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Complete List of Authors:	Kronberg, Rose-Marie; Swedish University of Agricultural Sciences, Department of Forest Ecology and Management Jiskra, Martin; CNRS, Geosciences Environment Toulouse, Observatoire Midi-Pyrenees Wiederhold, Jan; University of Vienna, Department of Environmental Geosciences Björn, Erik; Umeå University, Chemistry Skyllberg, Ulf; Swedish University of Agricultural Sciences, Forest Ecology

Methyl mercury formation in hillslope soils of Boreal forests – the role of forest harvest and anaerobic microbes

ROSE-MARIE KRONBERG[§], MARTIN JISKRA[#,|], JAN G. WIEDERHOLD[#,|], ERIK BJÖRN[‡], ULF SKYLLBERG[§]*

[§]Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden. ‡Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden [#]Soil Chemistry Group, Institute of Biogeochemistry and Pollutant Dynamics, ETH Zurich, CH-8092 Zurich, Switzerland. [|]Isotope Geochemistry Group, Institute of Geochemistry and Petrology, ETH Zurich, CH-8092 Zurich, Switzerland.

* Corresponding author phone: +46 (0)90-786 84 60; e-mail: ulf.skyllberg@slu.se

ABSTRACT

Final harvest (clear-cutting) of coniferous boreal forests has been shown to increase stream water concentrations and export of the neurotoxin methyl mercury (MeHg) to freshwater ecosystems. Here the spatial distribution of inorganic Hg and MeHg in soil as a consequence of clear-cutting are reported. A comparison of soils at similar positions along hillslopes in four 80-years-old Norway spruce (*Picea abies*) stands (REFs) with four similar subjected to clear-cutting (CCs) revealed significantly ($p<0.05$) enhanced MeHg concentrations (ng g^{-1}), MeHg areal masses (g ha^{-1}), and %MeHg of Hg_{TOT} in O horizons of CCs located between 1m and 41 m from streams. Inorganic Hg measures did not differ between REFs and CCs at any position. The O horizon thickness or bulk density did not differ, but at CCs the groundwater table and soil water content was significantly higher than at REFs. The largest difference in %MeHg of Hg_{TOT} (11 times, $p<0.003$) was observed in concert with significant increases in soil water content ($p<0.0002$) at intermediate hillslope positions (20-38 m from stream), outside the stream riparian zone. Incubation experiments demonstrated that soils having significantly enhanced soil pools of MeHg after clear-cutting also showed significantly enhanced methylation potential as compared with similarly positioned soils in mature reference stands. Addition of inhibitors demonstrated that sulfate reducing bacteria (SRB) and methanogens were key methylators. Rates of demethylation were not enhanced after clear-cutting. Our results suggest that enhanced water saturation of organic soils providing readily available electron donors stimulate Hg methylating microbes to net formation and build-up of MeHg in O horizons after forest harvest.

INTRODUCTION

The potent neurotoxin methyl mercury (MeHg) is of great concern in boreal landscapes where it forms and accumulates in aquatic food-webs. Concentrations of MeHg are high in pescivorous fish and in boreal landscapes the severity of this situation seems to have increased during the last decades.[1-3] Because MeHg imported by terrestrial runoff exhibits a significantly higher rate of bioaccumulation in costal sediments than *in situ* formed MeHg,[50] the formation and export of MeHg from terrestrial environments play a critical role in aquatic ecosystems.

In the 1990's it was demonstrated that MeHg formed in wetlands is an important source of MeHg in runoff to surface waters.[4] Different types of boreal wetlands have since then been identified as net producers of MeHg,[5,6] with rates of Hg methylation varying more than rates of demethylation.[7] So far Black Alder swamps is the only type of wetland shown to net degrade MeHg.[8] After microbial activity was demonstrated to be responsible for Hg methylation[9] several microbial communities have been identified to methylate Hg in wetland environments, including sulfate reducing bacteria (SRB), iron reducing bacteria (IRB), methanogens and firmicutes.[10-14]

While wetland soils are recognized as major sources, forested upland soils are generally considered as sinks for MeHg.[15] However, during the phase of forest harvest and time period of establishment of a new stand, studies have revealed forest soils to be significant sources of MeHg to surface waters and their biota.[16-22] Yet, some studies have found little or no effect from forest harvest on MeHg stream export.[23] Clear-cutting is the most common final harvest practice in boreal forests.[24] It results in a loss of evapotranspiration from trees which increases both the runoff and the level of the ground water table. Because of a large leaf area, this effect is greatest in coniferous forests[25] where the runoff has been reported to increase 48 to 107% after clear-

61 cutting,[21,22,25,26] mainly depending on the fraction of the watershed area harvested and its
62 topography (response in runoff). As a consequence of rising groundwater levels, discharge areas
63 with newly water saturated soils are extended up along lower sections of hillslopes and into local
64 depressions forming fringes and patches of new wetland-like habitats. Once inundated, the soil
65 organic matter quality of these normally well-drained forest soils may provide excellent substrate
66 as electron donors for anaerobic, Hg methylating bacteria.[27,28] Electron donors are also provided
67 by organic debris left after clear-cutting. Soil organic matter degradation is further excelled by
68 increased exposure to solar radiation,[29] which also has been shown to increase $Hg^0(g)$ photo-
69 emissions from soils after clear-cutting.[30]

70 In a recent parallel study[22] to the one reported here, we showed that: (1) the organic
71 horizon MeHg soil pool (g ha^{-1}) in average increased seven times two years after clear-cutting,
72 and (2) the stream MeHg export increased significantly after clear-cutting in undulating terrain.
73 An upscaling calculation demonstrated that in Sweden, where >95 % of the productive forest
74 area is managed, final harvest of boreal coniferous forest increases the MeHg export to aquatic
75 ecosystems by 12 - 20 % as compared to non-harvested forests.[22] This estimate, based on
76 watershed export data alone, narrows a previous one reported by Bishop et al.,[19] based on results
77 from a variety of studies.

78 To mitigate stimulatory effects of forest harvest on MeHg formation and stream export,
79 adjustment of forest management practices have been suggested, such as leaving zones of gallery
80 forest along streams and avoiding soil disturbance and compaction by heavy machinery.[19,34]
81 However, before such actions can be fully designed and implemented we need to better
82 understand processes and factors in control of MeHg formation in forest soil (before and after
83 harvest) and the spatial distribution of MeHg net producing “hot-spots” and their connections to

streams.[35] Here we report the effect of forest clear-cutting on the distribution of MeHg accumulated in organic soil horizons along hillslope transects, by comparing 80-years-old reference stands of Norway spruce (*Picea abies L. Karst*) with two-years-old clear-cuts of similar stands. The potential for MeHg formation and degradation in soil of clear-cuts and reference stands was further examined in incubation experiments where rate constants for potential Hg methylation and MeHg demethylation, k_m and k_d, were determined. Amendments of electron acceptors and metabolism-specific inhibitors were added to identify and quantify the role of different microbial communities for net MeHg formation in soil before and after forest harvest.

MATERIALS AND METHODS

Site descriptions. Four mature (>80-years-old) Norway spruce (*Picea abies* Karst.) reference stands (designated REFs) and four similar stands subjected to final harvest (clear-cutting two years prior to the study, designated CCs) were selected. Two REFs and two CCs were situated above the post-glacial marine limit (AML) of the ancient Baltic Sea, and two REFs and two CCs were situated below the post-glacial marine limit (BML). All sites were located in north-central Sweden and were part of a larger study[22] including a total of 20 watersheds (Figure S1, Text S1). Sites represented an area of 26 800 km^2 covered by northern boreal coniferous forests and corresponding to about 15% of the forested land in Sweden. Sites situated AML were located between 297 and 488 m.a.s.l. and sites BML between 16 and 113 m.a.s.l. To facilitate comparison with parallel studies, the site designation follows Kronberg et al.[22]

105 **Soil sampling and analyses of soil Hg and MeHg pools.** Sites REF1, REF2, CC2 and
106 CC3 situated the AML and sites REF1, REF4, CC2 and CC4 situated BML were selected for soil
107 sampling and determinations of Hg and MeHg soil pools. Site coordinates and topographic
108 characteristics are reported in Table S1, S2 (Supporting Information, SI). The organic (O)
109 horizon was collected in June 2011 (two years after clear-cutting) by cutting samples with a
110 specified volume from the side of dug pit with a steel knife. The O horizon was divided into the
111 top, non-humified Oe, and the underlying, humified Oa, horizon. The complete Oe horizon was
112 sampled and the top 15 cm of the Oa horizon. Depths of Oe and Oa and the underlying E horizon
113 and the level of the groundwater table (after 30 min to let the water stabilize) were measured in
114 the pit. In some pits large boulders prevented further digging and the groundwater level was set
115 10 cm below the maximum depth. Composite samples of Oe and Oa (comprised of five sub-
116 samples taken within a plot of 1 by 1 m^2) were taken at five positions (P1-P5) along one hillslope
117 transect (in total 22 – 93 m long) running from recharge to discharge areas and positioned
118 perpendicular to the first-order stream defining the watershed. Sample P1 was taken 1 m from
119 the stream in the riparian zone and P5 in the recharge area. Samples P2-P4 were taken in
120 between at arbitrary distances from stream to cover local depressions at intermediate positions
121 along hillslopes. Billberry (*Vaccinium myrtillus*) was the dominant plant in the field layer at
122 REFs with *Deschampsia flexuosa* and other grasses and herbs gradually taking over after clear-
123 cutting. Feather mosses (*Hylocomnium splendens, Pleurizium schreberi)* dominated the bottom
124 layer at both REFs and CCs. Soils were classified as Podzols[31] along hillslopes and Histosols (O
125 horizon > 40 cm), with a patchy distribution, in the riparian zone along streams (having a width
126 of about 2-8 m). Sampling positions at REFs and CCs were selected to be as equal as possible in
127 relation to topography and average hydrology prior to clear-cutting. This was indeed achieved as

judged by the thickness of the O horizon which proved to be very similar at REFs and CCs (Figure 1, Table S5). Samples were stored in a cooling bag while transported to the lab and then in a fridge at 4°C. Within 48 h samples were homogenized through a 4 mm cutting sieve, after removal of larger plant materials (roots) and woody debris. The soil was dried (45 °C to avoid losses of Hg) and the fresh soil bulk density was calculated as gram of dry soil mass per dm^{-3}. Analytical methods for the determination of Hg_{TOT}, MeHg, and geochemical parameters (pH, C, N, S) and water content are reported in Supporting Information (Text S2). Soil MeHg and Hg_{TOT} concentrations and areal masses were calculated for Oe and Oa horizons of REFs and CCs as arithmetic means of the 20 composite samples (P1-P5 at four REFs and four CCs, respectively). Data on MeHg and Hg_{TOT} areal masses (g ha^{-1}) for Oe and Oa sub-horizons were first summed for each sampling position (P1-P5) before calculating the arithmetic average for the complete O horizon of REFs and CCs. Data on concentrations of Hg_{TOT}, MeHg, %MeHg, and water mass-% (of fresh soil) for Oe and Oa sub-horizons were weighted (by the measured sub-horizon thickness) to calculate average values for the complete O horizons of CCs and REFs.

Soil sampling and determination of potential Hg methylation and MeHg demethylation rates. At four of the sites (REF1 AML, CC3 AML, REF4 BML, and CC4 BML) soils were re-sampled in August 2012 (three years after clear-cutting) for incubation experiments to determine Hg methylation and MeHg demethylation rate constants. Because no significant differences in % MeHg (of Hg_{TOT}) were observed between Oe and Oa sub-horizons at the first sampling occasion (in June 2011), the sampling in August 2012 was restricted to the top 10 cm of the O_a horizon. At CCs, samples were taken at three of the five positions decided at the sampling occasion in 2011: P1, P3 and P4 and at REFs samples were collected at two positions: P1 and P4. Samples were taken using a soil corer with a steel edge (10.5 cm in diameter),

immediately put in a ziplock plastic bag and kept in a cooling box on ice during transportation to the lab. The samples were stored at 4°C in refrigerator for one week.

Isotopically enriched $^{198}Hg(NO_3)_2$, ^{201}Hg-NOM (natural organic matter), and Me^{204}HgCl tracers were used in soil incubation experiments. The ^{201}Hg-NOM tracer is less available for methylation than the traditionally used $^{198}Hg(NO_3)_2$ tracer,[36] and can be assumed to be more relevant as a substrate in an organic forest soil where the complexation of Hg(II) to NOM thiol groups dominates the inorganic Hg speciation.[37,38,54] The ^{201}Hg-NOM tracer was prepared 5 days prior to the incubation.[36] Two days prior to the incubation, soil samples were homogenized by hand in a plastic bag (to avoid soil water losses) in the glovebox (95% N_2 and 5% H_2). This was done as gently as possible, basically removing roots and mixing the sample by loosening up the depth-related structures (layering) of the O horizon to provide a representative, mixed sample still maintaining most of its small-scale structure. Subsamples were taken out for determination of water content, total Hg (Hg_{TOT}) and MeHg concentrations (Text S2, SI). The latter two analyzes were done immediately to decide the quantity of Hg and MeHg tracers to be added (corresponding to 10 – 30 % of ambient). Ten grams of homogenized soil were weighed (by two decimals) into 50 mL Falcon tubes covered with aluminum foil. Amendments and isotopically enriched tracers were added to the soil in a minimum amount of deoxygenated water (enough to provide efficient mixing but still maintaining differences in water contents among soil samples). The soil was mixed thoroughly using a metal spatula. A subsample (t_{48}) was transferred to a second tube, weighed and incubated in darkness in the glovebox at 21±1°C. After 48 hours the t_{48} sample was frozen at -20°C. The first tube (t_0) was frozen on dry ice after each amendment was done, which took 10-15 minutes. All treatments were done in triplicate. Sulfate and freshly prepared amorphous iron hydroxide[39] (henceforth designated Fe(III)) were added as potential

bacterial electron acceptors for SRB and IRB, respectively. Molybdate (Na_2MoO_4) and bromoethanesulfonic acid (BES) were added as specific inhibitors of SRB and methanogens, respectively,[40] and azide (NaN_3) was used as a general microbial metabolic inhibitor.[41] Final concentrations in the samples were 50 μM of sulfate and molybdate, 10 mM of BES, 1 mol/L of Fe(III), and 100 mM azide. The CC4 P3 sample was excluded because of analytical problems.

Potential methylation and demethylation rate constants (d^{-1}) were calculated by equations (1) and (2), respectively, from masses of transformed ^{201}Hg (^{201}Hg-NOM tracer), ^{198}Hg ($^{198}Hg(NO_3)_2$ tracer) and $Me^{204}Hg$ ($Me^{204}HgCl$ tracer). Because of the difficulty to directly determine the pseudo-first order kinetics of Hg methylation (*dHg/dt*), and its dependency on the quantity of added Hg tracer, k_m in reaction (1) is commonly adopted as the potential methylation rate constant.[32] The demethylation rate constant is determined by pseudo first-order kinetics. Demethylation of $Me^{201,198}Hg$ formed during the course of the incubation experiment, as well as methylation of ^{204}Hg, were assumed negligible.

$$k_m = ([Me^{201,198}Hg]t_{48} - [Me^{201,198}Hg]t_0) / ([^{201,198}Hg\text{-tracer}]_{added} \times \Delta t) \quad (1)$$

$$k_d = -1 \times (\ln [Me^{204}Hg]t_{48} - \ln [Me^{204}Hg]t_0) / \Delta t \quad (2)$$

The $[MeHg]t_{48}$ and $[MeHg]t_0$ are the determined MeHg concentrations (ng Hg g^{-1}) for a given isotope at 48 hours (t_{48}) and at the start of the experiment (t_0). The $[^{201,198}Hg\text{-tracer}]_{added}$ is the initial concentration of isotope tracer and Δt is the incubation time (days).

Two-tailed Student's *t*-test for heteroscedastic distributed log-transformed data were used to compare soil data for REFs and CCs. Differences between controls and treatments of incubation studies were tested by ANOVA followed by Tukey multiple comparison test.

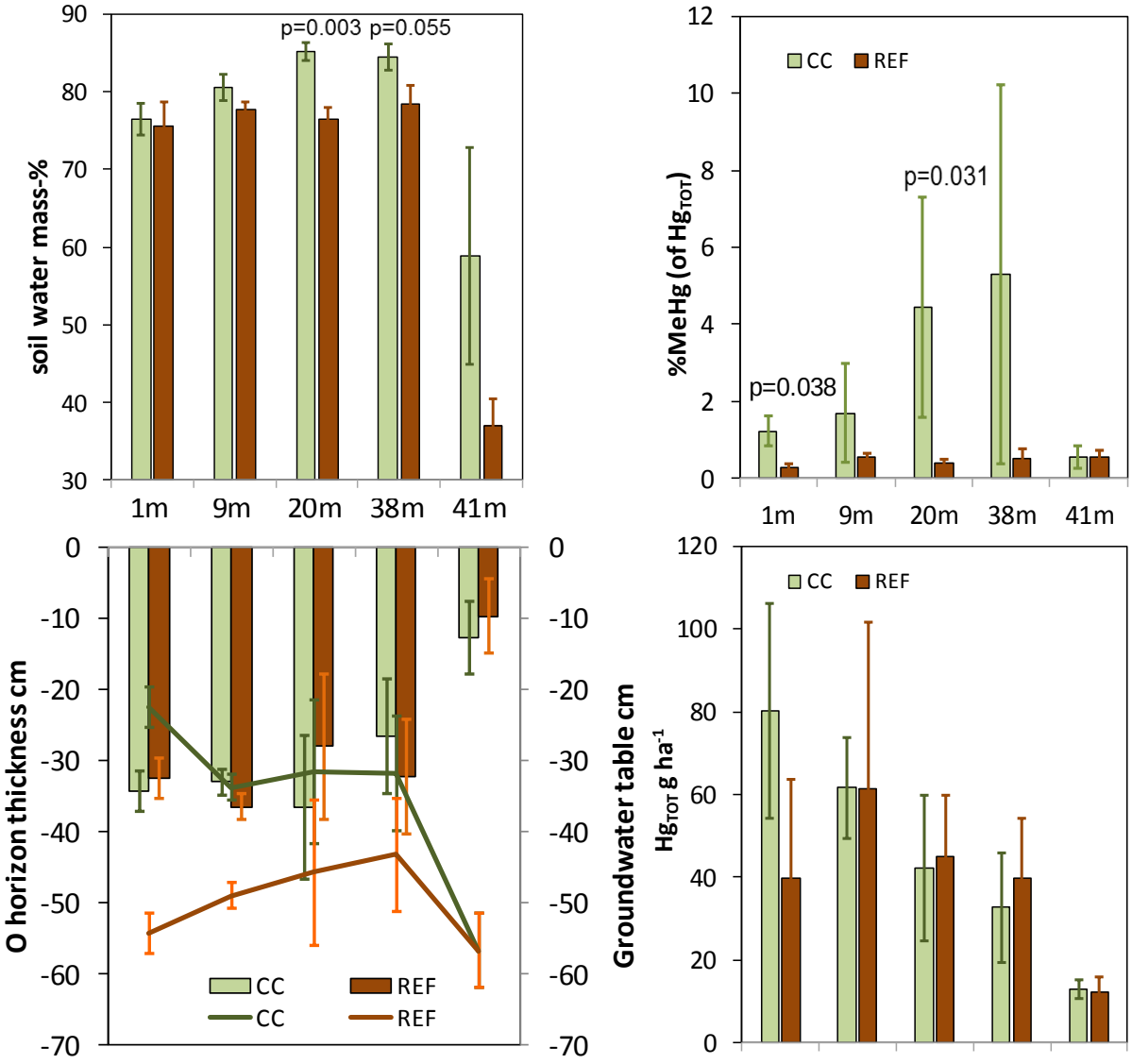
195 RESULTS

196 **Soil concentrations and areal masses of Hg_{TOT} and MeHg along hillslopes.** As previously
197 reported, [22] average O horizon Hg_{TOT} concentrations and Hg_{TOT} areal masses did not differ
198 between the REF (210 ng g^{-1} and 41 g ha^{-1}, respectively) and CC sites (220 ng g^{-1} and 48 g ha^{-1},
199 respectively) of this study. In contrast, MeHg concentrations ($p=0.002$) and areal masses of
200 MeHg ($p=0.006$) and MeHg in % of Hg_{TOT} ($p=0.003$) were significantly higher at CC (4.8 ng g^{-1},
201 1.1 g ha^{-1}, and 2.7%, respectively) than at REF sites (1.0 ng g^{-1}, 0.16 g ha^{-1}, and 0.4%,
202 respectively). Similar differences between REFs and CCs were reported for the sub-horizons Oe
203 and Oa (Figure S2, SI). In summary, concentrations and areal masses of MeHg in CCs were 9-12
204 times and 4-7 times higher that REFs in the Oe and Oa horizons, respectively, and 5-7 times
205 higher than REFs in the O horizon as a whole. Individual data for all sampling sites are reported
206 in the Supporting Information (Table S3, S4, SI).

207 The average thickness and bulk density of the O horizon was similar at REF (29±22 cm
208 and 64±19 g dm^{-3}) and CC (29±13 cm and 69±32 g dm^{-3}, Table S5, SI) sites. At sampling
209 positions P1-P4 (situated 1 – 38 m from stream) the average O horizon thickness varied between
210 26 and 32 cm at both REFs and CCs (Figure 1). Even if the thickness did not meet criteria for
211 peat formation (Histosols: >40 cm organic horizon) it is indicative of an average groundwater
212 table close enough to the surface to periodically saturate most of the O horizon. Further away, at
213 sampling point P5, well-drained Podzols with typical O horizons (REF: 10 cm, CC: 13 cm) were
214 developed. At the sampling occasion in June, in the middle of a dry spell, the groundwater at
215 CCs reached into the lower part of the Oa horizons, while at REFs the level was several dm
216 deeper (Figure 1, Table S5, SI).

In parallel to the groundwater level, the water content of the O horizon was enhanced at CCs and reached a maximum at some distance from the stream. Water contents were significantly higher at CCs as compared to REFs at sampling locations P3 ($p=0.03$) and P4 ($p=0.055$, Figure 1, Table S6, SI). At REFs there was no clear pattern for the soil water content with distance to stream. The soil organic carbon content was significantly higher at REFs, indicative of soil disturbance and mineral matter mixing into the O horizon after clear-cutting.

One meter from stream (P1) MeHg expressed as concentrations, areal masses and % of Hg_{TOT} were all significantly higher at CCs than at REFs (Figure 1, Table 1 and S6). A significant enhancement at CCs were also observed at position P3 (for MeHg concentrations and % of Hg_{TOT}). When the two sampling points P1+P2 and P3+P4 were grouped together (to improve statistical testing) the enhancement of MeHg at CCs (in relation to REFs) was most pronounced at intermediate positions (P3+P4) along hillslopes. MeHg concentrations were enhanced seven times ($p=0.009$) at P3+P4 and four times at P1+P2 ($p<0.04$) and %MeHg was enhanced 12 times ($p=0.003$) at P3+P4 (Table S6). Soil water contents were significantly enhanced at CCs at P3 ($p=0.003$) and P3+P4 ($p=0.0003$). Further up along the hillslope, moving into the recharge area (at P5), groundwater levels, soil water contents and MeHg measures (Figure1) all reached the lowest values along transects and differences between CCs and REFs were not significant. Concentrations and areal masses of Hg_{TOT} generally decreased by distance from the stream (Figure 1, Table 1 and S5) and showed no significant differences between REFs and CCs. Both C/N and C/S ratios (g/g) in the O horizon remained very similar: 28±10 and 240±72, respectively, at CCs, 30±9.4 and 240±59, respectively, at REFs.



238

239 **Figure 1.** Spatial pattern of mass-% soil water (upper left), O horizon thickness (bars) and
240 groundwater table (lines, lower left) and %MeHg of Hg_{TOT} and areal mass of Hg_{TOT} (right) for
241 the five sampling points (P1-P5) along hillslopes with average distances to stream denoted.
242 Average values ± SE are reported for reference stands (REF, N=4) and clear-cuts (CC, N=4).
243 Data are reported in Table S5 and S6, SI. All sampling positions were forested at REFs and prior
244 to harvest at CCs.

245 **Table 1.** Average (± SD) O horizon concentrations of total mercury (Hg_{TOT}) and methyl mercury
246 (MeHg) and areal masses of MeHg for composite soil samples taken at five hillslope positions
247 (P1-P5) at four sites. Figures in bold italics denote significant differences between references
248 (REF) and clear-cuts (CC). Data on %MeHg, areal masses of Hg_{TOT} (g ha^{-1}) and soil water and
249 soil organic carbon mass-% are reported in Table S6, SI.

Hillslope position	Hg_{TOT} (ng g^{-1})		MeHg (ng g^{-1})		MeHg (g ha^{-1})		250
	REF	**CC**	**REF**	**CC**	**REF**	**CC**	251
P1 1m (N=4)	170±53	210±95	***0.6±0.4^a***	***2.8±1.1^b***	**0.16±0.20^a**	**1.1±0.5^b**	
P2 9m (N=4)	220±59	250±17	1.3±0.5	4.0±5.8	0.26±0.14	0.94±1.2	
P3 20m (N=4)	220±35	190±46	***0.8±0.6^a***	***7.2±6.5^b***	0.13±0.09	2.0±2.4	
P4 38m (N=4)	220±21	200±35	1.3±1.1	7.9±6.0	0.20±0.13	1.2±1.1	
P5 41m (N=3)	190±10	230±54	1.0±0.6	1.2±1.2	0.05±0.03	0.06±0.04	
P1+P2 (N=8)	200±22	230±25	***0.9±0.2^a***	***3.4±1.5^b***	***0.21±0.06^a***	***1.1±0.3^b***	
P3+P4 (N=8)	220±10	195±14	***1.1±0.3^a***	***7.6±2.2^b***	*0.16±0.04^c*	*1.6±0.7^d*	
P1 to P5 (N=20)	210±43	220±54	***1.0±0.7^a***	***4.8±5.1^b***	***0.16±0.14^a***	***1.1±1.4^b***	

252

253 **Methylation and demethylation incubation studies.** Rate constants for the potential
254 methylation and demethylation, k_m and k_d, were determined in incubation experiments of soil Oa
255 horizon samples collected in August 2012, three years after final harvest. The water content of
256 soil samples taken in August 2012 (68-91%, Table S7,SI) were similar to samples taken in June
257 2011 (65-86%, Table S6, SI). Also similar to in June 2011, clear-cuts (CC3, CC4) demonstrated
258 higher %MeHg and higher water contents in positions P3 and P4 than in position P1 (Table S7,
259 SI). Soil samples at the reference stands (REF1 P1 & P3, REF4 P1) all showed lower water
260 contents and substantially lower concentrations of MeHg (and %MeHg of Hg_{TOT}) than all the CC
261 samples. Notable was the high concentration of MeHg (and %MeHg) and water content at

sample point P3 in REF4. This sample was affected by clear-cutting of the forest stand further up along the slope just a short distance from the sampling point, conducted one year before the sampling occasion. The clear-cutting effect noted for MeHg% at REF4 P3 was also reflected by the potential methylation rate constant k_m (0.029 d^{-1}), which fell into the range observed for the six CCs (0.014 – 0.582 d^{-1}). In contrast, the three true REFs showed much lower methylation rate constants (0.0001-0.007 d^{-1}, Table S7, SI). Statistical testing conducted with REF4 P3 considered to be affected by clear-cutting (and thus included as a CC) revealed that CC soils (N=7) had significantly higher concentrations of MeHg (p=0.003), %MeHg (p=0.010), k_m (p=0.048, one-tailed test) and water content (p= 0.017) than REFs (N=3), Figure 2. Potential demethylation rate constants showed no response to clear-cutting, as illustrated by similar ranges for CCs (0.006-0.081) and REFs (0.005-0.173), Table S7, SI.

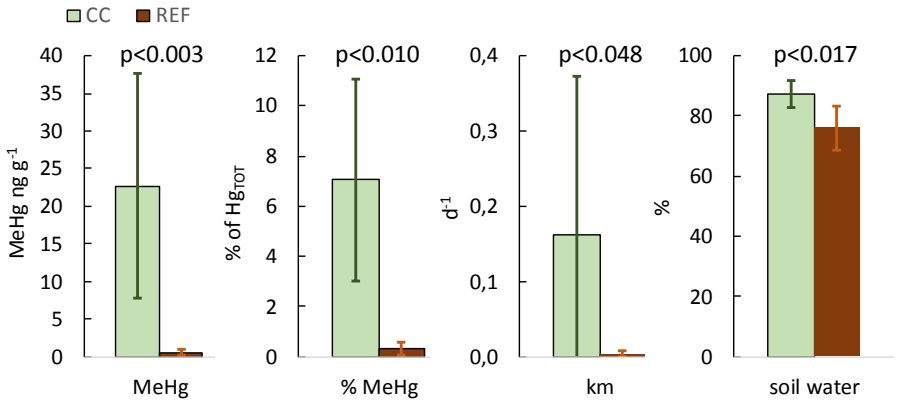


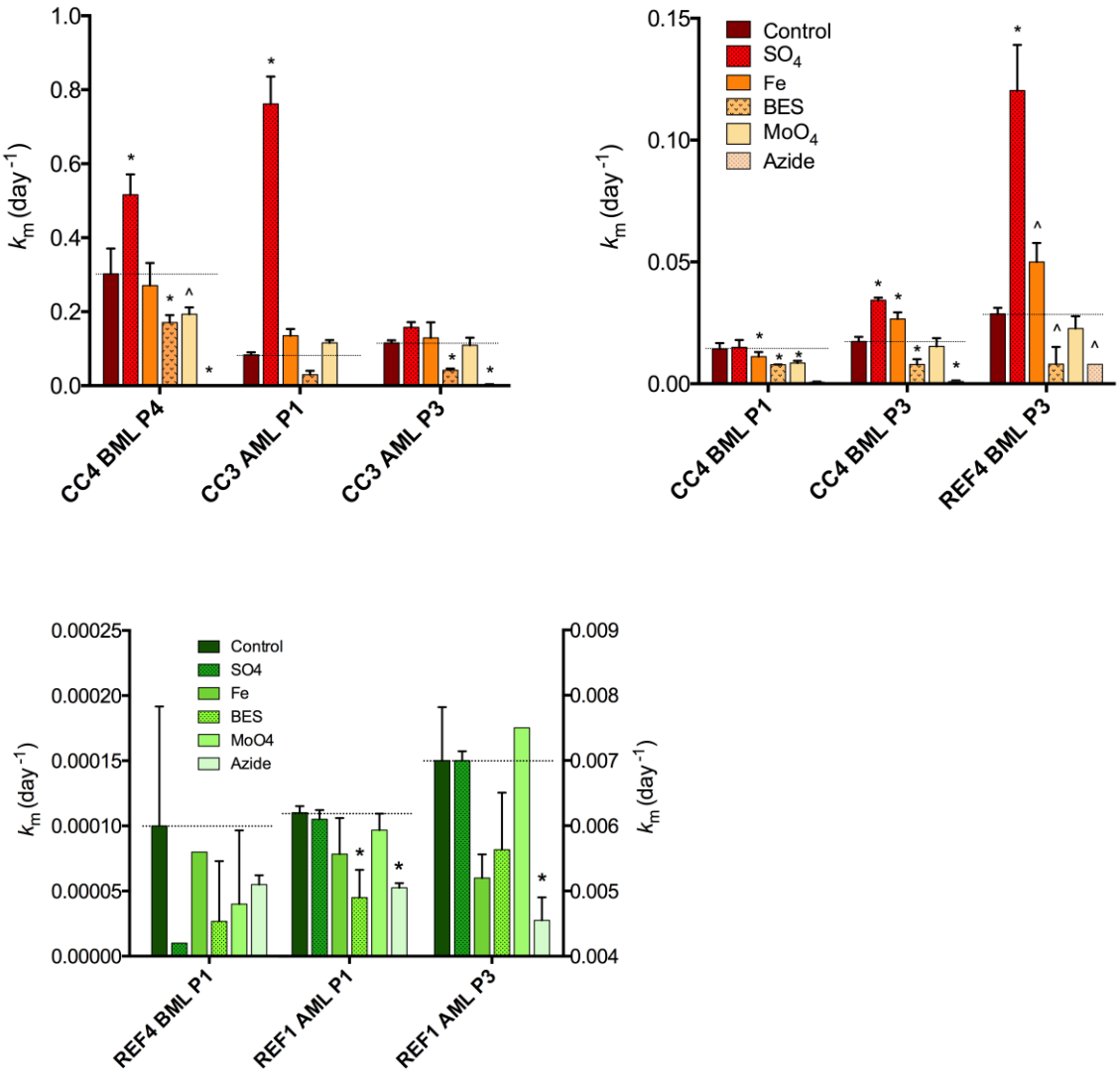
Figure 2. Average (±SD) concentrations of MeHg, %MeHg of Hg_{TOT} in soil prior to incubation, potential methylation rate constant (k_m) and soil water content (% of wet soil mass) for soil samples taken at clear-cuts (CCs, including REF4 P3 that was affected by clear-cutting, N=7) and reference stands (REFs, N=3). Data in Table S7, SI. Corresponding plots for log-transformed data are for clarity presented in SI, Figure S3.

Amendments of electron acceptors and inhibitors. In three of the five CC soils, and in the REF4 BML P3 soil (which was affected by clear-cutting), there was a significant ($p<0.05$) increase in k_m after addition of the potential electron acceptor sulfate (Figure 3). In two of these samples also addition of the electron acceptor Fe(III) enhanced k_m: one significantly ($p<0.05$; CC4 BML P3) and one almost significantly ($p<0.10$; REF4 BML P3). None of the REF samples were significantly affected by additions of sulfate and Fe(III).

The microbial inhibitor azide significantly ($p<0.05$) or almost significantly ($p<0.10$) decreased the k_m in all CC samples (including REF4 BML P3) and in two out of three REFs. Amendment with BES (specific inhibitor for methanogens) significantly ($p<0.05$) reduced k_m by almost half in four of the six CC samples (CC4 BML P1, P3 and P4, and CC3 AML P3). A significant decrease was also observed in REF1 AML P1. Further, in response to addition of the specific inhibitor of SRB (molybdate), k_m decreased significantly ($p<0.05$) in the CC4 BML P1 sample and almost significantly in the CC4 BML P4 sample ($p=0.06$).

The effect of isotope labeled inorganic Hg tracers with different availability for methylation was evaluated by comparing the relative differences in k_m determined from the $^{198}Hg(NO_3)_2$ and ^{201}Hg-NOM tracers. For the $Hg(NO_3)_2$ tracer, rate constants were about 20 times higher than for the Hg-NOM tracer (Figure S4), well in agreement with previous findings.[36] As noted from the figure the pattern of relative response to electron acceptor and inhibitor amendments was similar for the $^{198}Hg(NO_3)_2$ and ^{201}Hg-NOM tracers.

In contrast to the results for the methylation process, amendment did not have any clear effect on demethylation rates (Figure S5).



300 **Figure 3.** Potential methylation rate constants (k_m) determined for the $^{198}Hg(NO_3)_2$ tracer.
301 Control samples are compared with samples amended with electron acceptors (sulfate, Fe(III))
302 and microbial inhibitors (BES, molybdate, azide). The asterisk (*) denotes significant differences
303 ($p < 0.05$, ANOVA + Tukey multiple comparison test) and (^) marginally non-significant
304 differences ($p < 0.07$) from control (horizontal dotted lines). Error bars represent the standard
305 deviation for triplicates. In the lower figure REF1 AML P1 and P3 are plotted on the right axis.

306

DISCUSSION

Previous studies of boreal forest harvest have demonstrated increased MeHg concentrations in stream runoff and in downstream biota,[16-22] but the source of MeHg has not been clearly identified. Here we extend the findings from Kronberg et al.,[22] demonstrating increased MeHg pools in the O horizon after clear-cut, by reporting spatial distributions along recharge-discharge transects. Spatially, MeHg soil quantities were most enhanced at some distance from the stream, well outside the riparian zone, where also the water content of the O horizon was significantly enhanced (Figure 1). Thus, the most important factor for creating oxygen deficiency in soil: water saturation, likely played an important role to enhance soil MeHg after forest clear-cutting.

As a consequence of forest harvest, evapotranspiration significantly decreases[25] and the runoff was shown to be enhanced on average 62% at the CC sites above the ML of this study.[22] The extra water supply will extend the discharge area from the riparian zone patches of Histosols into better drained O horizons of Podzols in lower sections and local depressions of hillslopes. The water table positioned in the lower part of the Oa horizon at CCs (Figure 1) demonstrates the effect of increased water supply after final harvest. Even if the O horizon in average was as thick as 26 – 36 cm at sampling positions P2-P4 (located in average 9 to 38 m from streams), the development of E and Bhs and Bs horizons (diagnostic for Podzols) prove at least seasonally relatively well-drained conditions prior to clear-cutting. It has been demonstrated that experimental flooding of well-drained upland soil O horizons results in high rates of Hg methylation and MeHg accumulation in soils.[28,44] This observation has primarily been explained by a higher availability of electron donors (for methylating microbes) in relatively well-drained Podzol O horizons, as compared to the more recalcitrant organic matter accumulated in Histosols.[28,44,53] We therefore suggest that hot-spots for MeHg net formation after forest clear-

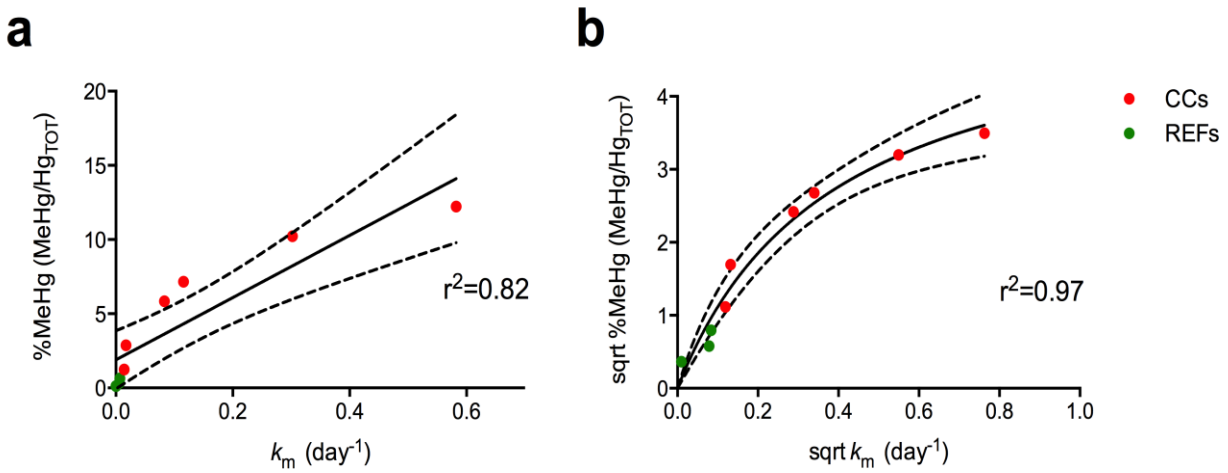
cutting are mainly to be found in lower sections (including local depressions) of hillslopes, at some distance from streams, where the combination of high availability of electron donors and increasing groundwater tables stimulates the activity of anaerobic microbes..

This picture also agrees well with our previous findings that stream MeHg concentrations[20] and annual MeHg export[22] is most clearly enhanced after forest harvest in undulating terrain with small riparian zones, as compared to flatter landscapes with a larger contribution from riparian zones and wetlands. The more or less continuous layer of organic soil, connecting near stream Histosols with O horizons of Podzols in lower sections of hillslopes, possess a high hydraulic conductivity and serves as permeable medium for lateral movement of water and its solutes from soils to streams under high-flow conditions.[43] Thus, through this medium, MeHg bonded to mobile organic matter[45] will readily be transported from the hot-spots of MeHg formation in soil to the stream.

Soil samples taken at positions along hillslopes, which were demonstrating significantly enhanced concentrations and areal masses of MeHg two years (Table 1, Table S6) and three years (Table S7) after clear-cutting, also showed significantly enhanced methylation rate constants (k_m), as compared to REFs (Figure 2). Thus, a positive relationship was observed between %MeHg (of Hg_{TOT}) in soils and k_m determined in the same soil after short-term (48 h) incubation (Figure 4). Similar relationships have been reported for sediments.[51] Because the k_m is a true measure of short-term MeHg formation, the relationship can been seen as a confirmation that the build-up of %MeHg in soils (2-3 years) after forest harvest indeed is due to an increased net formation of MeHg. Because demethylation rate constants (k_d) overlapped largely between CCs and REFs (Figure S4) it is concluded that the increased O horizon pool of MeHg after final

352 harvest is mainly caused by an enhanced rate of methylation and not by a decreased rate of
353 demethylation.

354



355

356 **Figure 4.** Relationships between %MeHg (of Hg_{TOT}) in soil samples prior to incubation and
357 potential Hg methylation rate constant k_m determined for clear-cuts (CCs, red symbols N=6) and
358 references (REFs, green symbols N=3). Linear model (a) and nonlinear model (b). The sample
359 REF4 BML P3 was excluded from the plot. Dotted lines display 95% confidence intervals.

360

361 It is reasonable to argue that factors in control of the longer-term build-up of MeHg in soils after
362 clear-cutting also were largely responsible for the high methylation rate (as compared to REFs)
363 as determined in the 48 h laboratory experiments. The considerable thickness of the sampled O
364 horizons of both REFs and CCs (29 cm in average) suggest they were regularly affected by water
365 saturation during periods of high flow events such as spring snowmelt and autumn rains, at least
366 during the last rotation period of the forest stand. As a consequence of forest harvest, the

367 groundwater table at CCs was further increased and reached into the lower part of the O horizon
368 even during a very dry period of the year (Figure 1). Thus it is expected that water saturated
369 conditions prevailed throughout most of the year at sampling positions P1-P4 at CC sites. This
370 study did not include genetic or molecular work to quantify abundance of different groups of
371 bacteria, but given the hydrologic conditions after forest harvest, it is expected that an active
372 community of anaerobic microbes were built-up in the water-saturated O horizon soils after
373 forest harvest.

374 In addition to microbial activity, the chemical speciation of Hg and MeHg is expected to
375 influence the absolute rates at which these forms transform in the soil.[56] Soil porewater chemistry
376 was not characterized in this study, but streams draining REFs and CCs generally showed a
377 dominance of Fe(II) over Fe(III) and sulfide was (barely) detected (>0.3 μM) in a few streams.[22]
378 A comparison of C/N and C/S ratios in soils reveal no differences between REFs and CCs.
379 Because sulfide readily reacts and becomes incorporated into NOM in permanent sulfate
380 reducing environments,[47,48] the C/S ratio provide a time-integrated measure of sulfate reduction
381 in organic soils. The production of sulfide was obviously not large enough to significantly
382 decrease the C/S ratio in soils of CCs below the values of the REFs (Table S5, SI).

383 In the O horizon soils of this study both Hg and MeHg are expected to be almost
384 exclusively complexed by NOM associated thiol groups (RSH).[37,38,47,54,57] Spectroscopic studies
385 of NOM from O horizon soils of Podzols and Histosols and dissolved OM in streams in the
386 region suggest that the concentration of RSH group make up on average 0.15 mass-% of organic
387 C[54,55]. Based on % soil organic C (Table S6, SI) and soil pH (3.8 (±0.3) in REFs and 4.2 (±0.2)
388 in CCs) in the soils, and an maximum concentration of dissolved inorganic sulfides of 1-2 μM in
389 soil porewater, even in the most anoxic riparian soils of the study area,[7,57] thermodynamic

calculations demonstrate that $Hg(SR\text{-}NOM)_2$ and MeHgSR-NOM complexes will constitute more than 95% of Hg and MeHg, respectively, in the soils of this study.[55]

Given the dominance of $Hg(SR\text{-}NOM)_2$ complexes in the soils, we expect the ^{201}Hg-NOM tracer to reflect the availability for methylating bacteria better than the $^{198}Hg(NO_3)_2$ tracer. However, since the chemical speciation of Hg (and MeHg) at ambient conditions in soils cannot be reliably simulated by any isotopic enriched tracer, there is currently no method available for accurate determination of actual Hg methylation or MeHg demethylation rates in soil. Therefore the relevance of the results of is study (and other incubation studies conducted in laboratory systems) relies on the clear demonstration that incubation of Hg-NOM and $Hg(NO_3)_2$ tracers in estuarine sediments at both micro[36] and mesocosm[50] scales, as well as in a wide range of wetland soils,[57] give very similar results on a relative scale when soils or sediments are compared. Thus the Hg-tracer method is highly relevant for comparative purposes. The results further suggest that other factors than the Hg speciation are in control of the large differences observed between REFs and CCs when regards to MeHg build-up in soils and k_m determined in laboratory experiments. In addition to the microbial activity, electron donors and acceptors need to be considered.

Potential electron acceptors (Mn (IV), Fe(III), SO_4^{2-}) and electron-donors (low molecular mass organic compounds and H_2) for anaerobic microbes were not determined in the soils, but amendments of electron acceptors and inhibitors during the incubation experiments can provide useful information on these aspects, as well as on the activity and identity of bacteria responsible for the methylation of Hg. By necessity additions of redox modulating constituents like Mn(IV), Fe(III) and SO_4^{2-} will affect the redox conditions in the incubation slurries. A caveat may be in place, since it cannot be ruled out that the amendments may be differently affected in different

413 soil samples, due to some variation in the composition of soil constituents and chemistry. That
414 being said, it should be noted that the soils in this study can be expected to represent a narrow
415 collection of biogeochemical conditions. As reflected by uniform % organic C, C/N and C/S
416 ratios, and the small contribution from reactive mineral components, we do expect the abiotic
417 soil components to module the effect of amendments in a much similar way in all samples. Thus
418 effects of amendments should be reliable, as reflected by the small errors of replicates which
419 provided significances although the data material was not very extensive.

420 The effect of the azide amendment demonstrated that Hg methylation was a biotic
421 process. SRB were indicated to be present in most CC soils as indicated by the stimulatory effect
422 of sulfate and inhibitory effect of molybdate (Figure 3). The effect of sulfate suggests this
423 electron acceptor may have limited the Hg methylating bacteria in CC soils. In contrast, sulfate
424 had no effect in any of the REFs and molybdate had an effect only in one REF. This may suggest
425 that the population of SRB was not very large, or at least not very active in the REF soils. The
426 inhibitory effect of BES in all CCs further points at methanogens contributing to MeHg
427 formation after clear-cut. BES only had an effect in one of the REFs, again implying that either
428 the activity and/or the population size of methanogens were small in REF soils. Notably, in two
429 of the clear-cut samples (CC4 BML P1 and P4) the significant inhibitory effects of BES and
430 MoO_4 were similar in size. This may indicate a syntrophic relationship between the SRB and
431 methanogenic communities, and that this interaction was stimulated after forest harvest.

432 Given that the combined inhibition of SRB by molybdate and inhibition of methanogens
433 by BES only halved the methylation rate constant, as compared to the control, it is reasonable
434 that IRB and/or firmicutes[13,14] also may have contributed to the increased Hg methylation after
435 clear-cut. The fact that Fe(III) hydroxide addition did not result in significant responses (with the

436 exception of one CC sample), suggests that if IRB were present they were not limited by electron
437 acceptors. Molybdate amendment proved SRB being responsible for the majority (up to 95%) of
438 MeHg formation in riparian zone wetlands from the same boreal landscape as in this study.[7] In
439 this study the role of methanogens was not tested. A stimulatory effect by sulfate addition has
440 been demonstrated in wetland soils in northern forest ecosystems,[49] but not before in forest soils.

441 In addition to quantifying soil pools of MeHg, this is the first study reporting potential
442 methylation and demethylation rate constants, including effects of electron acceptor and donor
443 amendments, related to forest harvest. In lack of process-oriented studies on Hg biogeochemistry
444 after forest harvest, it may prove relevant to compare our results with studies of wetland soils in
445 the same type of boreal landscape (and using the same incubation method). The range of
446 %MeHg reported for CCs (0.2 – 11.8 % of Hg_{TOT}, Table S3-S4) in this study falls well into the
447 range reported for boreal wetlands (2.3 – 17 %).[7] The REFs (0.1 – 1.1%) clearly had %MeHg
448 lower than boreal wetlands, while some of the CCs were just as high as the most net methylating
449 and MeHg exporting boreal wetlands. The k_m reported for REFs (0.0001-0.007 d^{-1}, Table S5)
450 was clearly much lower than in any of the boreal wetlands, whereas k_m values reported for CCs
451 (0.014 – 0.58 d^{-1}) were well in the range reported for boreal wetlands (0.002-0.10 d^{-1}).[7] The most
452 highly methylating CC soils showed five times higher k_m than the most highly methylating boreal
453 wetlands. Thus, while mature coniferous reference stands may show low rates of Hg methylation
454 and MeHg soil pools are low, rates may locally increase tremendously after clear-cutting at Hg
455 methylation hot-spots and O horizon pools of MeHg may reach levels similar to the highest net
456 methylating wetlands. The same stimulatory factors: availability of electron donors, acceptors
457 and nutrients suggested to explain hot-spots for Hg methylation in the transition zone between

458 uplands and fen wetlands[52] and in fens with intermediate nutrient status,[6,7] may apply also for
459 hot-spots after forest clear-cutting.

460 Since there were no indications that differences in Hg speciation or availability of
461 electron acceptors control the large differences in the concentrations of MeHg build-up in CC
462 soils and the much higher k_m, as compared to REFs, we argue that the increased water saturation
463 of soil, in concert with readily available organic electron donors[28,44] are the main factors
464 responsible for building up an active community of Hg methylating microbes in O horizons of
465 hillslope soils after forest clear-cut.

466 **Environmental implications of forest management practices.** Of utmost importance to
467 minimize MeHg export to aquatic ecosystems would be to avoid connecting Hg methylation hot-
468 spots established in lower sections in local discharge areas of hillslopes with draining streams
469 during clear-cutting operations. Connectivity is provided by driving tracks and by digging new or
470 clearing old ditches. These activities therefore should be minimized until a new forest stand has
471 been established. Previous studies suggest it may take 10 years or longer until the effect of forest
472 harvest on MeHg export returns back to pre-harvest levels.[18,20] Whole-tree harvest, where some
473 of the organic clear-cut debris is removed, may decrease this time window by limiting the input
474 of readily available electron donors in form of organic debris to anaerobic bacteria. Although
475 peaty soils located in the riparian zone along streams may be less prone to increased MeHg net
476 formation after forest harvest, the general recommendation to avoid driving close to streams[19,34]
477 would result in minimum export of MeHg from these regularly Hg methylating soils.

478

479

480 ASSOCIATED CONTENT

481 **Supporting Information**

482 The supporting information is available free of charge on the ACS Publications website. SI
483 contains Text S1-S3, Figure S1-S3 and Tables S1-S4.

484

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491

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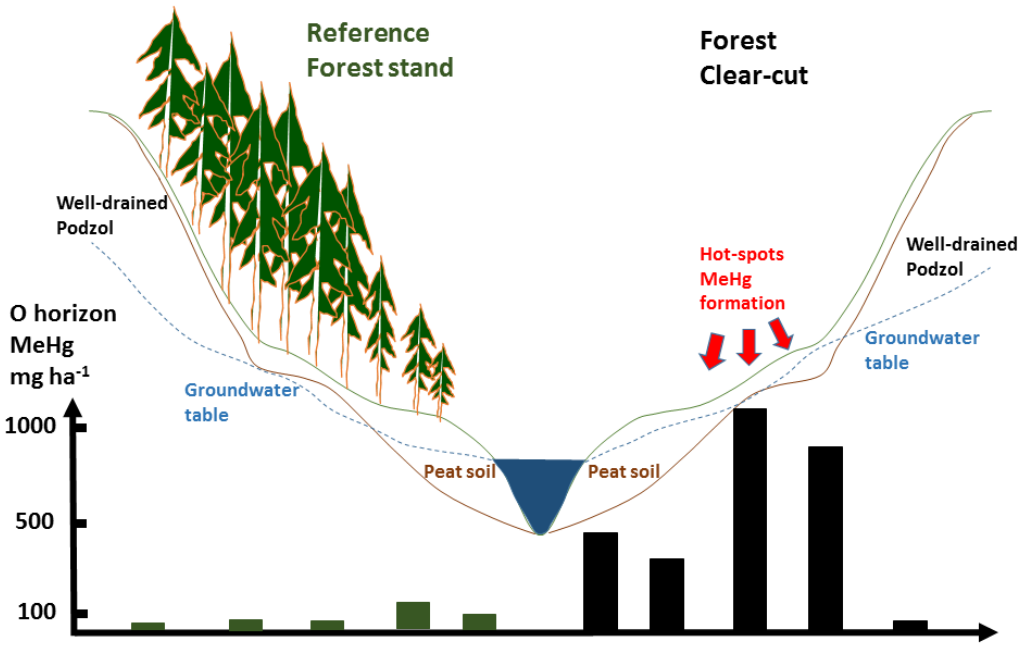

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