Methyl halide emissions from greenhouse-grown mangroves

Steven L. Manley,[1] Nun-Yii Wang,[2] Maggie L. Walser,[3] and Ralph J. Cicerone[2,3,4]

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[1] Two mangrove species, *Avicennia germinans* and *Rhizophora mangle*, were greenhouse grown for nearly 1.5 years from saplings. A single individual of each species was monitored for the emission of methyl halides from aerial tissue. During the first 240 days, salinity was incrementally increased with the addition of seawater, and was maintained between 18 and 28‰ for the duration of the study. Exponential growth occurred after 180 days. Methyl halide emissions normalized to leaf area were measured throughout the study and varied dramatically. Emission rates normalized to land area (mg m^{-2} y^{-1}), assuming a LAI = 5, yielded 82 and 29 for CH_3Cl, 10 and 1.6 for CH_3Br, and 26 and 11 for CH_3I, for *A. germinans* and *R. mangle*, respectively. From these preliminary determinations, only CH_3I emissions emerge as being of possible global atmospheric significance. This study emphasizes the need for field studies of methyl halide emissions from mangrove forests. **Citation:** Manley, S. L., N.-Y. Wang, M. L. Walser, and R. J. Cicerone (2007), Methyl halide emissions from greenhouse-grown mangroves, *Geophys. Res. Lett.*, *34*, L01806, doi:10.1029/2006GL027777.

1. Introduction

[2] An important source of halogens in the troposphere and stratosphere are the naturally produced methyl halides, methyl chloride (CH_3Cl), methyl bromide (CH_3Br) and methyl iodide (CH_3I). Their atmospheric breakdown products are important in stratospheric ozone destruction and tropospheric chemistry. *Saiz-Lopez and Plane* [2004] describe the tropospheric reaction mechanisms whereby inorganic halogen species (a) destroy ozone, (b) oxidize organic compounds such as dimethyl sulfide, c) influence the ratios of NO_2/NO and HO_2/HO and (d) by which inorganic bromine removes elemental mercury and (e) how iodine can cause fine particle formation, especially in the marine boundary layer. Detailed reaction schemes are also presented by *von Glasow et al.* [2004] and references therein.

[3] The magnitude of known atmospheric methyl halide removal mechanisms is much greater than known methyl halide sources [*Cox et al.*, 2005; *Montzka et al.*, 2003]. Although the marine environment (oceanic and coastal zones) have long been recognized as important natural producers of methyl halides, terrestrial ecosystems may be, equally if not, more important sources of methyl halides, especially CH_3Cl and CH_3Br. More recently, CH_3Cl, CH_3Br and CH_3I emissions have been measured from temperate coastal salt marshes [*Manley et al.*, 2006]. Global extrapolations suggest that they may contribute no more than 4% of the missing flux for CH_3Cl and CH_3Br, and 1% for CH_3I. These estimates are based on measurements with limited temporal and spatial components and assumed that mangroves, which replace temperate salt marshes in subtropical and tropical latitudes, had similar emission rates.

[4] Highly productive mangrove forests, or mangals, are situated in sandy and muddy tropical and subtropical sheltered coastal locals [*Hogarth*, 1999]. Although more structurally complex, mangals are the tropical equivalent of the temperate coastal salt marsh ecosystem. Mangroves are halophytic trees and woody shrubs that are exposed directly or indirectly to seawater halides. Their global range is approximated by the 20°C winter ocean isotherm, and as such cover approximately 60–70% of the coastline between 25°N and S latitudes [*Hogarth*, 1999]. Of the 50 to 75 recognized mangrove species, comprising 16 – 20 families, there are 4 major genera: *Rhizophora, Avicennia, Bruguiera* and *Sonneratia* [*Ellison and Farnsworth*, 2001; *Hogarth*, 1999; *Kathiresan and Bingham*, 2001].

[5] We reasoned that mangroves may be globally important producers of methyl halides because they are tropical, highly productive, and, more importantly, halophytic and subject to the periodic tidal inundation as are coastal salt marshes. Our approach was to obtain young red (*R. mangle*) and black (*A. germinans*) mangrove saplings, grow them in a greenhouse, mimicking certain features of their tropical habitats such as exposure to high temperature, seawater and waterlogged anoxic soil, and measure their in situ emission of methyl halides.

2. Materials and Methods

2.1. Mangrove Planting and Growth

[6] On 18 October 2002 (t = 0) 15 cm tall saplings of *R. mangle* (red mangrove) and *A. germinans* (black mangrove) were obtained from Mangroves Direct (Nova University) and 5 each planted in water tight bin containers (0.91 m × 0.91 m × 0.6 m depth) located in the UCI greenhouse. Four trees were located near each corner with the fifth in the center, all equally spaced from each other. The center plant was monitored for methyl halide emissions and growth throughout the study. The marginal plants were placed to minimize possible "edge effects" that might have occurred between the roots and the bin. As all of the plants grew, the outer plants began to crowd and grow over the container sides making their potential chambering unfeasible. Initially, growth of all plants were followed, however, as the marginal plants began to crowd

[1]Department of Biological Sciences, California State University, Long Beach, California, USA.
[2]Department of Earth System Science, University of California, Irvine, California, USA.
[3]Department of Chemistry, University of California, Irvine, California, USA.
[4]Now at National Academy of Sciences, Washington, D. C., USA.

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Table 1. Methyl Halide Emission for Greenhouse-Grown *A. Germinans* and *R. Mangle*

			Mean Methyl Halide Emissions Per Leaf Area,[a] ng cm^{-2} d^{-1}					
			CH_3Cl		CH_3Br		CH_3I	
Growth Period	Days After Planting	Salinity, ‰	A[b]	R[c]	A	R	A	R
1	60–150	increased 1 to 15	−6.2 (10)	11 (10)	−0.02 (0.10)	0.24 (0.40)	12[d] (4.2)	2.3 (2.0)
2	150–240	maintained 10–15	6.0 (6.0)	2.5 (0.5)	1.0 (1.1)	0.21 (0.26)	0.94 (1.6)	−0.03 (0.18)
3	>240	maintained, 18–28	4.5 (4.0)	1.6 (1.1)	0.56 (0.40)	0.09 (0.13)	1.4 (0.7)	0.58 (0.24)
Mean, all periods			1.5	5.3	0.52	0.18	4.8	1.0

[a](SD), n = 4 except for growth period 3 for *R. mangle* where n = 3.
[b]*A. germinans*.
[c]*R. mangle*.
[d]Significantly different from other growth periods ($p \leq 0.05$).

the center plant, they were trimmed to allow unrestricted growth and the continued chambering of the center plant.

[7] The bin was lined with EPDM rubber pond liner (PondGard®; Firestone, Akron OH). Each bin was filled (~340 kg) with sifted soil (1.3 cm screen) from a nearby freshwater marsh to which solid fertilizer (Scotts Turf Builder®, Marysville, OH; equivalent to 15 g-N, 1.5 g-P and 1.5 g-S per bin) had been thoroughly mixed [*Redeker and Cicerone*, 2004]. A hole (15 cm diameter ×30 cm deep) was dug and the bottom $^2/_3$ of the planted tree roots were surrounded by sand and soil with the upper third surrounded with "Aruba Puka Shells" (Seaflor®; CaribSea, Miami FL) to 3 cm above the surface. Freshwater of salinity <1‰ covered the soil-substrate for the first 60 days. The salinity was then increased by 5‰ increments with addition of seawater every month until reaching 15‰ (day 150) after which it was maintained between 10–15‰ for an additional 3 months (day 240). After this 8 month period, the salinity was maintained between 18–28‰.

[8] Supplemental lighting (350 μmole quanta m^{-2} s^{-1} at the soil surface, 12L/12D) was supplied to each bin from a separate metal halide lamp (1000 W; Venture Lighting, Racine WI). Prior to day 334, the greenhouse glass was tinted to reduce the ambient light by 50%, supplemental heating was not available except during the day from the artificial lighting (minimum winter night temperature was 14°C reached on several days) and cooling kept the summer high temperature of air below 35°C. After that date the tinting was removed and the greenhouse environment maintained by computer (GEM Link; QCOM, Irvine CA) at 19–32°C. Growth was monitored by measuring leaf surface area of the centered individual from which methyl halide emissions were also measured.

2.2. Methyl Halide Analysis

[9] Analysis was performed by GC-ECD as described by *Manley et al.* [2006]. Standards in N_2 (9.68 ppb CH_3Br and 479 ppb CH_3Cl; 25.4 ppb CH_3I) from Scott Specialty Gases were injected at low pressures to create a linear calibration curve with a range of absolute amounts bracketing sample amounts. Methyl halide flux was determined in the daytime between 10 AM and 2 PM by enclosing the center plant in an incubation chamber and periodically withdrawing a gas sample into a valved evacuated canister [*Redeker et al.*, 2000]. Artificial lighting was turned off during incubations to prevent excessive chamber temperatures. The bottom was sealed by the surrounding water (1–2 cm deep) and mud. Four different sized chambers were used to accommodate the growing plant: 9 L glass cylinder (14 cm I.D.), 40 L and 80 L polycarbonate cylinder (29.5 cm I.D.) and 450 L Plexiglas® cube. Control incubations were performed similarly by positioning the 9 L glass cylinders or 20 L polycarbonate cylinder (29.5 cm id) over the water/mud avoiding all aerial portions and visible roots of the plant. These chambers do not inherently produce methyl halides [*Redeker et al.*, 2000]. Incubation temperatures ranged from 24–37°C. Incubation sampling frequency and duration ranged from 0–15 min at 5 min intervals, to 0–90 min at 30 min intervals depending on the size of the chamber. The mean net methyl halide emission (chambered plant emissions minus chambered mud emissions) from each growth period for each mangrove was analyzed using one-way ANOVA (non-transformed) or the Kruskal-Wallis test when the data were non-parametric.

2.3. Tissue Halide Analysis

[10] The halide concentration of the leaf tissue was determined from sequential aqueous extractions of dried milled tissue using a specific ion meter/autotitrator in conjunction with the appropriate halide electrode and the Gran's known addition method [*Ralph and Manley*, 2006; *Gran*, 1952].

3. Results and Discussion

[11] After an apparent lag phase of growth of 170 days for both species, a steady and apparently healthy growth increase of 10% and 7.8% per week ensued for *A. germinans* and *R. mangle*, respectively. These growth rates are similar to those derived from the data of *Smith and Snedaker* [1995] for mean leaf index of *R. mangle* seedlings grown in outdoor containers: mean growth between days 99 and 144 ranged from 1–6% per week. Throughout the study all the plants appeared healthy and showed no evidence of chlorosis or disease. *A. germinans* and *R. mangle* developed their characteristic root types: pneumatophores (breathing roots) and stilt roots (support roots), respectively. The rate of leaf area increase declined slightly for both plant species between the last two dates of measurements (beginning at t = 355 for both species and ending at t = 473 for *A. germinans* and t = 510 for *R. mangle*) due to leaf senescence. New leaves, however, were continually being produced during this period.

Table 2. Yearly Methyl Halide Emission From Halophytes and Rice

Plant Species	CH_3Cl, mg m^{-2} y^{-1}	CH_3Br, mg m^{-2} y^{-1}	CH_3I, mg m^{-2} y^{-1}	Reference
A. germinans, period 3[a]	82	10	26	this study
A. germinans, entire study[a]	27	9.5	88	this study
R. mangle, period 3[a]	29	1.6	11	this study
R. mangle, entire study[a]	97	3.3	18	this study
Rice, field[b,c]	4.0	0.92	49	*Redeker et al.* [2000]
Rice, greenhouse[b,d]	2.2	0.70	65	*Redeker and Cicerone* [2004]
Spartina foliosa, field	12	2.8	7.6	*Manley et al.* [2006]
Salicorna virginica, field	12	3.2	2.0	*Manley et al.* [2006]
Batis maritima, field	1.4×10^3	230	16	*Manley et al.* [2006]
Frankenia grandifolia	2.6×10^3	340	25	*Manley et al.* [2006]
Pachycornia arbuscula, field	2.6	1.7	2.2	*Cox et al.* [2004]

[a]LAI = 5.
[b]Based on a single growing season of 110 days (±15 days).
[c]Emissions from rice (M202) minus control plot.
[d]Cultivar M202 grown in UCI greenhouse.

[12] Chamber temperature during the incubation of the mangroves had no measurable effect on methyl halide emissions. All the methyl halide emission measurements were weakly linearly correlated with incubation temperature for either *A. germinans* (r^2 from −0.10 to +0.17) or *R. mangle* (r^2 from −0.02 to +0.14).

[13] For the last two 90 min incubations of *A. germinans*, when leaf area was above 3,000 cm^2 (growth period 3), photosynthesis may have become CO_2 limited based on the known photosynthetic rate of 9.7 μmole CO_2 m^{-2} leaf area s^{-1} measured in the tropical midday sun (photon flux density = 2100 μmole m^{-2} s^{-1}) [*Sobrado*, 2000]. Our daytime greenhouse incubations, however, were done at a photon flux density of 300–350 μmole m^{-2} s^{-1} at the canopy top (daylight only; lighting turned off). We calculated that 40% of the entrained CO_2 would have been consumed. Examination of the methyl halide emission kinetics over these long time intervals yielded linear correlations of $r^2 > 0.9$. Carbon dioxide limitation did not appear to have affected methyl halide emissions. During the incubation periods, stomatal pores probably were not closed, because the plants were never subjected to water stress, and remained open to facilitate CO_2 uptake and methyl halide efflux. Methyl halide emissions from rice were similar during light and dark periods [*Redeker and Cicerone*, 2004].

[14] Mean methyl halide emissions for each growth period are shown in Table 1. Period 1 is when seawater was initially added incrementally to 15‰. The salinity was maintained between 10 and 15‰ during Period 2. Salinity was increased to 18‰ at the start of Period 3 and maintained between 18 and 28‰ throughout.

[15] The mean emission rates for a given methyl halide and mangrove at each growth period were not statistically different from each other ($p < 0.05$) except for the emission of CH_3I during the first growth period by *A. germinans*. The production of CH_3Cl by *A. germinans* was lower during growth period 1 as compared to the other growth periods at $p = 0.11$. Because of high variability, there was no significant difference between the two mangroves ($p \leq 0.05$) in the mean emission of a given methyl halide over the entire study. *A. germinans* tended to emit more CH_3I than *R. mangle* ($p = 0.13$). *R. mangle* tended to emit more CH_3Cl but with much less certainty ($p = 0.28$) because of its extremely high emission during growth period 1 compared with net uptake for *A. germinans*.

[16] During growth period 1 for *A. germinans*, net uptake of CH_3Cl occurred during 3 of the 4 measurements and net uptake of CH_3Br occurred for half the measurements. Relatively high net emissions of CH_3I were measured during this period. Net uptake of methyl halides by chambered plants was inferred because no concentration changes were detected, while net emissions occurred from the soil control. Seawater additions began during this period and presumably a transition in the soil microbial community was taking place. Also occurring during this period was increased root development with pneumatophores becoming evident from *A. germinans*. Undoubtedly the biological (microbial and faunal) and chemical composition of container soil was very different from that in a mangrove forest. Any processes occurring therein, affecting soil methyl halide emissions or uptake, are probably not representative of the natural condition. The dominant emission of CH_3Cl and CH_3Br from the chambered soil may have been a result of a changing microbial community and/or production from roots, although no visible pneumatophores were enclosed. Clearly CH_3I was being emitted from the aerial part of the plant.

[17] Except for the first day of measurement for *R. mangle*, there were net plant emissions for all three gases during growth period 1, with CH_3Cl dominant. Methyl halide emission was highest during this period and tended to decline with growth. During growth period 1, stilt roots emerged from the main trunk of *Rhizophora*. Control emissions were less from *R. mangle* controls than from *A. germinans* controls during growth period 1, perhaps because of less root development. Control emissions from both bins were more similar during the other two growth periods.

[18] Direct comparisons of methyl halide emissions between these mangroves and other plants can not be made because plant biomass was not measured and consequently emissions are not normalized to biomass. Estimates of emissions per land area can be made, however, using the above determinations of methyl halide emissions and estimates of leaf area index (LAI; single sided leaf area ÷ ground area) for each species (Table 2). This is a more accurate method of estimation rather than using the surface

area of the mud covered by the chamber because our plant density was not similar to natural mangrove stands. LAI estimated from our data (not shown) increased over time reaching a maximum during growth period 3: 1.9 for *R. mangle* and 2.6 for *A. germinans* which is low compared to field values, as would be expected from juvenile trees. LAI determinations for *R. apiculata* have been reported (mean ± SD) as 5.1 ± 0.11 ranging from 2.2 to 7.4 [*Clough et al.*, 1997], 4.4 ± 0.26 for a *R. mangle* dominated stand [*Sherman et al.*, 2003] and 5.7 ± 1.8 for a *R. mangle* basin type stand [*Araújo et al.*, 1997]. Although no LAI values for *Avicennia* could be found, a mixed mangrove stand of *R. mangle*, *Laguncularia racemosa* and *A. germinans* growing with *Concarpus erectus* (tropical buttonwood) had an LAI range of 0.8 and 7.0, with a mean of 4.0 [*Green et al.*, 1997].

[19] Methyl halide emissions normalized to land area were calculated using emission rates for these two species from growth period 3 and the mean emission rate for the entire study (Table 2). A LAI of 5 was used which approximates the average values determined from field studies. Growth period 3 was the longest period of the study and the most stable environmentally, with the maximum salinity maintained and temperature being controlled by computer for most of this period. During growth period 3 there was only a single instance of measured net uptake of a methyl halide; CH_3Br uptake by *R. mangle*. Emissions for this period may be a more accurate representation of what occurs naturally. The mean methyl halide emission by the two mangroves during period 3 were close to being significantly different for CH_3Cl and CH_3Br ($p = 0.10$), and for CH_3I ($p = 0.06$). The mean emissions for a given methyl halide by the two mangroves over the entire study were not significantly different.

[20] Using rates from growth period 3, calculated CH_3Cl emissions per land area (mg m^{-2} d^{-1}; LAI = 5) from these two mangrove species were within the range reported for coastal salt marsh plants. Mangrove emissions of CH_3Cl may contribute to the strong CH_3Cl emissions associated with tropical coastlines [*Yokouchi et al.*, 2000]. Methyl bromide emissions tended to be similar to the lower emitting salt marsh plants, while CH_3I emissions were similar to the high emitting salt marsh plants (Table 2). Methyl iodide emissions by mangroves during growth period 3 are not as high as that from rice which uses CH_3I as a means to rid itself of tissue iodide [*Redeker and Cicerone*, 2004]. Rice values, however, are based on a single growing season of approximately 110 days.

[21] Analysis of leaf tissue halide content collected over growth period 3 (n = 3) yielded the following results based on leaf dry weight: *A. germinans* 4.9% chloride, 1.1‰ bromide and 6.3 ppm iodide; *R. mangle* 9.0% chloride, 2‰ bromide and 13 ppm iodide. *Market and Jayasekera* [1987] reported the following dry weight concentration ranges from *R. mangle* leafs: 1–10% chloride, 0.1 to 1‰ bromide, and <6 ppm iodide. Our chloride values are comparable. Our analysis of leafs from a mature *R. mangle* tree from a natural population near Ft. Pierce Florida yielded 5% chloride, 0.8‰ bromide and 1.6 ppm iodide. The chloride and bromide values were significantly different between the two greenhouse-grown species at $p < 0.1$. The iodide values were not significantly different ($p = 0.35$). Although the leaf halide concentrations are greater in *R. mangle* during growth period 3, methyl halide emissions are greater in *A. germinans* during this period. These species have different strategies to tolerate and grow in high salinity conditions. *Avicennia* rids itself of excess salt by actively excreting it through its leaves. *Rhizophora* selectively excludes ions by ultrafiltration and stores excess ions in cell vacuoles [*Kathiresan and Bingham*, 2001]. The cell vacuoles of *Rhizophora* may sequester halides from methyl transferases, the enzyme responsible for methyl halide biosynthesis in plant tissue [*Rhew et al.*, 2003].

[22] An estimate of global mangrove methyl halide production can be made using the above determinations of methyl halide emissions, an LAI of 5 and an estimate of global mangrove surface coverage (2×10^5 km^2) [*Duarte et al.*, 2005]. The combined mean methyl halide emissions for both mangroves (ng cm^{-2} y^{-1}) for period 3 were used to calculate global mangrove emissions: 11 Gg CH_3Cl y^{-1}, 1.2 CH_3Br Gg y^{-1} and 3.7 CH_3I Gg y^{-1}. Using the combined mean methyl halide emissions for both mangroves (ng cm^{-2} d^{-1}) for the entire study to calculate global mangrove emissions yielded 12, 1.3 and 11 Gg y^{-1} for CH_3Cl, CH_3Br and CH_3I, respectively. Using the CH_3Cl global sink strength of 4005 Gg y^{-1} reported by *Montzka et al.* [2003], our preliminary analysis shows mangroves producing 0.3%. Using global sink strengths of 205 Gg CH_3Br y^{-1} and 258 Gg CH_3I y^{-1} [*Cox et al.*, 2005] indicate that mangroves contribute approximately 0.6% to global CH_3Br production and no more than 4% to global CH_3I production. From these preliminary determinations, only CH_3I emissions emerge as globally significant.

[23] This work represents the first measurements of methyl halide emissions in mangroves. Although caution must be taken applying emissions derived from a single individual grown in a greenhouse, to natural populations, methyl halide emissions from greenhouse-grown rice is very similar to that found in the field (Table 2). (We utilized the same bins and greenhouse used for the rice experiments.) The two species chosen were from 2 dominant mangrove genera. However, juvenile trees may have inherently different emission rates (per unit biomass or leaf area) than mature trees. We also did not take into account possible emissions from roots and stems, or the possibility of enhanced emissions during flowering [*Manley et al.*, 2006]. Clearly a more accurate determination must be conducted on natural populations. Tidal fluxes, rainfall, biogeochemical processes and community interactions would all affect the growth and physiology of the plant species and microbial populations present. As demonstrated for coastal salt marsh plants, accurate determinations of methyl halide production from ecosystems must involve measurements of a majority of species present as well as the amount of unvegetated area present [*Manley et al.*, 2006]. Also, plant species present in low numbers can be significant producers of certain methyl halides in the ecosystem. The task of measuring methyl halide emissions in the field will be arduous in part because of the large number of mangrove species.

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R. J. Cicerone, National Academy of Sciences, Washington, DC 20001, USA.

S. L. Manley, Department of Biological Sciences, California State University, 1250 Bellflower Blvd., Long Beach, CA 90840, USA. (slmanley@csulb.edu)

M. L. Walser, Department of Chemistry, University of California, Irvine, CA 92697, USA.

N.-Y. Wang, Department of Earth System Science, University of California, Irvine, CA 92697, USA.