# Plant mercury accumulation and litter input to a Northern Sedge-dominated Peatland

Ting Sun[1,2,3] and Brian A. Branfireun[4]

[1]School of Environmental Science and Engineering, Shandong University, Qingdao 266237, PR China

[2]Institute of Eco-Environmental Forensics, Shandong University, Qingdao 266237, PR China

[3]Department of Earth Sciences, University of Western Ontario, 1151 Richmond Street, London, Ontario, Canada

[4]Department of Biology, University of Western Ontario, 1151 Richmond Street, London, Ontario, Canada

*Correspondence to*: Ting Sun (tsun64@uwo.ca)

Abstract

Plant foliage plays an essential role in accumulating mercury (Hg) from the atmosphere and transferring it to soils in terrestrial ecosystems, while many studies have focused on forested ecosystems. Hg input from plants to northern peatland peat soils has not been nearly as well studied and is likely equally important from a mass balance perspective. In this study, we investigated the accumulation of atmospheric Hg by the dominant plant species, few-seeded sedge [*Carex oligosperma* Michx.], wire sedge [*Carex lasiocarpa* Ehrh], tussock sedge [*Carex stricta* Lamb.], and sweet gale [*Myrica gale* L.] in a boreal sedge-dominated peatland. Foliar Hg concentrations decreased early in the growing season due to growth dilution, and after that were subsequently positively correlated with leaf age (time). Hg concentrations were 1.4-1.7 times higher in sweet gale than in sedges. A leaching experiment showed that sweet gale leached less Hg but more bioaccessible dissolved organic matter (DOM) by mass than sedges. Leaching of Hg was positively related to the aromaticity of DOM in leachate, suggesting the importance of DOM with higher aromaticity in controlling Hg mobility. Annual inputs of Hg through senesced leaf material to peat soils were 9.88 mg/ha/yr, 1.62 mg/ha/yr, and 8.29 mg/ha/yr for sweet gale, tussock sedge, and few-seeded sedge/wire sedge, respectively. Future investigations into foliar Hg accumulation and input from other plant species to the sedge-dominated peatland are needed to estimate the annual Hg inputs precisely.

## 1 Introduction

Mercury (Hg), especially methylmercury (MeHg), is a global concern due to its potential toxicity and ubiquitous presence in the environment (Morel et al., 1998). Hg is emitted to the atmosphere from both natural (e.g., volcanoes, wildfires, geothermal activity) and anthropogenic sources (e.g., coal combustion, artisanal gold mining, incineration) (Schroeder and Munthe, 1998; Streets et al., 2011). Atmospheric Hg exists as gaseous elemental mercury (GEM, Hg(0)), reactive gaseous mercury (RGM, Hg(II)), and particulate-bound mercury (PBM, $Hg_p$) with GEM as the dominant species (> 95 %) (Schroeder and Munthe, 1998). RGM and PBM have shorter atmospheric residence time ranging from hours to days, whereas GEM has a longer atmospheric residence time of several months to a year and thus is transported globally (Schroeder and Munthe, 1998). These atmospheric Hg species are eventually deposited into aquatic and terrestrial ecosystems via wet deposition (precipitation, such as rain, snow, and fog) and dry deposition (particle settling or direct partitioning to vegetation, water, and soil surface, or direct absorption by vegetation foliage) (Lindberg et al., 2007). Hg dry deposition is a larger input than wet deposition to vegetated terrestrial landscapes, contributing 70 %~85 % of total Hg deposition (dry and wet deposition) in terrestrial ecosystems (Graydon et al., 2008; Risch et al., 2017; Risch et al., 2012; St. Louis et al., 2001; Wang et al., 2016; Zhang et al., 2016), and more than 70 % of Hg dry deposition is by vegetation litterfall/incorporation into soil organic matter (SOM) (Obrist et al., 2017; Wang et al., 2016).

Vegetation is generally considered a sink for atmospheric Hg, with the majority of Hg in vegetation leaves accumulated from the atmosphere (Jiskra et al., 2018; Obrist et al., 2017). Plant leaves accumulate Hg from the atmosphere mainly through stomatal uptake (Lindberg et al., 1992). Stamenkovic and Gustin (2009) suggested that the non-stomatal pathway of Hg deposition to the leaf cuticle and subsequently retention and incorporation into leaf tissue also plays an important role in accumulating atmospheric Hg. Plant roots are thought to generally act as a barrier of Hg transport from soils to shoots (Wang et al., 2015), and it has been shown that less than 10% of Hg in roots is transported to the aboveground portion of plants (Ericksen et al., 2003; Mao et al., 2013). Some studies have found that a great proportion of foliar Hg in halophytes in salt marshes was translocated from the root (Canário et al., 2017; Cabrita et al., 2019; Weis and Weis 2004). The plausible reason is that plants in the hydroponic growth system have fewer apoplastic barriers (i.e. Casparian bands and suberin lamellae) in root architecture than plants grown in contaminated soils (Redjala et al., 2011).

Forest ecosystems are important sinks of atmospheric Hg and have received widespread attention from researchers (Risch et al., 2012; St. Louis et al., 2001; Wang et al., 2016; Zhang et al., 2009); however, studies about foliar Hg accumulation in other plant

68  types in boreal peatlands ecosystems are few (see Moore et al., 1995) despite their critical role in the carbon  (Gorham, 1991)

69  and Hg cycles (Grigal, 2003). Boreal peatlands are a type of wetland that stores large amounts of Hg (Grigal 2003) and can be

70  major MeHg sources to downstream ecosystems (Branfireun et al., 1996; Mitchell et al., 2008; St. Louis et al., 1994), given their

71  anaerobic conditions, non-limiting amounts of inorganic Hg, and often available but limited amounts of sulfate (Blodau et al.,

72  2007; Schmalenberger et al., 2007) and bioaccessible carbon facilitating net MeHg production (Mitchell et al., 2008).

73  Elucidation of foliar Hg input from the dominant plant types to boreal peatlands is important to further estimate the supply of

74  bioavailable Hg(II) for net MeHg production.

75  Previous studies have found that the majority of Hg in plant leaves in wetlands was from the atmosphere (Brahmstedt et al.,

76  2021; Enrico et al., 2016; Fay and Gustin 2007) and nonvascular plants (e.g., fungi, lichens, and mosses) had higher foliar Hg

77  concentrations than vascular plants (Moore et al., 1995; Pech et al., 2022). Although foliar Hg concentration is lower in vascular

78  plants than in nonvascular plants, Hg mass input to peatlands may be substantial, given the greater litter input from vascular

79  plants than from nonvascular plants (Frolking et al., 2001). With more bioaccessible litter than bryophytes (Hobbie, 1996; Lyons

80  and Lindo, 2019), vascular plants also have a faster initial decomposition rate ($0.2$ $y^{-1}$) than bryophytes ($0.05$-$0.08$ $y^{-1}$) (Frolking

81  et al., 2001), leading to a rapid Hg release to the soil. Boreal peatlands are experiencing rising temperatures due to climate

82  change (IPCC, 2018) which is likely to both increase aboveground biomass in vascular plant-dominated peatlands (Tian et al.,

83  2020) and promote a shift from moss-dominated to more vascular plant-dominated plant communities (Buttler et al., 2015;

84  Dieleman et al., 2015; Weltzin et al., 2000) further affecting Hg deposition (Zhang et al., 2016). To date, the amount of

85  atmospheric Hg accumulated in dominant plants in the vascular plant-dominated (i.e., graminoid plants and shrubs) peatlands, an

86  important type of boreal wetlands (Rydin and Jeglum, 2013), is unknown.

87  Foliar Hg eventually enters peat soils via litterfall and is expected to follow the sequence: (1) wash-off of aerosols, particles, and

88  gases from leaf surfaces, (2) leaching of water-soluble components, and (3) incorporation into SOM after the microbial

89  decomposition of litter. Leaching is the initial phase of litter breakdown in aquatic environments and can rapidly release up to

90  30 % dissolved matter, primarily dissolved organic matter (DOM) within 24 h after immersion of litter (Gessner et al., 1999). It

91  has been established that dissolved organic matter (DOM) is closely related to Hg mobility in terrestrial and aquatic ecosystems

92  (Haitzer et al., 2002; Ravichandran, 2004; Kneer et al., 2020), given the strong affinity between Hg and reduced sulfur groups

93  (i.e., thiols) in DOM (Xia et al., 1999). DOM with higher aromaticity has more thiols ligands and has a stronger correlation with

94  Hg (Dittman et al., 2009). The rapid and abundant leaching of DOM, especially those with higher aromaticity from litterfall may

95   lead to large amounts of Hg leaching. The amount of rapidly released Hg during litter leaching is unknown and needs to be

96   elucidated because more recently deposited Hg appears to be more readily methylated than "old" Hg in peat soils (Branfireun et

97   al., 2005; Feng et al., 2014; Hintelmann et al., 2002). Despite previous studies showing that Hg mass in live leaf leachate is

98   insignificant compared to that on leaf surfaces and in SOM (Rea et al., 2001; Rea et al., 2000), litterfall generally lacks structural

99   integrity and likely leaches more Hg compared to live leaves.

100   The overall objective of this study is to link the vascular plant community (i.e., sedges and shrubs) to the peatland Hg cycle in a

101   vascular plant-dominated fen-type peatland. We use "sedge-dominated fen" instead of "vascular plant-dominated fen-type

102   peatland" hereafter, given that sedges are the primarily dominant plants in this study site (Webster and McLaughlin, 2010). The

103   specific objectives of this study are to:

104   (1) quantify the mass accumulation of atmospherically-derived Hg in leaves of dominant plant species in a sedge-dominated fen

105   over a growing season;

106   (2) estimate the Hg input from the litter of different plant species and through litter leaching to peat soils;

107   (3) clarify the role of DOM characteristics in controlling Hg leaching;

108   (4) estimate the annual areal loading of foliar Hg of different plant species to peat soils.

109   **2 Materials and methods**

110   **2.1 Study site**

111   Samples were collected from a sedge-dominated fen (10.2 ha) located in an 817 ha sub-watershed of the Lake Superior basin

112   near White River Ontario, Canada (48°21' N, 85°21' W). The growing season is roughly from June to September. The sedge-

113   dominated fen is mostly open and the vegetation community is dominated by three sedge species: few-seeded sedge [*Carex*

114   *oligosperma* Michx.]; wire sedge [*Carex lasiocarpa* Ehrh]; and tussock sedge [*Carex stricta* Lamb.] (Lyons and Lindo, 2019).

115   Sweet gale [*Myrica gale* L.] is the dominant shrub at this site (Lyons and Lindo, 2019; Palozzi and Lindo, 2017). The mean

116   species percent cover of few-seeded sedge, wire sedge, tussock sedge, and sweet gale from the sedge-dominated fen is 35.0 ±

117   21.79%, 0.3 ± 0.12%, 73.0 ± 18.81%, 44.8 ± 10.63% (average ± standard error (SE)), respectively (Palozzi and Lindo 2017).

118   Details of the study site and the characteristics of these plants are provided in the Supporting Information (SI). In this study, few-

119    seeded sedges and wire sedges were mixed during plant sample collection as they are indistinguishable in size and form from one

120    another when not in flower/seed, and frequently co-occur.

121    **2.2 Sample collection and analysis**

122    Five locations several hundred meters apart were selected in the sedge-dominated fen to serve as within-site replicates to account

123    for potential local-scale variability. These five locations were roughly evenly distributed over this study area. Approximately

124    fifty whole leaves of each few-seeded sedge/wire sedge, tussock sedge, and sweet gale were collected from each location using a

125    clean blade in the middle of June, July, August, and after senescence at the beginning of October 2018 in each location, totaling

126    60 samples. For the October sampling event, the sedge leaves were still standing with the lower sections green, and although

127    senesced, shrub leaves were sampled from the branch to ensure that there was no mixing with previous years' fallen leaves.

128    Disposable nitrile gloves were worn during the sample collection. All samples were double bagged with two polyethylene bags

129    and transported to the lab using a clean cooler. Leaves of each species that were collected from each location in October 2018

130    were divided for foliar total Hg (THg) analyses and a foliar Hg leaching experiment. Leaves were stored frozen until they were

131    returned to the university laboratory.

132    For estimation of annual biomass of senesced leaf, seven 0.25 m$^2$ (0.5 m × 0.5 m) plots several hundred meters apart were

133    selected at the end of August 2019 during senescence and before leaf off. All aboveground biomass of few-seeded sedge/wire

134    sedge and tussock sedge and all aboveground leaf biomass of sweet gale were collected separately using a clean blade from each

135    0.25 m$^2$ plot. All vegetation samples were stored by species in paper bags, transported to the lab, and then oven-dried at 60 °C

136    for a minimum of 48 h. Dried leaves of each species in each plot were sorted and weighed to estimate senesced leaf biomass of

137    each species for each plot. The senesced leaf biomass of each species per hectare per year was calculated and expressed as

138    mg/ha/yr.

139    **Foliar total mercury, C content and N content.** In the laboratory, leaf samples for chemical analyses were rinsed three times

140    with deionized water (18.2 MΩ cm), freeze-dried for 48 h, ground and homogenized, and then analyzed using a Milestone™

141    DMA-80 (EPA method 7473). Leaf C content (%C; w/w) and N content (%N; w/w) before and after the foliar Hg leaching

142    experiment was analyzed using a CNSH analyzer (Vario Isotope Cube; Elementar). The ratio of leaf C content and N content

143    (C:N) were calculated. Detailed information concerning analytical methods are described in the SI, including analysis of foliar

144    total Hg, %C, and %N.

145    **Foliar mercury leaching experiment.** The foliar leaching experimental procedure followed the design of Rea et al. (2000) and

146    Del Giudice and Lindo (2017). Senesced leaves of sedges and sweet gale collected in October 2018 were rinsed twice with 100

147    mL of deionized water (18.2 MΩ cm) to quantify particulate or loosely-bound Hg and DOM that can be easily removed/leached

148    from the leaf surface. This water was reserved for subsequent analysis. After rinsing, the leaves were oven-dried at a low

149    temperature (40 °C) for 48 h, and then the leaves of each species from each location were relatively evenly separated into three

150    groups and weighed, totaling 45 groups. These oven-dried senesced leaf samples were immersed in 150 mL of deionized water

151    in clean 250 mL PETG bottles. All PETG bottles were capped, double bagged, and incubated in the dark at room temperature

152    (~21 °C) for 48 h. Senesced leaf materials were gently swirled at the beginning of the leaching experiment to ensure complete

153    wetting. Following the leaching, the leachate was vacuum filtered through a 0.45 μm glass fiber filter into clean 250 mL PETG

154    bottles. Leachate from each sample was split into two aliquots. One was preserved by acidifying to 0.5 % (vol/vol) with high-

155    purity HCl for dissolved total Hg ($THg_{aq}$) analysis and stored in 250 mL PETG bottles; the other was stored in the clean 60 mL

156    Amber glass bottles and analyzed within 2 d for the quantity and characteristics of DOM. All samples were stored in the dark at

157    4 °C for further analysis. Method blanks of the leaching experiment were performed at the same time following the same

158    procedure.

159    Senesced leaf material was taken out of each PETG bottle, oven-dried at 40 °C for 48 h, and re-weighed after leaching. The dry

160    leaf weight before and after the leaching process was used to calculate the mass loss. These re-dried senesced leaf samples after

161    leaching were ground and homogenized before the measurement for %C and %N as described above.

162    The dissolved total Hg ($THg_{aq}$) concentrations in the rinse water and leachate were analyzed using Environmental Protection

163    Agency (EPA) method 1631. Dissolved organic matter is quantified analytically as dissolved organic carbon (DOC). DOC

164    concentrations in rinse water and leachate were measured using an iTOC Aurora 1030 (OI Analytical, College Station, TX,

165    USA) using the persulfate wet oxidation method. Details on the analytical procedures and QA/QC data for concentrations of

166    $THg_{aq}$ and DOC are provided in the SI.

167    DOM in leachate was characterized as specific ultraviolet absorbance at a wavelength of 254 nm ($SUVA_{254}$), an indicator of the

168    molecular weight (or size) and aromaticity (the content of aromatic molecules) of DOM (Weishaar et al., 2003). Higher $SUVA_{254}$

169    values suggest that DOM contains more high-molecular-weight and aromatic molecules (Weishaar et al., 2003). Fluorescence

170    excitation-emission matrices (EEMs) were also collected for calculating informative optical indices that reflect differences in

171    DOM characteristics in leachate. The reported EEMs were then converted to optical indices using R Software (R Core Team

172  2012). Three common indices were chosen in this study: the fluorescence index (FI), the humification index (HIX$_{EM}$), and the

173  biological index or 'freshness' index (BIX). Lower FI values (< 1.2) indicate that DOM is terrestrially derived (resulting from

174  decomposition and leaching of plant and soil organic matter) and has higher aromaticity, while higher FI values (> 1.8) indicate

175  that DOM is microbially derived (originating from processes as extracellular release and leachate of algae and bacteria) and has

176  lower aromaticity (Fellman et al., 2010; McKnight et al., 2001). High HIX$_{EM}$ (> 1.0) values reflect the high humification of

177  DOM and DOM is composed of more highly condensed and higher molecular weight molecules (Fellman et al., 2010; Hansen et

178  al., 2016; Huguet et al., 2009; Ohno, 2002). Higher BIX  values (> 1.0) reflect that more low-molecular-weight DOM was

179  recently produced by microbes (Fellman et al., 2010; Huguet et al., 2009). Details on the analytical procedures and QA/QC data

180  for SUVA$_{254}$, FI, HIX$_{EM}$, and BIX are provided in the SI.

181  **3 Statistical analysis**

182  Results were analyzed using IBM SPSS statistics software (IBM SPSS Inc. 24.0). The repeated-measures ANOVA was

183  performed to compare the difference in foliar THg concentrations among different plant species over the growing season and to

184  analyze the effect of leaf age on foliar Hg concentrations. Linear regressions were analyzed to examine the relationship between

185  foliar THg accumulation and leaf age. Differences in foliage quality (%C, %N, and C:N) were analyzed using a multivariate

186  ANOVA. One-way ANOVA was used to determine the effects of plant species on concentrations of THg$_{aq}$ and DOM quantity

187  and characteristics in leachate. The repeated-measures ANOVA, multivariate ANOVA, and one-way ANOVA were followed by

188  a *post hoc* test (Bonferroni's significant difference; honestly significant difference at the 95 % confidence interval). Weighed

189  least squares regression was used to examine the nature of the relationship between THg$_{aq}$ concentrations and SUVA$_{254}$ in

190  leachate. Data are presented as the mean ± standard deviation (SD). Coefficient of determination ($R^2$) and significance p-values

191  (p) are presented for linear regression fits, and $p < 0.05$ was considered significant.

192  **4 Results and discussion**

193  **4.1 Foliar mercury accumulation in peatland plants**

194  Foliar THg concentrations were related to time/leaf age ($F_{(3,36)} = 108.86$, $p < 0.001$) and plant species ($F_{(2,12)} = 51.85$, $p < 0.001$)

195  (Fig. 1). Based on *post hoc* tests, foliar THg concentrations were significantly different between plant species and between the

196  sampling months, except that there was no significant difference in foliar THg concentrations between June and August. The

197  mean foliar THg concentrations (n = 5) in June followed the sequence: few-seeded sedge/wire sedge < tussock sedge < sweet

8

198    gale. In July foliar THg concentrations decreased by 30 % (few-seeded sedge/wire sedge), 40 % (tussock sedge), and 47 %

199    (sweet gale), respectively. The decrease in THg concentrations is likely because of leaf growth dilution, although changes in leaf

200    biomass were not quantified as part of this study. Foliar THg concentrations were positively related to time after July (few-

201    seeded sedge/wire sedge: $F_{(1,13)} = 185.79$, $p < 0.001$, $R^2 = 0.93$; tussock sedge: $F_{(1,13)} = 200.87$, $p < 0.001$, $R^2 = 0.94$; sweet gale:

202    $F_{(1,13)} = 70.72$, $p < 0.001$, $R^2 = 0.84$). The mean foliar THg concentrations in October few-seeded sedge/wire sedge, tussock sedge,

203    and sweet gale were 1.7, 1.3, and 2.0 times higher than the initial concentrations in June. This result showed a clear pattern of

204    continuous THg accumulation in foliage in boreal peatland plant species over time as has been shown for forests (Laacouri et al.,

205    2013; Millhollen et al., 2006b; Rea et al., 2002), which can be attributed to foliar Hg accumulation from the air, given that plant

206    roots act as a barrier of Hg transport from soils to shoots (Wang et al., 2015). Further studies are needed to quantify the

207    contribution of atmospheric and soil Hg to foliar Hg.
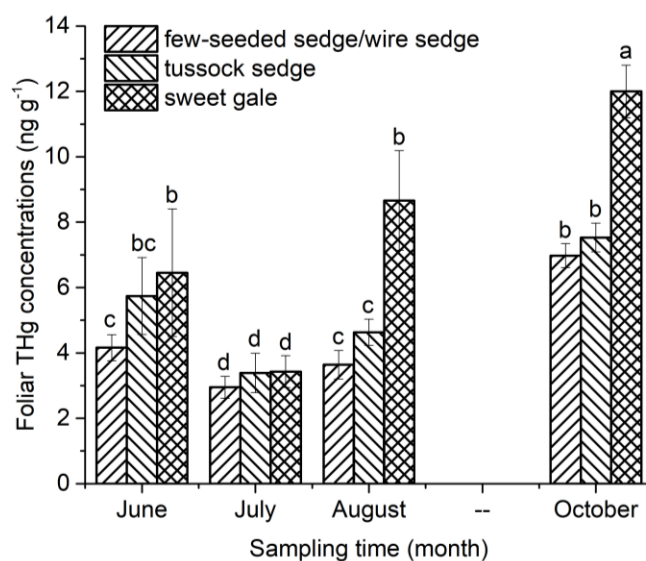


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209 **Figure 1 The intraseasonal trend in foliar total mercury (THg) concentrations (ng g$^{-1}$) of few-seeded sedge/wire sedge, tussock sedge,**

210 **and sweet gale (ng g$^{-1}$). All concentrations are expressed in dry weight. Error bars are ± SD (n = 5 for each species for each time**

211 **interval). The same letters above bars denote that values of foliar THg concentrations are not significantly different at the 0.05 levels.**

212    Mercury accumulation in leaves is affected by many factors, such as atmospheric Hg concentration, environmental conditions

213    (e.g., solar radiation and temperature), and biological factors (e.g., leaf age, plant species, leaf area, and leaf placement)

214    (Blackwell and Driscoll, 2015; Ericksen et al., 2003; Ericksen and Gustin, 2004; Laacouri et al., 2013; Millhollen et al., 2006a).

215    Since all samples were collected in the same location, factors such as atmospheric Hg concentration and environmental

216    conditions were deemed the same, leaving only biological factors as an explanation for differences.

9

**Leaf age.** Leaf age is an important biological factor in controlling foliar concentrations (Ericksen et al., 2003; Laacouri et al., 2013). The positive relationship between foliar THg concentrations and time after July suggests that leaves of all species here continued to assimilate atmospheric Hg over the growing season right up to senescence. Some studies have found that the rate of foliar Hg uptake decreased toward the end of the growing season (Ericksen et al., 2003; Laacouri et al., 2013; Poissant et al., 2008), which appears to be because of the decrease in photosynthetic activity at the end of the growing season (Koike et al., 2003). In this study, foliar Hg concentrations continue to increase right up to senescence. Although foliar Hg can transport to other plant organs, such as tree rings (Arnold et al., 2018; McLagan et al., 2022), and/or can be re-emitted into the atmosphere (Zheng et al., 2016; Yu et al., 2016; Yuan et al., 2019), the majority of foliar Hg by mass is generally incorporated into leaf tissue (Laacouri et al., 2013; Lodenius et al., 2003; Stamenkovic and Gustin, 2009). In addition, it is likely that less than 10% of Hg in roots was transported to the leaves (Ericksen et al., 2003; Mao et al., 2013).

**Plant species.** Plant photosynthesis, transpiration, growth rates, and leaf area are different among plant species (Antúnez et al., 2001; Laacouri et al., 2013; Millhollen et al., 2006b), and given that these are important controls on Hg accumulation, the differences among species found in this study are not surprising. The mean foliar THg concentrations in tussock sedge were 1.2 times higher than that in few-seeded sedge/wire sedge, and although not measured as part of this study, tussock sedge has a larger leaf area than few-seeded sedge/wire sedge (Newmaster et al., 1997). A larger leaf has a higher density of stomate and thus more leaf accumulation of atmospheric Hg (Laacouri et al., 2013; Millhollen et al., 2006; Stamenkovic and Gustin, 2009). A larger leaf area may also provide more adsorption sites for non-stomatal Hg uptake. Increased biomass corresponding with a bigger leaf area can offset the effects of stomate number on atmospheric Hg accumulation by leaves to a certain degree. A plausible explanation is that leaf biomass does not proportionally increase with leaf area and stomata, leading to a higher absolute Hg concentration in tussock sedge leaves than in few-seeded segde/wire sedge leaves. Kozlowski and Pallardy (1997) reported that leaves near the top of the canopy generally have higher rates of photosynthesis and stomatal conductance than those near the bottom of the canopy due to light saturation. Sweet gale had potentially higher stomatal conductance due to higher incident radiation and vapor pressure deficits than sedges that are lower to the saturated ground with tightly packed vertical leaves.

Concentrations of Hg in senesced leaves of few-seeded sedge/wire sedge, tussock sedge, and sweet gale (6.58 ng g$^{-1}$ to 12.77 ng g$^{-1}$) were lower than that reported in tree litter (21 ng g$^{-1}$ – 78 ng g$^{-1}$) in North-America and Europe (Laacouri et al., 2013; Obrist et al., 2021; Poissant et al., 2008; Rea et al., 2002; Wang et al., 2016) but similar to that previously reported for grasses and

244 herbaceous plants (~10 ng g$^{-1}$) (Moore et al., 1995; Olson et al., 2019). The foliar Hg concentrations for plant species in this

245 study increased 1.3-2.0 times over the growing season, which was smaller than that (3-11 fold) reported for trees (Laacouri et al.,

246 2013; Poissant et al., 2008; Rea et al., 2002). The above results further confirm that foliar Hg concentrations differ among

247 vegetation types (Demers et al., 2007; Moore et al., 1995; Obrist et al., 2012; Richardson and Friedland, 2015). It has been

248 suggested that Hg previously retained in leaves can be photo reduced to Hg$^0$ that is re-emitted to the atmosphere, and consistent

249 Hg$^0$ re-emission from the foliage is positively related to photosynthetically active radiation (PAR) (Yuan et al., 2019). The plants

250 in open boreal peatlands lacking a tree overstorey like that in this study would receive very high exposure to ultraviolet (UV),

251 which may result in a greater photoreduction of Hg previously retained in leaves and then Hg loss than tree leaves that are more

252 often shaded. Moreover, despite angiosperms having higher stomatal conductance due to fewer stomata but more numbers (de

253 Boer et al., 2016; Jordan et al., 2015), stomatal opening in dark-adapted leaves after light exposure was generally faster in

254 gymnosperms than in angiosperms but stomatal closing upon the darkness of light-adapted leaves was faster in angiosperms than

255 in gymnosperms (Xiong et al., 2018). This phenomenon may lead to a higher Hg concentration in trees (a type of gymnosperms)

256 than in sedges and sweet gales (two types of angiosperms). More studies are needed to elucidate this mechanism of foliar Hg

257 accumulation by different plant types.

258 **Leaf carbon, nitrogen and mercury.** Leaf %C, %N, and C:N were significantly different among plant species ($F_{(6,104)} = 59.64$, p

259 $< 0.001$) over the growing season ($F_{(9,124)} = 45.42$, $p < 0.001$) (Fig. 2). Based on *post hoc* tests, foliar %C, %N, and C:N was

260 significantly different between sweet gale and sedges (few-seeded sedge/wire sedge and tussock sedge) but not between few-

261 seeded sedge/wire sedge and tussock sedge. Foliar %C and %N were much lower in these sedges than sweet gale, which agrees

262 well with a previous study that deciduous shrubs (i.e., sweet gale) generally have a higher foliar %C and %N than grasses

263 (Wright et al., 2004). The fixation of nitrogen in sweet gale is in part attributed to sweet gale root nodules containing symbiotic

264 nitrogen-fixing (Newmaster et al., 1997; Vitousek et al., 2002) with this greater amount of available N leading to higher

265 photosynthetic capacity (Wright et al., 2004), thus, species containing a higher foliar %N are usually accompanied with a

266 higher %C.

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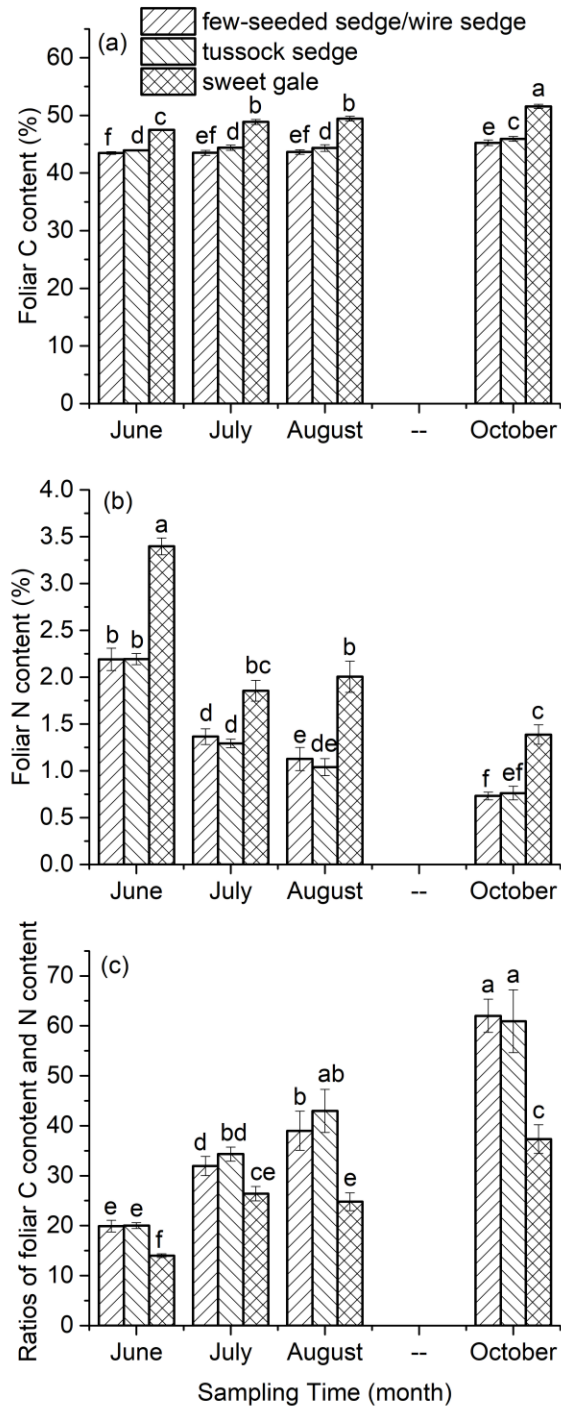
**Figure 2** The carbon content (%C) (a), nitrogen content (%N) (b), and the ratio of carbon content to nitrogen content (C:N) (c) over the 2018 growing season. Vertical bars are mean ± SD (n = 5). The same letters above bars denote that values of foliar THg concentrations are not significantly different at the 0.05 levels.

There were significant increases in foliar %C (few-seeded sedge/wire sedge: $F_{(3,9)} = 25.98$, $p < 0.001$; tussock sedge: $F_{(3,9)} = 20.56$, $p < 0.001$; sweet gale: $F_{(3,9)} = 115.90$, $p < 0.001$) but sharp decreases in foliar %N (few-seeded sedge/wire sedge: $F_{(3,9)}$

274  =354.20, p < 0.001; tussock sedge: $F_{(3,9)}$ = 252.36, p < 0.001; sweet gale: $F_{(3,9)}$ = 170.43, p < 0.001) over the growing season (Fig.

275  2). The strong decreases in foliar %N with leaf age can be attributed to the translocation of N from senescing leaves to new

276  leaves (Wang et al., 2003). A study found that approximately 77 % N, 57 % phosphorus (P), and 44 % potassium (K) were

277  translocated out of senescing leaves during mangrove leaf senescence (Wang et al., 2003). Foliar C is sequestrating continuously

278  over the growing season (Kueh et al., 2013). The element re-translocation and C sequestration in leaves may lead to the

279  foliar %C increase with time. The values of foliar C:N increased with time, which is a function of the decreases of foliar %N and

280  the increases of foliar %C.

281  Senesced leaf tissue with higher foliar %C and %N had higher foliar THg concentrations (%C and Hg: $F_{(1,13)}$ = 191.09, p < 0.05,

282  y = 0.78x − 28.20, $R^2$ = 0.94;%N and Hg: $F_{(1,13)}$ = 82.38, p < 0.05, y = 7.16x – 1.96, $R^2$ = 0.93) (Fig. 3a and 3b). THg

283  concentrations were negatively related to foliar C:N during senescence ($F_{(1,13)}$ = 175.10, p < 0.05, y = 0.18x – 18.33, $R^2$ = 0.86;

284  Fig. 3c). A previous study found soil Hg concentrations were positively related to soil organic C and N, and then given a possible

285  explanation that high C and N levels in soil reflect high vegetation productivity corresponding with high atmospheric Hg

286  deposition via litterfall (Obrist et al., 2009). Although the mechanism of these relationships between Hg concentrations and

287  contents of C and N in senesced leaves materials is still unclear, this study shows that Hg input via litterfall to soils can be

288  affected by C and N content in senesced leaves. More studies and data are needed to draw predictive conclusions.

289
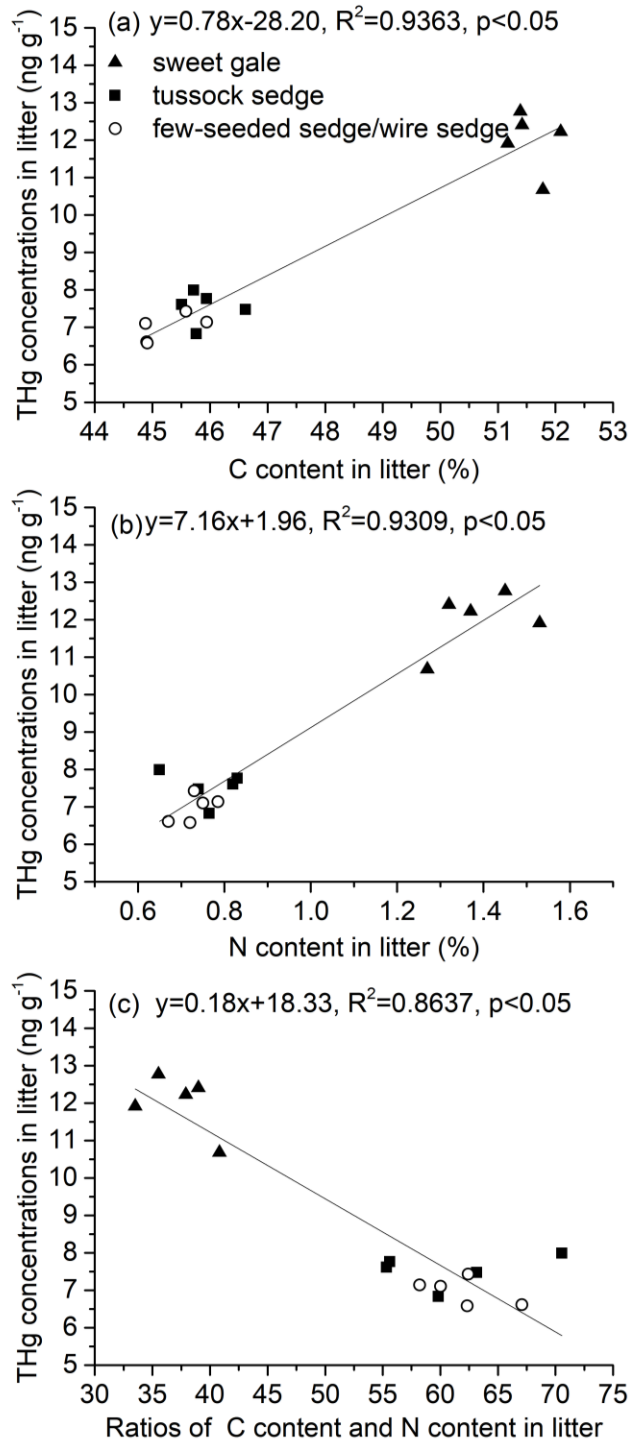
**Figure 3 Correlations between (a) THg concentrations and C contents, (b) THg concentrations and N contents, and (c) THg concentrations and ratios of C content and N content (C:N) in litter. All linear correlations are statistically significant (p < 0.05).**

**4.2 Mercury leaching from senesced leaves**

**Surface-rinsable mercury.** The mean mass of Hg from the surface rinse of senesced leaf material (expressed per gram of dry senesced leaf) was $0.02 \pm 0.01$ ng g$^{-1}$ and $0.01 \pm 0.00$ ng g$^{-1}$ (or $3.27 \pm 1.68$ ng L$^{-1}$ and $1.39 \pm 0.83$ ng L$^{-1}$, expressed per liter of

295  rinse water (18.2 MΩ cm)), respectively, indicating that mass of Hg that was loosely bound on the leaf surface was small relative

296  to the total senesced leaf tissue Hg concentration (8.83 ± 2.38 ng g$^{-1}$) representing on average only 0.4 % Hg (tussock sedge:

297  0.6 %; few-seeded sedge/wire sedge: 0.3 %; sweet gale: 0.3 %) of total THg mass.

298  **Leachable mercury.** The mean THg$_{aq}$ mass per gram of senesced leaf had significant differences between plant species ($F_{(2,41)}=$

299  11.55, p < 0.001; Fig. 4). Based on *post hoc* tests, there were significant differences in THg$_{aq}$ mass per gram of senesced leaf

300  between sweet gale and sedges (few-seeded sedge/wire sedge and tussock sedge) but not between few-seeded sedge/wire sedge

301  and tussock sedge. The senesced leaf of sweet gale leached the least Hg among these plant species, which is likely due to their

302  hydrophobic waxy cuticle that may both retain Hg, as well as protect the inner leaf material from leaching. Another plausible

303  explanation is that N was more easily released from sedges than C and it was the opposite for sweet gale, based on changes in

304  foliar %C and %N between before and after leaching (Table 1), whereas N groups in litter hinder the leaching of foliar Hg

305  (Obrist et al., 2009). Foliar %N of sweet gale increased after leaching, which is likely attributed to a large amount of loss of other

306  elements, such as K, Mg, and P, although they were not part of this experiment. Bessaad and Korboulewsky (2020) found that

307  60–79 % of K, 19–50 % of Mg, 22–30 % of P, and < 16 % of Ca and N were leached out from fully developed broadleaves

308  (collected in summer) during rainfall.
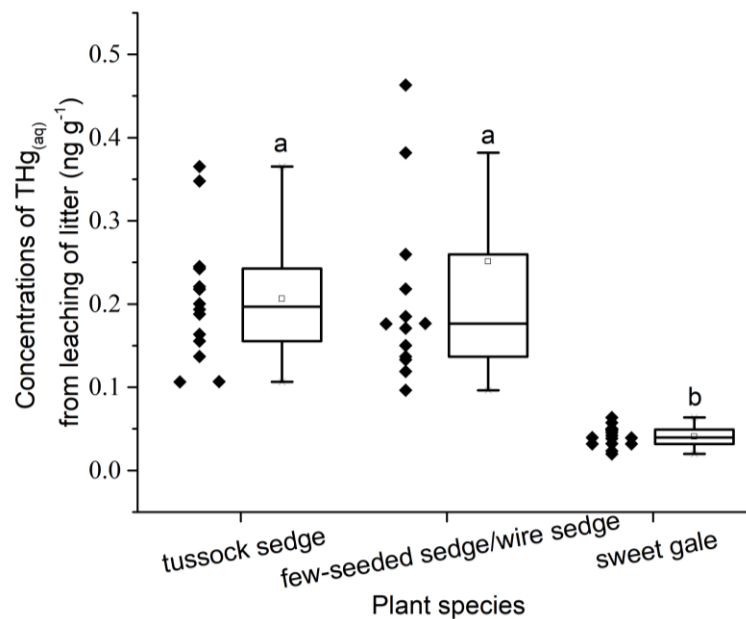


309

310  **Figure 4 Mass of mercury leached per gram of senesced leaf material (ng g$^{-1}$). Boxplot displays median (50th percentile; the inside line**

311  **of the box), first quartile (25th percentile; lower bound of the box), third quartile (75th percentile; upper bound of the box), whiskers**

312  **(all measures between 5th percentile and 25th percentile and between 75th percentile and 95th percentile; the straight line below and**

313  **above the box), and outliers (individual points outside of the percentile of 5th and 95th). n = 15.**

**Table 1 Changes of foliar carbon content (%C) and nitrogen content (%N) during leaching of litterfall. n = 15**

| | foliar %C | | | foliar %N | | |
|---|---|---|---|---|---|---|
| | before leaching | after leaching | change percentage (%) | before leaching | after leaching | change percentage (%) |
| sweet gale | 51.57 ± 0.36 | 51.03 ± 0.34 | −1.05 | 1.39 ± 0.10 | 1.50 ± 0.07 | −7.91 |
| tussock sedge | 45.91 ± 0.42 | 44.97 ± 0.54 | −2.05 | 0.76 ± 0.07 | 0.68 ± 0.10 | −10.53 |
| few-seeded sedge/wire sedge | 45.24 ± 0.49 | 43.83 ± 0.49 | −3.12 | 0.73 ± 0.04 | 0.64 ± 0.02 | −12.33 |

During experimental leaching, 3.0 %, 2.9 %, and 0.3 % of the total THg mass present in tussock sedge, few-seeded sedge/wire sedge, and sweet gale senesced leaf was leached, respectively. The percentages of Hg that leached from tussock sedge, few-seeded sedge/wire sedge leaves were 5.5 and 10.6 times higher than that from rinses, while the percentage of Hg that leached from sweet gale senesced leaf was similar to that from rinse water (0.3 %). Rea et al. (2000) reported that surface washoff of loosely bound and particulate Hg was a rapid and larger source of Hg in forest throughfall compared to continuously foliar Hg leaching from live leaves. It is likely because dry leaves lack structural integrity compared to live leaves in Rea et al.'s (2000) study, leading to more rapid leaching of soluble constituents (Gessner et al., 1999), including Hg, so the results of these prior studies are not directly comparable to this one. Further, although Hg leached from senesced leaf material was a small (< 5 % of foliar tissue Hg) but a measurable contributor to the mass balance, it is one that would be completely missed if material had been collected from a litter trap that had been exposed to rainfall for any period.

**4.3 The roles of dissolved organic matter properties in Hg mobility during litter leaching phase**

**The quantity and characteristics of DOM in leachate.** The mean mass of DOC leached per gram of senesced leaf material and the mass loss during senesced leaf material leaching was significantly different between plant species (leached DOC mass: $F_{(2,42)}$ = 34.95, p < 0.001; mass loss: $F_{(2,42)}$ = 11.62, p < 0.05) with a same sequence following: few-seeded sedge/wire sedge < tussock sedge < sweet gale (Fig. 5). The same sequence is in part because the loss of soluble carbons accounted for the majority of the mass loss during litter leaching (Del Giudice and Lindo, 2017). Mass loss of sweet gale (17.7%) was significantly larger than sedges (few-seeded sedge/wire sedge (8.1%) and tussock sedge (11.5%)). The released DOC accounted for 22.96 ± 14.85%, 23.73 ± 12.95%, and 17.03 ± 6.68% of mass loss during senesced leaf material leaching for few-seeded sedge/wire sedge,

tussock sedge, and sweet gale, respectively. Loss of other nutrients, such as dissolved organic nitrogen (DON) and dissolved

organic phosphorus (DOP) (Ong et al., 2017; Liu et al., 2018; Hensgens et al., 2020) and the inorganic components and other

elemental organic matter (Lavery et al., 2013; Jiménez et al., 2017) also contribute to the mass loss, despite these nutrients not

being measured.
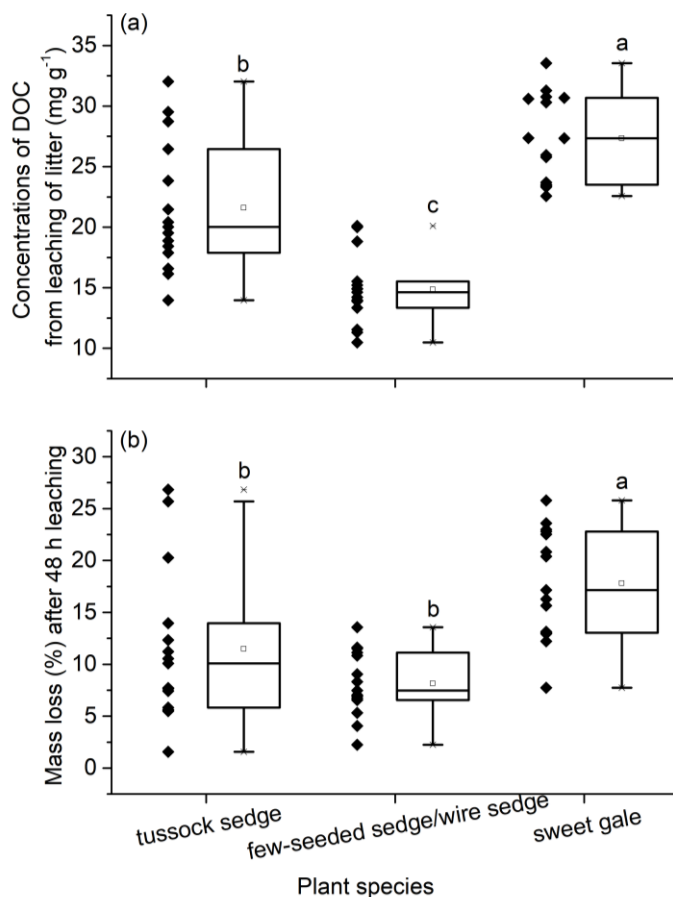


**Figure 5 Mass of dissolved organic carbon (DOC) leached per gram of senesced leaf material (mg g$^{-1}$) (a), and mass loss after 48 h leaching (b). Boxplot displays median (50th percentile; the inside line of the box), first quartile (25th percentile; lower bound of the box), third quartile (75th percentile; upper bound of the box), whiskers (all measures between 5th percentile and 25th percentile and between 75th percentile and 95th percentile; the straight line below and above the box), and outliers (individual points outside of the percentile of 5th and 95th). n = 15.**

Characteristics of DOM also varied among plant species (SUVA$_{254}$: $F_{(2,42)} = 24.02$, $p < 0.001$; FI: $F_{(2,42)} = 11.24$, $p < 0.001$;

HIX$_{EM}$:$F_{(2,42)} = 3.82$, $p < 0.05$; BIX: $F_{(2,42)} = 125.48$, $p < 0.001$) (Fig. 6 and Table 2). Based on *post hoc* tests, there were

significant differences in SUVA$_{254}$ and FI between sweet gale and sedges (few-seeded sedge/wire sedge) only and in BIX among

all plant species. FI (1.2-1.8) and BIX (<1.0) reflected that DOM in leachate was generally of plant origin, suggesting that the

microbially-derived OM was a smaller component. The mean value of SUVA$_{254}$ in leachate followed the sequence: tussock

349   sedge ≈ few-seeded sedge/wire sedge > sweet gale leaves, respectively, indicating that leached DOM from tussock sedge and

350   few-seeded sedge/wire sedge leaves had higher aromaticity and higher molecular weights than that from the sweet gale leaves.

351   $SUVA_{254}$ was negatively related to DOM concentrations ($F_{(1,43)} = 48.37$, $p < 0.001$, $y = -0.69x + 3.93$, $R^2 = 0.53$) when all plant

352   species were considered, suggesting that sweet gale prefers to release more amount of lower aromatic DOM.


353   Previous studies have found that characteristics of DOM controlled Hg mobility and methylation (Cui et al., 2022; Jiang et al.,

354   2018; Ravichandran 2004; Xin et al., 2022; Wang et al., 2022). Hg is tightly and readily bound to reduced sulfur groups (i.e.,

355   thiols) in DOM (Ravichandran, 2004; Xia et al., 1999). Mercury weakly binds to carboxyl and phenol functional groups in DOM

356   after all thiol groups are occupied at relatively high Hg concentrations (Drexel et al., 2002; Graham et al., 2012), which is

357   atypical in most natural environments in which Hg concentrations are relatively low. Higher terrestrial (plant-derived) DOM had

358   a greater DOM-Hg affinity (Wang et al., 2022). Additionally, DOM with higher aromaticity and molecular weight strongly

359   bonded with Hg(II), potentially because these DOM provide more sulfidic groups such as thiols (Dittman et al., 2009; Wang et

360   al., 2022). Therefore, terrestrial DOM and/or DOM with higher aromaticity and molecular weight may transport more Hg into

361   peat soils during the litter leaching phase.
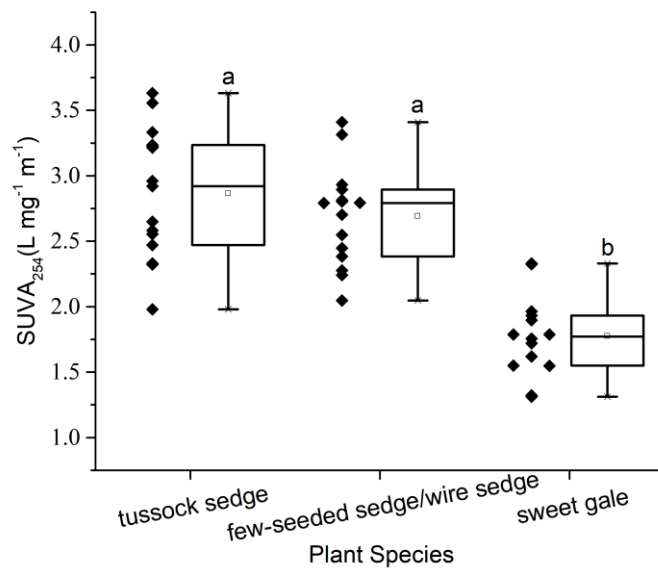


362

363   **Figure 6 Dissolved organic matter characteristics as measured by specific ultraviolet absorbance at the wavelength 254 nm (SUVA$_{254}$),**

364   **n = 15. Boxplot displays median (50th percentile; the inside line of the box), first quartile (25th percentile; lower bound of the box),**

365   **third quartile (75th percentile; upper bound of the box), whiskers (all measures between 5th percentile and 25th percentile and**

366   **between 75th percentile and 95th percentile; the straight line below and above the box), and outliers (individual points outside of the**

367   **percentile of 5th and 95th).**

368 **Table 2 The mean fluorescence indices of dissolved organic matter characteristics[a]**

| Index | Tussock sedge | Few-seeded sedge/wire sedge | Sweet gale |
|---|---|---|---|
| FI | $1.19 \pm 0.10$ | $1.31 \pm 0.09$ | $1.49 \pm 0.27$ |
| $HIX_{EM}$ | $0.16 \pm 0.03$ | $0.16 \pm 0.02$ | $0.19 \pm 0.03$ |
| BIX | $0.53 \pm 0.05$ | $0.63 \pm 0.06$ | $0.35 \pm 0.04$ |

369 [a]Lower values of the FI ($< 1.2$) suggest dissolved organic matter (DOM) has higher aromaticity and is primarily composed of
370 high-molecular-weight DOM, while high FI values ($> 1.8$) indicate that DOM has lower aromaticity and is mainly composed of
371 low-molecular-weight DOM. DOM with high $HIX_{EM}$ ($> 1$) values is composed of more highly condensed and higher molecular
372 weight molecules. In contrast, higher BIX ($> 1.0$) values reflect that more low-molecular-weight DOM is recently produced,
373 generally, by microbes. All indices are unitless, $n = 15$.

374 **Correlation between $THg_{aq}$ concentrations and $SUVA_{254}$ values in leachate.** The concentrations of soluble $THg_{aq}$ were
375 significantly related to $SUVA_{254}$ values ($F_{(1,41)} = 52.06$, $p < 0.001$, $y = 0.09x - 0.09$, $R^2 = 0.55$; Fig. 7). This result suggested that
376 DOM with higher aromaticity plays an important role in controlling Hg mobility (Ravichandran, 2004). The value of $R^2$ was
377 only 0.55, which can be attributed that the number of reduced sulfur groups in DOM far exceeds the amount of Hg in natural
378 environments and other factors, such as pH and sulfide may affect the binding between DOM and Hg (Ravichandran, 2004). In
379 this study, DOM with higher aromaticity may transport more Hg from litter to soils, and senesced leaves of sedges had a higher
380 potential in leaching Hg into peatland soils than the senesced leaves of sweet gales in this study.
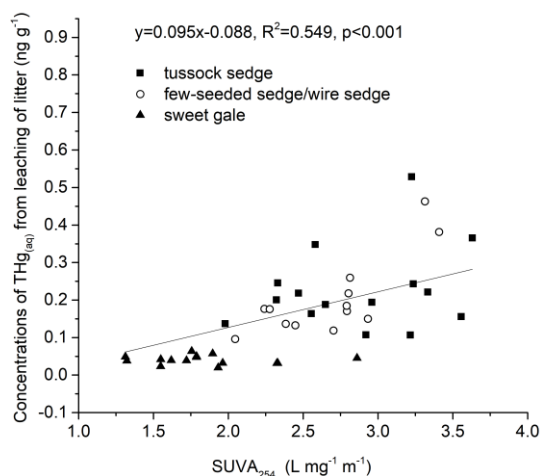


381

382 **Figure 7 Correlations between the mass of mercury leached per gram of senesced leaf material ($THg_{aq}$) and the specific ultraviolet**
383 **absorbance at the wavelength 254 nm ($SUVA_{254}$) in leachate.**

384 **4.5 Estimation of annual input of Hg via senesced leaves and rapid Leaching to peat soils**

385 The annual input of leaf biomass (mg/ha/yr) of few-seeded sedge/wire sedge into peat soils was 5.55 and 1.41 fold higher than

386 tussock sedge and sweet gale, while the annual inputs of Hg (mg/ha/yr) via sweet gale leaves were 6.29 and 1.22 fold higher than

387 via tussock sedge and few-seeded/wire sedge leaves in the sedge-dominated fen (Table 3). Annual total Hg input through

388 senesced leaves to peat soils were 1.56, 8.03, and 9.82 mg/ha/yr for tussock sedge, few-seeded sedge/wire sedge, and sweet gale,

389 respectively. The input of surficial Hg and leachable Hg accounted for 0.64 % and 0.37 %, 0.31 % and 3.20 %, and 2.86 % and

390 0.30 % of total foliar Hg input to peat soils annually for tussock sedge, few-seeded sedge/wire sedge, and sweet gale,

391 respectively. The majority of Hg in senesced leaves (> 96 %) was from the deposition of solid plant tissues in litter.

392

393 **Table 3 Annual input of senesced leaves, and senesced leaf Hg, surficial Hg, and leached Hg during leaching into peat**

394 **soils per hectare and per year in the sedge-dominated fen (mg/ha/yr).**

| Species | Senesced leaf biomass (mg/ha/yr) | Litter total Hg input (mg/ha/yr) | Washoff Hg input (mg/ha/yr) | Leachate Hg input (mg/ha/yr) |
|---|---|---|---|---|
| Tussock sedge | $2.07 \times 10^8$ | 1.56 | 0.01 | 0.05 |
| Few-seeded sedge/wire sedge | $1.15 \times 10^9$ | 8.03 | 0.03 | 0.23 |
| Sweet gale | $8.18 \times 10^8$ | 9.82 | 0.03 | 0.03 |
| Total | $2.17 \times 10^9$ | 19.41 | 0.07 | 0.31 |

395

396 Based on the data from the study growing season, the annual input of Hg in total via senesced leaves (19.40 mg/ha/yr) was 5-

397 22 % of litterfall in forest ecosystems (e.g., jack pine/black spruce/balsam fir forest, red maple/birch forest, Norway spruce

398 forest; 86-372 mg/ha/yr) (St Louis et al., 2001; Graydon et al.,2008; Shanley and Bishop, 2012), which can be attributed to those

399 forest ecosystems having both higher mean foliar Hg concentrations (21-51 ng g$^{-1}$) (Zhou and Obrist, 2021) and much greater

400 aboveground biomass and litterfall inputs (2000-3488 kg/ha/yr) (Graydon et al., 2008) than plants in this study. The overall

401 annual Hg inputs via these senesced leaves to peat soils in this sedge-dominated fen were 59 % of that via wet deposition using

402 the mean precipitation Hg input estimates from the Experimental Lakes Area (33 mg/ha/yr) for the years 2001-2010, which is in

403 the same general geographic region of Ontario (St Louis et al., 2019).

404 **5 Conclusions**

405 This study shows that the widely-observed pattern of foliar Hg accumulation from the atmosphere and changes in foliar Hg

406 concentrations over time are the same in peatland vascular plants as they are for forest trees and that the patterns are related to

407 time/leaf age and plant species. The THg concentrations in senesced leaves in this study are relatively lower than that in the

408 forest litterfall. Hg released from ubiquitous sedge litter during leaching is relatively more quickly than the much slower release

409 of tissue-associated Hg through the decomposition of plant tissues. Thus, the supply of inorganic Hg to sites of methylation in

410 peatlands has both fast and slow pathways that may shift under climate change, given that peatland plant species composition

411 and biomass will certainly change under climate change.

412

413 ■DATA AVAILABILITY

414 All data generated or analysed during this study are included in this published article and its supplementary information files.

415 ■SUPPLEMENT

416 The supplement related to this article is available online.

417 ■AUTHOR CONTRIBUTION

418 Ting Sun carried the project out, collected all samples, performed the leaching experiment, analyzed samples and data, and wrote

419 the manuscript. Brian A. Branfireun designed the experiments, provided supervision, and edited the manuscript.

420 ■COMPETING INTERESTS

421 The authors declare that they have no conflict of interest.

422 ■DISCLAIMER

429 ■ABBREVIATIONS

430 Hg, mercury; MeHg, methylmercury; GEM, gaseous elemental mercury; RGM, reactive gaseous mercury; PBM, particulate-

431 bound mercury; THg, total mercury; $THg_{aq}$, dissolved total mercury; SRB, sulfate-reducing bacteria; %C, carbon content; %N,

432 nitrogen content; C:N, the ratio of leaf C content and N content; dissolved organic matter (DOM); DOC, dissolved organic

433 carbon; $SUVA_{254}$, specific ultraviolet absorbance at a wavelength of 254 nm; EEMs, fluorescence excitation-emission matrices;

434 FI, fluorescence index; $HIX_{EM}$, humification index; BIX, biological index; soil organic matter (SOM); CRM, certified reference

435 material; RSD, relative standard deviation.

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