

Supplementary Materials for

Terrestrial discharges mediate trophic shifts and enhance methylmercury accumulation in estuarine biota

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Supplementary Materials and Methods

Chemical and biological analysis

Isotope selective determination of MeHg and total Hg in water, sediment and biota samples was done by isotope dilution analysis with gas chromatography or cold vapour coupled on-line to inductively coupled plasma mass spectrometry. Concentrations of nutrients (NH_4^+ , NO_2^- , NO_3^{2-} , PO_4^{3-} , and $\text{Si}(\text{OH})_4$) were determined using well-established standard methods and a Bran & Luebbe TRAACS 800 autoanalyzer. Concentrations of DOC were determined using a Shimadzu TOC-5000 high temperature catalytic oxidation instrument with NDIR detection. Concentrations of chlorophyll a and humic substances were determined by fluorescence spectrometry. The concentration of humic substance was determined using 350 nm as excitation wavelength and 450 nm as emission wavelength, which provides a measure of humic type aromatic compounds (61). The instrument was calibrated with quinine sulphate and the concentration of humic substances is thus expressed as quinine sulphate units. Integrated photosynthetic primary and heterotrophic bacteria production was calculated for 0–4.5 m water depth from photosynthetic primary production determinations by $\text{NaH}^{14}\text{CO}_3$ incubations at 0.01, 0.1, 0.5, 1, 2 and 4 m water depth in the mesocosms and heterotrophic bacterial production determinations by [^3H -methyl]-thymidine incorporation in dark of mesocosm water from 0.5 and 4 m. Deposition rates for autochthonous NOM were determined from sedimented material collected in sedimentation traps (8.5 cm \varnothing dimensions) placed at ~4 m water depth in each mesocosm during day 38 to 50. The collected material was freeze-dried and the total amount, %TOC, %N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ determined. The amount of deposited autochthonous and allochthonous NOM was then estimated from the total amount of deposited NOM and using a two-parameter $\delta^{15}\text{N}$ mixing model with the added terrestrial NOM and seston from each mesocosm as the two end members. The use of a $\delta^{13}\text{C}$ mixing model was first attempted but indicated >100% autochthonous NOM for all treatments. The δ

^{15}N parameter is more commonly used to assess the trophic level of organisms, and potentially unreliable to assess the contribution from different carbon sources since it is fractionated with increased trophic level. The fact that the different seston size fractions and *Bivalvia* from the different mesocosm treatments showed similar $\delta^{15}\text{N}$ values (8.03 and 7.21, respectively) do however support this approach in this particular case. The $\delta^{15}\text{N}$ value for the added terrestrial NOM was 3.53. Although uncertain, the estimated sedimentation rates indicate that the deposition rate of autochthonous NOM was highest for NP_{high} and similar for NP_{low} and TM. As expected the sedimentation rate of allochthonous and total amount of NOM were highest in TM (2.7–5.0 and 1.3–3.1 times, respectively, fig. S5).

Mercury mass balance calculations

We applied mercury mass balance calculations that allows to incorporate differentiated availability for methylation and bioaccumulation of specific geochemical pools and fluxes of Hg in ecosystems (Equations S1 and S2) (10). The model parameterisation, and a sensitivity analysis of its underlying assumptions, have previously been discussed in detail (10). We used the MeHg net formation and bioaccumulation factors (MeHg/Hg^{II} molar ratio and MeHg biota sediment accumulation factor (BSAF), respectively) determined in this study to predict the response in MeHg concentration in sediment, benthic biota and pelagic biota to simulated eutrophication and increased terrestrial discharge (induced by climate change) scenarios for the Öre Estuary in the Bothnian Sea off the Swedish north eastern coast. The MeHg/Hg^{II} molar ratio and MeHg BSAF input data for simulating present day conditions, eutrophication and increased terrestrial runoff scenarios were calculated from the NP_{low} , NP_{high} and TM treatments, respectively, using the average of the concentration values for each sample type, and assuming that $[\text{Hg}^{\text{II}}] = [\text{HgT}] - [\text{MeHg}]$ for all sample types. Total MeHg concentration in sediment ($[\text{MeHg}]_{\text{sed}}$; pmol g^{-1} d.w. in 0–1.5 cm) and specific contributions from different geochemical Hg pools were calculated using the MeHg/Hg^{II} molar ratios determined in the

mesocosm experiment, estimated loading rates of Hg^{II} and MeHg from catchment runoff and atmospheric deposition (mol input during 4 months; Table S4) and Hg^{II} and MeHg stored in the top 1.5 cm of the sediment as $\beta\text{-HgS(s)}$ and $\text{Hg}^{\text{II}}\text{-NOM}$ (mol). A MeHg fraction stored in sediment and not readily available for demethylation was calculated using a factor, $F_{[\text{MeHg}]_{\text{sediment}}}$, set to 0.30 of the sum of the other contributing sources. The magnitude of this factor was previously assessed based on measured MeHg/ Hg^{II} molar ratio for the $\text{Me}^{198}\text{Hg-NOM}_{\text{sed}}$ tracer (10).

The absolute amounts of MeHg (mol in 0–1.5 cm) were recalculated to a concentration (pmol g^{-1} d.w. in 0–1.5 cm) using a conversion factor C of 3.53×10^{12} g^{-1} d.w. ($C = (\text{density}_{\text{sed}} (\text{g w.w. cm}^{-3}) \times \text{d.w./w.w. ratio} \times \text{sediment depth (cm)} \times \text{estuary area (cm}^2))^{-1} \times 10^{12}$).

eq. S1. Calculation of MeHg concentration (pmol g^{-1} d.w.) in sediment.

$$\begin{aligned}
 [\text{MeHg}]_{\text{sed}} = C \times (1 + F_{[\text{MeHg}]_{\text{sediment}}}) \times & \left(\text{Hg}^{\text{II}}_{\text{atmospheric load}} \times \frac{\left[\frac{[\text{MeHg}]}{[\text{Hg}^{\text{II}}]} \right]_{204\text{Hg}^{\text{II}}_{\text{wt}}}}{\left(1 + \left[\frac{[\text{MeHg}]}{[\text{Hg}^{\text{II}}]} \right]_{204\text{Hg}^{\text{II}}_{\text{wt}}} \right)} + \text{Hg}^{\text{II}}_{\text{catchment runoff load}} \times \right. \\
 & \frac{\left[\frac{[\text{MeHg}]}{[\text{Hg}^{\text{II}}]} \right]_{204\text{Hg}^{\text{II}}_{\text{wt}}}}{\left(1 + \left[\frac{[\text{MeHg}]}{[\text{Hg}^{\text{II}}]} \right]_{204\text{Hg}^{\text{II}}_{\text{wt}}} \right)} + \text{Hg}^{\text{II}}_{\beta\text{HgS}} \times \frac{\left[\frac{[\text{MeHg}]}{[\text{Hg}^{\text{II}}]} \right]_{\beta 200\text{HgS}_{\text{sed}}}}{\left(1 + \left[\frac{[\text{MeHg}]}{[\text{Hg}^{\text{II}}]} \right]_{\beta 200\text{HgS}_{\text{sed}}} \right)} + \text{Hg}^{\text{II}}_{\text{Hg}^{\text{II}}\text{-NOM}} \times \frac{\left[\frac{[\text{MeHg}]}{[\text{Hg}^{\text{II}}]} \right]_{201\text{Hg}^{\text{II}}\text{-NOM}_{\text{sed}}}}{\left(1 + \left[\frac{[\text{MeHg}]}{[\text{Hg}^{\text{II}}]} \right]_{201\text{Hg}^{\text{II}}\text{-NOM}_{\text{sed}}} \right)} + \\
 & \left. \text{MeHg}_{\text{atmospheric load}} \times \frac{\left[\frac{[\text{MeHg}]}{[\text{Hg}^{\text{II}}]} \right]_{\text{Me}^{199}\text{Hg}_{\text{wt}}}}{\left(1 + \left[\frac{[\text{MeHg}]}{[\text{Hg}^{\text{II}}]} \right]_{\text{Me}^{199}\text{Hg}_{\text{wt}}} \right)} + \text{MeHg}_{\text{catchment runoff load}} \times \frac{\left[\frac{[\text{MeHg}]}{[\text{Hg}^{\text{II}}]} \right]_{\text{Me}^{199}\text{Hg}_{\text{wt}}}}{\left(1 + \left[\frac{[\text{MeHg}]}{[\text{Hg}^{\text{II}}]} \right]_{\text{Me}^{199}\text{Hg}_{\text{wt}}} \right)} \right)
 \end{aligned}$$

The total MeHg concentration (pmol g^{-1} d.w.) in biota ($[\text{MeHg}]_{\text{biota}}$) and specific contributions from different geochemical Hg pools were calculated using the predicted concentration of MeHg (pmol g^{-1} d.w.) in the sediment (0–1.5 cm) from each geochemical Hg pool calculated

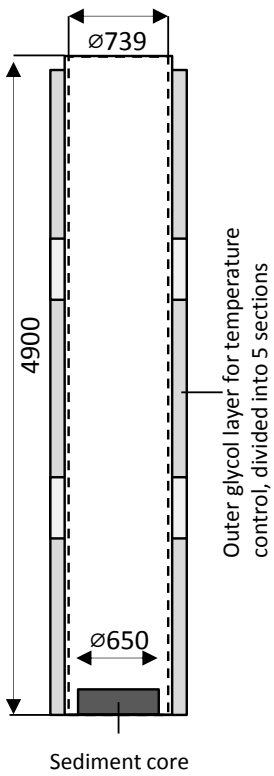
by Equation 1 (e.g. as $[\text{MeHg}]_{\text{Hg}^{\text{II}} \text{ atmospheric load}} = C \times \text{Hg}^{\text{II}} \text{ atmospheric load} \times \frac{\left[\frac{[\text{MeHg}]}{[\text{Hg}^{\text{II}}]} \right]_{204\text{Hg}^{\text{II}}_{\text{wt}}}}{\left(1 + \left[\frac{[\text{MeHg}]}{[\text{Hg}^{\text{II}}]} \right]_{204\text{Hg}^{\text{II}}_{\text{wt}}} \right)}$) and BSAF

determined using isotope enriched Hg^{II} and MeHg tracers in the mesocosm experiment, where e.g. $\text{BSAF}_{204\text{Hg}^{\text{II}}_{\text{wt}}}$ denotes BSAF for MeHg formed from the $^{204}\text{Hg}^{\text{II}}_{\text{wt}}$ tracer.

eq. S2. Calculation of MeHg concentration (pmol g⁻¹ d.w.) in biota.

$$\begin{aligned}
 [\text{MeHg}]_{\text{biota}} = & [\text{MeHg}]_{\text{Hg}^{\text{II}} \text{ atmospheric load}} \times \text{BSAF}_{204\text{Hg}^{\text{II}}_{\text{wt}}} + [\text{MeHg}]_{\text{Hg}^{\text{II}} \text{ catchment runoff load}} \times \\
 & \text{BSAF}_{204\text{Hg}^{\text{II}}_{\text{wt}}} + ([\text{MeHg}]_{\beta\text{HgS}} + [\text{MeHg}]_{\text{Hg}^{\text{II}}-\text{NOM}}) \times \text{BSAF}_{\text{average}(\beta^{200}\text{HgS}_{\text{sed}}, 201\text{Hg}^{\text{II}}-\text{NOM}_{\text{sed}})} + \\
 & [\text{MeHg}]_{\text{MeHg atmospheric load}} \times \text{BSAF}_{\text{Me}^{199}\text{Hg}_{\text{wt}}} + [\text{MeHg}]_{\text{MeHg catchment runoff load}} \times \\
 & \text{BSAF}_{\text{Me}^{199}\text{Hg}_{\text{wt}}} + [\text{MeHg}]_{\text{MeHg-Stored}} \times \text{BSAF}_{\text{Me}^{198}\text{Hg}-\text{NOM}_{\text{sed}}}
 \end{aligned}$$

Mesocosm - scale 1:50
(mm)



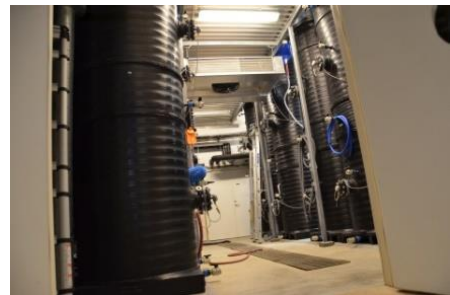
Sampling of sediment cores



Hg isotope tracer additions to intact sediment cores



Mesocosm facility – top floor



Mesocosm facility – bottom floor

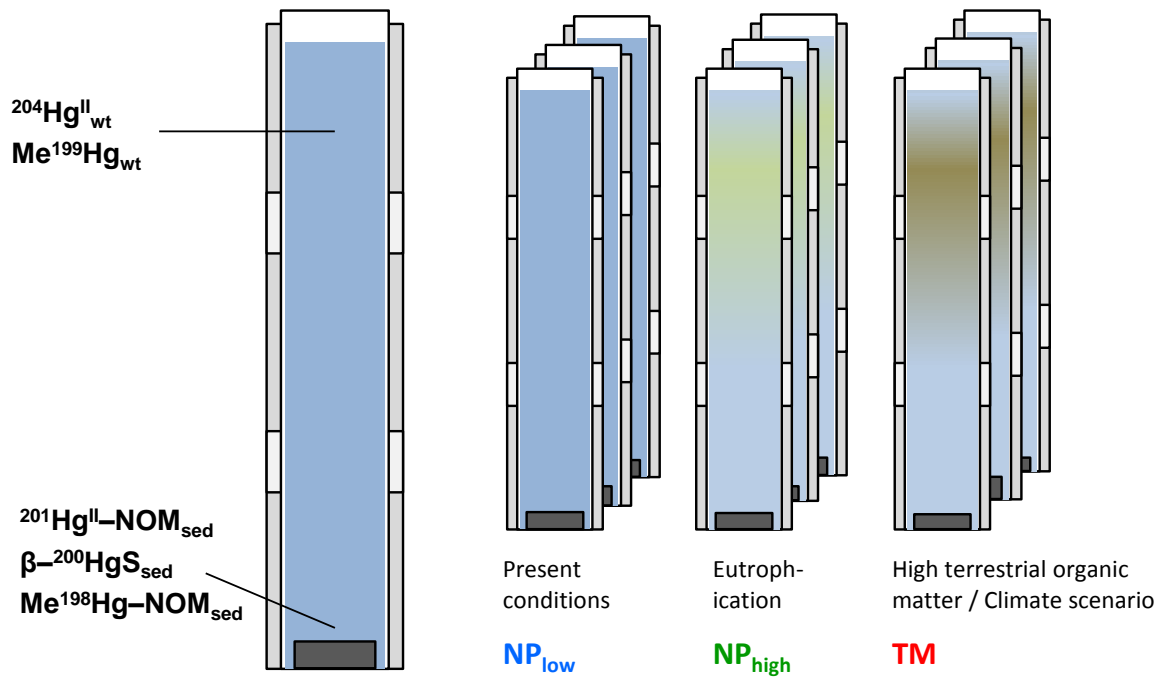


fig. S1. Schematic illustration of the mesocosm experiment setup.

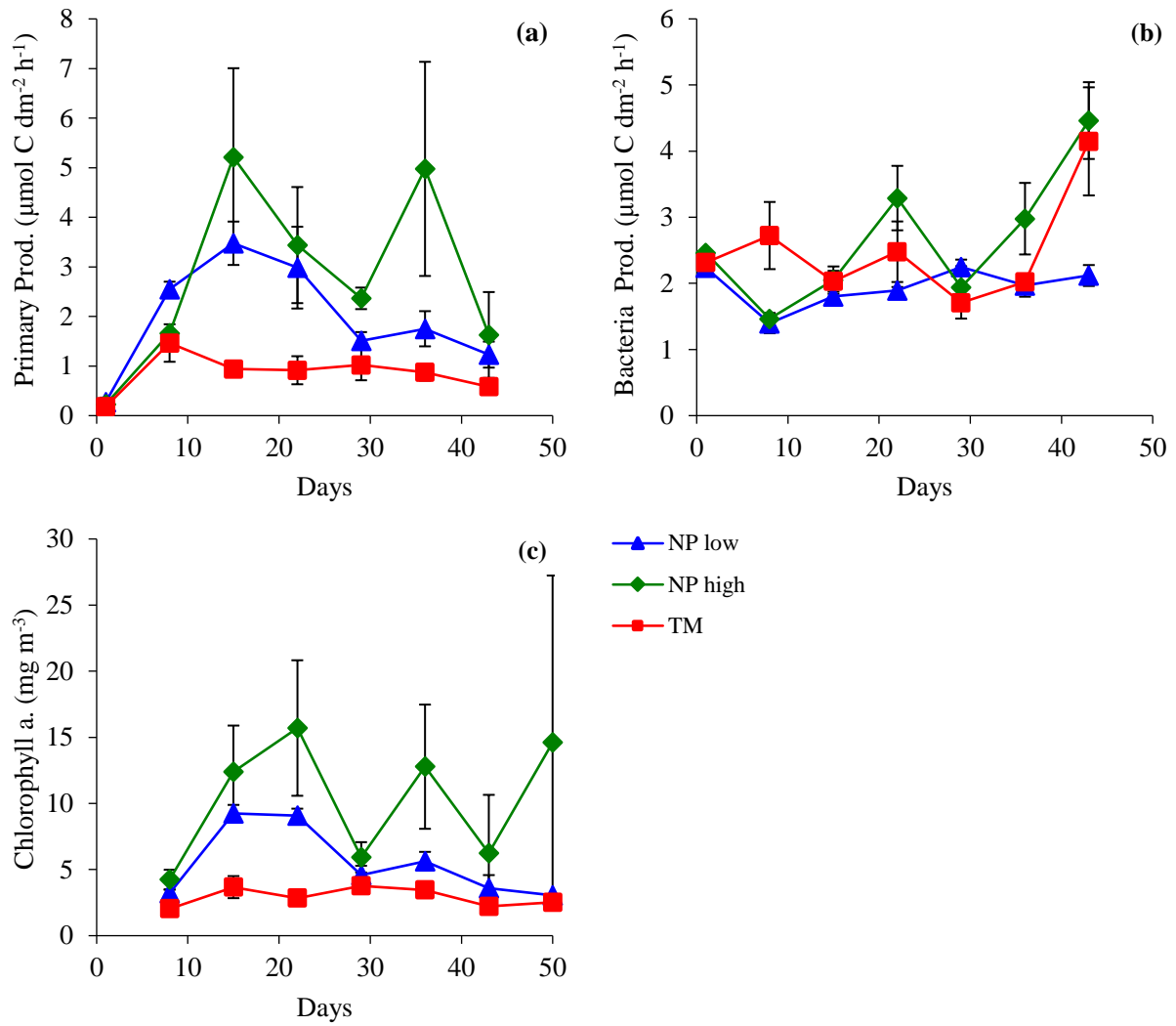


fig. S2. Pelagic biological productivity parameters. Average \pm SE ($n=3$) of rates of (a) photosynthetic primary production, (b) heterotrophic bacterial production, and concentration of (c) chlorophyll a, at different days in response to each of the three experimental treatments; Low nutrient input (NP_{low} , blue triangles), high nutrient input (NP_{high} , green diamonds), and terrestrial NOM input (TM, red squares). The data for the NP_{low} treatment was reproduced with permission from Jonsson *et al.* (10).

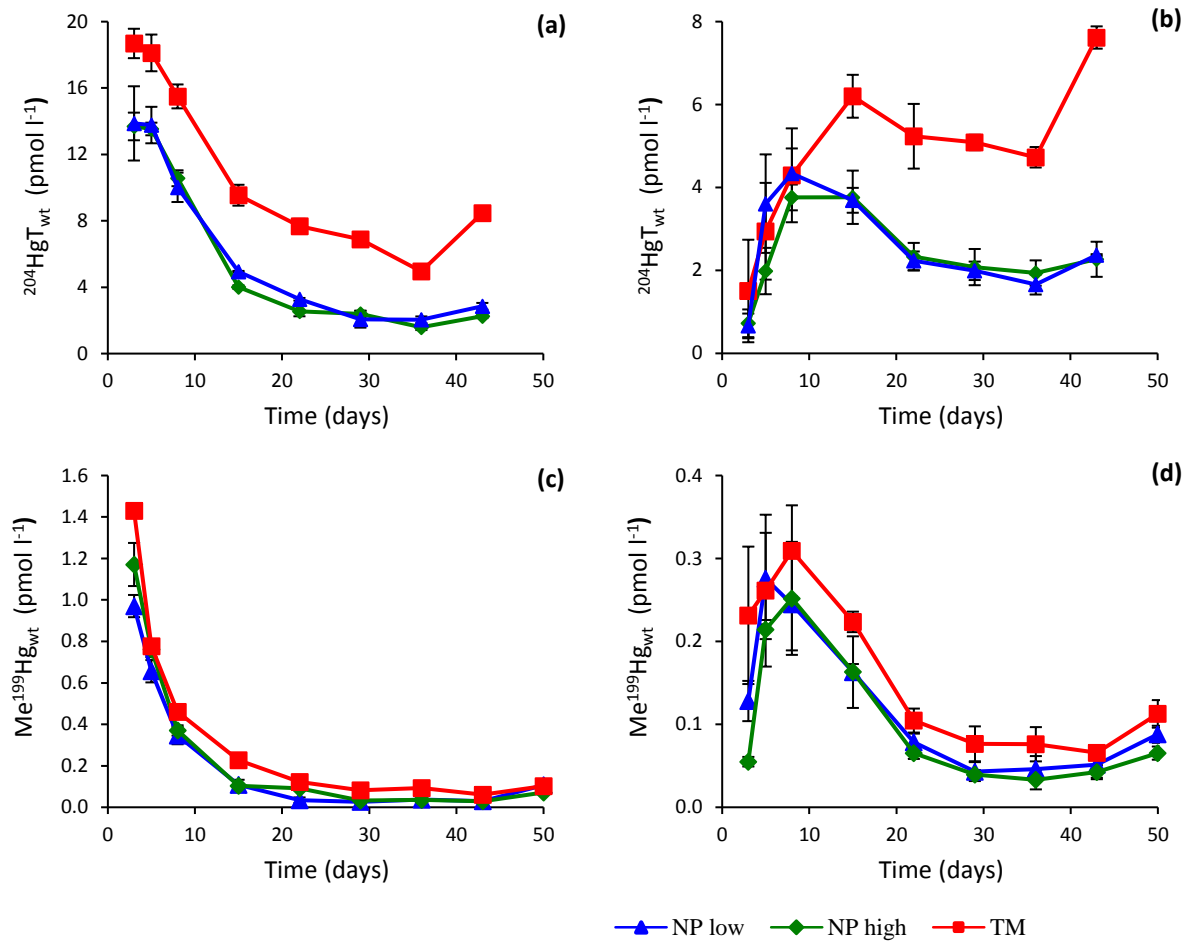


fig. S3. Total ^{204}Hg and Me^{199}Hg concentrations in water. Total Hg concentration in filtered ($0.45\ \mu\text{m}$) water for the $^{204}\text{Hg}^{\text{II}}_{\text{wt}}$ tracer (a) above (sampling at 1.5 m) and (b) below (sampling at 4m) the thermocline and MeHg concentration in water for the $\text{Me}^{199}\text{Hg}_{\text{wt}}$ tracer (c) above (sampling at 1.5 m) and (d) below (sampling at 4m) the thermocline during the course of the experiment (average of $n=3$ at each sampling occasion, SE is given as error bars). The different mesocosm treatments are indicated as: low nutrient input (NP_{low} , blue triangles), high nutrient input (NP_{high} , green diamonds), and terrestrial NOM input (TM, red squares) The data for the NP_{low} treatment was reproduced with permission from Jonsson *et al.* (10).

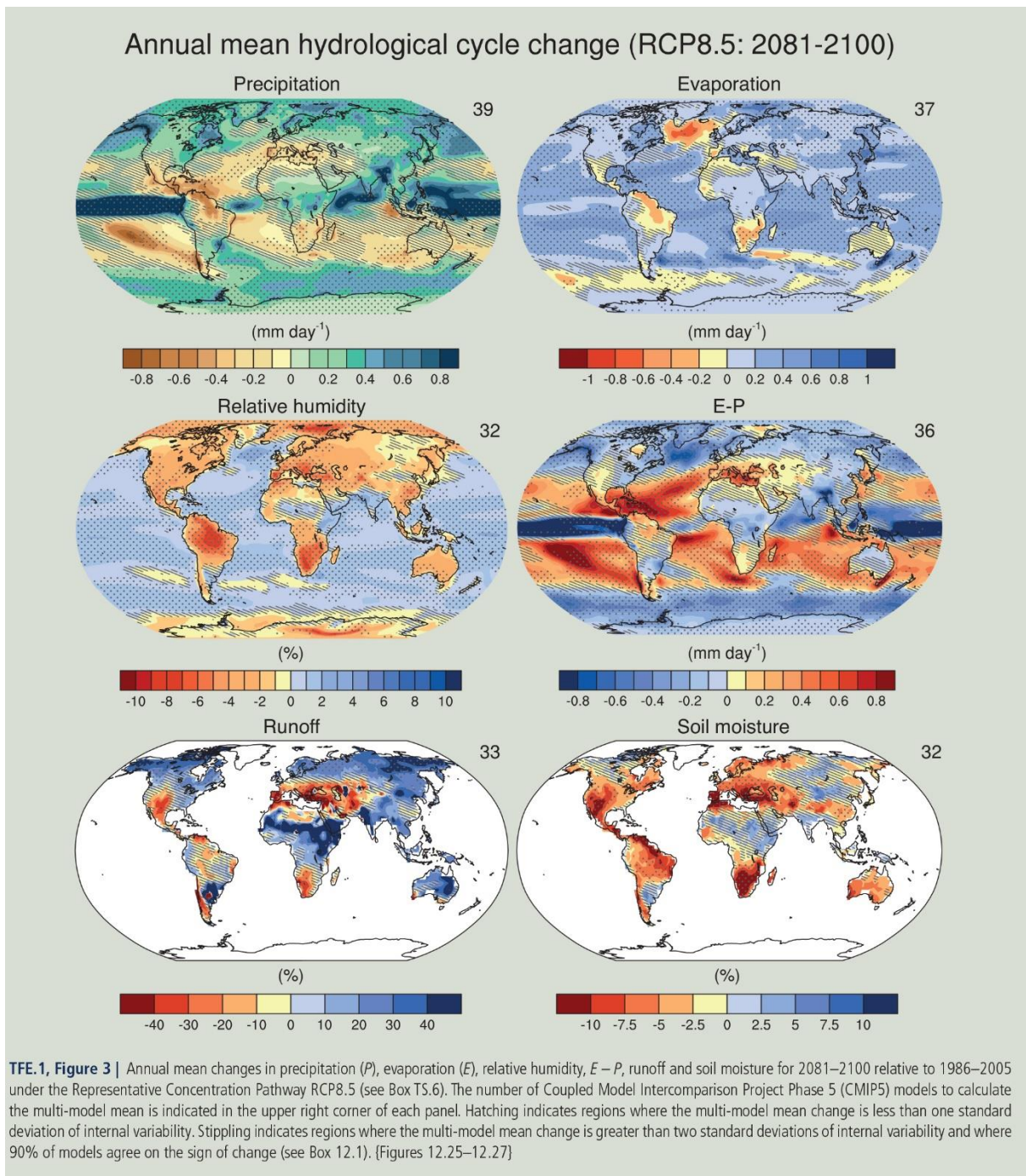


fig. S4. Projected changes in runoff (lower left figure) following climate scenario RCP8.5 (IPCC) (7). The figure is reproduced with permission from: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G. K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 33–115, doi:10.1017/CBO9781107415324.005. TFE.1, Page 45, Figure 3. https://www.ipcc.ch/pdf/assessment-report/ar5/wg1/WG1AR5_SummaryVolume_FINAL.pdf

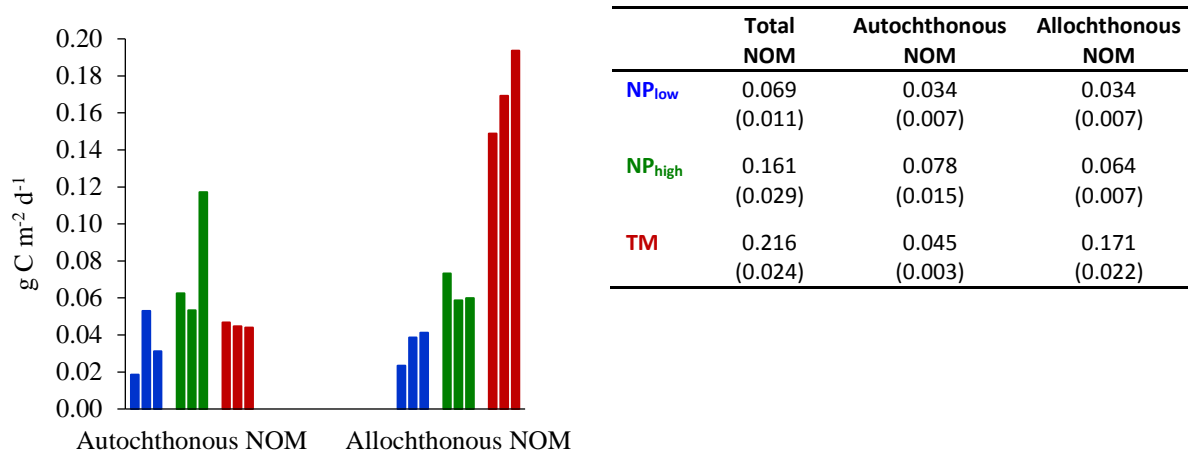


fig. S5. Sedimentation rate of NOM. Sedimentation rate ($\text{g C m}^{-2} \text{d}^{-1}$) for autochthonous and allochthonous NOM during day 38–50 for individual mesocosm tanks in response to each of the three experimental treatments; Low nutrient input (NP_{low} , blue), high nutrient input (NP_{high} , green), and terrestrial NOM input (TM, red). Average (SE) $n=6$ for each mesocosm treatment are given in the table.

table S1. Schematic description of the mesocosm experiment treatments.

Designation	Loading treatments	Anticipated treatment effects	Conceptually simulated scenario
NP _{low}	Moderate nutrients	Autotrophic pelagic food web Moderate primary production and sedimentation rate of autochthonous NOM	Present conditions in the Bothnian Sea
NP _{high}	High nutrients	Autotrophic pelagic food web High primary production and sedimentation rate of autochthonous NOM	Eutrophication scenario
TM	Low nutrients, High terrestrial organic matter	Heterotrophic pelagic food web Low primary production and sedimentation rate of autochthonous NOM, high sedimentation rate of allochthonous NOM	Scenario for increased terrestrial runoff induced by climate change

table S2. Chemical speciation calculations for Hg^{II} and MeHg in the mesocosm water phase.

	NP _{low}		NP _{high}		TM	
	M	permil	M	permil	M	permil
MeHgSR	9.1×10 ⁻¹⁴	1000	1.0×10 ⁻¹³	1000	1.6×10 ⁻¹³	1000
MeHgCl	1.9×10 ⁻¹⁷	0.21	2.2×10 ⁻¹⁷	0.22	3.1×10 ⁻¹⁷	0.20
MeHgOH	1.8×10 ⁻¹⁸	0.02	2.1×10 ⁻¹⁸	0.02	2.9×10 ⁻¹⁸	0.02
Hg(SR) ₂	1.7×10 ⁻¹¹	1000	2.5×10 ⁻¹¹	1000	3.3×10 ⁻¹¹	1000
HgCl ₂	9.5×10 ⁻²⁴	0.00	1.6×10 ⁻²³	0.00	1.6×10 ⁻²³	0.00
Hg(OH) ₂	1.6×10 ⁻²⁷	0.00	2.7×10 ⁻²⁷	0.00	2.7×10 ⁻²⁷	0.00
HgOHCl	2.5×10 ⁻¹⁸	0.00	4.1×10 ⁻¹⁸	0.00	4.1×10 ⁻¹⁸	0.00

Chemical speciation calculations were conducted using the thermodynamic constants provided in Skjellberg 2008(62) and input concentrations NP_{low}: 0.091 pM MeHg, 17 pM Hg^{II}, 60 mM Cl⁻ and 4.8 mg l⁻¹ DOC (assuming RSH = 0.15 mass-% of DOC(62)) and a pH of 7.55; NP_{high}: 0.10 pM MeHg, 25 pM Hg^{II}, 60 mM Cl⁻ and 4.8 mg l⁻¹ DOC (assuming RSH = 0.15 mass-% of DOC(62)) and a pH of 7.55 and TM: 0.16 pM MeHg, 33 pM Hg^{II}, 60 mM Cl⁻ and 5.6 mg l⁻¹ DOC (assuming RSH = 0.15 mass-% of DOC(62)) and a pH of 7.55.

table S3. Statistics for the treatment effects from repeated-measures ANOVA for eight response variables (Table 1) in mesocosm.

	χ^2	p	G-G ϵ	H-F ϵ
DOC	9.75	0.0066	0.4794	0.7997
Humic subst.	16.1	0.0001	0.4702	1
Autotrophic prod.	25.7	0.0102	0.3485	0.6429
Heterotrophic prod.	16.6	0.0117	0.6411	1
<i>Chl a</i>	40.3	0.0465	0.3172	0.6157
MeHg/Hg ^{II} molar ratio				
β - ²⁰⁰ HgS _{sed}	17.6	0.0452	0.4923	1
²⁰¹ Hg ^{II} -NOM _{sed}	9.4	0.7865	0.5704	1
²⁰⁴ Hg ^{II} _{wt}	16.4	0.0633	0.4723	1

table S4. Input data for the Hg mass balance calculations based on eqs. S1 and S2.

Source ¹⁾	Present conditions (NP_{low} treatment)				
	Input amount (mole) ²⁾	Hg tracer ³⁾	MeHg/Hg ^{II} ⁴⁾	BSAF ⁵⁾ plankton	BSAF ⁶⁾ bentic inv.
MeHg catchment runoff load	0.10	Me ¹⁹⁹ Hg _{wt}	0.094	14	310
Hg ^{II} catchment runoff load	1.07	²⁰⁴ Hg ^{II} _{wt}	0.026	2.7	73
MeHg atmospheric load	0.0043	Me ¹⁹⁹ Hg _{wt}	0.094	14	310
Hg ^{II} atmospheric load	0.36	²⁰⁴ Hg ^{II} _{wt}	0.026	2.7	73
MeHg sediment pool	0.081	Me ¹⁹⁸ Hg–NOM _{sed}		2.1	44
Hg ^{II} sediment pool	79				
	0.7 × 79	β– ²⁰⁰ HgS _{sed}	0.00049	0.38	16
	0.3 × 79	²⁰¹ Hg ^{II} –NOM _{sed}	0.0084	0.38	16
Eutrophication scenario (NP_{high} treatment)					
Source	Input amount (mole)	Hg tracer	MeHg/Hg ^{II}	BSAF plankton	BSAF bentic inv.
MeHg catchment runoff load	0.10	Me ¹⁹⁹ Hg _{wt}	0.076	25	258
Hg ^{II} catchment runoff load	1.07	²⁰⁴ Hg ^{II} _{wt}	0.027	3.2	42
MeHg atmospheric load	0.0043	Me ¹⁹⁹ Hg _{wt}	0.076	25	258
Hg ^{II} atmospheric load	0.36	²⁰⁴ Hg ^{II} _{wt}	0.027	3.2	42
MeHg sediment pool	0.090	Me ¹⁹⁸ Hg–NOM _{sed}		2.2	22
Hg ^{II} sediment pool	79				
	0.7 × 79	β– ²⁰⁰ HgS _{sed}	0.00067	0.38	8.3
	0.3 × 79	²⁰¹ Hg ^{II} –NOM _{sed}	0.0092	0.38	8.3
Scenario for increased terrestrial runoff induced by climate change (TM treatment)					
Source	Input amount (mole)	Hg tracer	MeHg/Hg ^{II}	BSAF plankton	BSAF bentic inv.
MeHg catchment runoff load	0.13	Me ¹⁹⁹ Hg _{wt}	0.084	91	284
Hg ^{II} catchment runoff load	1.39	²⁰⁴ Hg ^{II} _{wt}	0.045	8.4	46
MeHg atmospheric load	0.0043	Me ¹⁹⁹ Hg _{wt}	0.084	91	284
Hg ^{II} atmospheric load	0.36	²⁰⁴ Hg ^{II} _{wt}	0.045	8.4	46
MeHg sediment pool	0.087	Me ¹⁹⁸ Hg–NOM _{sed}		4.9	41
Hg ^{II} sediment pool	79				
	0.7 × 79	β– ²⁰⁰ HgS _{sed}	0.00040	0.38	19
	0.3 × 79	²⁰¹ Hg ^{II} –NOM _{sed}	0.0077	0.38	19

¹⁾ Geochemical Hg sources included in the Hg mass balance based on Equation S1 and S2.

²⁾ Hg input amount from each source as described in Materials and Methods and Jonsson *et al.* (10). The input amounts are expressed as “load” for each source in Equations S1 and S2.

³⁾ Mesocosm isotope tracer used to simulate each geochemical Hg source.

⁴⁾ MeHg/Hg^{II} molar ratio in sediment determined for each isotope tracer in the mesocosm experiment and used in Equation S1.

⁵⁾ MeHg Biota Sediment Accumulation Factor (BSAF) for plankton determined for each isotope tracer in the mesocosm experiment and used in Equation S2.

⁶⁾ MeHg Biota Sediment Accumulation Factor (BSAF) for benthic invertebrates determined for each isotope tracer in the mesocosm experiment and used in Equation S2.

table S5. Nutrient addition scheme for the mesocosms water phase.

Day	NP _{low}				NP _{high}				TM		
	NO ₃ ⁻	PO ₄ ³⁻	NH ₄ ⁺	(%)	NO ₃ ⁻	PO ₄ ³⁻	NH ₄ ⁺	(%)	NO ₃ ⁻	PO ₄ ³⁻	NH ₄ ⁺
1	1.0	0.077	0.18	(10%)	5.0	0.39	0.9	(50%)		0.025 ¹⁾	0.16 ¹⁾
3	1.0	0.077	0.18	(10%)	5.0	0.39	0.9	(50%)	1.0	0.077	0.18 (10%)
8	1.0	0.077	0.18	(10%)	1.0	0.077	0.18	(10%)	1.0	0.077	0.18 (10%)
9										0.012 ¹⁾	0.08 ¹⁾
12	1.0	0.077	0.18	(10%)	5.0	0.39	0.9	(50%)	1.0	0.077	0.18 (10%)
15	0.2	0.015	0.036	(2%)	5.0	0.39	0.9	(50%)			
18		0.015	0.036	(2%)		0.39	0.9	(50%)			
19										0.012 ¹⁾	0.08 ¹⁾
22	0.2	0.015	0.036	(2%)	5.0	0.39	0.9	(50%)			
33	0.2	0.015	0.036	(2%)	5.0	0.39	0.9	(50%)			
36					5.0	0.39	0.9	(50%)			
37										0.012 ¹⁾	0.08 ¹⁾

Nutrient values are given as the added concentrations (μM) in the 2000 liter mesocosm tanks. In brackets are given the percent values of the added concentrations relative to typical winter time nutrient concentrations in the Bothnian Sea ($10 \mu\text{M NO}_3^-$, $0.77 \mu\text{M PO}_4^{3-}$, $1.8 \mu\text{M NH}_4^+$). The nutrient addition scheme was fine tuned based on measured nutrient concentrations and no NO_3^- was therefore added on day 18 to the two NP treatments and not to NP_{low} on day 36.

¹⁾ Added nutrient concentrations to the TM treatment due to the presence of nutrients in the terrestrial NOM extract.