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Enrichment and molecular characterization of chloropicrin- and metam-sodium-degrading microbial communities

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Abstract Chloropicrin (CP) and metam sodium are commonly used as fumigants in agricultural soils in order to provide effective control of nematodes, soil-borne pathogens, and weeds in preparation for planting of high-value cash crops. Repeated application of these compounds to agricultural soils for many years may result in the enrichment of microorganisms capable of degrading them. In this study, a microcosm-enrichment approach was used to investigate bacterial populations that may be components of metam-sodium- and CP-degrading microorganisms in compost-amended soils. After 6 months incubation, with repeated application of metam sodium and CP, degradation was ≥70% faster in compost-manure-amended (CM) soil compared to ≤50% in the unamended soils. The accelerated fumigant degradation may have been due to the addition of compost or to the development of new microbial populations with enhanced degradation capacity. Denaturing gradient gel electrophoresis (DGGE) profiles of PCR-amplified regions of 16S rRNA genes were used to identify dominant bacterial populations responsible for the accelerated fumigant degradation. The DGGE results indicated that specific bacterial types had been enriched and these were similar to strains isolated from basal minimal media. Fragments from DGGE bands and colonies were cloned, sequenced, and compared with published 16S rRNA sequences. Cloned sequences were dominated by *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Mycobacterium* and uncultured bacterial species. The addition of organic amendment to soil during fumigation practices has the potential to increase the diversity of different microbial species, thereby accelerating fumigant degradation and reducing atmospheric emissions.

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Introduction

The repeated application of fumigants can result in soils with the capacity for accelerated degradation and diminished pest control. Adaptation of such soils to fumigants may be due to the selection of microbial populations with high degradative potentials (Verhagen et al. 1995; Van Dijk 1974; Van Hylckama and Janssen 1992). However, the stimulation of microbial degradation in adapted soil is difficult to evaluate because of the complexity of the soil environment and the lack of sensitive methods for identifying the responsible microbial populations. Gan et al. (1998a) showed that applying organic amendments can accelerate methyl isothiocyanate [(MITC) (primary active ingredient of metam sodium)] degradation in surface soil. They observed that the degradation of MITC was significantly enhanced in soils amended with composted manure compared to the unamended soil, and they attributed the increase in degradation rate to microbial transformation of MITC. Therefore, fumigant emissions can be minimized if best-management practices, such as application of organic amendments, are included to help reduce volatilization into the air. The benefits of adding organic amendments to soils, e.g. improvement soil nutrition, physicochemical conditions, and crop viability, are well documented (O'Hallorans et al. 1993; Hungalle et al. 1986). In addition, soil amendments have been found to be effective in reducing potentially harmful fumigant emissions (Gan et al. 1998b) and controlling soil-borne pathogens by stimulating antagonistic organisms (Akhtar and Malik 2000) or by producing toxic volatile compounds (Gamliel and Stapleton 1997). Applications of organic amendments have also been shown to increase the soil microbial biomass and stimulate microbial activity (Perucci 1990; Bandick and Dick 1999; Peacock et al. 2001).

The contribution of microorganisms to chloropicrin (CP) degradation was reported to be similar to that in MITC degradation (Gan et al.1999a). The authors of the study concluded that the significant contribution of microbial processes to CP degradation observed in soils from different climatic conditions suggested that CP-degrading microorganisms may be abundantly present in the soil environment. CP and MITC have been identified as potential replacements for methyl bromide in many field studies (Moldenke and Thies 1996; Trout and Agwa 1999), and biochemical and DNA methods were recently used to demonstrate the effectiveness of these agents as soil fumigants in a microcosm study (Ibekwe et al. 2001a).

Molecular analysis of microbial communities provides a means to study adapted bacteria that can be enriched from environmental samples. The application of denaturing gradient gel electrophoresis (DGGE) in microbial ecology has attracted great interest in the past few years (Muyzer and Smalla 1998), and is suitable for studying and monitoring enrichment cultures (Jackson et al. 1998; Santegoeds et al. 1996). Teske et al. (1996) used DGGE to identify the bacterial composition of a culture capable of sulfate reduction after exposure to oxic and microoxic conditions. DGGE was also used to show changes in the bacterial species composition in enrichment cultures prepared with various inoculum dilutions from compost-amended soil (Ibekwe et al. 2001b). In this study, bacterial populations in soil microcosms amended with composted manure during a 6-month incubation period were monitored by DGGE. Bacteria capable of degrading CP and MITC were identified following enrichment of the cultures. DGGE was then used to show changes in the dominant bacterial populations from the enrichment that may contribute to degradation of these compounds.

Materials and methods

Soil, organic amendments and chemicals

The soil used for this study, an Arlington sandy loam (coarse-loamy, mixed, thermic, Hapic Durixeralf), was taken from the top 10 cm at the University of California, Riverside Agricultural Experiment Station. There was no history of fumigant treatment on this plot. The soil had a pH of 7.2 and organic matter content of 1.10%. Moist soil was passed through a 4-mm sieve and stored at room temperature for 48 h before the start of the experiment. Composted steer manure (CM) purchased from a local supplier was used as an organic amendment. The CM contained 25% moisture. CP (99%) and metam sodium (dehydrate, 99%) were purchased from Chem Service (West Chester, Penn., USA). MITC was obtained from Sigma (St Louis, Mo., USA).

Microcosm experiment

Duplicate glass columns of 3905 cm^3 containing 3.6 kg soil and composted manure (4:1 w/w: dry wt) were packed to 75% capacity with soil or soil amended with CM. The experiment was conducted at 25°C. For all treatments, repeated applications of fumigants were made by injecting 100 µl pure CP and metam sodium (MITC precursor) at 30 cm depth into the soil columns once each week for the first 12 weeks of the study. Moistened air was flushed through the headspace of each column to keep the soil moist. During the last 12 weeks, the columns were spiked twice (week 16 and 20). After 24 weeks, the columns were stored at 4°C for 1 week, mixed and small portions taken for DNA extraction, plate count and degradation studies.

Enrichment cultures and degradation of CP and metam sodium in compost-amended soil

The enrichment cultures were prepared as described by El-Fantroussi (2000), with the modifications of CP and metam sodium as the sole carbon source. Ten grams of soil was added to 90 ml of basal minimal medium containing 93.50 mg $MgSO_4 \cdot 6H_2O$, 5.88 mg $CaCl_2 \cdot 2H_2O$, 1.15 mg $ZnSO_4 \cdot 7H_2O$, 1.16 mg H_3BO_3, 1.69 mg $MnSO_4 \cdot H_2O$, 0.24 mg $CoCl_2 \cdot 6H_2O$, 0.10 mg MoO_3, 2.78 mg $FeSO_4 \cdot 7H_2O$, and 0.37 mg $CuSO_4 \cdot 5H_2O$ l^{-1}. The solution pH was adjusted to 7.0. Flasks containing basal mineral salt medium with 0.01 mM CP and metam sodium, and 10 mg yeast extract l^{-1} were inoculated with amended soils obtained from microcosms 6 months after application of the two fumigants. After inoculation, the flasks were shaken for 10 min and dilutions of the cultures were plated onto solid basal mineral salt media. The plates were incubated at 28°C for 8 days and the resulting colonies were tested for degrading capacity as described below.

To evaluate the rate of CP and metam sodium degradation after the 6-month incubation, samples were divided into the following groups: amended samples with and without repeated fumigation for 6 months and unamended control soil. Microbial and chemical degradations were separated by sterilizing portions of the soils. Briefly, fumigated, non-fumigated CM-amended, and non-amended soil samples (500 g) were taken from each jar and autoclaved twice at 121°C for 60 min, with a 24-h interval between the first and the second autoclaving. Ten g (dry wt.) of the above samples with a 10% moisture content were placed in 21-ml headspace vials with 5 µl of acetone solution containing 100 µg CP and metam sodium μl^{-1} to give a final concentration of 50 mg of each fumigant kg^{-1} . The treated vials were immediately sealed with Teflon-faced butyl-rubber septa with aluminum caps and then shaken to achieve uniform fumigant distribution in the soil. The same procedure was used for non-sterilized soil. Fumigant was added to the soil samples under sterile conditions. The closed soil-sample vials were incubated in the dark at 20°C. Three replicate vials from each treatment were removed at 0, 8, 24, 96, 144, and 192 h and stored at

−20°C until the end of the experiment, when all samples were extracted. IN order to analyze the fumigant remaining in soil, samples were decapped when the soil was still frozen, anhydrous sodium (10 g) and 10 ml of ethyl acetate were added, and the vials immediately recapped. The samples were shaken for 1 h and vortexed for 2 min at room temperature. A portion of the supernatant was transferred into GC vials and analyzed using Gas chromatograph/electron capture detector/nitrogen-phosphorus-detector (GC/ECD/NDP). The recovery of fumigants by this method ranged from 95 to 100% according to preliminary experiments. Differences in the rate of fumigant degradation in autoclaved and non-steriled soils were attributed to microbial degradation. A first-order kinetic model was fitted to obtain the degradation rate coefficient k (day^{-1}). Degradation of CP and metam sodium in basal minimal media after 8 days of incubation was also determined as described above, except that 10 ml of liquid culture was used.

DNA extraction, PCR primers and DGGE analysis of soil

Total bacterial community DNA for DGGE analysis was extracted from each treatment column. About 500 mg of soil was placed in FastPrep tubes (BIO 101, Vista, Calif., USA) containing lysing matrix for the isolation of total DNA. PCR was first carried out using about 50 ng of template DNA with 10 pmol of primers 338f and 518r (Øvreas et al. 1997). Ready-To-Go PCR beads (Amersham-Pharmacia Biotech, Piscataway, N.J., USA) were used for PCR amplification. The final volume of the PCR mixture for bacterial 16S rRNA amplification was 25 µl . The PCR cycles used for amplification were as follows: 92°C for 2 min, followed by 30 cycles of 92°C for 1 min, 55°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 6 min.

DGGE was performed with 8% (wt/vol) acrylamide gels containing a linear chemical gradient ranging from 30 to 70% denaturant, with 100% defined as 7 M urea and 40% formamide. Gels were run for 3 h at 200 V with the Dcode Universal Mutation System (Bio-Rad Laboratories, Hercules, Calif., USA). DNA was stained with ethidium bromide and visualized by UV transillumination with a Polaroid camera. In order to determine the complexity of the microbial communities from CM-amended and unamended soils, a sevenfold dilution series of the soil samples was prepared in minimal salt media. Samples were homogenized for 1 h after which 500-µl portionsof each sample were used for DNA extraction as described above. PCR was run on the dilution samples (10^{-1}–10^{-5}) followed by DGGE. Major bands were excised for identification of bacterial species from both the original and the dilution samples. The bands were placed into sterilized vials with 20 µl of sterilized distilled water and kept overnight at 4°C to allow the DNA to passively diffuse out of the gel. Eluted rRNA (10 µl) was used as the DNA template with the universal bacterial primers described above. The sizes of the PCR products were checked on a 1.5% agarose gel, and the DNA was eluted and cloned into a pGEM-T Easy vector (Promega, Madison, Wis., USA) and transformed into *E. coli* JM109. Plasmid from the *E. coli* was isolated using the Qiagen Plasmid Mini Kit (Valencia, Calif., USA). The 200-bp inserts in pGEM-T were sequenced with the ABI PRISM Dye Terminator Cycle Sequencing Kit with AmpliTag DNA Polymerase, FS (Perkin Elmer). Sequence analyses were done using the BLAST database (National Center for Biotechnology Information: www.ncbi.nlm.nih.gov) to compare band sequences obtained from the experiment with sequences in the database.

Table 1 First-order degradation constants (k, d^{-1}), half-life ($t_{1/2}$), and correlation coefficients of fitting (r^2) for methyl isothiocyanate (MITC) and chloropicrin (CP) degradation in Arlington sandy loam soil amended with compost manure (CM), incubated and treated with fumigants for 6 months. *MS* Metam sodium

Fumigants	Matrix	k (d^{-1})	$t_{1/2}$ (days)	r^2
MITC	CM-amended	0.74±0.09	0.93	0.98
	CM-amended sterilized soil	0.18±0.03	3.80	0.92
	Unamended soil	0.07±0.01	10.0	0.92
	Unamended sterilized	0.05±0.01	13.9	0.88
	CM-amended and fumigated	1.19±0.06	0.58	0.99
	CM-amended + MS and sterilized	0.27±0.02	2.60	0.98
CP	CM-amended	1.12±0.20	0.62	0.99
	CM-amended sterilized soil	0.33±0.12	2.10	0.87
	Unamended soil	0.21±0.04	3.40	0.94
	Unamended sterilized	0.09±0.01	8.28	0.95
	CM-amended and fumigated	3.50±0.16	0.20	0.97
	CM-amended+CP and sterilized	0.59±0.08	1.18	0.98
CP[a]	Consortia 1	3.16±0.21	0.22	0.99
	Consortia 2	1.67±0.15	0.41	0.99
	Colony 1	0.13±0.02	5.47	0.94
	Colony 2	0.14±0.01	4.78	0.97
	Colony 3	0.14±0.01	4.79	0.97
	Colony 1, 2, 3	0.18±0.02	3.77	0.96

[a]CP Degradation was determined in broth incubated for 8 d at 20°C

Results

Degradation of chloropicrin

The rate of CP degradation was higher in the CM-amended soils than in the unamended soil, and significantly lower in the sterile soils (Table 1). The 8-day incubation study (Fig. 1a) showed that the degradation capacity of these samples was predominantly of biological origin. Based on the differences in degradation between sterile and non-sterile soils, it was estimated that microbial degradation accounted for 70, 57, and 83% of CP degradation in CM-amended, unamended, and CM-amended soil with repeated fumigation with CP, respectively. Detailed analysis of the degradation rate of CP showed that the disappearance of CP in unamended, CM-amended, CM-amended treated soils was well described by first-order degradation kinetics ($r^2 \geq 0.87$) (Table 1). The degradation of CP in soil increased significantly with the addition of CM, resulting in a fivefold increase in k (Table 1). In CM-amended soil repeatedly fumigated with CP for 6 months, the k was approximately 17 times greater than that in unamended soil (Table 1). For the unamended soil, the half-life of CP was 3.40 days compared to 10 days

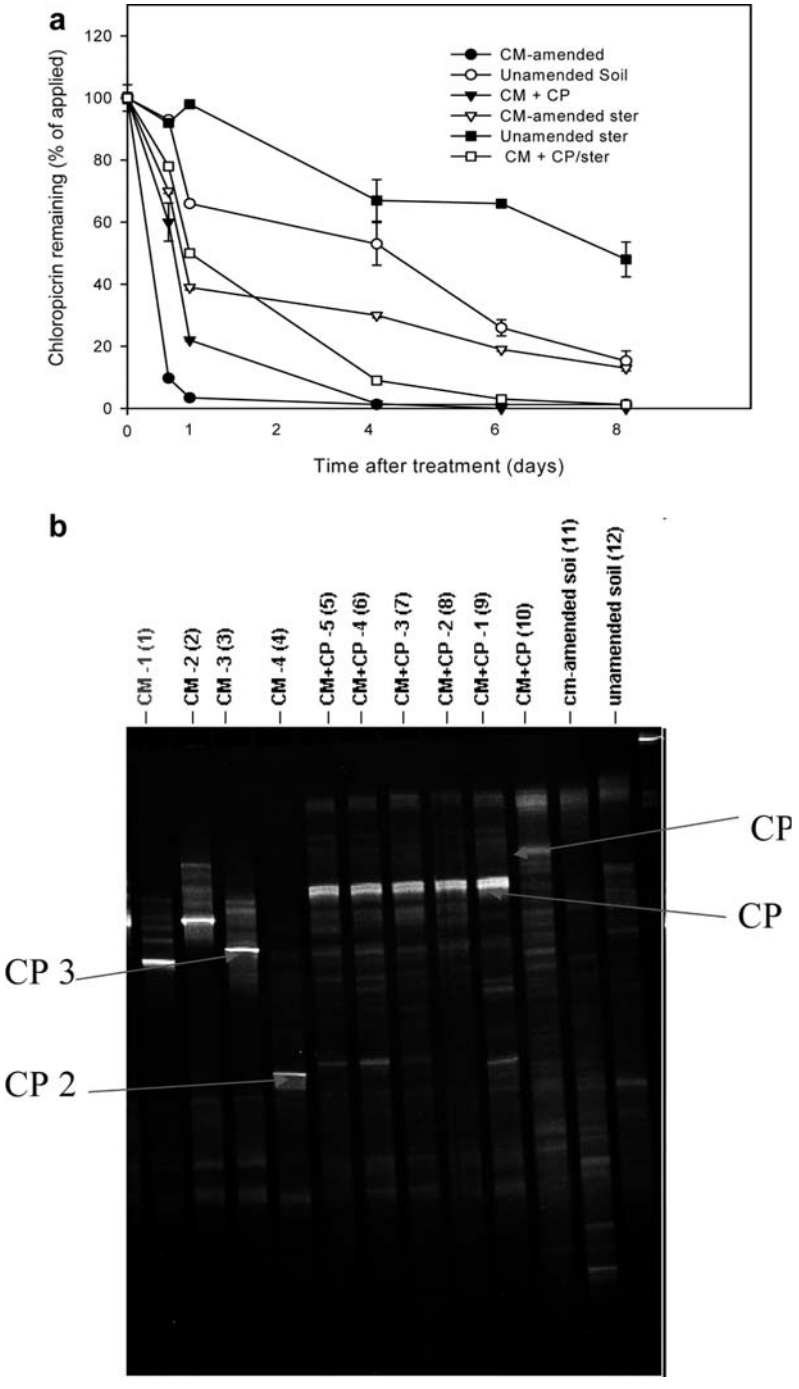


Fig. 1 **a** The dissipation of chloropicrin (CP) in Arlington sandy loam soil amended with composted manure (CM), amended with composted manure and repeated fumigation with CP for 6 months, and unamended soil. **b** Denaturing gradient gel electrophoresis (DGGE) patterns of CM-amended soil with repeated fumigation with CP for 6 months (*lane 10*), dilution series of the CP-fumigated soil from 10^{-5} to 10^{-1}CFU g^{-1} (*lanes 5–9*), CM-amended soil (*lane 11*) and microbial consortia from the CM-amended soil (*lanes 1–4*). The dilutions from *lanes 5–9* were plated on minimal salt media for identification of degrading communities and isolates. *Lanes 1–4* are communities from mineral salt media

for metam sodium (Table 1), and about 5 days for 1,3-D (Ibekwe et al. 2001b). Degradation of CP in sterile soil was significantly inhibited, with a degradation rate coefficient 2–6 times higher in non-sterile soil than in autoclaved soil (Table 1, Fig. 1a).

DGGE patterns of amended and unamended soils with and without CP (Fig. 1b) were analyzed to determine banding patterns that may reflect differences in microbial communities adapted to the fumigant and also associated with degradation of this fumigant. Comparison of the bacterial communities showed the banding patterns to be quite different between CM-amended soil treated with CP for 6 months (lane 10) and Arlington sandy loam soil with amendment (lane 11). With a fivefold serial dilution of the CM-amended soil treated with CP, the complexity of the banding pattern decreased with increasing dilutions (lanes 9–5 from 10^{-1} to 10^{-5} CFU g^{-1}). The dilutions were plated on minimal salt media in order to identify degrading communities and isolates. Degrading communities (microbial consortia) from mineral salt media are shown in Fig. 1b lanes 1–4. Four major bands were detected in the CP-treated soils (CP1, CP2, CP3, and CP4) and were indicative of detectable strains of the microbial communities that had developed during the 6-month period of the experiment. These bands may represent bacterial strains that degrade CP, and were the dominant microbial species in the CP-treated soils (Fig. 1b, and Table 2). Bands CP 1 and CP 4 included uncultured bacterium and *Pseudomonas* sp. and were unique to the CP-treated soils. A serial dilution assay was done in order to detect species that may not have been revealed by the banding pattern due to the complexity of the samples. The main bands of 16S rRNA amplified from compost-amended CP-treated soil were still visible after a 5-log dilution. The appearance of band CP 1 at 10^{-1} dilution and above indicated that this sequence was less efficiently amplified in the highly complex soil samples (Fig. 1b, lane 10). The uncovering of new banding patterns is typical in microbial communities when equally abundant species are diluted and the new communities are recovered in less complex communities.

For in-depth analysis of the strains constituting the CP-degrading communities, individual bacterial members of the CP-degrading community in mineral salts medium containing 1 mmol CP l^{-1} were cultured. Single strains from the community were isolated by selective cultivation, using CP as the sole carbon source. Three bacterial strains were isolated, and the strains were reanalyzed on DGGE gel in order to compare banding positions with the serial dilution gel. Taxonomic characterization of the three strains was based on 16S rRNA sequence analysis and showed that two isolates (colonies 1 & 2), belong to the genus *Pseudomonas*, having a sequence similarity to the species *Pseudomonas fluorescens* of 100%. Colony 3 was shown to have 100% sequence similarity to *Arthrobacter* sp. (data not shown). This strain was originally isolated from *Aplysina aerophoba* and *Aplysina cavernicola* (Hentschel et al. 2001), and is a major soil microorganism. The three bacterial strains were examined with regard to their degradative capabilities in liquid and solid mineral salts medium containing CP (1 mmol l^{-1}) as the sole carbon source. The degradation of CP by the three isolates individually and combined, as well as by microbial consortia obtained from two solid agar media after 8 days incubation with CP as the sole carbon source is indicated in Table 1 and Fig. 2. The degradation rate coefficient of CP in the consortia was nine times greater than that obtained from any individual colony or from the three colonies combined (Table 1). The half-life of CP was about 3 days for the combined colonies compared to >4 days for the individual colony. This was in contrast to the rapid degradation of CP by the consortia, which exhibited $t_{1/2}$ 0.5 days (Table 1). The community responsible for the accelerated degradation of CP in this study was thus more complex than the three isolates that were identified.

Degradation of metam sodium

The dissipation of metam sodium in unamended, CM-amended, and CM-amended with repeated fumigation soils was well described by first-order degradation kinetics

Table 2 Sequence analysis of bands excised from denaturing gradient gel electrophoresis (DGGE) gels derived from bacterial 16S rRNA extracted from fumigated soils

Band position	Related bacterial sequences	% similarity	Accession no
VP1	Uncultured bacterium	100	AF389450
VP2	Uncultured eubacterium	100	AF010042
VP3	Uncultured eubacterium WR 858	99	AJ292793
VP4	*Mycobacterium xenopi*	99	M61664
VP5	*Mycobacterium* sp. JLS	100	AF387804
VP6	*Bacillus* sp. SG-1	100	AF326373
CP1	Uncultured bacterium	100	AF389450
CP2	*Pseudomonas* sp.	100	AF205138
CP3	*Arthrobacter* sp.	100	AF197021
CP4	*Streptomyctes* sp.	99	AF131518
Colony 1	*Pseudomonas fluorescens*	100	AF228367
Colony 2	*Pseudomonas fluorescens*	100	AF228367
Colony 3	*Arthrobacter* sp.	100	AF218242

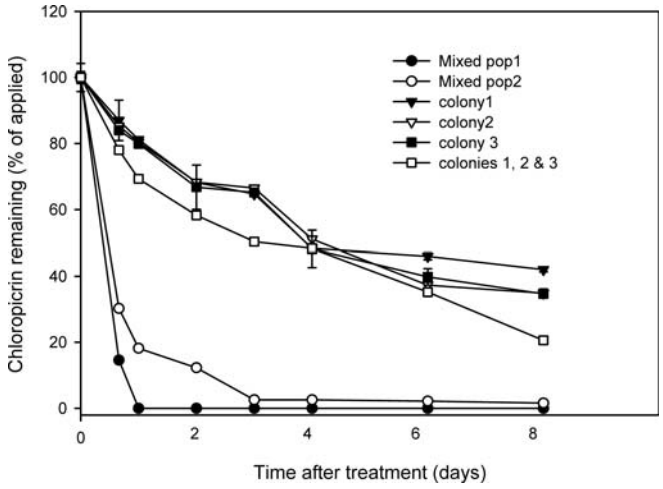


Fig. 2 The dissipation of chloropicrin (CP) in minimal salt media with CP as sole carbon source

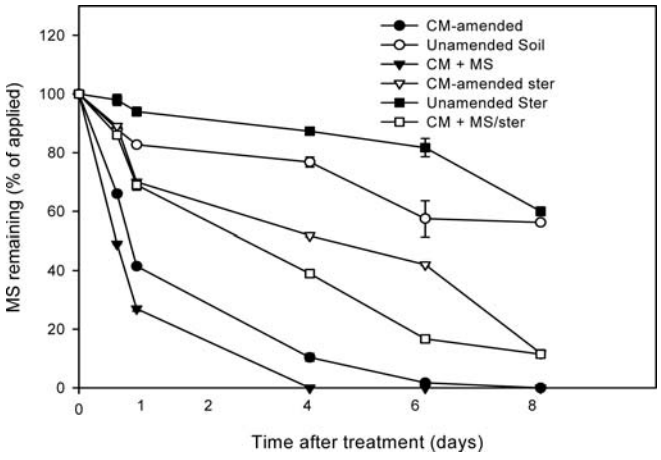
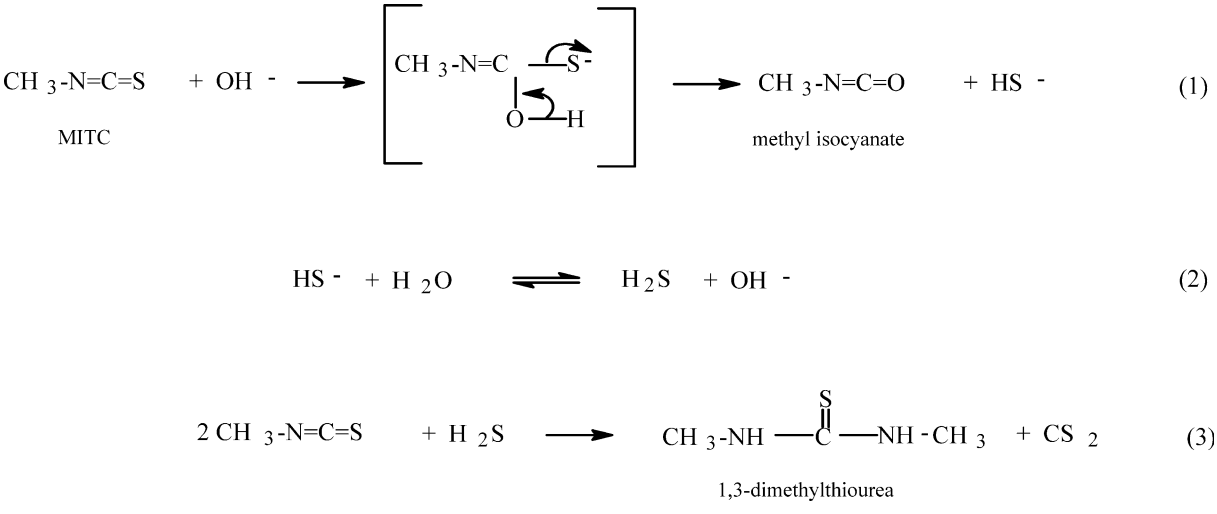


Fig. 3 **a** The dissipation of metam sodium in Arlington sandy loam soil amended with composted manure (CM), amended with composted manure and repeated fumigation with metam sodium (MS) for 6 months, and unamended soil

(r^2>0.88) (Table 1). The degradation of metam sodium in soil was accelerated with the addition of CM, and the *k* for metam sodium degradation in CM-amended soil was >10 times that in the unamended soil (Table 1; Fig. 3). In CM-amended samples with repeated fumigation with metam sodium for 6 months, the *k* was 17 times higher than that in freshly spiked unamended soil (Table 1; Fig. 3). This indicated an increase in the rate of metam sodium degradation resulting from amendment with CM and with repeated fumigation. Based upon these results, the combination of CM-amendment and repeated exposure to metam sodium appeared to accelerate the degradation of the latter in soil. This may be the result of the influence of specific microorganisms on biological activities resulting in rapid reaction rates. Metam sodium degradation in autoclaved soil was significantly inhibited compared to non-sterile soil within each treatment (Fig. 3, Table 1), suggesting some role of microorganisms in metam sodium degradation. Based on the differences in the rate of metam sodium degradation in autoclaved and non-autoclaved soils, it was estimated that microbial degradation accounted for 77% of metam sodium degradation in CM-amended soil and 28% in unamended soil. Incorporation of organic amendment to the degradation of metam sodium in soil significantly enhanced the degradation rate of the compound.

DGGE patterns of CM-fumigated soil with metam sodium after 6 months enrichment (data not shown) showed several conspicuous bands (VP1–VP7, Table 2). Sequence analysis from the DNA fragments excised from the major bands on the DGGE gels for identification showed that the sequences of VP1 and VP2 were most similar to those of uncultured bacterium (100%) (Table 2) that originated from landfill groundwater contaminated with chlorinated ethene (Brigmon et al. unpublished). Growth of *Mycobacteriun* sp. and *Bacillus* sp. on CM-amended soil with repeated fumigation with metam sodium was also observed.



Scheme 1

Discussion

In this study, we have shown that the contribution of microorganisms in CP degradation was more predominant than in 1,3-D isomers (Ibekwe et al. 2001b) and was similar to the results of previous reports for CP (Gan et al. 2000), and MITC (Gan et al. 1999b; Dungan et al. 2003). Degradation of CP was previously described by Castro et al. (1983), who showed that four species of soil *Pseudomonas* were able to dechlorinate CP to $CHCl_2NO_2$, CH_2ClNO_2, and CH_3NO_2 within 1 h. Our study is in agreement withthat of Gan et al. (2000), who reported CP half-lives of ≤4.5 days in unamended soils of differing organic matter content. In this study, the contribution of microorganisms to CP degradation was more predominant than to 1,3-D degradation (Ibekwe et al. 2001b), and more similar to MITC degradation (Gan et al. 1999a,b), and this study.

The positive association of fumigant degradation rate with organic amendment and repeated fumigation implies that, under typical fumigation conditions, metam sodium may degrade faster in soils with high levels of organic materials. The effects of adding manure (Dungan at al. 2003) and repeated application of metam sodium (Smelt et al. 1989; Verhagen et al. 1996) on increasing the rate of MITC degradation have been reported previously. Chemical reactions associated with the products of MITC hydrolysis in basic solution are outlined in Scheme 1 and as reported by Zheng et al. 2004.

The effects on degradation may be significantly higher if the total on-farm management strategy includes the use of organic mulch from the previous cropping season to provide composting materials that are mixed with soil before fumigation. For instance, if organic amendment is incorporated, the half-life of metam sodium would be <1 day for CM-amended and CM-amended soil with repeated fumigation compared to 10 days for the unamended soil (Table 1). This suggests a synergistic effect from the amendment on community composition. Since there was a significant impact of organic amendment on the metam sodium degradation rate, considerably more metam sodium should be degraded in the surface soil layer, if organic amendments are added, thanin the subsurface layers.

In this study, the differences between the degradation rate coefficients in non-sterile and sterile soils (unamended and CM-amended soil) were significantly different, with biological degradation contributing to higher metam sodium degradation (Table 1). Degradation of metam sodium was 1.4 to >4 times faster in non-sterilized than in sterilized soil (Table 1). Similar results were obtained by Dungan et al. (2003) and Taylor et al. (1996), who found tha, on average, MITC degradation was two times greater in non-sterile soil than in sterile soil. The addition of CM to the Arlington sandy loam soil considerably increased the rate of degradation over the non-amended soil (Table 1). Our study showed that degradation rate was faster in CM-amended soil with repeated fumigation than in CM-amended soil, but the percent biological degradation was the same. Previous studies have indicated that the addition of chicken manure to soil can result in a large CO_2 flux (Abdel Magid et al. 1993), suggesting that a large population of microorganisms had been added or that the introduced nutrients stimulated the indigenous microbial populations. In these experiments microbial degradation of metam sodium may be by catabolism, as suggested by Dungan et al. (2003), but continuous efforts in our laboratory to isolate organisms that utilize metam sodium as their sole C source have been unsuccessful. Therefore, MITC degradation may be cometabolically accomplished by the action of enzymes released from organic amendment.

The high rate of degradation of metam sodium and CP in CM-amended soils, relative to their degradation rates in the non-amended and autoclaved soil, suggests that these two compounds were subject to enhanced degradation, even though the degrading soil had not previously been exposed to fumigant. This suggests that the significantly higher degradation rate in the amended soil was predominantly the result of biological degradation, in addition to a much lower level of abiotic degradation as observed in the sterilized soil.

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