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Supplement of

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

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23   *Study site and characteristics of plant species.* Samples were collected from a sedge-dominated fen

24   (10.2 ha) located in an 817 ha sub-watershed of the Lake Superior basin near White River, Ontario,

25   Canada (48°21' N, 85°21' W). The weather data (air temperature and precipitation) was provided by

26   the Ontario Ministry of Natural Resources and Forestry, who installed a weather station and monitored

27   the environmental conditions in this study site. The growing season is from June to August. The mean

28   annual air temperature and precipitation from 2012 to 2018 were 1.7 °C and 721 mm, respectively. The

29   mean air temperature in the growing season in 2018 and 2019 was 15.82 ± 3.50 °C and 15.16 ±

30   3.38 °C, respectively. The total precipitation in the growing season in 2018 and 2019 was 243.9 mm

31   and 189.3 mm, respectively. The mean water table levels in the growing season in 2018 and 2019 were

32   –6.3 ± 1.0 cm, and –7.5 ± 3.9 cm, respectively. There were no significant differences in air temperature

33   and precipitation between the growing season in 2018 and 2019 (temperature: $F_{(1,182)} = 1.74$, p > 0.05;

34   precipitation: $F_{(1,182)} = 0.48$, p > 0.05). The sedge-dominated fen is surrounded by a mixed-wood

35   deciduous and coniferous forest with two small streams running along the northern and southwestern

36   edges.

37   The sedge-dominated fen is mostly open, and the vegetation community is dominated by three sedge

38   species: few-seeded sedge (*Carex oligosperma* Michx.), wire sedge (*Carex lasiocarpa* Ehrh), tussock

39   sedge (*Carex stricta* Lamb.) (Lyons and Lindo, 2019). Sweet gale (*Myrica gale* L.) is the dominant

40   shrub at this site (Lyons and Lindo, 2019; Palozzi and Lindo, 2017). The characteristics of these plants

41   are described in Newmaster et al. (1997). In brief, the few-seeded sedge is 40−100 cm tall; their leaves

42   are 1−3 mm wide, stiff, smooth, edges rolled in toward midrib and rounded in cross-section, and red-

43   tinged at the base. The wire sedge is 30−100 cm tall; leaves are arching, narrow, 1−2 mm wide, wire-

44   like, folded along the midrib, and angular. Tussock sedge is 40−140 cm tall; leaves are 3−6 mm wide,

45   lowest leaves reduced to bladeless sheaths. Sweet gale is a deciduous shrub, up to 1.5 m tall; leaves are

46   alternate, 3−6 cm long, toothed at the tip; sweet gale is a nitrogen-fixer and its root nodules contain

47   symbiotic nitrogen-fixing bacteria. In this study, few-seeded sedges and wire sedges were mixed during

48   plant sample collection as they are indistinguishable in size and form from one another when not in

49   flower/seed.

50 *Foliar total mercury, C content, and N content.* In the laboratory, leaf samples for chemical analyses

51 were rinsed three times with deionized water (18.2 MΩ cm) and then freeze-dried for 48 h. Freeze-

52 dried leaf samples were subsequently ground and homogenized with a stainless-steel blade grinder. All

53 powdered samples were stored in polyethylene bags for further chemical analysis. Precautions were

54 performed to avoid any cross-contamination during the process. Disposable nitrile gloves were worn

55 during sample handling. The blade grinder was thoroughly rinsed with deionized water (18.2 MΩ cm)

56 after each sample grinding and completely dried with Kimwipes® (Kimtech Science™). THg in leaves

57 was analyzed by thermal decomposition, amalgamation, and atomic absorption spectrometry using a

58 Milestone™ DMA-80 (EPA method 7473) with the National Research Council Canada, DORM-4 as the

59 Certified Reference Material (CRM) to validate instrument recovery and stability. Each analytical run

60 for THg included 10 % method blanks (empty sample boat), 10 % duplicates, and 20 % matrix spikes.

61 The detection limit for THg was 0.05 ng g^{-1}. All method blanks were below the detection limits. The

62 relative standard deviation (RSD) was 4.42 ± 3.60 % for all duplicate samples. Recoveries of THg for

63 matrix spikes and CRM (DORM-4) were 101.08 ± 3.08 %. All recoveries of matrix spikes and CRM

64 were comparable well with the certified values: 25 ng and 0.41 ± 0.04 mg kg^{-1}, respectively.

65 Leaf C content (%C; w/w) and N content (%N; w/w) before and after the foliar Hg leaching experiment

66 were analyzed using a CNSH analyzer (vario ISOTOPE cube; Elementar). The ratio of leaf C content

67 and N content (C:N) was calculated. Birch leaf Organic Analytical Standard (*Betula papyrifera* Marsh.)

68 was the CRM. Each analytical run for C and N included 10 % method blanks and 10 % duplicates (no

69 matrix spikes for C and N). The detection limits for C and N were 0.26 mg g^{-1} and 0.02 mg g^{-1},

70 respectively. All method blanks were below the detection limits. The RSDs of C and N were 0.28 ±

71 0.14 % and 4.73 ± 3.05 % for duplicate samples, respectively. Recoveries of CRM for C and N were

72 99.16 ± 0.30 %, and 101.62 ± 0.88 % of the certified values, respectively.

73 *Dissolved total Hg concentrations analyses and QA/QC.* The dissolved total Hg (THg$_{aq}$) concentrations

74 in the rinse water and leachate were analyzed using Environmental Protection Agency (EPA) method

75 1631. Samples were oxidized for 12 h with BrCl oxidation, neutralized using hydroxylamine, reduced

76 to Hg^0 by SnCl$_2$ reduction, purged onto gold traps, thermally desorbed in argon, and finally analyzed

77   by cold vapor atomic fluorescence spectroscopy (CVAFS, Tekran 2600, Tekran Inc., Canada) (Bloom

78   and Fitzgerald, 1988). The detection limit for THg_{aq} was 0.072 ng L^{-1}. The instrument, Tekran 2600,

79   was calibrated daily. Each analytical run included 10 % method blanks (deionized water), 10 % sample

80   duplicates, and 20 % matrix spikes. Check standards (made from 1000 ppm stock standard) were

81   included in every ten samples. All method blanks were below the detection limit. The RSD was 3.45 ±

82   2.48 % for all duplicate samples. Recoveries of matrix spikes and check standards were 101.09 ±

83   14.03 % and 104.60 ± 6.37 %, respectively.

84   *Dissolved organic matter concentration analyses and QA/QC.* Dissolved organic matter is quantified

85   analytically as dissolved organic carbon (DOC). DOC concentrations in rinse water and leachate were

86   measured using an iTOC Aurora 1030 (OI Analytical, College Station, TX, USA) using the persulfate

87   wet oxidation method. Dissolved organic carbon in the liquid was oxidized to CO_2 gas by the persulfate

88   wet oxidation and the amount of CO_2 was subsequently determined by measuring the infrared

89   absorbance of CO_2 gas. DOC concentrations in blanks were less than 1 mg L^{-1}. Each run included 10 %

90   deionized water blanks, 10 % sample duplicates, 10 % matrix spikes, and check standards. Deionized

91   water blanks were generally less than 1 mg L^{-1}. The RSD was 1.58 ± 1.83 % for sample duplicates.

92   Recoveries of matrix spikes and check standards were 101.04 ± 1.33 % and 104.60 ± 5.36 %,

93   respectively. Concentrations of THg_{aq} and DOM in both rinse waters and leachate are presented as the

94   mass of solute per mass of dry material.

95   *Characteristics of DOM and QA/QC data.* DOM in leachate was characterized as specific ultraviolet

96   absorbance at a wavelength of 254 nm ($SUVA_{254}$), an indicator of the molecular weight (or size) and

97   aromaticity (the content of aromatic molecules) of DOM (Weishaar et al., 2003). Higher $SUVA_{254}$

98   values suggest that DOM contains more high-molecular-weight and aromatic molecules (Weishaar et

99   al., 2003). Sample absorbance was measured at $\lambda = 254$ nm using a Horiba Aqualog® fluorescence

100   spectrofluorometer with a xenon lamp. $SUVA_{254}$ values were determined by dividing the absorbance at

101   254 nm by the DOM concentration of the same sample and multiplied by 100 and are reported in the

102   unit of L mg C^{-1} m^{-1} (Weishaar et al., 2003). There were no reference materials to assess method

103 performance, but 10 % of samples were run in duplicates. The RSDs of SUVA$_{254}$ were 0.62 ± 0.29 %

104 for sample duplicates.

105 Fluorescence excitation-emission matrices (EEMs) were also collected for calculating informative

106 optical indices that reflect differences in DOM characteristics in leachate using a Horiba Aqualog®

107 fluorescence spectrofluorometer with a xenon lamp. The ultrapure closed water blank was used to

108 correct the inner-effects of the Horiba Aqualog® fluorescence spectrofluorometer. Aqualog® directly

109 reported the fluorescence intensity as arbitrary units (A.U.). The reported EEMs were then converted to

110 optical indices using R Software (R Core Team 2012). Three common indices were chosen in this

111 study: the fluorescence index (FI), the humification index (HIX$_{EM}$), and the biological index or

112 'freshness' index (BIX). FI reflects DOM sources and characteristics with lower FI values (< 1.2)

113 indicating that DOM is terrestrially derived (resulting from decomposition and leaching of plant and

114 soil organic matter) and has higher aromaticity, while higher FI values (> 1.8) indicting that DOM is

115 microbially derived (originating from processes as extracellular release and leachate of algae and

116 bacteria) and has lower aromaticity (Fellman et al., 2010; McKnight et al., 2001). HIX$_{EM}$ is an indicator

117 of humic substance content or the extent of humification that converts lower-molecular weight organic

118 matter derived from animal and plant products to more condensed and higher-molecular-weight

119 organic matters by microbes. High HIX$_{EM}$ (> 1.0) values reflect the high humification of DOM and

120 DOM is composed of more highly condensed and higher molecular weight molecules (Fellman et al.,

121 2010; Hansen et al., 2016; Huguet et al., 2009; Ohno, 2002). BIX reflects the contribution of

122 autochthonous (or microbially derived) DOM with higher BIX values (> 1.0) reflecting that more low-

123 molecular-weight DOM was recently produced by microbes (Fellman et al., 2010; Huguet et al., 2009).

124 For FI, HIX$_{EM}$, and BIX, there were also no reference materials to assess method performance, but

125 10 % of samples were run in duplicates. The RSDs of FI, HIX$_{EM}$, and BIX were 7.27 ± 3.43 %, $2.05 \pm$

126 2.77 %, and 3.48 ± 3.50 % for sample duplicates respectively.

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