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Stimulation of Microbial Degradation of Methyl Bromide in Soil during Oxidation of an Ammonia Fertilizer by Nitrifiers

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To reduce volatilization of the fumigant methyl bromide (MeBr) from soil into the atmosphere, attempts were made to enhance microbial degradation of MeBr in soil by stimulating the activity of soil nitrifiers. Disappearance of MeBr in limed Arredondo soil (pH 7.70) treated with an ammonia-based nitrogen fertilizer, $(\text{NH}_4)_2\text{SO}_4$, was initially more rapid than in unlimed Arredondo soil (pH 5.5-5.7). Disappearance of MeBr in limed soil with or without treatment of $(\text{NH}_4)_2\text{SO}_4$ that received 20 $\mu\text{g/g}$ MeBr was more rapid than in the corresponding soil samples that received a higher rate of MeBr (50 $\mu\text{g/g}$). Due to higher nitrification activity in limed surface soil (0-15 cm depth) than in limed subsurface soil (15-30 cm depth), disappearance of MeBr in the surface soil with or without $(\text{NH}_4)_2\text{SO}_4$ treatment was also more rapid than in the corresponding subsurface soil. Both microbial and chemical degradation were involved in the MeBr degradation in soil, with chemical degradation possibly being the major factor. Contribution from microbial degradation was greater in soil treated with $(\text{NH}_4)_2\text{SO}_4$ than in untreated soil, and up to 57% of MeBr degradation was attributed to microbial degradation, mainly by the activity of nitrifiers during the oxidation of ammonia. Inoculation of an ammonia oxidation bacterium, *Nitrosomonas europaea* ATCC 19718, to soil greatly stimulated the initial degradation of MeBr. In conclusion, stimulation of MeBr degradation in soil can be achieved through liming and application of an ammonia fertilizer as well as through inoculation of an ammonia oxidation bacterium. Consequently, the rate of MeBr flux into the atmosphere may be reduced after fumigation is completed.

Introduction

Methyl bromide (MeBr) is presently the most important preplant soil fumigant commercially available (1). This chemical is used extensively in the United States in the production of many economically important crops for the management of plant-pathogenic nematodes, soil-borne fungi and bacteria, and weeds (2). This chemical is also used as a space fumigant for commodities, for structural pest control, and for quarantine and regulatory purposes. For preplant soil fumigation, MeBr is generally applied under a sheet of polyethylene plastic, which may remain in place until the crop cycle is completed.

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Methyl bromide is considered to be a highly potent depleter of the stratospheric ozone layer (3-5). The disruption of the ozone layer is believed to be due to the bromine atoms, released from MeBr in the stratosphere (3, 5). Consequently, the Montreal Protocol Treaty was amended to require developed countries to freeze production levels of MeBr at 1991 levels (5). The U.S. EPA has mandated a suspension of all use of MeBr in the United States by 1 January 2001 (1). This has triggered an intensive search for alternative chemicals and strategies to replace MeBr.

Due to its gaseous nature, after injection into soil for fumigation, MeBr rapidly diffuses through the soil pore space to the soil surface and then into the atmosphere (6-10). Since a plastic sheet typically covers the soil surface, the rate of emission into the atmosphere depends upon the thickness and density of the plastic, if other conditions are the same (7, 8). Other routes of disappearance from soil include chemical hydrolysis, methylation to soil organic matter through free radical reactions, and microbial degradation (6, 8, 11, 12). Yagi et al. (8) reported that up to 70% of the injected MeBr was degraded in soil. Shorter et al. (6) suggested that bacteria were responsible for the biological degradation of MeBr in soil.

Methyl bromide is a short-chained halogenated hydrocarbon. Microorganisms capable of utilizing short-chained halogenated hydrocarbons, including trichloroethylene (TCE) and MeBr, as a sole source of carbon and energy for growth have not been isolated, rather they are degraded through co-metabolic processes (13). These compounds, such as TCE, are oxidized by mono- and dioxygenases produced by bacteria specifically for initial oxidation of methane, toluene, phenol, 2,4-D, ammonia, etc. (13-19). Rasche et al. (18) first reported that some terrestrial and marine nitrifiers had the capacity to oxidize MeBr to formaldehyde and bromide ion. They concluded that ammonia monooxygenase produced by the nitrifiers that catalyzes the oxidation of ammonia to hydroxylamine was responsible for the oxidation of MeBr to formaldehyde. Oremland et al. (12) subsequently demonstrated that methane oxidation bacteria also had the capacity to co-oxidize MeBr by methane monooxygenase produced during the oxidation of methane to methanol. They also showed that methanotrophic soils that had a high capacity to oxidize methane degraded ^{14}C -labeled MeBr to $^{14}\text{CO}_2$.

Ammonia fertilizers are common nitrogen fertilizers. Even though stimulation of MeBr degradation in soil by ammonia fertilizers has not been reported, it is conceivable that, during the nitrification of ammonia in soil, MeBr also can be simultaneously oxidized by the ammonia oxidizers. As a result, degradation of the chemical in soil would be stimulated, and thereby the flux of MeBr into the atmosphere could be reduced. In this study, we reported the enhancement of the degradation of MeBr in soil pretreated with an ammonia fertilizer and stimulation of MeBr degradation in soil inoculated with a nitrifier, *Nitrosomonas europaea*.

Materials and Methods

Soil. Surface (0-15 cm depth) and subsurface (15-30 cm depth) soil samples were collected from an undeveloped forest site at the campus of the University of Florida. This site is about 100-200 m away from an agricultural experimental site that was used intensively for studying irrigation and fertilization. Soil samples were kept in plastic bags, stored in the dark at 4 °C, and used within 3 months after collection. Soil was classified to be Arredondo fine sand (loamy, siliceous, Hyperthermic Grossarenic Paleudult). Selected soil properties are shown in Table 1.

TABLE 1. Selected Properties of Arredondo Fine Sand

soil depth (cm)	pH		soil-water content (g/kg)	organic C (g/kg)	particle size distribution (%)		
	unlimed	limed			sand	silt	clay
0-15	5.50	7.70	65	11.8	92	7	1
15-30	5.70	7.74	53	7.8	93	5	2

Chemicals. Analytical-grade MeBr (99.5% purity) pressurized in a metal cylinder was purchased from Matheson Gases (Morrow, GA). Prior to use, MeBr was flushed through a vacuumed Tedler gas sampling bag (1-L size) (Supelco, Bellefonte, PA). After three volumes of MeBr had passed at 1 atm, the bag was closed. A gas-tight glass microsyringe was used for transferring a known amount of MeBr to soil. Analytical-grade ammonium sulfate [(NH₄)₂SO₄] (99.7% purity) was purchased from Fisher Scientific (Orlando, FL). All other chemicals were either analytical-grade or the highest grade commercially available.

Soil Treatment and Incubation. A total of 500 g of soil in a 1-L wide-mouthed glass Erlenmeyer flask was treated with 0.354 g of (NH₄)₂SO₄ to give a concentration of 150 μg of (NH₄)₂SO₄-N/g of soil, with or without liming. If limed, 0.75 g of CaO was added to the soil to give a pH value of 7.8 ± 0.1. All flasks were incubated in the dark at ambient temperature (23 ± 2 °C). Once a week, 10 g of soil was removed from each flask for the determination of soil pH. At intervals of 2-4 weeks, 10 g of soil was also removed for determination of nitrite and nitrate concentrations in soil.

After these soil samples were incubated for 28-42 days, a part of the soil samples (200 g) was autoclaved at 121 °C for 1 h. Ten grams of the soil samples (live and autoclaved) was transferred to a series of 22-mL headspace gas chromatography (GC) glass vials (Perkin Elmer, Norwalk, CT). A known amount of MeBr was introduced to the soil surface in a vial through a microsyringe, and the vial was immediately closed tightly with a Teflon-lined butyl rubber cap. Methyl bromide is much heavier than air. We found that escape of the gas into the atmosphere was negligible. After the vials were sonicated for 15 min, they were incubated in the dark at 25 °C. At predetermined time intervals, two vials from each treatment were removed and sonicated for 15 min before determination of MeBr residues by headspace GC as described below.

Analytical Procedures. Methyl bromide residues in the headspace GC vials, which contained 10 g of soil, were determined by a Perkin Elmer autosystem GC (Perkin Elmer, Norwalk, CT) equipped with a headspace autosampler, a flame ionization detector (FID), split-splitless injector, Turbochrom 4 software, and a 486 computer. GC parameters and operational conditions were as follows: column, RTX-1 (60 m × 0.32 mm, Supelco, Bellefonte, PA); detector, FID; detector temperature, 250 °C; oven temperature, 50 °C; carrier gas, He; flame gases, H₂/air; attenuation, 1; flow rates for He, H₂, and air, 2.74, 45, and 429 mL/min; and equilibrium cycle time, 5 min. Headspace parameters and operational conditions were as follows: sampling temperature, 28 °C; transfer line temperature, 28 °C; needle temperature, 28 °C; thermostat time, 15 min; injection time, 0.03 min; and withdrawal time, 0.2 min. Under these conditions, retention time for MeBr was 3.8 min.

Inoculation of *Nitrosomonas europaea* to Soil. The ammonia oxidizer, *N. europaea* ATCC 19718, was provided by M. Hyman (Oregon State University, Corvallis, OR). The organism was grown in the liquid culture medium (2 L) as described by Hyman et al. (20) to late log phase of growth. The bacterial cells were harvested by centrifugation, washed once, and suspended in 8 mL of buffer (2 mM MgCl₂, 50 mM

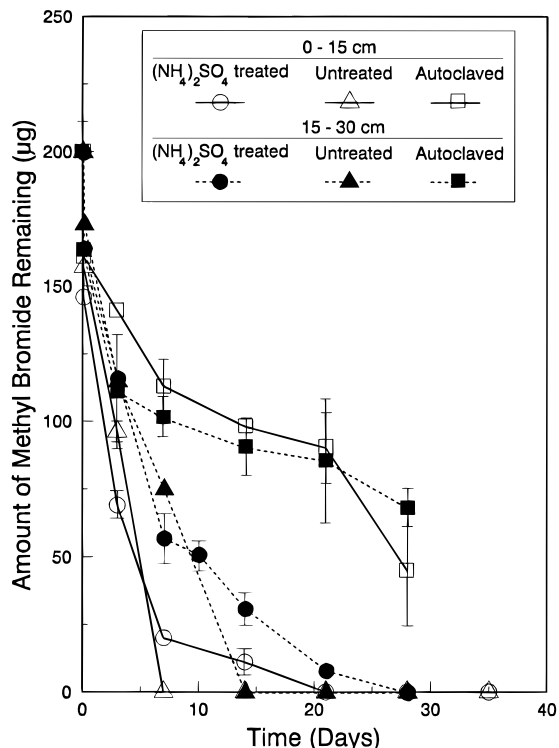


FIGURE 1. Disappearance of MeBr in surface (0-15 cm depth) and subsurface (15-30 cm depth) Arredondo soil (10 g) treated with 200 μg of MeBr (20 μg/g of soil). The soil samples were limed and treated with (NH₄)₂SO₄, limed but not treated with (NH₄)₂SO₄, or limed and autoclaved. Error bars represent 1 SD.

phosphate, pH 7.7) (21). The cell suspension was divided into two equal parts and transferred to glass vials. One vial was autoclaved to kill cells. A 0.2 mL sample of these suspensions was transferred to a series of headspace GC vials that contained 10 g of live or autoclaved soil [pretreated with (NH₄)₂SO₄ or untreated]. The oven-dry weight of the 0.2 mL cell suspension was 0.76 mg. After mixing, MeBr at the amount of 500 μg was injected into each vial, and the vials were immediately capped. The vials were incubated in the dark at 25 °C. At assigned time intervals, two vials from each treatment were used for the determination of MeBr residues by headspace GC.

Miscellaneous Analyses. Total soil bacterial populations were determined by a dilution-plate count method as described previously by Ou et al. (22). Ten grams of surface soil in 22-mL headspace GC glass vials was treated with various amounts of MeBr (0-2000 μg) and incubated in the dark at 25 °C for 16-18 h. Amounts of nitrite and nitrate formed in limed and unlimed soil samples were determined by conventional methods (23).

Results and Discussion

Degradation at a Low Concentration. At the amount of 200 μg of MeBr applied to 10 g of limed soil (20 μg/g of soil), MeBr in the live surface soil samples disappeared rapidly (Figure 1). During the first 3 days of incubation, MeBr in the (NH₄)₂SO₄ treated surface sample disappeared even more rapidly than in the untreated surface sample. Less than 40% and 50% of the applied MeBr remained after 3 days in the treated sample and the untreated sample, respectively. Methyl bromide in the untreated surface and subsurface samples disappeared linearly and completely disappeared in 7 and 14 days, respectively. Biphasic linear or near-linear disappearance patterns were observed for the two treated samples. In the first 7 days, MeBr in the two samples disappeared nearly

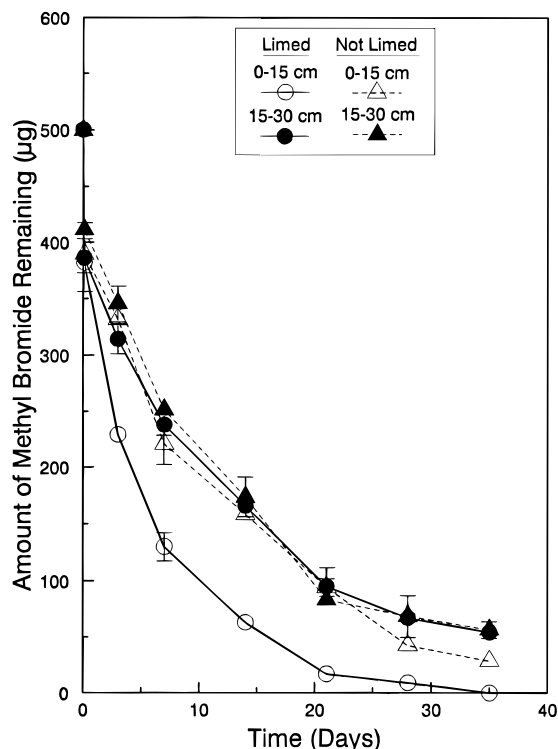


FIGURE 2. Disappearance of MeBr in $(\text{NH}_4)_2\text{SO}_4$ -treated surface (0–15 cm depth) and subsurface (15–30 cm depth) Arredondo soil. The soil samples (10 g) were treated with 500 μg of MeBr (50 $\mu\text{g}/\text{g}$ of soil) and were either limed or unlimed. Error bars represent 1 SD.

linear or linear; and after 7 days, the disappearance was still linear or nearly linear, but at lower rates. Methyl bromide in the treated surface and subsurface samples completely disappeared in 21 and 28 days, respectively. Furthermore, MeBr in the surface samples disappeared more rapidly than in the corresponding subsurface samples.

Biological degradation appeared to play a key role in the disappearance of MeBr in live soil. Disappearance of MeBr in the autoclaved surface and subsurface samples was significantly less than in live samples (Figure 1). A total of 23–34% of the applied MeBr still remained in the autoclaved samples after 28 days, whereas MeBr in live samples completely disappeared before 28 days. In contrast to the live samples, MeBr in the surface sample disappeared more slowly than in the subsurface sample. It was estimated that during the first 7 days of incubation, 35–57% and 13–22% of the degradation in live surface and subsurface samples, respectively, were attributed to microbial degradation. The contribution from biological degradation in surface soil was larger than in subsurface soil. However, it is commonly known that autoclaving may alter, to some extent, the chemical and physical characteristics of soil (24). It is not clear, however, whether autoclaving causes the soil to accelerate or to retard the chemical degradation of MeBr. However, our data suggest that autoclaving causes soil to accelerate the chemical degradation of MeBr, because disappearance rates of MeBr in autoclaved and live unlimed soil samples were similar (see Figure 3).

Degradation at a High Concentration. At the amount of 500 μg of MeBr applied to 10 g of soil (50 $\mu\text{g}/\text{g}$ of soil), MeBr in $(\text{NH}_4)_2\text{SO}_4$ -treated limed surface soil sample initially disappeared more rapidly than in the treated unlimed surface sample (Figure 2). In this limed sample, MeBr initially disappeared rapidly, with more than half of the applied fumigant disappearing in the first 3 days of incubation; whereas about half of the applied MeBr in the unlimed surface soil disappeared in the first 7 days of incubation. Methyl

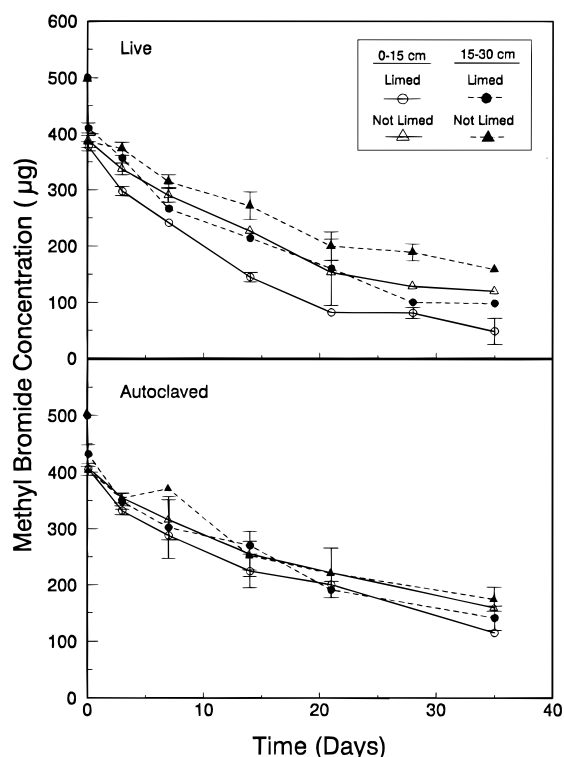


FIGURE 3. Disappearance of MeBr in surface (0–15 cm depth) and subsurface (15–30 cm depth) Arredondo soil (10 g) treated with 500 μg of MeBr. The soil samples were live limed or unlimed, or autoclaved limed or unlimed. These soil samples were not treated with $(\text{NH}_4)_2\text{SO}_4$. Error bars represent 1 SD.

bromide in the treated surface sample disappeared more rapidly than in the treated subsurface sample; whereas no difference was observed between the disappearance rates of MeBr in the subsurface limed and unlimed samples, with the exception in the first 3 and 7 days. In the first 3 and 7 days, MeBr in the limed subsurface soil disappeared significantly faster than in the unlimed subsurface soil. Methyl bromide in the limed surface sample completely disappeared in 35 days; whereas 6–11% of the applied MeBr still remained in the other samples.

Disappearance of MeBr in limed surface and subsurface samples, which were not treated with $(\text{NH}_4)_2\text{SO}_4$, was considerably slower than in the corresponding treated samples (Figures 2 and 3). Although disappearance of MeBr in $(\text{NH}_4)_2\text{SO}_4$ -treated unlimed samples was initially similar to the corresponding untreated samples, after 3–7 days, MeBr in the treated samples disappeared more rapidly than in the corresponding untreated samples. Furthermore, MeBr in untreated limed samples was degraded more rapidly than in the corresponding untreated unlimed samples (Figure 3). Methyl bromide in the limed and unlimed surface samples was degraded more rapidly than in the corresponding limed and unlimed subsurface samples.

The patterns of the MeBr disappearance in the unlimed surface and subsurface autoclaved soils treated at a rate of 50 $\mu\text{g}/\text{g}$ were generally similar to the corresponding soils treated at a lower rate, 20 $\mu\text{g}/\text{g}$ (Figures 1 and 3). After 21 days of incubation, when treated at rates of 20 and 50 $\mu\text{g}/\text{g}$, 44 and 45% of the applied MeBr in the unlimed surface samples and 43 and 43% of the applied MeBr in the unlimed subsurface samples were detected. Since no viable organisms and no active extracellular enzymes were present in these autoclaved soils, chemical degradation was solely responsible for the disappearance of MeBr. On the other hand, biological degradation appeared to play a role, in addition to chemical degradation, on the disappearance of MeBr in live soils,

TABLE 2. Change of pH Value and Formation of Nitrite and Nitrate in Limed and Unlimed Surface and Subsurface Soils Treated with $(\text{NH}_4)_2\text{SO}_4$

days	pH		NO_2 ($\mu\text{g/g}$)		NO_3 ($\mu\text{g/g}$)	
	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
Limed Soil						
0	7.70	7.74	TR ^a	TR	TR	TR
7	7.72	7.90	NM ^b	NM	NM	NM
14	7.31	7.64	NM	NM	NM	NM
21	6.82 ^c	7.62	4.6	6.8	13.5	5.2
28	7.81	7.69	NM	NM	NM	NM
35	7.74	7.72	9.6	9.1	11.3	10.4
42	7.58	7.64	NM	NM	NM	NM
Unlimed Soil						
0	5.50	5.70	TR	TR	TR	TR
7	4.92	5.15	NM	NM	NM	NM
14	4.79	5.08	NM	NM	NM	NM
21	4.76	5.05	NM	NM	NM	NM
28	4.98	5.24	2.3	1.3	TR	TR
35	4.79	5.08	NM	NM	NM	NM
42	5.11	5.32	3.2	2.3	TR	TR

^a TR trace amount, below detection limit. ^b NM, not measured. ^c After soil pH was determined, soil pH was readjusted with CaO.

especially in the surface samples. Disappearance of MeBr in live samples was more rapid than in corresponding autoclaved samples (Figure 3), with the exception of the unlimed subsurface sample. In this sample, the disappearance rate of MeBr was similar to the autoclaved unlimed subsurface sample. It was estimated that up to 37 and 21% of the MeBr disappearance in the limed $(\text{NH}_4)_2\text{SO}_4$ -treated surface and subsurface samples and up to 24 and 11% of the MeBr disappearance in the untreated limed surface and subsurface samples could be attributed to microbial degradation, respectively. Also microbial degradation could be responsible for up to 25 and 26% of the MeBr disappearance in the $(\text{NH}_4)_2\text{SO}_4$ -treated unlimed surface and subsurface samples and for up to 13 and 10% of the disappearance in the untreated unlimed surface and subsurface samples. Contribution from microbial degradation was evidently less important in untreated soils, especially in the unlimed untreated samples. The presence of $(\text{NH}_4)_2\text{SO}_4$ in unlimed soils should stimulate the nitrification activity, resulting in higher rates of MeBr degradation than in corresponding untreated samples. An increase in pH value from 5.8 to 7.7 may result in an increase in microbial activity, especially nitrification. At pH about 7.7, the prevalent form of ammonia is the molecular form. The molecular form of ammonia is responsible for inducing the production of ammonia monooxygenase by nitrifiers and serves as the substrate for this enzyme (21, 25). Ammonia monooxygenase produced by ammonia oxidizers during nitrification in soil can also contribute to the degradation of MeBr, as demonstrated in liquid media (18).

Based on the information from field studies (7, 8, 26), little or no MeBr volatilized into the atmosphere after 5-7 days of field injection of MeBr. This indicated that, within 5-7 days after injection, MeBr was either degraded in soil or volatilized. Thus, in order to have an effective stimulation of MeBr degradation in soil by biological means, it must occur within the first 5-7 days after the MeBr treatment.

Formation of Nitrite and Nitrate and pH Value Changes.

Oxidation of ammonia to nitrite by nitrifiers results in a rapid decrease in soil pH (27). This is caused by the oxidation of one molecule of ammonia, which results in the formation of five hydrogen ions (21, 25). Table 2 shows pH value changes with the formation of nitrite and nitrate in limed and unlimed surface and subsurface soils treated with $(\text{NH}_4)_2\text{SO}_4$ during

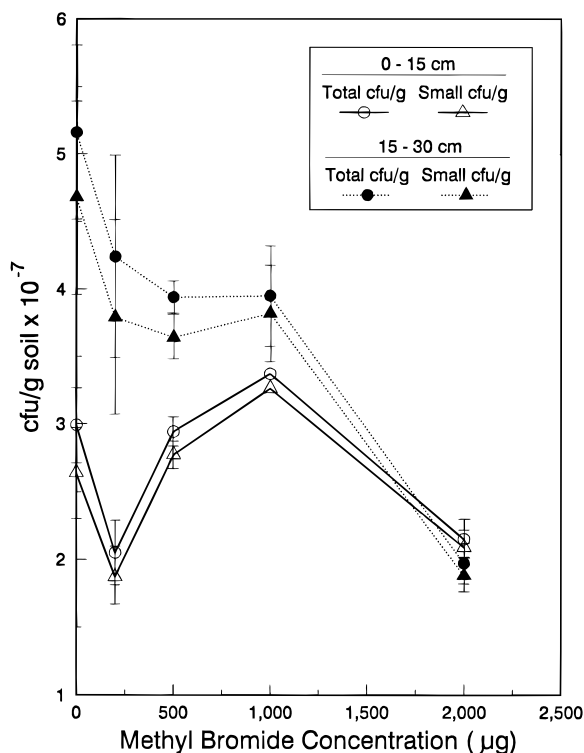


FIGURE 4. Total bacterial populations and the bacterial population that formed small colonies in surface (0-15 cm depth) and subsurface (15-30 cm depth) Arredondo soil (10 g) treated with various concentrations of MeBr for 16-18 h. These soil samples were limed but not treated with $(\text{NH}_4)_2\text{SO}_4$. Error bars represent 1 SD.

42 days of incubation. After 7 days of incubation, the pH value of the limed surface soil sample steadily declined, and after 21 days, the pH value had dropped to 6.82. Hence, soil pH was readjusted with CaO back to 7.70 following which the pH slowly decreased. For the limed subsurface sample, after a decrease in pH to 7.64 in 14 days, the pH value remained relatively unchanged between 14 and 42 days. For the unlimed soil samples, after an initial drop of pH values, little soil pH change was observed. The initial decline in soil pH was probably due to the application of $(\text{NH}_4)_2\text{SO}_4$. Higher soil pH values in limed samples reflected in higher rates of nitrification, especially in the surface sample. Even though nitrite and nitrate concentrations were determined at intervals of 2-4 weeks, it was clear that nitrification was higher in the limed surface sample than in the limed subsurface sample. This was reflected in the rapid decrease in pH in conjunction with a higher amount of nitrate formation. After readjustment of the soil pH on day 21, the rate of nitrification in the surface sample was slightly higher than the subsurface sample. Low levels of nitrite and trace amounts of nitrate were detected in unlimed surface and subsurface samples on days 28 and 42. Ou et al. (28) reported that nitrification in unlimed Arredondo surface soil collected from the same site slowly occurred after 28 days. Higher disappearance rates of MeBr in limed soil samples treated with $(\text{NH}_4)_2\text{SO}_4$, especially in the surface sample, were attributed to higher nitrification activity in these samples than in the corresponding unlimed samples. Higher nitrification also included higher activity of ammonia monooxygenase, which could result in a higher rate of MeBr oxidation.

Effect of Methyl Bromide on the Survival of Soil Bacterial Populations. Even though MeBr is toxic to all forms of living organisms including bacteria (1, 7), exposure of MeBr to soil as high as 200 $\mu\text{g/g}$ of soil (2000 $\mu\text{g}/10$ g of soil) for 16-18 h did not completely kill soil bacteria (Figure 4). In fact, in the

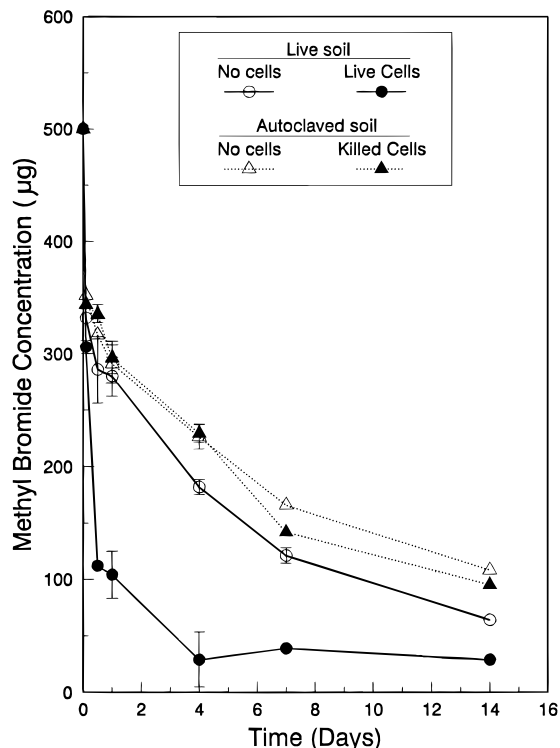


FIGURE 5. Disappearance of MeBr in limed surface (0–15 cm depth) Arredondo soil with or without inoculation of live cells of *Nitrosomonas europaea* and in autoclaved limed surface (0–15 cm depth) Arredondo soil with or without inoculation of killed cells of *N. europaea*. These samples (10 g) were treated with 500 µg of MeBr (50 µg/g of soil). Error bars represent 1 SD.

surface soil treated with 50 or 100 µg/g MeBr, total bacterial populations were either not inhibited or increased. At the rates of 20 and 200 µg/g, total bacterial populations were significantly inhibited, with a reduction of 31 and 28%, respectively. At 200 µg/g, it was likely that MeBr was toxic to bacteria at this high concentration. However, it was not clear as why total bacterial populations were reduced at 20 µg/g. In the subsurface soil, total bacterial populations were reduced in all MeBr-treated samples, with the sample treated with 200 µg/g in which total bacterial populations were severely reduced. A total of 88–97% of bacterial colonies formed in the agar plates were predominately small in size, ≤0.05 mm diameter. The fraction of small colonies generally increased with the increase in MeBr concentration, especially in the surface samples treated with 50 and 100 µg/g MeBr. In these samples, numbers of total small bacterial colony forming units (cfu) were larger than in the untreated surface sample. Methyl bromide in soil and water is subject to chemical hydrolysis to form methanol and bromide ion (11, 29). It was likely that an increase in the bacterial populations that formed small cfu was due to an increase in a specific bacterial population that degraded methanol. It was found that methanol degraders in the surface Arredondo soil predominately formed small cfu (Ou and Thomas, unpublished observation).

Enhancement of Methyl Bromide Degradation by *N. europaea*. It has been demonstrated that the ammonia oxidizer *N. europaea* ATCC 19718, during the oxidation of ammonia, also has the capacity to co-oxidize MeBr to formaldehyde and bromide ion (18). An investigation was conducted to determine whether or not inoculation of this ammonia oxidizer to Arredondo soil stimulated the degradation of MeBr. It was found that MeBr was initially rapidly degraded in the (NH₄)₂SO₄-treated limed surface soil inoculated with *N. europaea* (Figure 5) and after 4 days, only 8% of the applied MeBr remained in this sample. Whereas in

the same period, 36–46% of the applied MeBr remained in the soil samples that were either not treated with the bacterium, autoclaved, or autoclaved and inoculated with killed cells.

Monoxygenases and dioxygenases are involved in the initial degradation of aliphatic and aromatic hydrocarbons, phenol (30), as well as some pesticides such as 2,4-D, atrazine, and EPTC (31–33). Beside fertilizers, pesticides are often applied to agricultural fields to control unwanted pests. The herbicide 2,4-D is readily degraded in soils (34), and during the degradation may also stimulate the degradation of MeBr. Furthermore, soil humus is rich in phenolic constituents (35). Microbial degradation of soil humus, albeit slowly, may also stimulate the degradation of MeBr.

Acknowledgments

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Literature Cited

- (1) Noling, J. W.; Becker, J. O. *Suppl. J. Nematol.* **1994**, *26*, 573.
- (2) Ferguson, W.; Padula, A. *Economic effects of banning methyl bromide for soil fumigation*; USDA Economic Research Service, Agricultural Economic Report 677; USDA: Beltsville, MD, 1994.
- (3) Cicerone, R. J.; Stedman, D. H.; Stolarski, R. S. *Geophys. Res. Lett.* **1975**, *2*, 219.
- (4) Mellouki, A.; Talukdar, R.; Schmoltner, A. M.; Gierczak, T.; Mills, M. J.; Solomon, S.; Ravishankara, A. R. *Geophys. Res. Lett.* **1992**, *19*, 2059.
- (5) Watson, R. T.; Albritton, D. L.; Anderson, S. O.; Lee-Bapty, S. *Methyl bromide: Its atmospheric science, technology, and economics*; United Nations Environmental Programme, United Nations Headquarters: Nairobi, Kenya, 1992.
- (6) Shorter, J. H.; Kolb, C. E.; Crill, P. M.; Kerwin, R. A.; Talbot, R. W.; Himes, M. E.; Harris, R. C. *Nature* **1995**, *377*, 717.
- (7) Yagi, K.; Williams, J.; Wang, N.-Y.; Cicerone, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8420.
- (8) Yagi, K.; Williams, J.; Wang, N.-Y.; Cicerone, R. J. *Science* **1995**, *267*, 1979.
- (9) Yates, S. R.; Gan, J.; Ernst, F. F.; Mutziger, A.; Yates, M. V. *J. Environ. Qual.* **1996**, *25*, 184.
- (10) Yates, S. R.; Ernst, F. F.; Gan, J.; Gao, F.; Yates, M. V. *J. Environ. Qual.* **1996**, *25*, 192.
- (11) Gan, J.; Yates, S. R.; Anderson, M. A.; Spenser, W. F.; Ernst, F. F.; Yates, M. V. *Chemosphere* **1994**, *29*, 2685.
- (12) Oremland, R. S.; Miller, L. G.; Culbertson, C. W.; Connell, T. L.; Jahnke, L. *Appl. Environ. Microbiol.* **1994**, *60*, 3640.
- (13) Wackett, L. P. In *Microbial transformation and degradation of toxic organic chemicals*; Young, L. Y., Cerniglia, C. E. Eds.; Wiley-Liss, Inc.: New York, 1995; pp 217–242.
- (14) Arciero, D. M.; Vannelli, T.; Logan, M.; Hooper, A. B. *Biochem. Biophys. Res. Commun.* **1989**, *159*, 640.
- (15) Fox, B. G.; Borneman, J. G.; Wackett, L. P.; Lipscomb, J. D. *Biochemistry* **1990**, *29*, 6419.
- (16) Harker, A. R.; Kim, Y. *Appl. Environ. Microbiol.* **1990**, *56*, 1179.
- (17) Oldenhuis, R.; Oedze, J. Y.; van der Warrde, J. J.; Janssen, D. B. *Appl. Environ. Microbiol.* **1991**, *55*, 2819.
- (18) Rasche, M. E.; Hicks, R. E.; Hyman, M. R.; Arp, D. J. *J. Bacteriol.* **1990**, *171*, 5368.
- (19) Winter, R. B.; Yen, K.-M.; Ensley, B. D. *Biotechnology* **1989**, *7*, 282.
- (20) Hyman, M. R.; Sansome-Smith, A. W.; Shears, J. H.; Wood, P. M. *Arch. Microbiol.* **1985**, *143*, 302.
- (21) Rasche, M. E.; Hyman, M. R.; Arp, D. J. *Appl. Environ. Microbiol.* **1990**, *56*, 2568.
- (22) Ou, L.-T.; Davidson, J. M.; Rothwell, D. F. *Soil Biol. Biochem.* **1978**, *10*, 443.
- (23) Kenney, D. R.; Nelson, D. W. In *Methods of soil analysis, Part 2*; Page, A. L., Ed.; American Society of Agronomy, Inc.: Madison, WI, 1982; pp 643–698.
- (24) Skipper, H. D.; Westerman, D. T. *Soil Biol. Biochem.* **1973**, *5*, 409.

- (25) Wood, P. M. In *Nitrification*; Prosser, J. I., Ed.; Society of General Microbiology, IRL Press: Oxford, England, 1986; pp 39–62.
- (26) Yates, S. R.; Ernst, F. F.; Gan, J.; Gao, F.; Yates, M. V. *J. Environ. Qual.* **1996**, *25*, 192.
- (27) Alexander, M. *Introduction to soil microbiology*; John Wiley & Sons: New York, 1977.
- (28) Ou, L.-T.; Rothwell, D. F.; Mesa, M. V. *Soil Crop Sci. Soc. Florida Proc.* **1985**, *44*, 77.
- (29) Gentile, I. A.; Ferraris, L.; Crespi, S. *Pestic. Sci.* **1989**, *25*, 261.
- (30) Gibson, D. T. *Microbial degradation of organic compounds*; Marcel Dekker, Inc.: New York, 1984.
- (31) Nagy, I.; Schoofs, G.; Compennolle, F.; Prospect, P.; Vanderleyden, J.; De Mot, R. *J. Bacteriol.* **1995**, *177*, 676.
- (32) Shao, Z. O.; Behki, R. *Appl. Environ. Microbiol.* **1996**, *62*, 403.
- (33) Top, E. M.; Holben, W. E.; Forney, L. J. *Appl. Environ. Microbiol.* **1995**, *61*, 1691.
- (34) Ou, L.-T. *Soil Sci.* **1984**, *137*, 100.
- (35) Stevenson, F. J. *Humus Chemistry*; John Wiley & Sons: New York, 1982.

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