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Triple-Element Stable Isotope Analysis of Chloromethane Emitted by Royal Fern and Degraded by Club Moss

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Key Points:

- First triple-element isotopic characterization of plant CH_3Cl emission and degradation
- Plant degradation experiments suggest another yet unknown transformation pathway
- Important input data for future isotope based models to improve understanding of global CH_3Cl budget

Supporting Information:

Supporting Information may be found in the online version of this article.

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Abstract Chloromethane (CH_3Cl) is the most abundant natural chlorinated organic compound in the atmosphere playing an important role in catalyzing stratospheric ozone loss. Vegetation emits the largest amounts of CH_3Cl to the atmosphere but its source strength is highly uncertain leading also to large uncertainties in the global budget of CH_3Cl. Triple-element stable isotope analysis may help to reduce uncertainties because it provides additional process-level information compared to conventional quantification methods. In this study we performed experiments to obtain a first triple-elemental isotopic fingerprint (^2H, ^{13}C, ^{37}Cl) of CH_3Cl emitted by a relevant plant species (royal fern, *Osmunda regalis*). Isotopic values of all three elements showed considerable differences compared to isotopic values of industrially manufactured CH_3Cl which bodes well for future applications to distinguish individual sources. Isotopic analysis of potential precursors (rain, methoxy groups) of CH_3Cl in plants revealed no measurable change of hydrogen and chlorine isotopic ratios during formation which may provide a simpler route to estimate the isotopic composition of CH_3Cl emissions. Plant degradation experiments of CH_3Cl were carried out with club moss (*Selaginella kraussiana*) revealing significant isotopic fractionation for all three elements. The fractionation pattern characterized by epsilon and lambda is inconsistent with known biotic dechlorination reactions indicating a yet unreported biotic degradation mechanism for CH_3Cl. Overall, this study provides first insights into the triple-elemental isotopic fingerprint of plant emissions and degradation. The results may represent important input data for future isotope-based models to improve global budget estimates of CH_3Cl and to explore the yet unknown degradation pathways.

Plain Language Summary Chloromethane is the most abundant chlorinated organic compound in the atmosphere. It contributes to the destruction of the ozone layer that protects us from skin cancer and genetic damage. Currently, we do not have a good understanding of the sources and removal processes of chloromethane in the atmosphere. In this paper, we use a technique that takes advantage of the different varieties of a chemical element. These so-called isotopes behave differently during chemical reactions that lead to individual isotopic fingerprints depending on the source or removal process. We used isotopic fingerprints of all three chemical elements in chloromethane and showed that chloromethane produced by a plant (royal fern) differs substantially from chloromethane manufactured by industry. Other plant species such as club moss are able to remove chloromethane from the atmosphere but it is often not clear how this occurs. Isotopic analysis revealed that the studied club moss uses a unique, thus far unknown, way to break down chloromethane. This study demonstrates how information extracted from isotopic fingerprints will help to improve our understanding of sources and removal processes of chloromethane in the atmosphere. It can help to better predict how ozone destruction in the stratosphere affects us in the future.

1. Introduction

Chloromethane (CH_3Cl, methyl chloride) represents the largest natural source of chlorine in the atmosphere currently contributing about 17% of total chlorine (Engel et al., 2018). Chlorine together with other halogens and nitrogen compounds cause the depletion of the stratospheric ozone layer that absorbs most of the sun's ultraviolet radiation known to cause adverse effects in plants and animals (Ravishankara et al., 2009). The sources of CH_3Cl mostly are of natural origin (Carpenter et al., 2014) even though previously unrecognized minor anthropogenic sources have recently been reported (Keppler et al., 2017; Li et al., 2017; Thornton et al., 2016). Despite the large uncertainties in the global budget of CH_3Cl, plants have been suggested to be the major source of CH_3Cl (Carpenter et al., 2014). In this context, large emissions have been reported not only for tropical plants

(Blei et al., 2010; Gebhardt et al., 2008; Saito & Yokouchi, 2008; Xiao et al., 2010) but also for halophytic plants (Bill et al., 2002; Deventer et al., 2018; Harper et al., 2001; Rhew et al., 2003), fern species (Yokouchi et al., 2015), and crops (Harper et al., 1999) also occurring in temperate climate regions. Apart from producing and emitting CH_3Cl, plants are also known to degrade CH_3Cl with the help of bacteria colonizing the leaves (Bringel et al., 2019; Jaeger et al., 2018a; Krober et al., 2021; Nadalig et al., 2011; Saito et al., 2013) which often hampers a more precise quantification of sources and sinks because the net flux is influenced by both (Keppler et al., 2020a). The largest sinks of CH_3Cl are OH radical reaction in the troposphere as well as degradation in soils and oceans (Carpenter et al., 2014). Thus, large gaps in the budget of CH_3Cl persist because the magnitude of emissions from vegetation includes large uncertainties (Bahlmann et al., 2019), and the sink strength by plants is largely unknown.

The formation processes of CH_3Cl in living terrestrial plants have been investigated in a variety of previous studies (Bayer et al., 2009; Nagatoshi & Nakamura, 2007; Rhew et al., 2003; Schmidberger et al., 2010; Wuosmaa & Hager, 1990). Accordingly, biosynthesis of CH_3Cl and other halomethanes not only in plants but also in fungi, algae, and bacteria is primarily related to the so-called HOL genes (harmless to ozone layer), which generate production of halide methyltransferases. These enzymes catalyze production of halomethanes by combining a methyl group from the ubiquitous S-adenosyl methionine (SAM) with a halide ion in a nucleophilic substitution reaction. Even though the exact mechanisms of halomethane biosynthesis by methyltransferases are not fully understood yet their significance for contributing ozone-depleting compounds is undisputed.

Biotic degradation by living plants is, similarly as production, driven by enzymatic processes. Bacteria colonizing the phyllosphere but also other habitats such as the rhizosphere are able to use CH_3Cl as an energy source. A number of different bacterial strains utilizing CH_3Cl as an energy and carbon source have been identified in soils; for example, *Methylobacterium*, *Hyphomicrobium,* and *Aminobacter* (Doronina et al., 1996; McAnulla et al., 2001; McDonald et al., 2005). The metabolic pathway was discovered by Vanelli and colleagues (Vannelli et al., 1999) and first described for *Methylobacterium sp.* CM4 (now *Methylorubrum extorquens* CM4). Accordingly, CH_3Cl is dehalogenated by a methyltransferase/corrinoid-binding protein (CmuA), and the methyl group is transferred to a corrinoid cofactor. This corrinoid-bound methyl group is then further transferred by another methyltransferase (CmuB) to tetrahydrofolate (H_4F). Methyl-H_4F is oxidized to methylene-H_4F and subsequently to formate and CO_2 (Nadalig et al., 2014; Schäfer et al., 2007). In addition to the Cmu pathway, other yet uncharacterized metabolic pathways were identified in bacteria in the phyllosphere of fern trees such as *Friedmanniella* (Krober et al., 2021) or in bacteria from aquatic habitats such as *Leisingera methylohalidivorans* MB2 (Nadalig et al., 2014).

Stable isotope analysis may be beneficial to further elucidate those enzymatic production and consumption processes of CH_3Cl, and they have been applied in various studies (Jaeger et al., 2018b; Keppler et al., 2020b; Miller et al., 2004; Nadalig et al., 2014). Isotopic approaches also aimed at quantifying the contribution of emission and consumption of CH_3Cl by plants in the atmosphere (Rhew, 2011; Saito & Yokouchi, 2008; Saito et al., 2013; Weinberg et al., 2013) and in relation to the other sources and sinks (Bahlmann et al., 2019; Keppler et al., 2005; Keppler et al., 2020a). Isotopic analysis in CH_3Cl was shown to be particularly useful when the stable isotopes of all three available elements were investigated because different biotic and abiotic degradation processes showed distinct isotopic patterns which may help to identify or even quantify different processes (Horst et al., 2019; Keppler et al., 2020a). Particularly hydrogen and chlorine provided important information because, other than carbon, they were not involved in each reaction and hence did not undergo changes in their isotopic composition during such reactions. For instance, chemical decomposition via hydroxyl (OH) radical reactions showed large isotopic shifts for hydrogen (Keppler et al., 2018) but negligible shifts for chlorine whereas microbial degradation showed the opposite: small shifts for hydrogen isotopes but large shifts for chlorine isotopes (Keppler et al., 2020a). This could be explained with the underlying reaction mechanisms which either cleave the H-C bond (OH-radical reaction) or the Cl-C bond (microbial dechlorination). Keppler and co-authors (Keppler et al., 2020a) also used isotopic constraints to confirm that the two metabolic pathways of CH_3Cl dechlorination by *Methylorubrum extorquens* CM4 and *Leisingera methylohalidivorans* MB2 are actually different. This may have further implications because CM4 and MB2 might be representative for terrestrial and marine organisms, respectively. It was argued that, if the metabolic pathways are actually representative for each respective environment, this would provide a strong isotopic tool for the distinction of terrestrial and marine CH_3Cl degradation pathways.

Triple-element isotopic studies of CH_3Cl are, however, very scarce and have only been carried out for abiotic degradation pathways such as OH and Cl radical reactions (Keppler et al., 2020a) and hydrolysis and transhalogenation reactions (Horst et al., 2019). One study investigated microbial dechlorination pathways using cell extracts (Keppler et al., 2020a). Till now, there are no triple-element isotopic studies of emissions or degradation of CH_3Cl by living plants. Several studies applied dual element isotope analysis (stable isotopes of H and C) to disentangle degradation processes (Jaeger et al., 2018a; Nadalig et al., 2014) and to isotopically characterize emissions of CH_3Cl (Jaeger et al., 2018b) but stable chlorine isotope analysis has not been applied yet in this context.

Hence, the current study aimed at exploring the benefits of triple-element stable isotope analysis for the characterization of CH_3Cl emitted and consumed by vegetation. *Osmunda regalis* (royal fern) was used to isotopically characterize CH_3Cl production. *O. regalis* is a common fern species growing in temperate and subtropical regions. This fern species was chosen because it is known to produce large amounts of CH_3Cl (Jaeger, Besaury, Rohling, et al., 2018; Yokouchi et al., 2007, 2015). In addition, experiments with a club moss (*Selaginella kraussiana)* were carried out to investigate degradation of CH_3Cl and associated isotopic fractionation. Results are discussed in comparison to known fractionation patterns obtained in previous experiments with bacterial cell cultures. *S. kraussiana* has been used in this current study as it was observed in preliminary investigations at the Botanical Garden of Heidelberg University for its potential to strongly degrade CH_3Cl.

2. Materials and Methods

2.1. Sampling of Rainwater and Conversion of Chloride to CH_3Cl

Rainwater samples were collected at three different sites between April and June 2021 in order to determine the stable chlorine isotopic composition of the primary chlorine source for plants in terrestrial environments which are not located at the coast or close to halite deposits. Three sampling sites were chosen representing urban (Heidelberg, Leipzig) and rural (Rastenberg) environments that stretch along a transect of about 400 km in North-Western direction following the main wind direction in Germany. Two replicate samples were collected directly in the botanical garden of the city of Heidelberg from the roof of a greenhouse. Two replicate samples were taken at a rural site in central Germany (Rastenberg, Thuringia). Two more individual samples were collected in Leipzig, Saxony. All samples (1–2.5 L) were concentrated down to 30 mL by boiling. Containing Cl ions were precipitated as AgCl by adding $AgNO_3$. Next, AgCl was filtered off under safelight conditions and filters and iodomethane (CH_3I, 10 times excess concentration) sealed in borosilicate glass tubes. The tubes were placed in a furnace held at 80°C for 48 hr to react AgCl and CH_3I to CH_3Cl. The whole procedure followed established protocols (Eggenkamp, 2014; Gilevska et al., 2015).

2.2. Plant Sampling and Incubation Experiments

In preliminary field experiments using larger teflon-foil chambers (~11 l volume), we monitored several plants in the Botanical Garden of Heidelberg University for their potential to degrade CH_3Cl at ambient CH_3Cl concentrations (at around 500 to 600 pptv). The plants were not physically damaged before and during enclosure. We found several CH_3Cl degraders such as *Polystichum setiferum, Selaginella kraussiana, and Dryopterix filix-mas* that reduced the atmospheric concentration inside the chamber by up to 30% within 15–30 min yielding CH_3Cl degradation rates in the range of 1–5 ng g_{dw}^{-1} h^{-1}. For the current study, we chose *S. kraussiana* for investigation of degradation mechanisms with isotopic methods.

Fresh samples of *S. kraussiana* were collected in July and September 2019 and April 2021, respectively, at a greenhouse of the Heidelberg Botanical Garden. The greenhouse is characterized by high relative humidity (RH >90%). The whole stem of the living plant including attached leaves was cut and the sampled plant material was subsequently incubated in the lab to explore biotic degradation of CH_3Cl by plant-associated microbes. We did not expect any additional abiotic reactions to disturb the results because reactions such as hydrolysis previously showed rate constants of about 0.002 d^{-1} (Horst et al., 2019) which is several orders of magnitude slower than observed in previous microbial degradation experiments (Jaeger et al., 2018b). For incubation, the plant material was evenly distributed over nine vessels (~7–9 g wet weight plant material in each 170 ml custom-made glass cylinder). All vessels were sealed gas-tight at the same time under the fume hood to ensure all of them contain the same (lab-) air and therefore have the same initial CH_3Cl mixing ratio and isotopic ratios. After sealing, we

artificially increased the CH_3Cl mixing ratio of the vessels by injecting 2 ml of a diluted CH_3Cl reference (~1000 parts per million by volume (ppmv) CH_3Cl in N_2) into each of these vessels and waited for equilibrium to be established before measuring the initial CH_3Cl concentration $t = 0$ in each vessel. Concentrations were checked frequently and samples for isotope analysis were collected at selected time intervals.

Emission experiments were carried out with plant material of *O. regalis*. The samples of this plant were collected in July and September 2019. In contrast to *S. kraussiana,* the collected samples of *O. regalis* grew in the outside area of the Heidelberg Botanical Garden under the local climatic conditions. Whole fern fronds were cut off and brought to the lab for subsequent incubation. As for *S. kraussiana,* 7–9 g wet weight of *O. regalis* were put into the same 170 ml custom-made glass cylinders used for the degradation experiments and all vessels were sealed at the same time under the fume hood. The temperature in the lab (23.5 ± 1°C) was constant throughout the whole incubation period. Seven and eight experiments were run in parallel during the two experimental campaigns, respectively. Concentrations were checked frequently and samples for isotopic analysis were collected after the last concentration measurement (Figures S3 and S4 in Supporting Information S1).

Microcosm experiments as those described above represent very different environmental conditions for a plant compared to its natural habitat. It might be questioned whether the data collected in the laboratory is representative for field conditions. Isotopic data, however, seems to be quite robust in this context. For example, in a laboratory study Harper et al. (2001) measured a $\delta^{13}C$ of −65.7 ± 3.4 ‰ VPDB for CH_3Cl emitted by saltwort (*Batis maritima*) which is consistent with the values of −62 ‰ VPDB reported for emissions by this plant in the field (Bill et al., 2002) indicating a good agreement between emission data collected in the laboratory and in the field.

Blank measurements with reaction vessels only containing CH_3Cl without plant material in the vessels were carried out in order to evaluate whether any losses of CH_3Cl occurred, and if so, whether they caused isotopic fractionation. For this purpose, a CH_3Cl/N_2 gas mixture was filled into the vessels creating a final mixing ratio of 9.5 ± 0.5 ppmv. Samples were taken at $t = 0$ and after 25 hr ($t = 1$) which was the longest incubation period. Both the concentration in the reaction vessels and the stable carbon and chlorine isotope values of the blank samples were measured. Whereas the mixing ratio and the stable carbon isotopes only varied within analytical uncertainty, stable chlorine isotopes showed a slightly larger shift over time and a linear correction was applied as described more in detail (Text S2 in Supporting Information S1). Control measurements were also carried out with dried plant material to evaluate abiotic reactions that might occur alongside biotic reactions. In these experiments, we only observed a very small concentration decrease, which may not change the isotopic composition (Text S2 in Supporting Information S1).

2.3. Quantification of CH_3Cl

The CH_3Cl mixing ratio in each incubation vessel was determined at different times throughout the experiments. For quantification, gas chromatography–mass spectrometry (GC/MS) was used (HP 5973 mass selective detector; Hewlett-Packard/Agilent Technologies, Palo Alto, CA, USA). We extracted 100 µL headspace directly from the incubation vessel and injected it with a 100 µL gas-tight Luer lock syringe (SGE Analytical Science, Australia) onto the GC column (GS Gaspro 60 m × 0.32 mm; 1 mL/min constant flow, 150°C isothermal) using a split ratio of 5:1. Mass spectrometry was carried out in SIM mode using the masses $m/z = 50$, $m/z = 51$, $m/z = 52$, and $m/z = 53$.

The GC/MS system was calibrated by measuring a diluted reference (99.8% CH_3Cl in N_2, AirLiquide, France) at different mixing ratios with $n = 3$. For the samples obtained from the incubation of *S. kraussiana*, a four-point calibration in the range from 0 to 10 ppmv CH_3Cl was applied. Due to high CH_3Cl emission rates observed during incubation of *O. regalis*, we conducted a nine-point calibration in the range from 0 to 75 ppmv CH_3Cl for quantification of mixing ratios in the incubation vessels. At selected time intervals, samples for stable isotope analysis were taken. For this purpose, 25–30 ml of gas were removed from the incubation vessels, passed through an Ascarite® trap to remove CO_2, and subsequently transferred to 12 ml pre-evacuated exetainers to be analyzed at a later time for measurements of stable hydrogen, carbon, and chlorine isotope values of CH_3Cl. Furthermore, 15 mL of the CH_3Cl reference gas that was supplemented to the *S. kraussiana* incubation vessels was filled in exetainers for measuring the isotopic composition before changes due to degradation occurred.

2.4. Stable Hydrogen and Stable Carbon Isotope Analysis of CH_3Cl

Stable hydrogen and stable carbon isotopic ratios of CH_3Cl (expressed as δ^2H-CH_3Cl and $\delta^{13}C$-CH_3Cl) were measured by an in-house cryogenic pre-concentration unit coupled to gas chromatography (HP 6890, Agilent

Technologies, Palo Alto, CA) and isotope ratio mass spectrometry (IRMS) (Isoprime, Manchester, UK), as previously described (Greule et al., 2012; Keppler et al., 2020a; Nadalig et al., 2013). Deviating from the cited methods, an in-house modified interface was used which is based on the GC Combustion III Interface from ThermoQuest Finnigan (Bremen, Germany). For stable hydrogen isotope analysis of CH_3Cl, a ceramic tube reactor (length 320 mm, 0.5 mm i.d.) heated to 1,450°C was used for high-temperature conversion. For stable carbon isotope analysis the oxidation reactor (length 320 mm, 0.5 mm i.d.) contained Cu/Ni/Pt wires inside (activated by oxygen) and was operated at 960°C. The CH_3Cl working standard was calibrated against the IAEA standards NBS 22, LVSEC (carbon), VSMOW and SLAP (hydrogen) using TC/EA-IRMS (elemental analyzer-isotopic ratio mass spectrometry, IsoLab, Max Planck Institute for Biogeochemistry, Jena, Germany), yielding the following values: $\delta^{13}C$: −32.84 ± 0.06 ‰ VPDB (n = 11, 1σ) and $\delta^{2}H$: −140.1 ± 1.0 ‰ VSMOW (n = 10, 1σ). The H_3^+ factor, determined daily during this investigation (~2-month period), was in the range 5.22–5.35 ppm/nA.

2.5. Stable Chlorine Isotope Analysis of CH_3Cl

Stable chlorine isotope analysis was carried out by applying gas chromatography coupled via a heated transfer line to multiple-collector inductively coupled plasma mass spectrometry (GC-MC-ICPMS, Neptune, Thermo Fisher Scientific, Germany) according to previously published protocols (Horst et al., 2017; Renpenning et al., 2018). Up to 6 mL of gas collected during plant experiments were extracted from the vials using a 10 mL gas-tight syringe equipped with a valve (VICI precision sampling). In order to inject such large amounts of gas the GC was operated in splitless mode and equipped with a cryotrap as described in Keppler et al. (2020a). This cryotrap was cooled with liquid nitrogen to assure loss-less collection of the injected sample gas. After cryofocussing was complete, the trap was immersed in warm water (40°C) in order to release the analytes onto the chromatographic column (ZB1 Phenomenex, 60m, 0.32 ID, 2 mL/min constant flow, 30°C isothermal). For analysis of CH_3Cl obtained from conversion of Cl^- in rainwater samples, sealed borosilicate tubes were inserted into 120 mL serum bottles, flushed with nitrogen, and capped with gray PTFE-coated stoppers (Wheaton®). Before analysis these bottles were shaken to break the tube in the bottle. Several aliquots were injected in split mode (1:10) keeping the GC column at 120°C, which was sufficient to separate CH_3Cl and CH_3I. Two analyses of a reference-CH_3Cl were injected before each sample to determine the raw-$\delta^{37}Cl$ of each sample relative to this reference (see Equation 1). Raw-$\delta^{37}Cl$ values were then linked to SMOC and normalized to the currently used scale by applying a linear two-point calibration approach using organic in-house reference material (trichlorethylene TCE2 and TCE6, as well as CH_3Cl) (Horst et al., 2017).

2.6. Stable Carbon and Hydrogen Isotope Analysis of Plant Methoxy Groups

Stable hydrogen and carbon isotope values of methoxy groups ($\delta^{2}H$-OCH_3 and $\delta^{13}C$-OCH_3 values) were measured using the "HI method" described in previous investigations (Greule et al., 2012; Keppler et al., 2020b; Nadalig et al., 2013). Briefly, hydriodic acid (250 µL) was added to dried and milled *O. regalis* (30 and 100 mg for carbon and hydrogen isotopes, respectively) and *S. kraussiana* (25 and 65 mg for carbon and hydrogen isotopes, respectively) samples in crimp-top glass vials (1.5 ml). The vials were sealed with crimp caps containing PTFE-lined butyl rubber septa (thickness 0.9 mm) and incubated for 30 min at 130°C. After equilibration at room temperature, aliquots of the generated CH_3I in the headspace (carbon: 70 µL; hydrogen: 90 µL) were directly injected into a Hewlett Packard HP 6890N gas chromatography system (Agilent Technologies, Palo Alto, CA) coupled to a Delta^{PLUS} XL isotope ratio mass spectrometry system (ThermoQuest Finnigan, Bremen, Germany).

All $\delta^{2}H$-OCH_3 and $\delta^{13}C$-OCH_3 values were normalized by applying a two-point linear calibration approach using stable isotope values of the three working standards HUBG1, HUBG2 and HUBG3 which all were calibrated against international reference substances at the IsoLab of the Max Planck Institute for Biogeochemistry in Jena (Germany). The calibrated $\delta^{2}H$ values versus VSMOW were −102.0 ± 1.3 ‰ (n = 32, 1σ) for HUBG2 and −272.9 ‰ ± 1.5 ‰ (n = 11, 1σ) for HUBG3. The calibrated $\delta^{13}C$ values versus VPDB were −50.31 ± 0.16 ‰ (n = 14, 1σ) for HUBG1 and +1.60 ‰ ± 0.12 ‰ (n = 16, 1σ) for HUBG2. Details of the calibration procedure are provided in the studies by Greule and co-authors (Greule et al., 2019, 2020).

2.7. Reporting Isotopic Compositions and Isotopic Fractionation

Isotopic values are expressed in the delta notation according to the following equation:

$$\delta^{i}E\,(‰, Ur) = \frac{(R)\text{sample}}{(R)\text{standard}} - 1 \quad (1)$$

where ^{i}E indicates ^{2}H, ^{13}C, ^{37}Cl and R is the isotopic ratio $^{2}H/^{1}H$, $^{13}C/^{12}C$, and $^{37}Cl/^{35}Cl$. Delta values are expressed in per mil (‰) or rey (Ur) relative to the international standards VSMOW (Vienna Standard Mean Ocean Water) for hydrogen ($\delta^{2}H_{VSMOW}$), VPDB (Vienna Pee Dee Belemnite) for carbon ($\delta^{13}C_{VPDB}$), and SMOC (Standard Mean Ocean Chloride) for chlorine ($\delta^{37}Cl_{SMOC}$) (Brand et al., 2014). In this paper, isotopic values are reported in per mil notation.

Isotopic fractionation (ε) is usually calculated by using the Rayleigh equation which characterizes the constant change of the isotopic composition of the substrate reservoir due to a preferential loss of heavy or light isotopes caused by a reaction or process (Mariotti et al., 1981):

$$ln\left(\frac{\delta^{i}E+1}{\delta^{i}E_{0}+1}\right)\approx ln(f)\varepsilon_{x} \tag{2}$$

where $\delta^{i}E$ is the isotopic value ($\delta^{2}H$, $\delta^{13}C$, $\delta^{37}Cl$) of a compound after partial degradation, $\delta^{i}E_{0}$ is the initial delta value ($\delta^{2}H_{0}$, $\delta^{13}C_{0}$, $\delta^{37}Cl_{0}$), and f is the fraction of compound remaining after partial degradation. The Rayleigh equation derives the isotopic fractionation for first-order or pseudo-first-order reactions (van Breukelen, 2007).

Λ values (lambda) describe the ratio of the isotopic fractionation of two different isotopic systems (van Breukelen, 2007). Λ values should be determined graphically by plotting isotopic data of one element versus the isotopic data of another element determined from samples of the same experiment (Ojeda et al., 2019). When isotopic data was obtained in separate experiments, Λ-values may also be estimated according to the following relationship (van Breukelen, 2007):

$$\Lambda_{x/y}\approx\frac{\varepsilon_{x}}{\varepsilon_{y}} \tag{3}$$

where ε_{x} and ε_{y} are the isotopic fractionations of two different elements x and y determined for the same mechanism in a certain compound.

3. Results and Discussion

3.1. Emission Rates and Isotopic Composition of CH_3Cl by *Osmunda Regalis*

3.1.1. Emission Rates of CH_3Cl by *Osmunda Regalis*

Emission rates obtained during the experiments ranged from 0.8 to 22 µg g_{dw}^{-1} d^{-1} and were thus comparable to those reported in the literature for *O. regalis* (Jaeger et al., 2018b) and other species of the *Osmunda* family (Yokouchi et al., 2007, 2015) but were higher than median emissions reported for a tropical rainforest (0.72 µg g_{dw}^{-1} d^{-1} (Saito et al., 2008)). The relatively large range of emission rates may result from plant material of different growth stages and sampling time (July and September) which, despite this variability, provide a more realistic picture of emission of CH_3Cl from *O. regalis*. Due to our incubation method we also cannot completely exclude additional variation of emission rates due to wounding and stress. Chloride availability might also be considered a limiting factor because cutting the branches theoretically interrupts water and thus chloride supply. In our experiments we could not detect any signs of decreasing chloride supply. The CH_3Cl concentration in the reaction vessels was constantly increasing which indicates that chloride was available throughout our experiments (Figures S4 and S5 in Supporting Information S1). Overall, *O.regalis* was well suited for the planned experiments because it provided the high amounts of CH_3Cl necessary for isotopic analysis. Information regarding determination of emission rates is provided in Text S1 in Supporting Information S1.

3.1.2. Isotopic Composition of CH_3Cl Emitted by *Osmunda Regalis*

Chloromethane emissions collected from *O. regalis* were also measured for their isotopic composition. Average $\delta^{2}H$-CH_3Cl, $\delta^{13}C$-CH_3Cl, and $\delta^{37}Cl$-CH_3Cl values were -242 ± 39 ‰ VSMOW, -83.9 ± 4.2 ‰ VPDB, and -0.91 ± 0.84 ‰ SMOC, respectively (Figure 1). All data showed relatively small variations over the entire incubation period (up to 16 hr) and thus may be considered representative for CH_3Cl emissions by *O. regalis* (Figure 1). This consistency may also be an indicator that, in contrast to emission rates, stress, and wounding are not affecting the isotopic results. Measured $\delta^{37}Cl$ represent the first chlorine isotopic characterization of a natural source of CH_3Cl and hence these values provide a first estimate for the magnitude of $\delta^{37}Cl$ values of CH_3Cl emitted by plants. Stable hydrogen and stable carbon isotopic values largely agree with those obtained in a

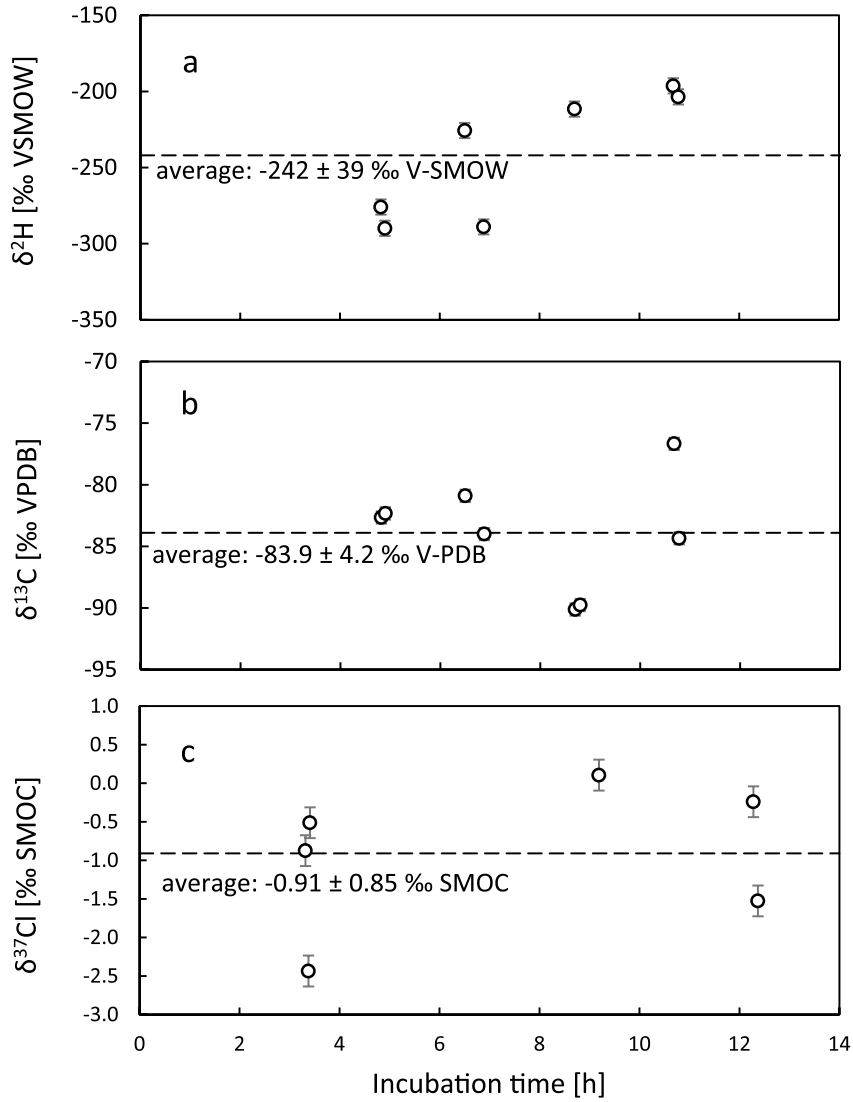

Figure 1. Isotopic values of hydrogen (a), carbon (b), and chlorine (c) of CH_3Cl emitted by *O. regalis*. Error bars indicate the overall uncertainty of the corresponding method which is usually 5 ‰ (δ^2H), 0.5 ‰ ($\delta^{13}C$), and 0.2 ‰ ($\delta^{37}Cl$) for a single analysis.

former study for the same fern species (Table 3): $\delta^2H = -202 \pm 10$ ‰ VSMOW and $\delta^{13}C = -97 \pm 8$ ‰ VPDB (Jaeger et al., 2018b). Other comparable experiments have, thus far, only been conducted in studies measuring stable carbon isotopes for a variety of higher living plants which showed a range of −56 ‰ to −114 ‰ VPDB (Saito & Yokouchi, 2008; Saito et al., 2008). In general, $\delta^{13}C$-CH_3Cl emitted by plants seem to be more depleted than in CH_3Cl emitted by other natural sources such as biomass burning, wood rotting fungi, and salt marshes (Bahlmann et al., 2019; Keppler et al., 2005; Saito & Yokouchi, 2008). Overall, isotopic values for all elements are slightly ($\delta^{37}Cl$) to more considerably (δ^2H, $\delta^{13}C$) depleted compared to their respective standards (SMOC, VSMOW, VPDB).

The measured isotopic values for hydrogen, carbon, and chlorine may serve as an isotopic fingerprint of CH_3Cl emitted by *O. regalis* and possibly also for other plants if isotopic values show comparable ranges. The strongly depleted δ^2H and $\delta^{13}C$ values of CH_3Cl emitted by *O. regalis* are clearly different from two available industrially manufactured CH_3Cl (δ^2H −127 ± 20 ‰ VSMOW and $\delta^{13}C = -45 \pm 15$ ‰ VPDB, working reference compounds used in the stable isotope labs at Heidelberg University and UFZ in Leipzig) or CH_3Cl emitted from salt marshes (δ^2H −185 ± 18 ‰ VSMOW and $\delta^{13}C = -49.1 \pm 3$ ‰ VPDB (Keppler et al., 2020b)). For stable chlorine isotopes no previous emission data is available but the slightly depleted values ($\delta^{37}Cl = -0.91 \pm 0.89$ ‰

Table 1
δ^{37}Cl Values of Chloride in Rainwater Samples Taken at Three Different Sites in Germany Between April and June 2021

	Sampling date	Volume (L)	Concentration (Cl^- mg/L)	δ^{37}Cl of Cl^-‰ SMOC	Σ ‰	*n*
Heidelberg 1a	27 April 2021	1.2	0.80	−0.28	0.04	3
Heidelberg 1b	27 April 2021	1.2	0.56	−0.21	0.03	3
Rastenberg 1a	5 June 2021	2.5	n.d.	−0.89	0.07	3
Rastenberg 1b	5 June 2021	2.5	n.d.	−0.94	0.02	3
Leipzig 1	7 June 2021 (2 mm)	1.2	n.d.	−0.52	0.09	3
Leipzig 2	20 June 2021 (14.8 mm)	2.5	n.d.	−0.09	0.08	3

Note. The uncertainty indicates the standard deviation for triplicate measurements of the corresponding sample.

SMOC) compared to seawater differ substantially from reported values of two industrially manufactured CH_3Cl samples (δ^{37}Cl-CH_3Cl = +6.0 ± 0.1‰ SMOC (Keppler et al., 2020a)). These results suggest that all, thus far, isotopically characterized sources of CH_3Cl may indeed be distinguishable when the isotopes of three different elements are measured and compared. Published studies investigating stable carbon isotopes in CH_3Cl provided first evidence that some major sources may be distinguishable based on their carbon isotopic composition alone. For instance, CH_3Cl from biomass burning showed a δ^{13}C-CH_3Cl value of −47 ‰ VPDB (weighted mean of C3 and C4 plants, range of −38 to −68 ‰ VPDB) (Czapiewski et al., 2002; Thompson et al., 2002) which is, on average, more enriched in ^{13}C compared to CH_3Cl emissions from living plants (−56‰ to −114‰ VPDB (Saito & Yokouchi, 2008; Saito et al., 2008)). Even higher δ^{13}C-CH_3Cl; that is, CH_3Cl was more enriched in the heavy isotope, were reported for emissions from oceans which covered a range of −12 to −43 ‰ VPDB and an estimated isotopic source signature of −36 ‰ VPDB (Bahlmann et al., 2019). Adding hydrogen and chlorine isotope analysis may provide additional isotopic tools to also identify and quantify those sources that are currently not distinguishable with stable carbon isotope analysis alone.

3.1.3. Isotopic Fractionation During Formation of CH_3Cl From Precursors

Isotopic fractionation during formation of CH_3Cl from their potential precursors was also investigated during this study by additionally determining the δ^{37}Cl of chloride in rainwater samples and the δ^{13}C and δ^{2}H of the plant methoxy pool which may be representative for the isotopic composition of the entire methyl pool in plants (Jaeger et al., 2018b). The δ^{37}Cl of chloride in rainwater samples varied between −0.94 ‰ and −0.09 ‰ SMOC which is consistent with the δ^{37}Cl of rainwater sampled in North America (Koehler & Wassenaar, 2010). There seems to be a tendency toward more depleted values for the rural site Rastenberg compared to the urban sites Heidelberg and Leipzig. Also, the rain event with higher precipitation (Leipzig 2, 14.8 mm) produced a more ^{37}Cl enriched isotopic value than the short precipitation event at Leipzig 1 (2 mm). The reason for this variation is unclear but it might be associated with additional heterogeneous reactions of chloride in the atmosphere such as the formation of HCl from primary sea salt aerosols. HCl is formed by acidification of the salt-containing aerosol by atmospheric sulfuric and nitric acid which is an isotope fractionating mechanism (Volpe & Spivack, 1994). Chloride in marine aerosols is produced by bubble bursting and wave-crest tearing mechanisms (Erickson & Duce, 1988) but most of this chloride is quickly removed from the atmosphere by wet or dry deposition (Koehler & Wassenaar, 2010). Hence HCl might be the largest source of chloride in the study area which is situated several hundred kilometers from the coast. The variability of δ^{37}Cl of chloride in rain in the studied region is small. The average of −0.49 ± 0.33 ‰ SMOC and the range of 0.85 ‰ may be considered as a first best estimate of δ^{37}Cl values in rainwater for South and Central Germany (Table 1).

Methoxy groups of plants have been shown to be direct precursors of methyl halides in abiotic as well as biotic reactions with abiotic reactions depending largely on temperature (Derendorp et al., 2012; Hamilton et al., 2003; Keppler et al., 2004; Wishkerman et al., 2008). The isotopic composition of plant methoxy groups might be considered representative for the entire methyl (CH_3) pool in plants. Although this assumption is a large simplification requiring further validation, as a first approximation it might be applied to indirectly characterize the stable hydrogen and stable carbon isotope composition of the methyl group in SAM, the precursor for enzymatic formation of CH_3Cl (Jaeger et al., 2018b; Keppler et al., 2004). *S. kraussiana* showed δ^{13}C-OCH_3 and δ^{2}H-OCH_3 values of −47.1 ‰ VPDB and −313 ‰ VSMOW, respectively (Table 2). For *O. regalis*, which was investigated for its CH_3Cl emissions in the current study, δ^{13}C-OCH_3 and δ^{2}H-OCH_3 values were found to be −60.8 ‰ VPDB and

Table 2
$\delta^{13}C$ and $\delta^{2}H$ Values of Methoxy Groups of the Two Selected Plant Species

	$\delta^{13}C$ [‰ VPDB]	σ ($n = 5$)	$\delta^{2}H$ [‰ VSMOW]	σ ($n = 5$)
S. kraussiana EW1	−46.76	0.19	−314.0	2.2
S. kraussiana EW2	−47.46	0.19	−312.5	2.1
O. regalis EW1	−60.84	0.20	−260.1	2.5
O. regalis EW2	−60.75	0.19	−257.5	2.4

Note. The uncertainty indicates the propagated error including the analytical uncertainty of measurements (90% Student factor) and the weighted analytical uncertainty of the scaling standards.

−259 ‰ VSMOW, respectively (Table 2). The $\delta^{2}H$ of *O. regalis* matches a previously published value of −259 ‰ VSMOW (Jaeger et al., 2018b). Generally, $\delta^{2}H$ of the two plants are within or close to the range of −200 to −296 ‰ reported for different plant material by Greule et al. (2012), while $\delta^{13}C$-OCH_3 are within the range of −40.5 to −77.2 ‰ published for different plant material by Keppler et al. (2004). However, the relatively negative isotopic values for carbon and hydrogen in methoxy groups suggest a large fractionation during plant growth. Rainwater and atmospheric CO_2 are the assumed sources of hydrogen and carbon in plants with approximate source values of $\delta^{2}H = -55$ ‰ (annual longterm average of the GNIP station Karlsruhe nearby Heidelberg (IAEA/WMO, 2021)) and $\delta^{13}C = -8.5$ ‰ (Schaeffer et al., 2008). Hence, the isotopic composition of methoxy groups and presumably the entire methyl pool of *O. regalis* indicates a shift of about −200 ‰ for $\delta^{2}H$-OCH_3

Table 3
Overview of Isotopic Data of CH_3Cl Obtained From Previous Emission and Degradation Experiments

Emission experiments							
	$\delta^{2}H$	$\delta^{13}C$	$\delta^{37}Cl$				
Species	‰ VSMOW	‰ VPDB	‰ SMOC			Comment	Reference
Tropical plants		−87.4 ± 12.3				average 12 tropical plants	Saito and Yokouchi, 2008
Fern trees		−61.9 ± 9.7				average 3 fern trees	Yokouchi et al., 2007
Osmunda regalis	−202 ± 10	−97 ± 8					Jaeger et al., 2018a, 2018b
Osmunda regalis	−242 ± 39	−83.9 ± 4.2	−0.91 ± 0.84				this study
Batis maritima (saltwort)		−65.7 ± 3.4					Harper et al., 2001
Solanum tuberosum (potato)		−62.7 ± 1.3					Harper et al., 2001
Phellinus pomaceus (mycelium of fungus)		−42.3 ± 0.7					Harper et al., 2001
Degradation experiments							
	ε_H	ε_C	ε_{Cl}	$\lambda_{H/C}$	$\lambda_{C/Cl}$		
Microbial degradation in soils	−29 to −54	−37 to −59					Keppler et al., 2020a, 2020b; Jaegeret al., 2018a; Miller et al., 2004
Marine bacteria	0 to −20	−76 to −92				unknown pathway	Keppler et al., 2020a, 2020b; Nadalig et al., 2014
Cyathea cooperi, Dryopteris filix-mas (ferns)	−8 ± 19‰	−39 ± 13‰		≈0.2			Jaeger et al., 2018b
Methylorubrum extorquens CM4	−35 ± 5	−42 ± 1		≈0.8			Nadalig et al., 2013 Nadalig et al., 2014
Hyphomicrobium sp. MC1	−27	−38					Nadalig et al., 2013
Methylorubrum extorquens CM4	−54 ± 8	−59 ± 6	−10.9 ± 0.7	0.6 ± 0.1	5.1 ± 0.1	Cmu pathway, terrestrial origin	Keppler et al., 2020a, 2020b
Leisingera methylohalidovorans MB2	−20 ± 5	−92 ± 16	−9.4 ± 0.6	0.9 ± 0.1	9.1 ± 0.1	unknown pathway, marine origin	Keppler et al., 2020a, 2020b
Selaginella kraussiana (club moss)	−77 ± 12	−10.8 ± 2.5	−5.7 ± 0.5	≈7.1	≈1.9		this study

Note. Rounded lambda values were calculated according to Equation 3 when experiments were conducted under similar experimental conditions.

and about −50 ‰ for δ^{13}C-OCH_3 values compared to the primary sources of hydrogen and carbon. For *S. kraussiana* this shift is about −260 ‰ for δ^{2}H-OCH_3 and −40 ‰ for δ^{13}C-OCH_3 values. The systematics of ^{2}H patterns of methoxy groups in plants are controlled by the plants source water (usually precipitation) and are related to the C1 metabolism including the transfer of the methyl group of SAM as it was recently proposed by Greule et al. (2021). As for carbon, both carbon fixation in photosynthesis and C1 biosynthesis; that is, the transfer of the methyl group of SAM and/or methionine, may largely contribute to carbon isotope fractionation.

These additional measurements (Tables 1 and 2) indicated that the δ^{2}H-CH_3Cl values of *O. regalis* are, within analytical uncertainty, indistinguishable from the δ^{2}H-OCH_3 values in *O. regalis* whereas δ^{13}C-CH_3Cl values of CH_3Cl released by *O. regalis* are about 23 ‰ depleted in ^{13}C compared to the methoxy pool in the investigated fern samples. Similarly to hydrogen isotopes, the δ^{37}Cl values of CH_3Cl emissions by *O. regalis* are indistinguishable from the potential precursor-δ^{37}Cl; the chloride in the collected rainwater. These results suggest that formation of CH_3Cl in plants cause rather small and negligible isotopic fractionation for hydrogen and chlorine. This might be explained with none of the three hydrogen atoms of the methyl group (stemming from SAM) exchanging or reacting during formation of CH_3Cl and presumably only negligible secondary isotope effects occur. For chlorine, the insignificant isotopic fractionation may be rationalized with the absence of any reaction of Cl ions until formation of CH_3Cl takes place. Apparently, uptake and transport of chlorine ions within *O. regalis* only cause small and insignificant isotopic fractionation. In contrast, results for carbon isotopes in CH_3Cl emitted by *O. regalis* indicate substantial isotopic fractionation of about −23 ‰, which is likely caused by a primary isotope effect due to bond cleavage of the methyl group from SAM or methoxy groups (Keppler et al., 2020b). It is conceivable that these observed isotopic fractionation pattern may also be found for other important plant species since formation of CH_3Cl is mainly linked to SAM as precursor and hence the formation mechanism and associated isotope effects might be similar.

3.2. Degradation and Isotopic Fractionation of CH_3Cl by *Selaginella Kraussiana*

3.2.1. Degradation Rates of CH_3Cl

The main objective of this part of the study was to explore triple-element isotopic analysis as a tool to identify potential reaction mechanisms. Current state of the art methods are not able to measure the δ^{2}H-CH_3Cl, δ^{13}C-CH_3Cl, and δ^{37}Cl-CH_3Cl values of ambient mixing ratios of CH_3Cl of 500–600 parts per trillion (pptv) and below. Therefore, degradation experiments were carried out by spiking reaction vessels containing *S. kraussiana* with CH_3Cl to produce a mixing ratio of 10 ppmv in the incubation vial. During incubation, absolute CH_3Cl mixing ratios in the flasks approximately halved within 24 hrs resulting in degradation rates between 0.6 and 1.5 μg g_{dw}^{-1} d^{-1}. To the best of our knowledge, there are no CH_3Cl degradation rates published yet for *S. kraussiana* but several preliminary and unpublished experiments with this plant showed rates in the same order of magnitude. CH_3Cl degradation by *S. kraussiana* is in the same order of magnitude as those rates previously observed for several fern species (0.3–17 μg g_{dw}^{-1} d^{-1}; Jaeger et al., 2018b) when spiked with 5 ppmv CH_3Cl. Observed rates are considerably higher than those observed for tropical trees (~0.1 ± 0.07 μg g_{dw}^{-1} d^{-1}; Saito et al., 2013) at ambient mixing ratios. Transformation of CH_3Cl by *S. kraussiana* followed pseudo first-order kinetics with rate constants of 17.5–19.9 d^{-1} (Figure S6 in Supporting Information S1) which is a prerequisite for the application of the Rayleigh equation (Equation 2). It should be mentioned at this point that the incubation in microcosms using a higher mixing ratio might cause changes in the reaction kinetics. Rate constants shown here might therefore not be extrapolatable to much higher or much lower mixing ratios and also degradation rates should be interpreted with caution. Isotopic fractionation, however, is less sensitive to such changes; that is, data reported for laboratory experiments carried out at higher concentrations agrees reasonably well with field data and hence this approach is widely used in the field of contaminant science and recommended by the EPA (Hunkeler et al., 2008).

3.2.2. Isotopic Fractionation in CH_3Cl Caused by Degradation by *S. Kraussiana*

Isotope analysis of CH_3Cl in the subsamples revealed considerable isotopic fractionation for all three elements (Figure 2). Isotopic hydrogen, carbon, and chlorine fractionation expressed as ε_H, ε_C, and ε_{Cl} was −77 ± 12 ‰, −10.8 ± 2.5 ‰, and −5.7 ± 0.5 ‰, respectively. Comparable triple-element isotopic data has not been published yet. One former dual-element isotope study investigated stable hydrogen and stable carbon isotopic fractionation in CH_3Cl degraded by various fern species (Jaeger et al., 2018b) when spiked with 5 ppmv CH_3Cl. Compared to

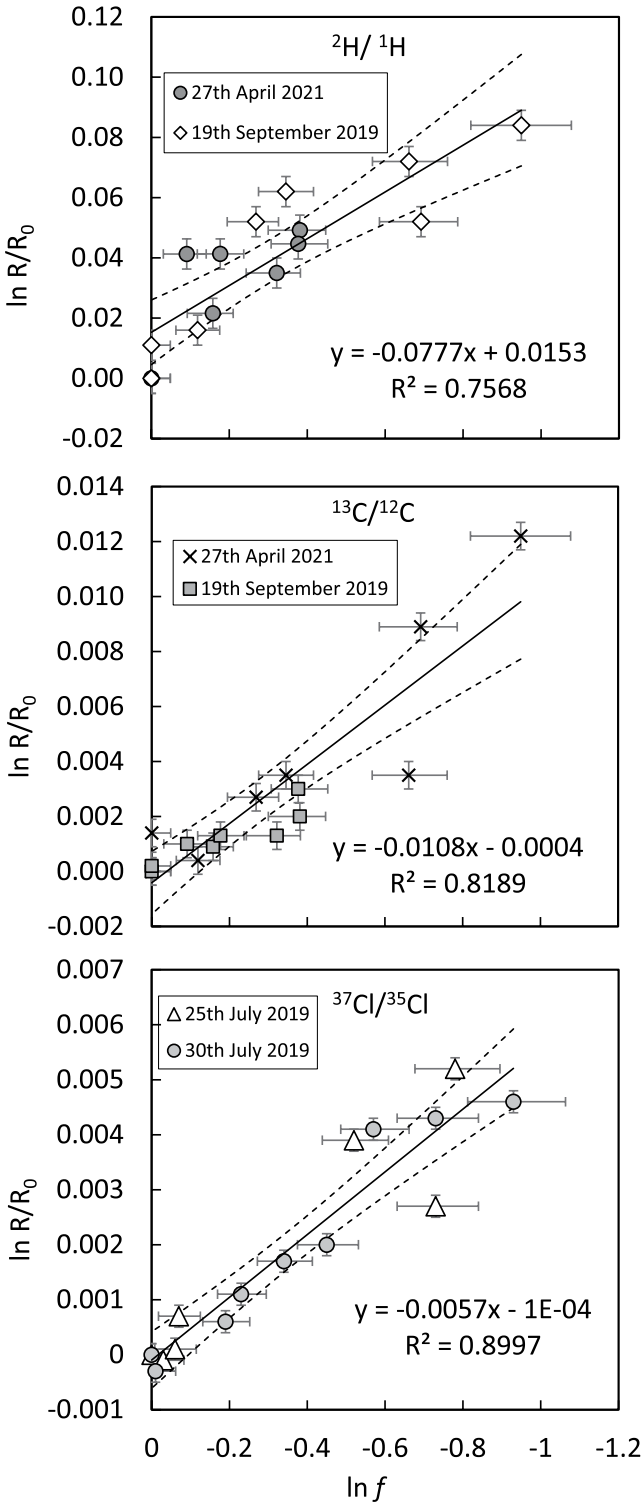

Figure 2. Rayleigh plots for stable hydrogen, stable carbon and stable chlorine isotopic fractionation in CH_3Cl during degradation by club moss (*S. kraussiana*). The linear regressions result from the combination of two separate experiments for which the slopes were indistinguishable based on the standard error. Error bars indicate the analytical uncertainty and the dashed lines show the 95% confidence intervals.

our study, isotopic fractionation was negligible for hydrogen (-8 ± 19 ‰) but considerably larger for carbon reporting an ε_C of -39 ± 19 ‰. Metagenomic investigations by Jaeger et al. (2018b) did not detect any genes associated to the only known chloromethane utilization pathway (Cmu) and suggested an unknown pathway for CH_3Cl breakdown in the fern phyllosphere. Jaeger et al., 2018a, 2018b also used lambda values (dual element isotopic ratios) to corroborate these findings. The $\Lambda_{H/C}$ of 0.2 was considerably smaller than the $\Lambda_{H/C}$ associated to the Cmu pathway (*Methylorubrum extorquens*, $\Lambda_{H/C} = 0.6$ to 0.8) (Keppler et al., 2020a; Nadalig et al., 2013, 2014). The assumption of a new mechanism was also supported by another CH_3Cl degradation study with plants (Kröber et al., 2021).

The largely differing $\Lambda_{H/C}$ of 7.1 obtained in the current study for degradation of CH_3Cl by *S. kraussiana* suggests that yet another pathway may be responsible for consumption of CH_3Cl. Degradation by the Cmu pathway supposedly starts with dehalogenation by nucleophilic attack (S_N2-type) which explains the relatively large ε_{Cl} of -10.9 ‰ reported for consumption of CH_3Cl by *M. extorquens* (Keppler et al., 2020a). The ε_{Cl} measured for CH_3Cl degradation by *S. kraussiana* in the current study is smaller (-5.7 ‰) but still within the range of ε_{Cl} for abiotic and biotic S_N2 reactions reported thus far (Horst et al., 2019; Keppler et al., 2020a; Westaway, 2007). In contrast to chlorine isotopes, parameters for fractionation of hydrogen and carbon isotopes (ε_H, ε_C, $\Lambda_{H/C}$, $\Lambda_{C/Cl}$) indicate a different reaction mechanism. For hydrogen the secondary isotope effect of -77 ‰ is the largest so far reported for CH_3Cl degradation in biological samples contrasting the small or even negligible ε_H for secondary hydrogen isotope effects found in other experiments (Table 3). Carbon, in contrast, yielded a rather small ε_C of -10.8 ‰ which is outside the range of -37 ‰ to -92 ‰ reported for degradation by other plants or microbes (Table 3). Hence the isotopic data does not support the hypothesis that dehalogenation in the current experiments follows a S_N2-type nucleophilic substitution reaction.

The magnitude of isotopic fractionation of all three elements resembles, however, a S_N1-type nucleophilic substitution mechanism even though, from a chemical point of view, such a reaction is rather unlikely for CH_3Cl (Schwarzenbach et al., 2003). Elsner et al. (2005), and references therein, give a range of about 0 to -30 ‰ for stable carbon isotopic fractionation and about -100 to -200 ‰ for stable hydrogen isotopic fractionation which fit quite well with our experimental data. These values differ substantially from those reported for a S_N2-type reaction ($\varepsilon_C = -30$ to -90 and $-50 < \varepsilon_H < +50$). Hence, these clearly different carbon and hydrogen isotope fractionation values may be considered indicative for S_N1 and S_N2 mechanisms, respectively, and those differences are closely linked to the transition state of the reacting molecule (Westaway, 2007). During a S_N1 type reaction a carbenium ion is formed before nucleophilic attack occurs whereas in a S_N2 type reaction nucleophilic attack and dehalogenation occur in a concerted reaction; that is, simultaneously. Halogenated methanes usually do not react via S_N1 in chemical reactions because a stable carbenium ion, other than in compounds with secondary or tertiary carbon-halogen bonds, may not be formed (Melander and Saunders, 1980; Schwarzenbach et al., 2003). Biochemical reactions, however, involve large proteins (enzymes) with charged active sites which stabilize ionic transition states such as the carbenium ion (Nelson & Cox, 2005; Warshel et al., 2006). Hence it may be conceivable that the methyl cation reaches a reasonably stable transition state in the enzyme-substrate complex and thus the reaction might indeed proceed via a S_N1 reaction as

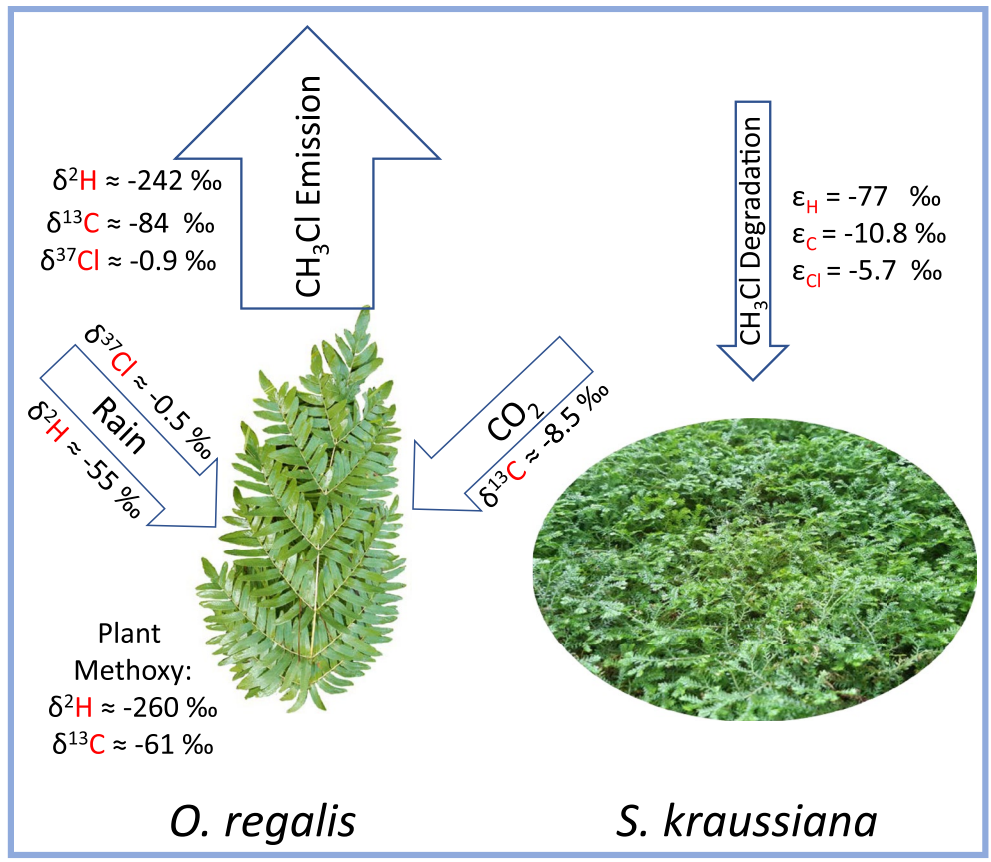

Figure 3. Emission and degradation of CH_3Cl by the two plant species *O. regalis and S. kraussiana* and associated isotopic fingerprints and isotopic fractionation, respectively. δ^2H and $\delta^{13}C$ values for primary precursors (rain, CO_2) were estimated for the location (Heidelberg) or taken from the literature, respectively. The sizes of the blue arrows (emission, degradation) indicate the approximate relative importance of plant emissions in general.

indicated by the isotopic data. Further evidence to prove this hypothesis is currently not available due to the rather exploratory nature of this study. In general, microorganisms and associated microbial key enzymes responsible for halogen cycling in the environment are still largely unknown. Future studies involving a much larger set of CH_3Cl degrading plants in combination with isotopic and metagenomics studies are required to further investigate the largely unknown degradation mechanisms of CH_3Cl by plants.

4. Implications

In this study we presented the first triple-element isotopic characterization of a natural source of CH_3Cl. Formation and emission of CH_3Cl by plants contributes more than 50% of this important ozone-depleting gas to the atmosphere (Carpenter et al., 2014). Quantifying emissions of CH_3Cl from plants, however, is associated with large uncertainties (1,430–2,650 Gg a^{-1} (Carpenter et al., 2014)). A balanced budget has not been achieved yet and a recent study argues against tropical plants being the major source of CH_3Cl to the atmosphere (Bahlmann et al., 2019). The isotopic data collected in this study represents an important step toward creating a triple-element isotopic database of sources and sinks of CH_3Cl which is a prerequisite for future isotope based global models to improve unbalanced budget estimates. The isotopic patterns of CH_3Cl formation and degradation of the two studied plant species are graphically summarized in Figure 3.

Whether the isotopic composition of CH_3Cl emitted by *O. regalis* is representative for other plant species remains speculative but in principle it may serve as a first best estimate for other CH_3Cl producing plants. CH_3Cl is, according to current knowledge, formed by similar precursors (rainwater, SAM) in all plants and hence the isotopic composition of CH_3Cl produced by other important plants might show similar isotopic patterns as those observed for *O. regalis*. If this hypothesis turns out to be true, the isotopic signatures of plant-produced CH_3Cl

should fall within a similar isotopic range for all plants. Similar source ranges in combination with strongly depleted isotopic values, in this case for carbon and hydrogen, bode well for future studies attempting to distinguish plant emissions from, for example, anthropogenic emissions and to quantify the amounts of produced CH_3Cl by the different sources (e.g., plants, soils, and fungi) and the fraction that is released to the atmosphere.

In this study we also investigated isotope effects caused during formation of CH_3Cl from potential precursors. The isotopic composition of direct precursors (SAM, rain) and emitted CH_3Cl revealed, within analytical uncertainty, no difference for hydrogen and chlorine isotope ratios and a measurable offset of about 20 ‰ for carbon isotopes. This absence of isotopic fractionation for chlorine also means that soil processes as well as uptake, transport, and conversion of dissolved Cl^- to CH_3Cl in the plant did not cause measurable isotopic fractionation throughout our experiments. Should emissions of other relevant plant species show similarly small or negligible isotopic differences between precursors and CH_3Cl, this might open up a much simpler way to isotopically characterize CH_3Cl produced by plants. This alternative approach would only require the collection of rainwater and plant samples of relevant species to estimate the isotopic composition of plant-emitted CH_3Cl without the need for laborious large-volume air sampling and preconcentration methods.

In comparison to CH_3Cl emission, the importance of CH_3Cl degradation by plants for the atmospheric budget is even more uncertain. For tropical forests some former studies reported degradation rates being at least one order of magnitude smaller than production rates with overall strong net emissions (Gebhardt et al., 2008; Saito et al., 2013). In addition, our results from degradation experiments and previous studies suggest that several transformation pathways exist causing distinct isotopic patterns (ε, λ). This fact may complicate the inclusion of degradation by plants in isotopic models because different isotope fractionation patterns may have to be considered for a variety of plants. These different isotopic patterns, however, will be clearly an asset in future microbial studies aiming at identifying different enzymatic pathways and CH_3Cl transformation processes.

Data Availability Statement

The data of this article is available via the data management portal of the UFZ (https://doi.org/10.48758/ufz.13388). The data of the manuscript was calculated and diagrams created with Microsoft Excel. Figures were created with Microsoft Power Point. The manuscript was prepared with Microsoft Word.

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