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Predominance of Biotic over Abiotic Formation of Halogenated Hydrocarbons in Hypersaline Sediments in Western Australia

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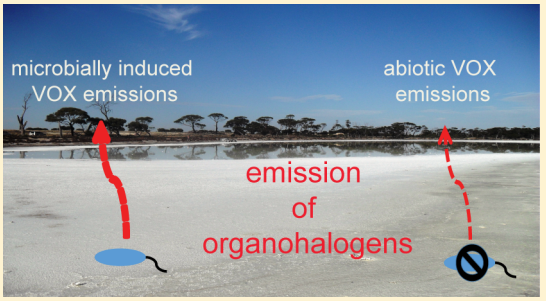
Supporting Information

ABSTRACT: Volatile halogenated organic compounds (VOX) contribute to ozone depletion and global warming. There is evidence of natural VOX formation in many environments ranging from forest soils to salt lakes. Laboratory studies have suggested that VOX formation can be chemically stimulated by reactive Fe species while field studies have provided evidence for direct biological (enzymatic) VOX formation. However, the relative contribution of abiotic and biotic processes to global VOX budgets is still unclear. The goals of this study were to quantify VOX release from sediments from a hypersaline lake in Western Australia (Lake Strawbridge) and to distinguish between the relative contributions of biotic and abiotic VOX formation in microbially active and sterilized microcosms. Our experiments demonstrated that the release of organochlorines from Lake Strawbridge sediments was mainly biotic. Among the organochlorines detected were monochlorinated, e.g., chloromethane (CH_3Cl), and higher chlorinated VOX compounds such as trichloromethane ($CHCl_3$). Amendment of sediments with either Fe(III) oxyhydroxide (ferrihydrite) or a mixture of lactate/acetate or both ferrihydrite and lactate/acetate did not stimulate VOX formation. This suggests that although microbial Fe(III) reduction took place, there was no stimulation of VOX formation via Fe redox transformations or the formation of reactive Fe species under our experimental conditions.



INTRODUCTION

Stratospheric ozone-degrading volatile organohalogen compounds (VOX) were for a long time considered as exclusively of anthropogenic origin. Since the beginning of the 1970s, natural sources of fluorinated, iodinated, brominated, and especially chlorinated hydrocarbons were explored.[1,2] Chloromethane (CH_3Cl) is the most abundant chlorinated hydrocarbon in the atmosphere with an estimated annual, global flux of about 2.8 Tg.[3] Due to their ozone-degrading properties the use of many volatile organohalogen compounds was strictly regulated by the 1987s' Montreal Protocol. Thereafter, natural sources of VOX started to gain attention and to date there is evidence of more than 5000 naturally produced halogenated hydrocarbons.[4] Natural sources include volcanoes, biomass burning, marine algae, plants, soils, and sediments, as well as microorganisms, and higher organized eukaryotes such as insects.[4]

The main natural abiotic sources of VOX in the atmosphere are volcanoes and biomass burning.[5,6] More than one hundred different organohalogen compounds were identified in fumaroles and lava gases of volcanoes with highest concentrations for chlorinated methanes, chloroethene, and chlorobenzene, reaching values of up to 100 ppb.[6] In 2001, annual fluxes of chloromethane from biomass burning and emissions in the atmosphere from different vegetation types were analyzed. A total flux of 0.9 Tg $year^{-1}$ was estimated for CH_3Cl to originate from biomass burning[3] suggesting that biomass burning is the main source of natural atmospheric chloromethane fluxes. The highest release was estimated for savannas with a global emission of 0.24 Tg $year^{-1}$.[5] Among natural biotic sources of VOX, the major contributors are marine algae, terrestrial fungi, and plants.[7−9] They use halogenating enzymes such as methyltransferases, haloperoxidases, and $FADH_2$-dependent halogenases for direct biotic organohalogen formation.[9−12] Knowledge on the different groups of halogenating enzymes was reviewed in 2009.[13]

In particular, soils and lake sediments play an important role in natural VOX emissions.[14−18] The exact mechanisms as well as the contribution of abiotic and biotic processes to total VOX emissions from soils and sediments are still largely unclear. For a temperate forest soil, the abiotic formation of methyl halides has been demonstrated.[19] For this system, it has been suggested that in the presence of Fe(III) and a halide ion, organic material is oxidatively degraded while the Fe(III) is reduced. In a second step, the oxidized organic compound is halogenated. In 2002, it was shown that H_2O_2 as an oxidizing agent increased the methyl halide release by more than 100%.[15] The authors suggested that a "Fenton-like" reaction might be involved and highlighted the importance of hydroxyl radicals for methyl

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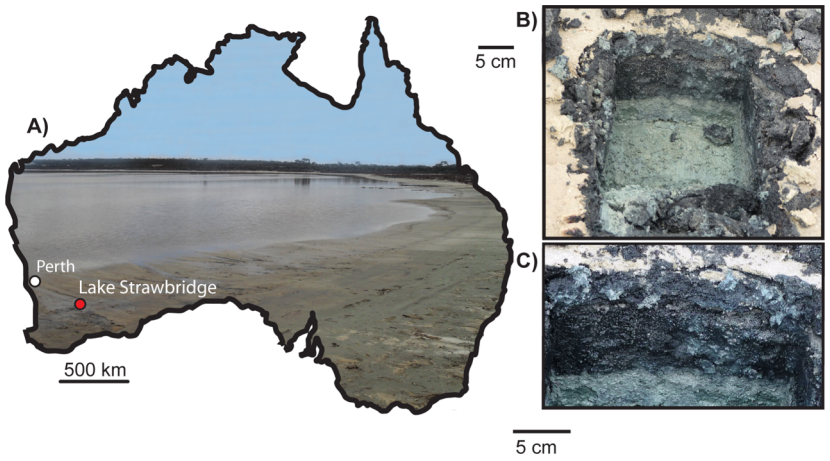

Figure 1. Location of the sampling site, Lake Strawbridge, in South Western Australia, and its sediments. (A) A map of Australia showing the sampling site Lake Strawbridge (red circle) and its proximity to Perth (white circle). Inset photograph shows Lake Strawbridge. (B) Sediments from Lake Strawbridge. (C) Vertical sediment profile from Lake Strawbridge.

halide formation in soils. The importance of redox reactions between iron and soil organic matter in the presence of halide ions for the formation of VOX and its precursors was further supported by additional studies.[20−22] Besides this abiotic formation of halogenated hydrocarbons via reactive Fe-species and hydroxyl radical initiated transformation of natural organic matter, the direct biological formation of VOX in soils and sediments via halogenating enzymes plays an important role.[13]

Compared to soils, where some mechanisms for VOX formation have been postulated, the knowledge on processes leading to natural VOX formation in salt lakes and their sediments is very scarce. This is surprising as salt concentrations up to the solubility limit of, e.g., NaCl found in hypersaline environments might promote high rates of organic matter halogenation.[23,24] The contribution of coastal salt marshes to atmospheric VOX fluxes has been demonstrated.[25] It was postulated that up to 10% of the atmospheric fluxes of chloromethane and bromomethane might originate from coastal salt marches.[25] The importance of salt marshes in the global VOX budgets was confirmed later.[26] In 2005, hypersaline lakes were suggested as a source of volatile chlorinated C_1 and C_2 hydrocarbons.[18] The authors quantified a release of trichloromethane, tetrachloromethane, and tetrachloroethene in the ng g^{-1} sediment range from pH neutral hypersaline lakes in Southern Russia. The highest rates of release were found for lakes with a high activity of halophilic microorganisms in combination with catalytic amounts of dissolved iron. This is in line with studies from Keppler and colleagues, who also showed the importance of iron for VOX formation, although in a purely abiotic process.[15,19] Whether the formation of VOX can be stimulated either by Fe(II)-oxidizing and Fe(III)-reducing bacteria producing reactive Fe-species or by microorganisms that use natural organic matter (NOM) as electron acceptor and form NOM radicals, is currently unknown. We hypothesized that microorganisms might stimulate abiotic organochlorine emissions by producing reactive Fe-species and NOM radicals which might contribute to organochlorine emissions via a "Fenton-like" reaction.

Recently, the importance of saline environments as a source for VOX has been shown on a broader scale by combining microcosm experiments with remote sensing, thus estimating emission rates for an area of 15 000 km^2 near the southern Aral Sea basin.[27] However, there are still large uncertainties within the global chloromethane budgets and especially the contribution of biotic versus abiotic reactions is poorly understood.[3] The goals of this study were therefore: (I) to quantify and identify the emission of VOX from a hypersaline lake sediment in Western Australia; (II) to elucidate the contribution of biotic and abiotic processes to VOX emissions; and (III) to define the role of microbial Fe-redox transformations on VOX emissions from salt lake sediments in microcosm experiments.

MATERIALS AND METHODS

Field Site and Sampling Procedure. Sediments were sampled from Lake Strawbridge (32.84°S, 119.40°E; WGS 84), a hypersaline lake in Western Australia. The sampling site was located at the northern shoreline of the lake (Figure 1). Three different sediment horizons could be distinguished visually, a whitish salt layer (0−1 cm depth), a blackish layer (1−10 cm depth), and a brighter greyish layer (>10 cm depth). Sediment cores (30 cm length) were taken in April 2013 by pushing a polypropylene tube (4.5 cm diameter) gently into the soft sediment. Before pulling the tube from the sediment, the top was closed with a butyl rubber stopper. The bottom of the tube was immediately closed with a rubber stopper after pulling the core from the sediment. Additionally, bulk material from the three layers was sampled for microcosm experiments. All samples were transported at 8 °C to the laboratory.

Setup of Sediment Microcosm Experiments. Microcosm experiments for quantification of VOX release were set up as triplicates in 20 mL GC vials, filled with 3.5 g wet sediment and 8.5 mL of incubation solution (NaCl solution of varying concentrations, corresponding to the different pore water salt concentrations measured in the field) and closed with PTFE (Polytetrafluoroethylene)-silicone septa. Sediment microcosms were prepared and incubated under oxic conditions for 60 min at 30 °C in the dark on a horizontal shaker at 25 rpm. Different experimental setups were chosen in order to elucidate the effect of (I) 5% (w/v) ferrihydrite,[28] (II) 100 μL of a stock solution containing 500 mM of each sodium lactate and acetate, (III) ferrihydrite + sodium lactate/acetate, and (IV) sterilized setups on VOX release from the microcosms. Sterilization was achieved either by the addition of NaN_3 (150 mM final concentration) or by autoclaving (twice at 121 °C for 30 min). VOX were quantified in the headspace using a Trace GC Ultra

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coupled to a DSQ II single quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, U.S.A.) at the University of Duisburg-Essen. Right after the GC measurements, samples were shaken and 500 μL of the sediment suspension of each microcosm were taken for the determination of Fe(II) and Fe(tot). In order to prevent reoxidation of Fe(II), the samples were immediately stabilized in 1.5 mL of 1 M HCl and stored at 5 °C until analysis via the spectrophotometric Ferrozine assay.[29,30] Before the analysis of Fe(II) and Fe(III), the acidified samples were centrifuged for 5 min at 20 230*g*. As the supernatant was not filtered, it might contain small colloidal particles potentially also containing some iron. After each sampling, the amount of incubation solution in the microcosms was readjusted and the headspace was exchanged with synthetic air for 90 s[31] before the vials were incubated again for 24 or 72 h.

Analytical Methods and Data Analysis. The sediment water content was determined by weighing wet sediment from the three different layers and subsequent drying at 105 °C until weight stability. After cooling to room temperature in a desiccator, the samples were weighed again to determine the dry weight. For pH measurements, 10 g of air-dried material were suspended in 25 mL of a 0.01 M $CaCl_2$ solution and pH was measured immediately, and after 2 h. Dried sediment samples were milled to a fine powder and the elemental composition of the different sediment horizons was analyzed by X-ray fluorescence (XRF). The total organic carbon (TOC) was determined in triplicates from ground sediment samples that were dried at 60 °C until weight stability using an Elementar Vario EL element analyzer. Ion chromatography (IC) and analyses of leachable organic carbon were performed with artificial pore water, modified after Emmerich et al.[32] One gram of dried, ground sediment sample was suspended in 20 mL of deionized H_20 in a 50 mL centrifuge tube and shaken on a horizontal shaker for 24 h at 150 rpm. Afterward, samples were centrifuged for 5 min at 3750*g*, and the supernatant was diluted 1:20 in deionized H_2O before filtration through a cellulose ester filter with a pore size of 0.45 μm. Major ions were quantified by ion chromatography. Leachable organic carbon was analyzed in duplicates using a High TOC Elementar instrument.

Concentrations of Fe(III) and Fe(II) in the sediments were analyzed in duplicates using sequential extraction,[30] and subsequent analysis of dissolved iron species using the spectrophotometric Ferrozine assay.[29] Concentrations of Fe(II) and Fe(total) were calculated from absorption values according to a standard calibration curve. The same was done for the headspace concentrations of CH_3Cl and $CHCl_3$. Student's *t* test was used to determine significant difference between the different experimental setups.

GC-MS Instrumental Parameters and ITEX Method. All measurements were performed using a Trace GC Ultra coupled to a DSQ II single quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, U.S.A.). A detailed description of the "in tube extraction method" (ITEX) and the GC-MS can be found in the Supporting Information (SI) and has been published before.[33]

Reagents and Standard Preparation. When not stated differently, all standards and solutions were set up with water from an analytical water purification system (Merck Millipore). EPA 624 standard calibration mix (100 μg mL^{-1} of each component in methanol) as well as trichloromethane and chloromethane were purchased from Sigma-Aldrich. Standards were prepared with a volume of 10 mL in incubation solution and final concentrations ranging from 1 to 1000 ng L^{-1}. Ammonium Fe(II) sulfate standards for the Ferrozine assay and hydroxylamine hydrochloride solution were prepared in 1 M HCl.

RESULTS

Sediment Characteristics and Geochemical Properties. Lake Strawbridge is a hypersaline, pH-neutral to slightly alkaline lake in Western Australia (Figure 1). Three different sediment layers could be distinguished visually. The salt layer (0−1 cm depth), followed by a blackish layer from 1 to 10 cm and a brighter greyish layer >10 cm. Table 1 gives an overview about the geochemical properties. The pH values decreased slightly with increasing depth and ranged from 8.3 in the salt layer to 8.1 in the layer >10 cm. The relative amount of Fe in % of the dry weight sediment determined by XRF was lowest in the top cm of the profile (0.3 weight%) and increased to 2.1 weight% in the layer >10 cm (Table 1). Acidic extraction confirmed the XRF data and showed that Fe concentrations increased with increasing sediment depth (Figure 2).

Table 1. Geochemical Characterization of Lake Strawbridge Sediments

sediment depth [cm]	leachable organic carbon [mg/L][a]	pH[b]	water content [%][c]	total Fe [%][d]	Cl [mM][e]	C_{org} [%][f]
0−1	2.2 ± 0.0	8.3	16.3	0.3	3234	0.1 ± 0.0
1−10	5.9 ± 0.1	8.2	23.8	0.9	2029	0.9 ± 0.1
>10	4.0 ± 0.0	8.1	21.1	2.1	816	0.1 ± 0.0

[a]Quantified in a sediment eluate by a High TOC Elementar Instrument (modified from Emmerich et al. 2012). [b]Measured in 0.01 M $CaCl_2$ after 2 h. [c]Dried at 105 °C until weight stability. [d][Weight % of dry sediment] quantified by XRF. [e]Quantified in a sediment eluate by ion chromatography. [f]Weight % quantified by a C/N analyzer using a HCl-titrated sample (modified from Emmerich et al. 2012). The standard deviations of duplicate (leachable organic carbon) and triplicate (C_{org}) measurements are given.

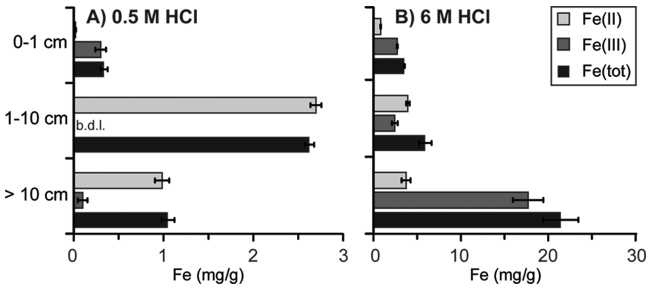

Figure 2. Concentrations of different Fe fractions in a sediment profile of Lake Strawbridge in mg/g dry weight sediment. (A) 0.5 M HCl extractable ("bioavailable" Fe). (B) 6 M HCl extractable ("crystalline" Fe). Error bars give standard deviations from duplicate measurements, b.d.l. = below detection limit.

Total iron concentrations differed considerably when comparing extractions with 0.5 and 6 M HCl. In the zone >10 cm, the 6 M HCl-extractable Fe was 20 times higher than the Fe extractable with 0.5 M HCl. Fe redox speciation was very similar in the salt layer for both 0.5 and 6 M HCl and showed high concentrations of oxidized iron, i.e., Fe(III), and low concentrations of reduced iron, i.e., Fe(II) (Figure 2). In

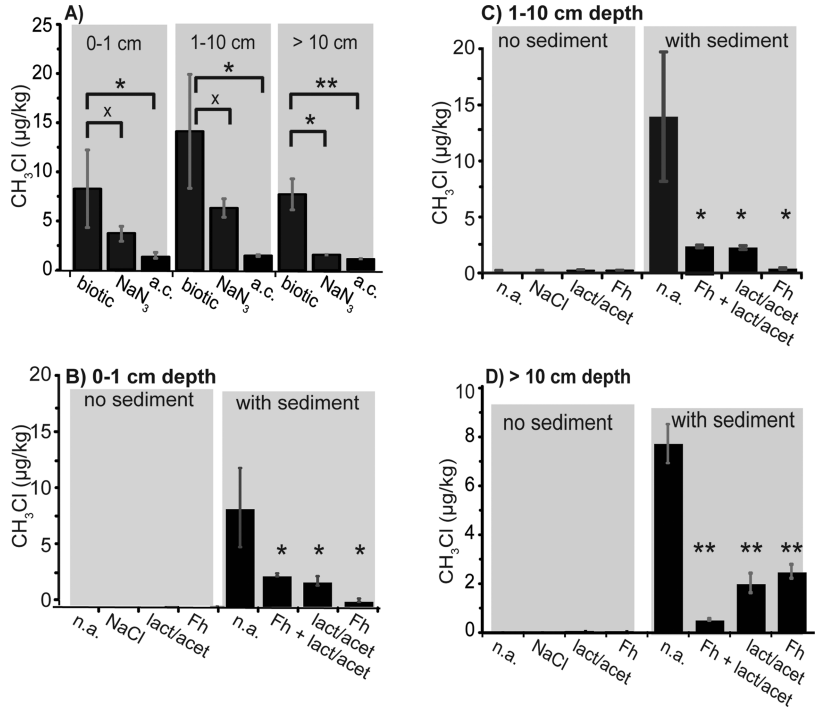

Figure 3. Biotic and abiotic chloromethane emission within the first 60 min of incubation from Lake Strawbridge sediments and the effect of addition of organic e-donors lactate/acetate (lact/acet) and/or ferrihydrite (Fh). (A) Effect of different sterilization techniques on CH_3Cl formation from sediments collected from different depth. Biotic = unsterilized sediment, NaN_3 = 150 mM sodium azide and a.c. = autoclaved sediment. (B–D) The effect of the addition of an organic e-donor and/or e-acceptor on CH_3Cl formation in bulk Lake Strawbridge sediments. n.a. = no additives, NaCl = incubation solution. All three amended setups were significantly (1 asterisk) or very significantly (2 asterisks) different from the setup without any additives. Significance was determined with a *t* test where $p < 0.01$ = very significant (**), $p < 0.05$ = significant (*) and $p > 0.05$ = not significant (x). Error bars indicate standard deviations from triplicate measurements. If error bars are not visible, they are smaller than the symbols.

the zone from 1 to 10 cm, Fe(III) concentrations determined by using 0.5 M HCl as extracting agent were below the detection limit, while 37% of the total iron was Fe(III) using 6 M HCl as extracting agent (Figure 2). In the sediments >10 cm depth, the iron redox speciation showed strong differences for the extractions with 0.5 and 6 M HCl. While 93% of the iron extracted using 0.5 M HCl was Fe(II) (and 7% Fe(III)), only 17% of the Fe extractable with 6 M HCl was Fe(II) and 83% Fe(III) (Figure 2).

Chloride was the most abundant halide ion reaching concentrations of >3 M in leachates of the surface salt layer and decreasing with sediment depth to ca. 0.8 M below 10 cm. TOC was highest in the layer between 1 and 10 cm (0.9 ± 0.1% of dry weight sediment) while concentrations of 0.1 ± 0.0 and 0.1 ± 0.0% were measured in the layers from 0 to 1 and >10 cm, respectively. Generally, TOC concentrations were below 1 wt % for all depth zones. Thus, the sediment studied can be considered carbon-poor. Leachable organic carbon ranged from 5.9 ± 0.1 mg L^{-1} in the depth from 1 to 10 cm to 2.2 ± 0.0 mg L^{-1} in the salt layer and 4.0 ± 0.0 mg L^{-1} in the greyish layer >10 cm.

Organochlorine Formation in Lake Strawbridge Sediments. The emission of chloromethane from Lake Strawbridge sediments in biotic and abiotic setups within the first 60 min of incubation is shown in Figure 3a. Highest emissions were found for the biotic setups and among them for the sediments from 1 to 10 cm depth which released 14.1 ± 5.8 µg chloromethane kg^{-1} dry sediment within the first 60 min of incubation. The salt layer emitted 8.3 ± 4.0 µg chloromethane kg^{-1} dry sediment and the layer >10 cm showed the lowest emissions of chloromethane with 7.7 ± 1.6 µg kg^{-1} dry sediment. When incubating an empty vial at the same conditions, the CH_3Cl concentration after 60 min of incubation was 0.4 µg L^{-1} headspace, while the chloroform emissions were below the detection limit.

Inactivation of microbial activities by adding NaN_3 lowered the emissions of CH_3Cl in the microcosms with sediments from the depth zone from 0 to 1 cm to 3.7 µg kg^{-1} ± 0.8 ($p > 0.05$) and for the layer from 1 to 10 cm to 6.3 ± 0.9 µg kg^{-1} ($p > 0.05$). In the depth zone >10 cm, the emissions were reduced to 1.6 µg kg^{-1} dry sediment ($p < 0.01$). The importance of biological processes for organochlorine formation at the field site became even more evident in the setups that were sterilized via autoclaving. Emissions from autoclaved sediments from the salt layer and from the layer from 1 to 10 cm showed emissions of only 1.45 ± 0.3 and 1.5 ± 0.09 µg kg^{-1} dry sediment and were both significantly lower than the emissions from the biotic setups ($p < 0.05$). Autoclaved sediments from >10 cm yielded only 1.2 ± 0.1 µg kg^{-1} and were statistically lower than the biotic setups ($p < 0.01$). The second most abundant quantified chlorinated hydrocarbon in the sediment was trichloromethane ($CHCl_3$). Although emissions were only detected within the layer from 1 to 10 cm, higher trichloromethane emissions in the biotic setups compared to the autoclaved and sodium azide amended setups confirmed that the chlorinated hydrocarbons released from this sediment are primarily of biotic origin (SI Figure S1). Trichloromethane emissions in general were lower compared to chloromethane emissions and reached only 1.8 ± 0.2 µg kg^{-1} dry sediment within the first 60 min of incubation.

After quantifying chloromethane emission from the sediments, we determined the effects of addition of an additional electron acceptor (ferrihydrite) and electron donor (lactate/

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acetate) on the emissions from the different sediment depth layers (Figure 3b–d). Control setups without sediments did not yield any organochlorines. We found that the addition of lactate/acetate or ferrihydrite or a combination of both did not stimulate chloromethane emissions (Figure 3b–d). The concentrations of chloromethane were even lower in the setups amended with ferrihydrite and/or lactate/acetate than in the nonamended setups. While the raw sediment (>10 cm) showed a chloromethane emission of 7.7 ± 1.6 μg kg^{-1}, the sediment amended with ferrihydrite and lactate/acetate released only 0.5 ± 0.0 μg chloromethane kg^{-1} sediment within the first 60 min of incubation. Similar results were obtained for the addition of lactate/acetate and ferrihydrite to sediments from the salt layer and the layer from 1 to 10 cm (Figure 3c,d). Even in the setups with extended incubation time (24 and 72 h), we did not see an increase in the CH_3Cl emissions. From this result, we conclude that the microorganisms responsible for the chloromethane formation did not need to adapt to the changed conditions after ferrihydrite and acetate/lactate addition and did not become more active over time.

When following the formation of chloromethane in the salt layer over time, we found that after a high initial formation of CH_3Cl, the emissions decreased significantly (Figure 4a).

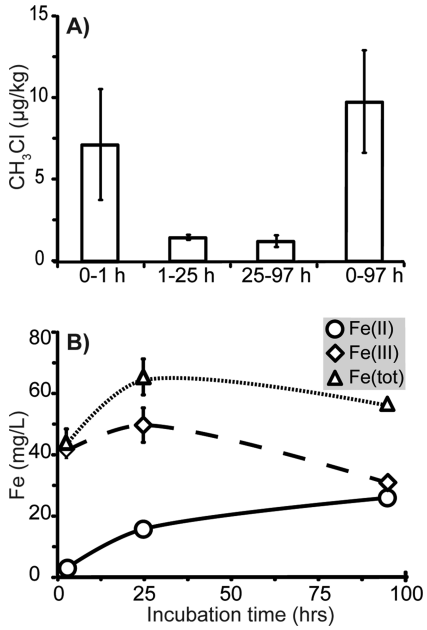

Figure 4. Time-dependent chloromethane formation and Fe-redox speciation in Lake Strawbridge sediments (collected from 0 to 1 cm depth). (A) CH_3Cl formation over time. Data from 0 to 97 h show the total accumulation of chloromethane (sum of the three other values). Error was calculated via error propagation. (B) Changes in sedimentary Fe-redox speciation over time during CH_3Cl release from the sediments. Error bars indicate standard deviations from triplicate measurements. If error bars are not visible, then they are smaller than the symbols.

Within the first 60 min 7.1 ± 3.4 μg chloromethane were emitted per kg dry sediment while between 1 and 24 h of incubation only 1.4 ± 0.2 μg kg^{-1} were emitted. This means that the total amount of CH_3Cl emitted between hours 1–24 is only 20% of the amount emitted within the first hour. When measured 3 days later, the total amount of emitted CH_3Cl between hours 25–97 (1.2 ± 0.4 μg kg^{-1}) was only 16% of the amount emitted within the first hour. The total accumulated emission of chloromethane within 97 h was 9.8 ± 3.1 μg kg^{-1} dry sediment. The high initial VOX release followed by a significant decrease in formation rates could at least partially be due to physical disturbance of the sediments during setup of the microcosms. Mixing of the sediment material could either lead to a direct release of surface-associated existing VOX or a mobilization of reactive compounds such as organics or halides from the sediment which would then stimulate organochlorine formation initially. However, as we did not observe the same initial organochlorine release in the abiotic and biotic setups, at least the release of sorbed organochlorines due to physical disturbance of the sediments is probably negligible.

Role of Fe Redox Changes for CH_3Cl Emissions in Lake Strawbridge Sediments. Whether and to what extent changes in Fe-redox speciation promote VOX formation was determined by following the Fe redox state during incubation of sediments from the salt layer. Fe(III) reduction occurred in our microcosms (sediments from 0 to 1 cm depth) since concentrations of Fe(II) in the aqueous supernatant of the microcosms increased from initially 2.4 to 15.3 mg L^{-1} after 25 h. At the end of the experiment (after 97 h) the concentration of Fe(II) in the microcosm increased to 25.9 mg L^{-1} (Figure 4b). However, no stimulating effect of the addition of ferrihydrite, lactate/acetate or both was found on VOX emissions from the salt layer. Thus, Fe-redox transformations seem to be of only minor importance for organochlorine emissions from the field site. During the incubation of the sterilized setups, we did not observe any changes in Fe redox speciation (data not shown).

DISCUSSION

Role of Biological Processes for Organochlorine Formation. The release of halogenated hydrocarbons was shown for all sediment layers with higher emissions in the biotic setups in comparison to the sterilized setups. Sterilization with either NaN_3 or by autoclaving lowered the organochlorine emissions significantly. This demonstrates (I) the importance of biological processes for organochlorine formation at the field site and (II) the suitability of both methods to inhibit biological processes in such microcosms. Nevertheless, the two used sterilization techniques alter the chemical and physical properties of the salt lake sediments in different ways which might affect (abiotic) organochlorine emissions from the sediments and explain the differences seen between the two sterilization techniques. While the NaN_3 concentrations used in our setups did not remarkably influence the pH, autoclaving might have had a greater impact on the sediment properties.[34] High temperature and pressure applied during autoclaving are expected to change the structure of soils and sediments and influence its surface structure.[35] New surface (sorption) sites can be formed and are exposed to the environment; substances previously stabilized within the sediment's bulk matrix are now exposed to the surrounding. These effects in combination with the high temperature present during autoclaving might have led to the desorption of atmospherically deposited organochlorines.[34,36] Especially as the autoclaving has been done several days before the start of the microcosm experiments, there might have been a loss of chloromethane (CH_3Cl) between the autoclaving and the first GC-MS measurements potentially explaining the lower organochlorine release from the autoclaved sediments in comparison to the NaN_3 treatment (Figure 3a).

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The results for trichloromethane ($CHCl_3$) emissions from biotic, autoclaved and NaN_3 sterilized sediments look slightly different than the results for chloromethane (CH_3Cl). Although the $CHCl_3$ data confirm the predominance of biotic over abiotic reactions in the emissions, the quantified concentrations in the NaN_3 treatment were lower than those from the autoclaved sediments. This is different from the results obtained for chloromethane and does not support the idea discussed above regarding the potential loss of atmospherically deposited organochlorines during autoclaving. However, the $CHCl_3$ results also demonstrate the suitability of the two applied methods to sterilize sediments and thereby differentiate biotic from abiotic processes in organochlorine emissions. Although we cannot fully exclude a possible chemical reaction between NaN_3 and trichloromethane leading to lower concentrations of $CHCl_3$ in the NaN_3 setups compared to the autoclaved ones, we did not find experimental evidence for such a reaction.

The concentrations measured in the sterilized setups were above the atmospheric background concentrations of 600 ppt,[37] indicating that abiotic processes contribute to the natural CH_3Cl emissions as well, although only to a minor extent compared to the biotic processes. A similar finding has been reported for temperate and boreal forest soils in which only a minor part of the natural halogenation of organic matter happened abiotically via a "Fenton-like" reaction.[38] Other studies investigating the formation of halogenated hydrocarbons in forest soils also indicated that the halogenation of organic matter is mainly a biotic process, although the exact contributions of biotic and abiotic processes have not been quantified in detail.[17,38,39] In contrast, for salt lake sediments the relative contribution of biotic and abiotic processes to the emissions of halogenated hydrocarbons have not been quantified so far.

Role of Fe-Redox Transformations, Sedimentary Fe Content and pH for Organochlorine Formation. Quantification of Fe redox species within the sediments showed low Fe concentrations with mainly Fe(III) and only small amounts of Fe(II) in the surface salt layer as it was described before for other hypersaline lake sediments.[32] The dominance of Fe(III) over Fe(II) in the salt layer is not surprising as the top sediment layer is probably oxic. The sediments below a depth of 1 cm contained considerable amounts of Fe(II) in the 0.5 M-HCl-extractable fraction, the so-called bioavailable Fe-pool. This suggests that microbial Fe(III) reduction takes place in Lake Strawbridge sediments. The presence of Fe(II) in the sediments of Western Australian salt lake sediments was observed before.[40] The higher crystalline Fe-phases (extracted with 6 M HCl), in particular in the zone >10 cm, contained mainly Fe(III) with low amounts of reduced iron (Figure 2b). This reflects the presence of higher crystalline Fe(III) mineral phases that are less favorable for microbial Fe(III) reduction (e.g., goethite and hematite).[32,41,42]

Previously it was shown that iron has a crucial impact on organochlorine emissions from forest soils as well as from hypersaline salt lake sediments.[18,19] Therefore, we investigated the effect of ferrihydrite addition on organochlorine emissions from our sediments. No stimulation of chloromethane emission by ferrihydrite addition was found, questioning whether a "Fenton-like" reaction as proposed earlier plays a major role in the formation of chlorinated hydrocarbons at the field site investigated here. This is surprising as the importance of iron was postulated for biotic, as well as for abiotic formation pathways of halogenated hydrocarbons from different environments.[18,22] In abiotic batch setups, Fe(III) and H_2O_2 were found as prerequisite for VOX emissions.[22] Interestingly, increasing Fe concentrations in these experiments did not lead to increasing emissions, while increasing H_2O_2 concentrations led to higher VOX concentrations. In the cited study, the promoting effect of Fe(III) and especially H_2O_2 as an oxidizing agent indicated that a "Fenton-like" reaction as postulated earlier by Keppler et al. might have been responsible for the abiotic VOX emissions.[15,22]

It has to be considered, however, that the pH in these studies was rather low (ranging from 2.4 to 4.1) while in our sediment the pH ranged from 8.3 to 8.1, suggesting the pH being a major controlling factor for abiotic VOX formation. This is supported by the fact that the emissions in the abiotic batch setups of the cited studies decreased with increasing pH values and above a pH of 3.7 trihalomethanes were no longer detected. The importance of dissolved iron for the biotic formation of VOX compounds was postulated under neutral pH conditions in a study with sediments from a hypersaline lake in Southern Russia.[18] The measured concentrations for trichloromethane were in the low ng g^{-1} sediment range and thus slightly lower than in our microcosms (SI Figure S1). However, the authors did not suggest any mechanism involving dissolved iron and microorganisms leading to VOX formation.

In summary, our microcosm experiments did not support our initial hypothesis of a stimulating effect of Fe on the emissions of organochlorines, in particular we did not find evidence that Fe(III)-reducing microorganisms can stimulate VOX formation via the formation of reactive iron species.

Importance of Salt Lakes in Comparison to Other VOX Emitting Field Sites. Our study suggests that Australian salt lake sediments contribute to global natural organochlorine emissions, and we have demonstrated that the emitted compounds (chloromethane and trichloromethane) are mainly of biotic origin. This is in line with previous results showing highest emissions of trichloromethane under warm and moist conditions from a temperate forest soil, indicating a strong microbial contribution to the emissions.[17]

While the emissions in our microcosms decreased with increasing incubation time, an increase in the trichloromethane emissions was found in forest soils from Germany and Denmark over 1 week of incubation.[22,43] One explanation for the differences observed might be that our salt lake sediments are very poor in organic carbon, which is a prerequisite for the formation of organochlorines. Alternatively, biotic and/or abiotic degradation of some of the formed chloromethane might play a role in our microcosms. The presence of reactive humic compounds or iron minerals is known to lead to abiotic degradation of CH_3Cl.[44,45] The biotic degradation of chloromethane has for example been shown for an aerobic, methylotrophic microorganism isolated from a beech forest in Ireland.[46] Since then, chloromethane degrading bacteria have been isolated from many environments, including lake sediments, estuarine, and marine samples. This makes it conceivable that CH_3Cl-degrading bacteria are ubiquitous in the environment.[47] Moreover, when comparing the studies mentioned above to our work, it has to be considered that the formation pathways of the two compounds investigated, i.e., chloromethane and trichloromethane, are different. While the formation of trichloromethane is based either on a "Fenton-like" reaction[21] or on an enzymatic formation via chloroper-

oxidases,[48] the formation of chloromethane in our study seems to be formed enzymatically via methyltransferases.[49]

Methyltransferases were shown to be responsible for direct enzymatic formation of CH_3Cl in the environment; this has been demonstrated first in the fungus *Phellinus pamaceus*.[49] Thereafter, the role of methyltransferases in the formation of chloromethane was shown for a wide range of organisms from algae to terrestrial bacteria and higher plants.[50,51] The biotic formation pathway of the second prominent chlorinated hydrocarbon detected in this study, i.e., $CHCl_3$, is different from the one described for CH_3Cl and consists of 2 individual steps. In the first step, chlorinating enzymes catalyze the formation of reactive halogen species, which react afterward nonspecifically with soil organic matter.[14] This process is widely distributed in the environment and the formation of organically bound chlorine was already shown in the 1990s.[52−54]

As the organisms that catalyze the enzymatic formation need a carbon source in order to maintain their metabolic activity, easily accessible organic carbon such as lactate/acetate might be limiting in our setups leading to the decrease in the net formation rate with increasing incubation time. However, our experiments did not show a promoting effect of lactate/acetate on organochlorine emissions suggesting that either organic carbon was not limiting, lactate/acetate does not support organochlorine formation or that the organochlorine producing organisms were outcompeted by other groups of microorganisms present in the sediments that used the lactate/acetate faster than the ones producing the chlorinated hydrocarbons. Although we did not see a promoting effect of the lactate/acetate addition on organochlorine formation in our setups, the importance of organic carbon for the emissions of chlorinated hydrocarbons from the field site became evident. The highest release of CH_3Cl and $CHCl_3$ were found from the organic rich depth zone from 1 to 10 cm, suggesting that organic carbon plays an important role in the emissions from the field site. The importance of high organic carbon concentrations in VOX formation was already shown for forest soils in the late 1990s.[14,55] Results from these studies showed highest emissions of trichloromethane from the top organic rich soil layer, while the emissions decreased with increasing soil depth.

Environmental Implications and Future Prospects. The data presented in this study contribute to a better understanding of the emission of halogenated hydrocarbons in the environment and fill some of the gaps in the global organochlorine budgets. Besides oceans and salt marshes,[25,56] hypersaline lake sediments also contribute to the global organochlorine emissions from saline environments. The quantification of the biotic and abiotic contributions to the net CH_3Cl emissions per kg dry weight sediment has not previously been presented in such detail for salt lake sediments. However, it has to be considered that in our study we measured concentrations of organochlorines in the headspace of sediment microcosms. Direct conclusions regarding global organochlorine fluxes into the atmosphere require additional field flux measurements from sediments and cannot be simply drawn from the concentrations quantified in our microcosms.

The impact of salt lake sediments and salt-affected soils to organochlorine emission is also important with regard to global warming and decreasing rainfall. The increasing salinization of freshwater lakes is of major concern in many arid and semiarid environments, including Australia, where 80% of the lakes are not freshwater but salt lakes,.[24,57] Freshwater lakes act as a sink for chloromethane and thus do not contribute significantly to the global CH_3Cl budgets,[58] whereas salt lakes seem to act as a source of organochlorines. Future scenarios predict that existing salt-affected landscapes increase in size and that there is also the formation of new areas suffering from salinization.[59] If these scenarios are realized, then there might be fundamental changes in the global VOX budgets with an increasing importance of salt lake sediments and salt affected soils.

This underlines the relevance of the investigation of saline environments in the global fluxes of halogenated hydrocarbons. Therefore, it would be of interest to get a more detailed picture of the VOX emissions from saline environments. Particularly, the estimation of annual global emissions of representative compounds such as chloromethane or trichloromethane from saline environments would be of interest. Future studies should therefore focus on arid and semiarid environments including the investigation of seasonal changes in the emission patterns of VOX compounds. Especially in combination with stable carbon and chlorine isotope analyses,[60] microcosm experiments and field measurements would broaden our understanding of natural halogenation processes and the formation pathways of the individual compounds in high saline environments.

■ ASSOCIATED CONTENT

Supporting Information

GC-MS instrumental parameters and ITEX method; and the effect of different sterilization techniques on trichloromethane emissions (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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