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Greenhouse gas production and emission from a forest nursery soil following fumigation with chloropicrin and methyl isothiocyanate

Kurt Spokas[a,*], Dong Wang[a], Rodney Venterea[b]

[a]*Department of Soil, Water, and Climate, University of Minnesota, 1991 Upper Buford Circle, 439 Borlaug Hall, St Paul, MN 55108, USA*
[b]*USDA-ARS, Soil and Water Management Unit, University of Minnesota, St Paul, MN 55108, USA*

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Abstract

Soil fumigation is commonly used to control soil-borne pathogens and weeds. Our aim was to examine the effects of soil fumigation with chloropicrin (CP) and methyl isothiocyanate (MITC) on CH_4, N_2O and CO_2 production and emission. These effects on a SE USA forest nursery soil were examined in field and laboratory experiments. Following field fumigation, CH_4 surface emissions and concentrations in the soil atmosphere were unaffected. Both fumigants increased N_2O emissions rates significantly compared to non-fumigated controls, and the effects were still evident after 48 d. These findings are in contrast to fertilizer-induced N_2O emissions, which generally return to background within 2 wk after application. Depths of N_2O production were different for the two fumigants as determined by soil gas sampling, suggesting fumigant-specific stimulation mechanisms. CO_2 emissions (0–15 d) were not altered significantly, although sub-surface CO_2 concentrations did increase following fumigation with CP or MITC and remained elevated for CP treatment on d 48. CP-induced N_2O production was also stimulated in aerobic laboratory incubation studies, with surface soils exhibiting 10 to 100-fold greater production rates. MITC and a combination of CP/MITC also stimulated N_2O production, but the effect was significantly less than for CP alone. MITC suppressed and CP did not effect CO_2 production in the laboratory incubation. By comparing sterilized to non-sterile soils, $>95\%$ of these effects appear to be of biotic origin.

Keywords: Soil fumigation; Trichloronitromethane; Nitrous oxide; Methane; Carbon dioxide; Methyl isothiocyanate

1. Introduction

Forest nurseries in the southern US are producing approximately 1.2×10^9 seedlings per year with 89% of the nurseries relying on chemical fumigation to control soil pathogens and weeds (Lantz, 1997). Methyl bromide (MeBr) has been the primary soil fumigant. As a result of the Montreal Protocol of 1995, the use of MeBr for fumigation is being curtailed and will eventually be eliminated. Therefore, other fumigants are being investigated to replace MeBr and are selected based on their efficacy toward a particular weed, insect, fungi, or pathogen at a site. However, the fumigant does not act solely on its target and can affect the entire soil microbial community.

The effects of fumigation on the soil microbial community are specific to the particular fumigant. Fumigation with MeBr reduces the aerobic bacterial population (Ridge, 1976) and substrate induced respiration (SIR) (Parthipan and Mahadevan, 1995; Lin and Brookes, 1999). Similar decreases in SIR have been observed with 1,3-dichloropropene and propargyl bromide, which are two potential MeBr replacements (Dungan et al., 2003). SIR rates are correlated with microbial counts and biomass for a specific soil type (Anderson and Domsch, 1978; Harden et al., 1993). There was no shift observed in proportions of Gram-negative and Gram-positive bacteria following MeBr fumigation (Ridge and Theodorou, 1972; Ridge, 1976).

Dazomet, a methyl isothiocyanate (MITC) precursor, caused a 50% reduction in total bacterial numbers in soil for at least 15 wk (Parthipan and Mahadevan, 1995; Charbol et al., 1988) and a reduction in nitrification rates (Lebbink and Kolenbrander, 1974). In contrast, chloropicrin (CP)

* Corresponding author. Tel.: +1 612 625 1798; fax: +1 612 625 2208.
E-mail address: kspokas@umn.edu (K. Spokas).

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increased the aerobic-Gram negative bacterial population 10-fold within 10 d following fumigation (Ridge, 1976; Kakie et al., 1978). In particular, *Pseudomonas fluoroscenes* flourished following CP fumigation, comprising over 70% of the aerobic population, compared to $<10\%$ in non-fumigated controls (Ridge, 1976; Rovira, 1976). This is of particular importance since some *Pseudomonas* spp have been shown to degrade CP (Castro et al., 1983). CP also increases the denitrifying bacteria population (Ishizawa and Matsuguchi, 1966) as well as reduces nitrification rates in soils (Kakie et al., 1978). It has been suggested by Dungan et al. (2003) that organic amendments reduce fumigant impacts on microbial population and diversity. However, decreased microbial population and diversity does not always affect soil functionality (Degens, 1998).

In addition to microbial community changes, there are also alterations in soil inorganic-N concentrations following fumigation. Soil ammonium (NH_4^+) concentrations have increased 10-fold following soil fumigation with CP (Winfree and Cox, 1958; Rovira, 1976), MITC (Hansen et al., 1990), or MeBr (Winfree and Cox, 1958). Increases in nitrate (NO_3^-) following application of CP and MITC have been observed to be soil type dependent (Ebbels, 1971; Hansen et al., 1990). Typically, these higher levels are assumed the mineralization of N from biomass killed by the fumigation (Jenkinson et al., 1972; Ridge and Theodorou, 1972; Hansen et al., 1990). The typical gain in inorganic-N from fumigation ranges from 5 to 10 kg N ha^{-1} (Lebbink and Kolenbrander, 1974). These nutrient increases have been postulated to cause increases in plant yield (e.g. Jenkinson et al., 1972) and vigour of conifer seedlings (e.g. Benzian, 1965). Soil nutrients could also be used as precursors in the complex cycles of greenhouse gas formation.

Flux of reactive trace gases from soil is the resulting balance of microbial consumption and production reactions (Hutchinson and Davidson, 1993). Alterations in microbial structure and diversity play an important role in the balance of greenhouse gas exchange, especially for CH_4 and N_2O fluxes (Kravchenko et al., 2002). Effects of soil fumigation on greenhouse gas exchange have only recently been addressed by Spokas and Wang (2003) who observed a 7-fold increase in N_2O emissions over 20 d following CP fumigation of a northern US nursery soil. To our knowledge, there have been no other studies on the effect of MeBr alternatives on greenhouse gas exchange. Our purpose of this study was to examine the effect of MITC and CP on the production and emission of N_2O, CO_2, and CH_4 in a southeastern US nursery soil using field and laboratory measurements.

2. Materials and methods

2.1. Experimental site

The field experiment was conducted at the Flint River State Nursery in Byromville, GA (32.169° N; 83.974° W), which was established in 1987 and is capable of producing 5×10^7 seedlings per year (Georgia Forestry Commission, 2003). The soil at the experimental site is a loamy sand of the Eustis series (siliceous, thermic psammentic paleudult). The experimental area was divided into 12 plots each measuring 9.1 m $\times$ 3.0 m with a minimum buffer zone of 1.5 m between plots. A randomized block design was used to position four replicates of three treatments dazomet (DAZ), chloropicrin/metam sodium (CPMS), and control. Dazomet and metam sodium are two field-applied fumigants that decompose rapidly in soil to MITC (Dungan and Yates, 2003) which is the active fumigant ingredient. Irrigation was applied for the first week ($\approx$1 cm d^{-1}) to provide a surface seal to reduce volatilization loss of fumigants.

2.2. Soil physical properties

Unfumigated soil was sampled at 15 cm intervals from 0 to 60 cm depth from random locations across the experimental area (Table 1). Soils were sieved (2 mm), homogenized by depth interval, and stored in a humidified and dark environment at 22 °C ($\pm$2 °C) until incubations could be performed (maximum 3 d of storage). Bulk density was determined using the core method (Blake and Hartge, 1986). Soil water content was determined by oven drying 10 g sub-samples 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 (Ann Arbor, MI) portable pH/EC/TDS/temperature probe. TOC was determined from loss on ignition (Nelson and Sommers, 1996). Soil texture was determined by the University of Minnesota Soil and Plant Testing Laboratory using the hydrometer method.

Table 1
Summary of soil physical properties at various depths sampled at the nursery site

Depth (cm)	ρ_b (g cm^{-3})	pH	θ_v (%)	Sand (%)	Clay (%)	Silt (%)	TOC (%)
0–15	1.26 $\pm$ 0.12	5.6	10.0 $\pm$ 1.9 a	86.2	7.3	6.5	1.37 $\pm$ 0.01 a
15–30	1.46 $\pm$ 0.27	5.9	14.6 $\pm$ 1.5 a	85.9	6.8	7.3	1.39 $\pm$ 0.01 a
30–45	1.63 $\pm$ 0.04	6.0	16.1 $\pm$ 0.6 b	86.2	6.5	7.3	1.39 $\pm$ 0.01 a
45–60	1.62 $\pm$ 0.01	5.0	11.0 $\pm$ 0.3 a	83.9	7.8	8.3	1.11 $\pm$ 0.01 b

ρ_b, soil bulk density; θ_v, Soil volumetric water content; and TOC, total organic C. Data are shown as the arithmetic means with the standard deviation ($n=3$); different letters indicate significant differences in the depth intervals ($P<0.05$), if no letters are present the data have are not significantly different for the different depth intervals.

Table 2
Summary of the microbial variables measured at the various depths of the nursery site

Depth (cm)	SMB (μg $C_{Biomass}$ g_{soil}^{-1})	SIR (μg $C_{Biomass}$ g_{soil}^{-1})	BRR (μg C-CO_2 $g_{soil}^{-1}d^{-1}$)	ARA (μg-O_2 g_{soil}^{-1} h^{-1})
0–15	259.0±23 a	31.5±2.2 a	1.56±0.36 a	1.38±0.18 a
15–30	224.0±12 ab	27.7±6.7 a	1.01±0.19 ab	0.87±0.16 ab
30–45	194.0±7 bc	28.0±8.9 a	0.54±0.11 bc	0.80±0.27 b
45–60	167.0±7 c	16.8±6.8 b	0.12±0.10 c	0.76±0.17 b

SMB, soil microbial biomass; SIR, substrate induced respiration; BRR, basal respiration rate; and ARA, aerobic respiratory activity. Data are shown as the arithmetic means with the standard deviation ($n=3$); different letters indicate significant differences for the depth intervals ($P<0.05$).

Inorganic-N forms was extracted with 2 M KCl and analyzed on a Lachat automated ion analyzer (Milwaukee, WI.). Inorganic-N concentrations were determined only in the field plots at 48 d.

2.3. Microbial biomass and activity measurements

Samples from each depth interval were analyzed for microbial biomass along with multiple indices of microbial activity (Table 2). Microbial biomass was estimated using chloroform fumigation-incubation (Jenkinson and Powlson, 1976). Sodium hydroxide traps of fumigated and non-fumigated samples were analyzed using a TOC instrument (Phoenix 8000, Tekmar-Dohrmann). The formula presented in Paul and Clark (1996) was used to calculate soil microbial biomass-C (SMB).

Substrate induced respiration (SIR) was determined by modifying the method of West and Sparling (1986). Sub-samples of 5 g (oven-dry basis) of field soil were placed in 125 ml serum vials and mixed with 2 ml of sterilized distilled water containing 50 mg ml^{-1} of glucose. Vials were sealed with butyl rubber septa and incubated at 22 °C (±2 °C). Headspace was sampled at 6 and 24 h and the quantity of CO_2 was determined by gas chromatography (GC). The rate of CO_2 production (P_{CO2}) was converted to SMB using the formula of Sparling and West (1990).

Basal respiration (BRR) rates were also measured (Sparling and West, 1990). This is the rate of CO_2 production with no nutrient or substrate additions. Sub-samples of 5 g (oven-dry basis) of field soil were placed in 125 ml serum vials. CO_2 produced from the soil was determined periodically by GC for 4 d. CO_2 evolution rates were determined after the incubations had reached steady-state production rates (typically after 24 h). BRR incubations were vented daily to prevent the excessive build-up of CO_2 and formation of anaerobic conditions within the vial.

Aerobic respiratory activity (ARA) was determined by adapting the method of Cannavo et al. (2002). Briefly, 10 g (oven-dry basis) of soil was placed in a sealed 125 ml serum vial. No rewetting or equilibrium time was employed in order to reflect ambient moisture conditions. Gas samples were withdrawn at 0, 24, 48, and 72 h after sealing and analyzed for O_2 using GC. Linear regression of O_2 concentrations versus time was used to calculate ARA.

2.4. Field flux and soil gas measurement

Greenhouse gas fluxes were measured utilizing static non-vented chambers (Spokas and Wang, 2003). Measurements were taken a minimum of three times d^{-1} and a maximum of 10 times d^{-1} at random locations within each plot. Fluxes were calculated from:

$$\text{flux} = V/A(\Delta C/\Delta t), \quad (1)$$

where V is the chamber volume (17.7 l), A is the enclosed surface area (0.12 m^2), and ($\Delta C/\Delta t$) is the slope obtained from linear regression of chamber headspace concentration versus time. Chamber concentration was determined in four gas samples taken at 0, 10, 20, and 40 min after placement. One out of 310 flux measurements (<0.4%) were discarded from the dataset since it did not exhibit a linear increase in headspace concentrations ($r^2<0.90$). Soil temperature (5 cm depth) and volumetric moisture content were recorded at each flux location using a combination soil moisture/temperature time-domain reflectometry (TDR) probe (Hydra Probe, Stevens Vitel, Chantilly, VA). This probe has an accuracy of ±0.01 (v/v) following soil-specific laboratory calibration. There were a total of 96 flux measurements of each treatment taken from 0 to 15 d and 4 sets of flux measurements of each plot taken on d 48.

Customized soil gas probes were constructed and installed at the center of each treatment plot. A 2.54 cm (ID) aluminum pipe was used to install 10 Teflon tubes (OD: 1.8 mm; ID: 0.71 mm) for soil gas sampling. Teflon tubings were installed through perpendicular drilled holes (1.8 mm) in the wall of the aluminum pipe and routed up the center of the pipe. Tubing ends were mated at the surface with a 10-port connector to aid in the sampling (Small Parts, Inc., Miami Lakes, FL). The aluminum pipe was filled with concrete to secure the tubing. The opposing end (bottom) of the aluminum pipe had a machined 60° point to reduce installation effort. Teflon tubing opening was trimmed flush to the outside edge of the aluminum pipe prior to installation. Samples were taken from 10, 25, and 50 cm depths for 0–15 d and 10, 25, 35, and 60 cm on d 48.

Soil-gas and flux samples were collected in empty 12 ml gas-tight glass vials (Exetainers©, Labco Ltd, UK) sealed with rubber septa. Gas samples (30 ml) were injected into each vial resulting in an over-pressure (≈2.5 atm). Vials were stored in a field cooler (without ice). These vials have been used to collect gas samples (e.g. Kelliher et al., 2002) and are known to

preserve samples in excess of 10 wk for N_2O analysis (Laughlin and Stevens, 2003). Integrity of the sample is ensured if the over-pressurize is still present in the vial when analyzed. A water lubricated glass syringe (BD; Franklin Lakes, NJ) was used to transfer gas samples from vials to the GC injection system and provided a reliable determination of sample integrity. At least a 10 ml gas sample was needed for an accurate analysis. Only 20 out of the 1200 (1.7%) vials collected in the field leaked after sampling (lack of over-pressure) and were excluded from the data. All vial samples were analyzed within 10 wk of collection.

2.5. Soil fumigant laboratory incubations

Effects of soil fumigation with CP and MITC on greenhouse gas production were examined in laboratory incubations. They were completed within 2 wk of soil collection so as to minimize the effects of prolonged storage and refrigeration on microbial activity (Ross et al., 1980; Zelles et al., 1999). Sub-samples (5 g) of field moist soil from each depth interval 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). Samples were treated with CP, MITC, a combination of CP and MITC, or no fumigants (control). The amounts of fumigants added were typical of field application rates for each chemical according to label directions (CP: 68 μg g^{-1}, MITC: 55 μg g^{-1}; assuming bulk density of 1.65 g cm^{-3} and 0.5 m treatment depth). Fumigants were injected through the septa. Vials were kept in the dark at 22 ± 2 °C. Temperatures were recorded every minute using a thermistor data logger (Onset Computer Corporation, Bourne, MA). All treatments were run in triplicate. Headspace O_2 concentrations in the vials were consistently above 175 ml l^{-1} at 10 d, indicating that headspace conditions were aerobic throughout the incubations. Identical incubations were performed on steam sterilized soil, following the procedures outlined in Ma et al. (2001). Steam sterilization alters inorganic extractable ion concentrations (Mn and K) as well as pH (Skipper and Westermann, 1973), thereby changing abiotic mineral interactions.

Greenhouse gas production rates were calculated based on the net difference in vial headspace concentration over 10 d and were reported on a soil dry weight basis. Previous studies (Drury et al., 1991; Spokas and Wang, 2003) showed that greenhouse gas production peaks by 10 d.

Before sampling the headspace of the vials, 10 ml of lab air was injected with a water lubricated glass syringe (BD; Franklin Lakes, NJ) through the septa, and the syringe was flushed three times. This ensured adequate mixing of headspace gases and also avoided potential errors with sampling under vacuum conditions. Ten milliliters was then withdrawn and sealed in the glass syringe, which was equipped with a one-way plastic stopcock (Cole-Palmer, Vernon Hills, IL). The gas sample was introduced into the GC system through a 14-port gas sampling valve (Valco Instruments, Houston, TX) controlled by a programmable computer board (Z-World Engineering, Davis, CA). Sample loops and associated connection tubing were initially evacuated to approximately 10 Pa. The pump was then isolated and the sample was injected into the loops to a minimum of 10 kPa overpressure. Sample loops were then vented to atmospheric pressure and all three loops were simultaneously injected onto three different GC columns connected to three different detectors (FID, ECD, and TCD). The column and instrument configuration is summarized in Table 3. Gas concentrations were corrected for dilution by 10 ml of lab air.

Table 3
Columns supplied by Alltech Associates (Deerfield, IL), carrier gas and oven conditions for the GC system

Detector	Column	Carrier Gas	Detected Components
TCD	CTR-1 (2 m×0.64 cm) Isothermal 50 °C	He 60 ml min^{-1}	CO_2, O_2, N_2
ECD	Porapak Q (2 m×0.32 cm) Isothermal 50 °C	Ar-5%CH_4 33 ml min^{-1} (45 ml min^{-1} make-up: Ar-5%CH_4)	N_2O
FID	Porapak N (2 m×0.32 cm) Isothermal 30 °C	He 32 ml min^{-1}	CH_4

2.6. Statistical analysis

Results presented for the laboratory incubations were arithmetic means of triplicate analyses and were expressed on an oven-dried soil basis. Data were analyzed using an ANOVA for independent samples to test for statistically significant differences using GraphPad InStat version 3.00 (GraphPad Software, San Diego, CA). If significant differences existed among fumigant treatments, as indicated by the F-ratio, the Tukey's Honest Significant Difference (HSD) test was performed to determine which fumigant interactions were significantly different from the control at the $P < 0.05$ level. Pair-wise correlations were also evaluated to determine significance at the $P < 0.05$ level. Daily averages ($n = 3$–10) of the measured fluxes were taken to represent the observed daily flux from that treatment. Soil gas concentration profiles were shown as the average ($n = 15$) of the 0–15 d measurements and the average ($n = 4$) of the readings taken on 48 d after fumigation.

3. Results

3.1. Soil physical properties and microbial activities

There was a significant decrease in TOC content with depth, and a significant increase in volumetric moisture

content at the 30–45 cm depth. This moisture increase was correlated to a plow pan formed by tillage and observed in the soil profile. No significant differences were observed in bulk density, pH, and texture as a function of depth (Table 1). The TOC data suggests uniformity in the top layers (0–45 cm) which was due to tillage before the fumigation experiment.

Significant decreasing trends in SMB ($r=-0.998$) and BRR rates ($r=-0.998$) with depth were observed (Table 2). No significant correlations with depth were observed in ARA ($r=-0.863$, $P=0.137$) or SIR ($r=-0.884$, $P=0.112$), but both properties significantly decreased in the lower depth intervals (Table 2). The data indicate that microbial activity and abundance changed with depth.

3.2. Field plot results

3.2.1. N_2O

An increase in N_2O flux was delayed for approximately 3 d in the DAZ treatment, whereas N_2O flux increased 4-fold for the CPMS treatment on the day of fumigation (Fig. 1A). Significant differences were observed in the N_2O flux between the control and CPMS treatments and the control and DAZ treatments. CPMS and DAZ N_2O fluxes were not statistically different throughout the 15 d. Total emissions integrated over the initial 15 d were 1.28 ± 0.72 mg N_2O-N m^{-2} for the control, 10.58 ± 7.09 mg N_2O-N m^{-2} for the CPMS, and 6.42 ± 5.04 mg N_2O-N m^{-2} for the DAZ treatment. Increases in the standard deviation of N_2O flux with time and treatments (Fig. 1A) suggest an increase in heterogeneity of the production source, which was not investigated further.

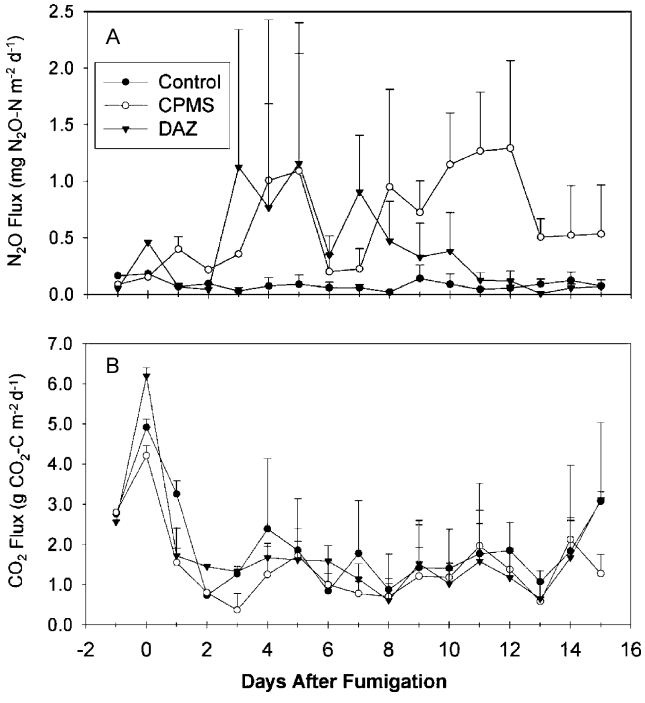

Fig. 1. Emission flux densities of (A) N_2O and (B) CO_2 for the three field treatments control, dazomet (DAZ), and chloropicrin/metam sodium (CPMS) treatments as a function of days after fumigation. Data presented are averages of fluxes taken each day ($n=3$–10) with bars representing the standard deviation.

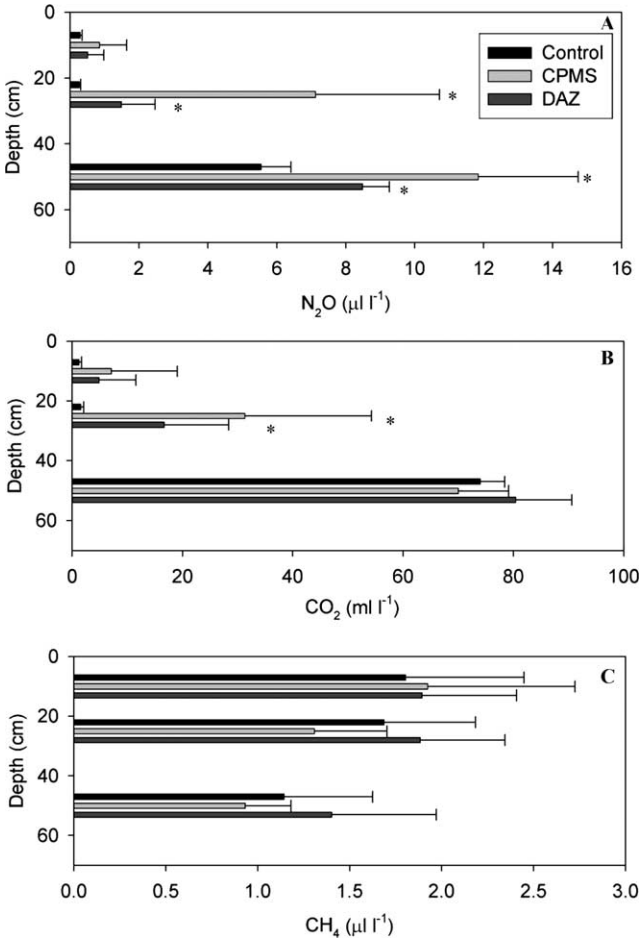

Fig. 2. Soil gas concentrations of (A) N_2O, (B) CO_2, and (C) CH_4, for the three treatments control, dazomet (DAZ), and chloropicrin/metam sodium (CPMS) treatments measured at 10, 25, and 50 cm depths. Data presented are averages of all measurements ($n=15$) with the bars representing the standard deviation. Symbol (*) indicates significantly higher concentration than the control ($P<0.05$).

Average N_2O soil gas concentrations are shown for 0–15 d (Fig. 2A) and 48 d after fumigation (Fig. 3D). There was reduced stimulation effect in the DAZ compared to the CPMS treatment. This is seen initially in lower subsurface N_2O concentrations in the DAZ plots compared to the CPMS (Fig. 2A), and then in the reduced effects on surface emission of N_2O at 48 d (Fig. 3A).

CPMS increased the NH_4^+ soil concentration 65-fold and DAZ doubled it (Table 4). Soil NO_3^- concentrations doubled in both treatments, but no significant differences were observed between the two fumigants.

3.2.2. CO_2

There were no significant difference in CO_2 flux between treatments ($P=0.802$) from 0 to 15 d after fumigation

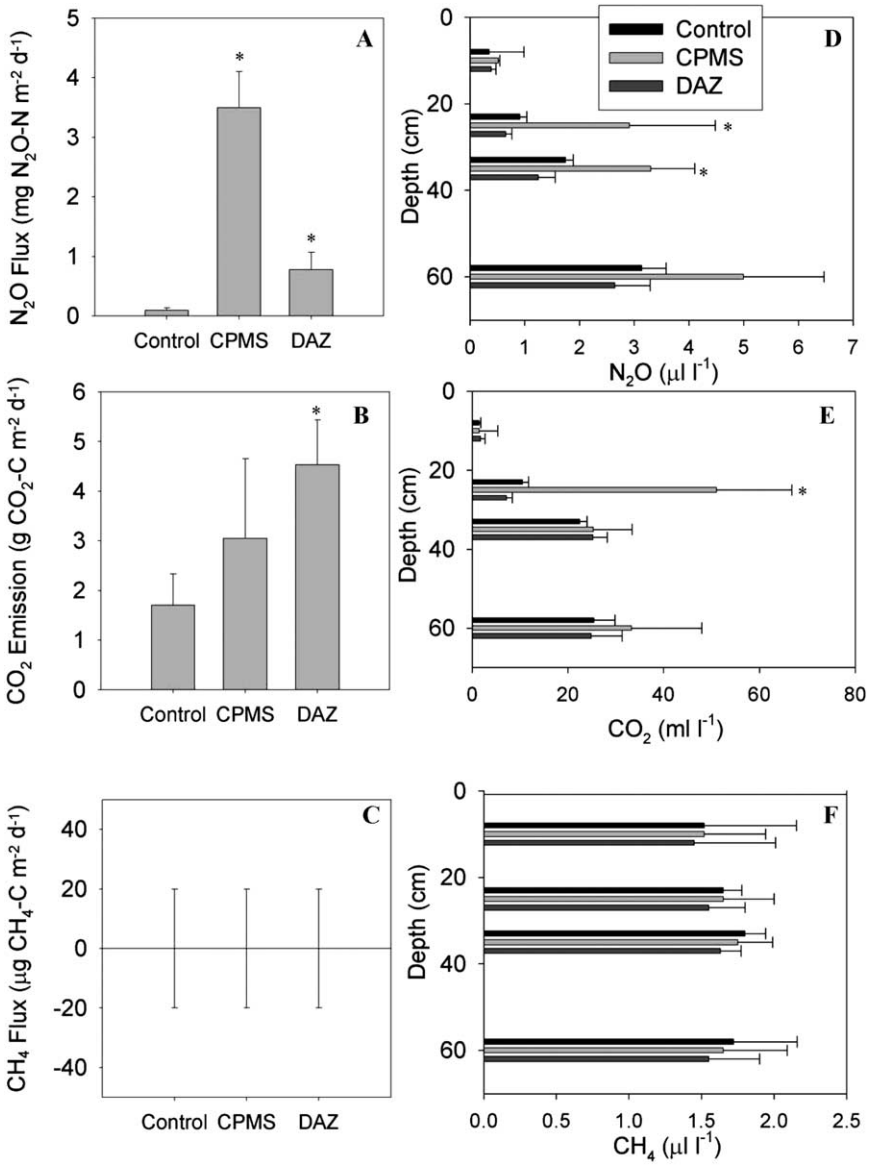

Fig. 3. Measured emission flux of (A) N_2O, (B) CO_2, and (C) CH_4 and soil gas concentrations of (D) N_2O, (E) CO_2, and (F) CH_4 measured at 48 d after fumigation. Data presented are averages of all measurements ($n=4$) as a function of depth are shown along with standard deviation. Symbol (*) indicates significantly higher concentration than the control ($P<0.05$).

(Fig. 1B). However, there were significant differences during the same period in the 25 cm CO_2 concentrations ($P<0.05$, Fig. 2B) with no significant differences manifested between the two fumigants. There were significant increases in the CO_2 flux from the DAZ plots 48 d after fumigation (Fig. 3B). At 48 d, the stimulation effect in subsurface CO_2 had disappeared in the DAZ treatment, but the 25 cm CO_2 concentration in the CPMS treatment was still elevated (Fig. 3E).

3.2.3. CH_4

Only one set of measurements (6 d after fumigation) showed a measurable CH_4 flux ($+30$ µg CH_4-C m^{-2} d^{-1}) for all treatments. All remaining fluxes were below detection limits of ± 20 µg CH_4-C m^{-2} d^{-1}. No significant differences were observed in CH_4 flux between fumigant and control plots ($P>0.05$) 0–15 d (data not shown) or 48 d following fumigation (Fig. 3C). Sub-surface CH_4 profiles displayed no significant differences between treatments (Figs. 2C and 3F).

Table 4
Inorganic-N analyses at 48 d after fumigation (mg N kg_{soil}^{-1})

Treatment	Ammonium	Nitrate
Control	0.44 ± 0.08 a	1.79 ± 0.07 a
DAZ	1.06 ± 0.06 b	4.10 ± 0.06 b
CPMS	28.52 ± 0.17 c	3.80 ± 0.05 b

DAZ, dazomet treatment and CPMS, chloropicrin/metam sodium treatment. Data are shown as the arithmetic means with the standard deviation ($n=3$); different letters indicate significant differences between treatment and control ($P<0.05$).

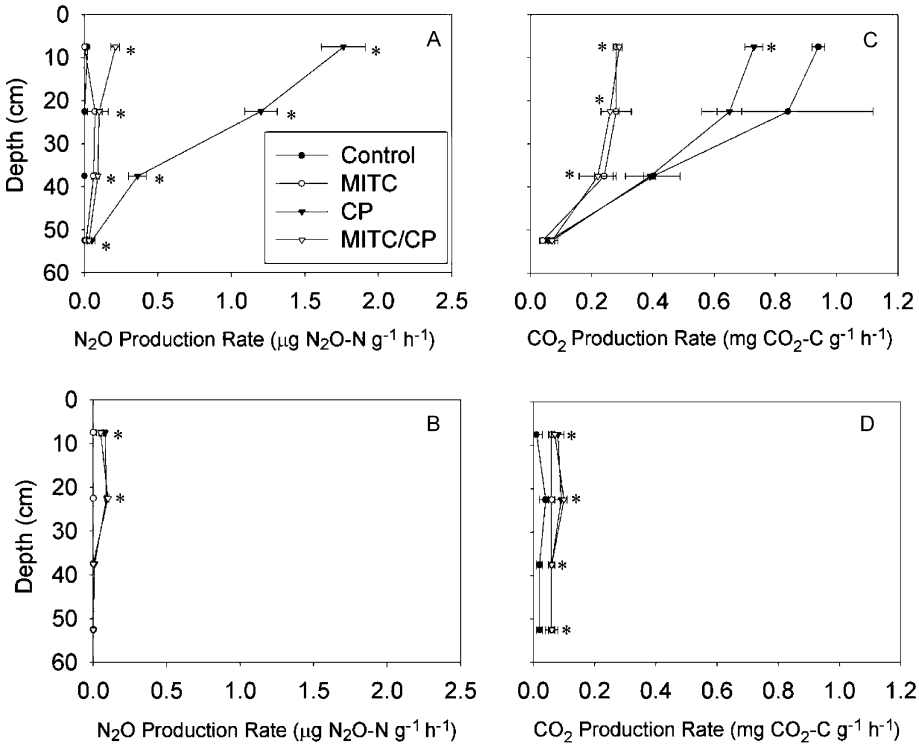

Fig. 4. Graphical representation of the measured (A) non-sterile and (B) sterile soil N_2O production and the (C) non-sterile and (D) sterile soil CO_2 production following 10 d incubations with the fumigant treatments control, methyl iosthiocyanate (MITC), chloropicrin (CP), and a combination of chloropicrin/methyl isothiocyanate (CP/MITC). Symbol (*) indicates significant difference ($P<0.05$) exists between the gas production rate of the fumigant treatment and the soil control.

3.2.4. O_2

O_2 illustrated a diffusional concentration profile and was consumed at depth. O_2 concentrations at 10, 25 and 60 cm were 200 ± 5, 180 ± 10, and 150 ± 10 ml l^{-1}. There were no significant differences observed between treatment and control plots.

3.3. Laboratory incubations

3.3.1. N_2O

In the lab incubations rates of N_2O production was high in surface soils (0–15 cm) and decreased with depth ($r=-0.987$). N_2O production rates in the controls were only correlated to ARA ($r=0.988$, $P<0.05$). The CP stimulated N_2O production rates were consistently significantly different from the controls at all depths (Fig. 4A). Stimulation by CP was correlated with SMB ($r=0.990$, $P<0.05$) and BRR rates ($r=0.999$, $P<0.05$), but not with SIR or ARA. There were significant differences between the controls and the MITC aerobic incubations at 10–45 cm (Fig. 4A). The stimulation from MITC is significantly lower than CP. There were significant differences between the non-sterile (Fig. 4A) and sterile (Fig. 4B) soils.

3.3.2. CO_2

In the laboratory incubations there was no effect on CO_2 production as a result of CP fumigation in the 10 d incubation, except for a reduction in the CO_2 production rate of the 0–15 cm depth (Fig. 4A). However, the CO_2 production rate was suppressed by MITC and MITC/CP fumigation ($P<0.05$) in surface soils (0–45 cm). The fumigation 'flush' of CO_2 was not seen in these trials as would be expected from the mineralization of killed biomass as in microbial biomass determinations (Jenkinson and Powlson, 1976). This was because the fumigant remained in contact with the soil during the 10 d incubation, unlike the procedure for microbial biomass which involves only a 24 h fumigation.

3.3.3. CH_4

There were no significant differences in any of the laboratory incubations for headspace CH_4 concentrations. 30 μg CH_4-C g^{-1} d^{-1} was the maximum production or minimum consumption rate that could be detected. There was no observed effect of the tested fumigants on CH_4 production or consumption in the nursery soil tested, which agrees with the field data.

4. Discussion

4.1. N_2O

Emitted N_2O is considered to be produced as a consequence of nitrification and denitrification reactions in the soil system (Firestone and Davidson, 1989). Our results

strongly suggest an aerobic source that could be important even in unfumigated forest soils. The presence of 30 ml l^{-1} O_2 has been shown to be an effective inhibitor of anaerobic denitrification in laboratory incubations (Kroeckel and Stolp, 1985; Drury et al., 1991). It has been shown that O_2 inhibits denitrifying enzyme activity and represses the synthesis of new denitrifying enzymes (Payne, 1973). Therefore, in our aerobic incubations with sieved soil (2 mm), it is very unlikely that the N_2O was the product of anaerobic denitrification. Because of the low aerobic respiratory activity (Table 2), it was unlikely that anaerobic microsites would have been formed.

Anaerobic conditions exist in aggregates at depth in the field soil profile even where aerobic soil gas conditions are observed (Van der Lee et al., 1999). Thus, increases in the N_2O production at depth could have resulted from anaerobic denitrification (Fig. 2A). For the DAZ plot, the highest concentration was at 50 cm and decreased with depth towards the surface, typical of a diffusion profile. The control plot illustrated the same trend, but at a lower magnitude. Both fumigants stimulated N_2O production at the 50 cm depth (Fig. 2A). Stimulation at depth (50 cm) of N_2O production was also recorded in N-fertilizer application studies (e.g. Müller et al., 2004) and could have been due to denitrification, but the exact mechanism was not confirmed. Coniferous forest soils generally possess low soil pH values (Ross et al., 1999) as at this nursery site (Table 1). Simek and Hopkins (1999) have shown that N_2O is the favored denitrification product over N_2 at pH <6 due to the pH sensitivity of N_2O-reductase (Knowles, 1981). This lower activity would mean more N_2O produced with less reduced to N_2 by N_2O-reductase.

In the CPMS plot, we measured the highest concentration at depth (50 cm), with a significant increase in the N_2O concentration at 25 cm and which was still present at 48 d following CP fumigation (Fig. 3A). This large increase would suggest a source of production at 25 cm, which is near the injection depth of the CP fumigant. This agrees with the laboratory incubations where aerobic production of N_2O was higher with CP than MITC, which could indicate different formation mechanisms for each fumigant.

In addition, 56% of the variability in the CP-induced N_2O flux was explained by surface soil moisture ($P=0.001$). No other significant relationship was recorded between any other flux with surface moisture or temperature. The relationship between CP-induced N_2O flux and soil moisture could be a result of the irrigation applied during the first wk to seal the surface soil for the fumigation treatment. Rainfall may influence microbial reaction rates (Harris, 1981) and N_2O emission (Weier, 1999). This is attributed to the limited diffusion of N_2O from the soil surface due to higher water filled pore space following precipitation events as well as the potential creation of anaerobic sites for denitrification (Glinksi and Stepniewski, 1985).

In all cases the co-application of MITC and CP reduced aerobic N_2O production by 40–92% compared to

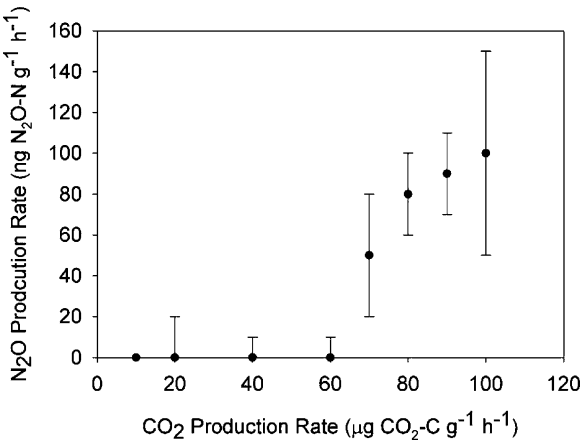

Fig. 5. Correlation of CO_2 and N_2O production rates in the sterilized soil treatments.

CP-induced N_2O. This reduction was less pronounced at deeper depths. This would suggest that the biota responsible for the aerobic stimulation effect was more susceptible to MITC than CP and more abundant in surface soil. This is expected since microbial biomass and populations typically decrease with depth (Fierer et al., 2003; Paul and Clark, 1996).

Minimal N_2O stimulation was observed in sterilized soil treatments (<100 ng N_2O-N g^{-1} h^{-1}, Fig. 4B). However, there was evidence of incomplete sterilization in the incubations, assuming CO_2 production represents biological activity. N_2O production was stimulated above a CO_2 respiration rate of 60 µg CO_2-C g^{-1} h^{-1} in the sterile tests (Fig. 5). If the highest CO_2 respiration rate is dropped from the dataset as an outlier, abiotic participation for all the soil incubations for N_2O production comprises less than 5% of the total. If the four highest CO_2 production rates are disregarded then there was no abiotic N_2O stimulation. The aseptic tests indicate there was a significant difference between the sterile (Fig. 4B) and non-sterile (Fig. 4A) stimulation effects ($P<0.05$). The stimulation effect was reduced in the sterile treatments, thus verifying the active role by soil microbes as initially suggested for N_2O stimulation seen with CP fumigation in northern US nurseries (Spokas and Wang, 2003). Aerobic sources for N_2O production have been reported by Smith and Thiedje (1979), Robertson and Tiedje (1987). The aerobic activity is probably of bacterial rather than fungal origin as eluded to in Robertson and Tiedje (1987), since numbers have been observed to be reduced by CP fumigation (Wilhelm et al., 1961; Locascio et al., 1997).

The delay in stimulation of N_2O emissions in the DAZ treatment is a consequence of the difference in conversion times to MITC between metam sodium (CPMS) and dazomet (DAZ) (Dungan and Yates, 2003). Soil gas profiles from the DAZ plots were no different to the control plots at 48 d (Fig. 3D). The CPMS treatment effects persisted until 48 d at the 25 and 35 cm depths, confirming long-term effects. The possibility exists that there were different

intermediate breakdown products formed that undergo slower mineralization and transformation, but these were not measured in this study.

Our observation that N_2O emission was still elevated 48 d after fumigation is striking, since most of the fertilizer induced agricultural N_2O emissions return to background rates 2 wk after application (e.g. Müller et al., 2004). This finding was further supported by the alteration of inorganic-N concentrations at 48 d, which have been reported for previous fumigation studies (e.g. Winfree and Cox, 1958). White (1986) determined that NH_4^+ released during organic matter decomposition persists longer in forest soils than agricultural soil types. This indicates that long-term changes in soil microbial community functionality need to be assessed before long term effects of the N_2O gas balance can be fully elucidated.

4.2. CO_2

No relationship was observed between the CO_2 flux and soil moisture or surface temperature ($P > 0.05$). The lack of significant differences in initial CO_2 fluxes could be due to the surface water seal that was induced through irrigation to aid the reduction of fumigant emissions. The additional moisture would have greatly influenced the surface microbial activity (Harris, 1981). The 0–15 d CO_2 fluxes might indicate the surface flux of CO_2 was largely dominated by the respiration activity of the surface heterotrophic microbial community and fumigant effects were not recorded by these flux measurements (Fig. 1B).

At 48 d after fumigation, the DAZ treatment flux was three times the control and the CPMS flux was twice the control (Fig. 3B). This would indicate that the DAZ treatment efficacy was higher as indicated by the higher CO_2 flux resulting from the mineralization of liberated biomass (Jenkinson and Powlson, 1976).

4.3. CH_4

Methane oxidation by methanotrophic bacteria (e.g. *Methylocystis* spp) in surface soils is the only known biological sink for CH_4. Methanotrophs can be inhibited by a variety of chemicals. Soil NH_4^+ and NO_3^- concentrations have been cited to increase following fumigation (Winfree and Cox, 1958; Hansen et al., 1990). This is important since NH_4^+ (King and Schnell, 1994) and NO_3^- (Priemé and Christensen, 1997) inhibit CH_4 oxidation. It was anticipated that there would be a decrease in CH_4 oxidation capacity following soil fumigation, with the elevated NH_4^+ and NO_3^- concentrations observed at 48 d. However, no differences were observed in any of the sub-surface CH_4 concentration or flux data as a function of treatment type. This leads to the conclusion that soil fumigation did not effect the net production or consumption of CH_4 at this field site.

4.4. Conclusions

Our purpose was to examine the effects of soil fumigation with CP and MITC as potential MeBr alternatives on greenhouse gas exchange at a forest tree nursery. Following CPMS fumigation, there was a significant effect on N_2O emissions which agrees with a study by Spokas and Wang (2003). In addition, we discover that MITC can cause a metabolic effect leading to increased N_2O production. N_2O flux rates were observed to increase 10-fold for both fumigants and persist for 48 d with CP fumigation. Less than 5% of N_2O production resulted from abiotic mechanisms by comparison with autoclaved soil. Results of this study indicate that the effects are fumigant and biota specific. These effects appear to be long lasting and might lead to substantial changes in greenhouse gas exchange over the entire year.

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