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Spatial variation in natural formation of chloroform in the soils of four coniferous forests

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Abstract Natural chloroform in soil gas below four coniferous forest sites was studied. High concentrations were found within narrow areas—Hot Spots—varying from ~25 to >400 m^2 in size, with chloroform concentrations being typically 20–100 times those in corresponding Low Spots.Attempts to localize Hot Spots by visual inspection with regard to type and density of vegetation failed. Possible differences between Hot and Low Spots could be emission, leaching or degradation of chloroform. However, emissions of chloroform from Hot Spots were ~10 times higher than from Low Spots and similarly the chloroform concentration in groundwater below a Hot Spot was ~10 times higher than below the corresponding Low Spot. No differences in chloroform mineralization rates were observed between sites and incubation of soil cores confirmed a larger net formation of chloroform in the Hot Spots. Various soil parameters were measured in order to compare the soil sampled from Hot and Low Spots. The halogenation degree of organic soil samples was in the same range, although slightly higher in the H-horizon of the Hot Spot. The chloroform formation potential of the soil organic matter showed differences between soil horizons but not between sites. The high levels of chloroform in the Hot Spots are probably best explained by differences in chloroform forming activity caused by an uneven distribution of yet unidentified microorganisms, since differences in soil organic matter quality and in emission, leaching and degradation of chloroform as well as a number of additional soil parameters could be completely ruled out.

Keywords Natural chloroform · Natural organohalogens · Soil · Spatial variation · Flux · Forest

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Introduction

Chloroform ($CHCl_3$) is a volatile chlorinated aliphatic compound. It is a common groundwater pollutant in urban areas as well as in rural areas (Squillace et al. 1999; Squillace et al. 2004). In Denmark, chloroform has repeatedly been found as

the single pollutant in groundwater beneath coniferous forests in concentrations often exceeding the local groundwater quality criteria of 1 μg/L (Laturnus et al. 2000; Jacobsen et al. 2007). The occurrence of chloroform in groundwater of otherwise fine quality indicates a natural origin and it is well established that chloroform is a natural substance, with only 10–25% of the annual flux of 0.3–1.0 Tg/yr to the atmosphere originating from human activity (Laturnus et al. 2002; McCulloch 2003; Worton et al. 2006). Atmospheric chloroform is present at concentrations of 10–15 pptv in background locations of the northern hemisphere (NOAA 2009) and based on atmospheric monitoring in Mace Head, Ireland, chloroform emissions in the western and central part of Europe seem to be highest from terrestrial regions in Ireland, Scotland and Scandinavia, while the emissions are much smaller from the dense populated areas (Ryall et al. 2001).

The presence of natural chloroform in temperate coniferous forests has been observed for more than 10 years (e.g. Hoekstra et al. 1998a, Hoekstra et al. 2001; Dimmer et al. 2001; Haselmann et al. 2002; Svensson et al. 2007) but even though the reported concentrations were always higher in the soil air than in the ambient air, highly varying concentrations and fluxes have been measured both within and between forests. Before the presence of natural chloroform in forest soil was known, Frank et al. (1989) reported chloroform concentrations as high as 14000 pptv at 30 cm depth, but with variations of more than one order of magnitude between and within three different forested areas. Similar concentrations in soil air of coniferous forests have been reported by Frank and Frank (1990) and Hoekstra et al. (1998a), also with variations as high as a factor of 10 within single forests. Haselmann et al. (2002) found lower concentrations in a temperate spruce forest, with only 2–10 times the atmospheric background in air samples taken at 0-10 cm depth. Hoekstra et al. (2001) and Dimmer et al. (2001) both investigated the flux of chloroform from forest soil and found differences of more than one order of magnitude between different vegetations.

The highly elevated concentrations in coniferous forest soils, and positive fluxes from these, are strong indications that a natural formation takes place in such soils. A more direct proof of chloroform formation in forest soil is the observed increase in the isotope fraction of ^{37}Cl in soil air chloroform, when $Na^{37}Cl$ was added to forest top soil (Hoekstra et al. 1998a).

The formation of chloroform in forest soil has been ascribed unspecific chlorination facilitated by extracellular enzymes excreted by fungi (Hoekstra et al. 1998a), although some fungi seem to produce chloroform intracellularly (Hoekstra et al. 1998b). Unspecific chlorination is known from research on macromolecular organochlorine, which is present in forest soils in concentrations of ~ 1 mg g^{-1} of soil organic matter (SOM) (Asplund et al. 1989; Asplund and Grimvall 1991; Hjelm et al. 1995; Öberg and Grøn 1998). This organic chlorine is supposed to be formed when chlorinating enzymes oxidize chloride to reactive halogen species like HOCl that react unspecifically with SOM. A pool of chlorinating enzymes have been shown to exist in forest soil (Asplund et al. 1993), and the formation of chloroform was suggested to be the result of a biotic reaction (1) followed by an abiotic reaction (2) (Hoekstra et al. 1998a):

$$H_2O_2 + H^+ + Cl^- \rightarrow HOCl + H_2O(\text{enzyme} - \text{catalyzed}) \quad (1)$$

$$HOCl + SOM \rightarrow CHCl_3 + \text{chlorinated SOM}\ (\text{non} - \text{enzymatic}) \quad (2)$$

The last reaction is expected to be similar to the formation of chloroform during the chlorination of drinking water (Rook 1977; Boyce and Hornig 1983; Deborde and von Gunten 2008). In addition to the enzyme-catalyzed reaction, Huber et al. (2009) recently demonstrated the abiotic formation of trihalomethanes from polyphenols added Fe(III), H_2O_2 and the increased formation of chloroform in soil when Fe(III) was added. The reaction seemed to take place mainly at very acidic pH (<3.7).

Although it seems well established that chloroform is formed in temperate coniferous forests, very different concentrations have been reported, also in similar forest types. Previously calculated global fluxes of chloroform to the atmosphere may therefore be uncertain and it may also be difficult to estimate expected chloroform concentrations in groundwater beneath forests. One purpose of our study was therefore to more systematically investigate spatial variation of soil chloroform between and within temperate coniferous forests. The second purpose was

to see if the spatial variation could be explained by variation in formation or removal of chloroform and if this could help to reveal the mechanism of formation, which is still on a very hypothetical level.

Materials & methods

Study sites

Two forests were chosen as study sites because monitoring wells with µg/L-concentrations of presumably natural chloroform were present in the vicinity. In Nordre Feldborg (NF), the study site (8°57′01″ E, 56°22′51″ N) is a stand of 40 year old Norway Spruce (*Picea abies*) as the dominating vegetation. No underwood is present, but the forest floor is partly covered with moss. The soil is sandy, with both diluvial and aeolian sand in the area, which has been forested since approximately 180 years. In Tisvilde Hegn (TH), the study site (12°03′40″ E, 56°02′22″ N) is a mixed stand dominated by older Scots Pine (*Pinus sylvestris*) and younger Norway Spruce with also a few Birch trees (*Betula pendula*) present. No underwood or moss is present. The soil is 200–400 years old aeolian sand, which has been forested since at least 150 years. The top sand is partly washed out, and the area is in the initial phase of a podzolization. Two additional forests near the town of Viborg (Fig. 1), Viborg Hedeplantage (VH) (9°22′14″ E, 56°25’37″ N) and Liseborg Plantage (LP) (9°21′33″ E, 56°25’42″ N), were included to confirm a general pattern of spatial variation. These forests were chosen because of problems with presumably natural chloroform in concentrations above the quality criteria in the nearby water works (Jacobsen et al. 2007). The two additional study sites are mixed stands with Norway Spruce as the dominating vegetation and apart from mosses no underwood is present. The soil is diluvial sand with well developed A-horizons. All four forests are temperate hemiboreal coniferous plantations and at all four locations, a 5–25 cm thick organic horizon has developed on top of the sandy soils. The organic horizon consists of a litter (L-) horizon followed by a fermentation (F-) horizon. Below the F-horizon, a humification (H-) horizon partly mixed with sand can be recognized.

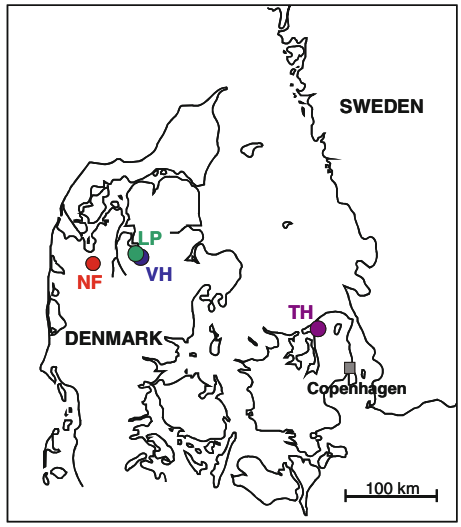

Fig. 1 Map of the location of the four study sites in Denmark. NF is Nordre Feldborg, LP is Liseborg Plantage, VH is Viborg Hedeplantage and TH is Tisvilde Hegn. See text for GPS-coordinates

Sampling dates

The field experiments and sample collections were done at a number of different field trips:

Soil and atmospheric air: May 2007, repeated in May 2009 (TH). October 2007 and May 2008 (NF). February 2008 and November 2008 (VH). November 2009 (LP).

Emissions of chloroform from soil: March, June and August 2009 (TH). March and June 2009 (NF).

Groundwater collection: ∼ every third week from July 2007 to July 2009 (Two wells in TH).

Soil for incubation in laboratory: May 2007 (TH).

Soil for degradation, total halogen and chlorination experiments: November 2008 (NF).

Soil for pH, Cl^- and Fe determinations: August 2007 (TH). November 2008 (NF).

Field studies

Soil air sampling

A stainless steel soil air lance was used to obtain soil air samples (Fig. 2a). The lance was forced into the soil and air was drawn through the screen of the lance at 40 cm depth using a membrane pump (Rietschle Thomas, Schopfheim, Germany) at a speed of ∼2 L min^{-1}. The soil air was led through a 200 mL

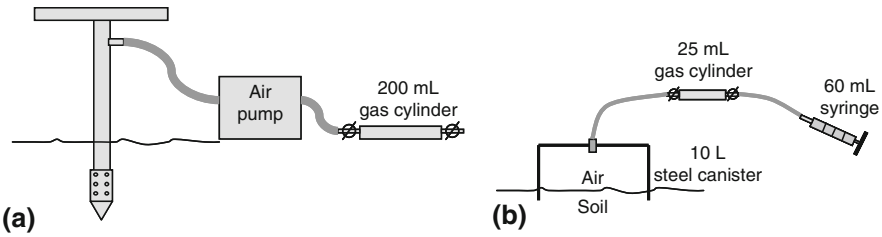

Fig. 2 **a** Setup for obtaining soil air samples from 40 cm depth. **b** Setup for measuring fluxes of gases from soil

stainless steel gas cylinder with two terminal valves. After 3 min the exit-valve was closed and the cylinder was pressurized to ~1.5 bar by continued pumping before closing the inlet valve (total sampling time ~5 min per sampling spot). The gas cylinders were brought to the laboratory and 15 mL pressure equalized subsamples were analyzed within 48 h. Reproducibility of the method was tested by analysis of consecutive gas samples, which showed a standard deviation <3% of the average value of three samples. Furthermore, possible errors due to chloroform interacting with the steel cylinders during storage was checked by comparing with samples collected in electropolished steel cylinders (Swagelok Company, Solon, OH) and in 30 mL glass cylinders. No errors were found. Prolonged storage up to 2 weeks resulted in only minor changes in chloroform and CO_2 content. Sorption of chloroform to the PE-tubing used to connect the pump with the air lance and the steel cylinder was undetectable during laboratory tests using ^{14}C-chloroform in aqueous solutions and the tubing did not release chloroform or other volatile halogenated compounds in detectable amounts. Atmospheric air samples (20 cm height) were sampled at each sampling date and location using the same membrane pump and procedure, but with the soil air lance detached.

In NF and VH, soil air samples were taken at two occasions, and two spots from the first sampling date were resampled on the second date. The sampling dates were chosen, according to the seasonal cycles of chloroform soil air concentrations (Albers et al., Submitted) and the chloroform concentrations were indeed similar at both dates at both sites: Average of the two NF spots in October 2007 was 13.0 ppvb and in May 2008 12.7 ppbv. In VH the average of the two spots was 19.3 ppbv in February 2008 and 18.1 ppbv in November 2008. Since the concentrations were very similar at both sampling dates, it was chosen to merge the chloroform concentrations before their presentation in Fig. 3. The risk of this leading to noticeable errors in the interval-based Fig. 3 seems very small.

Flux measurements

The emissions of chloroform and methyl chloride from the forest floor were investigated in NF and TH using a simple static chamber approach (Fig. 2b). Ten litre stainless steel canisters were equipped with valves and used as flux chambers. A 25 mL glass gas cylinder with two terminal valves was connected to a chamber and a 60 mL syringe. After pressing the edge of the canister ~1 cm into the soil, it was left closed for 2 h and a gas cylinder was then connected to the valve that was then opened. The syringe was drawn forth and back 20 times, in order to flush the gas cylinder and to mix the air in the flux chamber. The inlet valve of the gas cylinder was then closed and the gas sample pressurized to ~2 bars with the syringe piston. Samples were taken in duplets, with typical variation of 5–10% between replicates. The gas samples were brought to the laboratory and analyzed within 48 h. The flux from soil to the atmosphere was calculated from the increase in chloroform concentration during 2 h. Preliminary tests revealed that the increase during the 2 h was not completely linear (see supplementary material Fig. S1). For practical reasons it was not possible to make such time curve for each flux measurement, and the same curve was used for all measurements, despite the risk of small errors associated with this (e.g. caused by variations in temperature, soil moisture etc.). However, this should not significantly influence the main goal of making the flux measurements, which was a comparison of Hot Spots and Low Spots, since no systematic differences in such parameters were present between Hot and Low Spots. The first derivative of the fitting function at $t = 0$ was compared with the slope of the first order equation calculated from a single

point measurement and the flux at t = 0 could then be estimated from the flux calculated from the single measurements at t = 2 h.

The soil temperature and moisture was monitored on an hourly basis in TH, as described elsewhere (Albers et al., Submitted).

Shallow groundwater was sampled and the concentration of chloroform determined by purge&trap GC/ECD as previously described (Albers et al. 2008; Albers et al., Submitted).

Chemical analyses, gases

Pure $CHCl_3$ (>99.5%) for analytical standards was purchased from Sigma–Aldrich (Steinheim, Germany).

Air samples were analyzed for chloroform, CFCs and other C_1-organohalogens on a gas chromatograph equipped with an ECD detector (GC-8A, Shimadzu, Kyoto, JP). The analytical procedure was similar to that described by Busenberg and Plummer (1992) for chlorofluorocarbons (CFC) in age-dating of young groundwater. Briefly, the halocarbons were trapped at −30°C on a pre-column, which was then heated to 95°C. Separation of gas constituents was done on a 1.7 m packed column, Porasil-C, at 70°C. Pre-column back-flush technique was used to complete the analysis of each sample within 11 min. Using 15 mL of air sample, the limit of quantification (LOQ) for chloroform was ∼10 pptv and for methyl chloride ∼7 ppbv.

Analysis of O_2 and CO_2 in gas samples was done on a Mikrolab GC82 gas chromatograph equipped with a thermal conductivity detector (TCD) (Mikrolab, Aarhus, DK). Separation of the gas constituents was carried out at 60°C on 2 columns packed with molecular sieve 5A and Porapak C using helium (60 mL/min) as the carrier gas. The LOQ for CO_2 was 0.04%.

Chemical analyses, soils

pH was measured in a 2:1 water:soil slurry. Chloride and water extractable iron were determined in subsoil as follows: 5 g soil was shaken for 24 h with 5 mL MilliQ-H_2O, and the slurry was then centrifuged (3000×*g*, 10 min.). Chloride in the supernatant was determined by ion chromatography (Dionex DX500 IC). Total iron in the supernatant was determined spectrophotometrically at 520 nm after complexation of the reduced iron with 2,2′-bipyridine (Moss and Mellon 1942). The background absorption in the supernatant, constituting 10–20% of the total absorption at 520 nm, was subtracted from this.

Soil for the determination of total organic halogens was sampled with steel cylinders (H: 18 cm and Ø: 6 cm) in NF. The soil columns were stored cold and closed until arrival in the laboratory and the A_h-horizons from just below the H-horizons were then extracted with water for chloroform-determination as a quick test, to see differences in chloroform concentrations between soil columns. The different layers of the organic horizons were freeze dried and grinded to fine powder in a coffee mill followed by further grinding in an agate mortar. The powdered soil was then divided in two. One portion was taken for further analysis as it was (TX, total soil chlorine/bromine/iodine) and one portion was thoroughly washed three times with HNO_3/KNO_3 (0.02 M/0.2 M) solution (liquid:soil ratio of ∼30) and then once with MilliQ water to remove all inorganic halogen present. After freeze drying, this portion of the soil sample was analyzed similarly for halogens (TOX, total organic chlorine/bromine/iodine). The loss of organic chlorine in such a washing procedure has previously been shown to be less than 1% for organic horizons (Asplund et al. 1994; Silk et al. 1997). Total inorganic halogen (TIX) was calculated as (TX − TOX), which with the accuracy of the analytical procedure gave LOQ for TIX of ∼2 μg/g. A total of 24 soil samples (triplicate F- and H-horizons, NF Hot and Low Spot, TX and TOX) were then digested for the determination of total organic halogens using microwave-induced combustion (MIC), which was recently proposed to digest organic samples with complex matrices (Flores et al. 2007; Moraes et al. 2007). The conditions during the MIC-procedure were as follows: Triplicate soil samples (about 200 mg) were pressed as pellets using a hydraulic press set at 3 ton for 1 min for further digestion by MIC. Conditions were chosen according to previous work (Flores et al. 2008) using 6 mL of 50 mM $(NH_4)_2CO_3$ as absorbing solution. The heating program was 1400 W for 10 min (reflux step) and 0 W for 20 min for cooling. After combustion, the resultant solution was transferred to a polypropylene vessel and diluted with water to 30 mL. Accuracy was evaluated using certified reference material of coal (NIST 1632c) and spiked samples were used to

evaluate the recovery of halogens. After MIC, Cl was determined by ion chromatography and Br and I were determined by inductively coupled plasma mass spectrometry (ICP-MS). Operational conditions of halogens determination by IC and ICP-MS were selected according to literature (Flores et al. 2008).

Laboratory experiments

Net formation of chloroform

Eight soil cores were sampled in steel cylinders (H: 10 cm and Ø: 6 cm) in TH. The soil cores were incubated immediately at 10°C in the dark in 1 L closed glass jars with a sodium bicarbonate based plastisol-lined lid equipped with a luer lock port. This valve was connected directly to the injection port of the GC/ECD, for injection into the air-evacuated 15 mL sample loop. 2 × 0.2 mL air was sampled for CO_2 and O_2 analysis, respectively. The incubated jars were analyzed once a week during 5 weeks. Due to fairly high consumption of oxygen, we found it necessary to add 40 mL pure O_2 to each jar at the end of each analysis. The net formation was calculated from the average increase in chloroform concentration from day 6–34. Pilot studies with injection of chloroform to similar soil cores showed that equilibrium between soil cores and the surrounding air was established within 30–50 h.

Chlorination of soil samples

Freeze dried and powdered top soil samples were chlorinated chemically in order to detect any differences in chloroform formation potential between soil sites and horizons. Soil corresponding to 15.5 mg SOM was weighed into a 16 mL glass vial, 15 mL 0.1 M phosphate buffer (pH 4.0) was added followed by 50 mM NaOCl-solution to a final OCl^--conc. of 0.5 mM. The vials were then placed in the dark at 10°C, partly to mimic natural soil conditions, partly to minimize losses of chloroform to the small headspace and to the air, when taking subsamples for analysis. After 24 h, the reaction was quenched with 100 µL 1 M $Na_2S_2O_3$ and after centrifugation (1000 g, 15 min.), 100 µL of the supernatant was diluted to 125 mL with chloroform-free N_2-purged MilliQ-water and 30 mL of the solution was analyzed on purge & trap GC/ECD.

Mineralization of chloroform

A total of 30 g O-horizon, homogenized by hand and with roots and other greater particles removed, or 30 g 2-mm-sieved A-horizon were added to 600 mL glass jars equipped with a sodium bicarbonate based plastisol-lined lid with a 9 mm silicone-septum. All jars were equipped with a small glass container for CO_2-absorbing liquid (1 M NaOH) and 3 mL aqueous ^{14}C-$CHCl_3$ (ARC Inc., St. Louis, MO, radiochemical purity >99%) solution was then added to the soil in each jar (170000 DPM/mL, corresponding to a total of ~350 µg/kg soil wet weight, which corresponds to some of the highest concentrations found in natural soils). The lid was closed immediately after the addition of ^{14}C-$CHCl_3$ and a stainless steel needle with a 3-way valve was pierced through the septum and into the small glass container. After having added 3 mL of CO_2-absorbing liquid (1 M NaOH) to the container, the 3-way valve was closed. Because of its high volatility, some of the ^{14}C-$CHCl_3$ is likely to move from the soil into the CO_2-trap. It was therefore necessary to develop a method to distinguish between $^{14}CO_2$ and ^{14}C-$CHCl_3$ in the absorber fluid. The NaOH was changed every 3–10 days and the $^{14}CO_2$ originating from the mineralization of ^{14}C-$CHCl_3$ was separated from dissolved ^{14}C-$CHCl_3$ as follows: After thorough mixing, 1 mL of the absorber was added to each of two 2 mL centrifuge tubes (Sarstedt, Nümbrecht, Germany) containing either 1 mL H_2O or 1 mL 1 M $BaCl_2$. $BaCO_3$ was allowed to precipitate for 10 min at 5°C and all tubes were centrifuged (10000×*g*, 2 min.). 1 mL from each tube was then counted on LSC, and the total $^{14}CO_2$ released from the soil was calculated from the difference between the subsample with water added (containing both $^{14}CO_2$ and ^{14}C-$CHCl_3$) minus the subsample with $BaCl_2$ added (containing only ^{14}C-$CHCl_3$). Pilot experiments showed that $BaCl_2$ had no influence on the dissolved ^{14}C-$CHCl_3$ and that the method had high accuracy and reproducibility.

Fungi from a Hot Spot

Twenty fungal strains were isolated from a TH Hot Spot soil sample by placing 2-mm pieces of organic soil on soil agar plates. The agar was prepared by suspending 10 g air dried and homogenized O-horizon

and 15 g Noble agar in 1 L of MilliQ water followed by three-fold autoclavation. Inoculated plates were incubated at 15°C for several weeks. Emerging hyphae were purified on malt extract agar, and tested for production of chloroform in 100-mL serum bottles containing one g of homogenized O-horizon and 30 mL of water. The bottles were autoclaved three times, inoculated with three agar plugs of each strain, closed with sterile septa, and incubated at 20°C and 150 rev min^{-1}. The oxygen tensions in the headspaces were measured after 4 and 9 days. Extra oxygen (10 mL) was added on day 9. On day 14, the contents of the flasks were centrifuged and the chloroform concentration in the supernatant determined similarly to the analysis of chloroform in groundwater samples.

Statistical software

Spatial interpolation of chloroform concentrations in soil was performed with Surfer 8.02 (Golden Software Inc., Golden, CO). The Ordinary Linear Kriging method was used to perform interpolation between sample points.

Simple and multiple regressions on chloroform concentration (dependent variable) and CO_2 concentration or soil characteristics (independent variables) were performed using KyPlot Version 2.0 (KyensLab Inc.). Since data on neither chloroform concentrations in soil nor chloroform fluxes from soil were normally distributed, the non-parametric Spearman's rank correlation coefficient was used to determine if correlations were statistically significant.

Results

Preliminary investigations in the study areas revealed spatial variations in chloroform concentration in the soil air of up to two orders of magnitude between as well as within single stands. These surveys were done with tens to hundreds of metres between sampling points. Denser sampling with 1.5–10 m between sampling points revealed that the variation was not completely random, with points of high chloroform concentrations located close to each other (Fig. 3). These areas with high concentrations will from now on be termed "Hot Spots" whereas all other areas with chloroform concentrations lower than ~5 ppbv (but typically lower than 1 ppbv) in the soil air will be termed "Low Spots". In NF, the Hot Spot was rather large (at least 400 m^2) and only one Hot Spot was located within the 30 × 72 m area we

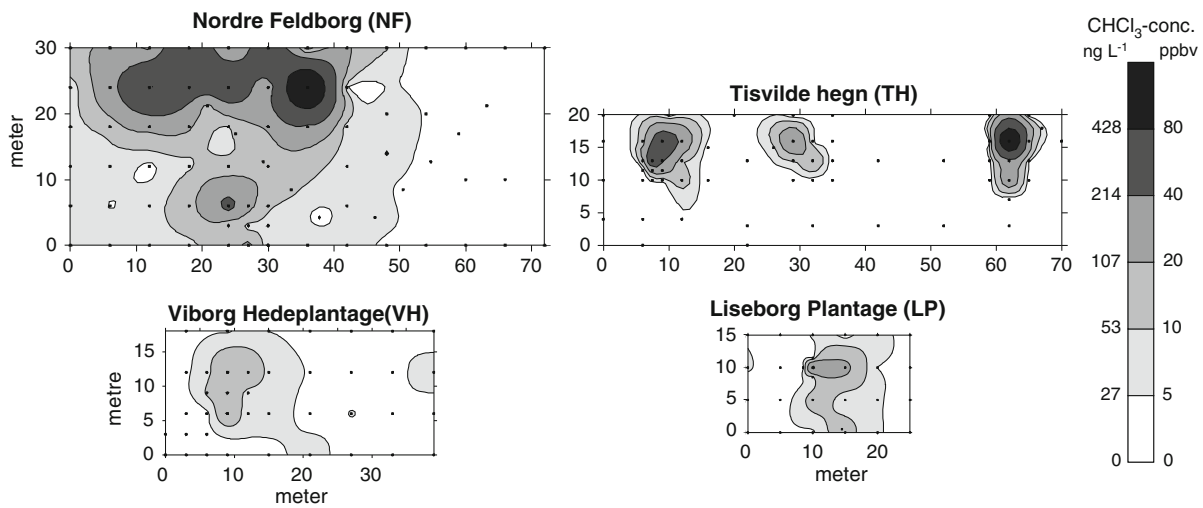

Fig. 3 Contour maps of Ordinary Kriging on chloroform concentrations (ppbv and ng L^{-1}) measured in soil air at 40 cm depth. Shaded colours show Hot Spots of chloroform with concentrations typically 100–1000 times the atmospheric background concentration. The white areas show Low Spots with typically ~10 times the atmospheric background concentration. The black dots show the location of the below ground air samples. The samples in TH were collected in May 2007. The samples in NF were collected in October 2007 and May 2008 (see Materials and methods). Samples in VH were collected in February and November 2008 and in LP in November 2009

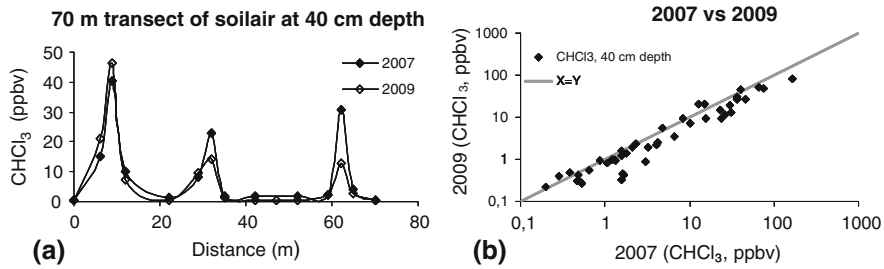

Fig. 4 **a** Transect in TH traversing the three Hot Spots. Soil air was sampled at 40 cm depth in May 2007 and May 2009. **b** Comparison of the 44 sampling points in TH that were sampled in both years. The 1:1 line is shown. Note the logarithmic scale on both axes

investigated. In TH, the Hot Spots were much smaller (25–50 m^2) and three Hot Spots were located within a 20 × 70 m area (Fig. 3).

In order to investigate whether the Hot Spot pattern was a general phenomenon in coniferous forests, two additional forests near Viborg, DK, were investigated for chloroform concentrations at 40 cm depth, and again an immense variation was found both between and within stands. Additional sampling around two sampling points having high chloroform concentration confirmed the presence of Hot Spots, having very narrow areas of distribution, similar to those in TH (Fig. 3). Although the chloroform concentrations in the Hot Spots were typically 20–100 times those in the corresponding Low Spots, the latter also showed increased chloroform concentrations compared to the atmospheric concentration (20 cm height), which was typically ∼0.06 ppbv, with a full recorded interval at all sampling dates of 0.034–0.18 ppbv. The lowest soil air concentration found was 0.22 ppbv but in general, chloroform concentrations in Low Spot soil air were between 0.4 and 1.0 ppbv.

A 70 m transect through the three Hot Spots in TH illustrates the huge variation within distances of just a few metres (Fig. 4a). Forty-four of the 60 sampling points in TH were marked during the first sampling in 2007 and these were re-sampled 2 years later showing very similar chloroform concentrations to what was found in 2007 (Fig. 4a, b).

Twenty fungal strains were isolated from the organic horizon of one of the Hot Spots in the TH study site. None of the strains produced chloroform when grown on homogenized O-horizon.

At 32 sampling points in TH, several soil parameters in addition to chloroform (CO_2 concentration in soil air, thickness of the organic horizon (varying from 3.5–20 cm) and furthermore pH (varying from 3.8–4.8), chloride (varying from 1.8–10.9 mg kg^{-1} soil) and total iron (varying from 0.12–3.5 mg kg^{-1} soil) in aqueous extracts of soil from 25 cm depth) were measured to try to explain the spatial variation in chloroform concentrations. As will be discussed later, these parameters could all be expected to influence the formation of chloroform. The correlations were very poor for all measured parameters, except for CO_2, where the Spearman′s rank correlation coefficient was significant ($p = 0.005$) although the R^2 of a simple regression was only 0.26. Performing multiple regressions with the different measured parameters as independent variables, did not result in any additional significant relationships.

Chloroform fluxes from soil

The higher concentrations of chloroform in the Hot Spots, in principle could be due to a relatively lower loss of chloroform through emission from the soil. In

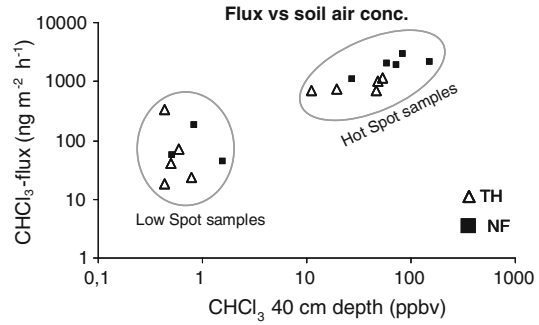

Fig. 5 Relationship between chloroform concentrations in the upper soil (40 cm depth) and chloroform fluxes from the exact same sample point in June 2009 in NF ($n = 8$) and TH ($n = 10$), respectively. Note the logarithmic scale on both axes

Table 1 Average (std. dev.) chloroform-fluxes in NF and TH and calculated emissions from the investigated forest areas, in which the distribution between Hot and Low Spots are known (estimated 40% Hot and 60% Low Spot in NF and 15% Hot and 85% Low Spot in TH)

	NF		TH	
	Low Spot	Hot Spot	Low Spot	Hot Spot
March 2009	16 (15)	214 (148)	40 (36)	86 (30)
Average forest soil emission	95		47	
June 2009	84 (59)	1998 (93)	98 (135)	864 (216)
Average forest soil emission	850		213	
August 2009			276 (437)	2676 (1584)
Average forest soil emission			636	

All values are in ng m^{-2} h^{-1}. $n = 5$. Values for each chamber in June and the exact location of the chambers can be seen in Supplementary Figs. S2 and S3

order to test this, flux measurements of chloroform were performed in both Hot and Low Spot areas in NF and TH. If the structural soil conditions are truly similar in Hot and Low Spots, some positive relationship would be expected between chloroform concentration in the upper soil and chloroform emission to the atmosphere, and this was indeed found to be the case at both sites (Fig. 5). The differences in emissions between Hot and Low Spot were slightly smaller in TH than in NF, especially in the winter measurement (Table 1).

The air samples collected during the flux-measurements in June (NF) or August (TH) were analyzed with respect to CO_2 and CH_3Cl in addition to chloroform. In NF, the chloroform flux showed no relationship to the flux of either CO_2 (Flux range = 39–150 mg m^{-2} h^{-1}) or CH_3Cl (Flux range = 104-4012 ng m^{-2} h^{-1}), with p = 0.79 and 0.99 for Spearman′s rank correlation, respectively. In TH the chloroform flux was slightly positively related to CO_2 (Flux range = 80–177 mg m^{-2} h^{-1}) with $p = 0.07$, while the CH_3Cl flux was too low to be determined.

The chloroform formed in the top soil is also subject to downwards movement towards the groundwater. A greater leaching and/or diffusion from the Low Spots could in principle cause the differences in chloroform concentrations between Hot and Low Spots. The shallow groundwater (0–50 cm below the groundwater table) did however show ~10 times higher chloroform concentration below a Hot Spot in TH than below the adjacent Low Spot, located 10 m away (Fig. 6).

Soil incubation study

Four Hot Spot and four Low Spot soil cores (0–10 cm depth sampled in TH (Fig. 7a)) were incubated for 5 weeks in the dark at 10°C to confirm that the

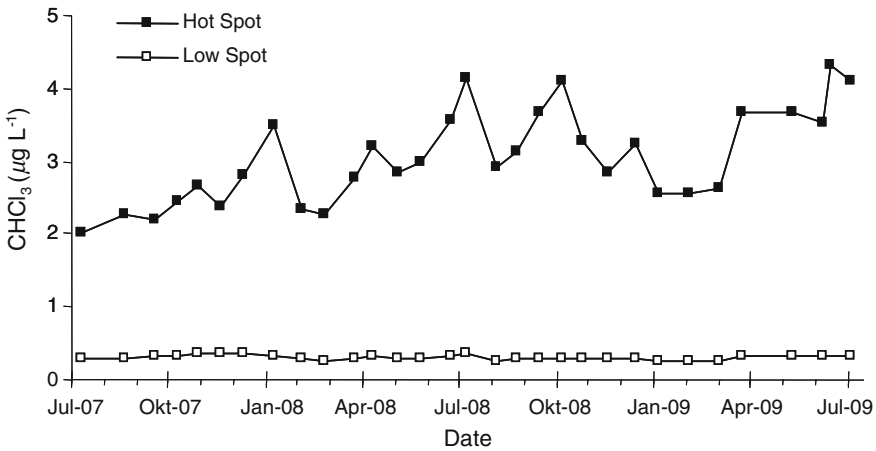

Fig. 6 Chloroform concentration in the shallow groundwater below a chloroform Hot Spot and the corresponding Low Spot in TH throughout 2 years. The distance between the two sampling wells is 10 m (see Supplementary Fig. S3 for exact location of sampling wells)

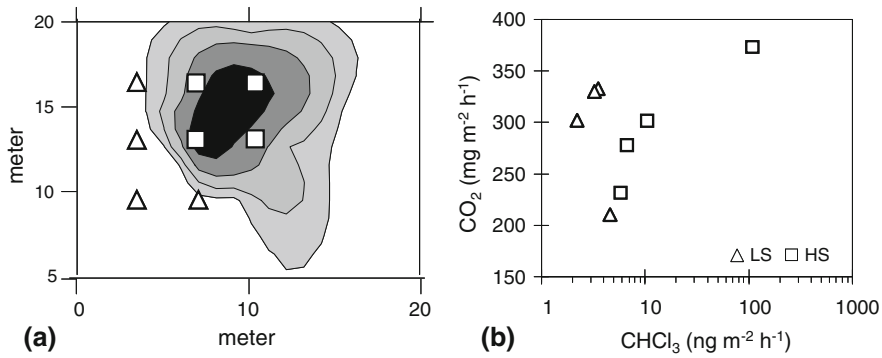

Fig. 7 a) Distribution of the eight 10 cm soil cores from TH used in the incubation study, four from the Low Spot (*triangles*) and four from one of the Hot Spot (*squares*) areas (Fig. 3). b) Results of the laboratory incubation study, presented as the average chloroform net production (ng m^{-2} h^{-1}) from day 6-34 (*first axis*). CO_2-production is also shown (*second axis*) from day 6-34. Note the semi-logarithmic scale

elevated concentrations and emissions found in the Hot Spot soil in vivo were due to a higher rate of chloroform net formation in the soil itself and not caused by e.g. input from vegetation or differences in leaching to deeper horizons. Differences in the net formation of more than one order of magnitude were found both within and between the Hot and Low Spot samples, but the net formation was in all four cases largest in the Hot Spot samples (Fig. 7b, first axis). No significant difference in CO_2-formation was observed between the Hot and Low Spot samples (Fig. 7b, second axis).

Degradation of chloroform

The higher concentrations, emissions and net formations of chloroform in the Hot Spots, point to a higher gross formation of chloroform in the Hot Spot soil. Degradation of chloroform in the soil cannot, however, be excluded to influence the distribution of Hot Spots and Low Spots and a few laboratory experiments using ^{14}C-labeled chloroform were performed to check if differences in aerobic degradation rates could be found. The results of these experiments did not show a larger mineralization of chloroform in the Low Spot, actually the opposite was seen with soil from the O-horizons and no difference was observable in the A-horizons (Table 2).

Total organic halogens

The F- and H-horizons of three soil columns from the Hot Spot and three soil columns from the Low Spot in NF were analyzed for total organic and inorganic chlorine, bromine and iodine to see if higher chloroform concentrations were related to a higher degree of halogenation of the soil organic matter (SOM) in general (Table 3). There seemed to be some trends in the halogen contents especially between soil layers, but differences between sites were minor.

The soil columns, from which the F- and H-horizons were isolated for total halogen analysis, also contained the top few cm of the A_h-horizon. This soil was analyzed for water-extractable chloroform, as described in the Materials and Methods section, in order to test, whether there was indeed a significant difference in chloroform concentration between those Hot and Low Spot soil samples, which were subsequently analyzed for total organic halogen. This test showed water-extractable chloroform concentrations

Table 2 Mineralization of added ^{14}C-chloroform in soils from NF Hot and Low Spot to ^{14}C-CO_2

Soil sample	O-horizon		A-horizon	
	Low Spot	Hot Spot	Low Spot	Hot Spot
Degradation rate, day 0–3	0.68 (0.67–0.70)	0.93 (0.92–0.93)	0.35 (0.32–0.37)	0.32 (0.31–0.32)

The degradation rate is given as average % per day (min. − max.) during the first 3 days of the experiment. n=2

Table 3 Results of the determination of total organic and inorganic halogens in samples from NF

	Hot Spot F-hor.	Low Spot F-hor.	Hot Spot H-hor.	Low Spot H-hor.
TOCl (SOM-basis)	580 (414–691)	560 (531–607)	623 (400–812)	533 (486–561)
TOCl (soil-basis)	469 (383–631)	515 (475–569)	347 (179–591)	176 (128–261)
TICl (soil-basis)	161 (50–217)	257 (242–285)	88 (37–185)	30 (4–61)
TOBr (SOM-basis)	78 (60–109)	63 (55–77)	95 (70–121)	93 (85–106)
TOBr (soil-basis)	61 (55–67)	57 (52–69)	48 (41–62)	29 (24–40)
TIBr (soil-basis)	BDL	BDL	BDL	BDL
TOI (SOM-basis)	23 (19–32)	16 (14–19)	24 (22–25)	25 (22–27)
TOI (soil-basis)	18 (17–20)	15 (13–17)	13 (8–22)	8 (6–11)
TII (soil-basis)	BDL	BDL	BDL	BDL

Three replicate soil samples were sampled for each soil type and each soil sample was analyzed in triplicates. Numbers in parenthesis are the range of the halogen content of the three independent soil samples. TO(X) = Total organically bound (chlorine/bromine/iodine). TI(X) = Total inorganic (chlorine/bromine/iodine). All values are in μg/g either on soil organic matter (SOM)-basis (TO(X)) or on soil basis (TO(X) and TI(X)). BDL means below the detection limit of ~2 μg/g

from 0.01–0.03 μg/kg in the Low Spot samples and 1.0–2.5 μg/kg in the Hot Spot samples, indicating that such a difference did exist.

Chlorination experiments

Another factor that may influence the formation of chloroform is the quality of the SOM regarding its chloroform formation potential. Attempts to characterize the organic matter from NF Hot and Low Spot were carried out, but elemental analyses of C, H and N-contents and solid state ^{13}C-NMR spectra showed no differences between Hot and Low Spot samples (results not shown). To detect differences in chloroform precursors, a chlorination study with the same 12 soil samples, which were used for total halogen determination, was performed (Fig. 8).

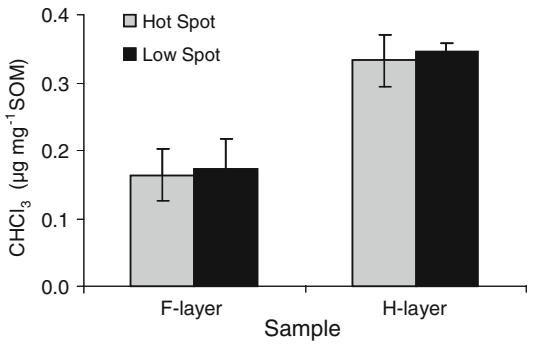

Fig. 8 Results from chlorination study with freeze dried and powdered F- and H-horizon soil samples from NF Hot and Low Spots. Error bars are the standard deviation of three replicates. Chlorination conditions: Soil = 1 g/L. $[OCl^-]$ = 0.5 mM. T = 10°C. pH 4.0. Reaction time = 24 h

Discussion

Chloroform in soil air

Our first surveys were done in order to see if the chloroform concentrations were different between areas with visible differences in vegetation type, density of vegetation, age of stands, light penetration through the canopy and moss coverage on the forest floor. Variations in chloroform concentrations in the soil air of almost two orders of magnitude were found in these surveys but surprisingly, similar variations were found within single stands with no visible differences. More detailed investigations revealed that the distribution of sampling points showing high concentrations of chloroform was not completely random, and the forest floor seemed to be divided into Hot Spots and Low Spots regarding the concentration of chloroform. When we recognized this pattern, systematic samplings with distances between sampling points as low as 1.5 m were performed, to demarcate the Hot Spots. The sizes of the Hot Spots varied depending on study site, but in TH and probably also in VH and LP, the Hot Spots seemed quite small, with areas from 25–50 m^2. The size and distribution of these Hot Spots were unchanged during a 2-year period. In NF, the Hot Spot was too large to be completely demarcated in this study, but

had an area of at least 400 m^2. Careful visual inspections were performed to note any differences in vegetation, understory, moss coverage etc., but the Hot Spots and Low Spots were indistinguishable by eye.

Chloroform concentrations in the soils of temperate coniferous forests have previously been investigated, and Hoekstra et al. (1998a) did a somewhat dense sampling campaign, with 16 sampling points within a 60 × 180 m area in a Dutch Douglas Fir forest. The campaign revealed spatial variation in chloroform concentration of ~one order of magnitude, with almost the full range of variation observed within 20 m, which was the distance separating the closest located sampling points. This could indicate that chloroform Hot Spots are also present in that forest. Similarly, the great variations in chloroform concentrations and fluxes reported by various authors are easier to interpret, if the occurrences of Hot Spots and Low Spots are widespread in forests in general.

Fluxes of chloroform

Emissions to the atmosphere

The emissions of chloroform from the forest floor were measured in Hot and Low Spots in NF and TH (Table 1). The fluxes of chloroform from the soil to the atmosphere were much higher during summer than during winter and in TH, the fluxes were much higher after a wet period of the summer (August; soil temperature ~14°C, soil moisture ~0.041 vol/vol both measured at 15 cm depth) than after a long dry period of the summer (June; soil temperature ~11°C, soil moisture ~0.010 vol/vol). For all dates measured, the fluxes of chloroform were much larger from the Hot Spots, as would be expected when the soil concentrations are much larger. This rules out the possibility that the increased concentrations in the Hot Spots are caused by larger emissions from the Low Spots.

Literature data on natural chloroform emissions from soil are rather scarce but in three previous studies, such emissions have been measured in the field. Hoekstra et al. (2001) reported average chloroform emissions of 10–110 ng m^{-2} h^{-1} from Dutch temperate forest soils with various vegetations (sampled in April), Hellén et al. (2006) reported average chloroform emissions of 100–800 ng m^{-2} h^{-1} in a Finnish boreal pine forest (sampled from April to June) and finally Dimmer et al. (2001) reported average chloroform emissions of 251 ng m^{-2} h^{-1} in a temperate Irish pine forest and ~16650 ng m^{-2} h^{-1} in a temperate Irish spruce forest (both sampled in September). Furthermore, Haselmann et al. (2000) calculated chloroform emissions of 3–160 ng m^{-2} h^{-1} from concentration gradients in soils of a temperate Danish coniferous forest. Except for the exceptionally high chloroform emission from the Irish spruce forest, our results seem comparable with the data presented in the literature. Our data show that the spatial variation in emissions is huge but also that it is somewhat systematic, which together with an apparent temporal dependence on temperature and moisture may point to the influence of yet unidentified microorganisms with well demarcated distributions in the soil. Variation in microbial activity was also mentioned by Hoekstra et al. (2001) as a possible explanation of the spatial variation in chloroform emission that they reported and patchiness in the distribution of litter degrading fungi is well known in forest soil (e.g. Osono 2007; Snajdr et al. 2008).

Chloroform and methyl chloride emissions showed no relationship as also reported earlier for forest and shrubland soil (Dimmer et al. 2001; Rhew et al. 2008). This was expected since the presumed modes of formation are very different, with methyl halides being formed either by direct bio-synthesis in fungi (Harper et al. 1988; Field et al. 1997) or by abiotic nucleophilic substitution of methoxy groups in pectin or lignin (Hamilton et al. 2003; Keppler et al. 2005).

As indicated by the large standard deviations in the flux experiment (Table 1), the spatial variation in chloroform emissions from soil to the atmosphere within both Hot and Low Spots is huge, and one should therefore be careful to calculate average forest emissions based on only 5 Hot and 5 Low Spot samples. The calculated average forest soil emissions in Table 1 nevertheless can give some idea of the average chloroform emission from the forest soil.

Leaching to the groundwater

The higher chloroform concentration in the Hot Spots could in principle be due to a lower leaching and/or diffusion of the chloroform towards the groundwater. As indicated earlier (Albers et al. 2008), the variation in chloroform concentration in top soil is reflected

throughout the unsaturated zone. Monitoring of the groundwater just below the groundwater table throughout 2 years showed that the concentration below the Hot Spot varied from 2.0–4.3 μg/L and below the Low Spot from 0.2–0.4 μg/L (Fig. 6). This strongly indicates that the transport of chloroform towards the groundwater is higher from the Hot Spot. Spatial variations in presumably natural chloroform concentrations in groundwater have been observed previously (Laturnus et al. 2000; Albers et al. 2008; Albers et al., Submitted), and the existence and distributions of chloroform Hot Spots in soil, that we present in this paper, may help to explain why large variations in natural chloroform in the groundwater will exist.

Formation and mineralization of chloroform

The soil incubation study with soil from TH confirmed a higher net formation of chloroform in the Hot Spot than in the Low Spot, but with huge variations between samples. Except for one sample, the Hot Spot samples showed much lower net formation than what would be expected from the flux measurements performed in the field. It is possible though, that even within a Hot Spot the variation is great when sampling only 28 cm^2 of soil as we did. Preliminary investigations actually showed that the chloroform concentration in the top organic horizon could vary by a factor of more than 10 within a distance of just a few decimetres (results not shown).

We wanted to check if differences in chloroform degradation rates influenced the distribution of Hot and a Low Spots. The study sites are all characterized by well oxygenated sandy soils (Albers et al., Submitted), and even though anaerobic niches might theoretically occur in the organic top soil after heavy rain events, any degradation that could cause the permanent difference between Hot and Low Spots would be expected to be aerobic. Abiotic degradation of chloroform is known in anoxic sediments (Kenneke and Weber 2003), but no abiotic aerobic pathway for the degradation of chloroform is known and the only known aerobic degradation pathway for chloroform is co-metabolic degradation by various oxygenase-expressing bacteria (Bartnicki and Castro 1994; Alvarez-Cohen et al. 1992; Chang and Alvarez-Cohen 1996). This degradation pathway leads to complete mineralization of the chloroform molecule and we therefore performed a mineralization experiment. The experiment revealed that a small mineralization of chloroform takes place in the forest soil, but more important here, the mineralization was not higher in the Low Spot than in the corresponding Hot Spot (Table 2). Mineralization can therefore not explain the differences in chloroform concentrations and since other (partial) degradation apart from mineralization is unlikely, higher degradation in the Low Spots seems not to explain the presence of the Hot Spots. In the literature, not much can be found on aerobic degradation of chloroform in natural soils, but our results of 0.3–0.9% mineralization d^{-1} are well in line with the only previously published data, where 3% of the added chloroform was converted to CO_2 within 5 days in a sandy loam, corresponding to 0.6% mineralization d^{-1} (Strand and Shippert 1986).

Soil characteristics

The various measurements of concentration, flux, formation and degradation of chloroform all pointed in the direction that the Hot Spots were the result of a higher gross formation of chloroform in the Hot Spot soil. Since the differences between Hot Spots and Low Spots could not be accounted for by visual observations in the field, measurements of various soil parameters were performed in order to narrow down the factors controlling the increased formation of chloroform in certain areas. An overview of these measurements is given in Table 4, together with a brief conclusion regarding the importance of each parameter. The bases of these conclusions are further discussed hereafter.

General microbial activity (CO_2)

CO_2 was measured in the soil air samples as well as in the flux chambers as an estimate of the general microbiological activity in the soil. It was the only parameter that showed a statistically significant relationship with the chloroform concentration. This positive relationship is pretty poor, though, and furthermore the incubation study with soil from TH showed no differences in CO_2-production between Hot and Low Spot soil. In NF, no relationship between chloroform and CO_2 seemed to occur, at all. The general microbial activity is therefore not the

Table 4 Summary with full ranges of all parameters measured in order to gain insight into the factors controlling the highly elevated chloroform concentration in the Hot Spots

Parameter	Location and sample type	*n*	Hot Spot	Low Spot	Conclusion
Identification of the $CHCl_3$ Hot Spots	All sites: Soil air concentrations. NF and TH: Fluxes to the atmosphere and groundwater, formation and degradation of $CHCl_3$ in soil in the laboratory.		High concentrations of $CHCl_3$ in soil air and high fluxes to the atmosphere and groundwater. High formation but low degradation of $CHCl_3$ in laboratory.	Much lower, but still increased conc. in soil air. Small positive fluxes to the atmosphere and groundwater. Low formation and degrad. of $CHCl_3$.	Higher gross and net formation of chloroform in Hot Spots, but also a small formation in the Low Spots.
O-horizon	TH: Thickness F+H-horizon	32	3.5–20 cm	5–14 cm	No difference/significance
CO_2	TH: Soil air	41	0.11–0.28%	0.11–0.22%	No difference/significance in NF. Slightly higher in TH Hot Spot?
	TH: Soil emission (August)	10	80–181 mg m^{-2} h^{-1}	81–148 mg m^{-2} h^{-1}	
	NF: Soil emission (June)	10	39–94 mg m^{-2} h^{-1}	50–125 mg m^{-2} h^{-1}	
	TH: Incubation of soil	8	231–373 mg m^{-2} h^{-1}	210–333 mg m^{-2} h^{-1}	
CH_3Cl	NF: Soil emission (June)	10	104–2400 ng m^{-2} h^{-1}	580–4000 ng m^{-2} h^{-1}	No difference/significance
pH	TH: pH_{H2O} in O-horizon	10	3.9–4.2	4.1–4.6	No difference/significance
	TH: pH_{H2O} in B-horizon	32	3.8–4.6	3.9–4.8	
	NF: pH_{H2O} in O-horizon	8	3.6–4.0	3.8–4.0	
Cl^-	TH: H_2O-extr. Cl^- in B-hor.	32	3.9–9.0 mg kg^{-1} soil	2.1–10.9 mg kg^{-1} soil	No systematic variation/ difference
	NF: Cl^- in F-horizon	6	50–217 mg kg^{-1} soil	242–285 mg kg^{-1} soil	
	NF: Cl^- in H-horizon	6	37–185 mg kg^{-1} soil	4–61 mg kg^{-1} soil	
Fe	TH: H_2O-extr. Fe in B-hor.	32	0.12–3.5 mg kg^{-1} soil	0.23–3.5 mg kg^{-1} soil	No difference/significance
Chlorination degree of SOM	NF: TOCl in F-horizon	6	0.41–0.69 g kg^{-1}	0.53–0.61 g kg^{-1}	Slightly higher in Hot Spot H-horizon?
	NF: TOCl in H-horizon	6	0.40–0.81 g kg^{-1}	0.49–0.56 g kg^{-1}	
Quality of SOM (chem. chlorination)	NF: $CHCl_3$ formed, F-horiz.	6	0.14–0.19 g kg^{-1}	0.13–0.22 g kg^{-1}	No difference between Hot Spots and Low Spots
	NF: $CHCl_3$ formed H-horiz.	6	0.31–0.36 g kg^{-1}	0.33–0.36 g kg^{-1}	

See the Materials and Methods section for further information on the various sample types. *n* is the total number of samples, in all cases distributed nearly or exactly identically between Hot Spots and Low Spots. The text should be consulted for further details leading to the brief conclusion concerning each parameter

single important parameter with regards to chloroform concentration in the soil.

General soil parameters

The thickness of the organic horizon and of individual organic horizons was measured since this is where the main chlorinating activity including chloroform formation has been found to occur in forest soil (Asplund et al. 1993; Hoekstra et al. 1998a; Haselmann et al. 2000; Albers et al., Submitted). The total thickness of the organic horizon showed a substantial variation (3.5–20 cm), but no difference in thickness or type of organic horizon was found between the Hot Spots and the Low Spots.

pH was measured, since both haloperoxidase enzymes and the natural unspecific chlorination processes in soil have been shown to be pH-dependent (Asplund et al. 1993; Vollenbroek et al. 1995; Öberg et al. 1996; Sheng and Gold 1997) and the same goes for abiotic formation (Huber et al. 2009). The pH was acidic at all sites and at all sampling points, but again, no difference could be observed between Hot and Low Spots.

Chloride is a substrate in the formation of chloroform but despite a large variation in the dataset, no systematic variation towards neither higher nor lower concentrations in the Hot Spots was found.

Water extractable iron was measured in the top soil, since an abiotic formation pathway of chloroform, which includes Fe(III) in the reaction, has recently been demonstrated in the laboratory (Huber et al. 2009). Other approaches for measuring iron (e.g. extraction with oxalate) could have been taken and the water-extractable iron is only a minor portion of the total iron. When the different soil samples are very similar regarding soil texture and pH as in our case, the approach gives an idea, though, whether or not iron could be an important parameter for the occurrence of chloroform Hot Spots and in this case it seemed not to be so. One possible hypothesis could be, however, that a sort of "background" abiotic formation of chloroform occurs in both Hot and Low Spots. This formation could then explain the concentration of $\sim$5 times the atmospheric background in the Low Spot areas, while microorganisms are responsible of the differences between the Hot and the Low Spots. This hypothesis would be interesting to test in future studies.

Total organic halogens

If chloroform formation is a result of unspecific chlorination caused by chlorinating enzymes (see e.g. Hoekstra et al. (1998a)), one might expect that the chloroform Hot Spots were the results of a general increase in chlorinating activity and that this increased activity was also reflected in a higher total organic chlorine concentration in the soil. Whereas for the F-horizons no difference was seen between Hot and Low Spot, on average 2 times as much TOCl was present in the Hot Spot H-horizons compared to the Low Spot H-horizons (Table 3). This difference is diminished, though, when the TOCl-concentration is recalculated on a SOM-basis, meaning that the difference in the degree of chlorination of the organic matter is not very large between Hot and Low Spot. The sample with the highest chloroform-concentration in the A_h-horizon (2.5 μg/kg soil) was actually the sample that showed the highest amount of TOCl in the H-horizon (812 μg/g SOM), but to finally conclude on this relationship, it would take a larger number of samples to be analyzed, since the variation in especially the Hot Spot is large. It should be mentioned here also, that in addition to the unspecific halogenation mechanism, TOCl is also suggested to be formed by incorporation of demethylated fungal chlorinated anisyl metabolites (CAMs) into SOM (Öberg et al. 1997; Hjelm et al. 1999). Since CAMs are formed in a specific intracellular halogenation reaction, this formation pathway for TOCl will not lead to the formation of chloroform and will therefore blur the relationship between TOCl and chloroform in soil. A more specific assay for determining unspecific chlorinating activity in the soils will probably be necessary to see if differences between Hot and Low Spots are caused by differences in unspecific chlorinating activity. Previous attempts to determine chlorinating activity in soil have been based on the extraction of chlorinating enzymes by phosphate-buffered salt solutions (Asplund et al. 1993; Laturnus et al. 1995). This is however not a very quantitative approach and the development of a new assay e.g. based on dilution of whole soil (Johnsen and Jacobsen 2008) would be necessary in order to make a quantitative comparison of chlorinating activity in Hot and Low Spots.

Bromine and iodine were almost exclusively present as organic halogen and in all cases too little

inorganic bromine and iodine was present to be detected. This is well in line with previously published data on bromine and iodine speciation in soil (Maw and Kempton 1982) and probably reflects the preference of the halogenating enzymes for bromine and iodine, which both have a lower oxidation potential than chlorine (Sheng and Gold 1997). Comparing with the literature, our findings of $\sim$600 μg TOCl g^{-1} SOM and an additional $\sim$100 μg TOBr + TOI g^{-1} SOM falls within the values of 100–1000 μg TOCl g^{-1} SOM found for O-horizons of temperate coniferous forests (Asplund et al. 1989; Hjelm et al. 1995; Johansson et al. 2001). How much of the TOCl reported in these studies actually being Br and I is unknown, since the TOCl was estimated from the group parameter TOX. Our results show that a significant pool of organic Br and I exists in the soil, and future studies should preferentially determine not just TOX, but rather the individual halogens, if possible.

Chemical chlorination

It is well known from the literature on chlorination of drinking water that the amount of chloroform formed upon chlorination of organic matter varies not only with the quantity but also the quality of the organic matter (e.g. Reckhow et al. 1990). Since no difference in soil or vegetation was visible between Hot and Low Spots, no major differences in chloroform precursors and hence chloroform formation potentials between Hot and Low Spot soil were expected, and the chlorination study confirmed this, since a difference between F- and H-horizon samples, but not between sites, was found.

Concluding remarks

In conclusion, we have systematically shown a huge spatial variation in chloroform concentration of more than two orders of magnitude within single stands in soils of temperate coniferous forests. Sample points with high chloroform concentrations are located close to each other in areas of varying sizes, with sharp borders towards areas with concentrations only 2–10 times higher than the atmospheric background. Chloroform concentration, emission, leaching and formation are all highest within these Hot Spots. This is important to bear in mind, both when interpreting the various published studies on natural chloroform in forests but also when designing future experiments. Furthermore our results stress that one should be very careful to use small datasets for calculating natural chloroform emissions to the atmosphere on a global, regional or even local scale.

Regarding the mechanism behind the formation of chloroform, we eliminated a number of causal relations between the chloroform Hot Spots and various parameters, potentially important for the formation of these Hot Spots. The spatial pattern, in addition to the positive influence of both soil temperature and soil moisture, supports the hypothesis that microorganisms are likely to be involved in the formation. On the other hand more than one mode of formation might co-exist. One possibility is that abiotic formation causes a background concentration as seen in the Low Spots. Fungal or other microbial activity could then explain the highly elevated concentrations in the Hot Spots. To fully conclude on this, further studies are needed. These should preferably manage to quantify chlorinating enzymes and/or chlorination potential of soils, more specifically than analyzing the TOCl contents of the soils or, if possible, to identify the responsible microbial species.

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