sites, which as explained previously, could be partitioning more IHg into the aqueous phase. The low boreal site also has the lowest C/N ratio. Peat with higher carbon content has the ability to retain more IHg than peat with lower carbon content (Yin *et al.*, 1997; Tjerngren *et al.*, 2012). It could therefore be the case that the peat cores from the low boreal site are less able to retain IHg, and as a result, they lose more IHg to the aqueous phase. Although DOC concentrations are slightly higher for the southern Ontario site for the duration of the experiment, results for all sites are very similar, and so any potential influences of DOC on IHg bioavailability (Miller *et al.*, 2006; Hammersmidt *et al.*, 2008; Graham, Aiken, & Gilmour, 2012) would likely be similar across all sites.

#### 2.4.4 Sulphate reduction versus sulphide and methylmercury production

Another interesting response that sets the low boreal site apart from the other two sites is the disparity between  $\text{SO}_4^{2-}$  reduction (inferred from solid sulphur accumulation), and MeHg production/accumulation. For both the subarctic and southern Ontario sites, there is a clear link between sulphur accumulation and % MeHg increase in column outflow. Higher values of sulphur accumulation are linked to higher values of % MeHg increase. However, for the low boreal site, despite high levels of sulphur accumulation in both the 5 and 30 ppm  $\text{SO}_4^{2-}$  treatments, % MeHg increase in cores and column outflow is only low to intermediate. In other words, the high  $\text{SO}_4^{2-}$  reduction for the low boreal site is decoupled from MeHg production.

The mechanism behind this result is unclear but could be linked to different SRB communities at the three sites. As explained previously, not all SRB have the ability to methylate Hg, and those that do don't necessarily methylate Hg at the same rate (King *et al.*, 2000; Ranchou-Peyruse *et al.*, 2009). Although the SRB community composition was not analyzed as part of this study, it is possible that the low boreal site hosts a variety of  $\text{SO}_4^{2-}$ -reducers, but that a higher proportion of these  $\text{SO}_4^{2-}$ -reducers are either non-Hg-methylating, or less efficient Hg methylators. This would lead to a high level of  $\text{SO}_4^{2-}$  reduction, but less actual MeHg production.

Another possibility for the decoupling of  $\text{SO}_4^{2-}$  reduction and MeHg production in the low boreal site is oxidative demethylation of MeHg by anaerobes. In anoxic soils  $\text{SO}_4^{2-}$ -reducers, methanogens, and other anaerobes can demethylate MeHg in an oxidative decomposition pathway as a by-product of their metabolism that is established for methylated carbon substrates (Oremland, Culbertson, & Winfrey, 1991; Barkay, Miller, & Summers, 2003). This process can become particularly significant in terms of net MeHg production if dissolved  $\text{S}^{2-}$  and solid phase reduced sulphur are higher (Marvin-DiPasquale & Agee, 2003). Since sulphur retention in solid phase peat and dissolved  $\text{S}^{2-}$  concentrations in column outflow were high in the low boreal site particularly for the 30



ppm  $\text{SO}_4^{2-}$  treatment, significant loss of produced MeHg to demethylation by anaerobes could be feasible for this site.

Interestingly, although there is a clear increase in sulphur accumulation in peat from the low boreal site subjected to the 5 ppm  $\text{SO}_4^{2-}$  treatment, dissolved  $\text{S}^{2-}$  in column outflow is lower than it is for the other sites that have comparatively lower sulphur accumulation in the 5 ppm treatment. It is possible that dissolved  $\text{Fe}^{2+}$  in outflow from this site was able to complex dissolved  $\text{S}^{2-}$ , thereby buffering the amount of dissolved  $\text{S}^{2-}$  in solution. The ability of Fe to buffer  $\text{S}^{2-}$  in solution has been demonstrated in other studies (Heijs *et al.*, 1998; Kanaya & Kikuchi, 2004) and is supported in this study by the higher concentration of Fe present in low boreal peat compared to the other two sites. The reason for the comparatively higher Fe concentrations at this site may in part be due to the underlying Precambrian bedrock (Webster & McLaughlin, 2010) which is richer in Fe deposits compared to the limestone bedrock of the other two sites (Givelet, Roos-Barraclough, & Shotyk, 2003; Corson & Campbell, 2013). The methylene blue method of  $\text{S}^{2-}$  analysis only measures dissolved  $\text{S}^{2-}$ , and so would not detect insoluble  $\text{FeS}_{(s)}$  complexes. The complexation of  $\text{FeS}_{(s)}$  could result in less free  $\text{S}^{2-}$  being available to form neutral dissolved HgS complexes needed for bacterial Hg uptake (Liu, Valsaraj, & Delaune, 2009; Ulrich & Sedlak, 2010). This would explain why % MeHg in outflow for the low boreal site is lower than would be expected based on high sulphur accumulation in the 5 ppm  $\text{SO}_4^{2-}$  treatment. In the 30 ppm treatment, there are larger concentrations of  $\text{S}^{2-}$  produced for the low boreal site, possibly due to the saturation of  $\text{Fe}^{2+}$  with  $\text{S}^{2-}$ , which would cause  $\text{S}^{2-}$  to remain dissolved in solution.

#### 2.4.5 Conclusions

Using this controlled laboratory study, I was able to investigate the effects of legacy  $\text{SO}_4^{2-}$  deposition on MeHg production potential in northern peatlands by taking advantage of the anthropogenically-influenced latitudinal gradient of  $\text{SO}_4^{2-}$  deposition across Ontario. Results suggest that as initially predicated, there exists a  $\text{SO}_4^{2-}$ -priming effect whereby past exposure to elevated  $\text{SO}_4^{2-}$  deposition primes the SRB community such that it responds more readily to further  $\text{SO}_4^{2-}$  inputs. However, future research would benefit

from elucidating the relationship between these proposed  $\text{SO}_4^{2-}$ -induced SRB community changes, and the ability of the community to produce MeHg.

It is also apparent that the geochemical composition of the peat itself must be taken into consideration when determining MeHg production potential, as even relatively small differences in Fe and carbon content for example can impact Hg biogeochemistry. It is important to keep in mind, however, that this research was carried out on a relatively small scale, and large scale landscape MeHg production has additional environmental complexities such as hydrogeologic setting (Demers *et al.*, 2013), temperature changes (Åkerblom *et al.*, 2013), and water table fluctuations (Coleman-Wasik *et al.*, 2015) to name a few. Chapter 3 will explore more landscape level influences of sulphur biogeochemistry on MeHg production. Regardless, this research suggests that the legacy effects of  $\text{SO}_4^{2-}$  on MeHg production in peatlands can persist long after  $\text{SO}_4^{2-}$  deposition has declined, and that these effects should be taken into consideration when developing remediation strategies for those wetlands impacted by significant  $\text{SO}_4^{2-}$  deposition.

## 2.5 References

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## Chapter 3

### 3 Methylmercury and Sulphur Accumulation in Pristine and Sulphate-amended Peats

This chapter focuses on broader, landscape level influences on methylmercury (MeHg) production and distribution in peatlands, with an emphasis on systems that are impacted by a sulphate ( $\text{SO}_4^{2-}$ ) source. Peatland transects that are impacted by both atmospheric, and point source deposition of  $\text{SO}_4^{2-}$  are investigated, and the factors controlling mercury biogeochemistry in these  $\text{SO}_4^{2-}$  impacted environments are discussed.

#### 3.1 Introduction

Mercury (Hg) is a ubiquitous, naturally occurring element with a complex and dynamic biogeochemical cycle. Background levels of inorganic Hg in the natural environment rarely exceed concentrations that constitute a health concern (Clarkson *et al.*, 2003), despite anthropogenic activities such as fossil fuel burning that have increased the pool of Hg in the atmosphere (Swain *et al.*, 1992). Of larger concern is the organic form of Hg known as methylmercury (MeHg). As an organic compound, MeHg readily bioaccumulates in organic tissue, such that the majority of Hg in organic tissue is found in the methylated form (Bloom, 1992). As MeHg is a known neurotoxin (Ratcliffe, Swanson, & Fischer, 1996), the factors that control its production and accumulation in natural environments are of particular concern.

Aquatic environments such as lake sediments (e.g. Krabbenhoft *et al.*, 1998), and wetlands (e.g. St. Louis, 1994) are known areas of MeHg production. One such environment is a peatland, a wetland characterized by a significant accumulation of organic soil (>40 cm in depth in the Canadian Wetland Classification Scheme [National Wetlands Working Group, 1997]). The inundated, nutrient-poor soils of northern peatlands promote slow decomposition and support bacterial anaerobic processes (Wood *et al.*, 1968; Compeau and Bartha, 1984; Kerin *et al.*, 2006). One of these groups of bacteria, the sulphate-reducing-bacteria (SRB), are principle methylators of Hg in freshwater ecosystems in particular (Compeau & Bartha, 1985). Their activity in peatland

soils is the reason that peatlands are well-established hotspots of MeHg production (Branfireun *et al.*, 1996; Mitchell, Branfireun, & Kolka, 2008a).

Sulphate-reducing bacteria (SRB) reduce sulphate ( $\text{SO}_4^{2-}$ ) to sulphide ( $\text{S}^{2-}$ ) in the process of breaking down organic matter. The supply of  $\text{SO}_4^{2-}$ , electron donors in the form of carbon compounds, and inorganic Hg are all important factors in determining SRB activity, and subsequent MeHg production in peatlands (Benoit *et al.*, 2003; Lambertsson & Nilsson, 2006). The supply of nutrients to these environments is heavily influenced by local hydrology. Uplands in particular can constitute a significant source of nutrients to these environments via runoff (Urban, Eisenreich, & Grigal, 1989; Mitchell, Branfireun, & Kolka, 2008b). The delivery of sulphur via upland runoff is particularly significant when it is in the form of interflow; that is, the runoff passes through the mineral horizon of the upland soil (Urban, Eisenreich, & Grigal, 1989) which is richer in minerals such as sulphur. Fresh inputs of organic matter such as those derived from forest litterfall can also be delivered to peatlands via upland runoff (Mitchell, Branfireun, & Kolka, 2008b), and organic carbon can facilitate the transport of Hg (Lee, Bishop, & Munthe, 2000; Eklöf *et al.*, 2012). This is due to the strong association of Hg with organic matter (Grigal, 2003), and more specifically, with reduced sulphur groups in organic carbon compounds that have a high binding affinity for Hg (Skylberg *et al.*, 2000; Drexel *et al.*, 2002).

However, in anoxic soils where  $\text{SO}_4^{2-}$ -reduction is elevated, the accumulation of  $\text{S}^{2-}$  can impact this binding affinity of Hg with organic matter. Sulphide, an inorganic form of reduced sulphur, also has a high affinity for inorganic Hg, and can compete with organic reduced sulphur compounds for inorganic Hg binding sites (Haitzer *et al.*, 2002; Skylberg, 2008). The formation of inorganic  $\text{HgS}_{(s)}$  complexes not only decreases the bioavailability of Hg for methylation by SRB (Benoit *et al.*, 1999; Skylberg, 2008), but it also decreases the mobility of Hg through the peatland due to precipitation of metacinnabar (Drexel *et al.*, 2002; Demers *et al.*, 2013). The topography of uplands, as well as the surrounding peatland, can also play an important role in determining hydrologic flow paths (Branfireun, Mitchell, & Kolka, 2009; Balliston, McCarter, & Price, 2018) and ultimately the delivery of nutrients to these systems. In addition to

uplands, groundwater also constitutes a source of nutrients to nutrient-limited peatland soils, and can help maintain the saturated, anoxic conditions required for SRB activity (Branfireun & Roulet, 2002). It is therefore the supply and delivery of nutrients, as well as the redox potential within peat soils that will ultimately determine Hg methylation potential.

Anthropogenic activities have altered both the hydrology of, and nutrient supply to, these systems and, as a result, Hg methylation potential. In southern Ontario, the burning of fossil fuels such as coal has increased the atmospheric deposition of both Hg and  $\text{SO}_4^{2-}$  due to urbanization and industrialization in the late 19<sup>th</sup> and 20<sup>th</sup> centuries (Givelet, Roos-Barraclough, & Shoty, 2003; Vet *et al.*, 2014). Mid-latitude regions in Ontario along the Canadian Shield such as Sudbury have also experienced increased levels of sulphur deposition in the form of acid rain. Intensive smelting of  $\text{S}^{2-}$  ores has increased sulphur dioxide ( $\text{SO}_2$ ) emissions in this region, although 90% emissions reductions in the 1970s have facilitated the recovery of surrounding ecosystems (Keller & Gunn, 1995; Keller *et al.*, 2001; Tropea *et al.*, 2010). Northern Ontario is more removed from these atmospheric sources of  $\text{SO}_4^{2-}$ , but mining operations in this region have the potential to leach  $\text{SO}_4^{2-}$  into the surrounding environment from oxidation of  $\text{S}^{2-}$  minerals in waste rock, as has been observed in other regions of resource extraction (Al, Martin, & Blowes, 2000; Berndt & Bavin, 2012)

The effects that long-term  $\text{SO}_4^{2-}$  addition have on wetlands has been studied from both microbial and geochemical perspectives (Branfireun *et al.*, 2001; Hoggarth, Hall, & Mitchell, 2015; Johnson *et al.*, 2016; Stickman *et al.*, 2016). However, the relationship between  $\text{SO}_4^{2-}$  and MeHg production in these peatlands from different  $\text{SO}_4^{2-}$  sources hasn't been investigated. With projected warming in northern peatlands (Bridgham *et al.*, 1995; Limpens *et al.*, 2008) that could cause more frequent water table draw-downs (Sheffield & Wood, 2008) and oxidation of previously reduced sulphur species (Coleman Wasik *et al.*, 2015), more  $\text{SO}_4^{2-}$ -rich peatlands could become persistent sources of  $\text{SO}_4^{2-}$  and potentially MeHg to the surrounding environment.

The objectives of this study were to determine if 1) there is a proportional relationship between sulphur and MeHg accumulation in peatlands along a  $\text{SO}_4^{2-}$  gradient from high to low atmospheric deposition, 2) otherwise  $\text{SO}_4^{2-}$ -limited peatlands that receive excess  $\text{SO}_4^{2-}$  from an anthropogenic point source display a similar sulphur-MeHg relationship to peatlands with higher atmospheric  $\text{SO}_4^{2-}$  deposition, and 3) organic carbon and inorganic Hg availability significantly influence MeHg accumulation across peatlands of different  $\text{SO}_4^{2-}$  exposure. I hypothesized that in peatlands receiving less  $\text{SO}_4^{2-}$ , the relationship between sulphur and MeHg accumulation in peat is positive and linear, with  $\text{SO}_4^{2-}$  availability being the main predictor of net MeHg production. In peatlands that receive more  $\text{SO}_4^{2-}$  either from the atmosphere or from anthropogenic point sources, I hypothesized that the relationship between sulphur and MeHg in peat is not proportional, but that MeHg accumulation is dependent instead upon inorganic Hg and organic carbon availability.

## 3.2 Methods

### 3.2.1 Field site descriptions

To address the first objective, three peatlands across a broad latitudinal gradient of  $\text{SO}_4^{2-}$  deposition in Ontario were sampled. These sites correspond to those sampled in Chapter 2, and constitute high, intermediate, and low levels of atmospheric sulphur deposition. In Ontario, sulphur deposition has historically been highest at southern latitudes due to industrialization, and the three chosen field sites represent different degrees of atmospheric sulphur loads along this gradient (refer to Figure 2.1). The most southerly site is the Sifton Bog in London, Ontario ( $42^\circ 58' 17.5''\text{N}$   $81^\circ 19' 30.8''\text{W}$ ), which represents a region of high historic atmospheric sulphur deposition. At this site, the raised portion of the bog consists of a shallow pond (~2 m depth) surrounded by a floating *Sphagnum* matt (Judd, 1957; City of London, 2009). The central portion of the bog is characterized by *Sphagnum* mosses, as well as shrubs such as leatherleaf, highbush blueberry, and large cranberry in the shrub kettle bog portion, and trees such as black spruce and tamarack in the treed kettle bog portion (Judd, 1957; City of London, 2009). The bog is surrounded by a lower-lying lag zone composed of a mixed forest swamp, with both deciduous

species such as maple, birch, and oak, and coniferous species such as pine, spruce, and tamarack (Judd, 1957; City of London, 2009). The forested swamp then transitions into an upland deciduous forest (Judd, 1957; City of London, 2009). Peat depth is highest near the center of the bog, with a maximum depth of ~10 m, while peat depth thins closer to the periphery of the bog, eventually transitioning into thin organic soils in the upland (Judd, 1957; City of London, 2009). This site will hereafter be referred to as the southern Ontario site.

The mid-latitude site which represents a region of intermediate historic atmospheric sulphur deposition is a poor fen in the White River, Ontario peatland complex (48°21'13.3"N 85°20'17.6"W). At this site, the portion of the fen closest to the upland has an average peat depth ranging from 0.05–1 m, while the central portion of the fen has an average peat depth of 0.5–3 m (Webster & McLaughlin, 2010). The surrounding boreal mixed wood upland forest consists mostly of white birch, balsam fir, and black spruce, while the fen is dominated by shrubs, *Sphagnum* mosses, and black spruce/tamarack trees (Webster & McLaughlin, 2010). This site is part of a long-term monitoring project maintained by the Ministry of Natural Resources and Forestry (MNR) as part of the White River Experimental Watershed Study. This site will hereafter be referred to as the low boreal site.

The most northerly site sampled that represents a region of low historic atmospheric sulphur deposition is a reference ladder fen at the DeBeers Victor Mine in the James Bay Lowlands (52°49'34.8"N 83°54'07.9"W). This site has been used in several other studies at the Victor Mine as a reference area (e.g. McCarter & Price, 2017a; McCarter & Price, 2017b). This peatland complex is characterized by 1.5–2.5 m of peat overlying mineral sediments (McCarter & Price, 2017), and includes a range of peatland types from carbonate-rich fens to mineral poor bogs (Corson & Campbell, 2013; Riley, 2011). Similar to the other sites, the fens in this region are dominated by *Sphagnum* mosses, as well as a significant abundance of *Carex* sedges and cotton grass (Leclair, Whittington, & Price, 2015; Riley, 2011). This site will hereafter be referred to as the subarctic reference site.



To address the second objective, two otherwise pristine peatlands impacted to varying degrees by point-source loading of  $\text{SO}_4^{2-}$  were sampled. These peatlands are also situated at the DeBeers Victor Mine site in the James Bay Lowlands. Both sites have been subjected to elevated  $\text{SO}_4^{2-}$  deposition in recent years. The first site has been experimentally exposed to high levels of  $\text{SO}_4^{2-}$  via simulated waste water additions containing ~30 ppm  $\text{SO}_4^{2-}$  in a nutrient polishing study conducted by McCarter, Branfireun, & Price (2017). This study was initiated in the summer of 2014, and waste water additions continued for 51 days. The site is a ladder fen, meaning it exhibits a pool-peat-rib-pool morphology, with the direction of water flow following a path down a slight elevation gradient perpendicular to the peat ribs (McCarter & Price, 2017a). The site is bound on both sides by two bogs and bound at the top by an upgradient pool (McCarter & Price, 2017a), to which the waste water additions in the McCarter, Branfireun, & Price (2017) study were added. Peat depth is highest near the top of the fen close to the addition pool (~2.1 m) and thins moving down the peat ribs (~1.73 m at the south end of the fen; McCarter & Price, 2017b). This site will hereafter be referred to as the experimental fen.

The second site is another ladder fen located to the northeast of the main waste rock stockpile at the mine, hereafter referred to as the northeast fen. This fen has served as a passive wetland treatment system for mine rock stockpile runoff since 2010 (Wood Environment and Infrastructure Solutions [WEIS], 2018). Runoff from the stockpiles contains as much as 400 ppm  $\text{SO}_4^{2-}$ , and as a result, the concentration of  $\text{SO}_4^{2-}$  in the northeast fen has increased, with  $\text{SO}_4^{2-}$  concentrations as high as 155 ppm at the proximal end of the fen closest to the waste rock stockpile observed (WEIS, 2018). The site is flanked to the north by a *Sphagnum* bog and to the south by the open pit of the mine. As such, it is within the cone of depression in the bedrock that has developed as a consequence of intensive de-watering of the mining pit, which has the potential to increase seepage losses from surrounding watersheds due to shifting hydraulic gradients (Leclair, Whittington, & Price, 2015).

### 3.2.2 Study design and sample collection

Peat samples were collected at all five sites according to slightly different sampling designs. At the southern Ontario and low boreal sites, peat samples were collected along a transect from the central portion of the peatland towards the upland to capture a range of sulphur concentrations. Sampling for the southern Ontario site and low boreal site took place in June and July 2018 respectively. At the low boreal site, sample started ~20 m from the edge of a small freshwater lake bordering the fen (Soulier Lake), and ended at the treed hillslope to the north of the fen. Each sample was taken ~15-20 m apart, collectively constituting a ~200 m long transect from the lake to the hillslope. At the southern Ontario site, sample collection started ~10 m from the edge of the small pond (Redmond's Pond) in the middle of the *Sphagnum* mat and ended at the forested swamp portion of the wetland before the transition to upland deciduous forest. Each sample was taken 10 m apart, collectively constituting a ~100 m long transect from Redmond's Pond in the centre of the bog to the forested swamp area. Figure C1 in Appendix C shows sampling maps for the southern Ontario bog and low boreal fen transects. At the subarctic reference site, three samples were taken in a ~1 m<sup>2</sup> area in the middle of the fen as this site was the least sulphur impacted and sulphur heterogeneity between samples was expected to be minimal.

Sample collection for the two SO<sub>4</sub><sup>2-</sup>-impacted fens at the DeBeers Victor Mine followed a more intensive sampling design, to ensure that the accumulation of the additional SO<sub>4</sub><sup>2-</sup> that was present as a result of SO<sub>4</sub><sup>2-</sup> loading was accurately captured. Sampling for the experimental fen and northeast fen took place in August 2018. At the experimental fen, sample collection started at the first peat rib closest to the pool used for SO<sub>4</sub><sup>2-</sup> additions in the McCarter, Branfiruen, & Price (2017) study, and ended at the seventh peat rib furthest from the SO<sub>4</sub><sup>2-</sup> addition pool. Three samples were taken along the length of each peat rib, for a total of 21 samples. Collectively the transect was ~115 m long. At the northeast fen, sample collection started at the west side of the fen ~25 m from the waste rock stockpile at the mine, and ended at the far east border of the fen ~1 km from the waste rock pile. The first four longitudinal locations sampled were ~100 m apart, while the remaining three locations were ~200–300 m apart. At each longitudinal location, four samples were

taken, three of which spread the width of the fen and the fourth was taken at the north edge of the fen in the surrounding bog, for a total of 28 samples. Figure C2 in Appendix C shows sampling maps for the two  $\text{SO}_4^{2-}$ -impacted Victor Mine transects.

Peat samples at each site were collected at a consistent depth of 10–20 cm after removing the top 10 cm of vegetation. Initial sample weights ranged from ~80–120 g. Nitrile gloves were worn to prevent any additional Hg contamination. Once removed, samples were immediately bagged and placed in a cooler until transport back to the on-site laboratory. Samples were then kept frozen at -20 °C during transport back to the Biotron at Western University, where they were then frozen at -80 °C until lyophilization.

### 3.2.3 Peat analysis

Samples were lyophilized for ~72–96 hours or until all water was sublimated from the peat, and were then thoroughly homogenized by pulse grinding in a stainless steel coffee grinder, ensuring sample integrity was maintained by cleaning with acetone in between samples. All elemental analysis on peat samples was performed in the Biotron Analytical Services Laboratory (Western University, London, ON). Total Hg (THg) analysis on solid peat samples was carried out using a Milestone Direct Mercury Analyzer (DMA)-80 following EPA method 7473 (U.S. EPA, 2007). Analytical duplicates, blanks, and 50 ng matrix spikes were run every 10 samples. The certified reference material (CRM) MESS-3 ( $0.091 \pm 0.009$  mg/kg Hg) was also run every 10 samples to validate instrument performance throughout the run. All matrix spike, duplicate, and CRM recoveries were required to fall within 20% of their expected value. All blanks were required to fall below the method reporting limit (0.24 ng). MeHg analysis on solid peat samples was performed by first digesting ~100 mg dry peat samples with 4.0 M  $\text{HNO}_3$ , followed by a microwave digestion for 4 h at 82 °C. Digestate was diluted with MilliQ deionized water before analysis on the Tekran® 2700 following the EPA 1630 (U.S. EPA, 1998) protocol. Inorganic Hg (IHg) concentration in solid peat samples was calculated as the difference between THg and MeHg concentration. This value represents all Hg complexes present in the sample that are not in the methylated form.

Analysis for % sulphur and carbon/nitrogen (C/N) ratios was performed using an Elementar vario ISOTOPE cube CHNS purge and trap chromatography system. Calibration of the instrument was validated each run with daily factor sample recoveries of sulfanilamide (41.85 % C, 18.62 % S, 16.27% N) which were required to fall within 10% of their targets. Consistent performance of the instrument was validated by including the CRM B2166 (48.09% C, 2.12% N, 0.17 % S) every 10 samples. CRM recovery was required to fall within 15% of the expected values. Analytical duplicates and blanks were run every 10 samples as well. Blanks consisted of empty tin boats used for sample packaging and were required to fall below the method reporting limit (MRL) which was 0.077 mg C, 0.006 mg N, and 0.017 mg S. Duplicate recoveries were required to fall within 20% of each other. Approximately 30 mg of dry sample was used, and a 5:1 ratio of sample to tungsten trioxide was used to ensure complete sulphur oxidation during analysis.

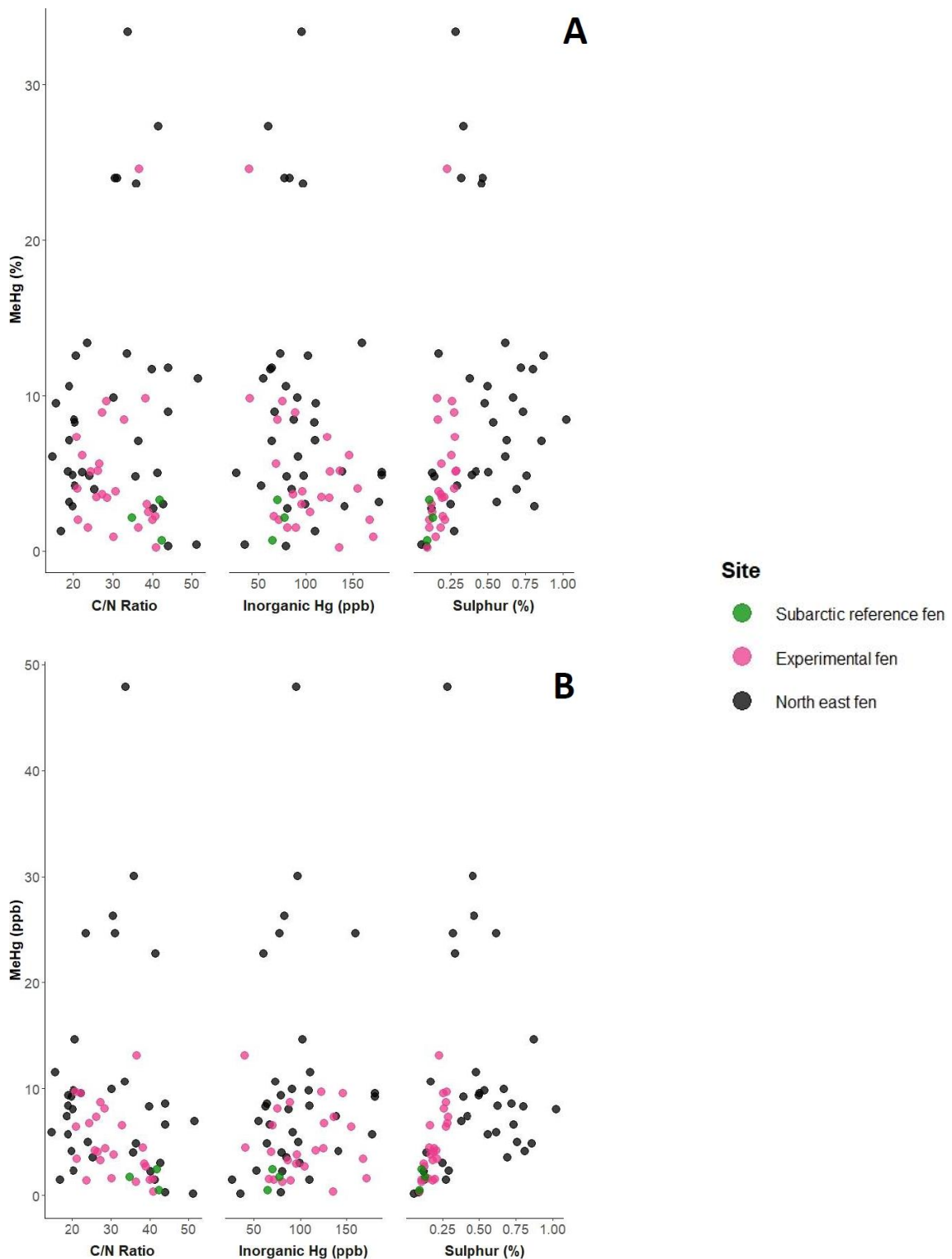
### 3.2.4 Statistical analysis

All statistical analyses were performed using the statistical computing software R version 3.6.1 (R Core Team, 2019). Separate linear regression models were fit for both % MeHg and MeHg data within each site, and with % sulphur, IHg concentration, and C/N ratio as explanatory variables. For the subarctic reference fen, within-site linear regressions were not run on any variables due to low sample number, but results for this site were included on plots for comparison. Backwards selection based on AIC values using the dredge function in the MuMIn package (Barton, 2019) was used to select the model of best fit. Separate linear regression models were also fit for MeHg and % MeHg data from all sites collectively, with % sulphur, IHg concentration, and C/N ratio as explanatory variables. The same backwards selection process was used to identify the model of best fit. The assumptions of normality and homoscedasticity were validated through residual histograms, QQ-plots, and standardized residual plots. Where needed,  $\log_{10}$  transformation was applied to absolute MeHg values to homogenize residuals. Site sampling maps were generated in R using the ggmap package (Kahle & Wickham, 2013) paired with Google maps satellite imagery. All other plots were created using the R package ggplot2 (Whickham, 2016).

## 3.3 Results

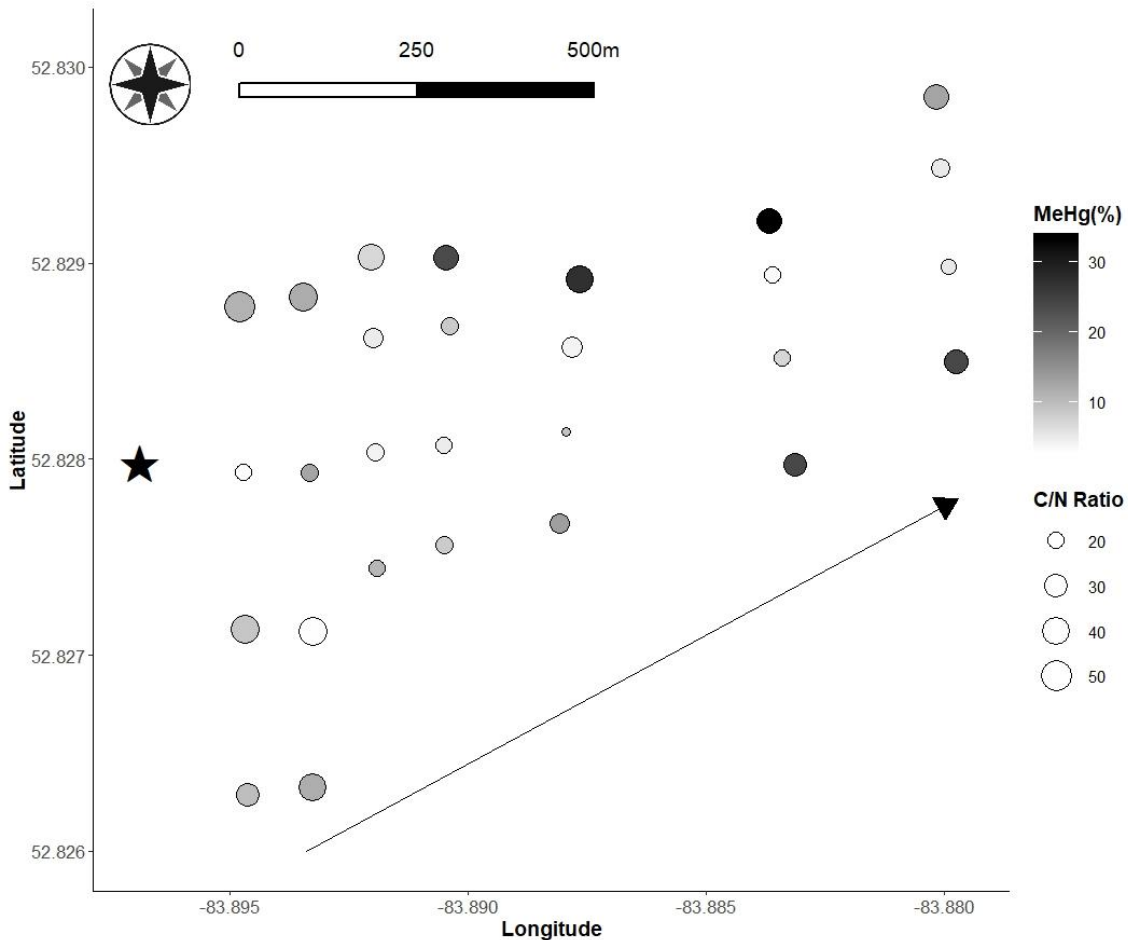
### 3.3.1 Sites impacted by anthropogenic sulphur point source

Geochemical relationships across sites impacted by a  $\text{SO}_4^{2-}$  point source are displayed for % MeHg and absolute MeHg concentration in Figure 3.1A and 3.1B respectively. At the northeast fen, the linear model with only % sulphur as an explanatory variable resulted in the best model fit for absolute MeHg concentrations. However, the overall model was not significant (Adj.  $R^2=0.02$ ,  $F(1,26)=1.48$ ,  $p=0.24$ ), which suggests that no linear relationship exists between any of the analyzed variables and MeHg concentration at this site. For % MeHg, the linear model with both % sulphur and IHg values as explanatory variables resulted in the best model fit, and resulted in overall model significance (Adj.  $R^2=0.16$ ,  $F(2,25)=3.58$ ,  $p=0.043$ ). However, neither IHg concentration nor % sulphur alone showed a significant relationship with % MeHg ( $p=0.071$  and  $p=0.07$  respectively). This suggests that while both variables together may provide some explanatory power with respect to % MeHg values, neither variable alone is linearly related to % MeHg at the northeast fen.



**Figure 3.1:** Scatter plots of (A) MeHg (%) and (B) MeHg (ppb) plotted against C/N ratio, inorganic Hg (ppb), and sulphur (%) for sites impacted by an anthropogenic point source of  $\text{SO}_4^{2-}$  and the subarctic reference fen. Each point represents a single observation from each site. Sites are identified by colour.

It is worth noting, however, that samples taken along the edges of the northeast fen displayed higher values of % MeHg, and C/N ratio compared to the samples taken within the fen. Samples on the edges of the fen were on average  $16.35 \pm 2.23\%$  MeHg compared to the lower average of interior samples ( $5.96 \pm 0.78\%$ ). Edge sample C/N ratios were on average  $33.69 \pm 2.42$  compared to interior samples ( $23.73 \pm 2.32$ ). However, the higher % MeHg in edge samples may in part be the result of lower IHg values in the edge samples ( $83.75 \pm 7.20$ ) compared to interior samples ( $116.42 \pm 11.01$ ) resulting in a larger fraction of MeHg/IHg. Additionally, while C/N ratio did not show any linear trend with MeHg, samples collected within  $\sim 120$  m of the waste rock piles had higher C/N ratios compared to samples collected further from the waste rock piles. These spatial trends are displayed in the Figure 3.2.



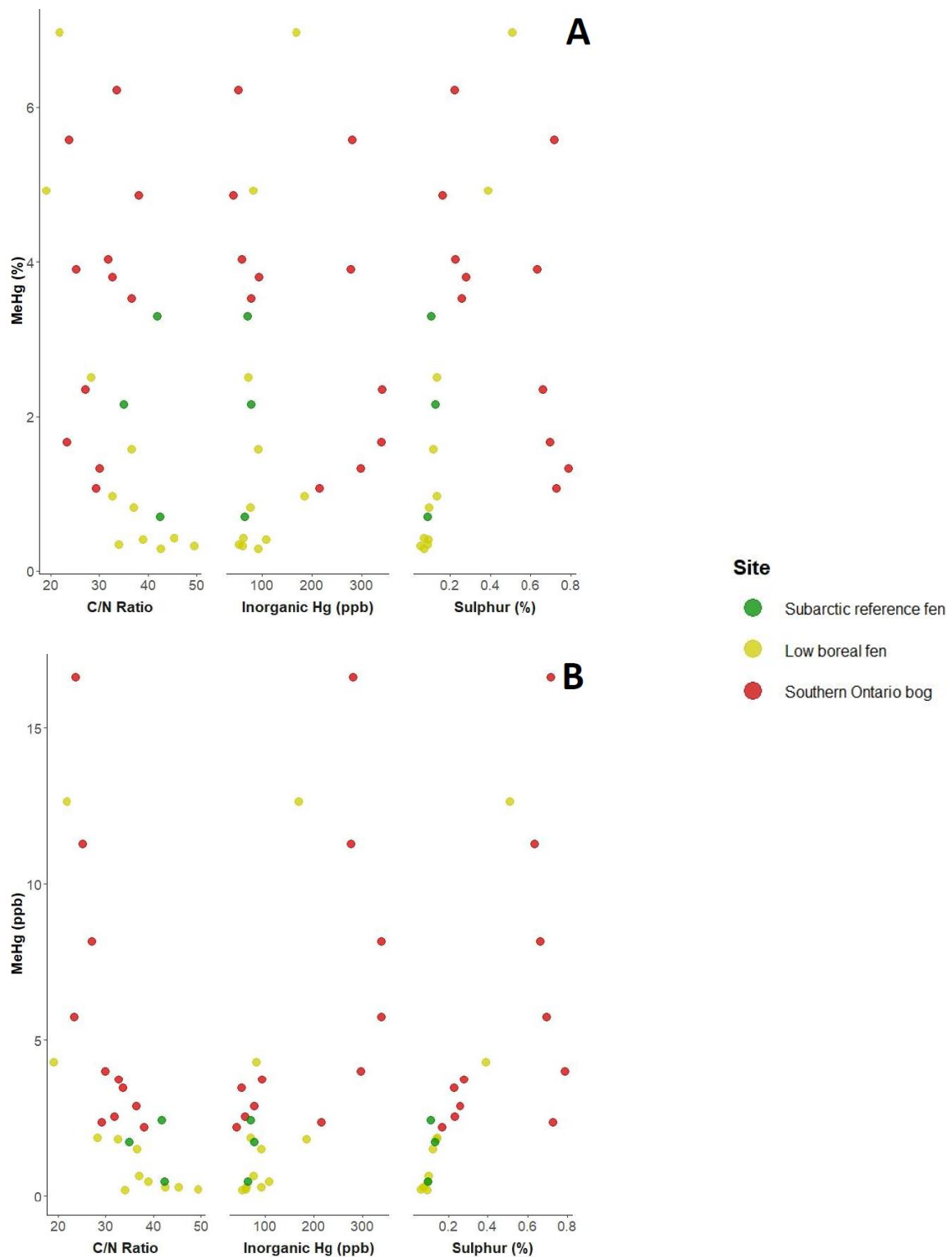
**Figure 3.2:** Spatial patterns of MeHg (%) and C/N ratio values in peat samples collected at the northeast fen transect. A scalebar and compass rose is provided for scale and direction at the top of the map. The star denotes the location of the waste rock stockpile, and the arrow indicates the direction of water flow in the fen.

At the experimental fen, MeHg concentrations were best predicted by the inclusion of both IHg and % sulphur as explanatory variables (Adj.  $R^2=0.69$ ,  $F(2,17)=22.36$ ,  $p<0.05$ ). However, only % sulphur showed a significant and positive linear relationship with MeHg concentration ( $p<0.05$ ). Similarly, both IHg concentration and % sulphur inclusion resulted in the best model fit for % MeHg (Adj.  $R^2=0.64$ ,  $F(2,17)=17.97$ ,  $p<0.05$ ), and both IHg ( $p=0.002$ ) and % sulphur ( $p<0.05$ ) showed significant linear relationships with % MeHg. The relationship between IHg and % MeHg was marginally negative ( $-0.04 \pm 0.01$ ), while the relationship between % sulphur and % MeHg was positive ( $31.73 \pm 5.53$ ). It should be noted that linear models were fit after the removal of an influential data point that had a much higher % MeHg and MeHg value (24.59% and 13.19 ppb respectively) compared to the rest of the samples (average of  $3.93 \pm 0.54\%$  and  $4.48 \pm 0.64$  ppb respectively). Once again, the inflated % MeHg value may in part be a result of the lower IHg concentration of this sample, which was 40.44 ppb compared to the rest of the samples (average of  $112.63 \pm 7.16$  ppb).

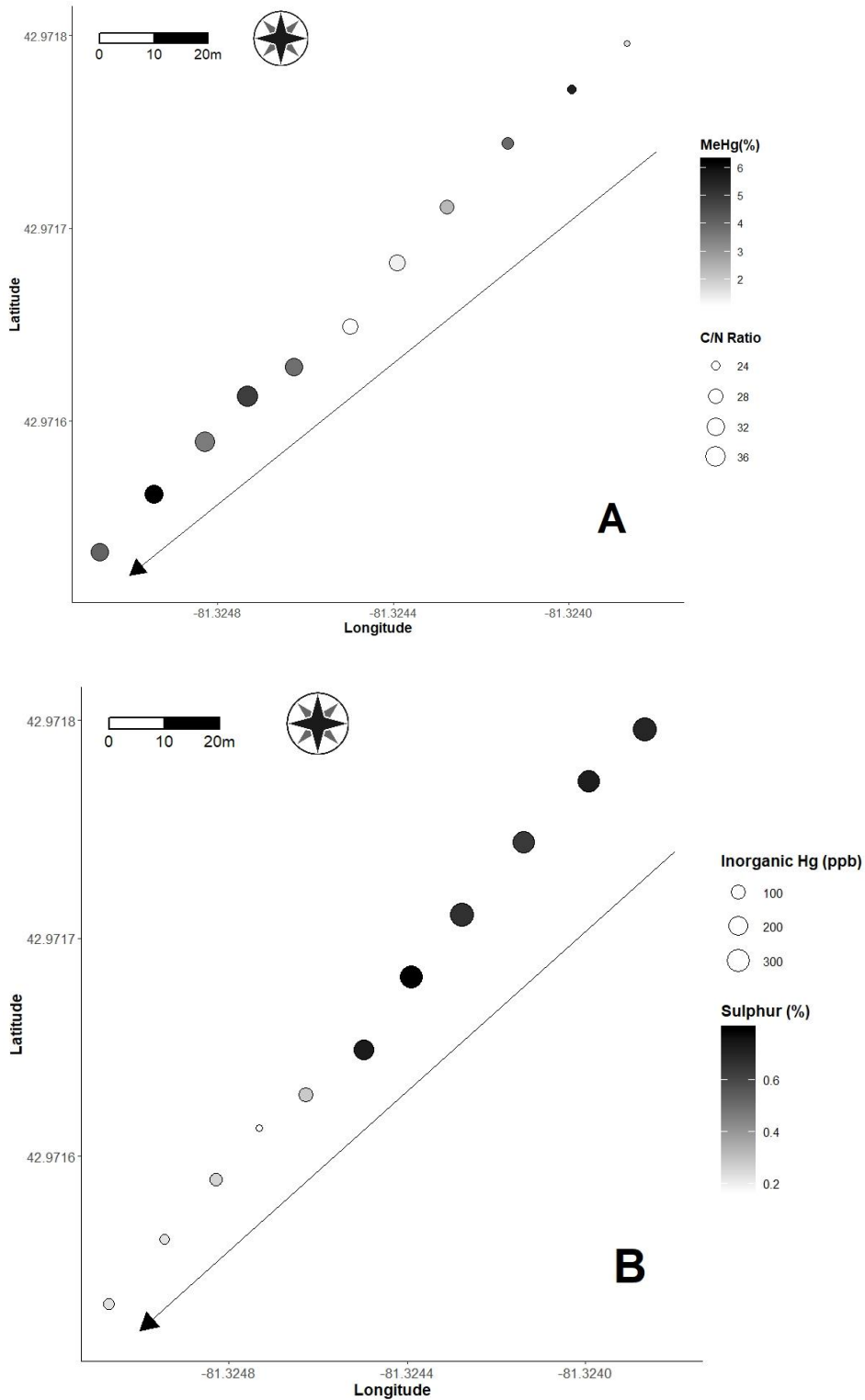
### 3.3.2 Sites impacted by atmospheric sulphur deposition gradient

Geochemical relationships across sites impacted by atmospheric  $\text{SO}_4^{2-}$  deposition are displayed for % MeHg and absolute MeHg concentration in Figure 3.3A and 3.3B respectively. At the southern Ontario site, the model of best fit for MeHg values included only C/N ratio as an explanatory variable (Adj.  $R^2=0.48$ ,  $F(1,9)=10.14$ ,  $p=0.011$ ), which had a negative relationship with MeHg concentration ( $-0.68 \pm 0.21$ ). For % MeHg, the inclusion of both C/N ratio and % sulphur as explanatory variables resulted in the model of best fit, but the overall model was not significant (Adj.  $R^2=0.39$ ,  $F(2,8)=4.24$ ,  $p=0.056$ ). Although there was no linear relationship between % sulphur and % MeHg, % sulphur values were elevated closer to the upland portion of the bog which did corresponded with higher % MeHg values. Percent MeHg was also elevated in the central portion of the bog, where C/N values were also comparatively higher. Inorganic Hg values were also comparatively higher closer to the upland portion of the bog, similar to % sulphur. These spatial trends are illustrated in Figure 3.4.





**Figure 3.3:** Scatter plots of (A) MeHg (%) and (B) MeHg (ppb) plotted against C/N ratio, inorganic Hg (ppb), and sulphur (%) for sites impacted by atmospheric  $\text{SO}_4^{2-}$  deposition and the subarctic reference fen. Each point represents a single observation from each site. Sites are identified by colour.



**Figure 3.4:** Spatial patterns of (A) MeHg (%) and C/N ratio and (B) sulphur (%) and IHg (ppb) values in peat samples collected at the southern Ontario transect. Scalebars and compass roses are provided for scale and direction at the top of both maps. Arrows indicate the direction of water flow in the bog.

At the low boreal fen, MeHg concentrations were best predicted by including C/N ratio, IHg concentration, and % sulphur in the model (Adj.  $R^2=0.83$ ,  $F(3,7)=16.73$ ,  $p=0.001$ ). However, none of these variables showed a significant linear relationship with MeHg concentration on their own. Percent sulphur was the best predictor of % MeHg and resulted in the best model fit (Adj.  $R^2=0.96$ ,  $F(1,9)=222.8$ ,  $p<0.05$ ), and showed a positive relationship with % MeHg ( $14.74 \pm 0.99$ ). It is important to note, however, that this relationship is largely driven by the two samples taken closest to the upland portion of the fen that showed much higher % MeHg and % sulphur than the rest of the samples. These samples also had comparatively lower C/N ratios and higher IHg concentrations.

### 3.3.3 Overall predictors of mercury methylation across sites

When the data from all sites were combined, the best predictors of MeHg concentration were C/N ratio and % sulphur (Adj.  $R^2=0.37$ ,  $F(2,81)=25.24$ ,  $p<0.05$ ). Percent sulphur displayed a positive linear relationship with MeHg concentration ( $p<0.05$ ), and C/N ratio displayed a negative linear relationship with MeHg concentration ( $p=0.003$ ). The best predictors of % MeHg were % sulphur and IHg concentration (Adj.  $R^2=0.15$ ,  $F(2,81)=8.29$ ,  $p=0.0005$ ). Percent sulphur displayed a positive linear relationship with % MeHg ( $p<0.05$ ), and IHg displayed a negative linear relationship with % MeHg ( $p=0.0013$ ). It is important to note, however, that while these relationships were significant, the adjusted  $R^2$  values for both models are relatively weak, which suggest that there is a high degree of variability that is not being accounted for by the model. This is likely the result of site-specific differences discussed previously.

## 3.4 Discussion

### 3.4.1 Within-site mercury geochemical relationships

As predicted, at the northeast fen, % sulphur did not show a proportional relationship with either % MeHg, or absolute MeHg concentrations. As can clearly be seen from Figure 3.1, the northeast fen has much higher values of % sulphur compared to the subarctic reference site. This suggests that this site is not  $SO_4^{2-}$ -limited, and MeHg production in this fen should be determined by the availability of other nutrients. Indeed,

% MeHg was best predicted by both IHg and C/N ratio, although there was no clear linear relationship between any one variable and % MeHg. As % MeHg can be used as a proxy for long-term MeHg production (Drott *et al.*, 2008; Bailey *et al.*, 2017), these results support the theory that MeHg production is more dependent on IHg concentration and C/N ratio than % sulphur at this site.

There is clearly higher % MeHg at the north and south edges of the northeast fen compared to the interior, which suggests that these areas are MeHg production hotspots. Hotspots of various biogeochemical processes can occur when hydrologic flow paths carrying limiting reactants in the process converge (McClain *et al.*, 2003). Indeed, spatial heterogeneity at peatland edges can preferentially deliver nutrients to localized areas thus creating hotspots of MeHg production (Branfireun, Heyes, & Roulet, 1996 ; Brown *et al.*, 2003). As C/N ratios were also much higher in the edge samples of the fen, the theory that carbon availability may be a more important regulator of MeHg production in this fen is supported. Carbon/nitrogen ratios have been used as an indicator of decomposition in soils, with higher C/N ratios indicating less decomposed organic matter (Kuhry & Vitt, 1996; Krüger *et al.*, 2015). With a greater supply of fresh organic matter, the availability of electron donors for SRB also increases (Tjergren *et al.*, 2012). The optimal supply of nutrients in these localized sampling locations, coupled with the higher position of the water table along the edges of the fen that would promote reducing conditions could explain these hotspots of MeHg production.

It is interesting that absolute MeHg concentrations are not linearly related to % sulphur values in peat, since reduced sulphur compounds in peat have a high affinity for Hg species (Skylberg *et al.*, 2000; Hesterberg *et al.*, 2001) and so MeHg accumulation in areas of high reduced sulphur would be expected. However, as evidenced by the higher level of % sulphur at this site, it is reasonable to assume that high  $\text{SO}_4^{2-}$  reduction, and therefore  $\text{S}^{2-}$  production, is occurring. High levels of dissolved  $\text{S}^{2-}$  can cause the re-partitioning of MeHg bound in peat organic matter to the aqueous phase (Skylberg, 2008). This would cause a decrease in MeHg accumulated in peat, and an increase in MeHg concentrations in pore water. Therefore, absolute MeHg concentrations may reflect MeHg partitioning within the fen while % MeHg values may reflect hotspots

where sufficient nutrients and appropriate redox condition are present that promote active MeHg production.

Lastly, the elevated C/N ratios close to the waste rock piles likely indicate an increase in inorganic carbonate minerals in this area rather than indicating a fresh supply of organic carbon. Since carbonate minerals are common in economic deposits (Al, Martin, & Blowers, 2000; Lu *et al.*, 2013) leaching of carbonate minerals into the fen could explain why C/N ratios of peat samples close to the waste rock piles are high. It is important to note that this could potentially be confounding with respect to the positive relationship between C/N ratio and % MeHg, but as % MeHg values are not elevated in these samples close to the waste rock compared to the edge samples, these higher C/N ratios don't seem to be associated with higher levels of MeHg production.

At the experimental fen MeHg concentrations were best predicted by % sulphur, while % MeHg was best predicted by both IHg concentration and % sulphur. Two years after the initial  $\text{SO}_4^{2-}$  additions to this fen by McCarter, Branfireun, & Price (2017), Twible (2017) measured % sulphur values in peat as high as ~0.25%. Similar elevated % sulphur values were measured in the current study, but as Figure 3.1 shows, % sulphur values at the experimental fen do overlap with subarctic reference fen values. What this suggests is that although this site has been subjected to elevated  $\text{SO}_4^{2-}$  loading, this site may now be in a recovery period in which sulphur loads in solid peat samples are returning to background levels. Indeed, Coleman Wasik *et al.* (2012) found that within six years of ceasing  $\text{SO}_4^{2-}$  additions to an experimental peatland, the sulphur pool in peat was similar to a control peatland.

The fact that both MeHg concentration and % MeHg were predicted by % sulphur values in this fen suggests that this site has reverted back to a  $\text{SO}_4^{2-}$ -limited system in which sulphur once again is proportionally related to Hg methylation. The reason for this could be due to  $\text{SO}_4^{2-}$  removal from the system from such processes as water table draw down events during warmer years that reoxidize reduced sulphur upon exposure to oxygen, and thus re-mobilize  $\text{SO}_4^{2-}$  in the fen (Coleman Wasik *et al.*, 2015). During periods of high water table and flow, it is possible that the fen could become a source of water to the

surrounding bogs (McCarter & Price, 2017a), and over time draw down events followed by periods of high water table could result in net loss of  $\text{SO}_4^{2-}$  from the fen. Lastly, the marginally negative relationship between IHg concentration and % MeHg is likely a reflection of the fact that when more Hg is in the methylated form, IHg values will inevitably be lower, and clearly it is % sulphur, not IHg concentration that is driving Hg methylation at this site.

At the southern Ontario site, neither % sulphur nor IHg concentration showed a proportional relationship with MeHg concentration or % MeHg. This result is similar to that of the northeast fen, likely because both of these peatlands have excess amounts of sulphur. As can be seen from Figure 3.3, the southern Ontario site has elevated % sulphur values as well as IHg concentrations compared to the subarctic reference site, likely due to the latitudinal gradient of sulphur (Vet *et al.*, 2014) and IHg (Givelet, Roos-Barraclough, & Shotyk, 2003) deposition in Ontario as a result of industrial activities such as coal burning in the south. This excess of Hg methylation reactants could explain why MeHg production and accumulation is less coupled to the supply of IHg and % sulphur.

The effect that C/N ratio has on Hg methylation at this site is more difficult to discern. Although C/N ratio showed no significant linear relationship with % MeHg values, C/N ratio did show a negative relationship with absolute MeHg concentrations. Rather than a reflection of lower Hg methylation coupled to higher C/N ratio, this relationship likely reflects preferential binding of MeHg in peat. Closer to the upland of the bog, C/N ratios were generally lower, but % sulphur values were generally higher. As the ratio of C/S decreases, the binding affinity of peat for Hg species increases due to the increased availability of reduced sulphur groups (Demers *et al.*, 2013). The fact that IHg values were also higher in this region is evidence for preferential Hg binding in this peat. It is also not surprising that % sulphur and IHg were higher closer to the upland because uplands can constitute significant sources of sulphur (Urban, Eisenreich, & Grigal, 1989; Mitchell, Branfireun, & Kolka, 2008a), and IHg (Demers *et al.*, 2013) to adjacent wetlands.

Both % MeHg values and C/N ratio were higher in the region closer to the central pond of the bog. The high C/N ratios in these samples again suggests the peat in this region is less decomposed (Kuhry & Vitt, 1996; Krüger *et al.*, 2015). Decomposition is much slower in peats that are consistently waterlogged as opposed to aerated (Whittington & Price, 2006; Ise *et al.*, 2008; Haynes *et al.*, 2017). Therefore, this region of the bog may support more saturated conditions that are conducive to  $\text{SO}_4^{2-}$  reduction and associated Hg methylation by SRB in addition to having a larger supply of organic carbon, resulting in the elevated % MeHg in this region.

At the low boreal site, % sulphur had a positive linear relationship with % MeHg, suggesting that sulphur is the limiting nutrient for Hg methylation at this site similar to the experimental fen. However, at the low boreal site, the higher adjusted  $R^2$  value for this relationship suggests that % sulphur is much more strongly coupled to % MeHg compared to the experimental fen. It is not surprising that this relationship is strong, as % sulphur values at the low boreal site are closest to those values observed for the subarctic reference site (Figure 3.3), and as such, it is likely sulphur-limited. This is expected, as this site is not near a significant point source of  $\text{SO}_4^{2-}$ , and is not in an area of high legacy atmospheric sulphur deposition like the southern Ontario site.

However, it is important to note that across the entire transect, all variables were relatively constant, except in the two samples in closest proximity to the upland, forested portion of the fen which had much higher values of % MeHg and % sulphur. These influential points drove the strong relationship between % sulphur and % MeHg. These samples also had relatively higher values of IHg and lower values of C/N ratio. Unlike bogs, fens still receive hydrological inputs from groundwater in addition to precipitation (Rydin *et al.*, 2013). When there is a break in slope in the transition from an upland to a lower-lying wetland, there can be an upwelling of groundwater at this interface (Winter, 1988). Groundwater can be a source of both Hg (Krabbenhoft & Babiarz, 1992) and  $\text{SO}_4^{2-}$  to the fen and can also help maintain anoxic conditions required for methylation (Branfireun & Roulet, 2002). This may explain why there is an abrupt shift from low, to high values of IHg, and % sulphur at these sample locations compared to the more gradual shift that was observed at the southern Ontario bog. Higher decomposition, and

subsequent Hg methylation at these sample sites in response to increased nutrients would explain the lower C/N ratios and higher % MeHg values in these samples. Conversely, the southern Ontario bog is a raised bog, and as such, the groundwater at this site is recharged by precipitation from the over-lying bog but groundwater at this site does not discharge to the bog due to the downwards hydraulic gradient (City of London, 2009).

### 3.4.2 Overall geochemical relationships

Methylmercury concentrations across sites were best predicted by both % sulphur and C/N ratio, while % MeHg values were best predicted by % sulphur and IHg concentrations. However, as previously mentioned, the low adjusted  $R^2$  values for both these models suggest that these relationships do not fully explain the overall variability in MeHg and % MeHg values, which emphasizes the site-dependent nature of geochemical relationships in this study. Nonetheless, the positive relationship between % sulphur and absolute MeHg, and the negative relationship between C/N ratio and absolute MeHg is likely a reflection of preferentially binding of Hg species to peat with lower C/S ratios (Demers *et al.*, 2013) as previously discussed. However, this relationship breaks down when sulphur is in excess and reduced sulphur binding sites are readily available, such as at the northeast fen.

Similarly, the overall positive relationship between % sulphur and % MeHg is mostly relevant for the experimental fen and low boreal site where sulphur is more limiting. For the more sulphur-enriched sites, namely the southern Ontario site and northeast fen, this relationship breaks down. The overall negative relationship between IHg and % MeHg likely reflects the fact that % MeHg is a derived value that will be higher when a greater proportion of Hg is in the methylated form and less is in the inorganic form. That being said, only samples from the experimental fen showed a significant negative relationship between IHg and % MeHg, which suggests that the experimental fen is the only site at which larger % MeHg values may be the result of lower IHg concentrations that inflate % MeHg values. It should also be noted that since the bioavailability of IHg for methylation is dependent on the geochemical speciation of Hg (Hsu-Kim *et al.*, 2013),



the ability of simple quantitative measurements of IHg in the solid phase used in this study to make inferences about Hg bioavailability is limited.

Lastly, it is interesting to note that while both the southern Ontario site and northeast fen had similar overall % sulphur values in peat samples, the northeast fen had the highest overall concentrations of MeHg and % MeHg values. As Coleman-Wasik *et al.* (2012) observed, newly added sulphur seems to be more readily available for bacterial metabolism as opposed to older, more recalcitrant  $\text{SO}_4^{2-}$  that has been repeatedly turned over by the microbial community. Since  $\text{SO}_2$  emissions in Canada have been declining since the 1980s (Government of Canada, 2012), it is possible that the deposited sulphur at the southern Ontario site has become more recalcitrant compared to the fresh  $\text{SO}_4^{2-}$  that is leaching from the waste rock at the northeast fen site. As a result, SRB usage of  $\text{SO}_4^{2-}$  and associated MeHg production is higher at the northeast fen.

### 3.4.3 Conclusions

Overall, there was no observed consistent universal relationship between sulphur and MeHg across all sites. As predicted, % sulphur was not a reliable predictor of MeHg production for sites where % sulphur was higher and therefore not limiting, which was the case at both the northeast fen and southern Ontario site. Percent sulphur did, however, show a positive, proportional relationship with MeHg production at sites where % sulphur was lower and therefore limiting, which was the case for both the experimental fen and low boreal site. Even though the experimental fen has been subjected to elevated  $\text{SO}_4^{2-}$  loading, it seems to have entered a recovery period in which sulphur has once again become limiting.

Mercury methylation at the northeast fen and southern Ontario site seemed to be somewhat associated with higher C/N ratios, suggesting the important role of organic carbon in Hg methylation when  $\text{SO}_4^{2-}$  is abundant. Therefore, areas where the impacts of anthropogenic  $\text{SO}_4^{2-}$  loading are combined with activities that increase the supply of organic carbon to these environments such as clear-cutting (Zhang *et al.*, 2016) will be especially susceptible to increased MeHg production. The results from the southern Ontario site also suggest that the effects of increased  $\text{SO}_4^{2-}$  deposition can persist long

after deposition has decreased, which has also been found in other studies (Coleman Wasik *et al.*, 2015; Strickman *et al.*, 2016). Management decisions for these impacted areas should therefore carefully monitor recovery with respect to sulphur accumulation and should be cautious to not consider legacy  $\text{SO}_4^{2-}$  effects in isolation from geochemical context.

### 3.5 References

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## Chapter 4

### 4 Conclusion

This final chapter draws overall conclusions for the research presented in this thesis. Limitations and implications for the present work are noted, and suggestions for future work are provided.

#### 4.1 Overall conclusions

This work demonstrates that Hg methylation in response to long-term  $\text{SO}_4^{2-}$  deposition in northern peatlands is not a simple function of the magnitude of the  $\text{SO}_4^{2-}$  addition itself, but a complex interplay between  $\text{SO}_4^{2-}$  addition and the biological and geochemical components of the peat. As Chapter 2 demonstrates, historic additions of  $\text{SO}_4^{2-}$  seem to increase the ability of peats to methylate Hg when supplied with further  $\text{SO}_4^{2-}$  inputs. This is presumably why the southern Ontario site showed the greatest % MeHg increase in column outlet waters over the subarctic and low boreal sites, which had only low to intermediate levels of legacy  $\text{SO}_4^{2-}$  deposition, respectively. However, the results from the field study suggest that total carbon content and C/N ratio as a measure of the ‘decomposability’ of the peat is equally as important as  $\text{SO}_4^{2-}$  availability when  $\text{SO}_4^{2-}$  is in excess.

Hot spots of MeHg production coincided with samples that were high in C/N ratio at both the southern Ontario and northeast fen sites, where sulphur concentrations were significantly higher than the reference fen. Several studies have likewise shown that the ability of  $\text{SO}_4^{2-}$  to stimulate Hg methylation is also dependent on the balance of other nutrients such as organic carbon (Lambertsson & Nilsson, 2006; Mitchell, Branfireun, & Kolka, 2008; Tjerngren *et al.*, 2012; Beck & Johnson, 2014), and therefore the effect that legacy  $\text{SO}_4^{2-}$  deposition has on MeHg production cannot be considered outside of the geochemical context of the peatland. The peat from the southern Ontario site used in the column experiments was sampled from the *Sphagnum* mat where C/N ratios were high, which may have contributed to this peat being able to sustain high levels of MeHg production.

At the low boreal site where  $\text{SO}_4^{2-}$  was more limiting, % MeHg in peat samples was much more strongly associated with % sulphur. The much higher  $\text{SO}_4^{2-}$  reduction in the low boreal peat in response to  $\text{SO}_4^{2-}$  additions in the column experiments further confirms that the low boreal site is a  $\text{SO}_4^{2-}$ -limited system. However, the subarctic reference fen is similarly a  $\text{SO}_4^{2-}$ -limited environment, but the  $\text{SO}_4^{2-}$  reduction in peat from this site in response to  $\text{SO}_4^{2-}$  addition was much lower. Since the low boreal site did have a higher measured Fe content, this could be the result of the  $\text{S}^{2-}$  buffering capacity of the low boreal peat (Heijs *et al.*, 1999; Bailey *et al.*, 2017), as mentioned previously, that allows  $\text{SO}_4^{2-}$  reduction to continue without  $\text{S}^{2-}$  inhibition.

Regardless of this higher  $\text{SO}_4^{2-}$  reduction however, the low boreal site still displayed a lower Hg methylation response compared to the southern Ontario site. The reason for this likely lies in bacterial community differences at these sites. First of all, the peat core sampled from the low boreal site for use in the column experiments was taken near the upland in a region where IHg and % sulphur were relatively higher, and C/N ratio was relatively lower. The high  $\text{SO}_4^{2-}$  reduction decoupled from high MeHg production in response to  $\text{SO}_4^{2-}$  inputs could thus reflect non-methylating SRB dominating these samples, potentially because these species are more efficient at using carbon (the limiting nutrient for this particular sample) compared to methylating species of SRB. Indeed, the Hg methylating efficiency of some SRB is linked to their ability to utilize certain types of carbon (King *et al.*, 2000), and carbon quantity/quality has been shown to be a determining factor in bacterial community structure of soils and sediments (Tian *et al.*, 2018; Graham *et al.*, 2018).

It is also possible that the different temperatures at these sites as a result of latitudinal differences are a determining factor of SRB communities. This has been shown to be the case in studies of wetland methanogens (Yavitt *et al.*, 2012; Wen *et al.*, 2017), and could explain some of the differences in MeHg production and accumulation at the different sites. However, bacterial community analysis would be needed to confirm that there are different communities at these sites, and further experimentation would be required to determine if these differences are truly a function of legacy  $\text{SO}_4^{2-}$  exposure, or if they are also dependent on differences in carbon availability and temperature between sites.

The effect of IHg concentration on MeHg at these sites is more difficult to quantify as IHg concentration did not seem to have a straight-forward relationship with MeHg accumulation in the field study nor MeHg production in the column experiments. This result is similar to that of Åkerblom *et al.* (2013) who found IHg concentrations were much less important for MeHg production in boreal peatlands compared to  $\text{SO}_4^{2-}$  concentrations. That being said, MeHg production is linked to the bioavailability of IHg, which is heavily dependent on the speciation of dissolved IHg with reduced sulphur compounds (Benoit *et al.*, 1999; Benoit *et al.*, 2001; Drott *et al.*, 2007). Because the speciation of IHg compounds was not analyzed in this thesis, it is difficult to conclude whether the excess IHg in column outflow for the low boreal site, or the excess accumulation of IHg at the southern Ontario site were in accessible forms and therefore had a more deterministic role in MeHg production and accumulation.

## 4.2 Implications

What the results of my research suggest is that the geochemical context and legacy of  $\text{SO}_4^{2-}$  deposition of a peatland need to be taken into account when considering how past or future sulphur additions will affect MeHg production. Although the subarctic reference fen which had the lowest legacy sulphur deposition also showed the lowest Hg methylation response to  $\text{SO}_4^{2-}$  inputs in the column experiments, the northeast fen, which is from the same latitudinal location, still had much higher % MeHg values overall compared to the other sites in the field study. This suggests that even pristine peatlands that may have a delayed response to  $\text{SO}_4^{2-}$  inputs can accumulate significant amounts of MeHg over time, and potential thresholds on MeHg accumulation are still unclear. Although MeHg concentrations in surface water from the northeast fen have been declining since 2012 (WEIS, 2017), the results from the northeast fen show that there still exist hotspots of MeHg production, and there is no guarantee that MeHg will stay sequestered in solid peat.

This is particularly relevant as the northeast fen is known to have a fluctuating water table (WEIS, 2017) which could increase aerobic decomposition under unsaturated conditions. This could potentially increase both MeHg release from peat (Haynes *et al.*,

2017; Haynes *et al.*, 2019), as well as re-oxidation of reduced sulphur, further stimulating MeHg production (Coleman Wasik *et al.*, 2015). With projected warming in northern peatlands that could lead to larger water table fluctuations (Sheffield & Wood, 2008), the increased release of MeHg and re-oxidation of reduced sulphur could occur in any of the studied peatlands. As the results of this study suggest, legacy exposure to elevated  $\text{SO}_4^{2-}$  deposition could increase the Hg methylation potential of peats in response to further  $\text{SO}_4^{2-}$  inputs, with the potential to create positive feedback loops in these systems if not given enough time to recover. In addition, the results from the column experiments for the low boreal site seem to point to the important role of SRB community structure in determining MeHg production potential. Therefore, peatlands affected by land use changes that could alter SRB community structure through changes in  $\text{SO}_4^{2-}$  (Strickman *et al.*, 2016) or organic matter (Tian *et al.*, 2018) deposition should be carefully monitored for changes in MeHg production.

### 4.3 Limitations and future work

While I was able to investigate the relationship between  $\text{SO}_4^{2-}$  deposition and MeHg production in both comparative laboratory and field studies, several of the suggested mechanisms to explain observations remain speculative. For the column experiments, the proposed  $\text{SO}_4^{2-}$  priming effect that would explain higher Hg methylation at the southern Ontario site would be confirmed through bacterial community analysis, as well as through a more extensive analysis of bacterial activity. Comparing SRB community structure between initial cores from each site, as well as whole community structure to include other known methylators such as iron-reducing bacteria (Fleming *et al.*, 2006; Kerin *et al.*, 2006), and methanogens (Kennedy, Rosen, & Wood, 1968; Hamelin *et al.*, 2011) could help explain differences in Hg methylation response between these sites. Because bacterial community structure and activity can vary across peatland nutrient gradients (Godin *et al.*, 2012; Preston *et al.*, 2012) and the chosen sites are slightly different in terms of successional stage, the bacterial communities specific to these peatlands could be causing some of the observed variation in Hg methylation, confounding the effect of legacy  $\text{SO}_4^{2-}$  deposition. Community analysis among peatlands

of similar nutrient status and different levels of legacy  $\text{SO}_4^{2-}$  loading could help link or discriminate between these two effects.

The analysis of microbial biomass in peat cores before and after column experiments was attempted using chloroform fumigation extraction (Vance, Brookes, & Jenkison, 1987; Gregorich *et al.*, 1990), but this method was not sensitive enough to identify small differences in microbial biomass specific to the SRB, which is the target group of interest. More sensitive and targeted approaches such as quantitative PCR (qPCR) using primers specific to SRB such as *dsrAB* (Geets *et al.*, 2006; Dar *et al.*, 2007) or *hgcA* and *hgcB* (Parks *et al.*, 2013) would be useful in future studies of this kind. In addition, bacterial priming in response to an introduced nutrient can be confirmed by measuring external cellular enzymes as a proxy for increased decomposition (Blagodatskaya, & Kuzyakov, 2008). This method has been used in several studies of wetland soils (Freeman *et al.*, 1995; Sjögersten *et al.*, 2011; Dunn *et al.*, 2014) and could be one method of confirming increased decomposition in peatland soils exposed to elevated levels of  $\text{SO}_4^{2-}$ .

In the field study, the aforementioned bacterial analysis along each transect could help explain the variation in MeHg accumulation between sites, as well as the variation within sites along some of the geochemical gradients that emerged in this study. More intensive sampling strategies for field studies of this kind are also needed, as this study indicates spatial variability along these peatland transects is relatively high. In addition, the variation in values such as C/N ratio is likely also high among different peatlands in the same region, and so more extensive sampling of peatlands in each region would allow for more general conclusions to be drawn rather than peatland-specific conclusions. Sampling pore water along these transects would also confirm some of the speculative conclusions that have been drawn about MeHg partitioning and sulphur speciation.

## 4.4 References

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# Appendices

## Appendix A: Supplementary Calculations

### Sample calculation: Southern Ontario

#### **Column cross sectional area:**

Inner diameter: 4.8 cm

$$\text{Area} = \pi r^2$$

$$= \pi(2.4)^2$$

$$= 18.065 \text{ cm}^2$$

#### **Conversions:**

Column cross sectional area = 18.065 cm<sup>2</sup>

18.095 cm<sup>2</sup>/1,000,000 cm<sup>2</sup>/ha = 0.000018095 ha

Length of experiment: 15 days

15 days/365 days/year = 0.041 year

#### **Mass of sulphur entering column during experiment:**

Average deposition rate from Vet *et al.* (2014): 6.5 Kg S/ha/year

6,500,000 mg S/ha/year X 0.000018095 ha X 0.041 year = 4.8223 mg S/ column/ 15 days

#### **Volume of SO<sub>4</sub> solution flowing into column during experiment:**

Average flow rate: 17.5 mL/h

17.5 mL/h X 24 h/day = 420 mL/day

15 days X 420 mL/day = 6,300 mL/column = 6.3 L/column/15 days

#### **Calculation of SO<sub>4</sub> solution concentration:**

4.8223 mg S/6.3 L = 0.765 mg/L (ppm) S

**Figure A1:** Calculation of SO<sub>4</sub><sup>2-</sup> solution concentration used in Chapter 2 experiments representing elevated atmospheric deposition rate. Raw values for deposition rates are taken from Vet *et al.* (2014) for the southern Ontario region, and are adjusted for column cross-sectional area, experiment duration, and flow rate.

**Sample calculation: Low Boreal site**

**Bulk density of three homogenized peat trials:**

130 g homogenized wet peat in 125 cm<sup>3</sup> : dry weight = 10.23 g

Bulk density = 10.23 g/125 cm<sup>3</sup> = 0.082 g/cm<sup>3</sup>

115 g homogenized wet peat in 125 cm<sup>3</sup> : dry weight = 9.092 g

Bulk density = 9.092 g/125 cm<sup>3</sup> = 0.073 g/cm<sup>3</sup>

100 g homogenized wet peat in 125 cm<sup>3</sup> : dry weight = 8.22 g

Bulk density = 8.22 g/125 cm<sup>3</sup> = 0.066 g/cm<sup>3</sup>

**Linear equation for bulk density extrapolated from trials:**

$y = 1851x - 20.9$  ( $R^2 = 0.99$ ) where:

y = wet peat weight in g

x = bulk density in g/cm<sup>3</sup>

**Calculation of wet peat mass in 125 cm<sup>3</sup> that yields target bulk density**

Target bulk density = 0.07 g/cm<sup>3</sup>

$y = 1851(0.07 \text{ g/cm}^3) - 20.9$

$y = \sim 110 \text{ g in } 125 \text{ cm}^3$

**Calculation of wet peat mass extrapolated for volume of column:**

Volume of column = 542.87 cm<sup>3</sup>

$542.87 \text{ cm}^3 / 125 \text{ cm}^3 = 4.34$

$4.34 \times 110 \text{ g} = 477.4 \text{ g of wet peat/column}$

**Figure A2:** Sample calculation of homogenized wet mass of peat used in columns for study sites in Chapter 2. Target dry bulk density is 0.07 g/cm<sup>3</sup>.

Low Range			Mid-low Range			Mid-high Range 1: 25 dilution			High Range 1:50 dilution		
[S <sup>2-</sup> ]	Abs.	SE	[S <sup>2-</sup> ]	Abs.	SE	[S <sup>2-</sup> ]	Abs.	SE	[S <sup>2-</sup> ]	Abs.	SE
0.03	0.006	5.3 X 10 <sup>-3</sup>	0.1	0.032	3.2 X 10 <sup>-2</sup>	1.3	0.036	1.2 X 10 <sup>-1</sup>	8	0.077	1.2 X 10 <sup>-1</sup>
0.05	0.014	3.3 X 10 <sup>-2</sup>	0.5	0.229	2.5 X 10 <sup>-1</sup>	3.5	0.103	3.1 X 10 <sup>-1</sup>	16	0.165	3.1 X 10 <sup>-1</sup>
0.07	0.019	1.9 X 10 <sup>-2</sup>	0.9	0.422	3.5 X 10 <sup>-1</sup>	5.7	0.162	4.0 X 10 <sup>-1</sup>	24	0.248	4.0 X 10 <sup>-1</sup>
0.09	0.026	2.4 X 10 <sup>-2</sup>	1.28	0.575	4.6 X 10 <sup>-1</sup>	7.9	0.233	6.2 X 10 <sup>-1</sup>	32	0.328	6.2 X 10 <sup>-1</sup>
<b>Linear calibration equation</b>											
<b>y=2.9x + 0.0136</b>			<b>y=2.155x + 0.017</b>			<b>y=33.84x + 0.075</b>			<b>y=95.52x + 0.442</b>		
<b>R<sup>2</sup>=0.986</b>			<b>R<sup>2</sup>=0.998</b>			<b>R<sup>2</sup>=0.998</b>			<b>R<sup>2</sup>=0.999</b>		

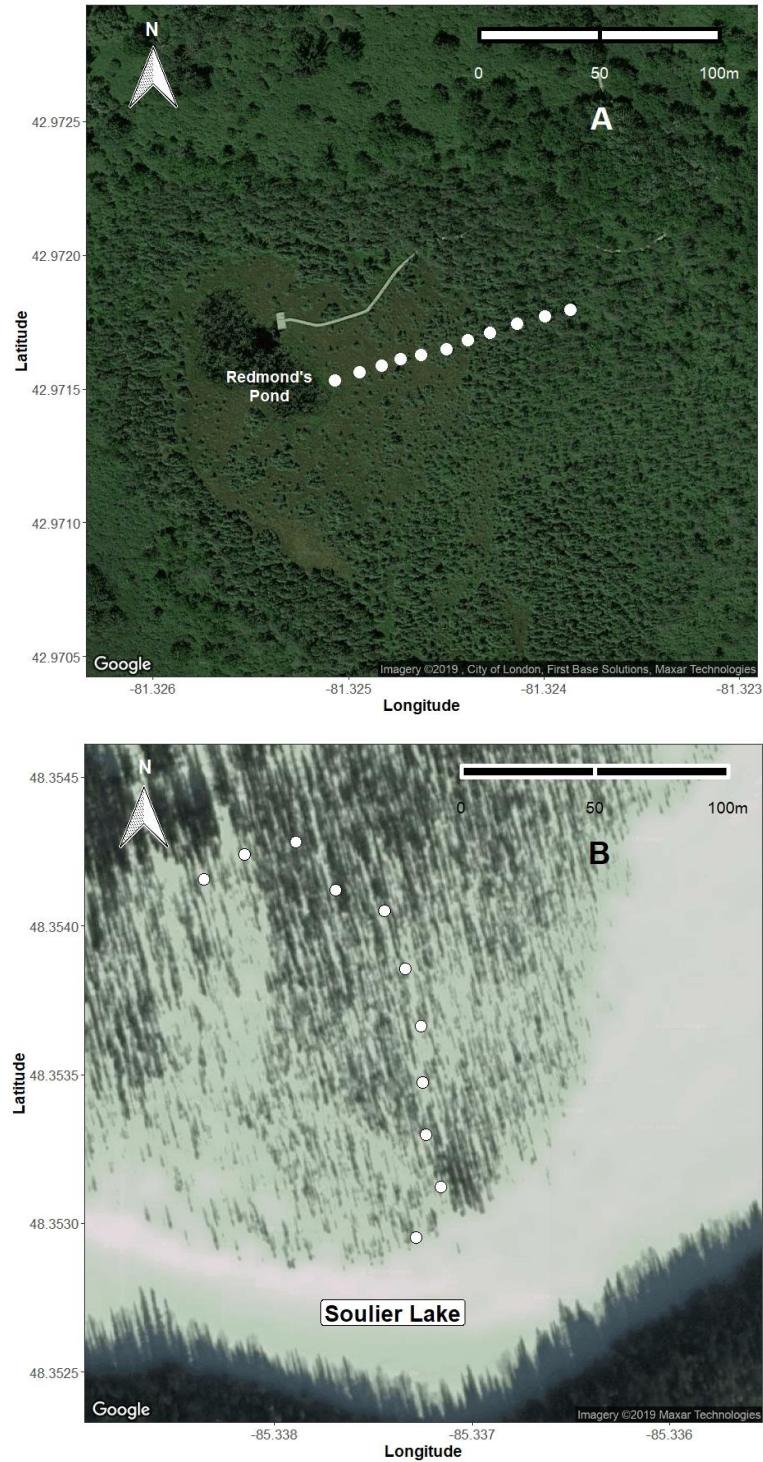
**Figure A3:** Sulphide calibration curve values. Sulphide standards are divided into four ranges based on the protocol of Cline (1969). Sulphide standards denoted as [S<sup>2-</sup>] are reported in ppm. Absorbance values (Abs.) for the corresponding standards are reported as the average of three absorbance trials with standard error run on the Horiba Aqualog® with the addition of the associated diamine reagent. The mid-high and high standard ranges required 1:25 and 1:50 dilutions of the diamine-S<sup>2-</sup> standard mixture according to the protocol of Cline (1969). Linear calibration equations for each standard range are reported with associated R<sup>2</sup> values.

## Appendix B: Supplementary photographs



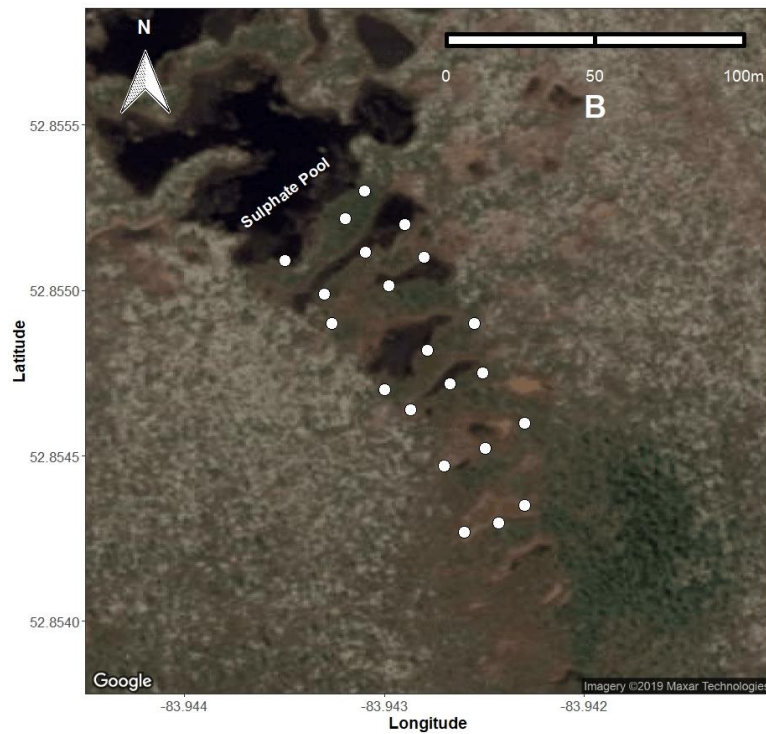
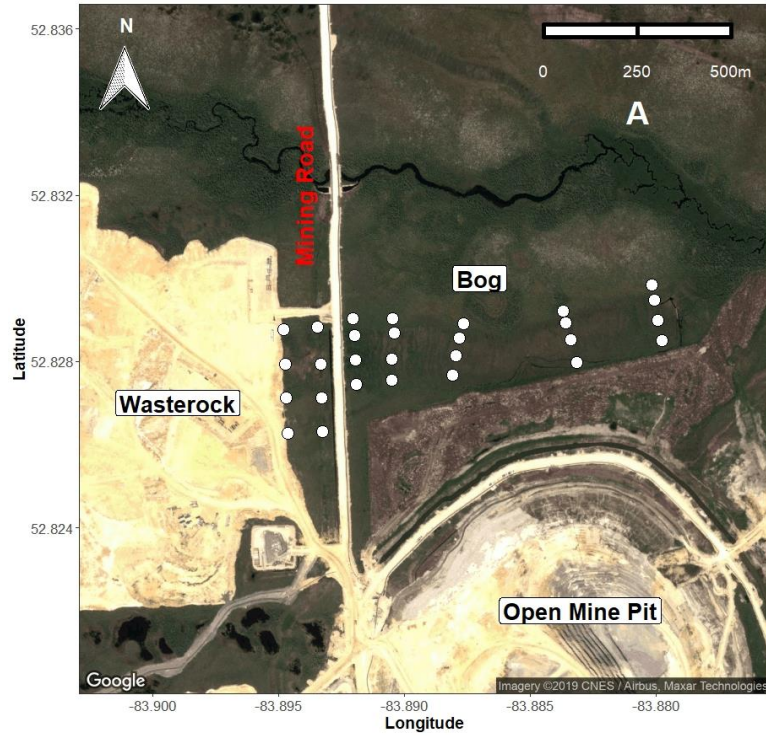
**Figure B1:** Experimental set-up for Chapter 2 column experiments.

## Appendix C: Supplementary site maps



**Figure C1:** Spatial map of the southern Ontario bog (A) and low boreal fen (B) sampling designs. Points represent locations where peat samples were taken. Scale bars are provided at the top of each map and relevant landscape features are labelled.





**Figure C2:** Spatial map of northeast fen (A) and experimental fen (B) samling designs. Points represent locations where peat samples were taken. Scale bars are provided at the top of each map and relevant landscape features are labelled



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