Production of methyl bromide by terrestrial higher plants

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Abstract. Methyl bromide (CH$_3$Br) is considered to play an important role in stratospheric ozone depletion, but its sources are currently not well defined. We have observed that Brassica plants can take up Br$^-$ from soil, produce CH$_3$Br and release it into the air. Emission of CH$_3$Br was detected from plants grown in natural soils containing 0.06-0.31 mg/kg Br$^-$; the emission increases with increasing soil Br$^-$ level. We estimate that cabbage produces 0.4 ± 0.2 Gg/yr, and rapeseed plants 6.6 ± 1.8 Gg/yr. Given the ubiquitous distribution of Br$^-$ in soil, CH$_3$Br production by terrestrial higher plants is likely a large source for atmospheric CH$_3$Br.

Introduction

Methyl bromide (CH$_3$Br, bromomethane) contributes more than a half of the total stratospheric bromine, and with an estimated ozone depletion potential (ODP) of 0.65, it is considered as one of the most important ozone depleting compounds [Wofsy et al., 1975; Yung et al., 1980; Khalil et al., 1993; Penkett et al., 1995; Schaufler et al., 1998]. The currently identified CH$_3$Br sources include production by the ocean [Penkett et al., 1995], biomass burning [Mano and Andreae, 1994], emissions from leaded gasolines [Thomas et al., 1997], and emissions during the use of man-made CH$_3$Br for fumigation [Majkuzi et al., 1995; Yagi et al., 1995; Yates et al., 1996]. The use of man-made CH$_3$Br is now targeted for imminent phase out. The known CH$_3$Br sinks include degradation in the troposphere [Wofsy et al., 1975; Mellouki et al., 1992], dissolution and degradation in the ocean [Butler and Rodriguez, 1996; Yvon-Lewis and Butler, 1997; Lobert et al., 1995], removal by soil [Shorter et al., 1995], and possibly degradation by green plants [Jeffers et al., 1998]. Although uncertainties exist, the current estimates of all the sinks (206 Gg/yr) are the large source of CH$_3$Br.

This discrepancy should increase further if CH$_3$Br removal by green plants [Jeffers et al., 1998] is taken into consideration. Since this missing fraction is even greater than that expected from the man-made source (42 Gg/yr) [Yvon-Lewis and Butler, 1997], it is of clear importance to identify it.

Experimental

Because previous studies indicated that plants of the Brassicaceae family have higher methyl transferase activity [Attieh et al., 1995], we first measured emissions of CH$_3$Br from Brassica plants that were grown in soil with different Br$^-$ concentrations. Soil Br$^-$ levels were adjusted by addition of KBr, and the initial levels were 0.4 (no KBr amendment), 3.4, 10, 30 and 100 mg/kg. The tested plants included 5 cultivated species: broccoli (Brassica oleracea var. botrytis), cabbage (Brassica oleracea var. capitata), mustard (Brassica juncea), Chinese cabbage (Brassica pekinensis) and pak-choi (Brassica chinensis), and 2 non-cultivated species: alyszum (Lobularia maritima) and wild mustard (Brassica juncea). Young plants of 5-20 g fresh weight were transplanted into 200-g soil in small glass jars and grown in a greenhouse for 4 weeks. To measure CH$_3$Br production, each plant container was placed into a 1.9-L glass jar and sealed with a metal cover for 24 h to allow the production of CH$_3$Br to accumulate. Fifty milliliters of the headspace was then withdrawn and analyzed for CH$_3$Br content on a Hewlett Packard 6890 GC equipped with a μECD. To determine whether the production was from soil or plant roots, soils that were treated with 100 mg/kg Br$^-$, and plant samples that were decapitated, were also analyzed for CH$_3$Br production. Analysis of CH$_3$Br was performed on a 30m x 0.53mm x 3μm AT-Q capillary column (Alltech, Deerfield, IL). Additional confirmation of CH$_3$Br was

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Figure 1. Measured daily \( \text{CH}_3\text{Br} \) emission rates (ng of \( \text{CH}_3\text{Br} \) per g of plant material, dry weight) from young \textit{Brassica} plants growing in soil containing different concentrations of \( \text{Br}^- \). a, Cultivated species; b, Non-cultivated species. \( \text{CH}_3\text{Br} \) production was measured by sealing plant containers into 1.9-L large glass jars for 24 h and analyzing 50 mL of the headspace for \( \text{CH}_3\text{Br} \) on a Hewlett Packard 6890 GC-ECD (Hewlett Packard, Wilmington, DE). The detection limit was about 1 pg/ml of \( \text{CH}_3\text{Br} \) on the HP 6890 GC-ECD. Calibration standards were prepared by adding \( \text{CH}_3\text{Br} \) (in 3 to 24 \( \mu \)L hexane) into 1.5-L closed glass containers (baked at 220°C before use) and then stirring with a magnetic bar for >1 h. The calibration curves were linear for the 0.05-2.0 ng/mL concentration range (\( r^2 \geq 0.99 \)). To ensure zero contamination, syringes were washed with acetone between injections and dried with \( N_2 \), and ambient air samples were frequently checked for background \( \text{CH}_3\text{Br} \). No \( \text{CH}_3\text{Br} \) was detected in the syringes or from the ambient air at the time of analysis.

As \( \text{CH}_3\text{Br} \) produced by plants could be degraded by soil or plants during the 24-h accumulation period, we conducted a separate experiment to determine \( \text{CH}_3\text{Br} \) degradation rate constant \( \mu \) (d\(^{-1}\)) under the experimental conditions. The measured values were then corrected by a factor of \( \mu/(1-e^{-\mu}) \) to yield the plant \( \text{CH}_3\text{Br} \) production rate. To obtain \( \mu \), untreated plant containers were sealed into glass jars, and a known amount of \( \text{CH}_3\text{Br} \) (about 2 mg/L) was introduced. The decrease of \( \text{CH}_3\text{Br} \) concentration in the headspace over 24 h was determined by GC analysis and fitted to a first-order model for calculating \( \mu \).

After measuring \( \text{CH}_3\text{Br} \) production, \( \text{Br}^- \) content in plants and soil was separately measured to determine plant uptake of \( \text{Br}^- \) from soil in which they grew. Plants were removed from the soil and rinsed, and the whole samples were then dried at 70°C for 24 h. The dried matter was ground and an aliquot (200 mg) of the powder was ashed in a crucible at 550°C after 1.5 mL 0.5M KOH was added. The ash was extracted with 20 mL water, and an aliquot was analyzed for \( \text{Br}^- \) concentration on a Dionex-100 Ion Chromatograph. The recovery of \( \text{Br}^- \) from plant tissues using this extraction method is near 100%. To determine soil \( \text{Br}^- \) concentration, 10 g soil was extracted with 20 mL \( H_2O \) by vigorously shaking the sample in a centrifuge tube for 1 h, and an aliquot of the supernatant was analyzed on the IC after centrifugation. The recovery of \( \text{Br}^- \) from soil for this method is greater than 95%.

To verify if plants grown under natural conditions can produce \( \text{CH}_3\text{Br} \), we further measured \( \text{CH}_3\text{Br} \) emission from broccoli, rapeseed (\textit{Brassica napus}), cabbage and wild mustard plants that were sampled directly from farm fields or in the wild. The plants were excavated with adhering soil and immediately transported to the laboratory. These plants, along with the original soil, were sealed into glass containers, and \( \text{CH}_3\text{Br} \) concentration in the headspace was measured after a 24 h accumulation period. The experimental and GC conditions were similar as given previously. The native \( \text{Br}^- \) level in the plant and soil samples were analyzed after \( \text{CH}_3\text{Br} \) measurement, using the same procedures described above.

Results and Discussion

Figure 1 shows the measured daily emission of \( \text{CH}_3\text{Br} \) from single \textit{Brassica} plants that were grown in soil containing various concentrations of \( \text{Br}^- \). Emission of \( \text{CH}_3\text{Br} \) per gram of dry plant mass increased proportionally as the soil \( \text{Br}^- \) level increased within the 0.4 - 100 mg/kg range, and the overall correlation coefficient was 0.89 ± 0.07. Emission made on a separate GC-ECD (HP 5890) using a 3m Heyesep Q packed column (Hewlett Packard, Wilmington, DE). The detection limit was about 1 pg/ml of \( \text{CH}_3\text{Br} \) on the HP 6890 GC-μECD. Calibration standards were prepared by adding \( \text{CH}_3\text{Br} \) (in 3 to 24 \( \mu \)L hexane) into 1.5-L closed glass containers (baked at 220°C before use) and then stirring with a magnetic bar for >1 h. The calibration curves were linear for the 0.05-2.0 ng/mL concentration range (\( r^2 \geq 0.99 \)). To ensure zero contamination, syringes were washed with acetone between injections and dried with \( N_2 \), and ambient air samples were frequently checked for background \( \text{CH}_3\text{Br} \). No \( \text{CH}_3\text{Br} \) was detected in the syringes or from the ambient air at the time of analysis.

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Figure 2. Correlation between daily plant \( \text{CH}_3\text{Br} \) production rates (ng of \( \text{CH}_3\text{Br} \) per g of dry plant material) and plant bromide contents (μg). Plant bromide was extracted after a dried subsample was oxidized in 1.5 mL of 0.5 M KOH at 550°C. The \( \text{Br}^- \) concentration in the water extract was analyzed on a Dionex-100 ion chromatograph using AS-14 column (Dionex, Sunnyvale, CA).
of CH$_3$Br was even detected from all plants growing in the unamended soil that contained only 0.4 mg/kg Br$^-$. All cultivated species and wild mustard produced CH$_3$Br at a similar rate (Figure 1A), and abyssin produced CH$_3$Br at a lower ($p = 0.01$) rate (Figure 1B). It was also observed that 23 to 35% of the produced CH$_3$Br was consumed during the 24-h accumulation period, with the specific values varying among species. Therefore, original CH$_3$Br production rates should be 23-35% higher than the measured values. This observation supports recent findings that soil and green plants can also degrade CH$_3$Br, acting as sinks for atmospheric CH$_3$Br. No CH$_3$Br was detected from Br$^-$.treated soil (without plants), or from Br$^-$.treated soil containing only plant roots. Thus, we conclude that CH$_3$Br was produced by and released from the aboveground part of the plants.

After analysis of Br$^-$ in soil and plants, we found that at the time of measurement, 95 ± 7% of the total soil Br$^-$ was present in the plants, regardless of the initial soil Br$^-$ concentration, and only a small fraction (<5%) remained in the soil. Plant Br$^-$ uptake was linearly ($r^2 = 0.96$) related to the soil Br$^-$ level, which coincided with a linear relationship ($r^2 = 0.91$) between CH$_3$Br production rates and plant Br$^-$ contents (Figure 2). Thus, we further conclude that the plants first obtained Br$^-$ from the soil in which they were grown, and then produced CH$_3$Br and subsequently released it into the air.

In a separate study, we confirmed that CH$_3$Br was produced from field grown broccoli, rapeseed, cabbage and wild mustard plants that were sampled in southern California. Emission of CH$_3$Br was detectable from all samples (Table 1). The Br$^-$ levels in these soils are substantially lower than the values reported for other regions or the average value of 1 mg/kg [Maw and Kempton, 1982; Flury and Papritz, 1993; Yuita, 1994]. Therefore, it can be expected that more CH$_3$Br emissions would occur if the soil Br$^-$ level were higher. Using the CH$_3$Br production rates for cabbage and rapeseed, for which the global biomass is known [FAO, 1997], and a linear relationship between the production rate and soil Br$^-$, we estimate that at 1.0 mg/kg soil Br$^-$ level, the global CH$_3$Br production by rapeseed plants alone would be 6.6 ± 1.6 Gg/yr and that for cabbage alone 0.4 ± 0.2 Gg/yr.

Our preliminary study also identified radish (Raphanus sativus) and turnip (Brassica rapa) as CH$_3$Br producers. Our results, along with the observation of the in vitro studies [Saini et al., 1995], suggest that the Brassicaceae family as a whole is capable of producing CH$_3$Br. The Brassicaceae family is an important component of the terrestrial plant biomass makeup with worldwide distribution as vegetables, oil crops, pastures, ornamental crops, weeds and wild species. It is therefore probable that CH$_3$Br production by this family alone may be substantial in quantity. Although not determined in this study, it is likely that many other terrestrial plants can also produce CH$_3$Br. For example, there have been observations that CH$_3$Br was produced, though at a lesser magnitude, by the leaf discs of many other plants [Saini et al., 1995]. Thus, the size of the CH$_3$Br source may be very large and therefore should be considered as the missing source or a large part of it.

Unlike compounds such as chlorofluorocarbons (CFCs), CH$_3$Br has both natural and anthropogenic origins. An understanding of the relative significance of the controllable (man-made) and uncontrollable (natural) sources is apparently critical for predicting the effectiveness of any regulatory action. The fact that soil contains an average of 1 mg/kg of Br$^-$ and 10-800 mg/kg of nonextractable bromine that can be gradually released as Br$^-$ upon weathering [Flury and Papritz, 1993; Yuita, 1994], or that the soil crust of Earth is a large Br$^-$ reservoir, is neglected in our current consideration of atmospheric CH$_3$Br cycles. Our study suggests that land plants may serve as a "sink" between soil Br$^-$ and stratospheric CH$_3$Br. This finding is also in agreement with the observed 1.2-1.4 northern to southern hemispheric ratio of atmospheric CH$_3$Br concentration [Lobert et al., 1995; Penkett et al., 1995]. Northern hemisphere has a greater landmass than southern hemisphere, and thus likely a greater capacity of plant production of CH$_3$Br. Noteworthily, soil also contains Cl$^-$ and to a lesser extent I$, and some plants, under in vitro or natural conditions, can transform Cl$^-$ to CH$_3$Cl and I$^-$ to CH$_3$I [Wuosmaa and Hager, 1990; Saini et al., 1995; Muramatsu and Yoshida, 1995]. Therefore, this property of terrestrial ecosystem may be broadly important in regulating the abundance of a number of halogenated compounds that are potentially significant ozone depleters, and should be better characterized.

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