Redefining the Role of Wetlands as Methyl Mercury Sources

Insights from Wetlands Before and After Restoration

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Abstract

Current literature identifies boreal wetlands as net sources for the potent neurotoxin methyl mercury (MeHg). Combined with national environmental aims of restoration of previously drained wetlands, there is a possible conflict between the ecological benefits of wetlands and their role as MeHg sources.

This thesis presents a four-year study of seven Swedish boreal wetlands of different nutrient status subjected to restoration measures. Wetlands were characterized according to climate/geography, vegetation type and ancillary chemistry. Mercury (Hg) biogeochemistry was assessed by determination of proxies for long- and short-term net MeHg production rates in soils, i.e., %MeHg (of total Hg) and potential methylation and demethylation rate constants (k_m and k_d). MeHg exports from each wetland catchment were calculated. In addition, each wetland was assessed as a net MeHg source or sink, based on mass balance budgets.

Results follow similar patterns for %MeHg, k_m/k_d and budgets among the wetlands. The nutrient status of the wetlands affect the net production of MeHg, with wetlands of intermediate nutrient status, i.e., poor-fen types of wetlands, having the highest %MeHg, k_m/k_d and the largest net output of MeHg. MeHg budget results showed that six out of seven wetlands were net MeHg sources. The MeHg output varied more among wetlands, than before and after restoration measures of an individual wetland. This suggests that the nutrient status of a wetland is more important to the MeHg production than the performed restoration measures. A nutrient-rich *Alnus glutinosa* swamp was a net sink for MeHg during the entire study period. A spatial analysis along a gradient into the *Alnus* swamp showed an increased degradation of MeHg in the swamp soil. Snapshot budgets from nine additional swamps suggest that net degradation of MeHg is a general phenomenon for *Alnus* swamps.

Results from this thesis have implications for forest managers and landscape planners. Previously drained wetlands can be restored based on informed decisions, avoiding restoration of poor-fen types of wetlands. In addition, *Alnus* swamps should be maintained and restored if possible, helping to mitigate the production of MeHg in boreal landscapes.

Keywords: methyl mercury, mercury, wetlands, methylation, demethylation, wetland restoration

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Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Tjerngren I., Karlsson T., Björn E., Skyllberg U. (2011).
Potential Hg methylation and MeHg demethylation rates related to the nutrient status of different boreal wetlands.
Biogeochemistry, [Online early access]. DOI: 10.1007/s10533-011-9603-1.
http://www.springerlink.com/content/w70n57l513118350/

II Tjerngren I., Meili M., Björn E., Skyllberg U.
Boreal wetlands as sources and sinks for methyl mercury before and after small-scale flooding.
In review in Environmental Science & Technology

III Kronberg R-M.*, Tjerngren I.*, Drott A., Björn E., Skyllberg U.
Net degradation of methyl mercury in alder swamps.
Submitted manuscript

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Abbreviations

C/N	Carbon/nitrogen ratio
Ca	Calcium
CH_4	Methane
Cl	Chloride
CO_2	Carbon dioxide
DOC	Dissolved organic carbon
e.g.	*Exemple gratia* (for example)
EHT	Site Edshult (*Alnus* swamp)
Fe	Iron
Fe(II)	Ferrous iron (reduced form)
Fe(III)	Ferric iron (oxidized form)
FeRB	Iron reducing bacteria
FeS(s)	Mackinawite
GDL	Site Grundsdal (artificial wetland)
GTN	Site Gästern (mesotrophic wetland)
H_2S(aq)	Dissolved hydrogen sulfide
Hg	Mercury
Hg^0	Elemental mercury
Hg_{inorg}	Inorganic divalent mercury
HgS(s)	Cinnabar
i.e.	*Id est* (that is)
k_d	MeHg demethylation rate constant
k_m	Hg_{inorg} methylation rate constant
KSN	Site Kroksjön (shallow lake)
LDN	Site Långedalen (nutrient gradient including LDNA & LDNB)
LDNA	Site Långedalen A (upstream bog)
LDNB	Site Långedalen B (downstream fen)
m.a.s.l.	Meter above sea level

MeHg	Methyl mercury
Mg	Magnesium
Mn	Manganese
MoO_4^-	Molybdate
$N_2(g)$	Nitrogen gas
Na	Sodium
NO_3^-	Nitrate ion
NOM	Natural organic matter
pH	$-\log[H_3O^+]$, measure of hydrogen concentration
PO_4^{3-}	Phosphate ion
S	Sulfur
S^{2-}	Sulfide
S-HYPE	Swedish Hydrological Predictions for the Environment Model
SKM	Site Storkälsmyran (riparian)
SO_4^{2-}	Sulfate ion
SOC	Soil organic carbon
SRB	Sulfate reducing bacteria
SRD	Site Sjöarödd (open fen)
$SUVA_{254nm}$	Specific UV-absorbance at 254 nm, measure of C quality

1 Introduction

1.1 Mercury

Mercury (Hg) is mobilized into atmospheric, aquatic and terrestrial compartments from deep reservoirs in the earth by natural, e.g., volcanic eruptions, and anthropogenic activities (Selin, 2009). The latter is dominated by release of Hg during burning of fossil fuels, mining and industrial activities. Globally, Hg levels in the environment have increased due to anthropogenic activities by a factor of 3 to 5, increasing the risk for human exposure (Selin, 2009). Natural events and anthropogenic activities emit different forms of mercury, but the organic methyl mercury (MeHg) is only emitted in minor amounts (Selin, 2009). However, MeHg is the dominating form of mercury found in fish (Clarkson & Magos, 2006). Hence, in combination with the persistent, toxic and bioaccumulating properties of MeHg, the cycling of mercury in the environment is of major interest. The biogeochemical cycling of Hg poses a challenge because it encompasses a number of reaction pathways, speciation transformations and environmental compartments such as the atmospheric, marine and terrestrial systems (Morel *et al.*, 1998). The elemental form of Hg (Hg^0) is volatile and can be airborne for years resulting in long-range transport of the compound causing high loads of Hg even in remote, pristine areas (Lindqvist & Rodhe, 1985). For instance, Hg levels in Arctic biota have been reported to be high enough to cause concern for the health of the local human population (Donaldson *et al.*, 2010). In Sweden, about half of the lakes contain fish with Hg levels exceeding the lower threshold limit of 0.5 mg Hg/kg fish fresh weight set by the European Union (The Commission of the European Communities, 2006; Lindqvist *et al.*, 1991).

1.2 Wetlands

There are a number of definitions of the term "wetland", mainly including criteria related to the presence of water, hydric soil conditions and hydrophytic vegetation (Warner & Rubec, 1997; National Research Council, 1995; Finlayson & Moser, 1991; Cowardin *et al.*, 1979). The research presented in this thesis is based on the definition used by the Swedish Wetland Inventory (SVI): "Wetlands are areas where water table for the main part of the year is close, below, at or above the ground level, including vegetation covered lakes. A site is called a wetland when at least 50 % of the vegetation is hydrophilic, i.e., water loving. An exception is periodically flooded shores along lakes, seas and rivers, which are classified as wetlands despite a lack of vegetation." (Gunnarsson & Löfroth, 2009).

In addition, there are a number of ways to distinguish among different types of wetlands. The following terms will be used in this thesis (Mitsch & Gosselink, 2000): *Bogs* are defined as peat-accumulating wetlands only receiving water from precipitation, thus, characterized by nutrient-poor vegetation. *Fens* are classified as peat-accumulating wetlands that receive some drainage from surrounding uplands, supporting slightly more nutrient-rich vegetation. *Swamps* are defined as wetlands with mineral soil, not accumulating peat. The vegetation is characterized by tree stands and relatively nutrient-rich understory vegetation. *Lakes* are classified as wetlands with open-water areas, where any of the above mentioned wetland types occur at the lake edges. In addition, the term *peatlands* is used to denote both bogs and fens, i.e., peat-accumulating wetlands.

Over the past centuries, about half of the world's wetlands have been lost mainly as a consequence of wetland drainage for agricultural purposes (Zedler & Kercher, 2005). Over time, awareness of the ecological importance of wetlands has increased. Main ecological functions of wetlands include serving as habitat for a diversity of flora and fauna, reducing the risk of flooding, recharging aquifers and improving the water quality (Mitsch & Gosselink, 2000). As a response to the decrease in wetland area across the globe, the intergovernmental Ramsar Convention, signed in 1971, urged their members to maintain the ecological character of chosen wetlands and apply a sustainable use to all of their wetlands (The Ramsar Convention on Wetlands). In Sweden, the national environmental goal known as "thriving wetlands" aims at maintaining wetland ecosystem functions and restoring 12 000 ha of wetlands by 2012 (Swedish Environmental Protection Agency).

1.3 Methyl Mercury & Wetlands

Numerous reports have identified wetlands as major MeHg sources on a landscape level (Hall *et al.*, 2008; Hurley *et al.*, 1995; Rudd, 1995; St.Louis *et al.*, 1994). In accordance with such reports, the creation of wetlands by flooding of boreal soils has been found to increase the production of MeHg (Hall *et al.*, 2005; St.Louis *et al.*, 2004; Kelly *et al.*, 1997). Furthermore, comparisons of reservoirs of different ages with natural lakes have showed significantly higher MeHg concentrations in reservoir water than natural lake waters (Montgomery *et al.*, 2000). Also, a comparison between temporary and permanent impoundments showed higher MeHg concentrations in both types as compared to reference waters (Brigham *et al.*, 2002). Noteworthy is that both of the two latter studies suggest that higher MeHg concentrations can last for 10-20 years after impoundment. Thus, even temporary impoundments can have long-term impact on MeHg levels in the ecosystem.

The reported increase in MeHg production in wetlands is explained by the wetland supporting factors promoting methylation of inorganic Hg (Hg_{inorg}), including anoxic conditions and high availability of palatable carbon (Ravichandran, 2004). Because wetlands are part of, and thereby have a hydrological connection to, large watersheds, there is a risk for MeHg that is produced in wetlands to be transported to downstream waters and ultimately end up in fish. Thus, the international aim of wetland restoration for ecological purposes may be in conflict with aims of reducing MeHg exposure to humans. Consequently, it is of utmost importance to understand the role of wetlands in Hg cycling in order to mitigate drawbacks related to MeHg production of wetland restoration.

1.4 Formation & Degradation of Methyl Mercury

MeHg levels in the environment are the result of a number of processes, including the production of MeHg from Hg_{inorg} (methylation) and degradation of MeHg (demethylation).

Methylation of Hg_{inorg} is mainly the result of biotic processes. Sulfate reducing bacteria (SRB) have been identified as main methylators in both sediments and soils (Gilmour *et al.*, 1992; Compeau & Bartha, 1985). There are also reports on iron reducing bacteria (FeRB) producing MeHg in natural environments (Fleming *et al.*, 2006; Kerin *et al.*, 2006). Although the methylation mechanism is not understood in detail, a number of factors have been identified as important (Figure 1). These factors include the composition and activity of the microbial community, which are dependent on the availability of nutrients, metabolic electron acceptors, redox conditions, and

temperature (Ullrich *et al.*, 2001). For instance, a number of reports demonstrate an increased MeHg production due to an increased SRB activity after addition of sulfate (SO_4^{2-}) to mesocosms (Mitchell *et al.*, 2008a; Jeremiason *et al.*, 2006) and freshwater sediments (Gilmour *et al.*, 1992). Likewise, the importance of carbon as an electron donor during SO_4^{2-} reduction for MeHg production has been demonstrated both directly by carbon stimulation (Mitchell *et al.*, 2008a) and indirectly through comparisons among environments with different carbon qualities and/or quantities (Windham-Myers *et al.*, 2009; Lambertsson & Nilsson, 2006; Hall *et al.*, 2005).

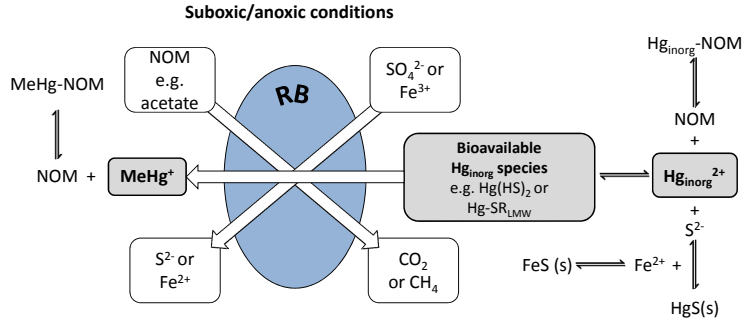


Figure 1. Schematic illustration of the theory behind MeHg production by SRB and FeRB. White solid lines illustrate bacterial reactions.

Methylation rates have been suggested to be limited by the amount of Hg_{inorg} available for bacterial uptake (Benoit *et al.*, 2003). The bioavailable amount of Hg_{inorg} has been shown to be affected by sulfide concentrations, organic matter, pH, and redox (Benoit *et al.*, 2003). Due to the affinity of Hg to sulfide, the speciation of Hg is largely governed by S^{2-}concentrations (Benoit *et al.*, 2003). Fe(II) additions have been shown to decrease MeHg production in wetland slurries due to the formation of mackinawite, FeS(s) (Mehrotra & Sedlak, 2005, Rickard & Luther, 2007). A number of dissolved, neutral mercury-sulfide and/or low-molecular weight mercury-thiol complexes have been suggested to be available for bacterial uptake (Schaefer & Morel, 2009; Skyllberg, 2008; Benoit *et al.*, 1999; Paquette & Helz, 1997). In addition, sulfur atoms have been shown to be the main binding sites for Hg in natural organic matter (NOM) (Skyllberg *et al.*, 2006). This strong binding explains the role of dissolved organic carbon (DOC) as transporting agent for Hg, illustrated by the coupling of DOC and Hg transport in stream water (Dittman *et al.*, 2010; Schuster *et al.*, 2008). The complexation of DOC with Hg_{inorg} has also been suggested to decrease methylation rates at high DOC concentrations (Winfrey & Rudd, 1990). The effect of pH on methylation rates is complex,

but generally lower pH has been linked to higher methylation rates in lake water and at the water/sediment interface (Gilmour & Henry, 1991; Winfrey & Rudd, 1990). Higher methylation rates at lower pH were explained by an increased bacterial uptake of Hg_{inorg} (Kelly *et al.*, 2003). However, decreased sediment methylation rates at lower pH have also been reported (Steffan *et al.*, 1988).

In contrast to methylation, a number of MeHg demethylation mechanisms are described. The reported abiotic mechanisms are dominated by photodemethylation in open waters (Sellers *et al.*, 1996). Biotic mechanisms include a "reductive" mechanism and an "oxidative" demethylation. Reductive demethylation involves resistance to Hg in bacteria carrying the *mer* operon where methane (CH_4) is the end product (Liebert *et al.*, 1999). This mechanism has been shown to occur in marine and freshwater environments (Pearson *et al.*, 1996; Dahlberg & Hermansson, 1995). Oxidative demethylation is performed by both methanogens and SRB (Oremland *et al.*, 1991). This mechanism has also been reported to occur in a diversity of environments such as marine, estuarine and freshwater sediments (Marvin-DiPasquale *et al.*, 2000; Marvin-Dipasquale & Oremland, 1998; Oremland *et al.*, 1995). Factors suggested to control biotic demethylation include composition and activity of the microbial community as well as bioavailability of MeHg (Marvin-DiPasquale *et al.*, 2000). Biotic MeHg demethylation has been suggested to increase with organic matter content, reduced sulfur species and higher rates of anaerobic metabolism (Marvin-DiPasquale *et al.*, 2000). In the same study, the oxidative demethylation mechanism was suggested to dominate at uncontaminated total Hg levels.

1.5 Objectives

The principal aim of this thesis was to determine if certain types of boreal wetlands are more prone to net MeHg production than others. The results will help forest managers and landscape planners to make informed decisions about what type of wetlands to restore. By studying the Hg biogeochemistry in seven Swedish wetlands with different nutrient status before, during and after restoration, the following questions were addressed:

- Are certain types of wetlands more prone to net MeHg production than others?
- What is the effect of restoration on production of MeHg in wetlands?
- What processes control production and degradation of MeHg in boreal wetlands?
- Is there a link between processes in wetland soils and stream-water exports?

In paper I, the wetlands were categorized according to nutrient status based on climatic parameters, vegetation types and ancillary chemistry such as pH, carbon quality (as determined by $SUVA_{254nm}$), iron (Fe), SO_4^{2-} and the carbon/nitrogen ratio (C/N). Soil concentrations of total Hg and MeHg were compared among the wetlands as well as potential Hg_{inorg} and MeHg demethylation rates.

In paper II, the strength of each wetland as a source or a sink for MeHg was determined by calculating stream-water export and mass balance budgets. Budgets were also calculated for Hg_{inorg}, DOC and SO_4^{2-}. Export and mass balance budget results were compared among the wetlands, with their corresponding Hg soil biogeochemistry and before/after restoration.

In paper III, the net degradation of MeHg in the *Alnus* swamp EHT was studied in more detail, both spatially along a gradient into the wetland and temporally over the years 2006 to 2010. A synoptic study of nine additional swamps and two contrasting peatlands was performed, to determine if *Alnus* swamps in general are sinks for MeHg.

2 Materials & Methods

2.1 Study Sites

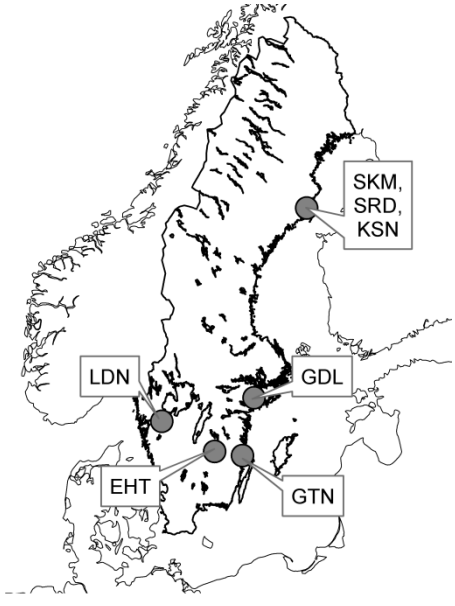


Figure 2. Location of the seven wetlands of this study. © Lantmäteriet, I2011/0032.

Soils and streams of seven boreal Swedish wetlands (Figure 2) were studied during 2006 to 2010. The wetlands were chosen in collaboration with the forestry companies Holmen Skog AB and Sveaskog AB, who were responsible for restoration of the sites.

All wetlands have clearly defined inlet and outlet streams, with outlet streams marking the outlet of the catchment. Details for the wetland catchments, including maps, are found in paper II. The specific wetlands

receive water from catchments consisting of various types of land use areas, including additional wetlands. Thus, the seven wetlands are henceforth termed "specific wetlands" to separate them from other wetlands within the catchments. Based on geographic location, climate, vegetation type and nutrient status, the specific wetlands were divided into three subgroups (Table 1).

Table 1. Summary of wetland characteristics including type of wetland, specific wetland and catchment area, geographic location and classification into subgroup. Temperature sum (°C) for 2007 to 2010 calculated as the annual sum ±SE of summarized daily mean air temperature exceeding 5°C.

Site	Wetland type	Specific wetland area (km^2)	Catchment area (km^2)	Location in Sweden	Tsum (°C)	Subgroup
SKM	Riparian	0.020	0.48	North	1954±34	Northern nutrient-poor
SRD	Open fen	0.084	1.2			
KSN	Shallow lake	0.26	1.0			
LDNA	Bog	0.078	0.91	Southwest	2595±69	Nutrient gradient LDN
LDNB	Fen	0.028	1.1			
EHT	*Alnus* swamp	0.042	0.58	Southeast	2356±46	Southern nutrient-rich
GTN	Mesotrophic lake	0.58	23		2721±72	
GDL	Artificial wetland	0.031	0.37		2598±28	

Below follows a brief description of the specific wetlands and their catchments. Detailed descriptions are found in paper I.

2.1.1 The Northern Wetlands

Figure 3. The northern wetlands (from left to right): site SKM, SRD and KSN.

The northern wetlands include sites Storkälsmyran (SKM), Sjöarödd (SRD) and Kroksjön (KSN) (Figure 3). The sites are closely situated (all three catchments are situated within an area of 4 km^2) and soils are dominated by Spodosols and Histosols (Soil Survey Staff, 2010). The three sites differ in terms of hydrological characteristics and type of land use as described below.

Site SKM is a forested riparian zone wetland. In October 2007, 0.032 km^2 of the western forested part of the catchment was harvested. In May 2008, the site was dammed causing a maximum of 50 cm increase in water table over the specific wetland during high-flow events. The tree layer is dominated by *Betula pubescens* and *Picea abies* while *Carex spp.* dominates the field layer. Before damming, there were only scattered pockets of peat soils with bottom layers dominated by *Sphagnum spp.* and *Polytricum spp.* After damming, *Sphagnum spp.* and *Polytricum spp.* dominated the bottom layer of the flooded wetland. The catchment surrounding the specific wetland SKM consists of semi-open wetlands and forested uplands dominated by *Picea abies* and *Pinus silvestris*.

Site SRD is a nutrient-poor open fen without open water areas. At the specific wetland SRD, the field layer is dominated by *Carex spp.* while the bottom layer is dominated by *Sphagnum fallax*. The maximum peat thickness is 3 m. The catchment surrounding the specific wetland SRD is dominated by open wetlands and uplands. Uplands are forested by *Picea abies* on Histosols. Site SRD was drained in the 1920s by the creation of a deep central ditch. Over time, the ditch was filled by erosion and peat formation. Two new ditches were created in June 2008 and 30 cm of peat was excavated from a 0.02 km^2 area near the outlet. In May 2009 a dam was created at the outlet, causing a water table increase of up to 50 cm during high flow events. The water level was then managed by drainage of the wetland during the winter season (November to April) and damming during the summer season (May to October).

Site KSN is a shallow lake which was drained in the 1920s for agricultural purposes. After abandonment in the 1950s, the water table increased due to soil

erosion filling up the ditch. As a consequence a peat thickness of 30 cm formed on the lake shore, with field layers dominated by *Carex rostrata* and bottom layers by *Sphagnum fallax*. On the lake, floating mats of *S. lindbergii* developed. Site KSN was dammed in June 2008 causing a 40 cm increase in water level at the outlet. The catchment surrounding the specific wetland KSN consists of semi-open wetlands forested by *Pinus silvestris*, Histosols covered by *Picea abies* and upland till soils forested by *Picea abies*, *Pinus silvestris* and *Betula pubescens*.

2.1.2 The Nutrient Gradient

Figure 4. The nutrient gradient site LDN (from left to right): the poor-bog part LDNA and the poor-fen part LDNB.

Site Långedalen (LDN, Figure 4) is considered a nutrient gradient as the upstream part consists of an ombrotrophic bog (LDNA) and the downstream part of a poor-fen (LDNB). The specific wetland LDN is a narrow valley-bottom wetland formed at the table mountain Hunneberg (70 m.a.s.l.). Catchment uplands are dominated by Spodosols and Inceptisols while Histosols dominate wetland areas. The upland tree layer is dominated by *Picea abies* and *Pinus silvestris*. The field layer of the poor-bog LDNA consists of *Calluna vulgari* and less nutrient-demanding *Carex spp.* while the bottom layer is dominated by *Sphagnum spp.* The poor-fen LDNB is sparsely covered with a tree layer consisting of *Betula pubescens*, *Alnus glutinosa* and *Picea abies*. The field layer is dominated by patches of broad-leaved grasses and more nutrient-demanding *Carex spp.* The bottom layer is dominated by *Sphagnum* mosses. Site LDN was drained in the 1950s. Restoration measures first took place in 2008 by harvesting *Pinus silvestris* stands intruding on the former open bog. In May 2009, two dams of 0.005 km^2 each were created upstream and downstream the open bog LDNA.

2.1.3 The Most Nutrient-Rich Wetlands

Figure 5. The most nutrient-rich sites (from left to right): site EHT, GTN and GDL.

The most nutrient-rich southern wetlands include site Edshult (EHT), Gästern (GTN) and Grundsdal (GDL) (Figure 5). These sites are the most nutrient-rich sites of the study, as determined by their vegetation and measured ancillary chemistry.

Site EHT is a productive *Alnus glutinosa* swamp. The upstream third of the specific wetland EHT is dominated by broad-leaved grasses, herbs (e.g., *Oxalis acetosella*) and ferns (e.g., *Athyrium filix-femina*). Tree layers include approximately 60 % *Alnus glutinosa* and 40 % *Picea abies*. The middle third of the specific wetland is dominated by tall ferns and *Scirpus sylvaticus*. *Alnus glutinosa* dominates the tree layer with only 10 % *Picea abies*. The downstream third of the specific wetland is dominated by broad-leaved grasses and nitrogen-demanding herbs such as *Urtica dioica*. In this area *Betula pubescens* dominates the tree layer while *Alnus glutinosa* is absent. The inlet water to EHT originates from a 0.084 km^2 upstream drained bog, 0.37 km^2 of drained peat soils and a 0.03 km^2 clear-cut. This generates acidic and NOM-rich water. During the entire study period the specific wetland EHT has been saturated with water but lacking open-water surfaces. Restoration measures were conducted in May 2008 by rerouting the secondary outlet towards the main outlet.

Site GTN is a mesotrophic wetland. The total catchment area is 23 km^2, i.e., by far the largest catchment of the sites in this study. The tree layer of the catchment upland is dominated by *Pinus silvestris* and *Quercus robur* while *Calluna vulgaris* dominates the field layer. The bottom layer of the uplands is dominated by *Pleurozium schreberi* feather mosses. Before drainage in the 1920s, site GTN was a eutrophic lake. The lake was restored by damming the outlet in October 2006, resulting in an increased open water area corresponding to more than half of the original lake area.

Site GDL is an artificial wetland created by flooding former agricultural pastures. The tree layer of the catchment is dominated by single species plantations and mixed *Pinus silvestris* and *Picea abies* stands. *Vaccinium*

shrubs and feather mosses dominate the field and bottom layers, respectively. Before restoration by damming of the outlet in spring 2007, the now flooded areas were dominated by thin organic horizons or grass vegetations on top of heavy clayey-silty soil. Only small areas of Histosols were present in the center of the present wetland. After restoration, little new vegetation has developed and the wetland is dominated by open water.

An extended study of *Alnus* swamps was performed in May 2009 and 2010. Detailed descriptions of the ten additional sites are found in paper III. Briefly, the study included stream-water sampling of nine additional swamps, eight of which were dominated by *Alnus glutinosa* and one swamp dominated by *Betula pubescens*. The field layers of the swamps were dominated by nutrient-demanding herbs and broadleaved grasses such as *Scirpus sylvaticus*. The *Alnus* swamps covered small areas (0.011 to 0.038 km^2) and they all had a distinct stream inlet and outlet.

Additionally, two contrasting peatlands were sampled: site LDN and site Ystebo. The latter was characterized as a *Betula* bog with a tree layer dominated by *Betula pubescens* and a bottom layer consisting of *Sphagnum* mosses.

2.2 Sampling & Chemical Analyses

Analyses of chemical elements/compounds in soil (Table 2) were performed to evaluate Hg biogeochemistry and nutrient status of each wetland. Soils were sampled in November 2006, September 2007 and 2008, and May 2009 at most sites. Details on soil sampling and soil sample pretreatment are found in paper I. Some key points regarding soil sampling are mentioned below. For instance, samples were usually collected in the central parts of the wetlands, in some cases closer to the outlet. The groundwater table at sampling occasion was used to define the top level of the soil core. To adjust for effects of water level fluctuations due to seasonal variations and restoration measures, sampling was done where the water table was within centimeters of the soil surface. In the laboratory, pretreatment of all soil samples was done under N_2(g)-atmosphere in a glovebox to reduce the risk of oxidation. As a further step to reduce the risk of measuring aerated samples, the top 2 cm of the soil sample was removed before starting soil pretreatment. Soils were homogenized using a glass stick. Pore water was extracted by centrifugation.

Table 2. Analyses performed on soil, water and precipitation samples. Details for soil and stream-water sample analyses are found in paper I while details for analyses of precipitation and shallow groundwater samples are found in paper II.

Sample fraction	Elements/compounds analyzed
Intact soil core	pH, $H_2S(aq)$
Homogenized soil core	Potential Hg_{inorg} methylation & MeHg demethylation rate (incubation studies), total Hg and MeHg
Solid phase	C, N, S, Fe
Pore & stream water	Total Hg and MeHg, DOC, DIC, $SUVA_{254nm}$, Fe(II)/(III)[1], anions (e.g., SO_4^{2-}, NO_3^-) and total element concentrations (Cl, S, Ca, Fe, Mg, Mn, Na)
Open field precipitation, snow & shallow groundwater	pH, Cl, SO_4^{2-}, total Hg, MeHg, DOC[2]

[1] Pore water only

[2] Shallow groundwater samples only

Stream-water samples were analyzed for elements/compounds related to the Hg biogeochemical cycle (Table 2) in order to evaluate the wetlands as sources and/or sinks for Hg_{inorg} and MeHg (paper II). Water samples from inlet and outlet streams were collected at 29 to 34 occasions for each wetland during 2007 to 2010, with emphasis on the growing season and high flow events. Details on water sampling and pretreatment are found in paper II. Sampling of open-field precipitation, snow and shallow groundwater was performed in 2010 and are described in detail in paper II. In addition, monthly data on Cl and SO_4^{2-}concentrations in precipitation (open field and throughfall) were obtained from the Crown Drip Measurement Network (Swedish Environmental Institute, IVL, Göteborg, Sweden). Monthly total Hg concentrations in precipitation were obtained from IVL. Sampling of inlet and outlet streams of eight additional *Alnus* swamps, one *Betula* swamp and one *Betula* peatland is described in detail in paper III.

Details for most analyses are found in paper I, whereas details on analyses of precipitation and shallow groundwater samples are found in paper II.

2.3 Incubation Studies

Incubation studies were performed on homogenized soil cores to determine the potential rates of Hg_{inorg} methylation and MeHg demethylation (Lambertsson *et al.*, 2001). Details are found in paper I.

Briefly, approximately 10 g of sample was weighted in two centrifuge tubes. In-house prepared aqueous species specific tracers of $Me^{204}HgCl$ and

$^{201}Hg(NO_3)_2$ (Snell *et al.*, 2000), corresponding to 10 % of the total MeHg and Hg_{inorg} concentrations, were added to each sample. One tube, denoted T<1h, was immediately put in the freezer while the other tube, denoted T48h, was incubated in the glovebox, at room temperature in the dark for 48h. Incubation was terminated by placing the T48h tube in the freezer. The frozen samples were thawed and an internal standard, $Me^{200}HgCl$, was added. Solid-liquid extraction was performed using $KBr/CuSO_4/H_2SO_4/CH_2Cl_2$ after which the MeHg species were transferred to an aqueous phase. MeHg was ethylated using $NaB(CH_2CH_3)_4$ in acetate buffer followed by preconcentration onto Tenax TA adsorption tubes. Analysis was performed using thermal desorption GC-ICPMS. Isotope dilution analysis was used to determine MeHg concentrations (Qvarnstrom & Frech, 2002). Both methylation and demethylation rates were described by first-order kinetic models, assuming negligible demethylation of $Me^{201}Hg$ and methylation of $^{204}Hg_{inorg}$ during the incubation time (Hintelmann *et al.*, 2000). Potential rates of Hg_{inorg} methylation (ng g^{-1} day^{-1}) were calculated using equation 1. Potential methylation rate constants (k_m, day^{-1}) were calculated using equation 2.

$$\text{Potential } Hg_{inorg} \text{ methylation rate} = ([Me^{201}Hg_{T48h}]-[Me^{201}Hg_{T<1h}])\times 0{,}5 \qquad (1)$$

$$k_m = (\text{Potential } Hg_{inorg} \text{ methylation rate}) / [^{201}Hg(NO_3)_{2tracer}] \qquad (2)$$

Potential MeHg demethylation rates (ng g^{-1} day^{-1}) and potential demethylation rate constants (k_d, day^{-1}) were calculated using equation 3 and 4, respectively.

$$\text{Potential MeHg demethylation rate} = ([Me^{204}Hg_{T<1h}]-[Me^{204}Hg_{T48h}])\times 0{,}5 \qquad (3)$$

$$k_d = -1 \times \ln([Me^{204}Hg_{tracer}]-([Me^{204}Hg_{T<1h}]-[Me^{204}Hg_{T48h}]))-\ln([Me^{204}Hg_{tracer}]) \qquad (4)$$

Molybdate (MoO_4^-), an inhibitor of SO_4^{2-} reducing bacterial activity, was also added to incubated soil samples collected in May 2009. Two levels of MoO_4^- were added, corresponding to 100 % and 500 % of ambient SO_4^{2-} concentrations in pore water.

2.4 Export & Mass Balance Budgets

Annual MeHg export budgets were estimated for each catchment. The strength of each wetland as a sink or source for MeHg was determined by calculating the net MeHg yield from estimated MeHg mass balances. Below follows brief descriptions for how catchment areas and water budgets were calculated. The

descriptions also include calculations for the export and mass balance budgets by combining catchment areas, water budgets and MeHg concentrations in inputs and outputs. Details for the calculations are found in paper II.

2.4.1 Catchment Areas

Catchment area delineation was determined using both GSD (Geografiska Sverigedata) elevational data from Lantmäteriet (Gävle, Sweden) in the software ESRI ArcGIS (Redlands, CA, USA) and field observations during high flow.

2.4.2 Water Budgets

For all sites, daily specific runoff data from nearby catchments were obtained as 1) measured specific runoff from stations included in the Swedish Meteorological & Hydrological Institute (SMHI, Norrköping, Sweden) network; and 2) modeled specific runoff using the hydrological catchment model Swedish Hydrological Predictions for the Environment (S-HYPE) (Lindstrom *et al.*, 2010) (SMHI). The model S-HYPE calculates a specific runoff for a certain type and size of catchment including input data such as land use, sub-basin area, soil type, precipitation, and elevation. Specific runoff for each wetland was chosen from catchments that were similar to the studied wetland catchments with regards to land use, distance to specific wetland and size of catchment. Thus, by using a set of 1 to 3 hydrological stations and 3 to 4 catchments subject to S-HYPE modeling for each wetland, the influence of variability in specific runoff data on export and chemical element/compound mass balance budgets was estimated. In addition, specific runoff was available from measurements at site GTN during 2007 to 2009 (Swedish Nuclear Fuel & Waste Management Co, SKB, Stockholm, Sweden).

Major water inputs to the specific wetlands include stream inlet, upland runoff and shallow groundwater (Figure 6). The specific runoff was used to calculate water input from stream inlets, upland runoff and shallow groundwater as well as stream water outlet from the specific wetland. The area contributing with shallow groundwater was calculated using Cl as a conservative element. The mass balance budget for Cl was considered valid if $\%Cl_{output}$ of input was ±10 %. In cases where the net Cl output (yield) exceeded 10 % of input, an additional input of shallow groundwater Cl was considered. Thus, by knowing the concentration of Cl in shallow groundwater (from analyzes of shallow groundwater samples at site SRD and KSN or stream-water concentrations at base flow at site GDL), the area contributing with shallow groundwater could be estimated (Wood & Sanford, 1995).

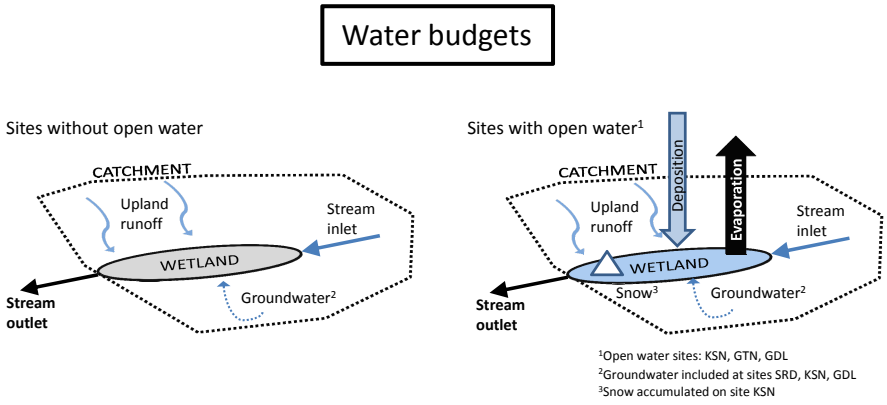


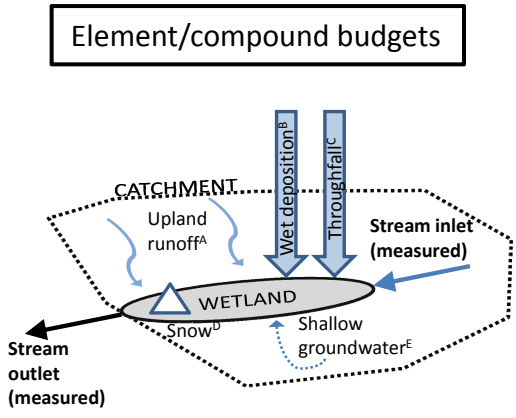
Figure 6. Illustration of water inputs (blue arrows) and outputs (black arrows) in wetlands without and with open water areas. The catchment area is delineated by a dotted black line while the specific wetland area is illustrated by a solid black line.

The main water output was the stream outlet, which was calculated in the same way as stream-inlet runoff. Because four of the wetlands (sites SRD, SKM, LDN and EHT) lacked open water surfaces, precipitation onto and evaporation from the specific wetland were assumed to be included in the applied specific runoff. The exceptions were wetlands with open water surfaces (sites KSN, GTN and GDL), for which precipitation and evaporation were included as separate fluxes in water budget calculations. Precipitation was assumed equal to measured precipitation at nearby stations in the SMHI network. Evaporation from open water surfaces at site KSN, GTN and GDL were estimated by a modified Penman equation using monthly average air temperature, latitude, altitude and wind speed (Linacre, 1993). Snow accumulated on the specific wetland was included in calculations for site KSN only, sites GTN and GDL did not have any snow accumulation.

2.4.3 Export & Mass Balance Budgets

The chemical element/compound concentrations determined in stream waters were assumed constant over a time-period centered on the sampling occasion. Thus, daily specific runoff was integrated over a time-period centered at the date of the sampling occasion. Export budgets for each catchment were calculated by multiplying the element/compound concentrations measured at the stream outlet with the integrated specific runoff for the catchment. Yields for each wetland were calculated from mass balance budgets by subtracting all element/compound inputs from the export (Figure 7). Thus, yields isolate the effect of the specific wetland on the element/compound. Element/compound concentrations were measured in inlet and outlet streams and in certain shallow

groundwater samples (sites KSN and SRD) and precipitation samples (Table 2).



Concentrations estimated from (included at sites):	*Element/compound*
[A]Runoff concentrations estimated from measurements of inlet stream water (all sites)	Hg_{inorg}, MeHg, DOC, Cl, SO_4^{2-}
[B]Wet deposition concentrations estimated from IVL data (all open sites)	Hg_{inorg}, Cl, SO_4^{2-}
[C]Throughfall concentrations estimated from IVL data (forested sites SKM & EHT)	Hg_{inorg}, Cl, SO_4^{2-}
[D]Snow concentrations estimated from snow sample measurements (site KSN)	Hg_{inorg}, MeHg, DOC, Cl, SO_4^{2-}
[E]Shallow groundwater concentrations estimated from groundwater sample analyses (sites KSN, SRD) and inlet concentrations at baseflow (site GDL)	Hg_{inorg}, MeHg, DOC, Cl, SO_4^{2-}

Figure 7. Illustration of inputs (blue arrows) and outputs (black arrows) in element/compound budgets for open and forested sites. Index refers to table with information on basis for concentration estimates. The catchment area is delineated by a dotted black line while the specific wetland area is illustrated by a solid black line.

2.4.4 Uncertainties in Budget Calculations

Export and mass balance budgets were calculated based on specific runoff data obtained from hydrological stations at nearby catchments or simulated runoff data. This approach introduces some uncertainties in the budget results and a more detailed discussion is found in paper II. For example, the relative standard deviation (calculated by using different sets of specific runoff data) for export budgets of Hg_{inorg} and MeHg, ranges from 2 to 26 %. However, the relative element/compound export for any given wetland in comparison to the other wetlands is the same regardless of what specific runoff data set are used.

Element/compound yields were calculated by subtracting inputs from outputs, isolating the specific wetland object. Because the areal extent of the specific wetland object in comparison to the catchment is small (below 10 % of the catchment for all objects except site KSN, 26 %), yields are less affected by

the applied absolute specific runoff data and more by the measured element/compound concentrations in inlet and outlet stream waters. This is also illustrated by comparing inlet and outlet element/compound concentrations, which give the same conclusion as the mass balance budget results as to whether a given wetland is a net sink or source for the element/compound (see chapter 3.5).

In summary, the main conclusions obtained from export and mass balance budgets are consistent with e.g., stream-water concentrations, despite the uncertainties in calculations.

2.4.5 Input-Output Snapshot Budgets

In paper III, input-output snapshot budgets were performed at nine additional swamps to test if net retention/degradation of MeHg is general to *Alnus* swamps. At each site, the inlet and outlet stream water was sampled within approximately one hour. At the same time flow was measured using salt dilution (Moore, 2005). Snapshot mass fluxes of Hg_{inorg}, MeHg and DOC were calculated by multiplying inlet or outlet flow with the corresponding concentrations. Snapshot yields were then calculated by subtracting input masses from output masses.

2.5 Statistical Analyses

In papers I-II, data were checked for normal distribution using Shapiro-Wilkinson test and homogeneous variance using Levene Statistics (Zar, 1996). In paper I, statistical analyses were performed to test for differences in concentrations of ancillary chemistry, soil Hg and MeHg, as well as potential Hg_{inorg} methylation and MeHg demethylation rates and rate constants. For normally distributed data with homogeneous variance, one-way analysis of variance (ANOVA) was used to test for significant differences within the dataset. When ANOVA yielded significant differences, differences between wetlands were explored by pair-wise comparison using post hoc Tukey's test. The corresponding nonparametric tests for data that were non-normally distributed with/without homogeneous variance even after log-transformation, were Brown-Forsythe and Welch for differences within the dataset, followed by post-hoc Games-Howell's test for differences between wetlands.

In paper II, Pearson correlations and linear regression were used to evaluate the relationship between wetland averages of annual MeHg exports, net MeHg yields, %MeHg (of total Hg in soil) and k_m/k_d. For all statistical analyses in paper I-II the software PASW Statistics 18 (SPSS, Inc, USA) was used.

In paper III, two populations of soil MeHg concentrations determined in September 2008 were formed: one population in the upstream part of the *Alnus* swamp (-40 to 250m) and one downstream (270 to 400m). Soil MeHg concentrations were normalized to soil organic carbon (SOC) content and annual population average. Data were tested for normal distribution and homogeneous variance using Shapiro-Wilkinson test and homogeneous variance using Levene Statistics. One-way ANOVA was used to test for a difference between the two populations at p=0.005. Statistical analyses were done using the software PASW Statistics 18 (SPSS, Inc, USA). In addition, linear regressions were used to analyze the relationship between %MeHg (of total Hg in soil), k_m and k_d with distance into the swamp. Linear regression was also used to analyze the relation between %MeHg and k_m/k_d. Data were checked for normality and homogeneous variance before linear regression analysis. For these tests, the software Minitab 16 (Minitab Inc., Saltsjöbaden, Sweden) was used.

3 Results & Discussion

3.1 Nutrient Status

The seven wetlands of the study were classified into three subgroups according to geographic location and climate, vegetation type, acidity, nutrient status, and ancillary chemistry (paper I). Briefly, the three northern sites SKM, SRD and KSN were characterized as acidic and nutrient-poor. Site LDN was characterized as a nutrient gradient, ranging from the acidic, nutrient-poor *Sphagnum* bog LDNA to the slightly richer *Carex* fen LDNB. The three southern sites EHT, GTN and GDL were the most nutrient-rich sites of the study.

The nutrient status of each wetland was linked to the Hg biogeochemistry based on the theory of SRB and FeRB being the main MeHg producers (Figure 1). Thus, chemical elements/compounds used to characterize the wetlands according to nutrient status include $SUVA_{254nm}$ (measure of carbon quality), total Fe and SO_4^{2-} (electron acceptors). In addition, pH illustrates acidity while C/N-ratio in soils and nitrate (NO_3^-) in outlet stream waters are measures of the general nutrient status. Phosphate (PO_4^{3-}) in outlet stream waters was also measured but was generally at or below the detection limit (2.11 μM). The chemical elements/compounds are summarized in Table 3 and 4 for soils and outlet stream waters, respectively.

It should be noted that the same patterns among sites can be seen when comparing levels of the chemical elements/compounds in the wetland soils and outlet stream waters. For example, the three northern nutrient-poor sites have low pH, high amounts of recalcitrant carbon (as reflected by a high $SUVA_{254nm}$), low Fe and SO_4^{2-}, and high C/N-ratios. In contrast, the three southern most nutrient-rich sites have high pH, low $SUVA_{254nm}$, and high Fe, SO_4^{2-} and C/N-ratios.

Table 3. Summary of chemical elements/compounds in wetland soils and soil pore waters used for characterization of wetlands. Average ±SE of soil pore water pH, $SUVA_{254nm}$, total Fe, SO_4^{2-}, and soil C/N-ratios. Data in paper I.

Site	Wetland type	pH	$SUVA_{254nm}$ (L mg^{-1} m^{-1})	Fe (µM)	SO_4^{2-} (µM)	C/N	Subgroup
SKM	Riparian	4.3±0.1	3.2±0.2	110±19	5.6±1.6	28±1.2	Northern nutrient-poor
SRD	Open fen	4.6±0.1	2.8±0.1	270±160	15±4.2	36±4.3	
KSN	Shallow lake	4.8±0.1	2.4±0.4	320±74	6.2±1.6	37±2.4	
LDNA	Bog	4.6±0.3	3.8±0.5	170±75	6.8±2.5	34±2.4	Nutrient gradient LDN
LDNB	Fen	5.1±0.2	3.7±0.0	560±300	7.5±1.1	21±1.8	
EHT	*Alnus* swamp	5.7±0.2	4.7±0.9	330±110	17±4.7	14±0.3	Southern nutrient-rich
GTN	Mesotrophic lake	5.6±0.1	3.0±0.5	570±170	17±8.6	19±2.8	
GDL	Artificial wetland	5.8±0.2	2.0±0.6	510±130	13±4.4	21±1.5	

Table 4. Summary of chemical elements/compounds in stream water outlets of the specific wetlands used for characterization of wetlands. Detection limit for NO_3^- is 0.81µM. Average ±SE for study period before restoration measures. Data in paper II.

Site	Wetland type	pH	$SUVA_{254nm}$ (L mg^{-1} m^{-1})	Fe (µM)	SO_4^{2-} (µM)	NO_3^- (µM)	Subgroup
SKM	Riparian	4.4±0.2	4.6±0.3	33±7.9	11±2.5	≤0.81	Northern Nutrient-poor
SRD	Open fen	4.9±0.2	4.1±0.2	32±6.0	21±4.9	≤0.81	
KSN	Shallow lake	4.7±0.2	4.0±0.2	18±2.5	15±1.8	≤0.81	
LDNA	Bog	5.1±0.1	4.1±0.2	17±2.5	31±7.0	0.86±0.0	Nutrient gradient
LDNB	Fen	5.0±0.1	4.1±0.2	17±2.3	29±6.7	≤0.81	
EHT	*Alnus* swamp	5.1±0.1	4.3±0.1	160±21	70±15	6.2±2.7	Southern nutrient-rich
GTN	Mesotrophic lake	6.2±0.0	3.6±0.1	52±6.1	130±12	1.6±0.30	
GDL	Artificial wetland	6.4±0.1	3.2±0.1	46±11	64±23	2.6±1.3	

In addition to the availability of electron donors and acceptors, SRB and FeRB activities are highly dependent on anoxic or suboxic conditions (Lovley, 1995; Gottschalk, 1979). To estimate the levels of anoxia in the wetland soils of this study, concentrations of reduced species of Fe and S were determined. Inorganic sulfides were only found in minor amounts (3.1±5.0 μM for all wetland soil pore waters, 2007 to 2009, paper I). However, considering frequent detection of the characteristic smell of $H_2S(g)$ during soil sampling, it is likely that the determination of inorganic sulfides were underestimated. Nevertheless, suboxic to anoxic conditions in all wetlands soils were indicated by the dominance of Fe(II) over Fe(III) in soil pore waters (paper I).

In summary, the seven wetlands of this study were divided into three subgroups with different nutrient status, all with suboxic to anoxic soils.

3.2 Soil Hg Biogeochemistry

In paper I, Hg biogeochemistry in soil was studied by measuring total concentrations of Hg and MeHg in the solid phase and pore waters. In addition, incubation studies were performed to determine the potential Hg_{inorg} methylation and MeHg demethylation rates.

In the literature, %MeHg (of total Hg in soil) is frequently used as a proxy for the long-term net production of MeHg in soils, although mostly in marine and brackish water sediments (Drott *et al.*, 2008; Fitzgerald *et al.*, 2007). Results presented in Table 5 clearly show a variation in %MeHg among wetlands with different nutrient status where wetlands of intermediate nutrient status, i.e., poor-fens like site LDNB, have the highest %MeHg.

Correspondingly, potential Hg_{inorg} methylation and MeHg demethylation rates determined during 48h laboratory incubation studies have been suggested as proxies for short-term methylation and demethylation rates, respectively (Drott *et al.*, 2008). It should be noted that potential rate constants for Hg_{inorg} methylation and MeHg demethylation, k_m and k_d, respectively, are better measures than the absolute potential rates because rate constants are independent of tracer concentration (Hintelmann *et al.*, 2000). Also, k_m and k_d cannot be quantitatively compared or used to calculate the net MeHg production because the constants are measured using different tracers, i.e., different tracer bioavailability and concentration. Nevertheless, distinct variations among wetlands of different nutrient status are found when qualitatively comparing the ratio of the rate constants, k_m/k_d (Table 5). In addition, %MeHg in soil roughly follows the ratio of k_m/k_d illustrating the link between long- and short-term net MeHg production proxies.

Table 5. Average ±SD of wetland proxies for net MeHg production in soil, 2007 to 2009: %MeHg of total Hg and k_m/k_d (paper I). Average ±SD for stream-water MeHg yield and export for the study period, 2007 to 2009 (paper II). A positive yield indicates that the wetland is net source for MeHg.

Site	Wetland type	%MeHg	k_m/k_d	Wetland yields (g/km^2)	Catchment export (g/km^2)
SKM	Riparian	5.1±3.1	0.32±0.5	0.56±0.4	0.12±0.05
SRD	Open fen	4.9±3.2	0.34±0.6	0.72±0.3	0.097±0.04
KSN	Shallow lake	4.5±3.4	0.65±0.8	0.55±0.2	0.23±0.04
LDNA	Bog	8.0±6.3	0.31±0.5	1.9±0.4	0.29±0.07
LDNB	Fen	17±9.3	0.80±0.8	6.2±4.4	0.41±0.18
EHT	*Alnus* swamp	6.3±6.8	0.56±0.5	-3.8±2.2	0.28±0.11
GTN	Mesotrophic lake	2.3±1.8	0.24±0.4	3.0±0.6	0.14±0.02
GDL	Artificial wetland	4.6±4.5	0.079±0.1	0.54±0.6	0.13±0.06

By adding MoO_4^- during incubation studies, SRB activity is inhibited allowing an estimation of the importance of SRB for MeHg production (Kerry *et al.*, 1991; Compeau & Bartha, 1987). In this way, FeRB have been identified as producers of MeHg at rates comparable to SRB in freshwater sediments (Fleming *et al.*, 2006; Kerin *et al.*, 2006). In this study, incubations of soil samples with the addition of MoO_4^- resulted in a drastic decrease in k_m by 81±15 % (average±SD), for all sites except SKM (paper I). This suggests that SRB are the main methylators of Hg_{inorg} in the wetland soils studied in this thesis. Interestingly, the northern site SKM had the lowest k_m and the lowest inhibitory effect of MoO_4^- (decrease of 18±38 %). This suggests that FeRB could be responsible for the production of MeHg at site SKM, a hypothesis that is further supported by low sulfide levels (paper I). During the MoO_4^- inhibition study, k_d was also determined. Due to the diversity of MeHg demethylation mechanisms, the varying effects of MoO_4^- addition on k_d were anticipated (-560±1600 % decrease compared to no MoO_4^- added). Noteworthy is the 73-fold increase in k_d at site LDNA after the high MoO_4^- addition (aiming at 500 % of ambient SO_4^{2-} concentration), which could be the result of an increased activity of competing methanogens once SRB were inhibited. Methanogens have been found to be capable of MeHg demethylation but not of Hg_{inorg} methylation (Pak & Bartha, 1998).

In summary, both long- and short-term proxies for net MeHg production vary with the nutrient status of the wetlands, with the poor-fen type of wetland LDNB having the highest rates of MeHg production. Results of incubation

studies with addition of MoO_4^- as a specific SRB inhibitor suggests that SRB are the main MeHg producers in six out of seven of the wetland soils studied in this thesis.

3.3 Stream-Water MeHg Budgets

3.3.1 Catchment MeHg Exports

Annual MeHg export from each wetland was calculated by multiplying MeHg concentrations in outlet stream waters with the outlet runoff integrated over a time period centered on the sampling occasion (paper II).

The annual MeHg exports from the seven wetlands in this study range from 40 to 647 mg MeHg/km^2 catchment, with the areal proportion of total wetland of the catchment area ranging from 6 to 49 %. The MeHg exports are thus well in agreement with other studies on boreal wetlands, ranging from 11 to 555 mg MeHg/km^2 (Larssen *et al.*, 2008; Selvendiran *et al.*, 2008; St.Louis *et al.*, 1996; St.Louis *et al.*, 1994). The MeHg exports also concur with studies on boreal catchments with <20 % wetland area (Porvari *et al.*, 2003; Lee *et al.*, 2000), temperate wetlands (Galloway & Branfireun, 2004) and northern and subtropic catchments with <20 % wetland area (Shanley *et al.*, 2008).

3.3.2 Wetland MeHg Yields

MeHg wetland mass balances were calculated by considering all inputs of MeHg to the specific wetlands (i.e., deposition, inlet stream water, shallow groundwater, upland runoff) and outputs (outlet stream water). The MeHg yield was calculated by subtracting all MeHg inputs from the MeHg export, isolating the specific wetland effect on the MeHg mass balance budget. Thus, MeHg yield is a measure of the strength of each wetland as a net source or sink for MeHg (paper II).

The contribution from each wetland to the catchment MeHg export for 2007 to 2010 is shown in Table 6. Results show that all wetlands are sources of MeHg, except the *Alnus* swamp EHT. The wetlands that are MeHg sources, contribute with 21 to 72 % of the exported amount of MeHg. Thus, the influence of wetlands on the export of MeHg is substantial even though the areal extent of the specific wetland in relation to the entire catchment is low (2.5 to 26 %). The results also show that the *Alnus* swamp EHT lowers the MeHg export by 82 %, illustrating that the net degradation of MeHg occurring in the swamp is of substantial magnitude.

Table 6. Summarized MeHg catchment export and wetland MeHg yield for the entire study period, 2007 to 2010. Average annual percentage wetland contribution ±SD to MeHg export (calculated as yield from wetland / export ×100). A positive value indicates that the wetland is a net source for MeHg. Percent areal extent of the specific wetland of the total catchment. Data modified from paper II.

Site	Catchment MeHg export Σ2007-2010 (mg)	Wetland MeHg yield Σ2007-2010 (mg)	Wetland contribution to MeHg export Average 2007-2010±SD (%)	Areal extent specific wetland of total catchment (%)
SKM	204	39	21±12	4.2
SRD	504	276	50±15	7.0
KSN	898	576	63±14	26
LDNA	1091	700	64±14	8.6
LDN	1715	1248	72±8.8	10
EHT	669	-543	-82±42	7.2
GTN	13390	6263	48±14	2.5
GDL	183	60	26±28	8.4

Both wetland MeHg yields and catchment MeHg export results varied among wetlands in the same way as soil proxies for net MeHg production data (Table 5). These patterns illustrate the linkage between soil processes and stream-water exports and yields. In fact, wetland site averages of both MeHg exports and yields were significantly correlated to the site average of %MeHg of total Hg in soil (Pearson correlations, R=0.83 and 0.86, for export and yield, respectively, $p<0.05$) (paper II). Accordingly, MeHg exports and yields followed the same pattern as site averages of k_m/k_d, but the correlations were weaker (Pearson correlations, R=0.70, p=0.052 for MeHg exports and R=0.64, p=0.12 for MeHg yields) (paper II). Thus, both results of soil and stream-water budgets from the seven boreal wetlands show that MeHg production and export vary with the nutrient status of the wetlands. The highest %MeHg in soil, the highest k_m/k_d, the highest MeHg yield and export in stream water were found at wetlands with intermediate nutrient status, i.e., poor-fens like LDNB. This corresponds to previous reports, where poor-fen areas have been found to have high rates of MeHg production, as determined by input-output budgets (St.Louis *et al.*, 1996) and pore water concentrations (Mitchell *et al.*, 2008b; Branfireun & Roulet, 2002). It has been suggested that high MeHg production is supported at poor-fen areas due to nutrients being transported by upwelling water from the mineral soil (St.Louis *et al.*, 1996).

In contrast, the northern more nutrient-poor wetlands (sites SKM, SRD and KSN) were relatively low net sources of MeHg, in absolute terms, in comparison to the other sites (Table 5). Results of soil analyses show that the same sites also had intermediate %MeHg and k_m/k_d (Table 5). The northern sites SKM, SRD and KSN were all characterized with low pH, C/N, high amounts of recalcitrant carbon (as determined by $SUVA_{254nm}$), Fe and SO_4^{2-} (Table 3 and 4). Thus, it was hypothesized that the intermediate rates of MeHg production found at sites SKM, SRD and KSN are due to their low nutrient status limiting the activity of the microbial methylation communities.

Among the most nutrient-rich sites, site GTN was a net MeHg source per areal unit of intermediate magnitude whereas site GDL was a lower net MeHg source (Table 5). As determined by soil analyses, both sites GTN and GDL had low %MeHg and k_m/k_d (Table 5). More specifically, it should be noted that site GDL had among the lowest k_m/k_d-ratios of the studied wetlands.

MeHg yields reveal that site EHT was a net sink for MeHg for four consecutive years (paper II). Soil data show that the effect of a high k_m at the nutrient-rich *Alnus* swamp EHT was counteracted by a high k_d resulting in an intermediate %MeHg (paper I). Thus, is may seem contradictory that an intermediate k_m/k_d and %MeHg in soil at site EHT was not companioned by an intermediate net MeHg yield (Table 5). However, site EHT receives high loads of MeHg from the upstream located bog and spruce forest (153 to 439 mg MeHg annually imported during the study period). This high MeHg input is degraded along a gradient moving further into the swamp, as discovered in a spatial analysis of soil data at site EHT (paper III). The result is lower MeHg in the outlet water as compared to the inlet water, and lower MeHg concentrations and k_m/k_d in soil moving further into the swamp. The results for %MeHg and k_m/k_d are averaged for samples from the entire swamp, thus capturing the gradient with high values in the upstream end of the swamp and yielding relatively high standard deviations. A more detailed discussion of the degradation of MeHg in the *Alnus* swamp EHT is found in chapter 3.6.

Based on the net MeHg degradation at site EHT and the comparatively low MeHg production at sites GTN and GDL, it is suggested that demethylation of MeHg is promoted over Hg_{inorg} methylation at wetlands with a higher nutrient status.

In summary, MeHg exports and yields at the different wetlands correspond to results of long- and short-term net MeHg production proxies in the corresponding soils. This points to a link between soil processes and stream-water MeHg budgets. Furthermore, the MeHg yield is related to the nutrient status of the wetlands, with a higher yield at wetlands with intermediate nutrient status.

3.4 Effects of Boreal Wetland Restoration on MeHg

The seven wetlands were all subjected to restoration measures, usually by damming the stream-water outlet. Details on restoration measures are found in paper II. Noteworthy is that in relation to the other sites, damming of the outlets at sites SRD, GTN and GDL were of larger scale. For all sites, except EHT, the visible effects of a higher water level after restoration included increased open water areas (ranging from more puddles to larger lakes) and changes in vegetation towards more hydrophilic plant types and mosses. Effects of restoration on the Hg biogeochemistry were evaluated by comparing MeHg yield results before and after restoration measures were conducted (paper II).

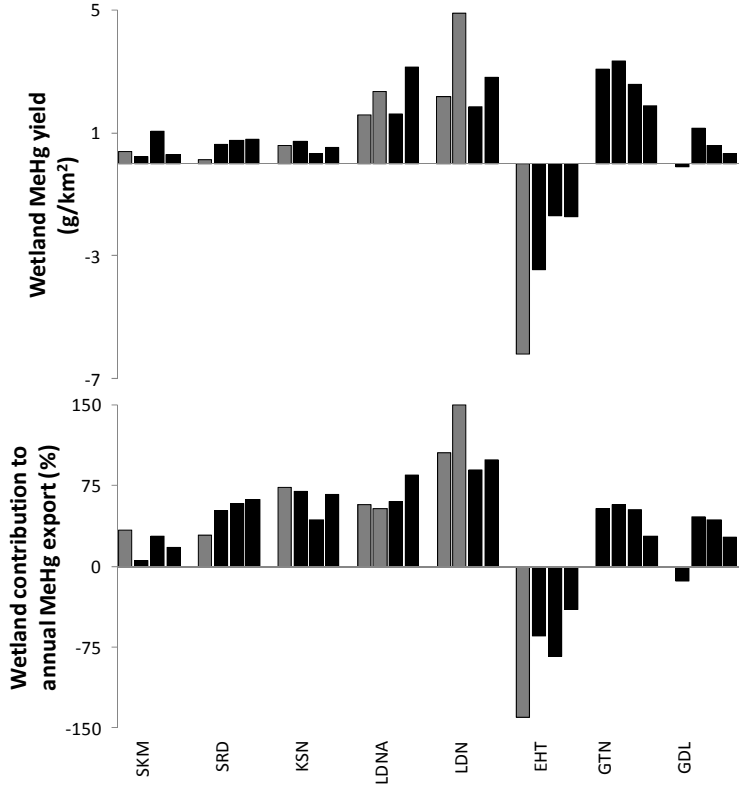


Figure 8. Annual wetland MeHg yields in g/km^2 (upper graph) and percent contribution to catchment export (lower graph) before ( ) and after flooding ( ). A positive value indicates that the wetland is a net source for MeHg. Wetlands are ordered, left to right, according to increasing nutrient status. Data modified from paper II.

Overall, results from the seven studied wetlands show that net MeHg production varied more among wetlands than before and after restoration of each wetland (Figure 8). This suggests that the nutrient status of these wetlands were more important to the net MeHg production than the performed restoration measures.

At the nutrient-rich southern sites GTN and GDL, a high MeHg yield was seen in year 1-2 after restoration followed by a decline (Figure 8). There are no data available from the pre-treatment period for these two sites, since they were restored just before the project start. However, the consistent pattern with a pulse in MeHg yield after restoration is in agreement with results from two studies of reservoir creation by flooding of boreal uplands at the Experimental Lakes Area (ELA), Ontario (Hall *et al.*, 2005; St.Louis *et al.*, 2004). Both studies include large-scale flooding, most closely resembling the restoration measures taken at sites GTN, GDL and SRD. At site SRD the water level was actively adjusted in roughly 6-month cycles. As expected, a consequence of restoring site SRD was an increased MeHg yield, but three years after restoration no decline in MeHg yield was yet seen (Figure 8). The pulsed MeHg production at both reservoir creation studies at ELA are explained by microbial MeHg demethylation dominating over methylation after a few years of inundation (Hall *et al.*, 2005; St.Louis *et al.*, 2004). Thus, the post-treatment study period at site SRD may have been too short to cover a decrease in MeHg yield. Alternatively, conditions at site SRD may promote methylation over demethylation for a longer time period after inundation, in contrast to conditions at sites GTN, GDL and the ELA-sites. In one of the ELA-studies, three different reservoirs were compared, showing that there were higher MeHg production in areas with higher organic carbon contents and more specifically, in areas with higher content of new and more labile carbon (Hall *et al.*, 2005). In a third study of boreal peatlands at ELA, Ontario, higher levels of MeHg in peat and peat pore water of impounded areas were explained by facilitated SO_4^{2-} transport to the peat surface where methylation occurred (Heyes *et al.*, 2000). Of all the northern sites, site SRD had the highest SO_4^{2-} export, in the range of the southern more nutrient-rich wetlands (paper II). The high SO_4^{2-} export was probably due to the oxidation of reduced sulfide during drainage periods. Thus, the nutrient status of site SRD may be high enough to support methylation, but not high enough to support microbial demethylation. In addition, the drainage/damming cycling probably caused corresponding cycles in sulfide oxidation/reduction, which has been suggested to promote SRB activity and hence, MeHg production (Branfireun *et al.*, 2001).

In contrast, no effect of restoration on MeHg yield was seen at the riparian wetland SKM (Figure 8). This was explained by the nutrient-poor conditions,

illustrated in outlet stream water by a low pH, high amounts of recalcitrant carbon (as reflected by a high $SUVA_{254nm}$) and low levels of SO_4^{2-}, Fe and other nutrients (Table 4).

A decrease in MeHg yield after damming of the outlet was seen at the shallow lake KSN (Figure 8). Damming resulted in an average increase in water level of about 40 cm, causing an increased open water area of the lake. Thus, decreased MeHg yield may be explained by photodegradation in the open water column, a well-known MeHg degradation process in humic lakes (Sellers *et al.*, 1996).

At the *Alnus* swamp EHT, restoration was performed by rerouting the secondary outlet which had little or no effect on net MeHg yield (Figure 8). The lack of restoration effects may be due to the *Alnus* swamp EHT being consistently wet, with the water table always 2-5 cm above the soil surface, even before rerouting of the secondary outlet.

Finally, evaluation of the restoration effects on MeHg at site LDN was difficult due to large inter-annual variations (Figure 8). Two dams were created at the site: at the main inlet and the outlet of the upstream bog part LDNA. The site was a consistent net source of MeHg during the study period.

In summary, the conducted restoration measures had less effect on MeHg production in the wetlands than the nutrient status of the sites. Thus, restoration of wetlands does not necessarily lead to an increased MeHg production, as found in most previously reported studies.

3.5 Annual Variations

MeHg was analyzed in inlet and outlet stream-water samples collected throughout the years 2007 to 2010, with an effort of more frequent sampling during high flow events.

Stream-water MeHg concentrations generally followed a seasonal pattern with higher concentrations during the summer months (Figure 9). This is consistent with reports from other boreal wetlands, where an increase in MeHg concentration is explained by higher summer temperatures promoting production of fresh organic matter, thus stimulating microbial activity and MeHg production (Bradley *et al.*, 2011; Selvendiran *et al.*, 2008; Galloway & Branfireun, 2004; St.Louis *et al.*, 1994).

The site specific differences between inlet and outlet MeHg concentrations (Figure 9) should be compared with the MeHg yields calculated in paper II. For instance, outlet MeHg concentrations are generally equal to inlet concentrations at site SKM, agreeing with MeHg yields showing that site SKM was at steady-state with respect to MeHg. Site EHT had higher inlet MeHg

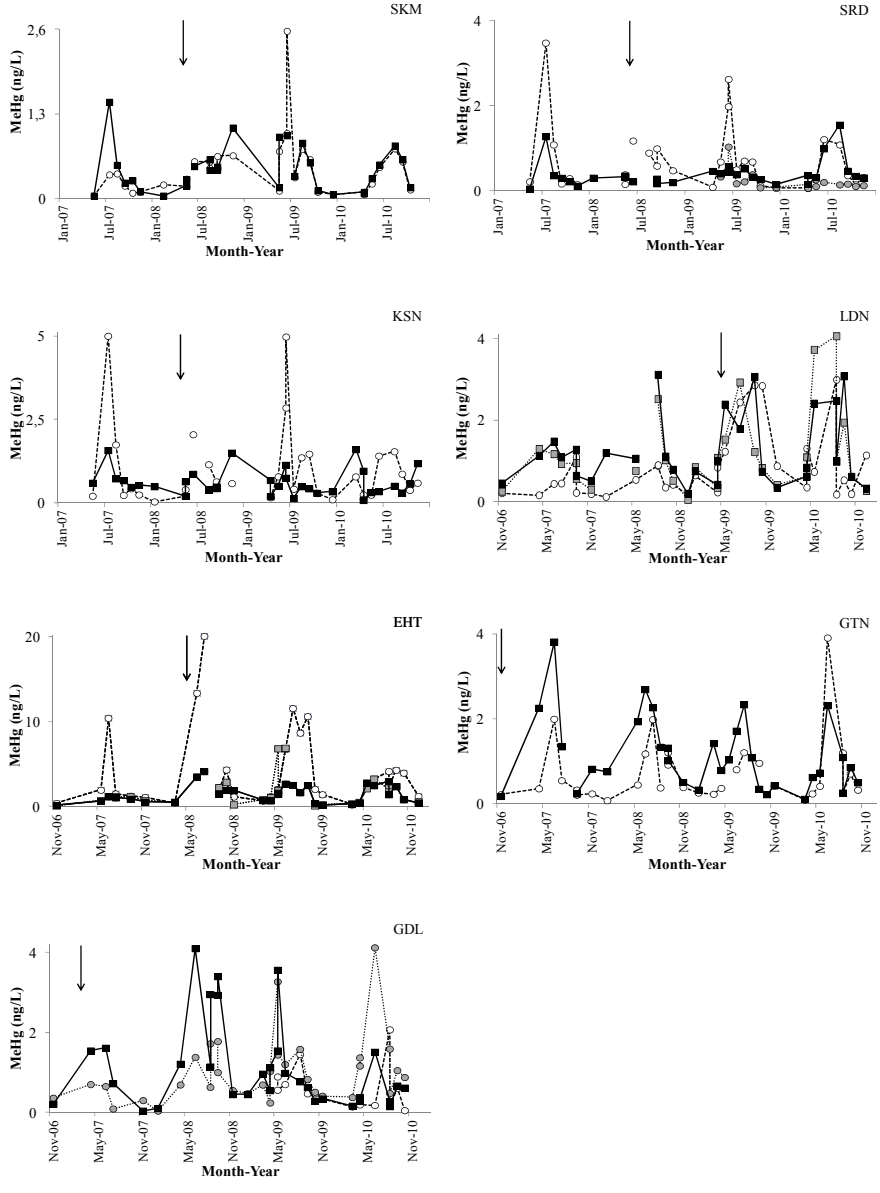


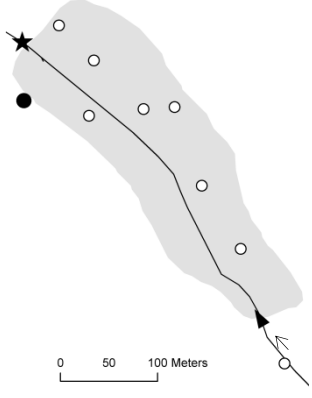
Figure 9. Annual variations of MeHg concentrations in main inlet (--○--), secondary inlet (···●···), main outlet (—■—) and secondary outlet (···□···). Arrows indicate time of restoration measures (details in paper II).

concentrations than outlet, reflecting the MeHg yields showing that site EHT is a net MeHg sink. In contrast, the rest of the sites had higher concentrations in outlet streams as compared to inlet and these sites were also net MeHg sources according to MeHg yield results. Thus, stream-water MeHg concentrations were more important for yield results than variability in water flow. This is further explained by the relatively low areal contribution of the specific wetland to the entire catchment area (<10 % for all sites, except site KSN, 26 %), illustrating that the difference between import and export (i.e., the specific wetland area) was mainly due to MeHg concentrations.

In summary, stream-water concentrations of MeHg showed seasonal trends consistent with literature. Furthermore, differences between inlet and outlet stream-water concentrations reflect the MeHg yields (paper II) identifying the sites as sources/sinks for MeHg.

3.6 *Alnus* Swamps are Net MeHg Sinks

The discovery of the *Alnus* swamp EHT as a net MeHg sink during the entire study period 2007-2010 resulted in extra focus on this particular wetland (paper III).



MeHg concentration main stream water outlet: 1.9±1.5

Sample	%MeHg	k_m/k_d
400m	1.4	0.98
350m	2.5	0.87
250m center	1.4	0.74
150m	4.3	1.2
100m	5.6	2.3
-80m	9.7	17

MeHg concentration stream water inlet: 5.9±7.8

Figure 10. Map of the specific *Alnus* swamp EHT with main inlet (▶), outlet (★) and secondary outlet (●). Solid black line denotes stream, small arrow indicates flow direction and sampling points are indicated by ○. The table includes results from the 2008 sampling campaign for %MeHg (of total Hg in soil), k_m/k_d, as well as 2008 average MeHg concentrations in main outlet stream water (top) and inlet stream water (bottom).

The spatial distribution of total Hg, MeHg, %MeHg (of total Hg in soil), k_m, and k_d were analyzed along the south to north gradient from inlet to outlet in the swamp. Soil concentrations of total Hg in the swamp varied considerably during the entire study period, 2007 to 2010 (paper III). However, total Hg concentrations showed no consistent pattern along the gradient into the swamp. In contrast, soil MeHg concentrations from the upstream sampling points (270 to 400m) were significantly lower as compared to the upstream sampling points (0.34 as compared to 0.66 ng MeHg g^{-1} SOC^{-1} annual $average^{-1}$, one-way ANOVA, p=0.00056). Thus, soil MeHg concentrations decreased along the gradient into the swamp.

The pattern is further illustrated by linear regressions which indicate that %MeHg and k_m/k_d decreased with distance from inlet (Figure 10). %MeHg was negatively correlated with distance into the *Alnus* swamp (R^2=0.69, p=0.020), while it was positively correlated to the ratio k_m/k_d (R^2=0.89, p=0.0001) (paper III). In agreement, k_d was positively correlated with distance into the Alnus swamp (R^2=0.83, p=0.004), while k_m only had a very weak correlation with distance R^2=0.25, p=0.25 (paper III). These results illustrate the link between soil processes and the MeHg yields, where EHT was a net MeHg sink during four years (paper II).

It was hypothesized that the net degradation of MeHg in the *Alnus* swamp EHT was due to the nutrient-rich conditions (paper III) supported by the N-fixating *Alnus glutinosa* stand (Dilly *et al.*, 1999). This nutrient-rich environment may promote demethylation of MeHg either by biotic or abiotic means. Either way, the demethylating processes in the *Alnus* swamp are supplied with DOC and MeHg from the upstream located spruce forest and bog that generated high loads of these compounds. More studies are currently performed trying to elucidate the role of demethylating microorganisms at site EHT (Kronberg et al.).

In addition, input-output snapshot budgets for MeHg indicated that the nine additional swamps were net sinks or at steady-state for MeHg. In contrast, the snapshot budgets indicated that two peatlands were large net MeHg sources. Thus, net MeHg degradation may be a general phenomenon in *Alnus* swamps (paper III).

In summary, *Alnus* swamps in general seem to act as net MeHg sinks. This can be used actively in landscape planning, for instance by preservation of *Alnus* swamps located downstream or in riparian zones which can help mitigate the negative effects of net MeHg production occurring in upstream, e.g., poor-fen, areas.

4 Conclusions

- Are certain types of wetlands more prone to net MeHg production than others?

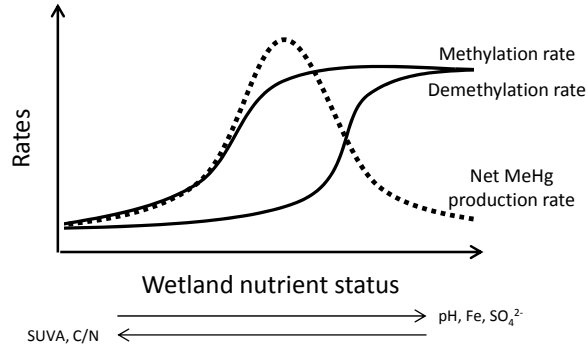


Figure 11. Conceptual illustration of main findings: MeHg production and degradation rates depend on the nutrient status of wetlands, with the peak net MeHg production occurring in wetlands of intermediate nutrient status.

The results presented in this thesis strongly suggest that there is a link between the nutrient status of a wetland and the production of MeHg (Figure 11). The highest net MeHg production, as determined through %MeHg (of total Hg in soil), potential Hg_{inorg} methylation and MeHg demethylation rates, and net MeHg yield in stream waters, occurred in wetlands with an intermediate nutrient status, i.e., poor-fens. In addition, the results from this thesis show that *Alnus* swamps are net sinks for MeHg, a finding that should be considered in future landscape planning and forest management.

- What is the effect of restoration on production of MeHg in wetlands?

Results presented in this thesis show that the nutrient status of the seven wetlands had a larger impact on the net MeHg production than the performed restoration actions. Furthermore, the results show a range of consequences when restoring forested boreal wetlands, contrary to current literature (e.g., Hall *et al.*, 2005; St.Louis *et al.*, 2004; Kelly *et al.*, 1997). Temporary peaks of MeHg production were found at more nutrient-rich sites while minor effects were found at less nutrient-rich sites. In addition, restoration of a shallow lake wetland caused an increased open-water area resulting in a decreased net MeHg yield.

- What processes control production and degradation of MeHg in boreal wetlands?

While processes behind the production and degradation of MeHg are complex, the results from the seven boreal wetlands studied in this thesis suggest that SRB are key producers of MeHg in these systems. However, more studies are needed to elucidate the processes behind MeHg production and degradation.

- Is there a link between processes in wetland soils and stream-water exports?

In this thesis, three main parameters were used to evaluate the potential MeHg production in the studied wetlands. The three parameters (k_m/k_d, %MeHg of total Hg in soil and stream-water budgets) all yielded similar results when compared among wetlands. Thus, this thesis points to a link between soil processes and stream-water exports despite the number of processes affecting and contributing to difficulties in quantifying each parameter.

5 Svensk sammanfattning: Boreala våtmarker – både sänkor & källor för metylkvicksilver

Ett stort antal vetenskapliga studier har visat att boreala våtmarker är stora källor för metylkvicksilver (MeHg) (Hurley *et al.*, 1995; Rudd, 1995; St.Louis *et al.*, 1994). MeHg produceras av mikroorganismer, främst sulfatreducerande bakterier (SRB), i våtmarker. (Ullrich *et al.*, 2001) Mekanismen bakom bildandet av MeHg hos bakterierna är inte helt klarlagd ännu, men vissa faktorer är kända. Exempelvis vet man att bakterierna behöver kol, sulfat, syrefria förhållanden och oorganiskt kvicksilver (Hg_{inorg}) för att kunna bilda MeHg. Dessa faktorer finns i boreala våtmarker, och eftersom våtmarkerna dessutom är del av större avrinningsområden är risken stor att det bildade MeHg transporteras vidare i vattendrag till sjöar och hamnar i fisk. Det finns alltså en risk för konflikt mellan miljömålet att restaurera våtmarker för deras ekologiska funktioner, samt att minska mängden MeHg i miljön.

I den här avhandlingen presenteras data från studier av sju boreala våtmarker i Sverige. Dessa våtmarker dikades under första halvan av 1900-talet och restaurerades i anslutning till studieperioden, 2007-2010. Data har samlats in från våtmarkernas jordar samt in- och utflödande vattendrag.

Resultaten visar att näringsstatusen hos en våtmark har betydelse för hur mycket MeHg som produceras i våtmarken (paper I-II). Den våtmark som producerade mest MeHg karaktäriserades som ett ”fattigt kärr” (lokal LDNB). Detta överensstämmer med tidigare studier, där bildandet av MeHg i fattiga kärr förklarats med att relativt näringsrikt grundvatten tränger upp från mineraljorden (St.Louis *et al.*, 1996). Generellt stämmer resultaten mellan jordar och bäckvatten överens; de våtmarker som hade hög bildning av MeHg i jordar var också stora källor till MeHg enligt analyser av bäckvatten (paper II).

Skillnaderna i MeHg produktion var större mellan våtmarkerna än före/efter våtmarksrestaurering (paper II). I de mer näringsrika våtmarkerna (lokaler

GTN och GDL) exporterades mer MeHg ut ur våtmarkerna under de två första åren efter restaurering, sedan sjönk MeHg-produktionen igen. Detta mönster stämmer med andra studier, där minskningen av MeHg-produktionen förklarats med en ökad nedbrytning av MeHg (Hall *et al.*, 2005). I en våtmark syntes ingen effekt av restaurering (lokal SKM), vilket förklaras med en generellt låg näringsstatus som inte antas kunna upprätthålla bakteriernas aktivitet i denna våtmark. Restaurering av en grund näringsfattig sjö (lokal KSN) minskade mängden MeHg, något som förklarades med en ökad areal öppen vattenyta vilket antogs ledde till ökad nedbrytning av MeHg med hjälp av UV-ljus. Denna process är väldokumenterad i humusrika sjöar (Sellers *et al.*, 1996).

Resultaten visar också att klibbalkärr (*Alnus glutinosa*) bryter ned MeHg (paper III). Detta visades i jordar och bäckvatten för ett specifikt alkärr (lokal EHT), samt i bäckvatten för åtta ytterligare alkärr med varierande mängd klibbal samt ett björkkärr. Restaurering av det specifika klibbalkärret påverkade inte nedbrytningen av MeHg. Upptäckten av alkärr som nedbrytare av MeHg kan användas i landskapsplanering och skogsskötsel, eftersom alkärr ofta förekommer nedströms eller i kantzoner. Om dessa alkärr bibehålls, eller dikade alkärr återställs, kan de användas för att minska mängden MeHg som produceras i uppströmsliggande våtmarker.

Sammantaget visar resultaten i denna avhandling på en mer nyanserad bild av våtmarker och MeHg, där näringsstatusen är avgörande för våtmarkernas roll som källa eller sänka för MeHg. Våtmarker av intermediär näringsstatus, såsom fattiga kärr, kan förväntas producera mycket MeHg medan mindre näringsrika våtmarker inte upprätthåller en lika hög bakteriell aktivitet och därmed producerar mindre MeHg. Mer näringsrika våtmarker kan dessutom upprätthålla en nedbrytning av MeHg som till viss del motverkar nybildningen. I denna grupp har alkärr visat sig ha unika egenskaper som nettonedbrytare av MeHg.

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