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Assessing the role of trichloroacetyl-containing compounds in the natural formation of chloroform using stable carbon isotopes analysis

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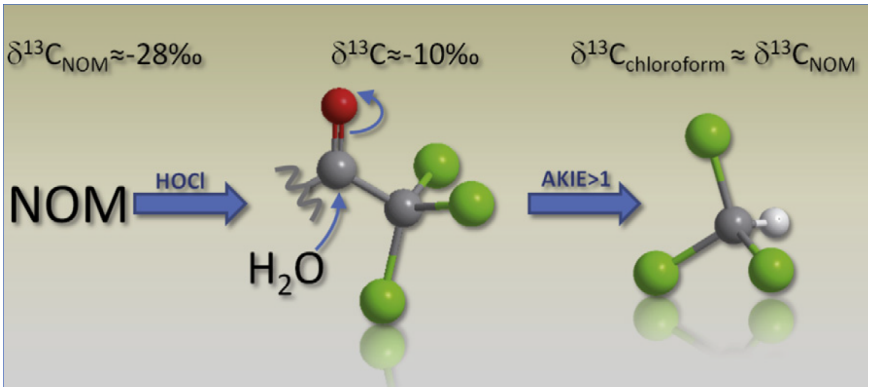
HIGHLIGHTS

- Trichloroacetyl-containing compounds (TCAc) and $CHCl_3$ were detected in forest soils.
- TCAc are much more enriched in ^{13}C compared to natural $CHCl_3$ and NOM.
- Natural $CHCl_3$ has a similar carbon isotope composition as NOM.
- The formation of $CHCl_3$ by hydrolysis of TCAc induced large ^{13}C-isotope fractionation.
- Our model confirms that TCAc are likely precursors of natural $CHCl_3$ in soils.

GRAPHICAL ABSTRACT



ABSTRACT

Chloroform ($CHCl_3$) is an environmental contaminant widely distributed around world, as well as a natural compound formed in various aquatic and terrestrial environments. However, the chemical mechanisms leading to the natural formation of chloroform in soils are not completely understood. To assess the role of trichloroacetyl-containing compound (TCAc) in the natural formation of chloroform in forest soils, carbon stable isotope analyses of chloroform and TCAc in field samples and chlorination experiments were carried out. The isotope analysis of field samples have revealed that the $\delta^{13}C$ value of natural chloroform ($\delta^{13}C_{mean} = -25.8‰$) is in the same range as the natural organic matter ($\delta^{13}C_{mean} = -27.7‰$), whereas trichloromethyl groups of TCAc are much more enriched in ^{13}C ($\delta^{13}C_{mean} = -9.8‰$). A similar relationship was also observed for TCAc and chloroform produced by chlorination of natural organic matter with NaOCl. The strong depletion of ^{13}C in chloroform relative to TCAc can be explained by carbon isotope fractionation during TCAc hydrolysis. As shown using a mathematical model, when steady state between formation of TCAc and hydrolysis is reached, the isotope ratio of chloroform is expected to correspond to isotope composition of NOM while TCAc should be enriched in ^{13}C by about 18.3‰, which is in good agreement with field observations. Hence this study suggests that TCAc are likely precursors of chloroform and at the same time explains why natural chloroform has a similar isotope composition as NOM despite large carbon isotope fractionation during its release.

Keywords:
Chloroform
Trichloroacetyl group
Isotope
Hydrolysis
Natural organic matter
Chlorination

1. Introduction

Chloroform ($CHCl_3$) has for a long time been considered as of anthropogenic origin only, classified as a Group B2 probable human carcinogen according to the World Health Organization

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classification scheme (WHO-IARC, 1999). Recently, the presence of chloroform in coniferous forest soil and groundwater has been demonstrated (Haselmann et al., 2000, 2002; Albers et al., 2008a,b). The frequent detection of chloroform in groundwater in absence of other anthropogenic contaminants suggests that chlo-roform may be formed naturally by biogeochemical processes (Laturnus et al., 2002). Numerous studies on natural organohalo-gens have suggested that enzymes such as chloroperoxidase (CPO) excreted by fungi could play an important role in biosynthe-sis of chlorinated organic compounds in soil (Urhahn and Ballsch-miter, 1998; Hoekstra et al., 1998a,b; van Pee and Unversucht, 2003). The current hypothesis is that CPO expresses a chlorinating activity by forming HOCl or other oxidized chlorine species (Hoek-stra et al., 1998a,b). Recently, Huber et al. (2009) have demon-strated that chloroform can also be formed abiotically, when organic matter is incubated with Cl^-, Fe^{3+} and H_2O_2.

As CPO is able to chlorinate the natural organic matter (NOM) likely via the formation of an oxidized diffusible intermediate like HOCl (Griffin, 1983), the chemical chlorination with sodium hypochlorite constitutes a good model system to mimic the enzymatic chlorination of NOM. Since NOM has a very complex chemical structure it is impossible to propose a unique reaction mechanism. Therefore, most studies have focused on the chlorination mechanism of simple model compounds such as phenol, substituted phenols, aliphatic β-dicarbonyl acids and glycosides (Rook, 1977; Boyce and Hornig, 1983; Gallard and von Gunten, 2002a,b; Dickenson et al., 2008). The chlorination of substituted aromatic compounds is presumed to take place through a halogenation by oxidized chlorine species on the activated aromatic carbon atoms. This step is followed by hydrolytic cleavage of the aromatic ring; further chlorination of aliphatic intermediates and finally the release of chloroform by hydrolysis. Boyce and Hornig have demonstrated that during the conversion of 1,3-dihydroxyaromatic substrates to chloroform the aromatic ring is broken and several chlorinated aliphatic intermediates are formed (Boyce and Hornig, 1983). Among these intermediates, several trichloroacetyl-containing compounds (TCAc) have been identified by GC/MS (Fig. 1) (Boyce and Hornig, 1983; de Leer et al., 1985).

TCAc can release chloroform either by nucleophilic substitution or by alkaline hydrolysis. Recently, Albers et al. have shown that TCAc are present in forest soils containing natural chloroform and the concentrations of TCAc and chloroform show a similar spa-tial variability (Albers et al., 2010a,b). They furthermore found the concentration of TCAc in soil profiles to decrease when the pH increases, suggesting natural TCAc to be stable only at acidic conditions.

Compound-specific isotope analysis (CSIAs) constitutes a potential tool to assess the mechanisms leading to formation of chloroform during chlorination of organic matter. This technique has recently been used to distinguish natural and anthropogenic sources of contaminants and to gain insights into the mechanisms of degradation of various pollutants (Aelion et al., 2010). Changes of the carbon isotope composition of organic compounds during formation and transformation processes can be attributed to a kinetic isotope effect due to the presence of a heavy isotope in the reacting bond(s), which is characteristic for the underlying reaction mechanism. Recently Arnold et al. (2008), have used CSIA to investigate the apparent ^{13}C kinetic isotope effect of the formation of chloroform during chlorination of selected model compounds to evaluate the functional group(s) in NOM responsible of chloroform formation. They suggest that compounds containing 1,1,1-trichloropropanone-like functional groups could be intermediates leading to chloroform.

The aims of this study are to characterize the isotope signature of natural chloroform and trichloromethyl groups of the TCAc in order to (i) gain better understanding of the role of TCAc in the formation of natural chloroform and (ii) constrain the carbon isotope signature of TCAc and chloroform in the natural environment in view of using isotope data for distinguishing different sources of chloroform. If TCAc is the precursor of chloroform, the isotopic signature of trichloromethyl groups of TCAc should be offset from that of chloroform by an amount that depends on the kinetic isotope effect associated with chloroform release. This study included a field study at forested sites where natural chloroform production is well established (Albers et al., 2010c,2011) and laboratory experiments to investigate the relationship between chloroform and trichloromethyl groups in TCAc in a well-defined closed system. In the field study, the isotope ratio of chloroform present in soil air was compared to the isotope ratio of trichloromethyl groups of TCAc in forest soil. The chlorination experiments were carried out under various pH (pH = 4, 7, 8) conditions as the release of chloroform by hydrolysis is pH dependant using soil material from one of the field sites and humic substances. As for the field study, the car-bon isotope ratio of chloroform and trichloromethyl-groups in TCAc was determined. Finally, a mathematical model of the carbon isotopic trends of chloroform and TCAc was established to assess the role of TCAc in the formation of chloroform and to better constrain the isotope signature of natural chloroform.

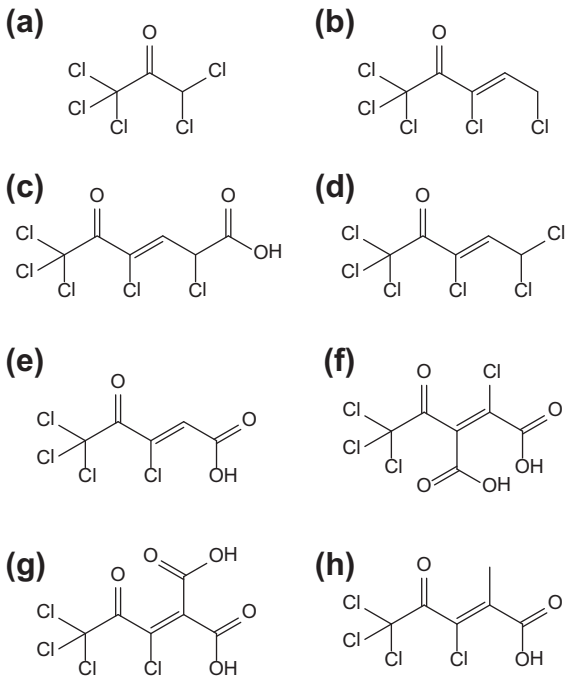

Fig. 1. Structures of trichloroacetyl-containing compounds formed by chemical chlorination of dihydroxyaromatic model compounds (a–d) (Boyce and Horning, 1983) and humic acid (e–h) (de Leer et al., 1985).

2. Materials and methods

2.1. Chemicals

The following chemicals were used as received: sodium hypochlorite (Sigma–Aldrich, available chlorine ⩾ 4%), phosphoric acid (Fluka, 85%), sodium dihydrogenphosphate monohydrate (Merck, >99%), disodium hydrogenphosphate dodecahydrate (Fluka, >99%), sodium sulfite (Sigma–Aldrich, >98%). Ultrapurified water (18.2 MΩ cm at 25 °C, Direct-Q UV-3 Millipore) was used to prepare the phosphate buffer solutions. Chloroform from Fluka (99.5%) and Acros Organics (99.8%) were used to prepare concentration and isotope standards. Humic substances used for chlorina-

tion experiments were obtained from the International Humic Substance Society: Suwannee river NOM (SRNOM), Nordic reservoir NOM (NRNOM), Pahokee peat humic acid (PPHA), Elliot soil humic acid (ESHA). Soil organic matter collected in the H and F horizons of a forest soil from Tisvilde Hegn (Denmark) and humic acids from the same forest NOM (FOHA) were also used for chlorination experiments. Humic acids were extracted by alkaline extraction with aqueous NaOH, followed by precipitation of humic acid at low pH and a desalting steps involving dialysis (Albers et al., 2008a,b).

The use of sodium hypochlorite to mimic CPO-catalyzed chlorination of NOM is based on the following arguments. Recently Breider et al. have shown that abiotic and CPO-catalyzed chlorination of humic substance produce trichloroacetic acid with very similar carbon isotope composition suggesting that the formation mechanisms of organochlorine from humic substances by abiotic and enzymatic chlorination are likely very similar (Breider and Hunkeler, 2011). Moreover, Kühnel et al. have shown using high resolution X-ray synchrotron diffractometry that only a narrow channels (Ø∼4 Å) connect the protein surface with the heme of CPO (Kuehnel et al., 2006). However, humic substances have molecular weights typically between ∼10 kDa up to ∼300 kDa and a diameter between ∼100 Å and ∼2000 Å (Christl et al., 2000). It can therefore be excluded that humic substances can reach the heme of CPO and react directly at the catalytic site. Hence we can conclude that the chlorination of humic substances can only occurs via the formation and diffusion of free HOCl from the enzyme.

2.2. Field sites and sampling

Field sampling campaigns were conducted in Denmark in June 2009 and July 2010 in two mixed Spruce (*Picea abies*) and Scots Pine (*Pinus sylvestris*) forests at the Tisvilde Hegn (THN) (56°02′N−12°04′E) and Viborg Hedeplantage (VBH) (56°25′N−9° 22′E) where natural chloroform production occurs (Albers et al., 2011). At these sites, chloroform production in soil varies spatially with hotspots of high production of limited spatial extent (20–400 m^2). In this study, soil-air samples were taken at one of the hotspots of each site that are equipped with a multilevel wells throughout the unsaturated zone (Albers et al., 2010c), denoted as THN and VBH hotspots. The top soil at the THN and VBH hotspots is constituted of an organic horizon mainly composed of partly degraded needles and branches. Soil-air samples for chloroform analysis were collected at 0.5 m depth using sorption tubes filled with 100 mg of Tenax TA (Supelco, Bellefonte, USA) connected to a membrane pump NMP05L (KNF, Balterswil, Switzerland) (Mead et al., 2008). Before sampling, the sorption tubes were conditioned at 200 °C during 120 min under N_2 flow of 40 mL min^{-1}, and sealed with a Teflon septum. After purging the internal volume of the sampling system at least two times, 3 L of soil-air were sampled at ∼200 mL min^{-1}. During sampling, soil-air was dried using a stainless steel cartridge filled with anhydrous sodium sulfate and the sorption tubes were cooled at ∼15 °C below ambient temperature using a Peltier device.

2.3. Preparation and analysis of field samples

Concentrations of chloroform in soil-air and TCAc in soil were measured by gas chromatography electron capture detector (8A, Shimadzu, Kyoto, Japan) according to the procedure described by Busenberg and Plummer (Busenberg and Plummer, 1992). These analyses were carried out in Denmark directly after field sampling. The samples preparation for TCAc concentration analysis was done as described previously by Albers et al. (2010b,c). Briefly, the soil samples were amended with chloroform free water in gas tight vials. Then, the soil-bound and water soluble TCAc present in soils were hydrolyzed by adding concentrated NaOH (pH ⩾ 12) solution and incubated during 24 h. The chloroform released by the hydrolysis of TCAc was analyzed by gas chromatography electron capture detector.

The concentrations and the carbon isotope measurements were conducted with subsamples of the same soils. For carbon isotope analysis of trichloromethyl groups of TCAc, the same procedure was used except 4 g of dried soil was mixed with ∼500 mL of pure water in a 1 L gas-tight bottle (Schott, Mainz, Germany) which was connected to a purge-and-trap system (Velocity XPT, Teledyne Tekmar Dohrmann, Mason, USA). For selected sample, the hydrolysis process was repeated and no further chloroform was detected (detection limit = 0.8 μg L^{-1}) indicating that the procedure leads to nearly complete hydrolysis. The purge gas stream of the purge-and-trap system was directed through the 1 L glass bottle equipped with a frit (Hunkeler et al., 2012). The sample was purged during 20 min at 150 mL min^{-1} and trapped at 30 °C with a VO-CARB 3000 trap (Supelco, Bellefonte, USA). In order to avoid satu-ration of the trap with water, the moisture was removed during the sample purge step with a Velocity XPT DryFlow trap heated at 100 °C. The carbon isotope ratios of chloroform formed by hydrolysis of TCAc was measured using gas chromatography (Trace GC Ultra, Thermo Fisher Scientific, San Jose, USA) coupled to a com-bustion interface and an isotope ratio mass spectrometer (IRMS)(Delta XP plus, Thermo Fisher Scientific, San Jose, USA). After the extraction step, the VOCARB 3000 trap was heated to 250 °C for 3 min. Chloroform was thermally desorbed and transferred to the GC at 1.7 mL min^{-1} and trapped in a cryogenic focuser (Optic 3 ATAS-GL, Veldhoven, The Netherlands) held at −100 °C during 3 min with liquid nitrogen before chromatographic separation. The chromatographic separation was carried out with a 60 m × 0.25 mm × 1.4 μm film thickness DB-VRX column (Agilent, Santa Clara, USA). The GC oven temperature program used was as follows: 6 min at 40 °C, 10 °C/min to 175 °C, hold for 1 min. Oxida-tion and reduction reactors of the combustion interface were respectively maintained at 940 and 640 °C.

To determine the $\delta^{13}C$ signature of natural gaseous chloroform, the sorption tubes containing chloroform from soil-air were analyzed using a thermal desorption system TDAS 2000 (CTC Analytics, Zwingen, Switzerland) with a Combi-PAL autosampler (CTC Analytics, Zwingen, Switzerland) connected to the GC/C/IRMS. The sorption tubes were flushed at ambient temperature with N_2 for 15 s and were desorbed at 200 °C during 1 min. Then, carbon isotopic ratios of chloroform were analyzed using the GC/C/IRMS method described above. The thermal desorption was carried out in splitless mode with cryogenic focusing. This approach mini-mizes the possibility for isotope fractionation, as the totality of the desorbed analyte is transferred to the GC–C–IRMS. To confirm that no isotopic fractionation occurs, a standard of chloroform with known $\delta^{13}C$ value (−53.8 ± 0.3‰, n = 3) was measured with this technique in laboratory. Gaseous chloroform was sampled during 15 min with sorption tubes at a flow rate of 200 mL min^{-1} corresponding to the flow rate used in the field. The obtained $\delta^{13}C$ value (−53.4 ± 0.3‰, n = 3). was not significantly different from the expected value (−53.8 ± 0.3‰, n = 3) according to a student's t-test (p = 0.18). The carbon isotopic composition of NOM was determined in triplicate using an elemental analyzer coupled with a Delta S stable isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany).

2.4. Chlorination experiments and analysis of laboratory samples

The different soil and humic substances were chlorinated with sodium hypochlorite using the following procedure: 4 mg of humic substance or 40 mg of soil was added in 42 mL vial containing

40.8 mL of 100 mM phosphate buffer and the reaction was initiated by adding 1 mL of 0.02 M aqueous solution of NaOCl. The vials were sealed with Teflon septum caps and agitated during 24 h at room temperature (~25 °C). After 24 h, samples were quenched with 200 µL of a 100 g L^{-1} aqueous solution of sodium sulfite to stop the reaction. The reaction was carried out at pH 4, 7 and 8. To analyze the concentration and the isotope signature of the trichloromethyl groups in TCAc, the samples were purged for 30 min with pure N_2 to remove chloroform formed during the chlorination and the pH was adjusted to ⩾12 with 6 M NaOH solution to hydrolyze TCAc and form chloroform. All samples were stored in the dark at 4 °C until concentration and carbon isotopes analysis.

The concentration of chloroform formed during chlorination experiments and by hydrolysis of TCAc was analyzed using gas chromatography (Trace GC Ultra, Thermo Fisher Scientific, San Jose, USA) with a quadrupole mass spectrometer (DSQII, Thermo Fisher Scientific, San Jose, USA). The analyses were carried out with 20 mL headspace vials containing 15 mL of sample. After equilibrating with agitation at 60 °C for 2 min, 500 µL of headspace from 20 mL vials containing 15 mL of sample were injected in split mode (1:10) by an autosampler (Combi-PAL, CTC Analytics, Zwingen, Switzerland) onto a 60 m × 0.32 mm × 1.8 µm film thickness Zebron ZB 625 column (Phenomenex, Torrance, USA). The injector temperature was 250 °C and the ion source temperature was 200 °C. The oven temperature was held at 150 °C for 5 min. The analyses were carried out in single ion monitoring mode using the following *m*/*z*: 48, 50, 83, 85, 118 and 120.

For carbon isotope ratios analysis of chloroform formed during chlorination experiments and by hydrolysis of TCAc, the GC/C/IRMS system described above was used except for the purge volume and N_2 purge flow rate, which were 25 mL and 40 mL/min during 10 min, respectively. In order to maximize the accuracy of measured $\delta^{13}C$ values, the aqueous samples were diluted to obtain constant peak amplitudes (5000 mV).

The hydrolysis of TCAc is assumed to be much slower than the chlorination steps. Thus in approximation hydrolysis can be considered as a simple one step reaction and thus the AKIE can be approximated using a Rayleigh approach. Therefore the isotope fractionation factor, α, for the formation of chloroform from TCAc was estimated using the following equation:

$$\alpha = \frac{\ln\left(\frac{R_{TCAc}}{R_{TCAc}f + R_{CF}(1-f)}\right)}{\ln(f)} + 1 \quad (1)$$

where R_{TCAc} is the isotope ratio of the trichloromethyl position in TCAc, R_{CF} is the isotope ratio of accumulated chloroform, and f is the remaining fraction of TCAc. Since only one carbon atom is present in chloroform, the fractionation factor α can be directly related to the apparent kinetic isotope effect (AKIE) according to:

$$\text{AKIE} = \frac{1}{\alpha} \quad (2)$$

3. Results and discussion

3.1. Concentrations and $\delta^{13}C$ of chloroform and TCAc in natural samples

The concentrations of chloroform in soil-air at 0.5 m depth in the THN and VBH hotspots varied within a range of 15–120 ppbv. The $\delta^{13}C$ values of chloroform measured in soil-air at a depth of 0.5 m in the THN and VBH hotspots ranged between −22.8 and −31.3‰ (Fig. 2). The $\delta^{13}C$ values of NOM from THN and VBH (−27.2 to −27.7‰) are in the same range as the $\delta^{13}C$ values of chloroform (Fig. 2). The total concentration of TCAc measured in four soil samples from THN and VBH ranged between 63 and 5565 µg $CHCl_3$/kg. The $\delta^{13}C$ values of the trichloromethyl component of TCAc varied between −9.2 and −10.2‰ for soil samples (Fig. 2). The variations of the $\delta^{13}C$ of chloroform in soil-air could be related to the combined isotope effect associated with diffusion in the unsaturated zone and the equilibration between soil-air and pore water. Modeling studies for CO_2 showed that in the unsaturated zone during transient conditions (e.g. related to rain events) isotope ratios of gaseous compounds can deviate from steady state values (Cerling et al., 1991; Nickerson and Risk, 2009). The $\delta^{13}C$ of chloroform is similar to that of NOM and distinctly different from the known range of anthropogenic chloroform (−43.2 to −63.6‰, Hunkeler et al., 2012) suggesting that chloroform in soil air originates from NOM. The trichloromethyl groups of TCAc present in upper soil horizons are considerably more enriched in ^{13}C compared to chloroform. Nevertheless, it is very likely that natural chloroform is released from TCAc during its decomposition in soils, since large carbon isotope fractionation is expected for the hydrolysis of TCAc (Arnold et al., 2008). In order to test this hypothesis and to better understand the mechanisms that could lead to the formation of chloroform from TCAc present in soils, chlorination experiments of NOM of different origins were carried out.

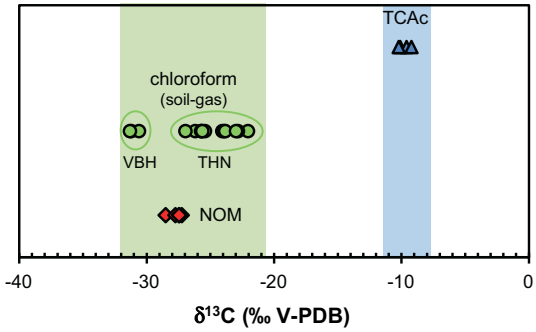

Fig. 2. $\delta^{13}C$ values of natural chloroform in soil–gas measured at VBH hotspot (n = 3, $\delta^{13}C_{mean}$ = −31.0‰) and THN hotspot (n = 12, $\delta^{13}C_{mean}$ = −24.6‰) at 0.5 m depth (green circles), $\delta^{13}C$ values of trichloromethyl groups of TCAc in THN and VBH soils (n = 4, $\delta^{13}C_{mean}$ = −9.8‰) (blue triangles), and NOM from THN and VBH hotspots (n = 5, $\delta^{13}C_{mean}$ = −27.7‰) (red diamonds). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Chlorination of NOM with hypochlorite

For all materials, the concentrations of chloroform and the total concentration of trichloromethyl groups in chloroform and TCAc increase with increasing pH whereas the TCAc concentration decreases (Fig. 3). This inverse correlation between chloroform and TCAc concentrations with pH could be due to the formation of chloroform by nucleophilic attack of the carbonyl C-atom of TCAc by OH^- or OCl^-, which are present at higher abundance at an elevated pH. At pH 4, the release of chloroform is more likely related to the nucleophilic attack of the carbonyl C-atom by H_2O. The increase of the total concentration of trichloromethyl groups with rising pH can be rationalized in terms of the reactivity of the ionized and un-ionized forms of functional groups in humic substances. Under alkaline conditions phenolic groups tend to be deprotonated. The electron donating character of the deprotonated O^- substituents tends to stabilize electron rich structures by resonance and thus activate the electrophilic substitution of the aromatic moieties in ortho and para positions (Rebenne et al., 1996; Gallard and Von Gunten, 2002b). The chlorination of ketone functional groups can also be activated under alkaline pH as the keto-enol equilibrium is shifted in the direction of the enolate isomer

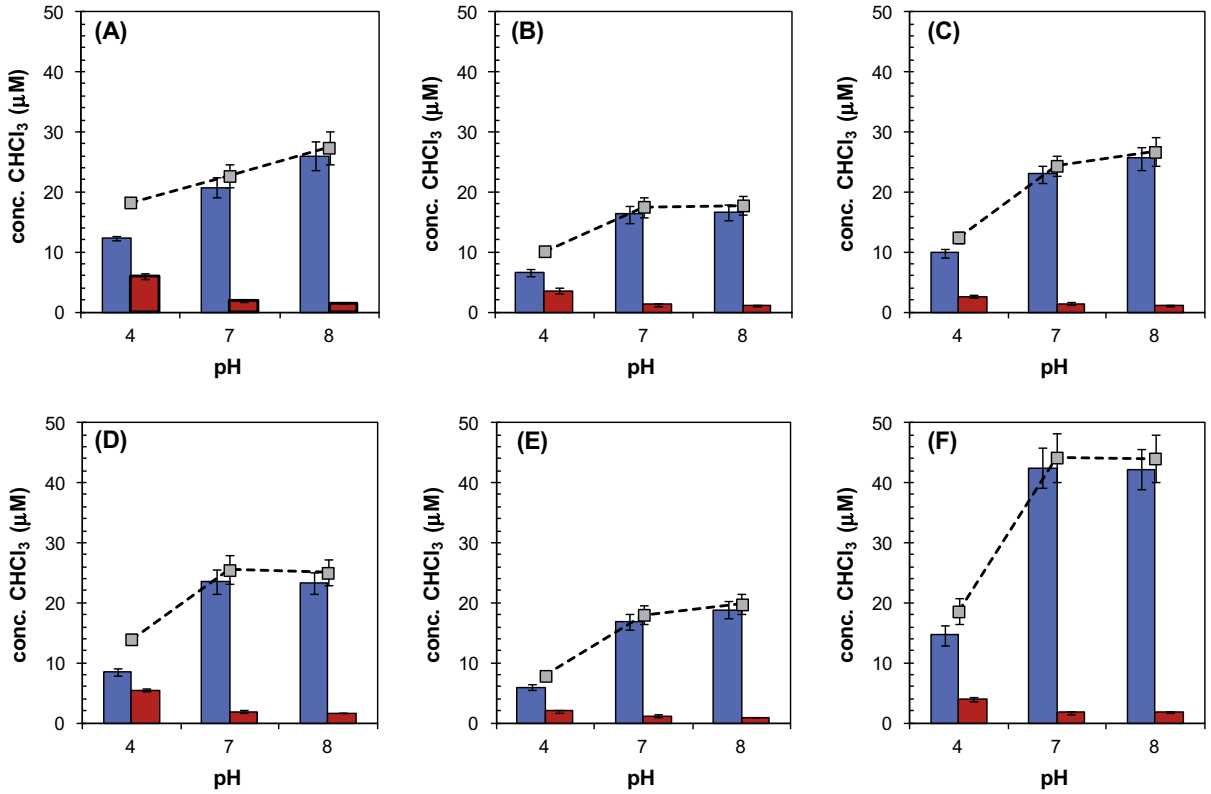

Fig. 3. Concentrations in µM of chloroform (blue bars), chloroform produced by hydrolysis of TCAc (red bars) and total concentration of trichloromethyl groups from chloroform and TCAc (grey squares) formed at pH 4, 7 and 8 by chlorination of NOM. (A) Forest soil organic matter (THN); (B) Elliot soil humic acid; (C) Suwannee river NOM; (D) Humic acid from forest soil (THN); (E) Pahokee peat humic acid, and (F) Nordic lake NOM. The error bars correspond to the standard deviation (1σ) of the concentrations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and thereby makes the carbon bond more susceptible to attacking electrophiles. The reactivity of HOCl depends of its speciation as function of pH. Therefore, the pH can indirectly strongly influence the chlorination rate and thus TCAc and chloroform production rates. The amount of chloroform released by chlorination of NOM of different origin at a given pH is likely determined by the relative amount of reactive functional groups in humic substances and the pH which controls the hydrolysis rate.

3.3. Carbon isotope signatures of chloroform and TCAc

The $\delta^{13}C$ values of chloroform and the trichloromethyl position of TCAc formed upon chlorination of NOM at pH 4, 7 and 8 are shown in Fig. 4. For all samples, the $\delta^{13}C$ values of chloroform are more depleted in ^{13}C compared to NOM. Chloroform released upon chlorination of NOM tends to be gradually enriched in ^{13}C with rising pH. Similar offsets are observed independent on the type of organic matter. With increasing pH, the $\delta^{13}C$ values of chloroform approaches the carbon isotopic signature of the original NOM. The $\delta^{13}C$ values of the trichloromethyl position of TCAc were only measured at pH 4 since at pH $\geqslant$ 7 there is not enough remaining TCAc for carbon isotope analysis. In contrast to chloroform, at pH 4 the trichloromethyl position of TCAc is enriched in ^{13}C compared to NOM with a difference in $\delta^{13}C$ of about 30‰ between chloroform and TCAc. The carbon-weighted mean $\delta^{13}C$ values of trichloromethyl groups formed upon chlorination (chloroform and TCAc) at pH 4 range between −28.3‰ and −36.6‰ (Fig. 4). The mean $\delta^{13}C$ values of trichloromethyl groups produced at pH 4 are similar to the $\delta^{13}C$ values of chloroform produced at pH 8 which suggest that at pH 8 almost all trichloromethyl groups formed upon chlorination have been converted to chloroform con-

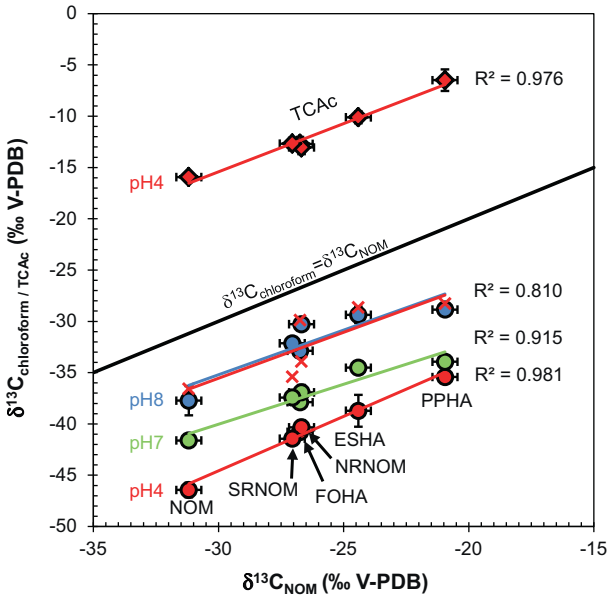

Fig. 4. $\delta^{13}C$ values of chloroform formed at pH 4 (red circles), pH 7 (green circles), pH 8 (blue circles) and trichloromethyl groups in TCAc formed at pH 4 (red diamonds) by chlorination of forest soil organic matter from THN (NOM), Suwannee river NOM (SRNOM), humic acid from forest soil (FOHA), Nordic lake NOM (NRNOM), Elliot soil humic acid (ESHA), and Pahokee peat humic acid (PPHA). The red crosses correspond to the carbon-weighted average $\delta^{13}C$ values of trichloromethyl groups formed upon chlorination at pH 4. The error bars correspond to the standard deviation (1σ) of the $\delta^{13}C$ values ($n = 3$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sistent with the concentration data. Furthermore, the mean $\delta^{13}C$ values of trichloromethyl groups are slightly depleted in ^{13}C compared to the $\delta^{13}C$ values of the natural organic precursors used for chlorination experiments. This small deviation of the $\delta^{13}C$ values may be due to (i) heterogeneities in ^{13}C distribution between the functional groups involved in the formation of trichloromethyl groups and the rest of the organic precursors (Schmidt, 2003; Galimov, 2006), (ii) an isotopic effect associated with a reaction step preceding the TCAc hydrolysis such as the cleavage of aromatic rings present in NOM, or (iii) an isotope sensitive branching of competing reaction pathways (Arnold et al., 2008).

The gradual enrichment of chloroform in ^{13}C with rising pH strongly suggests that the isotopic fractionation between NOM and chloroform is likely due to a pH-dependent reaction. As previously discussed, the concentration of chloroform formed upon chlorination increase with pH, whereas the concentration of TCAc tends to decrease (Fig. 3). Under alkaline conditions almost all trichloromethyl groups formed are converted into chloroform. Considering that the mean $\delta^{13}C$ value of the functional groups involved in the formation of trichloromethyl groups is close to the mean $\delta^{13}C$ value of NOM, the $\delta^{13}C$ value of chloroform must increase toward the $\delta^{13}C$ value of the precursor with the increasing extent of the hydrolysis. Therefore, the pH-effect observed on the $\delta^{13}C$ values of chloroform can likely result from different degree of hydrolysis of the trichloroacetyl groups of TCAc formed during the chlorination. The AKIEs calculated using Eq. (2) for the formation of chloroform from TCAc at pH 4 is 1.0183 ± 0.0002 for the chlorination of forest NOM and varies between 1.0142 and 1.0187 for the chlorination of humic substances. These AKIEs are in the same range as the AKIE determined by Arnold et al. for the alkaline hydrolysis of 1,1,1-trichloropropanone (AKIE = 1.014 ± 0.002) (Arnold et al., 2008), which is compatible with the hypothesis of chloroform release by hydrolysis of TCAc.

The chlorination experiments confirm that chloroform is released by hydrolysis of TCAc, which induces a large carbon isotopic fractionation. Contrary to the chlorination experiments, the $\delta^{13}C$ values of trichloromethyl groups in TCAc and chloroform in soils cannot be rationalized by a typical Rayleigh fractionation trend. Otherwise, both chloroform and trichloromethyl groups in TCAc should become increasingly enriched over time. Therefore, to better constrain the carbon isotope signature of chloroform and TCAc measured in soils, and to assess the combined effects of the simultaneous production and hydrolysis of TCAc, an isotopic model was developed in the following section.

3.4. Modeling of carbon isotopic trends of chloroform and TCAc

A mathematical model to assess the evolution of the $\delta^{13}C$ values of the trichloromethyl groups of TCAc and chloroform with time was established assuming a constant formation of TCAc in soil, the consumption of the trichloroacetyl groups by hydrolysis according to a first order rate law and no degradation of chloro-form in soil. Here we hypothesize that the formation of TCAc fol-lows a zero-order kinetic. In soils, NOM is present in excess and hence the reaction rate is not a function of the NOM concentration. Moreover, it can be assumed that HOCl is produced at a constant rate (for a given period within the year) and controls the rate of reaction. Thus, the TCAc concentration is governed by the follow-ing first order linear non-homogeneous differential equation:

$$\frac{\partial C}{\partial t} = P - k \cdot C \tag{3}$$

where C is the concentration of TCAc, P is the rate of formation and k the first order rate coefficient of degradation of TCAc. Solving Eq. (3) for an initial concentration of zero leads to the following equations for TCAc with trichloromethyl groups containing a ^{12}C and ^{13}C atom:

$$^{12}C_t = \frac{^{12}P}{^{12}k}(1 - e^{-^{12}kt}) \quad \text{and} \quad ^{13}C_t = \frac{^{13}P}{^{13}k}(1 - e^{-^{13}kt}) \tag{4}$$

^{12}P and ^{13}P are the rates of formation of TCAc with trichloromethyl groups containing a ^{12}C and ^{13}C atom, respectively. ^{12}k and ^{13}k are the first order rate coefficients of degradation of trichloroacetyl groups in TCAc containing a ^{12}C and ^{13}C atom at the trichloromethyl position, respectively. Hence the isotope ratio of TCAc ($R_{TCAc,t}$) evolves as follows:

$$R_{TCAc,t} = \frac{^{13}C_t}{^{12}C_t} = \frac{^{12}k}{^{13}k} \cdot \frac{^{13}P}{^{12}P} \cdot \frac{1 - e^{-^{13}kt}}{1 - e^{-^{12}kt}} \tag{5}$$

where,

$$\frac{^{12}k}{^{13}k} = \text{AKIE} \tag{6}$$

as

$$k = {}^{12}k + {}^{13}k \tag{7}$$

therefore,

$$^{12}k = \frac{k}{1 + \frac{1}{\text{AKIE}}} \quad \text{and} \quad ^{13}k = \frac{k}{1 + \text{AKIE}} \tag{8}$$

thus,

$$R_{TCAc,t} = \text{AKIE} \cdot \frac{^{13}f \cdot P}{^{12}f \cdot P} \cdot \frac{1 - e^{-\frac{k}{1+\text{AKIE}}t}}{1 - e^{-\frac{k}{1+\frac{1}{\text{AKIE}}}t}} = \text{AKIE} \cdot \frac{^{13}f}{^{12}f} \cdot \frac{1 - e^{-\frac{k}{1+\text{AKIE}}t}}{1 - e^{-\frac{k}{1+\frac{1}{\text{AKIE}}}t}} \tag{9}$$

where AKIE (AKIE = 1.0183 ± 0.0002) is the calculated isotope fractionation factor for TCAc hydrolysis of forest NOM (see Section 3.3), and ^{12}f and ^{13}f are the fractions of ^{12}C and ^{13}C atoms, respectively in NOM given by:

$$^{12}f = 1 - \frac{R_{NOM}}{R_{NOM} + 1} \quad \text{and} \quad ^{13}f = \frac{R_{NOM}}{R_{NOM} + 1} \tag{10}$$

where R_{NOM} is the measured isotopic ratio of forest NOM (R_{NOM} = 0.010925). The $\delta^{13}C$ values of TCAc and chloroform can be calculated using the following equations:

$$\delta^{13}C_{TCAc,t}(‰) = \left(\frac{R_{TCAc,t}}{R_{V-PDB}} - 1\right) \times 1000 \tag{11}$$

and

$$\delta^{13}C_{CHCl_3,t}(‰) = \delta^{13}C_{TCAc,t}(‰) - 1000 \times (\text{AKIE} - 1) \tag{12}$$

The $\delta^{13}C$ values of TCAc and chloroform were plotted versus $[TCAc]_t/[TCAc]_{steady\text{-}state}$ which was calculated using:

$$\frac{[TCAc]_t}{[TCAc]_{steady-state}} = 1 - e^{-kt} \tag{13}$$

where $[TCAc]_t$ and $[TCAc]_{steady\text{-}state}$ are respectively the concentration of TCAc at time t and at steady-state.

The results of the modeling show that chloroform and TCAc become progressively enriched in ^{13}C before reaching the steady-state ($[TCAc]_t/[TCAc]_{steady\text{-}state}$ = 1) (Fig. 5). The calculated carbon isotopic ratio of trichloromethyl groups of chloroform and TCAc at steady state are −28.2‰ and −9.9‰, respectively. These calculated $\delta^{13}C$ values agree well with the carbon isotopic ratios of chloroform and TCAc measured in the field and in the chlorination experiments, suggesting that TCAc likely plays an important role in the natural formation of chloroform. The simulation suggests that the natural chloroform evolves towards an isotopic signature close to the NOM from which it is derived despite substantial carbon isotope fractionation during release of chloroform thanks to

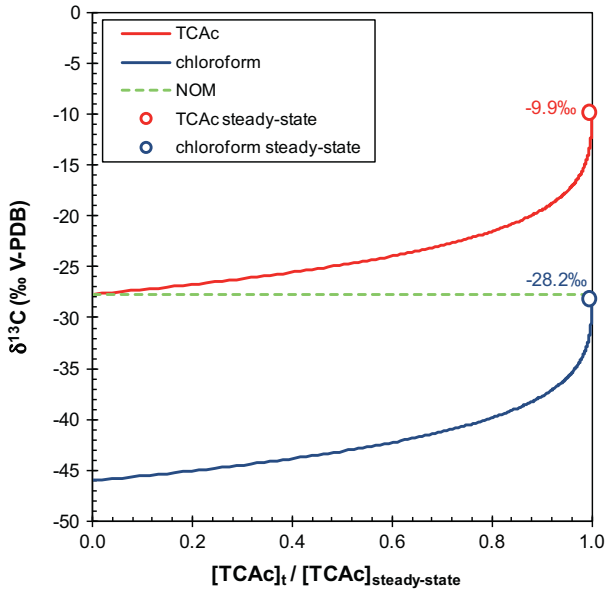

Fig. 5. Model of the carbon isotopic trends of chloroform (blue line), trichloromethyl groups in TCAc (red line), and forest NOM (green dashed line) versus $[TCAc]_t/[TCAc]_{steady\text{-}state}$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the presence of ^{13}C-enriched precursors. Such isotope patterns have to be expected at a steady-state situation because each trichloromethyl group that enters in the TCAc pool has to have the same isotopic composition as the chloroform that leaves the pool if there are no other major entries and if the formation of TCAc is not associated with isotope fractionation. The isotopic fractionation associated with reaction step(s) preceding the TCAc hydrolysis (e.g. chlorination, ring opening) could induced a deviation of the isotope composition of chloroform compared to NOM. In the case where the reaction step(s) preceding the hydrolysis would induced a normal isotope effect (KIE > 1), chloroform formed by hydrolysis would be depleted in ^{13}C compared to NOM. Indeed in the laboratory experiment at pH = 8 when nearly complete hydrolysis of TCAc is observed (Fig. 3), the CF is depleted by about 5‰ compared to NOM. Inversely, if the step preceding the hydrolysis of TCAc would involve an inverse isotope effect (KIE < 1) chloroform would be slightly more enriched in ^{13}C. The steady-state situation between the formation and the hydrolysis of TCAc could be perturbed during some periods of the year which could also lead to some variations of the chloroform isotope ratio around the average value of biomass. Even if the produced CF deviates from NOM by several ‰, it will still be distinctly different from anthropogenic CF (−43.2 to −63.6‰, Hunkeler et al., 2012).

4. Conclusions

Although the carbon isotopic signatures of chloroform and the trichloromethyl group in TCAc are very distinct, the chlorination experiments combined with a mathematical model have revealed that TCAc could play a fundamental role in the formation of chloroform in the terrestrial environment. The strong isotopic enrichment of the trichloromethyl group in TCAc indicates that a fraction of the trichloromethyl groups is released as chloroform by hydrolysis which will then equilibrate into soil-air. Using a mathematical model combined with field data, the present study shows that when the formation of TCAc and hydrolysis reach a steady state, the isotope composition of chloroform is expected to correspond to isotope ratio of NOM while TCAc should be enriched in ^{13}C. This study confirms that TCAc are reaction intermediates which are subsequently degraded in soil into chloroform, and explains why natural chloroform has a similar isotope signature as NOM despite a large carbon isotope fractionation during its release.

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