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Peat properties and mercury methylation in drained and restored boreal peatlands

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Abstract

Despite the societal demand for restoring drained boreal peatlands, a firm understanding of how drainage and restoration (through rewetting) affect the biogeochemistry of these ecosystems is missing. In addition to the intended ecosystem services, restoration may result in undesired effects and potential environmental threats, such as mercury (Hg) methylation. It is important to note that prolonged drainage can alter the peat properties, which makes the effects of restoration difficult to predict.

This thesis evaluates how drainage and restoration impact physical and chemical peat properties and net methylmercury (MeHg) formation. For each drained and restored peatland, a corresponding undisturbed natural reference peatland was sampled. Each peatland was sampled to a depth of 50 cm and analysed for carbon (C), nitrogen (N), $\delta^{13}C$, $\delta^{15}N$, organic matter content (OM), and dry bulk density (BD). The restored peatlands (and the natural peatlands) involved additional measurements of sulphur (S) XANES, along with the groundwater level and concentrations of inorganic mercury (IHg) and MeHg.

Peatland drainage was found to significantly alter the physical and chemical peat properties. The drained and restored peatlands, when compared with natural reference peatlands, were characterised by increased BD and C and N contents in OM, along with decreased OM contents, C:N ratios, and $\delta^{13}C$. Despite restoration efforts, the groundwater level of the restored peatlands remained five centimetres deeper than what was observed for natural reference peatlands. Although restoration failed to replicate the water table levels and redox conditions in natural peatland sites, the drainage and subsequent restoration increased concentrations of IHg and MeHg, as well as %MeHg of total Hg. The cumulative mass of MeHg and IHg in the top 50 cm of peat also increased after drainage and restoration. Thus, increasing the risk of MeHg and IHg export from peatlands to adjacent streams and lakes.

Keywords: boreal peatlands, peatland restoration, peat properties, carbon cycle, nitrogen cycle, peat decomposition, mercury methylation

Torvegenskaper och kvicksilvermetylering i dränerade och restaurerade boreala myrar

Abstract

Trots efterfrågan att restaurera dränerade myrar saknas fortfarande en gedigen förståelse för effekterna på de biogeokemiska processerna i torven. Utöver de avsedda ekosystemtjänsterna kan återvätning även leda till skadliga effekter och potentiella miljöhot, till exempel kvicksilvermetylering. Åtskilliga årtionden av dränerade förhållanden har också förändrat torvens egenskaper, vilket gör effekterna av restaurering på de biogeokemiska processerna svåra att förutspå.

Denna avhandling utvärderar effekterna av dränering och restaurering på torvens fysikaliska och kemiska egenskaper, samt hur återvätning påverkar kvicksilvermetylering. För varje dränerad och restaurerad myr som undersöktes provtogs en närliggande opåverkad referensmyr. Varje myr provtogs ner till 50 cm djup och analyserades för kol (C), kväve (N), δ^{13}C, δ^{15}N, innehållet av organiskt material (OM), och bulkdensiteten (BD). Mätningar och analyser på de restaurerade myrarna, med tillhörande referensmyrar, inkluderade också svavel (S) XANES, oorganiskt kvicksilver (IHg), metylkvicksilver (MeHg), samt grundvattennivån.

Dränering av myrar ledde till en signifikant påverkan på torvens fysikaliska och kemiska egenskaper. I de dränerade och restaurerade myrarna ökade BD samt innehållet av C och N, medan innehållet av OM, C:N kvot och δ^{13}C minskade relativt de naturliga referensmyrarna. Trots restaureringsinsatserna var grundvattennivån fortfarande fem centimeter lägre i de restaurerade myrarna än i de naturliga myrarna. Förekomsten av reducerade former av S relativt de oxiderade formerna var högre i naturliga myrar, vilket är i linje med en lägre grundvattennivå. Även om restaurering misslyckades med att återskapa hydrologin och redoxförhållanden relativt referensytorna så ökade nettoformeringen av metylkvicksilver i de restaurerade myrarna. Den kumulativa massan av MeHg och IHg i torvens översta 50 cm ökade också efter dränering och restaurering, vilket ökar risken för MeHg- och IHg-export från de restaurerade myrarna till närliggande bäckar och sjöar.

Nyckelord: Boreala myrar, våtmarksrestaurering, restaurering av myrar, torvegenskaper, kolcykeln, kvävecykeln, torvnedbrytning, kvicksilvermetylering

Dedication

Till Ardea – tack för ditt stöd och din kärlek!

Preface

‘The old man settled himself against the stern and shifted the sack that covered the bait box. He felt the pull of the fish and leaned back against it, quietly, as though it were something he had always known.’ – E.M.H.

In memoriam, Sven-Olov Eriksson

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List of publications

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

I. Smeds, J.*, Ehnvall, B., Liu, T., Bertilsson, S., Björn, E., Nilsson, M.B., Bishop, K., Skyllberg, U., Öquist, M.G. (2025). Effect of restoration on physical and chemical peat properties in previously drained boreal peatlands (submitted manuscript).

II. Smeds, J.*, Skyllberg, U., Nilsson, M.B., Björn, E., Bertilsson, S., Liu, T., Song, Y., Bishop, K., Öquist, M.G. (2025). Effects on net mercury methylation by restoration of previously drained boreal peatlands (manuscript).

III. Smeds, J.*, Nilsson, M.B., Ehnvall, B., Noumonvi, K.D., Bishop, K., Skyllberg, U., Öquist, M.G. (2025). Effects of drainage on physical and chemical peat properties in boreal peatlands (manuscript).

IV. Smeds, J.*, Öquist, M.G., Nilsson, M.B., Bishop, K. (2022). A simplified drying procedure for analysing Hg concentrations. *Water, Air, & Soil Pollution*, 233(6), 216.

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* Corresponding author.

The contributions of Jacob Smeds to the Papers included in this thesis were as follows:

I. Planned the study in collaboration with the co-authors, conducted the fieldwork and sample preparation, analysed the data, and wrote the manuscript in collaboration with the co-authors.

II. Planned the study in collaboration with the co-authors, conducted the fieldwork and sample preparation, conducted the laboratory analyses of total mercury and methylmercury, analysed the data, and wrote the manuscript in collaboration with the co-authors.

III. Planned the study in collaboration with the co-authors, conducted the fieldwork and sample preparation, analysed the data, and wrote the manuscript in collaboration with the co-authors

IV. Planned the study in collaboration with the co-authors, conducted the fieldwork and sample preparation, conducted the laboratory analysis of total Hg, analysed the data, and wrote the manuscript in collaboration with the co-authors.

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Abbreviations

Al	Aluminium
BD	Dry bulk density
C	Carbon
Ca	Calcium
CH_4	Methane
CO_2	Carbon dioxide
D-DPF	Densely forested drained peatland forest
Fe	Iron
Hg	Mercury
IHg	Inorganic mercury
IQR	Interquartile range
K	Potassium
KRI	Kulbäcksliden Research Infrastructure
LOI	Loss on ignition
MeHg	Methylmercury
Mg	Magnesium
Na	Sodium
O-DPF	Open drained peatland forest

OM	Organic matter
OPLS-DA	Orthogonal projections to latent structures – discriminant analysis
P	Phosphorus
PCA	Principal component analysis
S	Sulphur
Si	Silicon
TEA	Trollberget Experimental Area
VIP	Variables of importance
XANES	X-ray absorption near-edge structure spectroscopy

1. Introduction

Peatlands (or *mires*) are wetlands in which waterlogged conditions prevent plant material from fully decomposing. When the plant production rate exceeds the decomposition rate, undecomposed organic matter (OM) accumulates as peat, making peatlands a sink of atmospheric carbon (C; Clymo, 1965). Despite contributing to emissions of the potent greenhouse gas methane (CH_4), peatlands have a net cooling effect on the Earth's radiative forcing due to peat accumulation (Frolking and Roulet, 2007; Nilsson et al., 2008). These wetlands also contribute other valuable ecosystem services, such as biodiversity (Calvin et al., 2023) and surface water retention (Price, 1997).

Due to extensive drainage, about 12% of the world's peatland area has been degraded, including 50% of the peatland area in Europe (UNEP, 2022). To mitigate the degradation of peatlands by drainage, local governments and the EU have invested significant amounts of money in restoring drained peatlands (EU, 2024). The overarching aim of restoring peatlands through rewetting is to reinstate the ecosystem services associated with undisturbed natural peatlands. However, an extended period of drainage can alter the physical and chemical peat properties (Krüger et al., 2015; Laiho, 2006), which changes the preconditions for various anaerobic microbial processes (Bergman et al., 1998; Hu et al., 2020).

Besides the desired ecosystem services, the restoration of boreal peatlands can also yield undesired effects and potential environmental risks (Ojanen and Minkkinen, 2020; Tjerngren et al., 2012b). An example of a concerning externality of restoration is the transformation of inorganic mercury (Hg) into toxic methylmercury (MeHg; Gilmour et al., 2013). The MeHg formed in peatlands can enter streams and eventually reach lakes and marine systems (Thompson et al., 2023). Thus, the return to anoxic conditions associated with peatland restoration may result in highly undesired effects and conflicting ecosystem services.

Many of the biogeochemical processes, and thus the ecosystem services and/or potential environmental threats associated with peatlands, depend on the biogeochemistry in the superficial layer of peat (~50 cm; Eriksson et al., 2010a; Ivanov et al., 1981). Therefore, it is essential to understand how peatland drainage influences the physical and chemical properties of the uppermost peat layer if the biogeochemical effects of restoration are to be reliably predicted.

This thesis aims to: (1) evaluate the effects of drainage and restoration on physical and chemical peat properties (e.g., dry bulk density (BD) and C and nitrogen (N) contents); and (2) use this understanding to evaluate whether the restoration of previously drained peatlands has a significant impact on net MeHg formation.

1.1 Boreal peatlands

Peatlands are found in biomes where the climate is wet enough to sustain waterlogged soil conditions. With its positive water balance, the boreal climate zone is particularly suited for peatland formation, and high-latitude peatlands are essential to global C dynamics (Gorham, 1991; Yu et al., 2010). Boreal peatlands, which are characterised by long-term C fixation and slow peat decomposition, store a considerable share of the world's soil C (Hugelius et al., 2020; Yu, 2012). However, the processes governing peat decomposition lead to simultaneous emissions of carbon dioxide (CO_2) and CH_4 (Frolking et al., 2011). Under nutrient-rich conditions, peatlands may also emit the greenhouse gas nitrous oxide (Martikainen et al., 1993).

Peat accumulates when the primary production of plant material exceeds the decomposition rate (Clymo, 1965). The functioning of peatlands can be described through two principal depth layers: an active surface layer, known as the *acrotelm,* and a more inert underlying layer termed the *catotelm* (Figure 1; Ingram, 1978; Ivanov et al., 1981). Peat formation occurs in the biogeochemically active and aerobic acrotelm, where OM is partially decomposed. The underlying catotelm is characterised by anaerobic conditions and low peat decomposition rates (Ivanov et al., 1981). Peat enters the catotelm for long-term storage at the same rate as peat is formed in the acrotelm (Clymo, 1984). The boundary between the acrotelm and the catotelm (*mesotelm*; Clymo and Bryant, 2008) is not sharp and fluctuates depending on the groundwater level (Clymo, 1984).

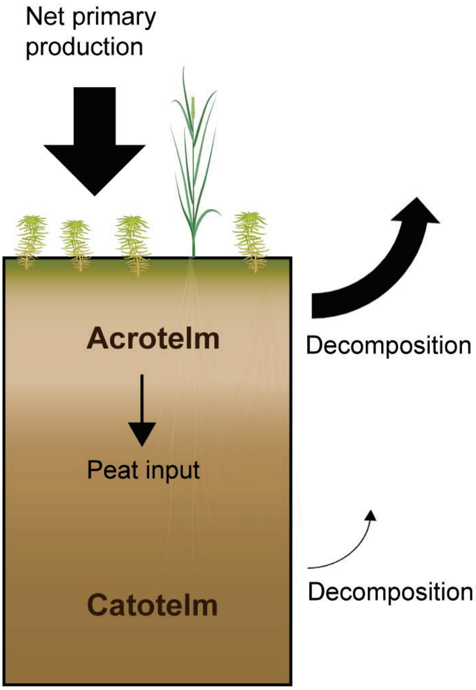

Figure 1. Net carbon accumulation in peatlands, as modelled through the net primary production, peat sequestration to the catotelm, and peat decomposition in the acrotelm and catotelm.

Minerogenic peatlands (fens) receive water from precipitation and surrounding watersheds. In contrast, ombrogenic peatlands (bogs) depend solely on precipitation and lack groundwater and nutrient inputs from an adjacent catchment (Rydin and Jeglum, 2013). The groundwater level significantly influences the peatland biogeochemistry, including the redox conditions (De Mars and Wassen, 1999).

Redox potential reflects the extent of oxidising or reducing conditions in a particular environment (Schlesinger and Bernhardt, 2020). OM serves as an electron donor, with the most rapid oxidation reactions occurring in the presence of molecular oxygen (Mitsch and Gosselink, 2015). As oxygen is consumed, other electron acceptors facilitate OM degradation in a hierarchical order based on energy yield (Mitsch and Gosselink, 2015). Denitrification is the most energetically favourable respiratory process once oxygen has been depleted (Schlesinger and Bernhardt, 2020). However, nutrient-poor (oligotrophic) peatlands may show limited nitrate availability, which will hamper denitrification (Klemedtsson et al., 2005). If nitrate is consumed and the redox potential drops, manganese, iron (Fe), and sulphate reduction can be used for OM decomposition (Mitsch and Gosselink, 2015). Fermentation is another pathway for decomposing complex organic molecules into simpler organic compounds and mineralisation (Schlesinger and Bernhardt, 2020). Fermentation products, such as acetate, hydrogen gas, and CO_2, are utilised during methanogenesis to produce CH_4 (Schlesinger and Bernhardt, 2020). The emission of CH_4 is a crucial process for the climate radiative forcing of peatlands, and can potentially outweigh the environmental effects of CO_2 sequestration (Frolking and Roulet, 2007; Nilsson et al., 2008). However, CH_4 formed in anoxic soil layers can be oxidised into CO_2 in the oxic peat layers closer to the surface (Hakemian and Rosenzweig, 2007), and thereby reduce the total CH_4 emissions tied to a fen or bog.

OM quality is another characteristic that influences peat decomposition, and hence, the rate of peat accumulation (Moore and Basiliko, 2006). High-quality organic polymers, such as polysaccharides, represent an accessible energy source for microorganisms (Serk et al., 2022). In contrast, inaccessible OM, such as aromatic polymers, tends to decompose more slowly (Leifeld et al., 2012). In the acrotelm, fresh, degradable compounds are decomposed, while more inaccessible polymers accumulate in the catotelm (Leifeld et al., 2012; Serk et al., 2022). Thus, deeper peat layers are characterised by substantial levels of inaccessible substrates with a low energy yield for heterotrophic respiration or fermentation (Nilsson and Öquist, 2013).

It is worth noting that many additional factors, including soil temperature and climate, pH, nutrient status, and plant community composition, influence the peat decomposition rate (Bergman et al., 1998; Von Lützow and Kögel-Knabner, 2009). These biogeochemical characteristics are part of a complex system that also affects the plant species composition at the site. As plant community composition is linked to long-term C fixation, any changes will also impact the peat accumulation rate, which highlights how these biogeochemical factors involve intricate interplay (Frolking et al., 2010).

Different species of *Sphagnum* mosses often dominate oligotrophic boreal peatlands as they thrive under nutrient-poor and moist conditions (Clymo, 1965). The water-holding capacity of *Sphagnum* spp. is up to 20 times its dry weight (Rydin and Jeglum, 2013), which helps maintain wet soil conditions. In addition, *Sphagnum* spp. influence the biogeochemical environment by storing nutrients and releasing hydrogen ions during water absorption (Rydin and Jeglum, 2013). Nutrient limitation and acidification further suppress the peat decomposition rate and decrease the cover of vascular plants (Rydin and Jeglum, 2013).

Vascular plants, such as sedges, demonstrate higher primary production rates, yet are often readily decomposed relative to *Sphagnum* spp. (Rydin and Jeglum, 2013). Sedges also fuel microbial activity through root exudation (Öquist and Svensson, 2002), and the presence of readily available C could promote processes such as methanogenesis and Hg methylation (Bergman et al., 1998; Windham-Myers et al., 2009). The aerenchyma of vascular plants can mediate oxygen transport to otherwise anoxic depths, which could enhance decomposition rates (Armstrong and Beckett, 1987; Philben et al., 2015). Vascular plants can also transport CH_4 from the depths of peatlands to the surface; this rapid transport bypasses the zones of potential methane oxidation, thus contributing to CH_4 emissions from peatlands (Joabsson et al., 1999).

Peatlands are complex systems in which long-term climate radiative forcing relies on the delicate balance between C fixation and greenhouse gas emissions (Frolking and Roulet, 2007; Nilsson et al., 2008). As such, these systems are vulnerable to external disturbance, and a large share of the original peatland area has been diminished due to anthropogenic activity (Calvin et al., 2023).

1.2 Drained peatlands

Over 50% of the peatland areas across Europe have been drained. This has mainly been done to increase the area of cultivable land for agriculture and forest production (UNEP, 2022). In Sweden, peatland drainage began in the late 19th century and peaked during the 1930s (Fahlvik et al., 2009). The drainage of peatlands continued during the second half of the 20th century, but government-funded drainage ceased in the 1990s (Fahlvik et al., 2009; Päivänen and Hånell, 2012). In numbers, 1.5-2 million hectares of the original 10 million hectares of Swedish peatlands have been drained (Fahlvik et al., 2009).

The groundwater level has a noticeable impact on the functioning of peatlands (Figure 2); for instance, as the groundwater level recedes, the depth of the acrotelm increases (Ivanov et al., 1981). The increased oxic conditions associated with this change stimulate microbial activity and peat decomposition, especially if fresh, high-quality OM is available (Freeman et al., 2001; Preston et al., 2012). However, drier conditions and increased plant root nutrient acquisition following peat decomposition may favour vascular plant species (Strack et al., 2006) and stimulate net primary production (Minkkinen et al., 2018).

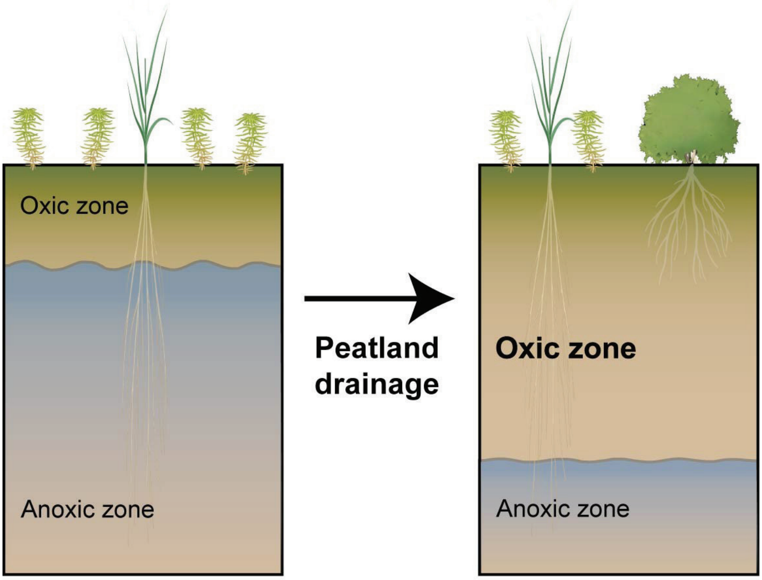

Figure 2. Drainage of peatlands will increase the depth of the oxic zone, thereby increasing the abundance of sedges and shrubs at the expense of *Sphagnum* spp.

The net effect of boreal peatland drainage on C accumulation remains an issue under debate. Some studies suggest that the soil remains a C sink following drainage (Minkkinen et al., 2018; Minkkinen and Laine, 1998a, 1998b; Turetsky et al., 2011), while other studies have found that the soil shifts to acting as a net C source (Hargreaves et al., 2003; He et al., 2016;

Simola et al., 2012). There is also debate over whether drained peatlands act as C sinks or sources on an ecosystem scale (Tong et al., 2024). Some argue that the increased photosynthetic capacity and dense tree stands that accompany drainage can compensate for the potentially enhanced soil C loss (Lohila et al., 2011; Meyer et al., 2013; Ojanen et al., 2013; Ratcliffe et al., 2019).

Although peatland soils may remain a C sink after drainage, the peat may undergo irreversible physical and chemical changes (Krüger et al., 2015). Drained peat can physically subside as the support from groundwater is lost, leading to an increase in dry bulk density (BD; Minkkinen and Laine, 1998b). Furthermore, increased peat mineralisation and a denser tree cover following drainage can further increase the BD (Minkkinen and Laine, 1998b). This alters the hydraulic properties of the peat and the groundwater dynamics of drained peatlands (Liu and Lennartz, 2019).

The degree of peat decomposition can be evaluated using chemical soil properties, such as OM content (Leifeld et al., 2011), along with the C and N contents in OM (Serk et al., 2022; Tfaily et al., 2014), C:N ratio (Leifeld et al., 2020), and δ^{13}C and δ^{15}N isotopic signatures (Krüger et al., 2015). Low OM content can indicate an increased peat mineralisation (Leifeld et al., 2011; Rogiers et al., 2008). The residual OM present after decomposition is often enriched in resistant polymers due to the preferential loss of readily degradable compounds (Drollinger et al., 2020; Serk et al., 2022). Recalcitrant compounds generally have higher C contents than less recalcitrant polymers (Reddy and DeLaune, 2008; Speight, 2017), and the C content in OM can reflect the degree of peat decomposition (Serk et al., 2022; Tfaily et al., 2014).

Both N content and the C:N ratio can serve as indicators of peat decomposition (Kuhry and Vitt, 1996; Malmer and Holm, 1984). Rapid C loss during the initial phase of decomposition can lead to relatively high N levels, which reduce the C:N ratio in residual peat (Kuhry and Vitt, 1996).

The δ^{13}C and δ^{15}N isotopic signatures have been used to assess the impact of drainage on peatlands (Drollinger et al., 2020, 2019; Groß-Schmölders et al., 2022, 2020; Krüger et al., 2015). The δ^{13}C and δ^{15}N signatures in vegetation result from fractionation during photosynthesis and plant nutrient acquisition (Rice and Giles, 1996). Decomposition further changes the relative abundances of ^{13}C and ^{15}N. For instance, the preferential use of readily degradable polymers, such as polysaccharides, and the accumulation of isotopically light lignin-enriched C, can decrease the levels of ^{13}C in peat (Benner et al., 1987; Serk et al., 2022).

An extended period of drained conditions can change the physical and chemical peat properties (Leifeld et al., 2020), and these conditions could influence various anaerobic microbial processes (Bergman et al., 1998; Graham et al., 2013). Hence, the impact of restoration on biogeochemical processes remains challenging to predict.

1.3 Restored peatlands

The EU and the governments of member states have allocated substantial resources to peatland restoration. EU member states have committed to restoring at least 20% of disturbed sea and land areas by 2030 and restoring every ecosystem in need of restoration by 2050 (EU, 2024). In Sweden, peatland restoration serves as an issue of political debate, as the Swedish government commits at least €12 million each year to wetland restoration. In 2024, more than €30 million was channelled towards wetland restoration (SEPA, 2024).

Due to the magnitude and costs of restoration activities, it is crucial to understand how changes in peat properties will affect critical biogeochemical processes after restoration. The short- and long-term effects of restoration on a range of biogeochemical functions remain uncertain, and may be difficult to predict since they do not necessarily mirror the conditions of natural peatlands (Kreyling et al., 2021).

Restoration through rewetting reintroduces anoxic conditions to peatlands and decreases the depth of the acrotelm (Figure 3). The decrease in redox potential associated with these changes can hamper peat decomposition rates and promote peat accumulation (Clymo, 1984). While long-term C sequestration may increase (Lucchese et al., 2010), restoration could also lead to undesired processes that become active under anoxic conditions, such as CH_4 production (Juottonen et al., 2012; Urbanová and Bárta, 2020). Increased CH_4 emissions could counteract the climate benefits associated with increased C sequestration, especially over a short- to mid-term time perspective following restoration (Günther et al., 2020). Another potential undesired impact of wetland restoration is the methylation of inorganic Hg (IHg) to toxic MeHg (Tjerngren et al., 2012b).

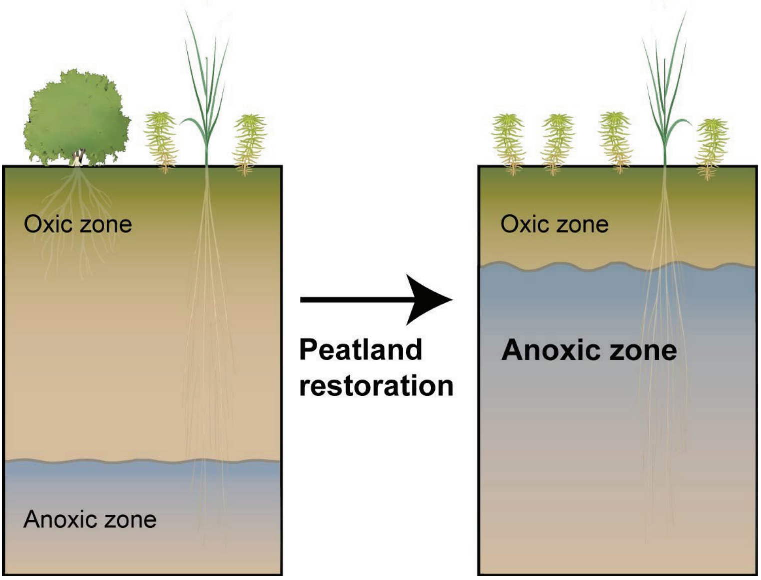

Figure 3. Restoration will decrease the depth of the oxic zone, increasing the abundance of *Sphagnum* spp. at the expense of vascular plants.

1.4 Mercury methylation

Mercury is a trace metal that occurs naturally in the environment at low levels, but can also be introduced into ecosystems from anthropogenic sources (Pirrone et al., 2010). Hg – when emitted into the atmosphere – can be transported long distances as the gaseous elemental form Hg(0) (Pacyna, 2020) before being deposited either as Hg(0) or the oxidised form Hg(II) (Jiskra et al., 2015). Hg that is deposited to soils, vegetation, and waters can undergo re-emission to the atmosphere as Hg(0), which will cause problems for years after initial emission (Amos et al., 2013).

The toxicity of the element increases when Hg is methylated to MeHg (Gilmour et al., 2013). MeHg is a potent neurotoxin that can impair cognitive development and cause symptoms such as memory loss, attention deficit, and decreased coordination abilities (Basu et al., 2023). When introduced into aquatic or marine food webs, MeHg bioaccumulates and can thus be present at high levels in apex predators via biomagnification (Nogara et al., 2019). As such, fish and seafood consumption accounts for a large share of human MeHg exposure (Sundseth et al., 2017). In Sweden, about half of the

~100,000 lakes contain fish with Hg concentrations that exceed the FAO/WHO guidelines for human consumption (Braaten et al., 2019; European Commission, 2023; Eklöf et al., 2024).

The high OM contents and low redox potential of peatlands favour net formation of MeHg, making these ecosystems a net source of MeHg to downstream environments (St. Louis et al., 1994). Methylation of Hg is primarily driven by anaerobic or facultative microorganisms that possess the *hgcAB* gene cluster; this includes, e.g., sulphate-reducing bacteria, iron-reducing bacteria, and methanogens (Compeau and Bartha, 1985; Gilmour et al., 2013; Parks et al., 2013). Redox conditions exert significant control over the abundance and activity of Hg-methylating species, with net methylation rates potentially increasing following the re-establishment of anoxic conditions associated with peatland restoration (Tjerngren et al., 2012b).

Another aspect that influences net Hg methylation is peatland nutrient status (Hu et al., 2020; Kronberg et al., 2012; Poulin et al., 2019; Tjerngren et al., 2012a, 2012a; Wang et al., 2020). Net methylation rates are highest in wetlands with an intermediate nutrient status, a C:N ratio of ~20 and pH of ~5 (Tjerngren et al., 2012b). These conditions favour Hg methylation over MeHg demethylation (Tjerngren et al., 2012a). Higher trophic status increases the demethylation rate, whereas methylation rates are low in nutrient-poor soils (Tjerngren et al., 2012a).

The methylation rate is also influenced by the bioavailability of Hg(II) and the availability of electron donors (Graham et al., 2013; Peterson et al., 2023). Hg in the environment can form complexes with OM, thiols, or other functional groups, and these chemical interactions can either inhibit or promote methylation depending on the binding affinity between ligands and inorganic Hg (Benoit et al., 2001; Gu et al., 2011; Holley et al., 2007; Poulin et al., 2019). Furthermore, Hg methylation depends on the accessibility of organic substrates for microbial activity (Graham et al., 2013; Mitchell et al., 2008). The most readily degradable organic molecules could be rapidly metabolised following peatland drainage, and poor substrate availability might impede microbial activity after restoration (Juottonen et al., 2012). However, root exudates of vascular plants can contribute to providing the necessary substrate (Ström et al., 2003), and the establishment of, e.g., sedges following restoration could increase microbial activity (Bragazza et al., 2015; Windham-Myers et al., 2009).

1.5 Knowledge gaps and thesis objectives

Despite the societal demand for restoring drained boreal peatlands, a firm understanding of the effects of drainage and restoration on boreal peatlands is missing. This thesis aims to improve the understanding of how drainage changes the conditions for biogeochemical processes after restoration by examining the physical and chemical properties of drained and restored peatlands. The collected data were then extended to investigate whether the restoration of drained peatlands has a significant impact on net MeHg formation.

Most of the desired ecosystem services and/or potential environmental threats of restoration depend on processes that occur in the top ~50 cm of peat (Bergman et al., 1998; Ivanov et al., 1981). Thus, elucidating the effects of drainage on superficial peat is essential for understanding the biogeochemical processes related to restoration.

The research covered in this thesis, which involved sampling 24 peatlands between latitudes 57° N and 64° N, moves beyond case studies to evaluate the effects of drainage and restoration at a regional scale. Redox-related processes are particularly sensitive to biogeochemical changes throughout the peat column (Eriksson et al., 2010a), and each 50 cm peat core was sliced into 25 two-centimetre discs. The analysed data, which were characterised by high spatial and vertical resolution, provided robust evidence for how drainage and restoration influence physical and chemical peat properties along with net MeHg formation.

To summarise, this thesis aims to: (1) evaluate how drainage and restoration affect physical and chemical peat properties, and (2) use any insights gleaned from the first stage to evaluate whether the restoration of previously drained peatlands has a significant impact on net MeHg formation. These aims were addressed in the four appended studies, referred to as Papers I-IV, in the following ways:

- Paper I assessed the impacts of more than 60 years of drainage followed by 6-9 years of restored conditions on key physical and chemical peat properties, i.e., peat BD, OM content, C and N contents in OM, and the δ^{13}C and δ^{15}N isotopic signatures in peat bulk samples. A total of 54 peat cores of 50 cm depth were extracted from 16 peatlands, organised into eight peatland pairs. For each restored peatland, an adjacent undisturbed natural peatland was sampled for reference. Each peat core was sliced into 25 two-centimetre discs to provide a high-resolution depth record.

- Paper II assessed whether restoration has a significant impact on net MeHg formation. The concentrations of IHg and MeHg, %MeHg of total Hg (a proxy for long-term net Hg methylation), groundwater level, and sulphur (S) X-ray absorption near-edge structure spectroscopy (XANES), along with *hgcA* gene abundance in the microbial community, were determined. The peat cores were also analysed for the total concentrations of aluminium (Al), calcium (Ca), Fe, potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), silicon (Si), and S.

- Paper III assessed the impacts of more than 100 years of drainage on boreal peatlands in terms of peat BD, OM content, C and N contents in OM, and $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures in peat bulk samples. Three 50-cm peat cores were extracted from eight peatlands (four natural and four restored), respectively. Each peat core was sliced into 25 two-centimetre discs.

- Paper IV evaluated the extent to which the sample drying procedure influences total Hg concentrations in peat samples. The methylation of Hg following restoration depends on the presence of IHg. However, the sample treatment process associated with total Hg measurements is costly and time-consuming, partly due to the need for freeze-drying to limit Hg loss. Thus, the research outlined in Paper IV examined an alternative, cost-efficient drying procedure that could potentially simplify the analysis of total Hg concentration in peat. Replicates from 203 peat samples were analysed for total Hg following freeze-drying and oven-drying at 60 ºC.

2. Methods

2.1 Site selection and description

2.1.1 Selection of restored and natural reference peatlands

A total of eight restored peatlands across Sweden were selected for Papers I-II. The chosen peatlands had been restored through rewetting between 2012 and 2015 within the *Life to ad(d)mire* project. The peatlands included both ombrogenic and minerogenic systems with an oligotrophic to intermediate (mesotrophic) nutrient regime across various geographical locations in Sweden.

For each restored peatland sampled for Papers I-II, an undisturbed natural reference peatland that was never subjected to drainage or restoration was chosen, with the prerequisite that the natural reference peatland was located within the same peatland area or complex. The distance between the restored and natural reference peatland varied between 150 and 1500 m, depending on site-specific characteristics. Another site selection criterion applied to both the restored and natural reference peatlands was that the peat depth exceeded 2 metres.

2.1.2 Description of the restored and natural reference peatlands

The eight restored peatlands included in Papers I-II were located between the latitudes of 57° N and 63° N in Sweden (Figure 4; Table 1). Of the studied peatlands, three, namely, Anderstorps Stormosse (57°19'N, 13°36'E), Store Mosse (57°16'N, 13°55'E), and Bredsjömossen (58°46'N, 15°12'E), are situated in southern Sweden. Anderstorps Stormosse is a bog where the vegetation is dominated by *Sphagnum* spp., sedges (*Eriophorum vaginatum*, *Carex* sp.), and ericaceous shrubs (*Calluna vulgaris*, *Erica tetralix*). The ericaceous shrubs were found to be more widespread in the restored area of the peatland, while the natural parts were dominated by *Sphagnum* spp. As was the case with all the other restored and natural reference peatlands sampled in Papers I-II, Anderstorps Stormosse was not forested. It was drained during the 1940s for peat extraction. However, peat was not extracted from the sampled area. In 2012, Anderstorps Stormosse was restored through the damming of the drainage ditches, which was the restoration method used for every restored peatland sampled within Papers I-II.

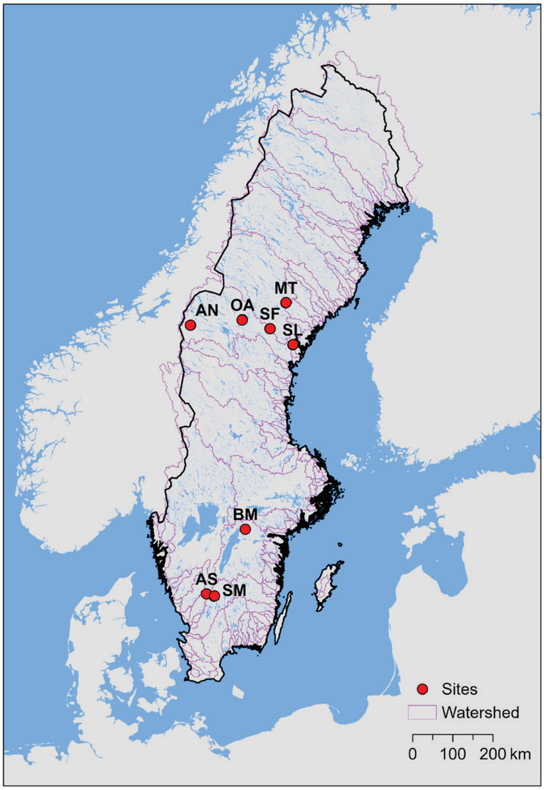

Figure 4. The geographical locations of the 16 peatlands sampled in Papers I-II. Each red point represents a pair of restored/natural reference peatland. The names of the sampled peatlands are Mossaträsk (MT), Öjsjömyrarna (OA), Årnnsjön Mire (AN), Stensjöflon (SF), Sör-Lappmyran (SL), Bredsjömossen (BM), Anderstorps Stormosse (AS), and Store Mosse (SM).

The peatland Store Mosse is a bog covered by *Sphagnum* spp., sedges (*Rhynchospora alba*, *Eriophorum* spp.), ericaceous shrubs (*Andromeda* spp., *Vaccinium* spp., *Calluna vulgaris*, *Erica tetralix*), and lichens (*Cladonia* spp.). Store Mosse was drained early in the 20th century and restored between 2013 and 2015.

The site Bredsjömossen is a fen with vegetation dominated by *Sphagnum* spp. and ericaceous shrubs (*Empetrum nigrum*, *Calluna vulgaris*). Bredsjömossen was drained during the 19th century and restored in 2014.

A total of five restored peatlands situated in northern Sweden, between the latitudes of 62° N and 63° N, were sampled. Notably, two of these peatlands, Ånnsjön Mire (63°18'N, 12°31'E) and Öjsjömyrarna (63°27'N, 15°05'E), are located at relatively high altitudes due to the proximity to the Scandinavian Mountains. Ånnsjön Mire is a fen located at 533 m.a.s.l. Vegetation at the site is dominated by *Sphagnum* spp., brown mosses (*Amblystegiaceae* family), and shrubs (e.g., *Calluna vulgaris*). Ånnsjön Mire was restored in 2012. Information on the site's drainage history is not available.

Öjsjömyrarna is a fen located at 459 m.a.s.l., with the vegetation dominated by *Sphagnum* spp. and brown mosses (*Amblystegiaceae* family). Information on the drainage history of Öjsjömyrarna is missing. The peatland was restored in 2012.

The remaining three peatlands, Stensjöflon (63°15'N, 16°28'E), Sör-Lappmyran (62°53'N, 17°34'E), and Mossaträsk (63°49'N, 17°18'E), are located in northeastern Sweden. The vegetation at the fen Stensjöflon is dominated by *Sphagnum* spp., sedges (*Eriophorum vaginatum*, *Carex pauciflora*), and shrubs (*Betula nana*, *Empetrum nigrum*, *Andromeda polifolia*). Stensjöflon was drained in 1926 and restored in 2012.

Sör-Lappmyran is a fen with vegetation dominated by *Sphagnum* spp., sedges (*Eriophorum vaginatum*, *Carex pauciflora*), and water trefoil (*Menyanthes trifoliata*). The drainage ditches at this site were established during the 1950s and 1960s, and the peatland was restored in 2012.

Mossaträsk is a fen dominated by *Sphagnum* spp., sedges (*Carex rostrata*, *Carex pauciflora*, *Eriophorum vaginatum*), shrubs (*Andromeda polifolia*, *Betula nana*), and cloudberry (*Rubus chamaemorus*). Information on the drainage history of Mossaträsk is missing. It was restored in 2013.

Table 1. Mean annual temperature (1990-2020), mean annual precipitation (1990-2020), average pH (±SE), and Köppen climate zone classification (e.g., Dfa/Dfb (Humid Continental Climate) or Cfb (Temperate Oceanic Climate)) at the sites Mossaträsk (MT), Öjsjömyrarna (OA), Årnnsjön Mire (AN), Stensjöflon (SF), Sör-Lappmyran (SL), Bredsjömossen (BM), Anderstorps Stormosse (AS), and Store Mosse (SM). Each peatland site involved a paired restored/natural reference peatland (Papers I-II).

Site ID	*Temperature (C°)*[a]	*Precipitation (mm y^{-1})*[a]	*pH restored*	*pH natural*	*Köppen classification*[b]
MT	3.7	583	4.3 ± 0.06	4.3 ± 0.03	Dfa/Dfb
OA	3.7	522	4.9 ± 0.13	5.1 ± 0.05	Dfb
AN	2.1	898	4.5 ± 0.27	4.1 ± 0.06	Dfb
SF	3.2	577	4.4 ± 0.03	4.4 ± 0.01	Dfa/Dfb
SL	5.0	743	4.9 ± 0.42	4.7 ± 0.35	Dfa/Dfb
BM	7.1	565	3.9 ± 0.04	3.9 ± 0.03	Cfb
AS	6.4	773	4.2 ± 0.13	4.2 ± 0.13	Cfb
SM	6.4	773	4.0 ± 0.04	4.1 ± 0.06	Cfb

[a] Data acquired from smhi.se
[b] Peel et al. (2007)

2.1.3 Description of drained and natural reference peatlands

A total of four drained peatlands were sampled for Paper III. Four natural peatlands were sampled as reference sites (Figure 5). Of the drained peatlands, two belonged to the Kulbäcksliden Research Infrastructure (KRI; 64°09'N, 19°33'E; Noumonvi et al., 2023) at the Hälsingfors drained peatland forest. The drained peatland forest at the Hälsingfors site is divided into two areas: a densely forested area (dense drained peatland forest; D-DPF) and an open forested area (open drained peatland forest; O-DPF). Prior to drainage in the late 19th century, the D-DPF and O-DPF areas were both oligo-mesotrophic fens (Tong et al., 2024). At present, the D-DPF site is covered by downy birch (*Betula pubescens*) and Norway spruce (*Picea abies*), which account for 56% and 37% of the tree density, respectively. Ground vegetation at D-DPF site is dominated by shrubs, e.g., *Vaccinium vitis-idaea* and *Vaccinium myrtillus* and forest mosses, e.g., *Pleurozium schreberi*. At the O-DPF site, Scots pine (*Pinus sylvestris*) dominates the above-ground vegetation and *Sphagnum* spp. dominates the ground vegetation (Tong et al., 2024).

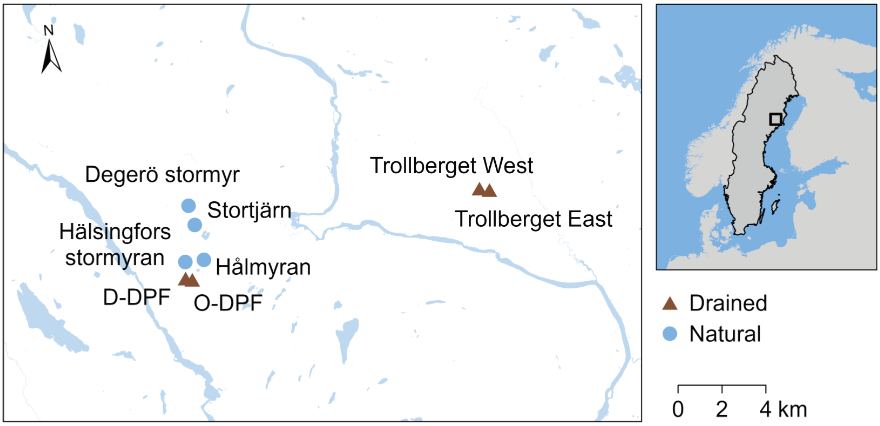

Figure 5. The geographical locations of the eight peatlands (four drained peatlands and four natural reference peatlands) sampled in Paper III (64° N, 19° E). Brown triangles represent drained peatlands, and blue dots denote natural reference peatlands.

The research described in Paper III also included two drained peatlands that belong to the Trollberget Experimental Area (TEA; 64°14'N, 19°46'E); this area is located ~15 km from KRI (Laudon et al., 2021). The TEA includes oligotrophic fens that were drained during the early 20th century. The samples from TEA were collected before the peatland was restored in 2020. Samples were collected from two different ends of TEA, e.g., the sites TEA-East and TEA-West. Neither site TEA-East nor TEA-West showed tree cover, and the vegetation was dominated by *Sphagnum* spp., ericaceous shrubs (*Andromeda polifolia*., *Calluna vulgaris*), and sedges (*Rhynchospora alba*, *Eriophorum* spp; Laudon et al., 2021).

In the research for Paper III, four natural reference peatlands within KRI were sampled. These sites include the oligotrophic fens Degerö Stormyr, Hålmyran, Stortjärn, and Hälsingfors. These natural sites all lie within a connected peatland complex within a 3 km radius (Noumonvi et al., 2023). The vegetation at all four sites was dominated by *Sphagnum* spp. and sedges (e.g., *Scheuchzeria palustris*, *Eriophorum vaginatum*, *Carex rostrata*). A more detailed site description of the KRI peatlands is available in the research by Noumonvi et al. (2023).

2.1.4 Other sampling sites

For Paper IV, archived peat samples from natural peatlands along the Gulf of Bothnia in northern Sweden were used (63°40-64'N, 20°20-50'E). These peatlands fell within the Sävar Rising Coastline Mire Chronosequence, which is located in the Swedish county of Västerbotten in the Bothnian Bay Lowlands. The samples represent fens of different ages and nutrient status ranging from oligotrophic to mesotrophic (Ehnvall et al., 2023; Wang et al., 2020). The vegetation at the sites was dominated by *Sphagnum* spp. and sedges (e.g., *Carex limosa*, *Eriophorum vaginatum*, *Carex rostrata*) in the lawn community. A comprehensive description of the sampling sites can be found elsewhere (Ehnvall et al., 2023; Wang et al., 2020).

2.2 Peat sampling, groundwater measurements, and sample processing

2.2.1 Peat sampling

A total of 54 peat cores were sampled for Papers I-II, while 24 peat cores were sampled for Paper III. In Papers I-II, three 50-cm peat cores were collected at each restored and natural peatland site. The Ånnsjön Mire site was an exception, as six peat cores were collected from both the restored and natural peatland. The additional sampling at Ånnsjön Mire enabled equal representation of the three principal geographical regions sampled within the research project, namely, Jämtland (northwestern Sweden), Västernorrland (northeast), and Småland/Östergötland (south). As was the case in Papers I-II, three peat cores were collected from each of the drained and natural reference peatland in Paper III.

Peat cores were collected from the lawn level at both natural, drained, and restored peatlands using a 55-cm circular stainless-steel corer (Ø16 cm) as described by Clymo (1988) with certain modifications (Figure 6). A 60-cm square corer (8x8 cm) as initially described by Jeglum et al. (1991) was used to extract the three peat cores sampled from the D-DPF site; this was done because sampling with the circular corer proved unsuccessful at the site. The extracted peat cores were frozen within four hours of sampling and stored at -18 °C until further processing.

Following the extraction of peat cores, peat that would be used to determine the abundance of the Hg-methylating gene *hgcA* at depths of 5, 15, 25, 35, and 45 cm was collected (Paper II). A custom-made plastic board with cut-out slits was used to achieve the correct depth, and a disinfected set of tweezers was used for sampling. Collected peat was put in a 2 ml cryotube and placed in a liquid nitrogen-cooled Air Liquid Voyager Container (TITAN Containers, Bussy-Saint-Georges, France) for transportation. Samples were stored at -80 ºC until further processing.

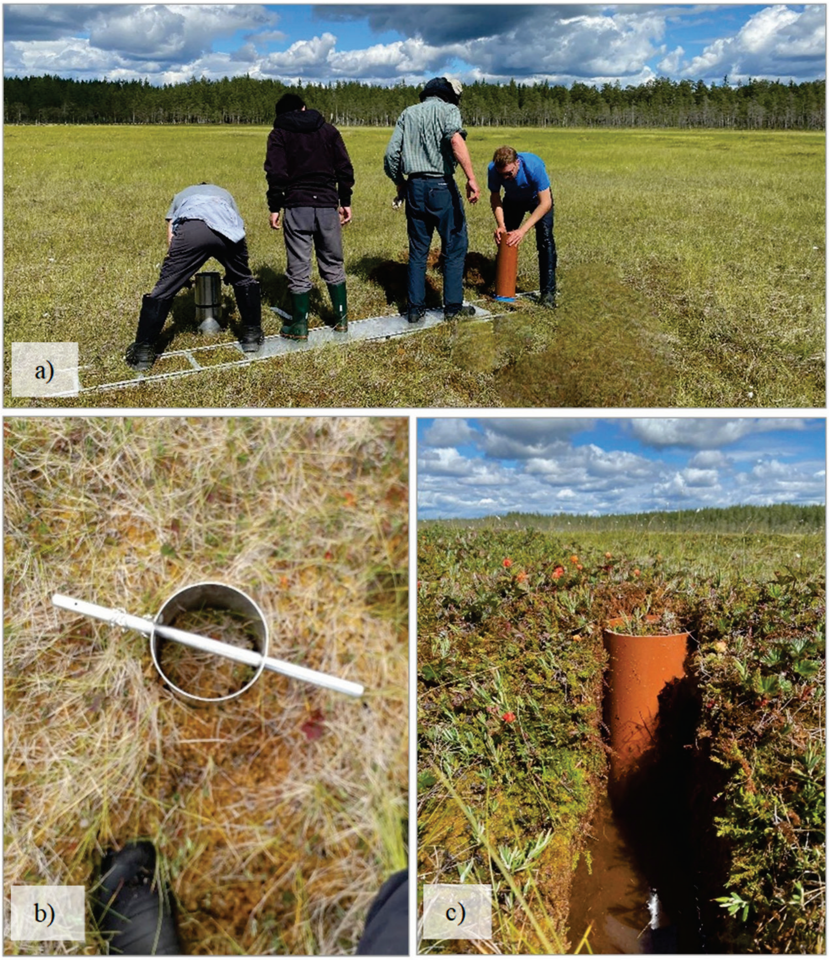

Figure 6. Peat cores were sampled using a stainless-steel corer (a, b) and a plastic tube to achieve a depth of 50 cm (a, c).

2.2.2 Groundwater level and pore water pH measurements

The groundwater level was measured continuously at the peatlands sampled in Papers I-II during two growing seasons (spring 2022 – autumn 2023). A groundwater well was installed within a one-metre radius of the spots from which peat cores had been extracted. Thus, three groundwater loggers (Levellogger Junior, Solinst Ltd., Georgetown, Ontario, Canada) were deployed at each restored and natural reference peatland (n = 54).

The beginning of the growing season was defined as the first five-day series with a daily mean air temperature that exceeded 5ºC. Similarly, the end of the growing season was defined as the consecutive occurrence of five days with a mean air temperature below 5ºC. Due to the lack of daylight during the late autumn, October 31 was used as an end date for the growing season even if the temperature-based criterion was not met.

Groundwater was always measured with the peatland surface (defined as the moss surface) serving as the reference point. As *Sphagnum*-based peat tends to swell or shrink depending on the water saturation, the groundwater loggers were attached to a 0.25 m^2 floating rig.

A handheld Greisinger GMH 5550 device (Greisinger, Regenstauf, Germany) was used to measure the pH of the superficial peat pore water (top ~5 cm of the groundwater). The pH measurements of peat pore water were conducted in the field following the installation of groundwater loggers.

2.2.3 Sample processing

The 50-cm peat cores were sliced into 25 discs with a width of 2 cm using a bandsaw (BAS 318 Precision WNB, Metabo, Nürtingen, Germany) equipped with a stainless-steel blade. Peat cores were sliced while still frozen in a cooling room (4 °C) to minimise any thawing (Figure 7). Samples from the D-DPF site were subsampled into 11 discs in the field (e.g., depths of 0-2.5 cm, 2.5-7.5 cm, 7.5-12.5 cm, ... , 42.5-47.5 cm, and 47.5-50 cm; Paper III). These peat subsamples were stored frozen (-18 °C) until further processing.

The peat samples collected for Papers I-III (n = 1908) were oven-dried at 70 °C until a constant weight was achieved (~72 h). A subset of the replicates (n = 240) used in Paper II were freeze-dried in addition to the oven-dried subsample. The 203 replicates for Paper IV were both freeze-dried and oven-dried at 60 °C until a constant weight was achieved (~96 h; Smeds et al., 2022).

Oven-dried peat samples were placed in a desiccator for cooling. The dry samples were weighed, and the BD was calculated using the known volume. Before chemical analysis, each sample was homogenised in a zip-lock bag, after which approximately one gram of dry peat was milled to a fine powder using an IKA Tube Mill Control, version 1.4 (Staufen, Germany).

Figure 7. The 50-cm peat cores were sliced (a) into 25 two-centimetre discs (b) using a bandsaw with a stainless-steel blade.

2.3 Laboratory analyses

2.3.1 Loss on ignition

The loss on ignition (LOI) values were determined for the peat samples used in Papers I-III (n = 1908). The already dry samples were dried again overnight before a four-hour furnace routine (550 °C). Loss of dry matter was input as peat OM content.

2.3.2 Chemical analyses

Oven-dried samples were analysed for C, N, $\delta^{13}C$, and $\delta^{15}N$ (n = 1908; Papers I & III). C and N contents was determined using an Elemental analyser (Flash EA 2000, Thermo Fisher Scientific, Waltham, MA, USA), while the $\delta^{13}C$

and δ^{15}N isotopic abundances were determined using an Isotope ratio mass spectrometer (DeltaV, Thermo Fisher Scientific; Werner et al., 1999). The vast majority of the C and N in peat is found in the OM. Therefore, the C and N contents were normalised to the quantity of organic content by dividing the $C\%_{weight}$ and $N\%_{weight}$ by the fraction of peat OM content derived from LOI. However, $C\%_{weight}$ and $N\%_{weight}$ were used for calculating the cumulative C and N mass.

Freeze-dried samples representing depths of 4-6, 14-16, 24-26, 34-36, and 44-46 cm were analysed for total Hg (Hg-tot) and MeHg ($n = 240$; Paper II). To evaluate the effects of drying, both oven-dried (60 °C) and freeze-dried samples were subjected to Hg-tot analyses in Paper IV ($n = 203$). For every fifth peat sample, a reference sample was analysed (NIST 1515 – Apple Leaves Standard Reference Material®, ERMCD-281 – Rye Grass ERM®). Hg-tot was analysed using a Milestone Direct Mercury Analyzer 80 instrument (Milestone Srl, Sorisole, Italy).

The Hg-species, which were necessary for calculating MeHg content, were analysed using Inductively Coupled Plasma Optical Mass Spectrometry (ICP-MS; TD-100 Markes-GC 7890B Agilent, ICP-MS 7700 Agilent, Santa Clara, CA, USA; Paper II). The samples that underwent MeHg quantification were prepared and analysed along with certified reference material (ERM-CC580 – Estuarine Sediment ERM®). A detailed description of the sample preparation and extraction of Hg-species can be found in Paper II.

The IHg content was calculated by subtracting the MeHg content from Hg-tot. The percentage of MeHg of Hg-tot (%MeHg) was determined by dividing the MeHg content by the Hg-tot content.

Freeze-dried peat samples were analysed for S species by X-ray absorption near-edge structure spectroscopy (XANES). Samples at depths 14-16, 24-26, and 34-36 cm were selected from one peat core from every restored or natural peatland ($n = 42$; Paper II). Each sample was analysed for oxidised S species (sulphonate and sulphate), reduced S species (thiols (RSH), organic sulphide (RSR), organic disulphide (RSSR)), and intermediate S species (sulphoxide and sulphone). Analyses were performed at the Beijing Synchrotron Radiation Facilities (BSRF), following Song et al. (2018).

Peat samples from restored and natural reference peatlands were analysed for total concentrations of Al, Ca, Fe, K, Mg, Na, P, Si, and S (Paper II). For each sampled peat core, samples corresponding to depths of 4-6, 14-16, 24-

26, 34-36, and 44-46 cm were selected for analysis (*n* = 240). Approximately 0.1 g of dry peat was digested in HNO_3 and H_2O_2 for 30 minutes at 180 ºC (Mars 6 Microwave, CEM Corporation, Matthews, NC, USA). Samples were then analysed using Inductively Coupled Plasma Optical Emission Spectrometry (ICO-OES; Varian Vista Ax Pro, Palo Alto, CA, USA). A detailed description of the sample digestion procedure and analysis can be found in Ehnvall et al. (2023).

2.3.3 *hgcA* methylating gene abundance

The presence of microorganisms that are able to methylate Hg was quantified in samples representing depths of 5, 15, 25, 35, and 45 cm in the restored and natural reference peatlands (*n* = 240; Paper II). Targeting the *hgcA* gene abundance, qPCR was performed by employing the primers ORNL-Delta-HgcA-F (CATGCAGATGTGGTTGATGC) and ORNL-Delta-HgcA-R (GCCAACTACAAGMTGASCTWC). Thus, the *hgcA* methylating gene from microorganisms within the Deltaproteobacteria class was captured (Christensen et al., 2016). A detailed description of the methodology can be found in Paper II.

2.4 Statistical analyses

2.4.1 Linear mixed-effects model and ANOVA

A linear mixed-effects model (Equation 1), combined with ANOVA, was used to evaluate differences in various peat variables between restored and natural reference peatlands (Papers I-II). In Paper I, differences between restored and natural reference peatlands were tested for BD, C and N contents in OM, C:N ratio, $\delta^{13}C$, $\delta^{15}N$, and OM content. In Paper II, differences between restored and natural reference peatlands were tested for %MeHg, *hgcA* gene abundance, and Al, Ca, Fe, IHg, K, MeHg, Mg, Na, P, Si, and S concentrations.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + C_k + e_{ijk} \qquad [1]$$

In Equation 1, Y_{ijk} denotes a variable at depth i below the peatland surface for the peatland class j (restored or natural) at a given site k (peatland pair).

The general mean value of a given variable is represented by μ, while α and β are the fixed effects of mire class and peat depth, respectively. The interaction effect of mire class and peat depth is denoted as $\alpha\beta$. The equation also includes a random effect, represented by *C,* and the general error term *e*. The analysis involved the assumption that consecutive samples in a peat profile show a degree of autocorrelation and were thus treated as dependent measurements. The R function *corCAR1* (R version 4.4.2, R Core Team, 2024; Pinheiro et al., 2025) was used to model consecutive samples through an autoregressive process. The linear mixed-effects model was performed using the R function *lme* in the *nlme* package (Pinheiro et al., 2025).

To filter out site-to-site differences, z-scores normalised to each peatland pair were calculated at each 2-cm depth level (Equation 2).

$$z = \frac{x-\mu}{\sigma} \qquad [2]$$

At a given depth, Z is the z-score, while x is the observed value and μ is the average value for a given variable. The average value and standard deviation (σ) were calculated using the six peat cores sampled at each peatland pair.

For the peat cores that were analysed at 2-cm depth-wise resolution, only peat layers at depths of 4-50 cm were included in the linear mixed-effects model, multivariate analyses, or other statistical methods to separate peat from the peatland vegetation (Papers I & III). Data from the site Store Mosse were excluded due to potential disturbance associated with previous peat extraction (Papers I-II). It is noteworthy that the results for cases in which Store Mosse data were included and excluded did appear similar, but the inclusion of the site data enhanced the differences in physical and chemical peat properties between restored and natural reference peatlands. As a conservative measure, Store Mosse was excluded from all statistical analyses (Papers I-II).

After the linear mixed-effects model had been conducted, an ANOVA was used to evaluate the effect of peatland class (restored or natural) on each variable. If the peatland class demonstrated a significant effect ($p < 0.05$) on the linear mixed-effects model, post-hoc tests were performed at each peat depth to test the effect of peatland class. Due to the presence of repeated tests, i.e., 23 and five post hoc tests in Papers I and II, respectively, p-values were adjusted for multiple testing using Bonferroni correction (Dunn, 1961).

2.4.2 Multivariate statistical analyses

Multivariate statistical analyses performed in the research underlying this thesis included principal component analysis (PCA; Jolliffe, 2002; Papers I-II) and orthogonal projections to latent structures – discriminant analysis (OPLS-DA; Paper III; Trygg and Wold, 2002). All of the multivariate analyses were performed using SIMCA, version 17 (Sartorius Stedim Data Analytics, Umeå, Sweden).

Due to pronounced site-to-site differences, z-scores normalised to each peatland pair were used in the PCA that involved physical and chemical peat properties (Equation 2; Paper I). However, the original data were used for the PCA in Paper II due to the low reproducibility (low Q^2) when using z-scores. Only data corresponding to peat profiles of five depth-wise observations were used in the PCA performed in Paper II. In addition to the data for methods described in section 2.4.1, data on *mcrA* (methanogenic) and *mxaF* (methanotrophic) gene abundance from Liu et al. (2025, manuscript) were used. The data describing *hgcA, mcrA,* and *mxaF* gene abundance were $\log_{10}$-transformed before statistical analyses (Paper II).

Prior to the statistical evaluation of the drained and natural reference peatlands (Paper III), data from the peatland D-DPF were interpolated to yield 25 data points for each peat core. The site D-DPF was sampled using an alternative peat sampler since the standard peat corer proved unsuccessful at the intensely drained D-DPF. Therefore, peat cores from site D-DPF were subsampled in the field and could not be sliced at the same vertical resolution as the other peat cores. Consequently, the peat cores collected from D-DPF were sliced in into 11 discs rather than the 25 discs for samples from other peatlands. The 11 discs from the site D-DPF represented the following depths: 0-2.5 cm; 2.5-7.5 cm; 7.5-12.5 cm; 12.5-17.5 cm; 17.5-22.5 cm; 22.5-27.5 cm; 27.5-32.5 cm; 32.5-37.5 cm; 37.5-42.5cm; 42.5-47.5 cm; and 47.5-50 cm. The data were interpolated to represent 25 depth layers corresponding to depths of 0-2 cm, 2-4 cm, … , and 48-50 cm. To obtain the depth layer 0-2 cm, 100% of the observed value at depth 0-2.5 cm was used. To convert the depth layers 0-2.5 cm and 2.5-7.5 cm to 2-4 cm, 25% of the observed value at depth 0-2.5 cm and 75% of the observed value at depth 2.5-7.5 cm were used. Similarly, 100% of the observed value at a depth of 2.5-7.5 cm was used to represent the depth 4-6 cm. The same routine was followed throughout the entire 50-cm profile, and this interpolation of the data collected from site D-DPF ensured that each peat core was represented with 25 data points. Thus, each peatland was equally represented in the multivariate statistical analysis. The peat depth profile from site D-DPF was plotted separately from data from other sites in the results section to demonstrate the observed values rather than the interpolated results.

An OPLS-DA model was fit to the data to distinguish variables characterising drained and natural reference peatlands (Paper III). In the OPLS-DA, the response variable (Y) was the data class (drained or natural), while the predictor variables (X) were BD, C and N contents in OM, C:N ratio, $\delta^{13}C$, $\delta^{15}N$, and OM content. In the OPLS-DA model, variation is separated into a predictive component, i.e., the part of X contributing to the class separation, and an orthogonal component representing variation in X unrelated to the data class. Variables co-vary with the response variable according to loadings on the predictive axis (*pq[1]*). In contrast, systematic noise unrelated to the data class label varies along the loadings on the orthogonal axis (*poso[1]*). Variables of importance (VIP) for the predictive component were calculated to determine significant covariations between the data classes. VIP scores exceeding one were considered to contribute significantly to predicting the response variable. The OPLS-DA model was validated using seven-fold cross-validation (Trygg and Wold, 2002).

2.4.3 Other statistical methods

The groundwater level differences between restored and natural reference peatlands were tested using a Wilcoxon Signed-Rank Test for pairwise samples (Paper II). The same statistical test was applied to evaluate the statistical significance of differences in Hg-tot concentration in oven- and freeze-dried peat samples (Paper IV).

Differences in reduced and oxidised S species between restored and natural reference peatlands were evaluated using pairwise t-tests (Paper II). As a result of repeated tests, all of the obtained p-values were adjusted using Bonferroni correction (Dunn, 1961).

Pearson correlation coefficients were calculated for %MeHg, MeHg and IHg concentrations to the variables BD, C and N contents in OM, C/N, $\delta^{13}C$, $\delta^{15}N$, gene abundance of *hgcA, mcrA,* and *mxaF*, OM content, reduced and oxidised S species, and concentrations of Al, Ca, Fe, K, Mg, Na, P, S, and Si (Paper II). To filter out site-to-site differences, z-scores normalised to each peatland pair (Equation 2) were used to calculate the Pearson correlation coefficient. Data from both peatland classes were included in the calculation of linear correlations. The significance of each correlation coefficient was tested using a t-test. The fact that repeated tests were performed (one test at each depth layer) meant that the obtained p-values were corrected using Bonferroni correction (Dunn, 1961).

A linear regression model was established to predict the Hg-tot concentrations in freeze-dried peat samples using oven-dried samples (Paper IV). The linear regression model was validated using 10-fold cross-validation (Lachenbruch and Mickey, 1968). Please see Paper IV for a detailed description of the cross-validation procedure.

3. Results

3.1 Physical and chemical peat properties in restored, drained, and natural peatlands

The restored peatlands showed significantly higher BD levels, as well as C and N contents in OM, than the natural reference peatlands (Linear mixed-effects model and ANOVA; Paper I). In contrast, the natural reference peatlands were characterised by higher OM content, C:N ratio, and δ^{13}C levels than restored peatlands. Except for δ^{13}C, differences between restored and natural reference peatlands were found below 20 cm depth of the 50 cm peat cores.

Principal component analysis assessed how the restored or natural peatland contributed to certain physical and chemical peat properties (based on z-scores normalised to each peatland pair). The resulting PCA plot demonstrates how components one and two explain 53% and 13% of total variance, respectively (R^2X=0.67; Q^2=0.33; Figure 8).

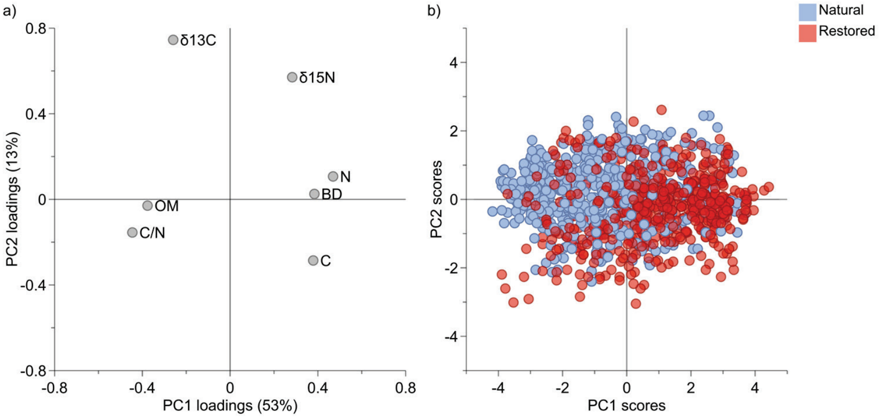

Figure 8. PCA (a) loadings and (b) scores based on z-scores normalised to each peatland pair of physical and chemical peat properties in restored and natural reference peatlands (Paper I).

The drained peatlands (Paper III) showed significantly higher C contents in OM and a higher BD than the natural reference peatlands. In contrast, $\delta^{13}C$ was significantly lower in the drained peatlands compared to the natural reference peatlands. An OPLS-DA found these three characteristics to have the highest explanatory power for the differences between drained and natural reference peatlands ($VIP_{predictive}>1$; $R^2X=0.73$; $R^2Y=0.28$; $Q^2=0.28$; Figure 9).

To evaluate whether the peatland characterised by the most prominent drainage, D-DPF, had some influence on the entire data set, a second OPLS-DA model was applied to all of the data except for the points corresponding to D-DPF ($R^2X=0.82$; $R^2Y=0.26$; $Q^2=0.25$). This second model provided similar results; that is, drained peatlands demonstrating significantly higher C content in OM and significantly lower $\delta^{13}C$ levels than the natural reference peatlands ($VIP_{predictive}>1$). However, there was no significant difference in BD between drained and natural reference peatlands when D-DPF data were excluded from the model. Instead, the results revealed that the drained peatlands have significantly lower OM contents than natural reference peatlands without D-DPF.

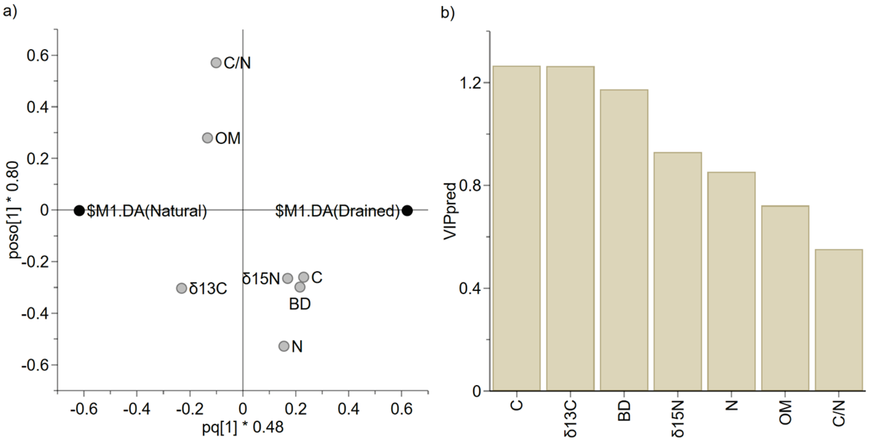

Figure 9. OPLS-DA (a) loadings and (b) VIP predictive scores based on observed values of physical and chemical peat properties (Paper III).

3.1.1 Peat dry bulk density

The average BD in the restored peatlands was 0.088 g cm^{-3} ± 0.0025 standard error (SE) and 0.076 g cm^{-3} ± 0.0019 SE in the natural reference peatlands (Table 2). The BD increased throughout the 50 cm depth profiles for both restored and natural reference peatlands (Figure 10a). However, the restored peatlands showed significantly higher BD, relative to the natural peatlands, at depths of 36-40 cm and 42-50 cm (results from the Linear Mixed-Effects Model and ANOVA; Paper I).

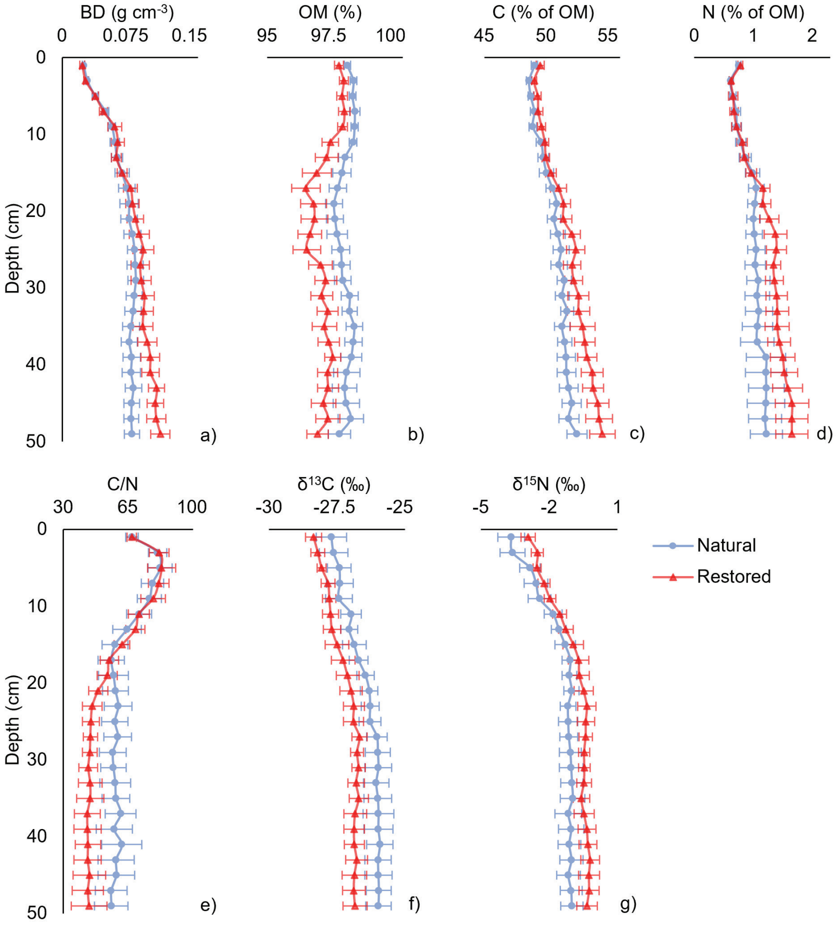

Figure 10. Average depth profiles (±SE) of (a) dry bulk density (BD), (b) organic matter content (OM), (c) carbon content in OM (C), (d) nitrogen content in OM (N), (e) C/N, (f) δ^{13}C (‰), and (g) δ^{15}N (‰) in restored and natural reference peatlands (Paper I).

Table 2. Average values (±SE) of dry bulk density (BD), organic matter content (OM), carbon content of OM (C), nitrogen content of OM (N), C:N ratio (C/N), $\delta^{13}C$ (‰), and $\delta^{15}N$ (‰) in restored and natural reference peatlands (Paper I) or drained and natural reference peatlands (Papers I & III).

Site	*BD ($g\ cm^{-3}$)* *Average (SE)*	*OM (%)*	*C (% of OM)*	*N (% of OM)*	*C/N*	*$\delta^{13}C$ (‰)*	*$\delta^{15}N$ (‰)*
Paper I							
Restored	0.088 (0.0025)	97.3 (0.093)	52.0 (0.19)	1.26 (0.043)	53.2 (1.64)	-27.0 (0.061)	-0.76 (0.089)
Natural	0.076 (0.0019)	98.2 (0.074)	50.9 (0.14)	1.02 (0.043)	61.9 (1.66)	-26.2 (0.076)	-1.34 (0.099)
Paper III							
Drained	0.13 (0.0059)	96.2 (0.71)	56.4 (0.40)	1.58 (0.055)	39.9 (1.28)	-27.5 (0.19)	-0.23 (0.13)
Natural	0.091 (0.0030)	97.3 (0.12)	53.4 (0.22)	1.31 (0.040)	46.0 (1.88)	-26.6 (0.082)	-0.96 (0.10)

The average BD in the drained peatlands was 0.13 g cm^{-3} ± 0.0059 SE and 0.091 g cm^{-3} ± 0.0030 SE in the natural reference peatlands (Table 2). One of the drained peatlands (D-DPF) showed a particularly high BD (Figure 11a), which contributed to the significant difference in terms of BD between drained and natural reference peatlands ($VIP_{predictive}=1.17$; Figure 9; Paper III).

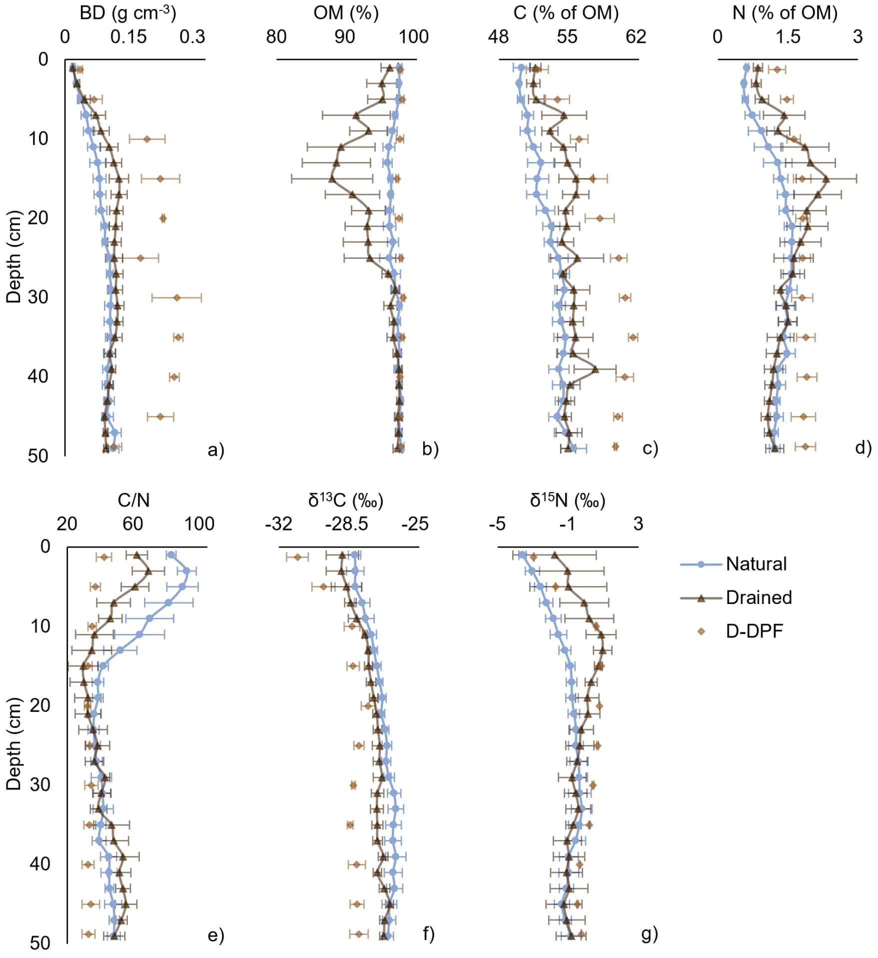

Figure 11. Average depth profiles (±SE) of (a) dry bulk density (BD), (b) organic matter content (OM), (c) carbon content in OM (C), (d) nitrogen content in OM (N), (e) C/N, (f) $\delta^{13}C$ (‰), and (g) $\delta^{15}N$ (‰) in drained and natural reference peatlands. The site D-DPF (drained) is plotted separately due to a different depth-wise resolution than what was the case for other sites. The D-DPF data are plotted as an average of the three peat cores sampled from the site (Paper III).

3.1.2 Peat organic matter content

The average peat OM content in the restored peatlands was 97.3% ± 0.093 SE and 98.2% ± 0.074 SE in the natural reference peatlands (Table 2; Paper I). The patterns in how OM content evolved with depth were similar for restored and natural reference peatlands, although the restored peatlands showed a distinct OM minimum between depths 18-26 cm (Figure 10b). Compared to the natural reference peatlands, restored peatlands showed significantly lower OM contents at depths of 22-26 cm, 34-36 cm, and 38-48 cm.

The average OM content in the drained peatlands was 96.2% ± 0.71 SE and 97.3% ± 0.12 SE in the natural reference peatlands (Table 2; Paper III). Notably, two of the drained peatlands (TEA-East and TEA-West) showed particularly low OM contents, which contributed to high variability within the drained peatland class (Figure 11b). However, there was no significant difference in OM content between the drained and natural reference peatlands (Figure 9).

3.1.3 Carbon content in organic matter and cumulative C mass

The average C content in OM in the restored peatlands was 52.0% ± 0.19 and 50.9% ± 0.14 in the natural reference peatlands (Table 2; Paper I). The C content in OM increased with depth for both restored and natural peatlands (Figure 10c). However, the C content in OM was significantly higher in the restored peatlands than in the natural reference peatlands at depths of 22-24 cm and 36-42 cm. The uppermost 50 cm of restored peatlands also showed a significantly higher average cumulative C mass (21.0 kg m^{-2} ± 1.54 SE) than what was observed at the natural reference peatlands (18.1 kg m^{-2} ± 1.84 SE; $p = 0.042$).

The average C content in OM in the drained peatlands was 56.4% ± 0.40 SE and 53.4% ± 0.22 SE in the natural reference peatlands (Table 2; Paper III). The drained peatlands showed high variability (Figure 11c), although the drained peatlands demonstrated significantly higher C content in OM than what was observed for the natural reference peatlands ($VIP_{predictive}=1.27$; Figure 9). The average cumulative C mass in the top 50 cm of drained peatlands was 33.9 kg m^{-2} ± 8.25 SE in the drained peatlands and 22.6 kg m^{-2} ± 1.45 SE in the natural reference peatlands. However, no significant differences in C mass were observed between the drained and natural reference peatlands (*p* = 0.26).

3.1.4 Nitrogen content in organic matter and cumulative N mass

The average N content in OM in the restored peatlands was 1.26% ± 0.043 SE and 1.02 ± 0.043 SE in the natural reference peatlands (Table 2; Paper I). The restored peatlands showed significantly higher N contents in OM at depths of 20-28 cm, 36-38 cm, and 44-46 cm compared to the natural reference peatlands (Figure 10d). The average cumulative N mass in the top 50 cm of peat was 0.56 kg m^{-2} ± 0.082 SE in the restored peatlands, and 0.39 kg m^{-2} ± 0.86 SE in the natural reference peatlands. There was no significant difference between the restored and natural reference peatlands regarding cumulative N mass in the top 50 cm of peat (*p* = 0.090).

The average N content in OM in the drained peatlands was 1.58% ± 0.055 SE and 1.31% ± 0.040 SE in the natural reference peatlands (Table 2; Paper III). The drained peatlands showed a distinct maximum in average N content in OM at a depth of about 15 cm (Figure 11d), although there was no significant difference between the two peatland classes (Figure 9). The average cumulative N mass in the top 50 cm of peat was 0.99 kg m^{-2} ± 0.29 SE in the drained peatlands, and 0.58 kg m^{-2} ± 0.064 SE in the natural reference peatlands. There was no significant difference in cumulative N mass in the top 50 cm of peat between the drained and natural reference peatlands (*p* = 0.26).

3.1.5 C:N ratio

The average C:N ratio in the restored peatlands was 53.2 ± 1.64 SE and 61.9 ± 1.66 SE in the natural reference peatlands (Table 2; Paper I). The average depth profiles from 0-20 cm showed similar patterns across both restored and natural reference peatlands (Figure 10e). At depths of 22-28 cm, 36-38 cm, and 44-46 cm, the restored peatlands showed significantly lower C:N ratios than the natural reference peatlands.

The average C:N ratio in drained peatlands was 39.9 ± 1.28 SE and 46.0 ± 1.88 SE in the natural reference peatlands (Table 2; Paper III). Although indications of a lower C:N ratio at the surface of drained peatlands were noted (Figure 11e), no significant difference between the two peatland classes was found (Figure 9).

3.1.6 $\delta^{13}C$ isotopic signature

The average $\delta^{13}C$ in the restored peatlands was -27.0‰ ± 0.061 SE and -26.2‰ ± 0.076 SE in the natural reference peatlands (Table 2; Paper I). The average $\delta^{13}C$ profiles increased from the peatland surface of both restored and natural reference peatlands, with this trend particularly evident in the top 25 cm (Figure 10f). The restored peatlands had significantly lower $\delta^{13}C$ values than the natural reference peatlands at depths of 4-18 cm, 20-42 cm, and 44-48 cm.

The average $\delta^{13}C$ in the drained peatlands was -27.5‰ ± 0.19 SE and -26.2‰ ± 0.082 SE in the natural reference peatlands (Table 2; Paper III). The average $\delta^{13}C$ depth profiles increase from the peatland surface to a depth of approximately 20 cm for both peatland classes (Figure 11f). However, the $\delta^{13}C$ in drained peatlands was significantly lower than in the natural reference peatlands ($VIP_{predictive}$=1.26; Figure 9).

3.1.7 $\delta^{15}N$ isotopic signature

The average $\delta^{15}N$ in the restored peatlands was -0.76‰ ± 0.089 SE and -1.34‰ ± 0.099 SE in the natural reference peatlands (Table 2; Paper I). The $\delta^{15}N$ signature across the peat profile followed a similar pattern to that reported for the $\delta^{13}C$ signature; that is, an increase from the surface to a depth of approximately 25 cm in both restored and drained peatlands (Figure 10g). However, there were no significant differences in $\delta^{15}N$ between the two peatland classes.

The average $\delta^{15}N$ in the drained peatlands was -0.23‰ ± 0.13 SE and -0.96‰ ± 0.10 SE in the natural reference peatlands (Table 2; Paper III). While the drained peatlands showed a $\delta^{15}N$ maximum at a depth of approximately 10 cm, the natural reference peatlands showed an increasing trend for the average $\delta^{15}N$ signature in the top ~25 cm of peat (Figure 11g). It is noteworthy that there was high variability in average $\delta^{15}N$ signature among the drained peatlands, and there was no significant difference between the drained and natural reference peatlands (Figure 9).

3.2 Groundwater level and long-term redox conditions in restored and natural peatlands

3.2.1 Groundwater level

The median groundwater level in the restored peatlands was 13 cm (IQR: 9.3), while it was 8.4 cm (IQR: 11) in the natural reference peatlands (Figure 12a; Table 3; Paper II). As such, the restored peatlands had a significantly deeper groundwater level compared to the natural reference peatlands ($p < 0.001$).

Table 3. Median (IQR) groundwater level (GWL) for all of the sampled restored and natural reference peatlands (total), along with individual pairs of restored and natural reference peatlands at the sites Mossaträsk (MT), Öjsjömyrarna (OA), Ånnsjön Mire (AN), Stensjöflon (SF), Sör-Lappmyran (SL), Bredsjömossen (BM), and Anderstorps Stormosse (AS; Paper II).

Site	*Total GWL (cm)* *Median (IQR)*	*MT*	*OA*	*AN*	*SF*	*SL*	*BM*	*AS*
Restored	13 (9.3)	15 (5.0)	14 (8.8)	14 (2.9)	9.0 (5.6)	20 (14)	13 (15)	7.8 (4.3)
Natural	8.4 (11)	3.1 (4.3)	9.2 (6.8)	5.0 (3.3)	7.5 (6.9)	6.9 (8.5)	15 (17)	19 (13)

3.2.2 Reduced and oxidised sulphur species

Among the restored peatlands, reduced S species, as determined by S XANES, contributed 62% ± 4.3 SE of total S, compared to 65% ± 5.2 SE in the natural reference peatlands (Figure 12b; Paper II). The oxidised S species in restored peatlands contributed to 31% ± 2.7 SE of total S, and 30% ± 3.5 SE in the natural reference peatlands. The remaining ~10% were intermediate S species. The only significant difference in S speciation was found at a depth of 24-26 cm, where the natural reference peatlands showed a higher fraction of reduced S species as compared to restored peatlands (14-16 cm: $p = 1.00$; 24-26 cm: $p = 0.036$; 34-36 cm: $p = 0.36$).

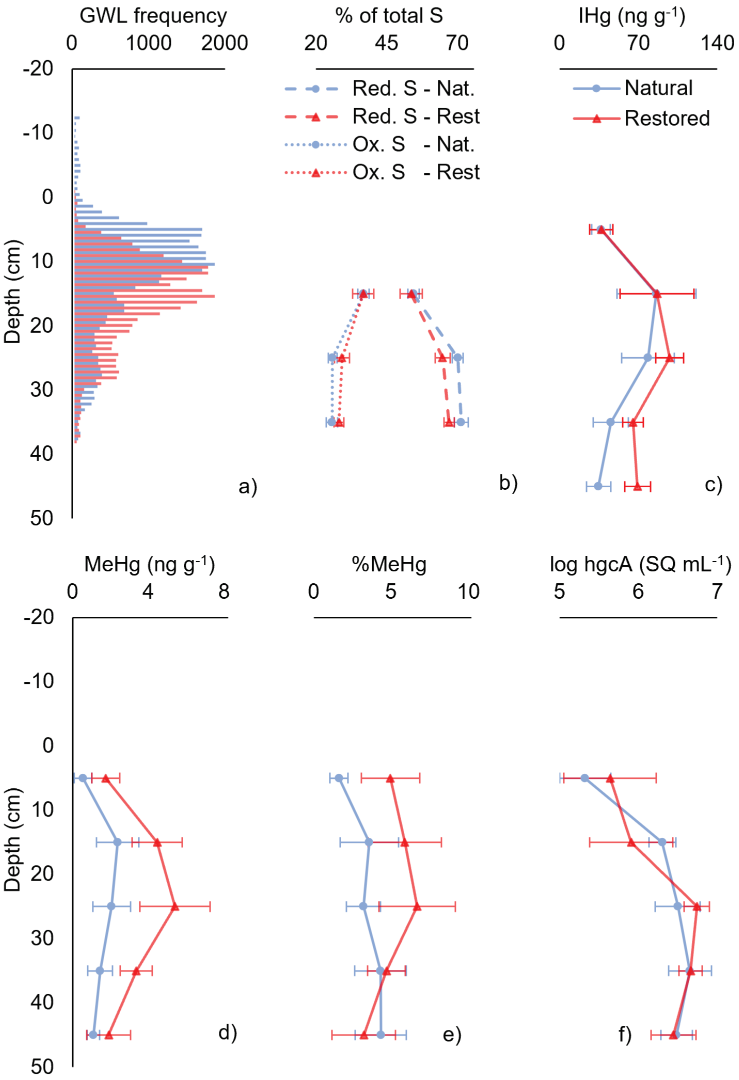

Figure 12. (a) Histograms of average groundwater level (GWL frequency) in restored and natural reference peatlands, where depth zero denotes the peatland surface. Average depth profiles (±SE) of (b) reduced and oxidised sulphur species, (c) inorganic mercury concentrations (IHg), (d) methylmercury concentrations (MeHg), (e) % methylmercury of total mercury (%MeHg), and (f) *hgcA* gene abundance (copy number per mL sample) in restored and natural reference peatlands (Paper II).

3.3 Inorganic Hg, MeHg, and other chemical characteristics in restored and natural peatlands

3.3.1 Inorganic Hg content

The average IHg concentration in the restored peatlands was 71 ng g^{-1} ± 6.0 SE and 56 ng g^{-1} ± 6.9 SE in the natural reference peatlands (Table 4; Paper II). Both peatland classes demonstrated a similar average IHg profile, which involved consistent average IHg concentrations at depths of 4-6 cm and 14-16 cm (Figure 12c). However, the IHg maximum in restored peatlands was observed at a depth of 24-26 cm, while the natural reference peatlands showed an IHg maximum at a depth of 14-16 cm. The IHg concentrations for the two peatland classes then increasingly diverged with depth, as the restored peatlands demonstrated significantly higher IHg concentrations than the natural reference peatlands at a depth of 44-46 cm. The restored peatlands also had a significantly higher average cumulative IHg mass (3.1 mg m^{-2} ± 0.35 SE) in the top 50 cm of peat than what was observed for the natural reference peatlands (2.1 mg m^{-2} ± 0.29 SE).

Table 4. Average (±SE) inorganic mercury concentration (IHg), cumulative IHg mass in the top 50 cm of peat (Cum. IHg), methylmercury concentration (MeHg), cumulative MeHg mass in the top 50 cm of peat (Cum. MeHg), and % methylmercury of total mercury (%MeHg) in restored and natural reference peatlands (Paper II).

Site	*IHg (ng g^{-1}) Average (SE)*	*Cum. IHg (mg m^{-2})*	*MeHg (ng g^{-1})*	*Cum. MeHg (mg m^{-2})*	*%MeHg*
Restored	71 (6.0)	3.1 (0.35)	3.3 (0.47)	0.13 (0.023)	5.1 (0.60)
Natural	56 (6.9)	2.1 (0.29)	1.6 (0.22)	0.056 (0.0083)	3.4 (0.44)

The IHg concentration positively correlated with C and N contents in OM and $\delta^{15}N$, using z-scores normalised to each peatland pair (Table 5; Paper II). In contrast, the IHg concentration was found to be negatively correlated with OM content and $\delta^{13}C$. Consistent with the changes in the physical and chemical peat properties, most of the significant correlations were found at depths of 34-36 cm and 44-46 cm. No significant correlations were found between IHg concentration and BD, C:N ratio, gene abundance of *hgcA*, *mcrA*, and *mxaF*, or Al, Ca, Fe, K, MeHg, Mg, Na, P, S, and Si concentrations.

Table 5. Pearson correlation coefficients for inorganic Hg concentration (IHg) versus dry bulk density (BD), organic matter content (OM), carbon content in OM (C), nitrogen content in OM (N), C:N ratio (C/N), $\delta^{13}C$, and $\delta^{15}N$. The correlation coefficients were determined using z-scores, with each observation normalised to the six peat cores (three natural and restored) sampled at each peatland pair. Significant correlation coefficients ($p < 0.05$) are indicated in bold (Paper II).

Peat depth (cm)	*BD*	*OM*	*C*	*N*	*C/N*	$\delta^{13}C$	$\delta^{15}N$
4-6	0.20	-0.07	**0.39**	**0.39**	-0.34	-0.15	0.06
14-16	0.22	-0.24	0.34	0.32	-0.16	-0.19	0.18
24-26	0.24	-0.23	0.33	0.21	-0.28	-0.24	0.19
34-36	0.25	**-0.52**	**0.50**	0.26	-0.22	-0.30	0.13
44-46	0.34	**-0.44**	**0.40**	0.35	-0.33	**-0.39**	**0.39**

3.3.2 MeHg content and *hgcA* gene abundance

The average MeHg concentration in the restored peatlands was 3.3 ng g^{-1} ± 0.47 SE and 1.6 ng g^{-1} ± 0.22 SE in the natural reference peatlands (Table 4; Paper II). Similarly to IHg, MeHg concentrations reached a maximum at a depth of 24-26 cm in the restored peatlands and at a depth of 14-16 cm in the natural reference peatlands (Figure 12d). The restored peatlands showed significantly higher MeHg concentrations than the natural reference peatlands at depths of 24-26 cm and 34-36 cm. The restored peatlands also showed significantly higher average cumulative MeHg mass in the top 50 cm of peat (0.13 mg m^{-2} ± 0.023 SE) than the natural reference peatlands (0.056 mg m^{-2} ± 0.0083 SE).

The average %MeHg in the restored peatlands was 5.1% ± 0.60 SE and 3.4% ± 0.44 SE in the natural reference peatlands (Figure 12e; Table 4; Paper II). The %MeHg was significantly higher in the restored peatlands than in the natural reference peatlands at a depth of 4-6 cm. There were no significant differences between the restored and natural reference peatlands in terms of *hgcA* gene abundance.

MeHg concentration was found to positively correlate with BD as well as C and N contents in OM (Table 6). A negative correlation between MeHg concentration and $\delta^{13}C$ was observed. No significant correlations between MeHg concentration and the C:N ratio, $\delta^{15}N$, gene abundance of *hgcA*, *mcrA*, and *mxaF*, OM content, or Al, Ca, Fe, IHg, K, Mg, Na, P, S, and Si concentrations were found. There was also no significant correlation between MeHg and IHg concentration.

%MeHg, in addition to demonstrating a positive correlation with MeHg and a negative correlation with IHg, showed a negative correlation with $\delta^{13}C$ (significant at a depth of 34-36 cm) and positive correlation with *hgcA* gene abundance (significant at a depth of 4-6 cm).

Table 6. Pearson correlation coefficients for methylmercury concentration (MeHg) versus dry bulk density (BD), organic matter content (OM), carbon content in OM (C), nitrogen content in OM (N), C:N ratio (C/N), $\delta^{13}C$, and $\delta^{15}N$, inorganic Hg concentration (IHg), and *hgcA* gene abundance. The correlation coefficients were determined using z-scores, in which each observation was normalised to the six peat cores (three natural and restored) sampled at each peatland site. Significant correlation coefficients ($p < 0.05$) are indicated in bold (Paper II).

Peat depth (cm)	*BD*	*OM*	*C*	*N*	*C/N*	*$\delta^{13}C$*	*$\delta^{15}N$*	*IHg*	*hgcA*
4-6	-0.16	-0.21	0.10	0.02	0.00	-0.06	0.00	0.21	0.26
14-16	**0.44**	-0.34	0.25	**0.40**	-0.18	-0.08	0.25	0.28	0.22
24-26	0.06	-0.36	0.32	0.27	-0.22	**-0.38**	0.05	0.09	-0.28
34-36	0.08	-0.17	0.31	0.08	-0.10	**-0.54**	0.10	0.16	-0.30
44-46	0.14	-0.28	**0.38**	0.23	-0.11	-0.29	-0.03	0.23	0.20

3.3.3 Other chemical characteristics

No differences between the restored and natural reference peatlands were observed regarding the total concentrations of Al, Ca, Fe, K, Mg, Na, P, S, and Si, or pore water pH. The interrelations among these variables were assessed via a PCA based on observed values (R^2X=0.37; Q^2=0.22; Figure 13). The first and second components explained 22% and 16% of the variance, respectively. The first component was largely influenced by factors such as C:N ratio, OM content, and C and N contents in OM, while the second component was more affected by elements such as Al, P, and S.

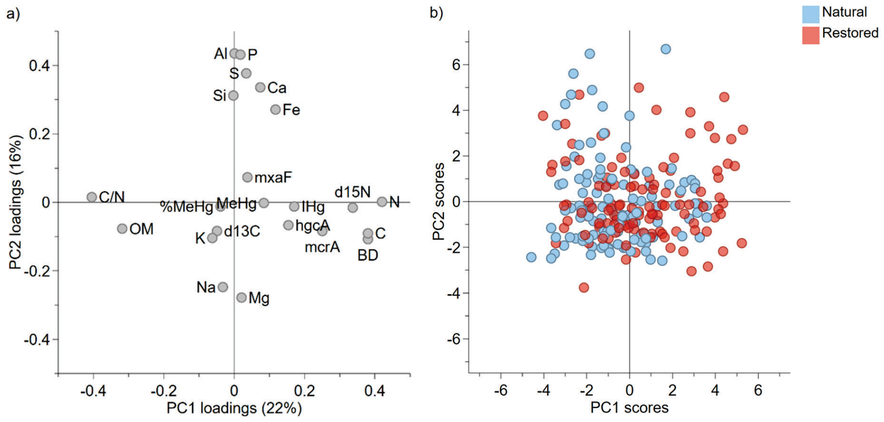

Figure 13. PCA (a) loadings and (b) scores based on observed values from restored and natural reference peatlands (Paper II).

3.4 Loss of total Hg from peat samples during oven-drying

Samples dried in a 60 ºC oven for 96 h showed a median Hg-tot deficit of 4.2% (IQR: 13) relative to freeze-dried samples (Table 7; Paper IV). The deficit corresponds to a Hg loss rate of 19 ng kg^{-1} h^{-1}. The difference in Hg-tot concentrations in peat samples as a result of the two tested drying methods was significant ($p = 0.017$).

A regression line of 'log $[Hg]_{\text{oven-dried}}$' vs 'log $[Hg]_{\text{freeze-dried}}$' was fitted (Figure 14). The regression line did not significantly differ from a 1:1 line regarding slope (95% c.l.; −0.02, 0.17) or intercept offset (0.90, 1.0).

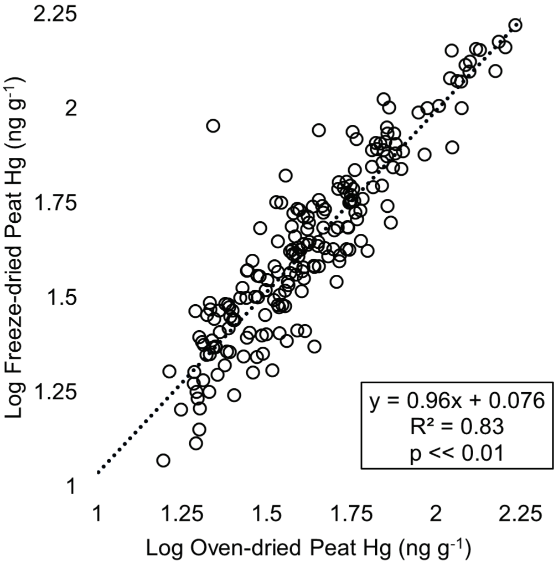

Figure 14. Linear regression of log Hg-tot concentration (nanogram per gram) in oven-dried peat samples (x) vs. log Hg-tot concentration in freeze-dried peat samples (y; Paper IV).

Table 7. Summary of the results regarding loss of total mercury (Hg-tot) in peat samples following 60 ºC oven-drying, namely, R^2 value of the linear regression 'observed log $[Hg]_{\text{oven-dried}}$' vs 'observed log $[Hg]_{\text{freeze-dried}}$', linear regression slope offset, linear regression intercept offset, p-value (Paired Wilcoxon Signed-Ranks Test), median Hg-tot deficit relative to freeze-dried samples (%), and Hg-tot loss rate during the 96h drying routine (nanogram per kilogram peat per hour; Paper IV).

Statistic	*Result*
R^2 value of the linear regression	0.83
Slope offset	0.040
Intercept offset	0.076
P-value, Paired Wilcoxon Signed-Ranks Test	0.017
Median Hg-tot deficit (%) in oven-dried samples	4.2
Hg-tot loss rate (ng kg^{-1} h^{-1})	19

Following a 10-fold cross-validation, the equation of the linear regression line and 'log $[Hg]_{\text{oven-dried}}$' (x) was used to predict 'log $[Hg]_{\text{freeze-dried}}$' (y) (Figure 15). The data set of predicted Hg-tot concentrations in freeze-dried samples yielded an R^2 of 0.83. The R^2 of observed log $[Hg]_{\text{oven-dried}}$ versus log $[Hg]_{\text{freeze-dried}}$ was also 0.83.

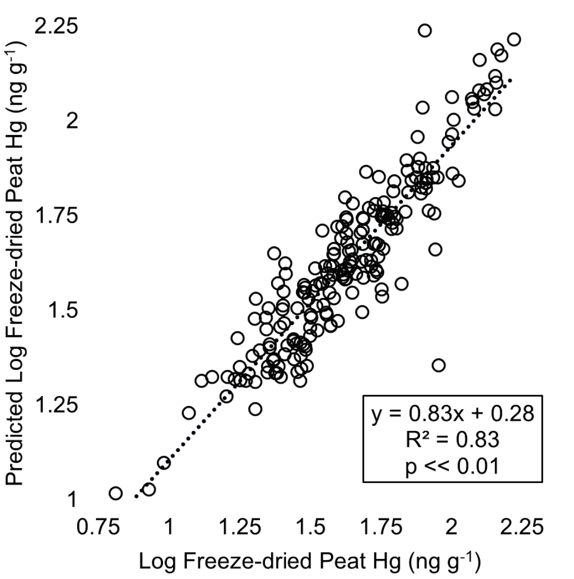

Figure 15. Linear regression of 'observed log Hg-tot concentration (nanogram per gram) in freeze-dried peat samples' (x) vs. 'predicted log Hg-tot concentration in freeze-dried peat samples' (y; Paper IV).

4. Discussion

4.1 Impacts of drainage and restoration on physical and chemical peat properties

An extended period of drained conditions significantly altered the physical and chemical peat properties of the investigated peatlands (Figures 10-11; Papers I & III). Drainage increases the acrotelm depth, and peat previously sequestered in the catotelm becomes exposed to oxygen, potentially increasing the peat decomposition rate (Clymo, 1984; Fenner and Freeman, 2011). A deeper groundwater level also affects the plant community composition (Strack et al., 2006), further affecting the peat formation rate and peat properties (Moore and Basiliko, 2006; Moore et al., 2007).

The drainage-induced changes to peat properties have altered the preconditions for the extent and distribution of microbial communities after restoration. Therefore, it cannot be assumed that the biogeochemical processes present in restored peatlands will closely resemble those of natural, undisturbed systems. Hence, past changes to the peatland environment must be considered to thoroughly understand the effects of restoration.

4.1.1 Increased dry bulk density in drained and restored peatlands

Both the drained and the restored peatlands showed significantly higher BD than the corresponding natural peatlands (Figures 10-11; Papers I & III). Drainage decreases the support provided by water-filled pore spaces, potentially leading to subsidence and compaction in the underlying peat layers (Liu and Lennartz, 2019; Minkkinen and Laine, 1998b). The degradation of plant fibres further reduces the water-holding capacity and porosity of peat (Boelter, 1969; Menberu et al., 2021). In addition, decomposition could increase the mineral content and proportion of fine particles in the peat (Chambers et al., 2011). This dynamic could be reflected in the investigated restored peatlands, with the results showing that OM content decreased as BD increased in the peat at depths between 36-50 cm (Figure 10; Paper I). Interestingly, there were no differences in BD or OM contents above a depth of 20 cm in the restored peatlands (Figures 10 & 16; Paper I). This suggests that peat may have formed after historical drainage, either due to restoration or a reduced drainage effect over time, as ditches can become less effective over time if not maintained (Hökkä et al., 2000; Robinson, 1986).

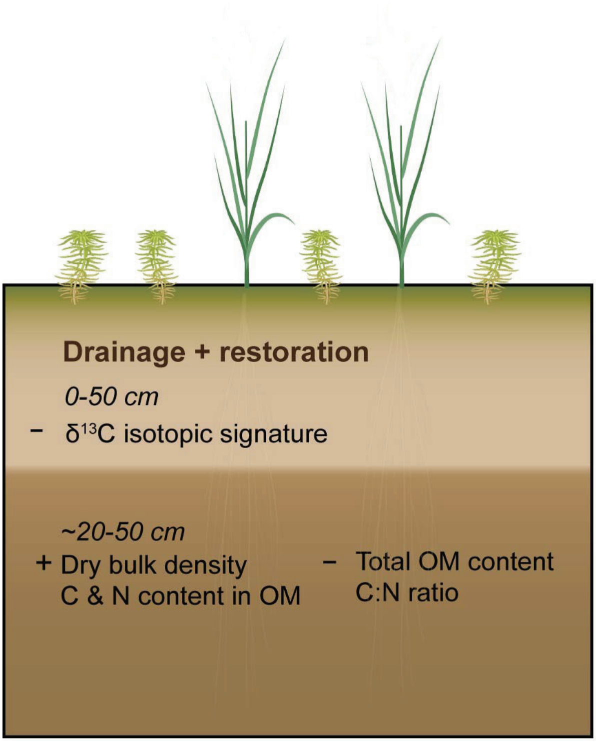

Figure 16. Drainage and the subsequent restoration caused a decrease in $\delta^{13}C$ at depths between 0-50 cm, an increase in dry bulk density and carbon and nitrogen contents in organic matter at depths between ~20-50 cm, and a decrease in organic matter content and the C:N ratio at depths between ~20-50 cm relative to the natural reference peatlands.

Peat at ~50 cm depth in the drained (Paper III) and the restored peatlands (Paper I) is possibly affected by older peat layers predating the corresponding depth at the natural peatlands (Minkkinen and Laine, 1998b). However, any potential age difference between the *i*) drained and natural peat cores and *ii*) restored and natural peat cores is ultimately not relevant for the purpose of this thesis, which focuses on the implications of these alterations in the top 50 cm peat. Regardless of peat age, the observed increase in BD across the drained and the restored peatlands has implications for biogeochemical process through, e.g., reduced gas diffusion and oxygen availability (Fujikawa and Miyazaki, 2005), and changes in the plant community composition (Cong et al., 2023; Noble et al., 2017).

The significantly higher BD among drained peatlands relative to natural peatlands was mainly attributed to the results from a single site, namely, the densely forested peatland D-DPF (Figure 11a; Paper III). The high BD measured in the D-DPF samples did not coincide with a decrease in OM content, suggesting that peat compaction by the dense forest stand, rather than increased mineral content, caused the high BD. Similar peat compaction has been reported at other forested drained peatlands (Liu et al., 2020; Minkkinen and Laine, 1998b; Nykänen et al., 2020; Turunen et al., 2024).

4.1.2 Altered chemical peat properties in drained and restored peatlands

Changes in the hydrological regime affect the chemical peat properties by affecting the peat decomposition rate (Krüger et al., 2015), plant community composition (Strack et al., 2006), and related processes (Minkkinen and Laine, 1998b). Such changes could be reflected in the C and N contents in OM and the $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures (Biester et al., 2014; Krüger et al., 2015; Leifeld et al., 2020). At both the drained and restored peatlands, OM was enriched in C and depleted in the ^{13}C isotope relative to the respective natural reference peatlands (Figures 10-11; Papers I & III). In addition, the restored peatlands also showed increased N content in OM and a decrease in the C:N ratio relative to natural reference peatlands (Figure 10; Paper I).

The increased C content in OM could reflect the preferential loss of readily degradable compounds following drainage, e.g., carbohydrate-based polymers over more degradation-resistant structures, such as aromatic and aliphatic compounds (Drollinger et al., 2019; Nilsson and Öquist, 2013; Serk et al., 2022; Tfaily et al., 2014). The C content of carbohydrate-based polymers is generally lower than in, e.g., aromatic and aliphatic polymer forms (Reddy and DeLaune, 2008; Speight, 2017). Hence, despite C loss, the initial stages of peat decomposition may lead to an increase in C content in OM (Serk et al., 2022; Tfaily et al., 2014).

The $\delta^{13}C$ isotopic signatures associated with the drained and the restored peatlands could also reflect an enrichment in recalcitrant compounds (Benner et al., 1987). Synthesis of readily degradable compounds, such as polysaccharides, results in a slight ^{13}C enrichment relative to more recalcitrant compounds (Benner et al., 1987; Rice and Giles, 1996; Serk et al., 2021). Therefore, the preferential loss of readily degradable organic compounds could explain the ^{12}C enrichment in peat environments that are experiencing increased decomposition rates. Changes to chemical peat properties other than $\delta^{13}C$ only occurred at depths between ~20-50 cm (Figure 10; Paper I), while differences in the $\delta^{13}C$ signature between restored and natural peatlands were consistent throughout the entire 50-cm peat cores. It is possible that the $\delta^{13}C$ signature still reflects differences in plant community composition between restored and natural reference peatlands, particularly since the median groundwater level was found to be five centimetres deeper at the restored peatlands.

The initial stages of peat decomposition could also be reflected in changes of the N content in OM and the $\delta^{15}N$ isotopic signature (Groß-Schmölders et al., 2020; Kuhry and Vitt, 1996; Malmer and Holm, 1984). This is because the concurrent loss of, e.g., C related to the decomposition of polysaccharides increases the relative N content in OM and decreases the C:N ratio (Kuhry and Vitt, 1996; Leifeld et al., 2020). In oligotrophic boreal peatlands, the N released during peat degradation is often immobilised through uptake by microorganisms and the vegetation (Eriksson et al., 2010b; Granberg et al., 2001). As net N mineralisation is unlikely at C:N ratios above ~21 (Klemedtsson et al., 2005), the increase in N content and decrease in C:N observed in the restored peatlands is likely explained by the loss of C.

Aerobic microbial decomposition has been suggested to preferentially utilise the lighter ^{14}N isotope over ^{15}N, thereby increasing $\delta^{15}N$ (Asada et al., 2005; Groß-Schmölders et al., 2020). Changes in $\delta^{13}C$ may be explained by a similar dynamic, as the preferential use of the kinetically more favourable ^{12}C relative to ^{13}C has been proposed to raise $\delta^{13}C$ levels (Drollinger et al., 2020; Nykänen et al., 2020). However, a prerequisite for such interpretations is that the ^{12}C and ^{14}N isotopes utilised by microorganisms are lost from the residual peat to an extent that enables measurements from bulk peat samples, which can be problematic. Conceivably, tracking such changes by measuring the $\delta^{13}C$ in bulk peat should not generate a clear signal, given the multitude of polymeric structures, each with inherent isotopic signatures (Serk et al., 2022).

The altered chemical peat properties must be considered when evaluating the effects of restoration since substrate availability significantly influences microbially mediated processes (Juottonen et al., 2012). For instance, the reintroduction of anoxic conditions will promote anaerobic processes such as methanogenesis (Abdalla et al., 2016) and Hg methylation (Tjerngren et al., 2012b). Despite favourable redox conditions, these processes may be affected by the lack of high-quality substrate (Bergman et al., 1998; Graham et al., 2013; Mitchell et al., 2008), particularly as the most significantly degraded OM was found below the long-term groundwater level in restored peatlands. However, anaerobic processes can also be stimulated by root exudates from vascular plants, such as sedges (Öquist and Svensson, 2002; Ström et al., 2003). Hence, increased sedge cover over *Sphagnum* spp. could promote CH_4 production and Hg methylation after restoration.

4.2 Impacts of drainage and restoration on inorganic Hg content and net MeHg formation

4.2.1 Increased inorganic Hg content in restored peatlands

The restored peatlands showed higher cumulative IHg mass in the uppermost 50 cm of peat and higher IHg concentrations at 44-46 cm depth, in comparison to the natural reference peatlands (Figure 12c; Table 4; Paper II). The retention of Hg – relative to major elements such as C, H and O – during peat decomposition over extended periods of drained conditions could increase IHg concentrations; this has also been observed in other studies (Becher et al., 2023; Golovatskaya and Lyapina, 2009). Furthermore, this dynamic (i.e., increased IHg concentrations) is consistent with the negative correlations between IHg concentration and both OM content and $\delta^{13}C$, along with the positive correlation between IHg concentration and C content in OM (Table 5). As discussed in section 4.1.2, oxygenation through drainage can lead to the loss of readily degradable compounds, such as polysaccharides (Serk et al., 2022). An increase in BD further increases the cumulative mass of IHg in the uppermost 50 cm of peat in the restored peatlands relative to the natural reference peatlands.

Larger losses of IHg from the natural reference peatlands during the period when the drainage affected the restored peatlands may have contributed to the observed difference in IHg between the restored and natural reference peatlands (Jiskra et al., 2015; Li et al., 2023; Osterwalder et al., 2017). Even after restoration, consistent patterns of deeper groundwater level and a lower fraction of reduced S species were observed in the restored peatlands. These aspects provide a strong indication of the reducing conditions present in the natural reference peatlands, and the redox difference was most likely even more enhanced during the drainage period. The reduction of Hg(II) to Hg(0) and subsequent losses of gaseous Hg(0) occur naturally under the anoxic conditions present at peatlands (Jiskra et al., 2015; Li et al., 2023; Osterwalder et al., 2017). If the redox potential increases due to drainage, the rate of Hg(II) reduction is expected to decrease, possibly contributing to the higher IHg concentrations among the restored peatlands at 44-46 cm of depth.

4.2.2 Increased net MeHg formation in restored peatlands

The availability of electron donors influences the activity and abundance of microorganisms capable of methylating Hg (Graham et al., 2013; Hu et al., 2020; Mitchell et al., 2008). The enhanced MeHg concentration found in the degraded peat layers of restored peatlands (depths 24-26 and 34-36 cm; Figures 12d & 17) was a surprising finding, as these layers could be expected to show depleted levels of the most bioavailable organic molecules. Consistent with this, the MeHg concentration was negatively correlated with $\delta^{13}C$ (Table 6), as a low $\delta^{13}C$ value may indicate losses of labile organic compounds (Benner et al., 1987; Drollinger et al., 2020). Notably, the presence of MeHg results from both Hg methylation and demethylation (Tjerngren et al., 2012a), with demethylation a process that could also be driven by microbial activity (Barkay and Gu, 2022). However, the more oxidative redox conditions indicated at the restored peatlands would be expected to increase rates of MeHg demethylation (Barkay and Gu, 2022).

The bioavailability of Hg(II) is another key factor for Hg methylation (Peterson et al., 2023; Poulin et al., 2019). Here, it is essential to note that the IHg concentration does not necessarily reflect the bioavailable pool of Hg(II) (Benoit et al., 2001; Peterson et al., 2023). Higher rates of Hg(II) reduction in the natural peatlands during historical drainage at the restored sites would lead to a smaller pool of bioavailable Hg(II), as reduction and methylation represent two distinct processes competing for Hg(II) substrate (Poulin et al., 2019; Wang et al., 2021, 2020). Therefore, a higher bioavailability of Hg(II) would explain the increased net MeHg formation in the restored peatlands (compared to natural peatlands) at depths 24-26 cm and 34-36 cm.

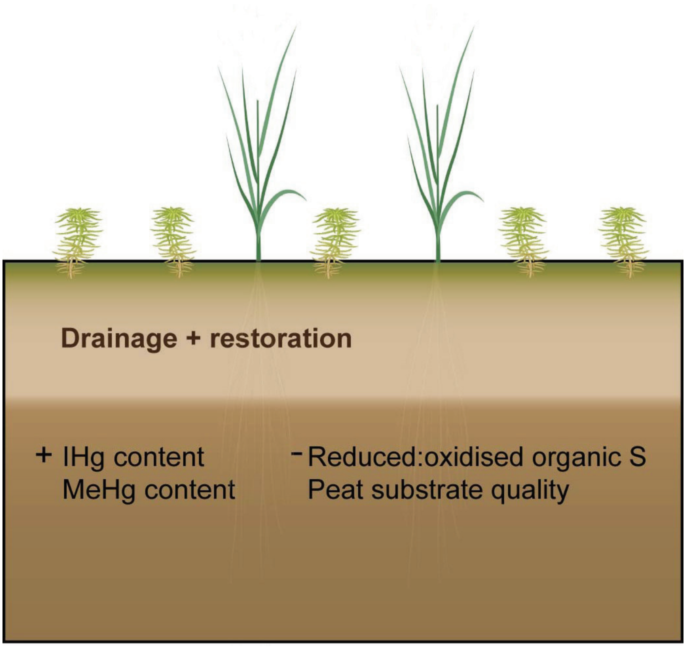

Figure 17. An extended period of drained conditions, followed by restoration, increased the cumulative masses and average concentrations of IHg and MeHg in the uppermost 50 cm peat relative to natural reference peatlands (denoted by a plus sign). The oxidising conditions during drainage likely increased the peat decomposition rate, decreasing the peat substrate quality in the restored peatlands, particularly in the ~20-50 cm depth layer (denoted by a minus sign). Despite the restoration efforts, the ratio of reduced to oxidised organic sulphur species was lower in the restored peatlands compared to the natural reference peatlands. Although restoration did not fully restore the redox conditions of natural peatlands, restoration likely shifted the redox conditions enough to favour net MeHg formation.

Peat depleted in readily degradable compounds, as indicated at depths below ~20 cm in the restored peatlands, may serve as a poor substrate for microbial activity (Bergman et al., 2020). However, microbial activity could also be stimulated by the readily degradable C of root exudates from vascular plants (Bergman et al., 2000; Bragazza et al., 2015). Increased oxygen availability and plant nutrient acquisition after drainage can enhance the cover of vascular plants, such as sedges, over *Sphagnum* (Eriksson et al., 2010b; Strack et al., 2006). While *Sphagnum* cover generally increases after restoration (Laatikainen et al., 2025), the restored peatlands had a median groundwater level five centimetres below the natural reference peatlands. Thus, the restored peatlands could still show increased vascular plant cover and root exudate supply, which can stimulate microbial activity and methylation of Hg (Windham-Myers et al., 2009). This could be a crucial factor underlying the increase in net MeHg formation at the restored peatlands. The role of root exudates would be particularly important in the rhizosphere near the peatland surface (a depth of 4-6 cm), where %MeHg increased across the restored peatlands (Figure 12e; Paper II).

Both net MeHg formation as well as plant community composition are strongly influenced by the peatland nutrient regime (Roth et al., 2021; Wang et al., 2020). However, the restored and natural reference peatlands did not differ with respect to total concentrations of Al, Ca, Fe, K, Mg, Na, P, S, or Si (Paper II). These elements mainly enter peatlands through minerogenic water (Ehnvall et al., 2023), and each peatland pair is located within the same watershed; this could explain the lack of differences between restored and natural reference peatlands. In addition, no significant linear correlations were found between these elements and MeHg concentration. If net MeHg formation were closely related to nutrient regime, a closer relationship to, e.g., P or Mg, would be expected (Figure 13). However, it is noteworthy that the total concentration of an element in the peat matrix does not necessarily reflect the bioavailability (Kooijman et al., 2020).

To summarise, several factors may have contributed to the increased net MeHg formation in the restored peatlands compared to the natural reference peatlands (Figure 12). For example, an increased bioavailability of Hg(II) in the restored peatlands relative to the natural reference peatlands may have contributed to an enhanced net MeHg formation. It is also possible that vegetation changes and an increased supply of root exudates may have contributed to the enhanced net MeHg formation in the restored peatlands.

The significant increase in IHg and MeHg mass in the top 50 cm of the restored peatlands is expected to affect Hg export to downstream ecosystems (Bishop et al., 1995, 1997; Fahnestock et al., 2019; Thompson et al., 2023). While Hg concentrations in Swedish freshwater fish have declined since the 1970s, fish with Hg concentrations above safe limits for human consumption are still found in about half of Sweden's 100,000 lakes (Braaten et al., 2019; European Commission, 2023; Eklöf et al., 2024). This emphasises the importance of thoroughly understanding how long-term drainage affects the conditions for net MeHg formation to minimise any undesired effects of peatland restoration.

5. Conclusion

Over 60 years of drained conditions across boreal peatlands in Sweden have drastically changed the physical and chemical properties in the uppermost 50 cm of peat. More specifically, long-term drainage changed the preconditions for the microbial activity and biogeochemical processes that are expected to recommence as soon as drained peatlands are restored. Therefore, we need a more nuanced understanding of the dynamics at play, as it cannot be assumed that the biogeochemical interactions and ecosystem services in recently restored peatlands will mirror those of natural peatlands.

About a decade after restoration, the drainage-induced changes to peat properties were still evident at depths of ~20-50 cm. In the uppermost ~20 cm of peat, the restored peatlands showed similarities to the natural peatlands. This indicates recent peat accumulation, either due to restoration and/or reduced drainage effects, as the original ditches may lose their function over time.

Drainage and subsequent restoration significantly increased the concentration of IHg and net MeHg formation in the top 50 cm of peat. Increased peat decomposition during the drained conditions, along with Hg retention, could explain the increase in IHg concentration. In addition, the increased redox conditions due to drainage may have decreased Hg(II) reduction and loss of elemental Hg(0) in relation to the natural reference peatlands. Reduction and methylation are two distinct processes competing for Hg(II) substrate, which may have decreased the pool of bioavailable Hg(II) in the natural peatlands. If so, more readily available Hg(II) may have contributed to increased net MeHg formation in the restored peatlands relative to the natural reference peatlands. Also, a potential increase in vascular plant cover and root exudate supply after drainage and restoration may have stimulated the activity of microorganisms that methylate Hg.

While restoration of peatlands can provide several important ecosystem services, restoration may also worsen an issue which is already of concern. About half of Sweden's 100,000 lakes contain fish where the Hg concentrations exceed the EU's recommendations for human consumption. The research presented in this thesis found that restoration increased net MeHg formation, thereby increasing the risk of MeHg export to adjacent streams and lakes. This must be considered when planning and executing large-scale restoration of peatlands.

6. Future perspectives

Future studies are needed to further understand the effects of drainage and restoration on peat properties, C dynamics, and net Hg methylation. The specific areas of research listed below may prove pivotal to better understanding the processes and mechanisms which yielded the results discussed in this thesis.

- How are the altered physical and chemical peat properties in drained and restored peatlands linked to specific changes in the composition of polymeric peat structures? The physical and chemical peat properties evaluated in this thesis offer a proxy for the degree of peat decomposition, but do not link the effects of drainage and restoration to specific molecular moieties.

- How are changes in the physical and chemical properties of peat related to the composition, activity and size of the microbial community in restored peatlands across various time scales? The effect of reintroducing anoxic conditions on the activities of methanogenic, methanotrophic, methylating and demethylating microbial communities is a particularly interesting avenue of research.

- The increased C and Hg mass in the top 50 cm of peat from restored sites should be assessed with regard to time in addition to peat depth. In other words, obtaining reliable dates for specific peat layers could clarify whether peat accumulation started before restoration and if the drainage ditches lost their function after the initial drainage effect.

- Decipher the connection between soil C and Hg contents to direct measurements of atmospheric exchange and discharge to surface waters. While the Hg, C, and greenhouse gas balance have been intensely studied in case studies, further research is needed to understand the effects of restoration on a larger spatial scale. It would be particularly interesting to assess whether the increased net MeHg formation after restoration influences MeHg levels in adjacent streams and lakes.

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Popular science summary

Peatland restoration has become an intensely debated issue, but a thorough understanding of the processes in restored wetlands is missing. We also do not fully understand the processes that occur in peatlands drained peatlands. Hence, several important questions need to be answered if society is to have an evidence-based debate about peatland restoration.

For context, peatlands are restored for several reasons. Examples include increasing biodiversity, improving surface water quality, and reducing greenhouse gas emissions. However, restoration activates several processes in peatlands, and all of these need to be accounted for. Even if a peatland is restored for a specific purpose, for example, increased carbon storage, several other processes will be restarted by restoration. Hence, undesired consequences can potentially outweigh the positive effects of restoration.

One clear undesired effect of peatland restoration is mercury methylation, which is the transformation of inorganic mercury to the more toxic form methylmercury. Mercury is methylated in environments characterised by low oxygen levels, which peatland restoration creates. The methylmercury formed in peatlands can eventually reach streams and lakes. Unfortunately, methylmercury accumulates throughout the food chain, and humans are exposed to mercury through the consumption of fish. In fact, about half of Sweden's 100,000 lakes have fish with mercury levels that exceed the recommendations for human consumption.

To thoroughly understand the effects of restoration, the research covered in this thesis started by investigating whether drainage caused significant changes to peatlands. This understanding was then used to assess the impact of restoration on net methylmercury formation. The results showed that drainage and restoration increase net methylmercury formation, increasing the risk of mercury export to nearby streams and lakes.

Populärvetenskaplig sammanfattning

Trots efterfrågan att restaurera dränerade myrar saknas fortfarande en gedigen förståelse för effekterna på de kemiska processerna i torven. Utöver de avsedda ekosystemtjänsterna kan återvätning även leda till skadliga effekter och potentiella miljöhot, till exempel kvicksilvermetylering. Detta innebär att oorganiskt kvicksilver omvandlas till den giftigare formen metylkvicksilver.

Åtskilliga årtionden av dränerade förhållanden har förändrat torvens egenskaper, vilket gör effekterna av restaurering på kemiska processer svåra att förutspå. Denna avhandling utvärderar effekterna av dränering och restaurering på torvens kemiska egenskaper, samt hur återvätning påverkar kvicksilvermetylering.

Dränering av myrar ledde till en stark påverkan på torvens kemiska egenskaper. Även om restaurering misslyckades med att återskapa grundvattennivån relativt opåverkade myrar ökade kvicksilvermetyleringen i de restaurerade myrarna. Således ökar restaurering risken för kvicksilverexport till närliggande bäckar och sjöar.

Knappt hälften av Sveriges sjöar redan har fisk med kvicksilvernivåer som överstiger rekommenderade gränsvärden för mänsklig konsumtion. Den ökade produktionen av metylkvicksilver till följd av restaurering bör tas i beaktning när myrar restaureras.

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IV

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A Simplified Drying Procedure for Analysing Hg Concentrations

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Abstract Mercury (Hg) in peatlands remains a problem of global interest. To mitigate the risks of this neurotoxin, accurate assessments of Hg in peat are needed. Treatment of peat that will be analysed for Hg is, however, not straightforward due to the volatile nature of Hg. The drying process is of particular concern since Hg evasion increases with the temperature. Samples are, therefore, often freeze-dried to limit Hg loss during the drying processes. A problem with freeze-drying is that cost and equipment resources can limit the number of samples analysed in large projects. To avoid this bottleneck, we tested if drying in a 60 °C-degree oven could be an acceptable alternative to freeze-drying. We both freeze-dried and oven-dried (60 °C) 203 replicate pairs of peat samples, and then examined the differences in total Hg concentration. The Hg concentration differed significantly between the two drying methods with a median Hg deficit in oven-dried samples of 4.2%. Whether a 4.2% deficit of Hg depends on one's purpose. The lower median Hg concentration in oven-dried samples has to be weighed against the upside efficiently drying large sets of peat samples. By freeze-drying a subset of the samples, we fitted a function to correct for Hg loss during oven-drying ($y = 0.96x + 0.08$). By applying this correction, the freeze-drying bottleneck could oven-dry large-scale inventories of total Hg in peatlands with results equivalent to freeze-drying, but only have to freeze-dry a subset.

Keywords Sample preparation · Drying procedure · Freeze-drying · Oven-drying · Mercury · Peat

1 Introduction

Mercury (Hg) is a potent neurotoxin contaminating a large portion of the soils and lakes in the world. Hg is a naturally occurring element, although anthropogenic emissions today equal three times the natural emissions to the atmosphere (Futsaeter & Wilson, 2013; Streets et al., 2019). The volatile nature of gaseous elemental mercury also enables long-range transport of Hg, making it a global

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pollutant, and even remote areas are subject to Hg contamination (Åkerblom et al., 2014).

Increased awareness of Hg exposure has led to a systematic and successful international action to mitigate global Hg pollution. As a result, atmospheric Hg concentrations have been declining in recent decades (Obrist et al., 2018; Zhang et al., 2016). Hg deposition, however, continues to pose a serious environmental threat. Once introduced to an ecosystem, Hg will accumulate in the food chain, leading to possible human exposure. Hg dynamics in anoxic environments are particularly relevant to understand, because such conditions promote the formation of the toxic compound methyl mercury (MeHg) most prone to bioaccumulation (Branfireun et al., 2020; Wu et al., 2019). In the boreal biome, peatland ecosystems with high MeHg production potential constitute the major source of MeHg to the aquatic food chain (Bishop et al., 2020).

Inorganic Hg is the source for microbially mediated production of MeHg. In order to estimate and monitor potential MeHg production, it is crucial to have exact and reliable methods for quantifying Hg concentrations in peat. However, peatlands are strongly heterogeneous environments with a distinct microtopography resulting in a large variability in the accumulation of Hg, both spatially and vertically. Surveying total Hg in peatlands thus requires large sample sets. The volatile nature of elemental Hg also brings a risk of losing Hg when preparing and pre-treating samples for analysis (Martínez-Cortizas et al., 1999). A severe bottleneck for processing large Hg sample sets is drying, since high temperatures increase the risk of Hg volatilization, especially the volatile species elemental Hg (Hg(0)) (Fernández-Martínez et al., 2005; Hojdova et al. 2015). Freeze-drying of soil samples is therefore a common practice (Yang et al., 2017). Freeze-drying is done at low temperatures (i.e. – 50 °C) and, hence, may better preserve Hg in the sample. Despite the fact that freeze-drying prevents temperature-induced loss of Hg(0), it should be noted that also freeze-drying may cause sample processing artefacts. High vapour pressure at vacuum may result in Hg loss (Martínez-Cortizas et al., 1999). Freeze-drying is however seen as the best option available and the "gold standard" for drying soil samples, but it is at the same time an expensive and time-consuming method. Equipment resources can thus limit the number of samples analysed in many projects.

Existing reports of Hg loss when oven-drying peat soil, as compared to freeze-drying, are few (Hojdová et al., 2015; Norton et al., 1997; Roos-Barraclough et al., 2002). The objective of this study was, therefore, to examine how well drying of peat soils at 60 °C for 96 h replicates freeze-drying in terms of sample Hg concentration. If the deviation in measured Hg after drying at 60 °C is insignificant, or systematic, relative to the results after freeze-drying, then oven-drying could be a faster, more affordable, and acceptable alternative to freeze-drying. Using 203 sample replicates and subsequent statistical analysis, our study constitutes a thorough investigation of the effects of oven-drying on Hg concentration. It is an important complement to the existing literature (Norton et al., 1997; Martínez-Cortizas, A., Pontevedra-Pombal, X., García-Rodeja, E., Nóvoa-Muñoz, JC., Shotyk, W. 1999; Roos-Barraclough et al., 2002; Fernández-Martínez et al., 2005; Hojdová et al., 2015; Yang et al., 2017) on sample preparation prior to Hg analysis of peat soil.

2 Methods

2.1 Sampling and Subsampling

Peat cores were sampled in June–August 2019 from eight peatlands (fens) located within a distance of 10 km near Umeå, Sweden [63°54′N, 20°36′E]. For detailed site descriptions, see Wang et al. (2020). A cylindrical corer of 16 cm in diameter was used to extract 50-cm-deep peat cores with the mire surface as a reference level (modified from Clymo, 1988). The cores were kept in air- and watertight PVC tubes sealed with plastic caps and transported to the lab within 6 h after sampling. The samples were then placed at – 18 °C and stored until subsampling.

Within 2 weeks after sampling, the cores were sliced into 2-cm (16 cm in diameter) discs using a bandsaw with a stainless-steel blade. Slicing was done in an – 18 °C freeze room, both to yield undisturbed discs and also to avoid potential volatilization of Hg from the peat samples associated with thaw. The discs were then split into two half discs, generating two replicates of each sample. One was used for freeze-drying and one for oven-drying at 60 °C. Due to a very high total number of samples along with equipment resource limitations, only a subset of the samples were freeze-dried. The rest of the samples were dried in a 60 °C oven for 96 h (until constant weight). To monitor the potential loss of total Hg during drying, 203 sample pairs from the same 2-cm disc (replicates) were both freeze-dried and dried at 60 °C in an oven. The oven-dried samples were placed in a desiccator for cooling immediately after drying to avoid moisture absorption during cooling.

The dry samples were homogenized by hand in a zip-lock bag to ensure a sample representative of each half disc. One gram of dry sample was then ground in a tube mill (IKA Tube Mill Control) during 10-s intervals to avoid heat generation during milling.

2.2 Analytical Methods

Analysis of total Hg was done using a Direct Mercury Analyzer (DMA 80) (Milestone, Shelton, CT, USA). Each peat sample was analysed in replicates and a certified reference sample was analysed every fifth sample for validation of the analytical precision (NIST 1515 – Apple Leaves Standard Reference Material® and ERMCD-281 – Rye Grass ERM® Certified Reference Material). The total Hg concentrations of the certified reference material had a precision within 2% of the mean total Hg concentration (95% confidence interval) of the reference value. The mean of our measurements underestimated the reported value of the standard by 5% and 4% respectively for NIST 1515 and ERMCD-281.

2.3 Statistical Methods

To test the hypothesis that Hg peat soil concentration measurements after oven-drying (60 °C) did not differ from the concentration determined after freeze-drying, we employed the Lin concordance correlation coefficient. This method is based on the assumption that one measurement technique represents “a gold standard “ (i.e. freeze-drying in our study), against which all other alternatives must be evaluated (Lin, 1989). This statistic is commonly used in medical and clinical studies for testing a new method when there is already an established methodology (King et al., 2007; Lin, 1989) (Microsoft Excel was used for statistical analysis and graphics).

In addition to a Pearson regression model, the Lin concordance correlation coefficient (CCC) also accounts for the agreement with a 45° line (Lin, 1989). To obtain the CCC, the Pearson correlation coefficient is multiplied by the deviation from the 45° line (C_b; Eq. (2)). The CCC has values between − 1 and 1, similar to the Pearson correlation coefficient, but the CCC only attains a perfect positive or negative correlation if the Pearson correlation coefficient equals unity and the absolute deviation from the 45° line is zero (Fig. 1) (King et al., 2007; Lin, 1989).

The relationship between the Lin and the Pearson correlation coefficients can be written as in Eqs. (1–4). In Eqs. (2–4), υ measures the offset (Fig. 1b) in relation to the scale and ω represents the scale shift (Fig. 1c).

$$\rho_c = \frac{2\sigma_{12}}{\sigma_1^2 + \sigma_2^2 + (\mu_1 - \mu_2)^2} = \rho C_b \tag{1}$$

$$\mathrm{C}_b = \frac{2}{\omega + \frac{1}{\omega} + \upsilon^2} \tag{2}$$

$$\omega = \frac{\sigma_x}{\sigma_y} \tag{3}$$

$$\upsilon = \frac{\mu_x - \mu_y}{\sqrt{\sigma_x \sigma_y}} \tag{4}$$

2.4 Wilcoxon’s Signed-Rank Test for Paired Samples

The data were log-transformed to attain normality. The Hg concentration in oven-dried samples was however still not normally distributed (Shapiro–Wilk test (Shapiro & Wilk, 1965)). We therefore employed the non-parametric tests equivalent to the *t*-test to test for a difference between the two drying methods; Wilcoxon’s signed-rank test for paired samples (Wilcoxon, 1945). The null hypothesis indicates no difference between the samples, while the alternative hypothesis is a difference with a significance level of 0.05.

2.5 Linear Regression Model and Validation

To predict Hg concentrations from freeze-dried samples using oven-dried samples, we fitted a linear function to the log-transformed Hg concentrations. The *Y*-value is thus freeze-dried samples and *X*-value oven-dried samples.

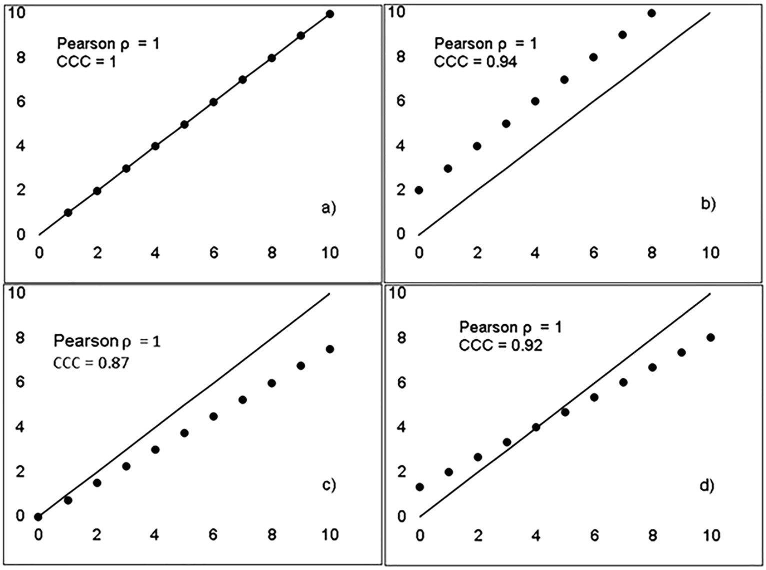

Fig. 1 **a** The theoretical patterns of correlation when the Lin concordance correlation coefficient (CCC) and the Pearson are both unity. **b** to **d** are examples of when the Pearson correlation coefficient is also unity but fails to detect a deviation between two methods, whereas the CCC does indicate a deviation.

To avoid over-fitting and to cross-validate our results, we employed *K*-fold cross-validation for our data set (Lachenbruch & Mickey, 1968). Our data set (203 sample pairs) was randomly assigned into 10 discrete groups of which seven of these groups had 20 members and three of them 21 members ($K = 10$). Nine of the discrete groups were used as a training data set to fit a function to predict the 10th group for validation. The model was looped ten times until all groups had served as a validation set. By combining the predicted values from each of the ten cross-validation runs, we generated a parameter of predicted Hg concentration from freeze-dried samples. The predicted Hg concentrations were then compared to the observed Hg concentrations in freeze-dried samples to validate the model.

2.6 Moisture Content of Oven-Dried and Freeze-Dried Samples

The hygroscopic nature of peat can alter the accuracy of dry bulk density determinations, and hence the concentration of a substance, in the samples. We therefore tested for a difference in water loss between oven-dried and freeze-dried samples using a non-parametric correspondent to a pairwise *t*-test (Wilcoxon's signed-rank test for paired samples). The data were not normally distributed, according to a Shapiro–Wilk test, and thus we used non-parametric statistics.

In addition to the Wilcoxon signed-rank test, we calculated the ratio between water loss during oven-drying and freeze-drying for each sample pair. Using all ratios, an average ratio was calculated to get a single quotient to compare oven-drying and freeze-drying. Identical water loss between the two drying methods would then yield an average quotient of 1.

3 Results

The Lin correlation coefficient yields a value of 0.91. The CCC uses both the offset (0.038) and scale shift (0.95) to calculate the C_b (1.0). The C_b is the correction factor that is multiplied by the Pearson correlation coefficient to obtain the CCC. While the CCC is suited for the comparison of two methods, it does not define the significance of a potential difference between the two methods. In Fig. 2, the regression line of log [Hg] from oven-dried vs. freeze-dried peat samples is not significantly different from a 1:1 line in terms of either offset (95% c.l.; – 0.02, 0.17) or slope (0.90, 1.0).

One-tailed Wilcoxon paired signed-rank test showed a significant difference between the two drying methods at a 95% significance level ($p = 0.017$). The median Hg deficit in oven-dried samples was 4.2%, with quartiles of − 13% and 15%. The negative value indicates a negative Hg concentration deficit (i.e. gain). A deficit of 4.2% corresponds to a Hg loss rate of 19 ng kg^{-1} h^{-1} when drying samples in a 60 °C oven for 96 h.

Equation (5) stems from linear regression of Hg concentrations in freeze-dried (Y) and oven-dried (X) peat samples. Through this equation, Hg concentrations in freeze-dried samples can be predicted using oven-dried peat samples (see also Fig. 2).

$$y = 0.96x + 0.08 \tag{5}$$

By using K-fold cross-validation, we generated a dataset of predicted Hg concentrations in freeze-dried samples (Lachenbruch & Mickey, 1968). The result was an R^2 of 0.83. For reference, this R^2 value is similar to the R^2 value of the regression 'oven-dried Hg concentration' vs 'freeze-dried Hg concentration' which was also 0.83 (Fig. 3).

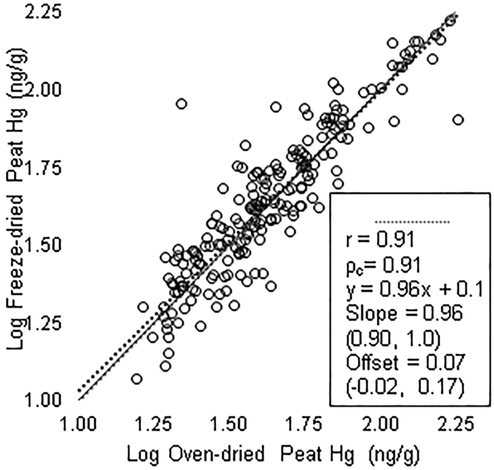

Fig. 2 Regression of oven-dried vs. air-dried peat log Hg concentration. The full line is a reference line with a 1:1 ratio. The dashed line is the regression line. r = Pearson's correlation coefficient. ρ_c = Lin's concordance correlation coefficient. y = linear regression line equation. For *Slope* and *offset*, the 95% confidence interval is reported in parentheses (− 0.02, 0.17)

Residuals of the regression between predicted and actual freeze-dried Hg concentrations were plotted to further examine our model (Figs. S1–2). Though our residuals are not normally distributed ($p = 0.05$; Shapiro–Wilk test (Shapiro & Wilk, 1965)), the residuals are symmetrically distributed around zero. There is thus no subspan in our range of investigated concentrations where our model is particularly weak (Table 1).

Based on a comparison of the water lost during drying, a pairwise Wilcoxon signed-rank test did not result in any significant difference in water lost during drying between freeze-dried and oven-dried samples ($p = 0.52$, two-tailed, the average weight of freeze-dried samples being 0.042% larger than oven-drying.). A Pearson correlation between log-transformed water loss on freeze-drying vs. oven-drying indicated a correlation coefficient (r^2) of 0.94 ($p < < 0.001$).

4 Discussion

Using oven-drying as an alternative to the more resource-demanding freeze-drying comes with the concern about Hg loss through volatilization in a heated oven (Hojdová et al., 2015;

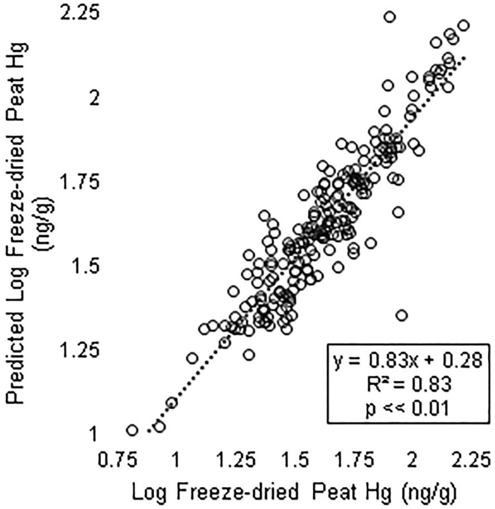

Fig. 3 Logarithm of observed Hg concentration from freeze-dried samples vs. predicted Hg concentrations of freeze-dried samples

Table 1 Summary of results

Statistical measure	Result
Pearson correlation coefficient ρ	0.91
Lin concordance correlation coefficient ρ_c	0.91
Accuracy C_b	1.0
Scale shift ω	0.95
Offset υ	0.038
One-tail paired Wilcoxon signed-rank test, *p*-value	0.017
Median Hg deficit, oven-dried samples (%)	4.2
Hg loss rate (ng kg^{-1} h^{-1})	19

Roos-Barraclough et al., 2002). The average 4.2% lower Hg concentrations found in 60 °C oven-dried peat samples relative to freeze-drying indicates that oven-drying does cause some Hg loss. It is likely that the loss of Hg we observed stems from the release of elemental Hg in our samples. Gaseous elemental Hg is volatile that is easily released into the atmosphere when exposed to temperatures approaching 100 °C (Windmöller et al. 1996), but it is evident that volatilization significantly affecting Hg determination in peat samples can occur at even lower temperatures.

Although some other comparisons of freeze-drying and oven-drying have been reported in the literature, few have looked specifically into the drying of organic soils, and specifically peat soils. Hg loss from oven-drying compared to freeze-drying of organic soil samples reported in the literature is scattered around zero (Table 2). Two of the four previous studies experienced similar Hg loss (0–5%) found in our study.

There are also studies of Hg loss when drying other soil types besides peat/organic soil. Some studies did not observe any Hg loss from mineral sediment samples dried in the 50–60 °C range (Crecelius et al., 1975; Mudroch & MacKnight, 1994). Crecelius et al. (1975) even dried estuarine sediments from Puget Sound, WA, USA, at 80 °C for 48 h without significant loss of Hg. For materials other than soil, e.g. animal tissue and wood studies, no Hg loss has also been reported after drying at 60 °C, as compared to freeze-drying (Schmidt et al., 2013; Yang et al., 2017). The variation in loss of Hg during oven-drying clearly indicates the importance of chemo-physical properties of the sample matrix in controlling the difference in [Hg] loss between oven- and freeze-dried samples.

Even if oven-drying gave a significant difference in peat [Hg] concentrations, 45% of the oven-dried samples had higher Hg concentration than the freeze-dried samples, which indicates that other factors than the drying temperature influenced our result (Fig. 1). A major source of the variation is most likely the variation between sample duplicates, i.e. the difference between analytical replicate samples. As mentioned in the method section, the paired samples used for comparing freeze- and oven-drying stem from a 2-cm slice of a 16-cm diameter disc split in two. It is possible that centimetre-scale differences in the peat influence the sample duplicates. Other studies have reported significant differences in Hg concentration between vegetation species growing side to side (Rydberg et al., 2010). We therefore stress the importance of careful homogenization before analysing peat samples for Hg.

Table 2 Loss of Hg when oven-drying organic soil samples reported in the literature and this study. Loss of Hg is defined as the Hg deficit after drying relative to freeze-frying if not otherwise specified

Study	Soil	Temp. (°C)	Drying time	Hg loss
Norton et al., 1997*	Peat	50	7 days	3.4% (relative 30 °C drying)
Roos-Barraclough et al., 2002†	Peat	60	5 days	~0%
Hojdova et al. 2015‡	Oe forest soil horizon	105	3 days	Significant Hg gain
Hojdova et al. 2015‡	Oa forest soil horizon	105	3 days	Significant Hg loss
This study	Peat	60	4 days	4.2%

*Norton et al. reported two values for Hg loss on drying from peat depths of 52.5 and 55 cm. The value found in the table below is an average of these two results.

†Although Roos-Barraclough et al. (2002) had several treatments for their peat samples, this value is from their experiment with unfertilised peat.

‡Value reported from uncontaminated soils.

It could also be discussed if 60 °C is the optimal temperature to dry peat in an oven. Drying at room temperature or at a maximum of 40 °C are two methods that have been proposed (de Groot et al., 1982; Ettler et al., 2007; Fernández-Martínez et al., 2005; Higueras et al., 2003). The problem is however that peat samples of our size contain around 100 g of water, with a high abundance of microorganisms in the samples. A week-long period for the peat samples to dry risks substantial microbial activity with concomitant biogeochemical changes, especially since room temperature, or slightly higher, is an optimum temperature window for many microorganisms found in peat soil (Liu et al., 2018; Schuster, 1991). Hg reduction by bacteria reducing Hg(II) to volatile Hg(0) is undesirable when preparing samples (Kritee et al., 2008; Zhou et al., 2020). Contrary to Hg evasion, cases have also been documented where samples dried in the open air have absorbed Hg from the laboratory surroundings (Roos-Barraclough et al., 2002; Yang et al., 2017). Overall, the downsides of drying at low temperatures (20–50 °C) have to be balanced against the risk of Hg evasion at higher temperatures such as 60 °C.

Another cause for concern when drying peat samples is the absorption of moisture after drying. The risk of water vapour condensation and subsequent moisture absorption is particularly high when warm samples cool down at room temperature. However, even if following standard procedures, i.e. the oven-dried samples were immediately put in a desiccator for cooling, the risk of moisture absorption needs to be monitored. In the case of water absorption after oven-drying, the oven-dried samples would have been heavier than freeze-dried samples after cooling. The net loss of water, i.e. water lost during drying plus potential moisture absorption, did not differ significantly (oven-dried samples being 0.042% higher than freeze-dried samples) between oven-dried and freeze-dried samples This number is also small in relation to the difference in Hg concentration of 4.2%. Moisture absorption and subsequent increase in density was thus not an explaining factor for the lower Hg concentration in oven-dried peat samples but might be a source of noise in these data.

To facilitate analysis of large sample volumes on total Hg concentrations in organic soils, we explored the possibility of freeze-drying a subset of the oven-dried samples to predict the Hg concentration in freeze-dried peat. Since the relationship between oven-dried and freeze-dried samples is under the influence of other effects than the drying method (i.e. subsampling), the model would likely perform better if the effects of drying were isolated. Linear modelling to predict Hg concentrations stemming from freeze-dried samples therefore seems like a reasonable approach to adjust the Hg concentration in oven-dried peat. This methodology will simplify the processes of analysing the total Hg in peatlands. To use peatlands as an archive for past atmospheric Hg concentration or to assess the amount of Hg stored in the soil, sampling is necessary both depthwise and spatially. This renders a large number of samples where an evident bottleneck is the drying procedure. Our study offers a way of navigating around this obstacle, which will lead to more accurate assessments of Hg in peatlands and a step towards mitigating the risks of Hg exposure.

5 Conclusion

Oven-drying of peat samples at 60 °C resulted in a median 4.2% Hg loss (− 13%, 15% quartiles). Whether this is acceptable or not depends on the purpose of the study. Equipment resource limitations could be a bottleneck to large-scale surveys of Hg content, especially in organic soils. Using oven-drying instead of freeze-drying could increase the number of samples analysed, which ultimately has to be weighed against the uncertainty that the risk of Hg loss brings. Using freeze-drying on a subset of samples could allow for the correction of any potential bias between the two methods.

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Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval The authors have no relevant financial or non-financial interests to declare.

Competing Interests The authors declare no competing interests.

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Despite the societal demand to restore drained boreal peatlands, a firm understanding of the effects of drainage and restoration on the biogeochemistry of these ecosystems is lacking. In addition to the intended ecosystem services, restoration may result in undesired effects and potential environmental threats, such as formation of the toxic methylmercury. This thesis examines the effects of drainage and restoration on peat properties and uses this understanding to assess the impact of restoration on net methylmercury formation.

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