Stable bromine isotopic composition of methyl bromide released from plant matter

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Abstract

Methyl bromide (CH_3Br) emitted from plants constitutes a natural source of bromine to the atmosphere, and is a component in the currently unbalanced global CH_3Br budget. In the stratosphere, CH_3Br contributes to ozone loss processes. Studies of stable isotope composition may reduce uncertainties in the atmospheric CH_3Br budget, but require well-constrained isotope fingerprints of the source end members. Here we report the first measurements of stable bromine isotopes ($\delta^{81}\text{Br}$) in CH_3Br from abiotic plant emissions. Incubations of both KBr-fortified pectin, a ubiquitous cell-stabilizing macromolecule, and of a natural halophyte (*Salicornia fruticosa*), yielded an enrichment factor (ϵ) of $-2.00 \pm 0.23\text{‰}$ (1σ , $n=8$) for pectin and $-1.82 \pm 0.02\text{‰}$ (1σ , $n=4$) for *Salicornia* (the relative amount of ^{81}Br decreased in CH_3Br compared to the substrate salt). For short incubations, and up to 10% consumption of the salt substrate, this isotope effect was similar for temperatures from 30 up to 300°C. For longer incubations of up to 100 hours at 180°C the $\delta^{81}\text{Br}$ values increased from -2‰ to 0‰ for pectin and to -1‰ for *Salicornia*. These $\delta^{81}\text{Br}$ source signatures of CH_3Br formation from plant matter combine with similar data for carbon isotopes to facilitate multidimensional isotope diagnostics of the CH_3Br budget.

Keywords: $^{81}\text{Br}/^{79}\text{Br}$; enrichment factor, abiotic formation, pectin, halophyte

1. Introduction

Methyl bromide (CH_3Br) is the most important source of Br radicals in the stratosphere. It accounts for 15% of the ozone depletion potential caused by halogen species (Butler, 2000). Consequently, the use of CH_3Br as a fumigant is scheduled to be phased out by 2015 under amendments to the Montreal Protocol (Montzka et al., 2011). However, methyl bromide is also released naturally from oceans (King et al., 2002), biomass burning (Andreae and Merlet, 2001), salt marshes (Rhew et al., 2000), wetlands (Varner et al., 1999), fungi (Harper, 1985), and several plant species (Gan et al., 1998, Wishkerman et al., 2008). The main sinks are believed to be uptake by oceans (Butler et al., 2007) and soils (Shorter et al., 1995) as well as reaction with OH radicals in the atmosphere (Saltzman et al., 2004). Although most of these source and sink processes appear to be relatively well investigated, albeit facing upscaling challenges common to most bottom-up approaches, the global budget shows an imbalance of 32 Gg/a corresponding to ca. 25% of the known annual emissions (Yvon-Lewis et al., 2009). Hence, sources are either underestimated and/or sinks are overestimated.

Emissions from plants may add considerably to the CH_3Br atmospheric budget. Warwick et al. (2006) ran an inverse model of currently observed mixing ratios in the atmosphere and identified a terrestrial source from tropical vegetation. Biotic and abiotic reaction mechanisms have been described. For the biotic reaction pathway, living plants are producing methyl halides in their cells as a result of enzymatic reactions (Saito and Yokouchi 2006 and references therein). Blei et al. (2010) performed flux measurements on branches and leaves in a southeast Asian tropical rainforest and found emissions up to $3 \text{ ng} \cdot \text{h}^{-1} \cdot \text{gdw}^{-1} \text{ CH}_3\text{Br}$ (gram dry weight). Large scale airborne measurements over the South American tropical rainforest showed small net fluxes because of local sinks such as photolysis and soil uptake (Gebhardt et al., 2008). Both studies demonstrate that vegetation in the tropics might be an important source of methyl bromide to the atmosphere.

Apart from biotic production of living plants, an abiotic reaction pathway has been described for CH_3Br following a nucleophilic substitution reaction ($\text{S}_{\text{N}}2$) (Hamilton

et al., 2003; Keppler et al., 2004). The methoxy groups of pectin and lignin, both abundant cell-stabilizing macromolecules, react with halide ions dissolved in the tissue water of the plants (Khan et al., 2001) (Fig. 1a) or present as hydrogen bonded ions on the pectin molecule (Fig 1b). Up to 52% of the halide ions may be H-bonded to functional groups of the organic molecules (Myneni, 2002). These halide ions form acids, either with protons from the unesterified carboxylic acid group of the pectin molecule (McRoberts, 2011) (Fig 1a) or from the surrounding water (Fig 1b). The acids can cleave the methoxy group from the pectin molecule in a nucleophilic substitution reaction with a methyl halide as one product. The same reaction pathway has been suggested for bromide salts (Hamilton et al., 2003).

Fig. 1. Suggested chemical reaction pathway for the formation of methyl halides from pectin: (a) CH_3Br formation from pectin and salt modified after McRoberts (2011) and (b) from H-bonded bromide.

This abiotic reaction pathway was suggested to be an important source to the atmospheric methyl halide budget (Keppler et al., 2005; Wishkerman et al., 2008). It accounts for both low temperature emissions from dead and senescent plant material (Derendorp et al., 2012) and, together with the lignin methoxy groups, for biomass burning (Andreae and Merlet, 2001; van der Werf et al., 2006). For methyl chloride ~20-25% of the estimated total budget might come from biomass burning and a similar amount from senescent plants and plant litter (Keppler et al., 2005; Saito and Yokouchi, 2008). For methyl bromide approximately 10% of the

atmospheric budget might originate from biomass burning (Yvon-Lewis et al., 2009). While vegetation-based emissions of CH₃Br have the potential to reduce the estimated imbalance between sources and sinks in the atmospheric budget, low-temperature formation from senescent and dead plant matter has not yet been included in the global budget because quantification is difficult.

The current estimates for plant emissions of CH₃Br are based largely on small-scale concentration or flux measurements (Gebhardt et al., 2008; Blei and Heal, 2011) and are not well constrained. Hence stable isotope techniques are starting to be explored to improve the source apportionment of methyl halides. Carbon-13 analysis on atmospheric samples was first shown for CH₃Cl by Rudolph et al. (1997) and since then it was applied in various studies to characterize source (Thompson et al., 2002; Keppler et al., 2004; Saito and Yokouchi, 2008) and sink signatures (Gola et al., 2005; Sellevag et al., 2006). A first $\delta^{13}\text{C}$ isotope based budget estimate was accomplished by Keppler et al. (2005). Recently, the hydrogen isotopic composition of CH₃Cl released from halophyte plant species was investigated (Greule et al., 2012).

Isotope studies on CH₃Br are much scarcer mainly because of its low atmospheric mixing ratio and small emission rates from its sources. Signatures of $\delta^{13}\text{C}$ have been determined for fumigation products (McCauley et al., 1999), salt marshes (Bill et al., 2002) and tropospheric air (Bill et al., 2004; Bahlmann et al., 2011). However, $\delta^{13}\text{C}$ values of salt marsh emissions (-43‰ VPDB) and the troposphere (-42.3.1‰ VPDB) are difficult to distinguish. Industrial CH₃Br products have more negative $\delta^{13}\text{C}$ values (-54‰ VPDB).

Bromine has two stable isotopes ⁷⁹Br (51.69%) and ⁸¹Br (49.31%) with an average ⁸¹Br/⁷⁹Br ratio of 0.954 (e.g. Wieser et al., 2013). The small mass difference of 2.5% causes only minor but measureable isotope fractionation. Two previous studies reported $\delta^{81}\text{Br}$ values of -0.80‰ to +3.35‰ SMOB (Standard Mean Ocean Bromide) for deep groundwaters of the Siberian Platform (Shouakar-Stash et al., 2007) and -4.3 to -0.4‰ SMOB for industrially produced brominated organic

compounds (Carrizo et al., 2011) thus giving a $\delta^{81}\text{Br}$ range of ca. 8‰. Two recent articles reported isotope enrichment factors (ϵ) of -0.2‰ to -0.76‰ for microbial debromination of brominated phenols (Bernstein et al., 2013) and -0.5‰ to -2.7‰ observed for Grignard reagent formation (Szatkowski et al., 2013) showing the potential to identify certain processes and reactions by using Br isotope analysis.

A method to measure bromine isotopes in CH_3Br was recently established (Horst et al., 2011) and the first $\delta^{81}\text{Br}$ isotope values for ambient tropospheric CH_3Br are in the range of -0.47 to +1.75‰ SMOB (Horst et al., 2013). Despite the small $\delta^{81}\text{Br}$ range, the high-precision values could be used to identify a degradation trend and to start considering the influence of potential sources on the tropospheric $\delta^{81}\text{Br}$ composition of CH_3Br .

Here we report the first measurements of bromine isotopes in CH_3Br derived from incubations with thermally-treated plant matter to explore its feasibility to aid in addressing the abiotic plant source. We suggest a potential $\delta^{81}\text{Br}$ source range and investigate the fractionation process caused by this abiotic production pathway of CH_3Br in senescent plants and dry plant litter.

2. Method

2.1 Plant sample and pectin

For our experiments we used KBr-fortified apple pectin (Sigma Aldrich) and dried leaf samples of the halophyte *Salicornia fruticosa* collected in tidal areas on Sardinia, Italy. The fortified pectin was prepared in the following way: Potassium bromide (KBr, Sigma Aldrich) solution (1.5 mg KBr, 100 mL) was placed in a beaker and heated to 50°C. Pectin (10 g) was slowly added while stirring. Then the formed gel was mixed (2 min) with an Ultra-Turrax® blender and transferred onto an aluminum foil tray and cooled. After freezing, the pectin was lyophilized and ground. *Salicornia* leaves, in total ca. 250 gdw from many different plants, were dried at room temperature and ground. Analysis of methoxy and (blank) salt concentrations was carried out prior to the incubation experiments. To determine

the methoxy content the functional groups of esters and ethers of pectin were reacted with hydriodic acid to form alkyl iodides which were analyzed by gas chromatography – mass spectrometry (GC-MS). Salt concentrations were quantified using X-ray fluorescence. Both methods are described in detail in McRoberts (2011).

2.2 Incubation experiments

To investigate the instantaneous CH_3Br emissions and the corresponding $\delta^{81}\text{Br}$ isotope values pectin and *Salicornia* samples were heated at temperatures ranging from 30°C to 300°C. Higher temperatures were not tested because methyl halide emission from this reaction pathway was expected to be of minor importance. Bjorkman and Stromberg (1997) reported losses of chlorine of 20-50% by 400°C, which was attributed to HCl volatilization. Sharma et al. (2001) showed that only negligible amounts of methoxy groups are left after 10 min pyrolysis of plant material at 300°C. This agrees with the studies of Hamilton et al. (2003) and Keppler et al. (2004) showing that pectin-related CH_3Cl formation terminated at 300°C within the tested time span.

The plant material was filled in 4.5 mm inner diameter (1/4 inch) x 150 mm stainless steel tubes and placed into an oven (Fig. 2). The inlet of the tube was connected to a mass flow controller delivering 20 mL/min N_2 gas. The outlet discharged into a liquid-nitrogen-cooled stainless steel loop (2 mm inner diameter (1/8 inch) x 500 mm) for trapping the emitted gases. This method of quantitative cryo-trapping has been previously shown to preserve the isotope composition of the sample (Thornton et al., 2013) and breakthrough was not observed for flow rates up to 50 mL/min. Hamilton et al. (2003) demonstrated that incubation of pectin under nitrogen atmosphere yields the same amounts of CH_3Cl as in air. Consequently incubations were accomplished with nitrogen as a carrier gas to avoid difficulties due to co-trapped O_2 . The incubated sample size was 20 mg for pectin and 100 mg for *Salicornia* for temperatures of 150°C to 300°C. At low temperatures up to 500 mg pectin or 2 g *Salicornia* were incubated in order to trap sufficient CH_3Br for isotope

analysis. After collection the loops were sealed with Swagelok® endcaps until quantitative analysis.

Fig. 2. Experimental setup for heating plant samples and cryogenic collection of instantaneously produced gases.

Two different experimental studies were carried out: a temperature series and a time series, in both cases designed to collect CH₃Br. That is, sampling of the formed CH₃Br was performed during a relatively small interval of the reaction progress in terms of substrate consumption, as opposed to accumulative sampling during a longer reaction interval. For the temperature series, different samples of pectin and *Salicornia* were incubated for one hour at a set of temperatures spanning 110 to 230°C. For lower temperatures, incubation/ collection times were prolonged (2 days for 70°C, 7 days for 50°C and 21 days for 30°C). At 300°C, the incubation time was 15 min including a 5 min pre-heating time which was not collected. For the time series, samples of pectin and *Salicornia* were incubated at 180°C for 290 and 90 hours, respectively. In certain time intervals, samples from the gas stream were collected, sealed with Swagelok® endcaps, and stored until quantification and isotope analysis.

2.3 Quantification

The cryoloops with the samples were connected to a gas-chromatography quadrupole mass spectrometry system (GCqMS) for quantification and purification. The complete procedure is described in detail in Thornton et al. (2013). Briefly, the GCqMS (HP5972A, Agilent®, Santa Clara, CA, USA) was equipped with a packed column (60/80 Carbopack 195 B/1% SP-1000 (polyethylene glycol); 8' x 1/8" steel column) for separation of the trapped compounds. The CH₃Br peak was heart-cut and trapped again in a liquid nitrogen cooled stainless steel loop. A custom-made split directed 5% of the gas stream to the MS for quantification. The MS was operated in selective ion mode for CH₃Cl and CH₃Br (*m/z* 50, 52, 94 and 96). The purified sample was sealed and stored in the freezer at -20°C until Br isotope analysis. Analytical uncertainty for the combined quantification/purification method was usually < 5% (Thornton et al., 2013).

2.4 Bromine isotope analysis

The bromine isotopic composition of the plant emitted CH₃Br was determined using gas-chromatography (HP 5890 Series II, Agilent®, Santa Clara, CA, USA) hyphenated with multi-collector inductively coupled plasma mass spectrometry (Isoprobe®, GV Instruments, Manchester, UK). The whole GC-MCICPMS method is described in detail in Horst et al. (2011). Briefly, the samples were extracted from the cryoloops using a 10 mL gas tight syringe (Hamilton®, Reno, USA) and septa fitted at both ends of the loop. The sample was then injected into the GC system, which was equipped with a DB-624 column (10 psi, 70°C isothermal). There was usually sufficient sample amount available to allow triplicate analyses. From the GC column, samples were transferred into the plasma and ionized. Two detectors (AX0 and H1) were aligned for simultaneous detection of the masses 79 and 81. Results were recorded into an Excel® spread sheet and integrated offline using a custom-created Matlab® code. The purification and injection procedure does not cause measurable shifts in the isotopic signature (Thornton et al., 2013)

The Br isotope ratio ($R = {}^{81}\text{Br} / {}^{79}\text{Br}$) of a sample versus the ratio of a standard reference material is reported in per mil (‰) as:

$$\delta^{81}\text{Br} = (R_{\text{sample}} / R_{\text{reference}} - 1) \quad (\text{Eq. 1})$$

Bromine isotope data are referenced versus Standard Mean Ocean Bromide (SMOB). Our internal laboratory standard is a commercially available CH_3Br (Sigma Aldrich® Sweden AB, Stockholm, Sweden, Catalog No. 65950) with a $\delta^{81}\text{Br}$ of $+0.12 \pm 0.02\text{‰}$. The KBr used for fortification of pectin has a $\delta^{81}\text{Br}$ of $+0.06 \pm 0.06\text{‰}$. Referencing of both materials versus SMOB was carried out as a commercial service by the Environmental Isotope Laboratory at the University of Waterloo (Canada) using continuous-flow isotope-ratio mass spectrometry (Shouakar-Stash et al. 2005).

2.5 Isotope fractionation systematics

The Rayleigh equation was used to investigate isotope fractionation processes. This equation is commonly used to investigate the partitioning behavior of isotopes between a substrate and a product reservoir as one reservoir decreases in size (Kendall and Caldwell, 1998). The remaining isotopic composition of the substrate and the instantaneous isotopic signature of the product are given in delta notation by equations 2 and 3 (e.g. Hoefs, 2004):

$$\delta_S \sim (\delta_{0s} + 1000) \cdot f^{(\varepsilon/1000)} - 1000 \quad (\text{Eq. 2})$$

$$\delta_P \sim ((\delta_{0s} + 1000) \cdot f^{(\varepsilon/1000)} - 1000) + \varepsilon \quad (\text{Eq. 3})$$

where δ_{0s} is the initial delta value of the substrate pool, f is the fraction of the remaining substrate, and δ_S is the delta value as a function of f . The isotope enrichment factor is denoted ε , and δ_P is the delta value of the instantaneously formed product. The ε value is a close approximation for the difference $\delta_P - \delta_S$ when the isotope fractionation is small (as in the current case for bromine isotopes) (Hoefs, 2004).

3. Results

3.1 Plant matter and blank

Salicornia and pectin were analyzed for methoxy and (blank) salt concentrations prior to the incubation experiments (Table 1). We monitored both Cl (emitted as CH₃Cl) and Br (emitted as CH₃Br) concentrations during our experiments (Fig. EA-1, Electronic Annex) although the subsequent interpretation focuses on Br and its isotopes. The chloride content for pectin was estimated from the cumulative yields of the time series experiment assuming 100% conversion of Cl⁻ to CH₃Cl. The bromide content was calculated from the added amount of KBr. Although pectin was only fortified with KBr, substantial production of CH₃Cl was observed (Fig. EA-1). This is likely due to the commonly used industrial extraction method for pectin from plant matter which uses acids such as HCl (e.g. Nanji and Chinoy, 1934). We tested for blank emissions by incubating unfortified pectin at 180°C for 20 min. We found 118 µg gdw⁻¹ Cl emitted from pectin (emitted as CH₃Cl) and 0.16 µg gdw⁻¹ Br (emitted as CH₃Br, gdw = grams dry weight). Whereas the production of CH₃Cl was considerable, the CH₃Br production was low (0.07%) compared to yields from fortified pectin incubations for the same temperature and incubation time. Therefore, we assume that blank emissions of CH₃Br are negligible for the interpretation of Br isotopic values of fortified pectin samples.

Tab 1 Halide and methoxy content of the plant samples

mg/g	pectin	Salicornia
methoxy	82	16.8
chloride	^a 1.7	174
bromide	^b 6.7	0.75

^a value was estimated from cumulative CH₃Cl emissions

^b amount of added Br

3.2 Temperature series experiment

For the first experimental series, incubations were carried out for different samples of *Salicornia* and KBr-fortified pectin for temperatures ranging from 30 to 300°C. The corresponding incubation times ranged from 21 d to 15 min respectively. The $\delta^{81}\text{Br}$ of CH_3Br that was emitted from both types of plant matter is largely similar over the tested temperature range (Fig. 3). Pectin emissions show an average $\delta^{81}\text{Br}$ of $-1.94 \pm 0.23\text{‰}$ (n=8). The 300°C sample shows a significantly higher value of $-1.38 \pm 0.21\text{‰}$. At this temperature, ca 50% of the substrate Br (KBr salt) was converted compared to < 10% conversion of the KBr salt at the lower temperatures. The emission rates were determined for some of the temperature steps (Table EA-1). The $\delta^{81}\text{Br}$ of CH_3Br emitted from *Salicornia* has an average of $-1.82 \pm 0.02\text{‰}$ (n=4). Not more than 11% of the substrate salt was transformed at the highest temperature (300°C) in this case. The $\delta^{81}\text{Br}$ of salts in *Salicornia* could not be measured for this study but might be similar to the Br isotopic composition of the salt used to fortify pectin ($\sim 0\text{‰}$). Halophytes are exposed to marine salts with a $\delta^{81}\text{Br}$ of 0‰ SMOB and have been shown to accumulate that salt (Zafrilla et al., 2010). Consequently, an isotopic shift of ca. -2‰ was observed in the studied temperature range for both fortified pectin and *Salicornia*, with large fractions remaining ($f > 0.9$; Eq. 2 and 3).

Fig. 3. Pectin and *Salicornia fruticosa* $\delta^{81}\text{Br}\text{-CH}_3\text{Br}$ values as a function of incubation temperature. The temperature range was from 30°C to mimic near-ambient emissions and up to 300°C to mimic emissions from smoldering biomass/vegetation burning. The values represent emissions during large fractions of remaining salt ($f > 0.9$) (except for the 300°C pectin sample) to allow estimation of the isotope enrichment factor ε (without effects of substantial shifts in isotopic composition of the Br source reservoir). Error bars represent the standard deviation for $n=3$ isotope analyses. The 30°C value represents single isotope analyses.

3.3 Time-series experiment

Samples of pectin and *Salicornia* were continuously incubated at 180°C and discrete subsamples of the emitted gas were collected after certain time intervals, representing instantaneously formed CH_3Br . Emission rates and Br isotope composition were determined for CH_3Br (Fig. 4; rates of CH_3Cl emission in the Electronic Annex, Fig. EA-1). The rates of CH_3Br formation were highest in the initial phase of the incubation, for *Salicornia* decreasing from 15.8 to 0.5 $\mu\text{g gdw}^{-1}\cdot\text{h}^{-1}$ Br (microgram Br per hour and gram dry-weight of plant matter) after 90 hours

(calculated from CH₃Br emissions). For fortified pectin, rates decreased from 643 to 2.6 µg gdw⁻¹·h⁻¹ Br after 100 hours. Thus, initial rates from the pectin incubation were higher and decreased faster than the rates obtained from *Salicornia*. In pectin 85% of the initial substrate Br was converted to CH₃Br after 100 h, whereas only 30% was transformed in *Salicornia* (Figure 4b). This may be explained by the lower methoxy content (by a factor 5) and the considerable high chloride content in the latter material, thus putting a stoichiometric constraint on the reaction, i.e. Cl is limiting the availability of methoxy groups for Br.

The Br isotopic composition changed over time. Both *Salicornia* and pectin-derived CH₃Br show the most depleted values of ~ -2‰ in the initial phase of heating when the production rates are highest. After 10 h (for pectin) to 20 h (for *Salicornia*) δ⁸¹Br values become less depleted and maintain the isotopic composition over the remaining time. Pectin-derived CH₃Br samples show δ⁸¹Br values of ca 0‰ at later incubation stages. Incubations with pectin were continued until 290 hours but no further changes in δ⁸¹Br values were observed. Br isotopic values of CH₃Br from *Salicornia* increase to values of ~ -1‰ within the tested time range. Taken together, the results show a decrease in CH₃Br production at longer incubation times, which is accompanied by increasingly higher isotopic values in the product.

Fig. 4. (a) Rates of Br (released as CH_3Br) emissions at 180°C from *Salicornia fruticosa* and fortified pectin for the individual samples (time series). (b) Cumulative amounts of Br released as CH_3Br from *Salicornia* and pectin (as % converted from the salt). Amounts between the individual samples were interpolated. The dotted grey line marks the theoretical complete conversion of Br salt to CH_3Br (c) Measured instantaneous $\delta^{81}\text{Br}$ values in CH_3Br from *Salicornia* and pectin.

4. Discussion

4.1 Evaluation of isotope fractionation behavior

The isotopic values changed over the course of the time-series experiment (Fig. 4c). The most likely reason is the gradual emptying of the salt substrate pool and the accompanying ^{81}Br enrichment in the remaining substrate pool. To investigate this, the Rayleigh equations 2 and 3 were used to calculate the $\delta^{81}\text{Br}$ value of the remaining salt substrate and the $\delta^{81}\text{Br}$ value of the CH_3Br instantaneously emitted at any given time. The initial delta value of the KBr salt was known ($\delta_{0s} = +0.06\text{‰}$ SMOB), as was the instantaneous $\delta^{81}\text{Br}$ of CH_3Br (δ_P) for large fractions remaining ($f > 0.9$), based on the temperature series (excluding the data point for incubation at 300°C). The average enrichment factor was derived from the difference $\delta_P - \delta_s$ between the product and the substrate, yielding an ϵ of $-2.0 \pm 0.23\text{‰}$ (1s, $n=8$) for pectin and $-1.8 \pm 0.02\text{‰}$ (1s, $n=4$) for *Salicornia* (assuming a salt $\delta^{81}\text{Br}$ of 0‰). The Rayleigh plots in Fig. 5 show the theoretical development of Br isotopic signatures of the salt substrate pool (black lines) and the instantaneously produced CH_3Br (grey lines) assuming that the obtained isotope enrichment factors are constant over the whole range of f . Figure 5 also illustrates the measured samples from the time series experiment. For *Salicornia*, only 30% of the substrate was transformed ($f = 0.7$) and the $\delta^{81}\text{Br}$ of the instantaneous product CH_3Br follows the Rayleigh curve within analytical uncertainty for most of the samples. Pectin-derived CH_3Br follows the Rayleigh curve for the instantaneous product until ca. 50% of the substrate is consumed, but for small remaining f the $\delta^{81}\text{Br}$ values are lower than predicted by the Rayleigh model. Fitting of the Rayleigh curve to the pectin data leads to a ϵ of only -0.9‰ ($R^2 = 0.78$). Aside from not matching the data from the temperature series, this ϵ of -0.9‰ and the initial product $\delta^{81}\text{Br}$ of -1.9‰ would force the salt $\delta^{81}\text{Br}$ to -1.0‰ (in contrast to the measured $\delta^{81}\text{Br}$ of $+0.06\text{‰}$). Moreover, the temperature-series ϵ was derived from 4 to 7 individual samples incubated at different temperatures whereas the time series was carried out using one sample of plant matter only. Hence, this smaller ϵ is rejected in favor of the obtained ϵ of -2.0‰ for pectin and -1.8‰ for *Salicornia*.

It is unclear why we observed this atypical enrichment pattern for the pectin samples, and if a reaction process, or combination of more than one reaction, exists that would produce such a pattern. We were not able to analyze the KBr salt pool, during its gradual depletion, to confirm that the reaction progressed as anticipated (with respect to f), and investigate its concomitant isotopic evolution. The methods for isotope analysis (Horst et al., 2011) and cryo-sampling (Thornton et al., 2013) have been tested rigorously for isotope-conservative characteristics; these have been found not to induce any systematic shifts in the $\delta^{81}\text{Br}$ of the sample. It is possible that the very long incubations of plant matter caused unexpected changes in the reaction, and the isotopic composition, of a type not reported in the literature so far. Another conceivable explanation is a non-steady state isotope effect due to an emerging rate limiting step or change of reaction mechanism (Maggi and Riley, 2009). The $\delta^{81}\text{Br}$ of the pectin data points at $f = 0.1 - 0.2$ (Figure 5) would, if assumed to be accurate and representative of the studied reaction, result from a larger isotope fractionation than that of the first two data points (f in the range 0.5 - 0.9) because production of isotopically lighter CH_3Br plotting below the Rayleigh curve would result in an even stronger enrichment of heavier isotopes in the salt substrate. The observed pattern could potentially indicate a shift in bromide source pools (e.g. from hydrogen bonded to dissolved bromide; Fig. 1a vs. 1b reaction mechanisms) and in the associated ϵ value, or the emergence of an additional parameter acting as a hitherto masked rate-limiting step (Elsner et al., 2005).

Fig. 5: Rayleigh plots calculated using Equation 2 for the salt substrate (black) and using equation 3 for the CH₃Br as a product (grey) for pectin (dashed, $\epsilon = -2.0\text{‰}$) and Salicornia (straight, $\epsilon = -1.8\text{‰}$) respectively. The grey graphs represent the isotopic value of the instantaneous product. Enrichment factors were obtained from the temperature series experiment. The included samples are the results from the time series experiment.

4.2 Isotopic fingerprint of a potential source signature

The major objective of this study was to constrain the endmember $\delta^{81}\text{Br}$ signature for CH₃Br produced with pectin as the methoxy-group source. This mechanism may be equally important to the formation of CH₃Br from lignin in woody plants, which is active at high temperatures, e.g. during smoldering biomass burning, and has been suggested to add about 10% of CH₃Br to the atmospheric budget (Andreae and Merlet, 2001). Pectin is the dominant source from non-woody plants at ambient and higher temperatures (up to ca. 300°C), and pectin-based sources such as decomposition of senescent plant material can be expected to be widespread (Hamilton et al., 2003).

The isotope signature of the low-temperature emissions is represented by the $\delta^{81}\text{Br-CH}_3\text{Br}$ in the temperature series experiment, i.e. -1.8 to -2.0‰ . The $\delta^{81}\text{Br-CH}_3\text{Br}$ values were almost constant over the investigated temperature range of 30 to 300°C for $f > 0.9$. Environmental shifts of this isotope endmember are difficult to predict, but could result from e.g. a reduction of f due to conversion of inorganic bromide to organobromine compounds (Leri and Myneni, 2012). However, the herein studied reaction is by itself robust with respect to the $\delta^{81}\text{Br-CH}_3\text{Br}$. To illustrate, a sample incubated at 110°C revealed no significant shift in $\delta^{81}\text{Br-CH}_3\text{Br}$ when incubated for a time period of 1 h (where $f \sim 0.9995$) compared to 33 h ($f \sim 0.96$; average $\delta^{81}\text{Br}$ of $-2.1 \pm 0.12\text{‰}$, $n=5$). Indeed, eq. 3 gives that the instantaneous $\delta^{81}\text{Br-CH}_3\text{Br}$ changes only by ca 0.2‰ until $f = 0.9$. At ambient temperatures the reaction would be even slower. Emitted CH_3Br would have a constant $\delta^{81}\text{Br}$ for almost a year (at a temperature of 30°C), if our data were extrapolated with the approximation that the reaction rate at least doubles with every 10 K of temperature (according to the Arrhenius equation; e.g., Atkins, 2001).

High temperature CH_3Br emissions from e.g. biomass burning may exhibit a different ϵ value from that obtained in this study, since the reaction mechanism and the methoxy source (e.g. lignin for woody plants instead of pectin) may not be the same. Furthermore, such emissions may have a more variable $\delta^{81}\text{Br}$ signature due to the temperature effect on the reaction rate, and the corresponding consumption of the salt (i.e. large decrease in f in eq. 2 and 3). To illustrate, 50% of the KBr salt was consumed already after 10 min incubation of pectin at 300°C , resulting in a $\delta^{81}\text{Br}$ of -1.4‰ (instead of -2.0‰ for $f > 0.9$). Another effect that adds variability to the $\delta^{81}\text{Br-CH}_3\text{Br}$ is the decomposition of methoxy groups at high temperatures, thus halting the methylation reaction. This was tested at 300°C by three samples in sequence. Whereas 50.6% of the KBr was consumed during the first 10 min, only an additional 0.4% were transformed during the next 10 min, and as little as 0.07% in the third 10 min interval. Complete consumption of the salt could not be achieved and the cumulative $\delta^{81}\text{Br-CH}_3\text{Br}$ is likely to stay close to -1.4‰ for this sample. However, most terrestrial plants (except for halophytes) have Br concentrations at

the lower ppm range and thus all Br might be completely converted to CH₃Br before all of the methoxy groups are decomposed. Therefore we suggest a range of 0 to – 2‰ SMOB for high temperature conversion, which comprises both plants with low and higher Br contents.

4.3 A two-dimensional view of CH₃Br with existing $\delta^{13}\text{C}$ and $\delta^{81}\text{Br}$ signatures

Relatively few isotope studies have been carried out on ambient methyl bromide largely due to the substantial analytical challenges. Fingerprints of $\delta^{13}\text{C}$ have been determined for industrially produced CH₃Br (McCauley et al., 1999), salt marsh emissions (Bill et al., 2002) and atmospheric CH₃Br (Bill et al., 2004; Bahlmann et al., 2011). Fingerprints of $\delta^{81}\text{Br}$ have been measured previously for industrial (Horst et al., 2011) and atmospheric CH₃Br (Horst et al., 2013). Figure 6 shows the current state of combined $\delta^{13}\text{C}$ - $\delta^{81}\text{Br}$ isotopic ranges for potential sources based on all available measurements to date. The $\delta^{13}\text{C}$ values for CH₃Br released during biomass burning from rice plants have been measured to range from –46 to –53‰ (Komatsu et al. 2005). These data are consistent with previous data by Keppler et al. (2004) showing that ¹³C-depleted methoxy groups are the major parent organic matter of methyl chloride (CH₃Cl) formation. The carbon pool (methoxy groups) of CH₃Br formation is considered to be the same as for CH₃Cl. Although the isotopic shift may be different (Komatsu et al. (2004) observed 8‰ difference between $\delta^{13}\text{C}$ values of CH₃Br and CH₃Cl) because of differing reaction kinetics, the relative difference between low and high temperature emitted CH₃Br might be similar and the $\delta^{13}\text{C}$ -CH₃Cl values are a good first estimate for what $\delta^{13}\text{C}$ values to be anticipated for CH₃Br.

Fig. 6. Reported isotopic ranges of $\delta^{13}\text{C}$ and $\delta^{81}\text{Br}$ in methyl bromide in ambient atmosphere and from various source reservoirs. Stable carbon isotope values for plant emissions are estimated from CH_3Cl analyses (Keppler et al., 2005 and references therein). The $\delta^{81}\text{Br}$ values for low and high temperature emissions are related to a source signature (salt) of ~ 0 ‰ SMOB.

The currently known and assumed ranges of stable carbon and bromine isotopes in CH_3Br for different sources and reservoirs are summarized in Figure 6. Whilst $\delta^{81}\text{Br}\text{-CH}_3\text{Br}$ values of industrial sources are within the range of those recently measured for the atmosphere (Horst et al., 2013) $\delta^{81}\text{Br}\text{-CH}_3\text{Br}$ values released from dry plant matter at low and high temperatures are up to 4‰ lighter. These results might already imply that, if emissions from senescent and dead plants including biomass burning are globally significant, then a substantial kinetic isotope effect should be associated with the decomposition of CH_3Br in the environment. The isotopic effects associated with perceived major sinks such as uptake by the oceans, soils and reaction of OH radicals in the troposphere which could cause isotope shifts

need to be studied. Horst et al., (2013) calculated a field-based $\delta^{81}\text{Br}$ enrichment factor of $-4.7 \pm 3.7\text{‰}$ based on $\delta^{81}\text{Br}\text{-CH}_3\text{Br}$ measurements in atmospheric samples. The ϵ might represent the combined isotopic shift caused by these major sinks. Gola et al., (2005) found a stable carbon isotope enrichment factor of 59‰ for a CH_3Cl reaction with OH radicals. Both enrichment factors would have to be considered in order to find the original source signatures and to calculate the corresponding fraction remaining. This assessment only provides a first overview of known source ranges, but it shows that two-dimensional isotope analysis of $\delta^{13}\text{C}$ and $\delta^{81}\text{Br}$ might be able to resolve potential sources from known atmospheric signatures. It is also important to note that two potentially large sources – the oceans and living plants – have not been investigated yet for both $\delta^{81}\text{Br}\text{-CH}_3\text{Br}$ and $\delta^{13}\text{C}\text{-CH}_3\text{Br}$ values. Future investigations, including constraining the stable bromine and carbon isotope signatures of these sources and the most important sinks, has the potential to improve our picture of the global CH_3Br budget. Furthermore, recent development of $\delta^2\text{H}$ analysis in CH_3Cl emitted from plants (Greule et al., 2012) now opens the door for three-dimensional isotope analysis of methyl halides.

5. Conclusions

This study investigated the stable bromine isotopic composition of abiotically emitted CH_3Br from plant matter. Results show that the Br isotopic composition of the formed methyl bromide can be expected to be relatively constant over a wide temperature range and for incomplete reactions. The enrichment factor of ca. -2‰ (incomplete reaction) makes it a useful tool to identify emissions from halophytic plants. Similar fractionations may also be assumed for non-halophytic plants. Emissions from biomass burning possibly comprise a wider range of ca 0 to -2‰ for the cumulative product if Br-salt conversion approaches completion and provided the salt $\delta^{81}\text{Br}$ is 0 ‰ SMOB. *Salicornia* showed a similar enrichment factor as observed for pectin when heated at the same temperature. Therefore we suggest that this enrichment factor is characteristic for the pectin-salt reaction pathway in natural plant matter. However, CH_3Br emissions from other plant

species should be studied to confirm these findings. Furthermore it would be important to investigate concentrations and isotopes of CH₃Br formed from woody plant matter at high temperatures in order to get an insight into the magnitude of formation from the lignin-salt production pathway. Together with the findings of this study, a combined source signature for both pathways could be determined. This in combination with other stable isotopes such as carbon or hydrogen might provide a clearer picture of the dimension of this source and its importance for the atmospheric CH₃Br budget.

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Electronic Annex for

**Stable bromine isotopic composition of methyl
bromide released from plant matter**

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Content:

Table EA-1: quantification of CH₃Br emissions (expressed as Br) for the
temperature series experiment

Fig. EA-1: emission of Cl and Br released as CH₃Cl and CH₃Br for the
time series experiment

Table EA-1: Bromine emission rates ($\mu\text{g gdw}^{-1}\text{h}^{-1}$ Br) and the total emitted mass of bromine (emitted as CH_3Br) for selected incubation steps from the temperature series. The fraction remaining (f) indicates the fraction of salt remaining in the plant matter after the emission of bromine in the form of CH_3Br .

$^{\circ}\text{C}$	pectin $\mu\text{g gdw}^{-1}\text{h}^{-1}$ Br	$\mu\text{g Br tot.}$	f	Salicornia $\mu\text{g gdw}^{-1}\text{h}^{-1}$ Br	$\mu\text{g Br tot.}$	f	incubation hours
30	0.003	1.7	>0.99			>0.99	504
70	0.2	27.0	>0.99	0.001	0.2	>0.99	168
110	3.4	3.4	>0.99	0.1	0.1	>0.99	1.0
180	643.3	321.6	0.95	15.8	7.9	0.99	0.5
300	18925.6	3785.1	0.53	262.2	52.4	0.89	0.2

Fig. EA-1. (a) Rates of Cl and Br released as CH_3Cl and CH_3Br emissions from *Salicornia fruticosa* and fortified pectin for the individual samples (180°C experiments). (b) Cumulative amounts of Br and Cl released as CH_3Cl and CH_3Br from *Salicornia* and pectin (as % converted from the salt). Amounts between the individual samples were interpolated. The dotted grey line marks complete conversion of Cl/Br salt to CH_3Cl / CH_3Br .