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Effects of soil fumigants on methanotrophic activity

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Abstract

Negative impacts on methane (CH_4) oxidation capacity have already been observed for a variety of agronomic practices, but the effect of soil fumigation on CH_4 oxidation has not been investigated. Fumigation is a common practice in agricultural crop and nursery seedling protection. Soils from various agricultural experiment stations, forest nurseries, and a landfill were evaluated for effects of 1,3-dichloropropene (1,3-D), methyl isothiocyanate (MITC), and chloropicrin (CP) on CH_4 oxidation capacities. All three fumigants significantly reduced CH_4 oxidation rates in historically non-fumigated soils (>50%). 1,3-D enhanced CH_4 oxidation in 3 out of 5 previously fumigated soils and MITC increased CH_4 oxidation rates in all historically MITC-fumigated soils compared to controls. CP universally decreased oxidation capacity regardless of fumigation history. These results support the conclusion that CH_4 oxidation effects are fumigant specific and that prior fumigation history plays a vital role in determining the impact on CH_4 oxidizer community functionality, which may have implications on the global CH_4 cycle.
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Keywords: Methanotrophs; Enhanced biodegradation; Fumigation history; Methane oxidation

1. Introduction

Atmospheric methane (CH_4) is second to carbon dioxide (CO_2) in importance as an anthropogenic greenhouse gas, even though the rate of increase in atmospheric concentration has slowed to less than 0.1% in the past 15 yr (Dlugokencky et al., 1998) and has leveled off over the last 7 yr (Simpson et al., 2006). Aerated soils represent the only identified biological sink for CH_4, therefore any changes to the capacity of this sink will impact global concentrations (Reeburgh et al., 1993; King et al., 1998). Methanotrophic bacteria residing in soil can use CH_4 as a carbon source. Magnitude of the total terrestrial soil sink for CH_4 is uncertain, but is estimated at 30 Tg CH_4 yr^{-1} (Smith et al., 2000). Agricultural practices of fertilization (Mosier et al., 1997; Schimel and Gulledge, 1998), pesticide use (Topp, 1993; Priemé and Ekelund, 2001), and cultivation (e.g. Mosier et al., 1997; Hütsch, 1998) have decreased CH_4 oxidation rates in soils. However, effects on CH_4 oxidation as a

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consequence of soil fumigation have not been documented.

Surface emission (or uptake) of CH_4 is a balance between oxidation, production, and transport within the soil system (e.g. Castro et al., 1994; Bradford et al., 2001). The range of measured CH_4 oxidation potentials for several soil types are between 12 and 12,000 ng CH_4-C kg^{-1} h^{-1} (Conrad, 1999). Methanotrophs are believed to be ubiquitous in nature. However, landfill cover soils possess extremely high CH_4 oxidation capacities, with rates exceeding 150×10^6 ng CH_4-C kg^{-1} h^{-1} (Börjesson et al., 1998). Diffusion of CH_4 and oxygen are rate limiting factors for CH_4 oxidation in soils (Bradford et al., 2001), but oxygen is not limiting if CH_4 is of atmospheric origin (Mancinelli, 1995). Exact makeup of the methanotrophic consortium responsible for consumption/oxidation of low (atmospheric) concentrations of CH_4 is not known (Bull et al., 2000; Saari and Martikainen, 2003), but kinetic analyses illustrated variability in functional composition of the community with depth (Crill, 1991; Bender and Conrad, 1992). Exact microbes responsible for atmospheric consumption in aerobic soils are unknown due to difficulty in culturing and identification (Priemé et al., 1996; Saari and Martikainen, 2003), but CH_4 oxidation is believed to be accomplished mainly by methanotrophs and ammonia oxidizers (Bédard and Knowles, 1989). Therefore, different soils will possess different capacities based on the microbial community, nutrient availability, salt concentrations, and redox potential.

Net emission of CH_4 is the balance of consumption and production reactions which are complex interactions of both carbon and nitrogen element cycles. As an example, CH_4 oxidation in soils is mediated by two soil enzymes: CH_4-monooxygenase (methanotrophic bacteria) and ammonia-monooxygenase (nitrifying bacteria) (Bédard and Knowles, 1989). Oxidation of CH_4 by nitrifying bacteria is due to similar molecular size and shape of ammonia (NH_4^+) and CH_4, but nitrifiers cannot grow exclusively on CH_4 (requiring the presence of NH_4^+, Bédard and Knowles, 1989). This shared substrate couples these two biogeochemical cycles (Firestone and Davidson, 1989; Schimel and Gulledge, 1998). This coupling is vital because soil fumigant effects on nitrogen nutrient have already been documented (Winfree and Cox, 1958; Bending and Lincoln, 2000).

Impacts of soil fumigation on CH_4 oxidation have not been fully identified because the microbial consortium responsible for CH_4 oxidation and the effect of soil fumigants on these microbes are not known (Priemé et al., 1996; Saari and Martikainen, 2003). Some species of soil biota are able to survive fumigation and actually proliferate due to lack of microbial competition (Ridge, 1976; Rovira, 1976; Miller et al., 1997). Recent studies have shown enhanced biodegradation of fumigants after repeated application (e.g. Smelt et al., 1989; Warton et al., 2003), and have shown active roles of soil microbes in the degradation of applied fumigants (e.g. van Dijk, 1974; Miller et al., 1997). Biological activities of various fumigants are known to be different (e.g. amine and thiol nucleophilic substitution reactions versus cell wall permeability changes), but also are not well documented (Schwarenback et al., 1993). Impacts on soil microbial communities following fumigation have been shown to be fumigant dependent (e.g. Lebbink and Kolenbrander, 1974; Ibekwe et al., 2001). Results of these and other studies indicate that fumigation reduces overall microbial populations and affects diversity. Impacts on soil functionality, or the net impact of the microbial activity, have not been determined, but are a critical link because decreases in microbial diversity and numbers have not directly correlated with diminished levels of microbial activity (Degens, 1998; Boon et al., 2003; Seghers et al., 2003). Soil functionality is a major factor controlling net CH_4 exchange (Bradford et al., 2001).

Methyl bromide (MeBr) has inhibited CH_4 oxidation in field trials at high concentrations ($>10{,}000$ ppmv) (Oremland et al., 1994), whereas in laboratory cell suspensions MeBr has been degraded by methanotrophic communities (*Methylococcus* and *Methylomonas*) (Colby et al., 1975; Schaefer and Oremland, 1999). Inhibition of CH_4 oxidation by MeBr in the field study was most likely the result of substrate competition, since the CH_4-monooxygenase enzyme can also degrade MeBr. No conclusive fumigant impacts have been observed for CH_4 in field flux measurements due to limited methanotrophic activity in pre-fumigation and/or control plots (Oremland et al., 1994; Spokas et al., 2005).

Our primary purpose in this study was to quantify the effects of soil fumigants on soil CH_4 oxidation in laboratory microcosms. Significant effort has been made to find chemical fumigants that do not destroy the ozone layer but preserve the efficacy of MeBr as a soil fumigant (Majewski et al.,

1995; Yagi et al., 1995; Wang and Yates, 1998). Three registered soil fumigants were included in this study (UNEP, 1998): chloropicrin (CP), methyl isothiocyanate (MITC), and 1,3-dichloropropene (1,3-D). The effects of soil fumigation on CH_4 oxidation capacity were determined at ambient CH_4 concentrations for a variety of soils with different CH_4 oxidation capacities and fumigation histories.

2. Soil collection sites

Soils were selected to ensure a range of textures, CH_4 oxidation capacities, and fumigation histories in laboratory incubations. Soils from two forest nurseries, four agricultural research stations (two with a history of soil fumigation), and a landfill cover soil were evaluated for effects of soil fumigants on CH_4 oxidation capacities (Table 1). Soil microbial numbers and activity are controlled by a number of environmental (e.g. pH, climate, soil nutrient availability, soil texture, and moisture) as well as biotic factors, but organic carbon availability is often cited as the most dominant factor (Wardle, 1992). Therefore, populations of methanotrophs will vary as a function of nutrient and agronomic practices (fertilizer, cultivation), as well as soil type and climate. Representative northern (Hayward, WI) and southern (Flint River, GA) forest nurseries in the US were selected to capture differences in microbial and soil characteristics between geographical locations. At nursery sites, equivalent native soils (non-nursery management)

Table 1
Field sites for soils examined[a]

Site location	Latitude	Longitude	Soil description	Fumigation history	Soil management
Forest nursery					
Hayward State Nursery (Hayward, WI)	N46.0	W91.3	Sandy, mixed, frigid, entic haplorthod		
A. Native Soil				None	Grassland
B. Conifer nursery area				MITC, MeBr, CP	Nursery
C. Deciduous nursery area				MITC, MeBr, CP	Nursery
Flint River Nursery (Byromville, GA)	N32.2	W84.0	Siliceous, thermic psammentic paleudult		
A. Native soil				None	Grassland
B. Conifer nursery area				MeBr, CP, MITC	Nursery
Agricultural soils					
Becker, MN	N45.5	W93.8	Sandy, frigid, entic hapludoll	MITC	Agricultural
Lamberton, MN	N44.3	W95.3	Fine loamy, mixed, mesic typic haplustoll	None	Agricultural
St. Paul, MN	N44.9	W93.1	Fine loamy, mixed, udic haploboroll	None	Grassland
Tifton, GA	N31.5	W83.5	Fine loamy, siliceous, thermic plinthic paleudult		
A. Fumigated				MITC, MeBr, CP, 1,3-D	Agricultural
B. Non-fumigated				None	Agricultural
Landfill cover soil					
Raleigh, NC	N35.8	W78.5	Clayey, kaolinitic, thermic typic kanhapludult	None	Landfill cover soil

[a]MeBr is methyl bromide, MITC is methyl isothiocyanate, CP is chloropicrin, and 1,3-D is 1,3-dichloropropene.

were also collected. Exact fumigation histories of the nursery sites were undocumented, but past fumigants used are listed in the order of application frequency (Table 1). This limited history coupled with the native soil samples was used to assess any chronic impacts past fumigation might have had on CH_4 oxidation capacities. For the Hayward site, soil was also collected from conifer and deciduous seedling areas, since tree species impact soil microbial diversity (Grayston and Campbell, 1996).

Three of the four agricultural stations were selected near our laboratory to limit soil transport and storage issues. One of these locations (Becker, MN) had historical use of MITC soil fumigant for potato production. The other two locations in Minnesota were active (Lamberton, MN) or formerly active (St. Paul, MN) corn-soybean fields (Table 1). The St. Paul site was converted into a grassland area for over 60 yr, prior to this conversion the area was under cultivation and row crop production. The fourth agricultural site, located in Tifton, GA, permitted the evaluation of chemical fumigation on soil functionality, since the sole management difference between the two soils sampled at this site was the use of chemical fumigation. Otherwise, they possessed identical cropping, fertilization, and cultivation histories.

Landfill cover soil was utilized due to the adapted high level of methanotrophic activity observed in landfill soils from continual exposure to elevated CH_4 levels (e.g. Boeckx et al., 1998; Börjesson et al., 1998). Due to its high CH_4 oxidation capacity, this soil is a good candidate to evaluate the effectiveness of any stimulation or inhibition effects exhibited by evaluated fumigants.

3. Materials and methods

3.1. Soil physical properties

Soil samples (0–15 cm) from each site were collected at least 1 yr after the last fumigation (if occurred at site). Typically, a composite sample was taken from four locations all within a 1 m^2 area, sieved (2 mm), homogenized, and stored at 22 °C (±1 °C) in a humidified environment until laboratory incubations could be performed. Roots were removed during the sieving process. Moisture content, pH, weight fractions of sand, silt, and clay, and total organic carbon (TOC) were measured (Table 2). Water content was determined by mass loss from oven drying at 105 °C for 24 h. Soil pH was measured in a 1:1 (v:v) slurry of soil and deionized water using a Hanna Instrument portable pH/EC/TDS/temperature probe. TOC was determined following the loss on ignition method (550 °C, 1 h) and converted to organic carbon utilizing the factor 1.724 in Nelson and Sommers

Table 2
Physical and chemical properties of investigated soils[a]

Site location	pH	θ_w (%)	Sand (%)	Silt (%)	Clay (%)	TOC (%)
Forest nurseries						
Hayward, WI						
A. Native soil	5.8	1.46±0.1	82.5	8.0	9.5	0.65±0.03
B. Conifer nursery area	6.8	5.02±0.2	83.7	9.0	7.3	1.12±0.01
C. Deciduous nursery area	5.3	7.11±0.1	83.7	9.0	7.3	2.64±0.04
Byromville, GA						
A. Native soil	5.9	4.6±0.1	87.5	6.8	5.8	1.37±0.01
B. Conifer nursery area	5.6	7.0±0.1	86.2	7.3	6.5	1.86±0.01
Agricultural soils						
Becker, MN	5.8	5.63±0.1	92.0	3.3	4.8	1.18±0.01
Lamberton, MN	6.1	22.8±0.5	45.9	25.7	28.5	3.80±0.18
St. Paul, MN	6.5	15.9±0.1	21.4	58.9	19.6	5.19±0.01
Tifton, GA						
A. Fumigated	5.8	7.5±0.2	93.0	4.0	3.0	1.02±0.19
B. Non-fumigated	5.4	7.3±0.1	93.0	4.0	3.0	1.08±0.03
Landfill cover soil						
Raleigh, NC	4.5	31.6±0.2	22.2	66.7	11.1	7.03±0.23

Data are shown as the arithmetic mean with the standard deviation ($n = 3$).
[a]θ_w = soil moisture content by weight, and TOC = total organic carbon.

(1996). Soil texture was determined by the University of Minnesota Soil Testing Laboratory using a hydrometer method.

3.2. Soil fumigant laboratory incubations

Laboratory incubations were all carried out within 2 weeks of soil collection, to avoid decreases in microbial activity due to storage and refrigeration (Ross et al., 1980; Zelles et al., 1999). Sub-samples (5 g) of field moist soil (Table 2) were placed in sterilized 125 mL serum vials (Wheaton Glass, Milville, NJ) and sealed with Teflon-lined butyl rubber septa (Agilent Technologies, Palo Alto, CA). Teflon-lined septa were required due to absorption of fumigants by unlined butyl rubber septa. These incubations were similar in design to that of Chan and Parkin (2001), except soil moisture values were not optimized. Instead incubations were conducted on field moist soils (Table 2). Incubations were performed aerobically with CP, MITC, and 1,3-D, as well as a soil control (no fumigants) at $22 \pm 2\,^{\circ}C$. Fumigants were injected into the sealed vial at typical field application rates for each chemical (CP: $68\,\mu g\,g^{-1}$, MITC: $55\,\mu g\,g^{-1}$, 1,3-D: $50\,\mu g\,g^{-1}$; assuming a bulk density of $1.65\,g\,cm^{-3}$ and 0.5 m soil treatment depth). Triplicate lab controls were also prepared to account for fluctuations in the laboratory CH_4 concentrations (range for all incubations: 2.0 ± 0.3 ppmv). Enriched CH_4 level (100 ppmv) and sterilized soil incubations were also run exclusively on the landfill cover soil, since other soils tested would not experience elevated CH_4 concentrations and landfill soil possessed maximum methanotrophic activity.

3.3. Gas sampling and analysis

Headspace air samples were collected at a minimum of five separate time samplings through the 2- or 10-d incubations. CH_4 concentration was determined on a gas chromatographic (GC) system (HP-5890, Agilent Technologies, Palo Alto, CA), with a flame ionization detector and a 0.5 mL gas sampling loop (Spokas et al., 2005). The same GC system also analyzed for CO_2 and O_2 on thermal conductivity detectors using multiple sample loops (Spokas et al., 2005). The GC system was calibrated using commercially certified gas standards (Scott Specialty Gases, Troy, MI).

3.4. Statistical analysis

Results presented were arithmetic means of triplicate samples, and CH_4 oxidation rates were expressed on a soil oven-dried basis. CH_4 oxidation rate was determined from the linear decrease in headspace CH_4 concentration over 10 d, with the exception of the landfill cover soil incubation which was for 2 d. This modification was needed due to higher methanotrophic activity of landfill soil. Linear regression analysis for CH_4 oxidation rates (zero-order kinetics) has been performed in other studies (e.g. Reay et al., 2001; Gebert et al., 2003; Seghers et al., 2003; Börjesson et al., 2004), and is justified based on observed linear decreasing concentrations over the 10 or 2 d incubation periods (data not shown). Net CH_4 production is indicated by a positive number and net oxidation by a negative number.

Data were analyzed using an analysis of variance (ANOVA) procedure for independent samples to test for statistically significant differences using MINITAB (Minitab, Inc., State College, PA). If significant differences existed among the factors, as indicated by the *F*-ratio, the Tukey's Honest Significant Difference (HSD) test was performed to determine which pair-wise interactions were significantly different at the $P < 0.05$ levels. Soil effects were not evaluated over the range of soils; rather each treatment was compared to individual soil controls since microbial populations are different for each ecosystem collected. In addition, incubations of soils from each of the sites were conducted independently and therefore cannot be compared statistically.

4. Results/discussion

4.1. Soil properties

Strong relationships were observed between soil moisture and TOC content ($r = 0.92$, $P < 0.05$) and between clay and moisture content ($r = 0.61$, $P < 0.05$). Strong negative relationships were observed between sand and TOC ($r = -0.94$, $P < 0.05$) and between sand and moisture content ($r = -0.86$, $P < 0.05$). Soil texture and OM content are important in determining soil moisture and soil moisture potential, which determine the true availability of water to microbes and plants. There was a relationship ($r = 0.63$, $P < 0.05$) between the basal CO_2 respiration rates (data not shown) and the TOC

content of the soils across the various ecosystems, and exclusion of the landfill cover soil improved the relationship ($r = 0.83$, $P < 0.05$). This is expected since there is a strong linkage between the available organic carbon and microbial activity (Wardle, 1992).

4.2. Nursery soils

Two nursery soils were tested in this study: Hayward State Nursery (WI) and Flint River Nursery (GA) (Table 1). For the incubations presented here, the non-fumigated native (non-nursery) soil consistently had higher CH_4 oxidation rates, -8 to $-10\,ng\,CH_4\text{-}C\,kg^{-1}\,h^{-1}$, than the managed forest nursery soil at both sites (Figs. 1 and 2, "control"). This is in agreement with prior studies which also documented unmanaged systems exhibiting higher CH_4 oxidation potentials (e.g. Bender and Conrad, 1992; Chan and Parkin, 2001). Additionally, tillage (Mosier et al., 1997) and mineral fertilizers (Seghers et al., 2003) have been shown to decrease soil CH_4 oxidation capacities and historically were used at the nurseries, whereas the natural soils collected at the sites would not have had this fertilization and tillage history. Nursery soils (both conifer and deciduous in Hayward, conifer only in GA) did exhibit net CH_4 production (Figs. 1 and 2). Loss of oxidation capacity in the nursery soils compared to the native (non-nursery) soils at both sites could be due to the nursery soil management practices (e.g. cultivation, fertilization, or pesticide application).

Significant differences in fumigant effects were observed between native soil and the two nursery sites at Hayward (Fig. 1), which further emphasizes the importance of past history (or chronic effects) on the observed effects of fumigants on methanotroph activity. 1,3-D and MITC significantly increased CH_4 oxidation activity in the Hayward nursery soils following application (Fig. 1), but this was not observed in the non-nursery soil.

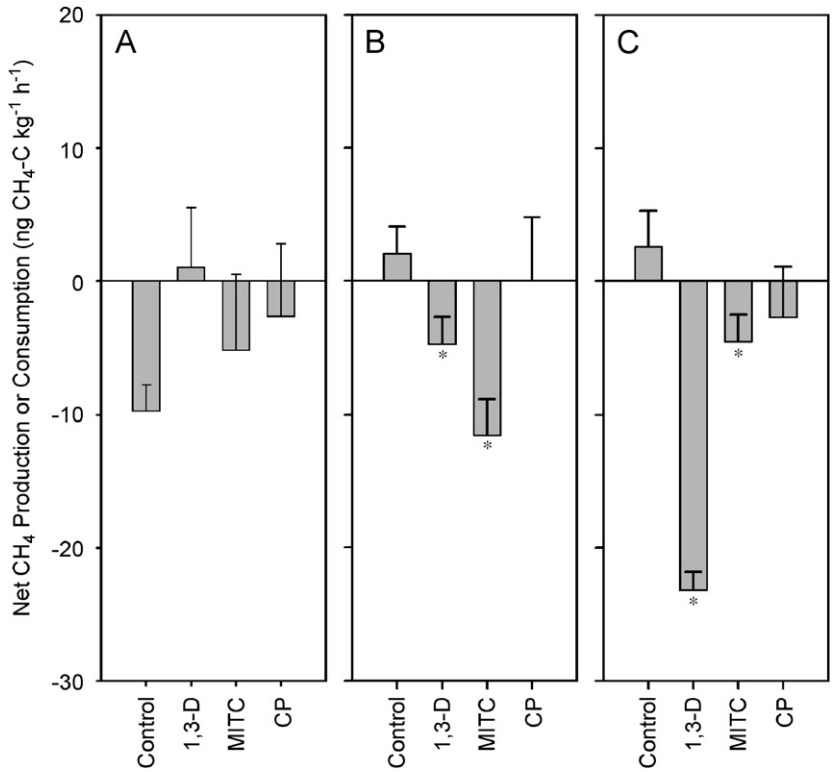

Fig. 1. Methane oxidation rates for the 4 fumigant treatments (no fumigants (Control), 1,3-dichloropropene (1,3-D), methyl isothiocyanate (MITC), and chloropicrin (CP)) for soil samples from Hayward State Nursery (Hayward, WI): (A) native soil (non-nursery), (B) conifer seedling nursery, and (C) deciduous seedling nursery area. Data presented are averages of measurements ($n = 3$) with error bars representing one standard deviation, * represents those fumigant treatments that are statistically different from control incubation ($P < 0.05$).

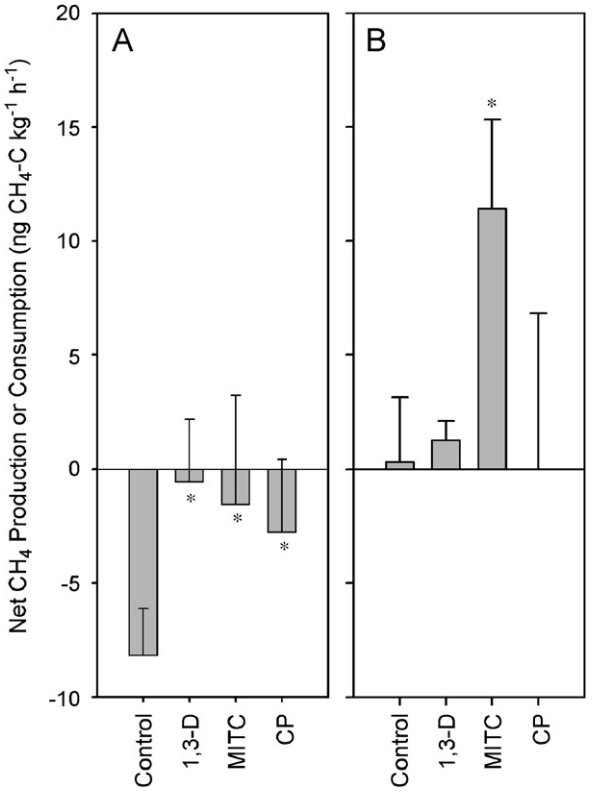

Fig. 2. Methane oxidation rates for the 4 fumigant treatments (no fumigants (Control), 1,3-dichloropropene (1,3-D), methyl isothiocyanate (MITC), and chloropicrin (CP)) for soil samples from Flint River Nursery (Byromville, GA): (A) native soil (non-nursery) and (B) nursery soil. Data presented are averages of measurements ($n = 3$) with error bars representing one standard deviation, * represents those fumigant treatments that are statistically different from control incubation ($P < 0.05$).

This change in functionality is very significant. Differences in the responses of native and nursery soil could be related to fumigation histories. Repeated fumigation leads to alterations in the microbial diversity favoring those organisms that are preferential degraders of soil fumigants (van Dijk, 1974; Verhagen et al., 1996). The potential exists for 1,3-D cometabolism by CH_4 monooxygenase, particularly since it has been shown that the methanotrophs (e.g. *Methylosinus*) are capable of biodegrading 1,2-dichloropropane which is found in commercial formulations of 1,3-D (Bosma and Janssem, 1998) and might be able to degrade 1,3-D. In addition, methanotrophs have been shown to degrade other chlorinated species (Bosma and Janssem, 1998; Scheutz et al., 2004). No statistical differences were observed in the reduction of CH_4 oxidation from CP fumigation in native and nursery soils at Hayward (Fig. 1), which is in agreement with Ibekwe et al. (2001) who observed that CP had less impact on microbial diversity compared with other fumigants when applied at equal concentrations.

Contrary to the observed results at Hayward (Fig. 1), 1,3-D and MITC did not stimulate CH_4 oxidation at the GA nursery site (Fig. 2). In fact, the only statistically significant difference was stimulation of CH_4 production from MITC in GA nursery soil. MITC was not used as heavily historically at the GA nursery as compared to Hayward. But no conclusive data exists to document this difference besides site managers' recollections. The mechanism of observed CH_4 production is unknown; especially since all incubations were aerobic (all incubations were above 18.5% O_2 at 10 d). However, aerobic CH_4 production has been observed in other agricultural soil incubations (e.g. Chan and Parkin, 2001).

4.3. Agricultural soils

Soils from three Minnesota agricultural experiment stations and one research station in Tifton, GA were tested. The three Minnesota stations were: Becker, MN which has a history of fumigant application (Fig. 3(A)), and Lamberton, MN and St. Paul, MN which do not have a fumigation history (Figs. 3(B) and (C)). There were two locations sampled at the GA research station: one with a history of fumigant application and another without (Figs. 4(A) and (B)). This permitted a comparison of chronic effects resulting from past chemical fumigation on CH_4 oxidation, since the soils had experienced similar agronomic practices with the exception of fumigation.

In Becker soil (Fig. 3(A)), a significant stimulation effect of 1,3-D on CH_4 oxidation was observed in previously MITC-fumigated soils, but 1,3-D suppressed oxidation activity in non-fumigated agricultural soils (Lamberton and St. Paul; Figs. 3(B) and (C)). Data from the three MN field stations indicate a significant reduction in the CH_4 oxidation rates as a result of fumigant application. All three fumigants significantly reduced CH_4 oxidation in Lamberton and St. Paul soils, however, only CP significantly reduced CH_4 oxidation at Becker. This could be related to the historical fumigant application that occurred at Becker, and these results are in agreement with those found for the Hayward nursery soil (Fig. 1). We also observed

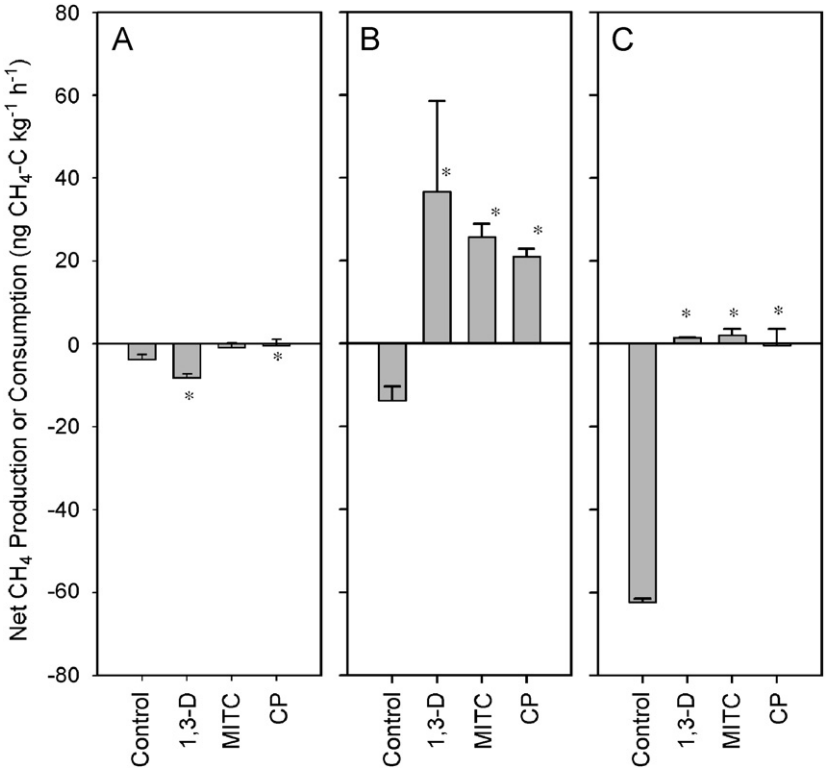

Fig. 3. Methane oxidation rates for the 4 fumigant treatments (no fumigants (Control), 1,3-dichloropropene (1,3-D), methyl isothiocyanate (MITC), and chloropicrin (CP)) for soil samples from Minnesota Agricultural Research Stations: (A) Becker, MN (with fumigation history), (B) Lamberton, MN (no fumigation history), and (C) St. Paul, MN (area with no tillage for 60+ years, no fumigation history). Data presented are averages of measurements ($n = 3$) with error bars representing one standard deviation, * represents those fumigant treatments that are statistically different from control incubation ($P<0.05$).

that the highest oxidation rates occurred in grassland soil (Fig. 3(C)) versus a soil under active agricultural management, which is in agreement with other studies (e.g. Mosier et al., 1997; Hütsch, 1998) and those differences shown above between native and nursery soil.

Fumigated Lamberton soil exhibited CH_4 production (Fig. 3(B)). As mentioned earlier, CH_4 production has been observed in other soil incubations (Yavitt et al., 1995; Chan and Parkin, 2001), and periods of CH_4 emissions have been observed in field plots following infiltration events (Delgado and Mosier, 1996). CH_4 production is attributed to the presence of anoxic microsites within the aerobic soil, which permits anaerobic CH_4 production. Lamberton soil possessed the highest clay and moisture content of agricultural soils used in this study (Table 2), and soil moisture has been cited as a variable in the creation of anaerobic sites within soil aggregates by limiting oxygen diffusion (Glinksi and Stepniewski, 1985). These factors could contribute to the observed CH_4 production.

At the Tifton, GA site, CH_4 oxidation was observed in the non-fumigated soil (Fig. 4(A)) and CH_4 production was observed in historically fumigated soil (Fig. 4(B)). Although these differences were not statistically significant ($P>0.05$), changing from CH_4 consumption to production indicates an important shift in the soil functionality regardless of the numerical significance. There were no significant differences observed between fumigant treatments and the control in any of these incubations, resulting from the similarity between the measurement standard deviation and low CH_4 oxidation/production rates. This limits the strength of conclusions drawn from this data. However, it does appear that there is a chronic negative impact on CH_4 oxidation capacity resulting from soil fumigation, in addition to the acute effects already documented in this study. The effects on these GA soils is opposite

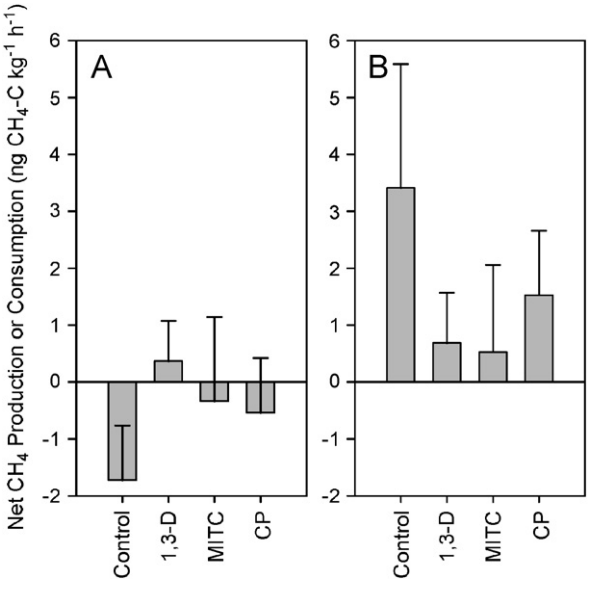

Fig. 4. Methane oxidation rates for the 4 fumigant treatments (no fumigants (Control), 1,3-dichloropropene (1,3-D), methyl isothiocyanate (MITC), and chloropicrin (CP)) for soil samples from Tifton, GA Agricultural Research Station: (A) no fumigation history and (B) with fumigation history. Data presented are averages of measurements ($n = 3$) with error bars representing one standard deviation, * represents those fumigant treatments that are statistically different from control incubation ($P < 0.05$).

of soils from Becker, and could potentially be due to transport, soil moisture, or differences in microbial activity and diversity. However, these impacts were not further investigated in this study.

4.4. Landfill cover soil

This soil exhibited the highest oxidation rates observed for control incubations in this study at $-416 \pm 4\,\text{ng}\,CH_4\,kg^{-1}\,h^{-1}$ (Fig. 5(A)) as well as the highest TOC content (Table 2). However, this oxidation rate is still below what has been found in other studies of landfill soils where rates have exceeded $-1.50 \times 10^8\,\text{ng}\,CH_4\text{-}C\,kg^{-1}\,h^{-1}$ (Börjesson et al., 1998). Multiple factors may have contributed to the lower observed value in this study. First, the collection period occurred during the winter season. Colder temperatures have been correlated with lower methanotroph activity and population density (Gebert et al., 2003; Börjesson et al., 2004). Second, this cover soil had a low pH value (Table 2; pH = 4.5), which has also been linked to lower methanotroph activity (Hütsch et al., 1994; Chan and Parkin, 2001). Optimal pH for methanotrophs is currently believed to be around neutral pH (Amaral et al., 1998). Third, these experiments were conducted on field moist soils, and the landfill soils were especially wet. Soil moisture is a primary variable affecting CH_4 oxidation rates, where 78% of the variability in CH_4 oxidation rates in forest soils has been correlated to soil moisture values (Castro et al., 1994).

Three different incubations were performed with the landfill cover soil. Non-sterile soil was incubated at ambient (≈ 2 ppmv) and elevated (100 ppmv) CH_4 levels (Figs. 5(A) and (B)), and steam sterilized landfill cover soil was incubated at ambient CH_4 levels to confirm that the effect was biotic in nature (Fig. 5(C)). At ambient CH_4 levels, landfill soil had a high CH_4 oxidation rate ($-416 \pm 4\,\text{ng}\,CH_4\text{-}C\,kg^{-1}\,h^{-1}$) and the oxidation rate was approximately 30% higher under elevated CH_4 levels ($-553 \pm 9\,\text{ng}\,CH_4\text{-}C\,kg^{-1}\,h^{-1}$) with no fumigant treatment. The increase in oxidation rate with elevated headspace CH_4 has been seen in other studies (e.g. Chan and Parkin, 2001), but the increase observed here was not as dramatic as increases documented in prior studies. Differences may be related to the depleted methanotrophic activity by the factors mentioned above.

In contrast to other soils where CP consistently inhibited oxidation to a greater extent than the other fumigants, the landfill soil incubation with CP had the highest residual oxidation rate of fumigants tested under both headspace concentrations (Figs. 5(A) and (B)), but still was significantly reduced from the control. All three fumigants significantly reduced the CH_4 oxidation capacity (in excess of 95% suppression) for both ambient and elevated CH_4 concentrations (Figs. 5(A) and (B)). Sterilized soil treatment confirmed that the consumption of CH_4 in this landfill cover soil is biologically mediated since the oxidation of CH_4 ceased in sterile soil compared to the non-sterile control (Figs. 5(A) and (C)). Fumigants also showed no significant abiotic effects on CH_4 production or consumption through these sterilized tests (Fig. 5(C)).

Boeckx et al. (1998) also used agricultural and landfill cover soils to evaluate effects of pesticides on CH_4 oxidation. They did not observe any significant impacts of tested pesticides on methanotrophic activity in landfill cover soil, but they observed reductions in CH_4 oxidation rates for agricultural soils. This could indicate that the soil fumigants tested in this study are a stronger

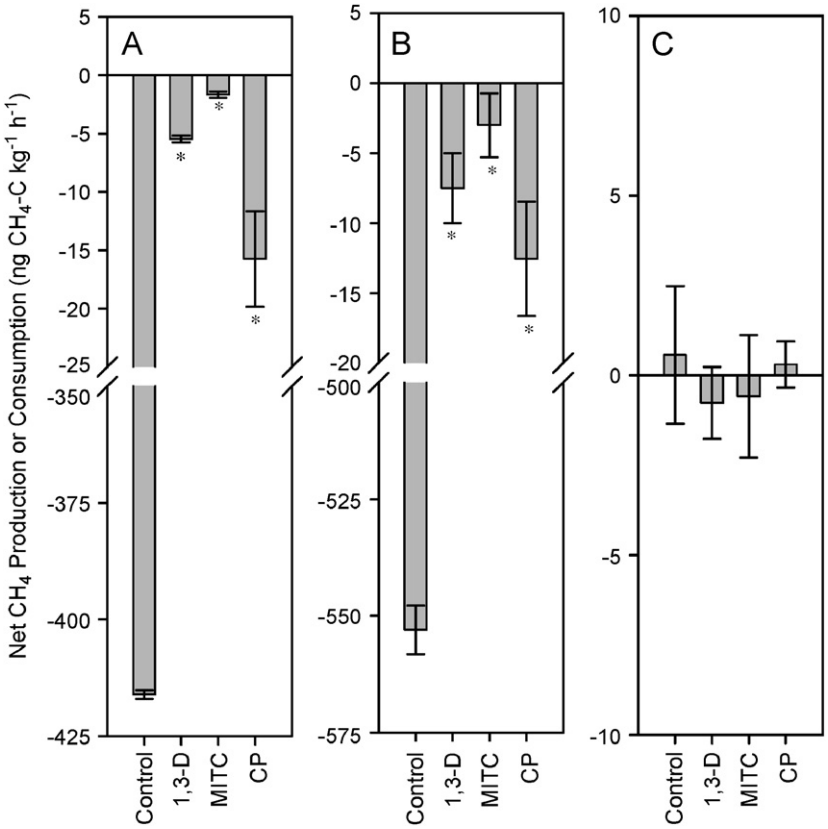

Fig. 5. Methane oxidation rates for the 4 fumigant treatments (no fumigants (Control), 1,3-dichloropropene (1,3-D), methyl isothiocyanate (MITC), and chloropicrin (CP)) for soil samples from landfill cover soil (Raleigh, NC): (A) ambient methane concentration, (B) elevated methane concentration (100 ppmv), and (C) ambient methane concentration with steam sterilized soil. Data presented are averages of measurements ($n = 3$) with error bars representing one standard deviation, * represents those fumigant treatments that are statistically different from control incubation ($P < 0.05$).

suppressor of CH_4 oxidation than the pesticides examined by Boeckx et al. (1998), which is expected since soil fumigants have a stronger effect on soil microbial populations than pesticides (Ibekwe et al., 2001).

5. Conclusions

The main emphasis of this work was to document effects of soil fumigants on CH_4 oxidation potentials. All three fumigants tested significantly reduced CH_4 oxidation rates in historically non-fumigated soils. 1,3-D enhanced CH_4 oxidation in 3 out of 5 previously fumigated soils and MITC increased CH_4 oxidation rates in all historically MITC-fumigated soils compared to controls. CP universally decreased oxidation capacity regardless of the fumigation history. Precise mechanisms of inhibition are not known, although the alteration in population and activity of the methanotrophic bacteria is hypothesized, as has been found in other studies (e.g. Reay et al., 2001). There are three potential explanations for effects observed in this study:

1. Soil fumigation increases soil inorganic-N levels (Winfree and Cox, 1958). High ammonia levels have been cited as inhibitors of CH_4 oxidation activity (Hütsch et al., 1994), and CH_4 oxidation capacity of a system has been negatively correlated to N-nutrient status (Schimel and Gulledge, 1998; Chan and Parkin, 2001). In the GA nursery plots, inorganic-N nutrients were uniformly doubled for ammonia and nitrate following fumigation with MITC, whereas the combination of MITC and CP fumigation increased the observed NH_4^+ soil concentration 65 times (Spokas et al., 2005). N-nutrients were not evaluated in these incubations or at the Hayward site, so direct confirmation of these increases

cannot be made for the laboratory incubations conducted here.

2. There could be a direct chemical interaction of the fumigants with the CH_4-monooxygenase enzyme. This is possible due to the non-specific character of the CH_4-monooxygenase enzyme (Bédard and Knowles, 1989; Boeckx et al., 1998).
3. Lastly, soil fumigants could have high toxicity towards methanotrophs. This is the most likely explanation, especially due to the extreme nature of suppression seen in the landfill cover soil and the observation that MITC and 1,3-D in previously fumigated soil tended to increase methanotrophic activity. This increase in activity suggests that repeated application of fumigants favors methanotrophic species that utilize fumigants as an energy source (Smelt et al., 1989) and are therefore resistant to the biocidal action of fumigants. However, due to the difficulty in isolating soil-dwelling methanotrophs (Priemé et al., 1996; Saari and Martikainen, 2003), this has not been demonstrated.

Effects measured in this study are acute in nature, and the chronic impacts have not been analyzed. Data from the Tifton, GA agricultural site and comparisons of the nursery and native soils indicate that fumigants and/or soil management have chronic effects on atmospheric CH_4 oxidation capacities that do warrant further investigation. These chronic effects must be quantified before the overall impact of fumigants on global biogeochemical cycles can be estimated. These results coupled with observations documenting the stimulation in nitrous oxide production from fumigant use (Spokas and Wang, 2003; Spokas et al., 2005) indicate that fumigant selection should be based not only on lack of ozone depleting properties but also on their total environmental impact on soil microbial activity. Such chronic effects may have significant impacts on the magnitude of CH_4 oxidation globally.

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