Microbial Populations and Enzyme Activities in Soils Fumigated with Methyl Bromide Alternatives

Mary E. Stromberger, Susanne Klose, Husein Ajwa,* Tom Trout, and Steve Fennimore

ABSTRACT

Methyl bromide (MeBr; CH_3Br) use for soil fumigation will be banned in 2005 due to its ozone depleting properties. Potential alternative chemicals to replace MeBr include chloropicrin (CP; CCl_3NO_2), 1,3-dichloropropene (1,3-D; $C_3H_4Cl_2$), iodomethane (IM; CH_3I), and propargyl bromide (PrBr; C_3H_3Br). The goal of this research was to assess changes in soil fungal populations, microbial biomass C (MB_C) and respiration, nitrification potential, and enzyme activities after fumigation with MeBr and alternative fumigants. Four formulations of alternative fumigants (CP, InLine [61% 1,3-D plus 33% CP], Midas [50% IM plus 50% CP], and PrBr) were applied at commercial rates through drip irrigation systems to two field plots located in main strawberry production areas in California, USA. Soil samples (0–15 cm) were taken at 1, 4, and 30 or 37 wk after fumigant application. Fumigation with MeBr plus CP and the alternative chemicals eliminated soil-borne fungal pathogens in soil and reduced culturable fungal populations up to 4 wk post fumigation. Soil microbial respiration decreased with fumigant application and was the least (>40% reduction relative to the control) in the PrBr treatment 1 wk after fumigation, while soil MB_C was not affected by fumigation. The activities of acid phosphatase and arylsulfatase were generally lower in fumigated soils over the 30- or 37-wk study, and those of β-glucosidase and dehydrogenase were lower up to 4 wk past fumigation. Potential nitrification rates were substantially reduced (>55% reduction relative to the control) by the fumigants, but rates recovered toward the end of this study. Results of this study suggested that fungal populations and the activities of acid phosphatase and arylsulfatase were more sensitive to fumigation with the tested MeBr and the alternative fumigants than total microbial biomass, microbial respiration, nitrification, and the activities of dehydrogenases and β-glucosidase. Short-term impacts of MeBr and its alternative fumigants on microbial activities and enzymatic processes suggest that all the tested fumigants have the potential to alter important microbial and enzymatic functions such as nutrient cycling.

Methyl bromide is a highly effective preplant soil fumigant that has been used for at least 60 yr to control insects, nematodes, weeds, and pathogens in more than 100 crops, including strawberries (*Fragaria* × *ananassa* Duch.). Stringent regulations on MeBr consumption by the Montreal Protocol (Montreal Protocol, 2000) have stimulated research to find alternative fumigants (Ajwa et al., 2003). Potential alternatives to MeBr are CP, 1,3-D, IM, and PrBr (Ajwa et al., 2003). Previous research found that CP has high biocidal activity against fungal pathogens, but is not as effective against nematodes as MeBr (Wilhelm and Pavlou, 1980). Therefore, a mixture of MeBr and CP is usually applied to control soil-borne fungal pathogens such as Verticillium wilt (*Verticillium dahliae* Kleb.) and weeds (Wilhelm et al., 1961). Recent research found that an emulsifiable concentrate (EC) formulation of CP applied through drip irrigation systems at rates > 200 kg ha^{-1} provided consistent cost-effective pest and weed control in strawberry production (Ajwa et al. 2002a, 2002b; Duniway, 2002; Fennimore et al., 2003; Haar et al., 2003; Ajwa and Trout, 2004). The fumigant 1,3-D is highly effective to control nematodes, but has relatively low activity against fungal pathogens and weeds (Noling and Becker, 1994). Thus, the biocidal activity of 1,3-D and CP can be enhanced when combined in a formulation of 61% 1,3-D and 33% CP as found in InLine. Iodomethane provides equivalent or superior control of soil pests (Becker et al., 1998) and it does not contribute to the depletion of the ozone layer because it is photolyzed before it reaches the stratosphere (Solomon et al., 1994; Ohr et al., 1996). Propargyl bromide also demonstrated good potential as soil fumigant and has been evaluated for crop production (Yates and Gan, 1998; Ajwa et al., 2001, 2003).

Fumigants, in general, cause marked alterations in soil microbial populations and enzyme activities and can contribute to physical, chemical, and biochemical changes in soil (Sinha et al., 1979; Domsch et al., 1983; Anderson, 1992; Zelles et al., 1997). Among the key enzymatic reactions in soils are those catalyzed by dehydrogenases (EC 1.1) reflecting the total oxidative activities of soil microorganisms essential in cell metabolism and in organic matter degradation in soil. Dehydrogenases are entirely intracellular enzymes. Other important soil enzymes, such as phosphatases, glucosidases, and sulfatases belong to the group of hydrolases, and may exist intracellular and extracellular as free or accumulated (stabilized) enzymes. Acid phosphatase (EC 3.1.3.2) catalyzes the hydrolysis of a variety of organic phosphomonoesters and is therefore important in soil organic P mineralization and plant nutrition. The enzyme β-glucosidase (EC 3.2.1.21) catalyzes the hydrolysis of cellobiose, and thus plays a major role in the initial phases of the decomposition of organic C compounds. Arylsulfatase (EC 3.1.6.1) is believed to be partly responsible for S cycling in soils as it participates in the process whereby organic sulfate esters are mineralized and made available for plants.

Soil fumigation with chloroform decreased dehydrogenase activity, potential nitrification, microbial biomass, phospholipids fatty acids, but had little or no effect

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Abbreviations: 1,3-D, 1,3-dichloropropene; CP, chloropicrin; EC, emulsifiable concentrate; IM, iodomethane; MB_C, microbial biomass C; MeBr, Methyl bromide; PN, *p*-nitrophenol; PrBr, propargyl bromide; TPF, Triphenyl formazan; TTC, triphenyltetrazolium chloride.

on the activities of xylanase, β-glucosidase, and alkaline phosphatase (Zelles et al., 1997). Chloroform fumigation increased arylsulfatase and urease activities (Klose and Tabatabai, 1999a, 1999b), and decreased other amidohydrolases, glycosidase, and phosphatases activities (Klose and Tabatabai, 2002a, 2002b, 2002c). Soil fumigation with MeBr stimulated ammonifying bacteria and N mineralization, but significantly reduced soil fungi for 36 wk, inhibited dehydrogenase activity for 2 yr, and reduced CO_2 evolution and nitrification for 45 wk (Malkomes, 1995). Among the fumigants studied, MeBr had the greatest impact while 1,3-D had the least impact on soil microbial communities. Soil fumigation with metam sodium resulted in persistent changes (at least 18 wk) in heterotrophic activity and fatty acid composition of the microbial communities, indicating that fumigants potentially alter nutrient cycling and pollutant degradation in soils (Macalady et al., 1998). Kandeler et al. (1996) suggested that the composition of the microbial community strongly affects the potential of a soil for enzyme-mediated substrate catalysis. Consequently, changes in microbial diversity in fumigated soils may also reduce microbial functionality.

The research presented here is part of a study to evaluate the effectiveness of drip-applied MeBr alternative fumigants to control pathogens and weeds while maintaining high strawberry yields in California, USA (Ajwa et al., 2002a). The main objectives of this study were to assess the impacts of drip fumigation with CP, InLine, Midas, and PrBr relative to a control and a standard MeBr + CP on the soil microbial biomass and respiration, nitrification potential, and several enzyme activities under a strawberry production system. Acid phosphatase, arylsulfatase, and β-glucosidase were selected because they play a key role in the turnover of P, S, and C compounds, and dehydrogenase activity because it reflects the total oxidative activities of soil microorganisms. The alternative fumigants included in this study represent the actual formulations that likely will be used by growers for strawberry production.

MATERIALS AND METHODS

Fumigant Formulations

The fumigants used in this study were commercial grade formulations. An EC formulation of CP (trichloronitromethane) (CP-EC, 96%) was provided by Niklor Chemical Co., Long Beach, CA. InLine, an EC formulation of 1,3-dichloropropene plus chloropicrin (61% 1,3-D and 33% CP), was provided by Dow AgroSciences, Redeck, NC. Midas, an EC formulation of a mixture of iodomethane and chloropicrin (50% IM and 50% CP) was provided by Arvesta Corporation, San Francisco, CA. Propargyl bromide (80%) was provided by Albemarle Chemical Company, Baton Rouge, LA. Methyl bromide (a mixture of 67% MeBr and 33% CP) was provided by Tri-Cal Inc., Hollister, CA.

Site Description and Treatment Application

The research sites selected were located in representative strawberry production areas of California, USA. The field studies were conducted in the central coastal region at Watsonville (121°50′ W long., 36°54′ N lat.) and in the southern coastal region at Oxnard (119°123′ W long., 34°146′ N lat.) in 2000 and 2001. Soils at both locations had not been fumigated for the past 2 and 3 yr before this experiment for Watsonville and Oxnard site, respectively. The soil in Watsonville was classified as an Elder sandy loam (coarse-loamy, mixed, thermic, Cumulic Haploxeroll) with 62% sand, 26% silt, and 12% clay, pH values of 7.8 (soil/water, 1:2.5) and 7.1 (soil:0.01 *M* $CaCl_2$, 1:2.5), and 6 g kg^{-1} organic C. The soil in Oxnard was classified as a Hueneme sandy loam (coarse-loamy, mixed, superactive, calcareous, thermic, Oxyaquic Xerofluvents) with 60% sand, 28% silt, and 12% clay, pH values of 7.8 (in H_2O) and 7.4 (in 0.01 *M* $CaCl_2$), and 7 g kg^{-1} organic C. The past 50-yr average annual precipitation was 582 and 385 mm at Watsonville and Oxnard, respectively. The average annual temperature at Watsonville ranged from 19.5°C (max) to 10.7°C (min). Corresponding values for Oxnard were 21.2 and 10.7°C. Throughout the study (August 2000 to May 2001), the plots at Watsonville received, on average, a higher precipitation and had a lower temperature than the Oxnard site (Fig. 1).

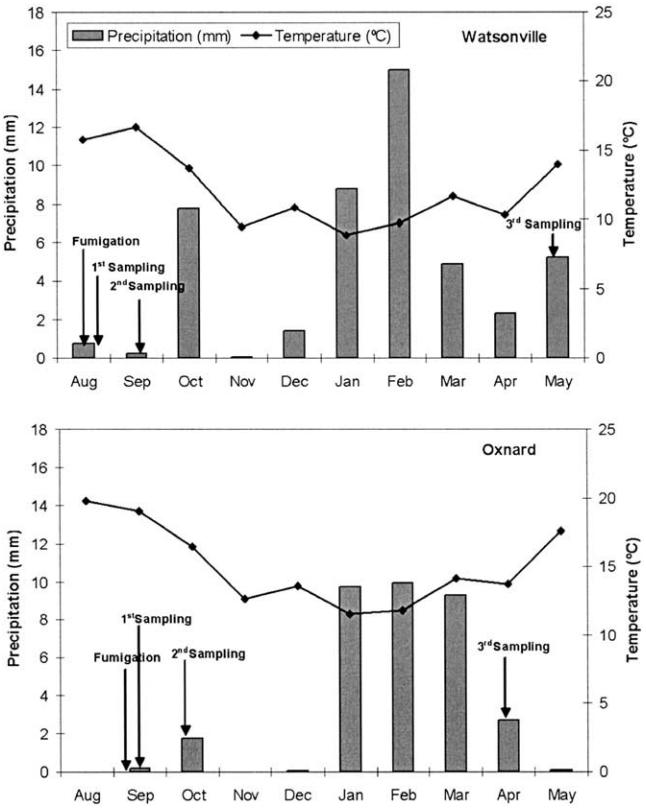

Fig. 1. Average monthly air temperature and precipitation at Watsonville and Oxnard soils over the experimental period from August 2000 to May 2001. Arrows indicate dates for fumigation and sampling.

Commercial cultural practices for the area were followed as described by Ajwa and Trout (2004). California strawberry growers completely recultivate the soil between successive strawberry crops. This involves removal of old plants, plastic mulch, and drip tape, then about 10 tillage operations (over 20 equipment passes) including deep ripping, and two irrigations are performed before the new beds are ready to plant. Soil beds (33 m long) were formed in Watsonville at 132 cm center-to-center (81 cm wide × 30 cm high) and in Oxnard at 173 cm center-to-center (122 cm wide × 30 cm high). Slow release fertilizer (12N-13P-18K) was applied to soil at the rate of 500 kg ha^{-1}. Drip irrigation systems used consisted of two drip tapes (Netafilm Streamline 60; Netafilm, Fresno, CA),

Table 1. Fumigant rate and application method used at the two study sites in Watsonville and Oxnard, California, USA.

Treatment†	Rate	Application method‡
	kg a.i. ha^{-1}	
Control	0	drip
MeBr+CP (67%+33%)	420	shank
PrBr (80%)	202	drip
InLine (60.8% 1,3-D+33.3% CP)	448	drip
Midas (50% IM+50% CP)	448	drip
CP-EC (96%)	336	drip

† Abbreviations: MeBr, Methyl bromide; CP, chloropicrin; EC, emulsifiable concentrate; 1,3-D, 1,3-dichloropropene; Midas, iodomethane (IM); PrBr, propargyl bromide.

‡ Shank injection was at a soil depth of 20 cm and drip fumigation was applied in 50 L m^{-2} irrigation water.

with emitters spaced 30 cm apart and an emitter flow rate of 1.5 L h^{-1} at 70 kPa. Each drip tape was placed 10 cm (in Watsonville) and 30 cm (in Oxnard) from the bed center at a soil depth ranging from 2 to 5 cm. Fumigation treatments were randomized in a complete block design with four field replicates per treatment at each site. Fumigant rates and application methods were selected according to the label recommendation of each chemical (Table 1). The soils in Watsonville and Oxnard were fumigated on 10 Aug. and 1 Sept. 2000, respectively. At the time of fumigation, the average daily soil temperature within the raised bed ranged between 16 and 20°C, and the average volumetric soil water content was less than 18% (33 kPa soil matric potential). About 4 wk after fumigation, bareroot strawberry [*Fragaria* X *ananassa* Duchesne, variety "Diamante" (Watsonville) and "Camarosa" (Oxnard)] was transplanted.

At the Oxnard site, strawberries are grown over 9 mo after which fields are cultivated for the next strawberry growing season and only gypsum and inorganic N, P, and K fertilizers are applied annually to the soil. At the Watsonville site, soil cultivation is less intensive than that at Oxnard. Manure (4.4 Mg ha^{-1} chicken manure), gypsum (4.4 Mg ha^{-1}), and inorganic fertilizers (N, P, K) are usually applied bi-annually. In addition, cover crops are planted for 3 to 4 mo during the 10 mo break after each 12 to 13 mo long strawberry growing season.

Soil Sampling and Analysis

Topsoil (0–15 cm) was collected in 2000 1 wk after fumigation (i.e., August 17 and September 8 at the Watsonville and Oxnard site, respectively), 4 wk after fumigation (at strawberry planting) and at the peak of fruit production at 30 and 37 wk (April and May, 2001) at the Watsonville and Oxnard site, respectively (Fig. 1). Three composite samples, two adjacent to the drip tapes and one from the center of the bed, were collected from five locations in the bed of each treatment, and pooled to one sample. Soils were passed through a 2-mm mesh sieve and stored at 4°C for microbiological analyses, which were completed within 3 wk of sampling. A subsample of the sieved soil samples was air-dried for physical and chemical analysis. Soil water content was determined by drying 50 g of moist soil to constant mass at 105°C. Laboratory measurements were performed in duplicate. The particle-size distribution was measured on air-dried samples by pipette analysis (Kilmer and Alexander, 1949), and the pH in 0.01 *M* $CaCl_2$ solution and in H_2O after 1 h (soil/solution, 1:2.5). Total C and N contents were determined on <180-μm samples by a flame photometrical method using a CHN-Analyzer (model Leco CHN-600, St. Joseph, MI).

Identification of Culturable Soil Fungal Groups

Culturable soil fungal groups (i.e., groups that are most efficient in nutrient utilization) of control and fumigated soils were determined by the soil dilution plate method as described by Parkinson (1994). Field moist soils (10-g oven-dry equivalents) were dispensed in 90 mL of 0.1 *M* phosphate buffered saline solution (pH 7.2, 0.7% NaCl) followed by shaking at 150 rpm for 10 min. Further serial dilutions were made from the initial 1:10 dilution, and 0.1-mL aliquots of the final dilutions (10^{-5} and 10^{-6}) were plated on Czapek-Dox with yeast extract and streptomycin for fungi isolation. Cultures were incubated at 27°C for 14 d, and dominant culturable fungi colonies were isolated and purified on Czapek-Dox agar. Fungi were identified by macroscopic and microscopic morphological characteristics (Watanabe, 1994; Davet and Rouxel, 2000). The soil was also analyzed for survival of *Verticillium dahliae* Kleb., a common soil-borne fungal pathogen, as described by Nicot and Rouse (1987). *Verticillium dahliae* Kleb. was studied because it is the most difficult pathogen to control in strawberry fields in California.

Soil Microbial Biomass and Respiration

Microbial biomass C was determined by the chloroform-fumigation incubation method (Jenkinson and Powlson, 1976). The soil moisture was adjusted with distilled water to 50% of the water-holding capacity and allowed to equilibrate in a desiccator at room temperature. After fumigation with ethanol-free chloroform for 24 h, the headspace CO_2 concentrations were measured after incubation at 28°C by gas chromatography using a Shimadzu GC-8A gas chromatograph (equipped with a 2-m PoropakQ column, operated at 70°C) (Shimadzu Scientific Instruments Inc., Columbia, MD). Microbial biomass C was calculated using a k_C of 0.41 (Voroney and Paul, 1984). Microbial respiration was determined by headspace analysis of incubated nonfumigated soils, and expressed as a rate (μg CO_2–C g^{-1} soil h^{-1}) after subtraction of CO_2 values obtained from empty serum bottles.

Enzyme Assays

Dehydrogenase (EC 1.1) activity was assayed by incubating 5 g of moist soil with 5 mL of triphenyltetrazolium chloride (TTC) solution [0.8%, dissolved in Tris buffer (0.1 *M*, pH 7.6)] at 30°C for 24 h. Controls contained only 5 mL of Tris buffer (0.1 *M*, pH 7.6). Triphenyl formazan (TPF) produced was extracted with methanol and estimated colorimetrically (Alef, 1995). The activities of acid phosphatase (EC 3.1.3.2), β-glucosidase (EC 3.2.1.21) and arylsulfatase (EC 3.1.6.1) were assayed in moist soils (equivalent to 1 g air-dried soil), which were incubated for 1 h at 37°C at their optimal buffer pH and substrate concentration as described by Tabatabai (1994). The product of all reactions, *p*-nitrophenol (PN), was measured by a colorimetrical procedure (Tabatabai, 1994).

Potential and Basal Nitrification

Nitrification potential was determined by aerobic incubation in the presence of NH_4^+ substrate. Soil (100 g) was added to a 250-mL plastic container, and the moisture content was adjusted to field capacity. Soils were allowed to equilibrate for 3 d at room temperature before the addition of 2 mL of $(NH_4)_2SO_4$ solution (25 mg mL^{-1}). The containers were loosely capped and incubated at 28°C for 10 d. Soils then were extracted in 1 *M* KCl for 1 h, filtered, and analyzed for NO_3^-–N to determine the potential nitrification activity. Concentrations of NO_3^-–N in soil extracts were analyzed by a rapid flow

analyzer (model Alpchem RFA-300, Clackamas, OR). To determine the "basal" nitrification activity, a second set of soils were incubated in the absence of $(NH_4)_2SO_4$ solution and analyzed for background levels of NO_3–N.

Statistical Analysis

All results reported are averages of duplicated assays and analyses, and are expressed on a dry soil basis. Soil water content was determined after drying at 105°C for 48 h. The data, evolving from four replicates per treatment, were analyzed by the analysis of variance procedure of SAS (SAS Institute, 2000) to determine the effect of fumigation on soil MB_C, respiration, and enzyme activities at each sampling date. When significant ($P \leq 0.05$) treatment differences were found, protected LSD values were calculated to separate the treatment means.

RESULTS

Microbial and enzyme activities and major culturable fungal groups were determined in soil samples taken before fumigation. Results of this sampling date were similar to the untreated control at 1 wk past fumigation (data not shown).

Culturable Soil Fungal Groups

Major culturable fungal groups were isolated from the control and the fumigated plots at Watsonville and Oxnard at all three sampling dates. The fungal groups isolated at 1 and 4 wk after fumigation showed similar morphological characteristics, and fungal isolates did not diverge from fumigated and nonfumigated soils at peak strawberry production (37 and 30 wk after fumigation for Watsonville and Oxnard, respectively). Therefore, the results of culturable soil fungi isolated at 4 wk after fumigation were selected to represent the general trend in the short-term response of the microbial community to pesticide addition (Table 2).

A consortium of 6 to 7 culturable fungal groups was isolated from the nonfumigated soils at Watsonville and Oxnard, including *Circinella muscae*, *Verticillium lecanii*, *Mucor hiemalis*, and *Mucor plumbeus*, which were found at both sites (Table 2). At both sites, fumigation with MeBr alternatives markedly reduced culturable fungi populations in soil compared with the untreated control. Soil culturable fungal groups differed between the two sites and among the treatments. Fungal groups like *Rhizoctonia* spp. were isolated from the soils fumigated with InLine, Midas, and CP-EC, while *Trichoderma* spp. were found in the soils fumigated with MeBr + CP and CP-EC. At the Watsonville site, between 0 (e.g., PrBr treatment) and 7 (e.g., MeBr + CP treatment) different fungi groups were isolated from the fumigated plots. At the Oxnard site, culturable soil fungi were reduced to two dominant groups in the plots fumigated with PrBr, InLine, and Midas, and no isolates were found in the CP-EC treatment. Generally, *Rhizopus* spp. isolates were found in soils fumigated with PrBr and Midas (Table 2). Before fumigation, the soils had medium to low populations of *Verticillium dahliae* Kleb (7 and 3 viable microsclerotia g^{-1} soil in Watsonville and Oxnard sites, respectively). After fumigation, viable microsclerotia of *V. dahliae* micro were not detected in any of the fumigated soils throughout the growing season (data not shown).

Table 2. Dominant fungal groups isolated from sandy loam soils in coastal California, USA, fumigated with methyl bromide and alternative fumigants (4 wk past fumigation).

	Site	
Treatment	**Watsonville**	**Oxnard**
Control	***Circinella***	
	Circinella muscae	***Circinella muscae***
		Codinaea parva
		Mortierella ambigua
	Mucor hiemalis	***Mucor hiemalis***
	Mucor plumbeus	***Mucor plumbeus***
		Rhizoctonia spp.
	Verticillium fungicola	
	Verticillium lecanii	***Verticillium lecanii***
MeBr+CP	***Arthrobotrys***	
	Codinaea parva	
	Mortierella ambigua	
	Mucor hachijoensis	
		Paeci lomyces farinosus
	Phomopsis	
	Rhizopus	
	Trichoderma	
		Ulocladium botrytis
PrBr	**n.f.i.†**	***Rhizopus***
		Ulocladium botrytis
InLine	***Mortierella***	
	Pithomyces maydicus	
	Rhizoctonia	
		Trichoderma aueroviride
		Verticillium lecanii
Midas		***Cephaliophora***
		Rhizopus
	Rhizoctonia	
	Strachybotrys elegans	
	Thielaviopsis paradoxa	
CP-EC	***Rhizoctonia***	**n.f.i.†**
	Trichoderma pseudokoningi	

† **n.f.i. = no fungi isolates found with three different platings on multiple dilutions of soil.**

Microbial Biomass and Respiration

The soil MB_C significantly ($P \leq 0.05$) decreased in all fumigated plots relative to nonfumigated control plots 1 wk after fumigation at the Watsonville site only (Fig. 2). Soil MB_C was already higher in May 2001 at 37 wk past fumigation (260–310 mg C kg^{-1} soil) than in the previous fall at 1 wk past fumigation (120–190 mg C kg^{-1} soil) at the Watsonville site, whereas MB_C was relatively similar across treatments (140–200 mg C kg^{-1} soil) during the study at the Oxnard site (Fig. 2).

In contrast to MB_C, microbial respiration was significantly inhibited at both sites up to 4 wk following the application of soil fumigants (Fig. 3). At the Watsonville site at 1 wk past fumigation, respiration was lower in plots receiving InLine and PrBr compared with the other treatments and the control. At Week 4, soil respiration was also lower in plots fumigated with CP and Midas compared with the MeBr and nonfumigated treatments following the order: control > MeBr + CP > CP-EC = Midas > InLine = PrBr. By the end of the study, respiration was similar among all plots, except for those fumigated with CP-EC, where respiration was significantly greater compared with plots receiving InLine and Midas. At the Oxnard site, fumigation of soil

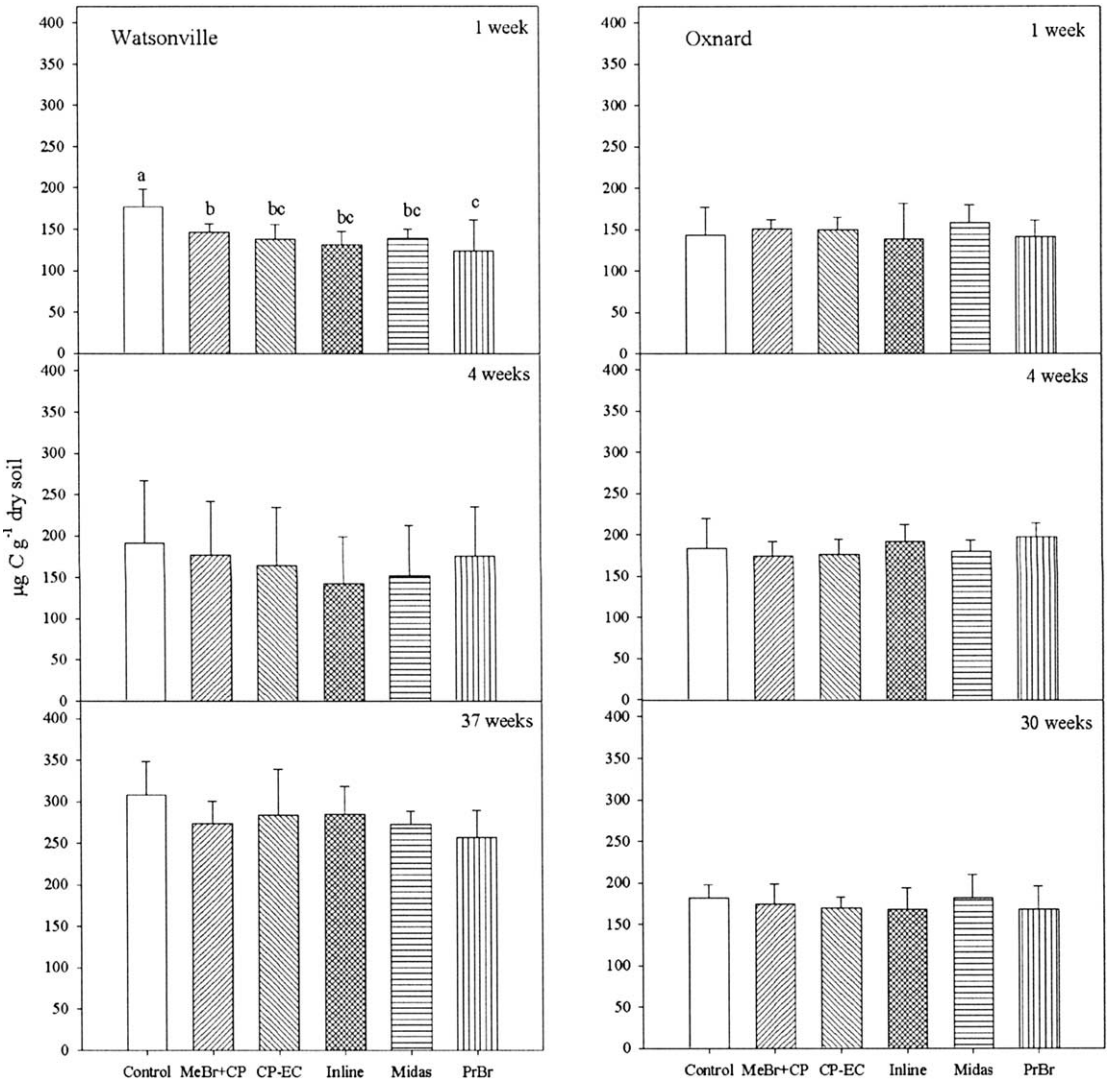

Fig. 2. Microbial biomass C of Watsonville and Oxnard soils 1 wk after fumigation with methyl bromide (MeBr) or an alternative (Week 1), at planting (Week 4), and at peak strawberry growth (37 or 30 wk). For each sampling date, means followed by a different letter are significantly different at $P < 0.05$. Absence of letters indicates no significant differences. Error bars represent the standard deviation, $n = 4$. CP chloropicrin; EC, emulsifiable concentrate; PrBr, propargyl bromide.

with MeBr or its alternatives resulted in lower microbial respiration up to 4 wk past fumigation compared with the nonfumigated soil (Fig. 3). Among the fumigants studied, respiration was lowest in plots treated with PrBr, but there were no significant differences among treatments by the end of the study (i.e., 30 wk past fumigation).

Enzyme Activities

Overall, sensitivity of soil enzyme activities to a particular fumigant differed between the Watsonville and the Oxnard sites. At the Watsonville site, soil acid phosphatase activity was significantly ($P \leq 0.05$) lower in all fumigated plots (with the exception of Midas at Week 1) compared with the control throughout the 37-wk study (Fig. 4). The response of phosphatase activity, however, was similar among all fumigants. At the Oxnard site, soil phosphatase activity was only inhibited in plots receiving CP-EC and PrBr compared with the control and other fumigant treatments. Inhibition of phosphatase activity by CP-EC and PrBr also lasted throughout the 30-wk study (Fig. 4).

Arylsulfatase activity at the Watsonville site was lower in all fumigated plots compared with the control over the 7- to 9-mo study (Fig. 5). At Week 1 past fumigation, arylsulfatase activity was significantly ($P \leq 0.05$) lower in plots receiving InLine compared with MeBr + CP and PrBr. At Week 4 past fumigation, arylsulfatase activities decreased in the following order: control > PrBr > MeBr + CP = CP-EC > InLine = Midas. At 37 wk past fumigation, arylsulfatase activities remained significantly lower in fumigated plots, except in plots receiving PrBr, which already had similar arylsulfatase activity values as the control. At the Oxnard site, fumigated plots also showed significantly ($P \leq 0.05$) lower arylsulfatase activities than control plots, and differences in the sensitivity of this enzyme activity toward the various fumigants were observed (Fig. 5). One week

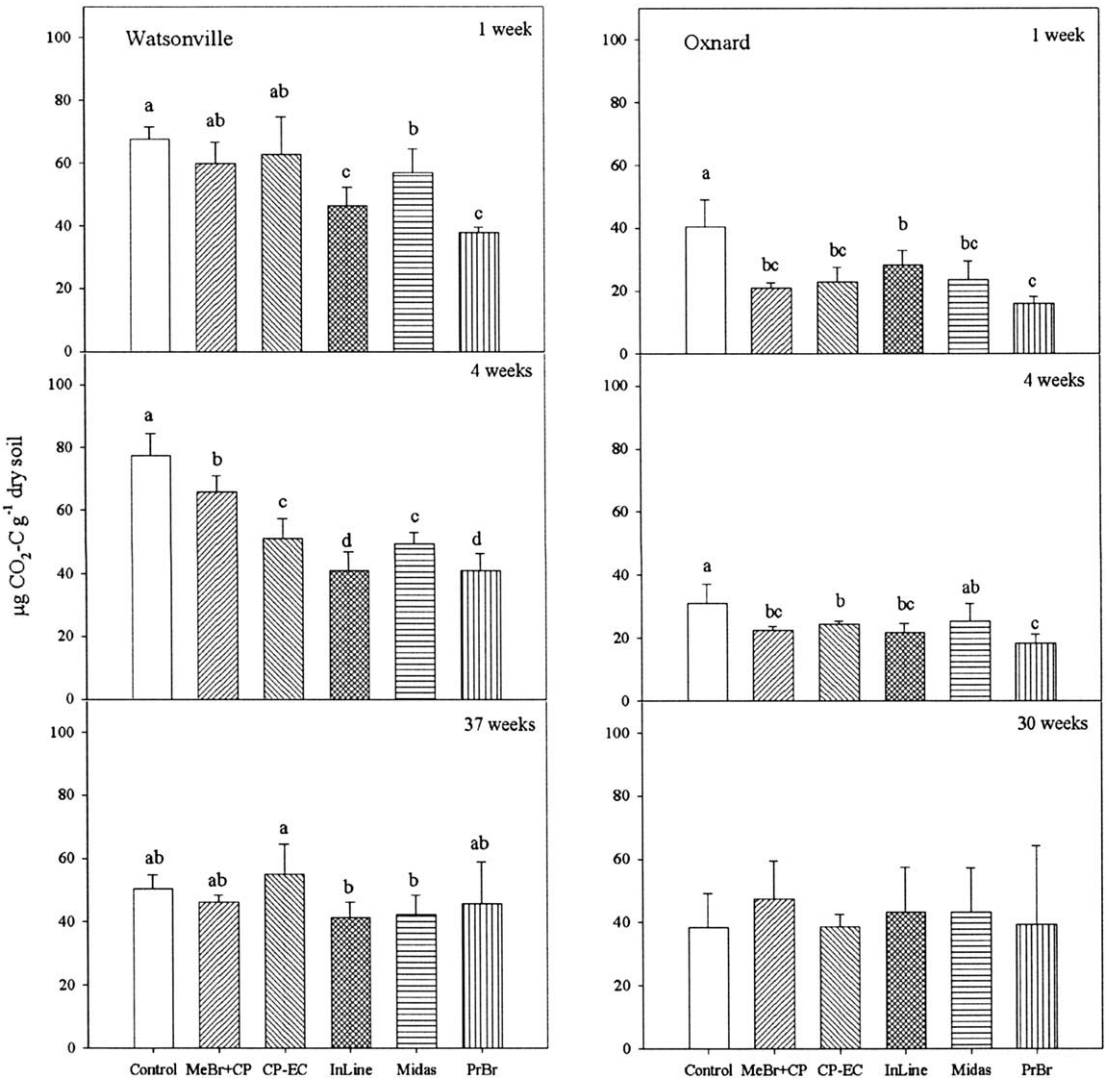

Fig. 3. Microbial respiration in Watsonville and Oxnard soils 1 wk after fumigation with MeBr or an alternative (Week 1), at planting (Week 4), and at peak strawberry growth (37 or 30 wk). For each sampling date, means followed by a different letter are significantly different at $P < 0.05$. Absence of letters indicates no significant differences. Error bars represent the standard deviation, $n = 4$. CP chloropicrin; EC, emulsifiable concentrate; PrBr, propargyl bromide.

after fumigation, arylsulfatase activity in fumigated plots significantly decreased in the order PrBr > MeBr + CP > InLine = Midas ≥ CP-EC, following a similar trend as observed for soils at the Watsonville site. At 4 wk past fumigation, there were no differences among the fumigants, with the exception of the CP-EC treated plots where arylsulfatase activity was significantly lower than in the Midas and PrBr plots. By Week 30 past fumigation, arylsulfatase activities were similar among the fumigant treatments.

In contrast to the activities of acid phosphatase and arylsulfatase, β-glucosidase activity was only affected by soil fumigation at the Watsonville site (Fig. 6). At Week 1 after fumigation, β-glucosidase activity was significantly lower in all fumigated soils with the exception of Midas fumigated soils. At Week 4, the activity of this enzyme was inhibited in all fumigated plots compared with the control soil following the order: control > Midas ≥ MeBr + CP = CP-EC = PrBr > InLine. Although β-glucosidase activity initially was lowest in plots receiving InLine, the activity of this enzyme recovered in all treatments toward the end of the study. In contrast to the activities of acid phosphatase and arylsulfatase, β-glucosidase activity was lower in the Oxnard soils (25–30 mg PN kg^{-1} soil h^{-1}) compared with the Watsonville soils (35–60 mg PN kg^{-1} soil h^{-1}), and significant impacts of the fumigants were not detected at any sampling date (Fig. 6).

At the Watsonville site, dehydrogenase activity was markedly lower in all fumigated plots compared with the control 1 wk after the applications and followed the order: control > Midas ≥ InLine = MeBr + CP > CP-EC = PrBr (Fig. 7). At 4 wk past fumigation, dehydrogenase activity remained lower in all fumigated plots, especially in those receiving CP-EC and InLine. By 37 wk, there were no significant differences in dehydrogenase activity among the control and the fumigated soils. At the Oxnard site, fumigation with MeBr + CP and alternative fumigants significantly inhibited dehydrogenase activities compared with the control plots 1 wk after

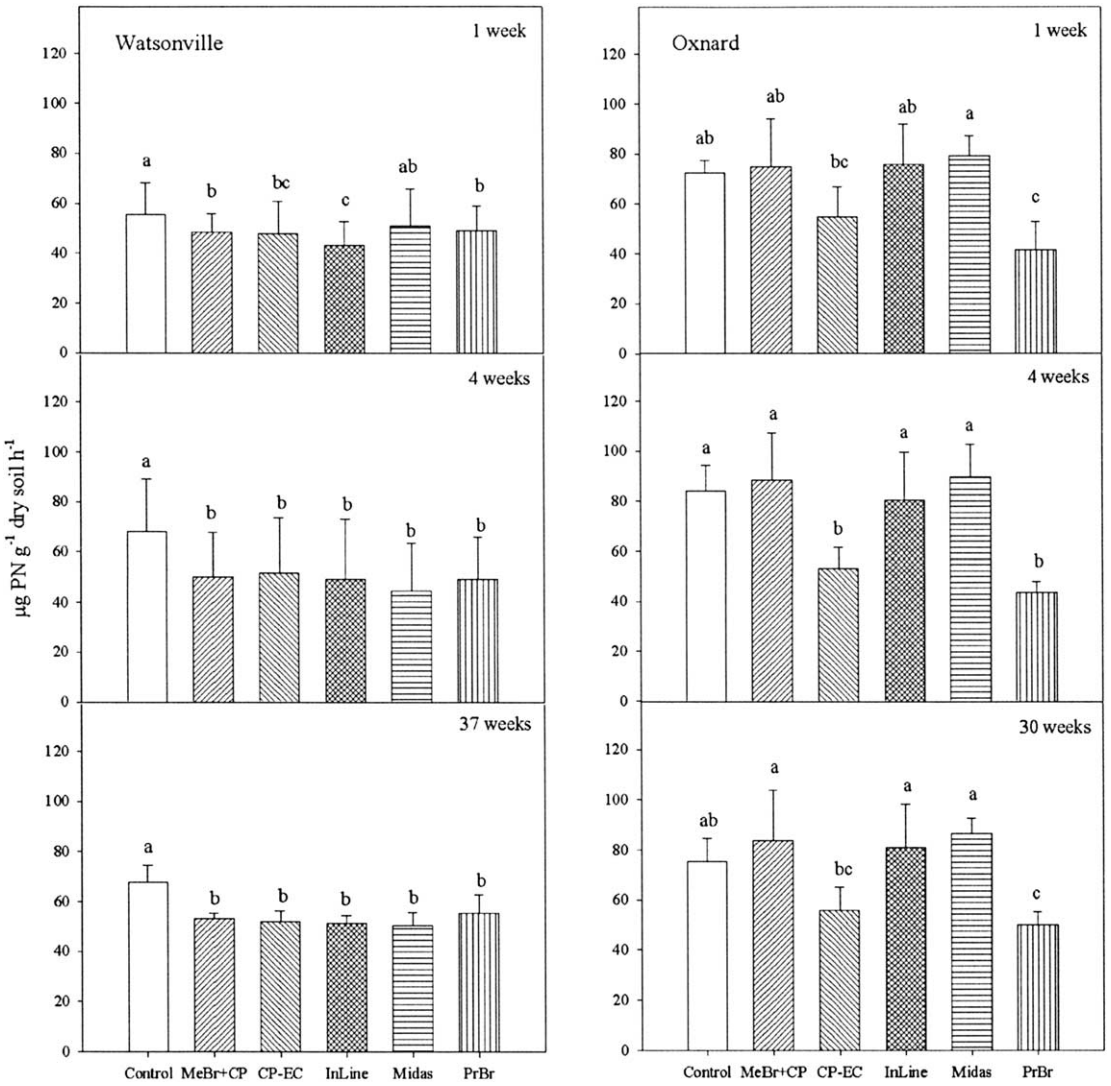

Fig. 4. Soil acid phosphatase activity in Watsonville and Oxnard soils 1 wk after fumigation with MeBr or an alternative (Week 1), at planting (Week 4), and at peak strawberry growth (37 or 30 wk). For each sampling date, means followed by a different letter are significantly different at $P < 0.05$. Error bars represent the standard deviation, $n = 4$. CP chloropicrin; EC, emulsifiable concentrate; PrBr, propargyl bromide.

the application (Fig. 7). Among the fumigants studied, lowest dehydrogenase activities were observed in plots fumigated with InLine and PrBr. By Week 4, soil dehydrogenase activities were similar among the control, MeBr + CP, and the Midas fumigated plots, but activity values remained lowest in soils fumigated with PrBr, InLine and CP-EC. As observed at the Watsonville site, there were no differences in soil dehydrogenase activities between the control and the fumigated treatments at the end of the study.

Potential and Basal Nitrification

Compared with the control soils, potential nitrification was significantly inhibited by fumigation at Weeks 1 and 4 after fumigation at both sites (Fig. 8). The decrease in potential nitrification in soil at Week 1 followed the order: control > MeBr + CP > CP-EC = InLine = Midas = PrBr. At the Watsonville site at Week 1, basal and potential nitrification was greater in MeBr + CP-fumigated plots than in plots fumigated with other fumigants (Fig. 8). Basal nitrification also was greater in the MeBr + CP-treated soil than in control soils (Weeks 1 and 4). At Week 4, basal and potential nitrification increased in the CP-EC-fumigated plots to levels similar to those observed in MeBr + CP-fumigated plots. By the end of the study, no differences in basal or potential nitrification were observed between the control and the fumigated soils.

At the Oxnard site, fumigation with MeBr + CP or its alternatives significantly depressed potential nitrification relative to the levels observed in control plots at Weeks 1 and 4 (Fig. 8). The response of potential nitrification rates to the different fumigants followed the same order as observed for the Watsonville site. Potential nitrification was significantly higher in MeBr + CP fumigated soils compared with alternative fumigants at Week 1. The latter treatments did not differ significantly at this sampling date. At Week 4, potential nitrification remained inhibited compared with controls, but NO_3^-–N levels were markedly greater in plots fumigated

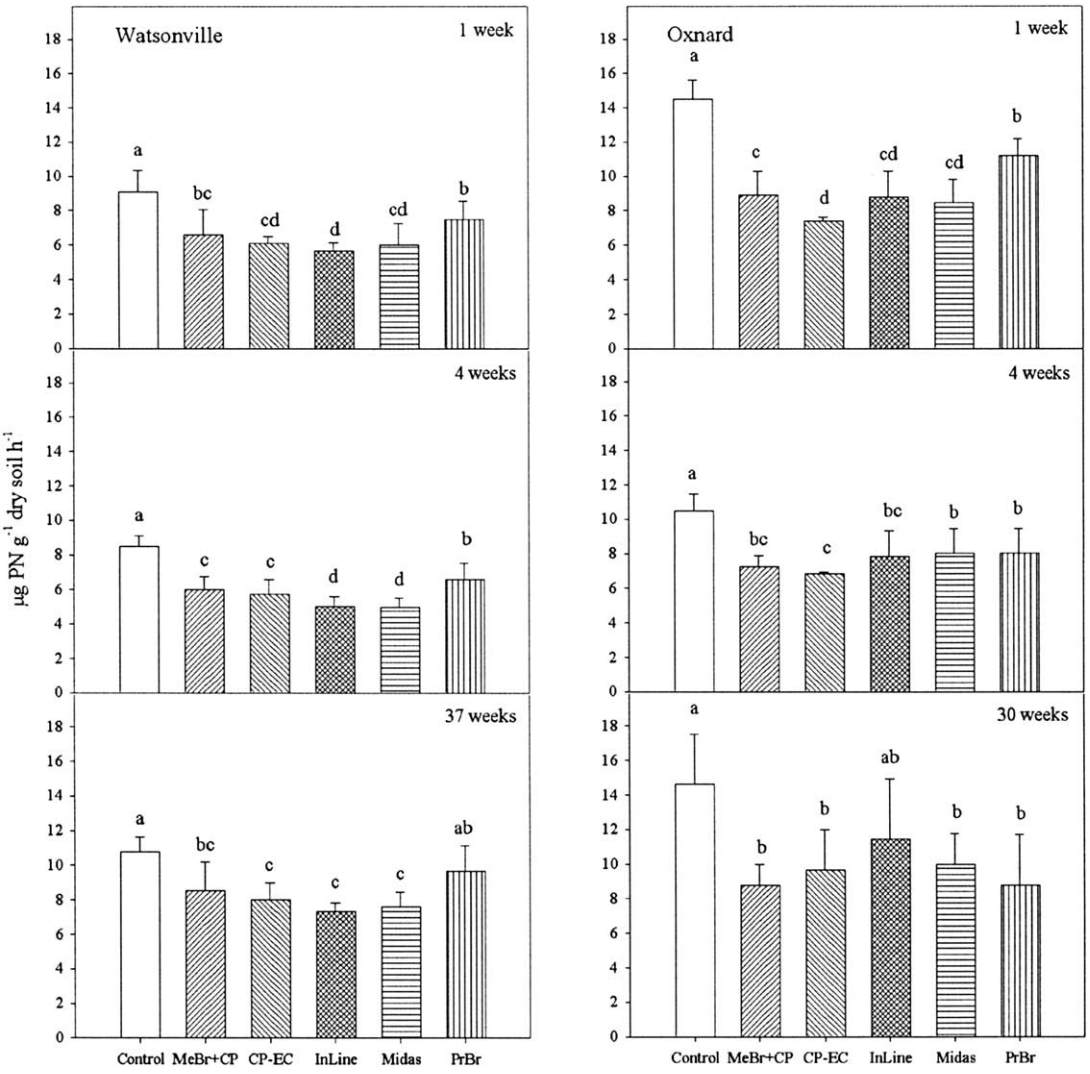

Fig. 5. Soil arylsulfatase activity in Watsonville and Oxnard soils 1 wk after fumigation with MeBr or an alternative (Week 1), at planting (Week 4), and at peak strawberry growth (37 or 30 wk). For each sampling date, means followed by a different letter are significantly different at $P < 0.05$. Error bars represent the standard deviation, $n = 4$. CP chloropicrin; EC, emulsifiable concentrate; PrBr, propargyl bromide.

with CP-EC compared with the other alternative fumigants. Among the fumigants tested, potential nitrification in soils followed the order CP-EC > Midas = MeBr + EC = InLine = PrBr. Basal nitrification increased in all plots fumigated with alternative chemicals, in particular in the CP-EC and Midas plots at 4 wk past fumigation. By Week 30, no differences in basal or potential nitrification were observed among any of the treatments.

DISCUSSION

The soils used in this study were very similar with respect to their physical and chemical properties but differed in regard to climatic conditions and management practices. The site at Watsonville is characterized by a higher average precipitation, a lower average temperature, and a longer strawberry growing season compared with the Oxnard site. Furthermore, soil cultivation at the Watsonville site is less intensive than that at Oxnard, and includes the application of manure, gypsum, and mineral fertilizers and planting of cover crops for 3 to 4 mo during the 10 mo break between each strawberry growing season (12–13 mo). Intensive soil cultivation practices at the Oxnard site relative to the Watsonville site may have resulted in lower labile C pools.

Variations in C availability may have been a significant factor contributing to the differences in culturable fungi in the indigenous culturable microbial populations (control plots) found between the two study sites. Patterns in soil fungi isolated from plots fumigated with alternative pesticides were variable and probably depended on location, management related differences in soil properties, and strawberry variety. Furthermore, the results may also indicate that the culture-dependent technique utilized to determine microbial groups in fumigated soils was unable to detect fungi that are resistant or able to degrade these pesticides or microorganisms that were nonculturable under the specific conditions of this assay. Tanaka et al. (2003) reported that viable counts of bacteria remained unchanged by fumi-

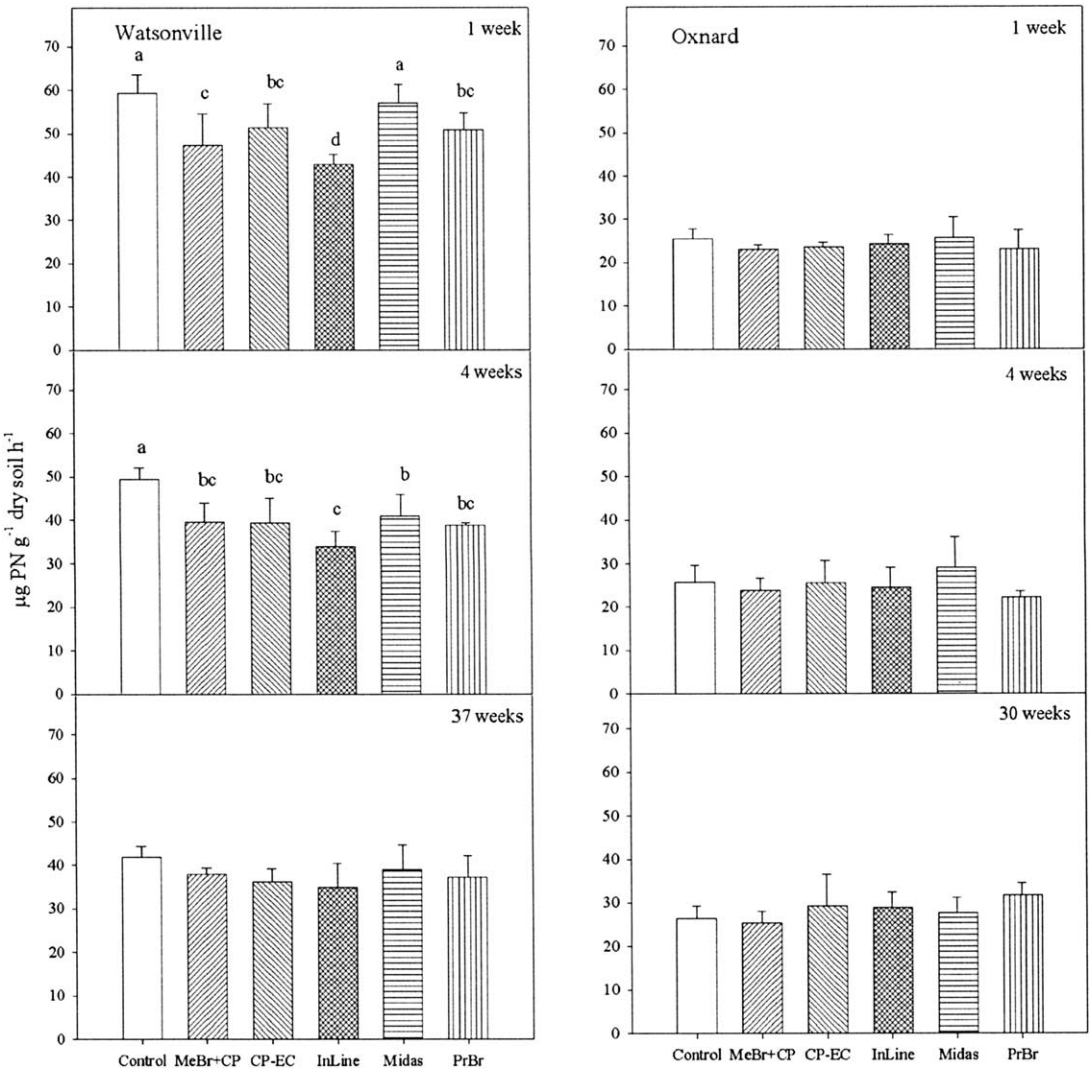

Fig. 6. Soil β-glucosidase activity in Watsonville and Oxnard soils 1 wk after fumigation with MeBr or an alternative (Week 1), at planting (Week 4), and at peak strawberry growth (37 or 30 wk). For each sampling date, means followed by a different letter are significantly different at $P < 0.05$. Absence of letters indicates no significant differences. Error bars represent the standard deviation, $n = 4$. CP chloropicrin; EC, emulsifiable concentrate; PrBr, propargyl bromide.

gation with MeBr and CP, while those of fungi decreased. In contrast, soil fumigation with CP decreased culturable numbers of most soil microbial groups by 20 to 80% (Itoh et al., 2000). Studies on the effect of metam sodium (sodium methyl dithiocarbamate) on soil microbial populations have shown contrasting results ranging from only small changes in the populations of spore-forming bacteria, gram-negative bacteria and fungi (Itoh et al., 2000) to a reduction in the size of total and culturable bacteria by 50 to 90% (Toyota et al., 1999).

The effect of soil fumigation on microbial populations depends on the biocidal action of the fumigant, soil properties, crop species and variety, and on pathogen-beneficial microorganism relationships in the rhizosphere (Sinha et al., 1979; Duniway et al., 2003). Sinha et al. (1979) reported that the success of the fumigant Vapam (metam sodium) in controlling soilborne infections by *Pythium* and *Rhizoctonia* was partly due to an increase in the populations of antagonists of these pathogens in fumigated soils. Many bacteria, especially *Pseudomonas* spp. from rhizospheres in fumigated soils revealed antibiosis to fungal pathogens such as *Verticillium*, *Phytophthora*, *Pythium*, *Rhizoctonia*, and *Fusarium* (Duniway et al., 2003).

Generally, soil fumigation with MeBr and the four selected alternative fumigants significantly decreased microbial respiration up to 4 wk past fumigation but not microbial biomass C. In the same research trials, Klose and Ajwa (2004) reported similar results 3-d post fumigation with MeBr and the potential alternatives CP-EC, InLine, Midas, and PrBr. Total microbial biomass contents are apparently not sensitive enough to reflect possible changes in the size and thus, potential functioning of microbial populations. The low response of microbial biomass to soil fumigation may be related to the lethal effect of fumigants on sensitive microbial populations, promoting the growth of resistant species or species that are able to utilize these fumigants as a C and energy source. The latter may feed on cell debris, leading to restructuring of soil microbial populations as indi-

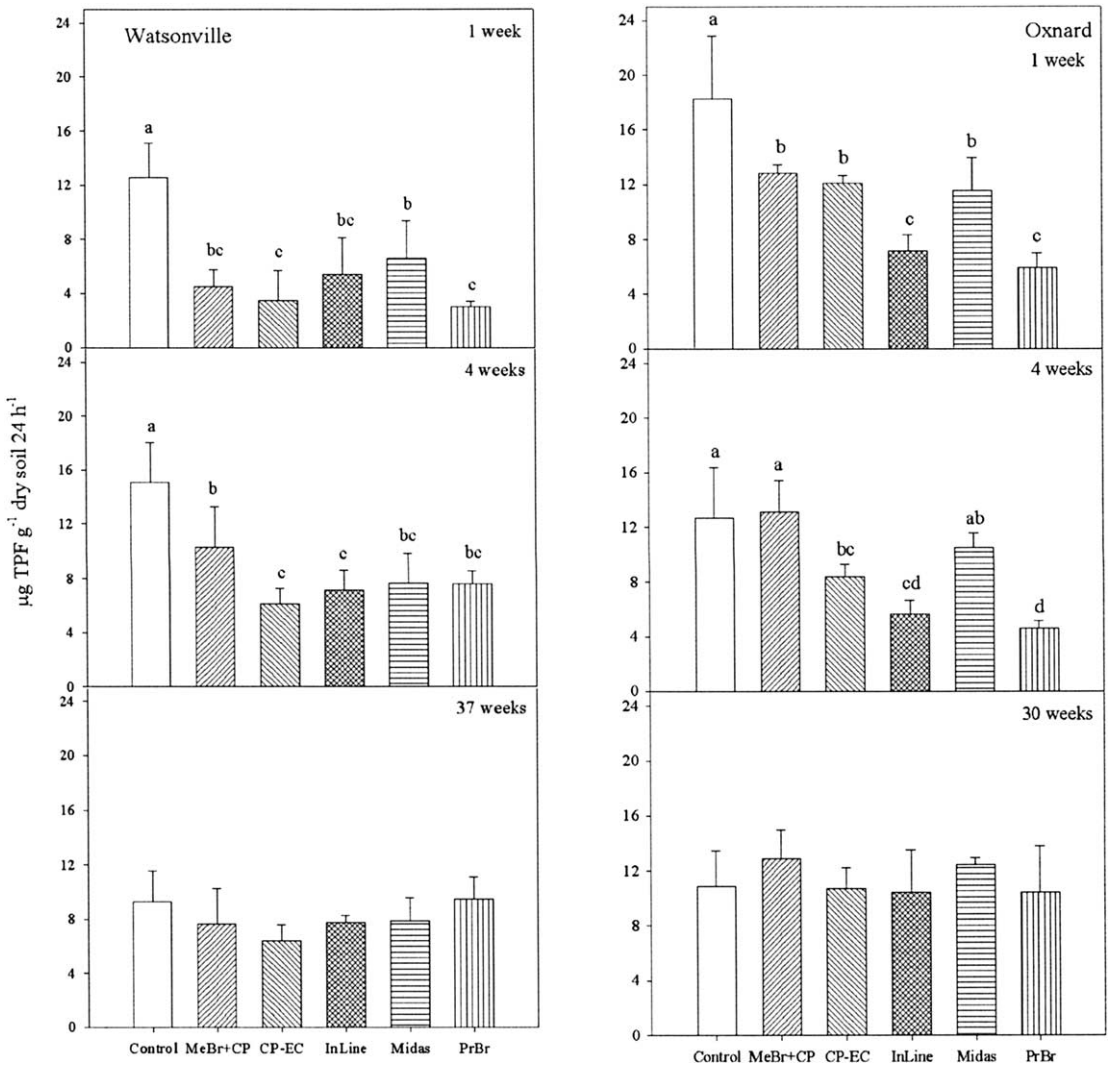

Fig. 7. Soil dehydrogenase activity in Watsonville and Oxnard soils 1 wk after fumigation with MeBr or an alternative (Week 1), at planting (Week 4), and at peak strawberry growth (37 or 30 wk). For each sampling date, means followed by a different letter are significantly different at $P < 0.05$. Absence of letters indicates no significant differences. Error bars represent the standard deviation, $n = 4$. CP chloropicrin; EC, emulsifiable concentrate; PrBr, propargyl bromide.

cated elsewhere (Macalady et al., 1998; Ibekwe et al., 2001; Orosco et al., 2001). Sinha et al. (1979) reported that fungi and *Rhizobia* populations were drastically reduced in fumigated soils, while populations of bacteria, actinomycetes, and *Azotobacter* gradually increased over 45 d of incubation. In contrast to our findings, a decrease in the microbial biomass contents was reported in soils fumigated with MeBr and CP (Tanaka et al., 2003) or chloroform (Zelles et al., 1997).

Lower respiration rates of fumigated soils relative to the control in our study indicate a general decline in the microbial activity caused by all fumigants studied. Microbial respiration in fumigated soils recovered at 37 or 30 wk after fumigant application at the Watsonville and Oxnard sites, respectively. In the same study, repeated fumigation (e.g., second year of application) with MeBr and alternatives resulted in decreased microbial respiration in fumigated soils compared with a nonfumigated control (Klose and Ajwa, 2004). These findings are consistent with the high sensitivity of respiration measurements of soils treated with heavy metals and pesticides (Kandeler et al., 2000; Tu, 2003).

Because microbial functional diversity includes many different metabolic processes, enzyme activities that control key metabolic pathways in soil can be measured and used as an index for microbial functional diversity (Nannipieri et al., 2002). Our study showed that soil fumigation initially reduced acid phosphatase and arylsulfatase activities, which are involved in the mineralization of high-molecular-weight P and S compounds, respectively. Also, fumigation initially reduced β-glucosidase and dehydrogenase activities, which are involved in the mineralization of low-molecular-weight C compounds. Acid phosphatase and arylsulfatase activities, however, remained small throughout the 30- to 37-wk experiment while β-glucosidase and dehydrogenase activities recovered within the strawberry planting (4 wk) and peak harvest periods (30–37 wk).

The decrease in acid phosphatase activity after fumigation may be due to the release of orthophosphates

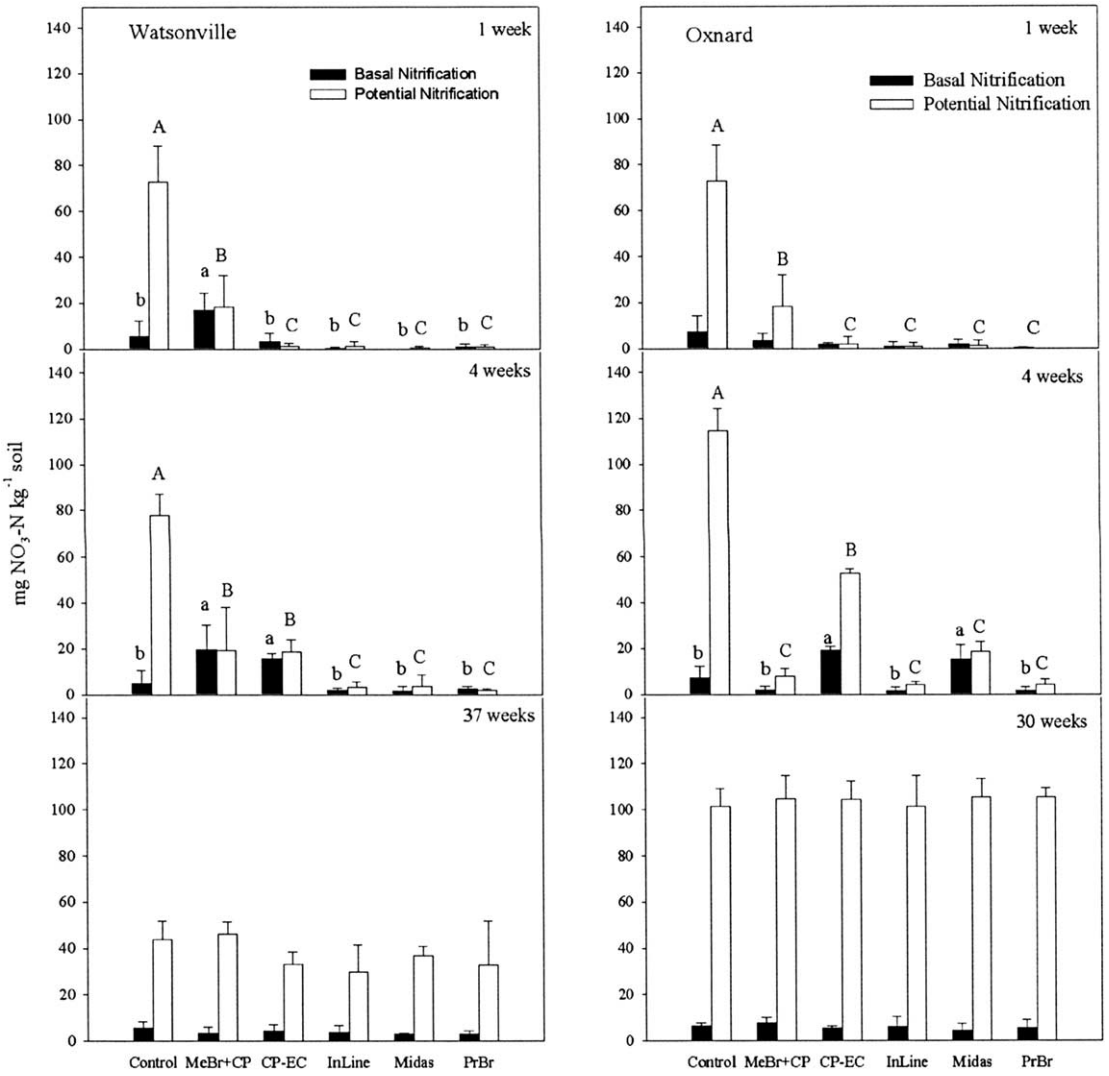

Fig. 8. Basal (no NH_4^+ substrate amendment) and potential (with NH_4^+ substrate amendment) nitrification in Watsonville and Oxnard soils 1 wk after fumigation with MeBr or an alternative (Week 1), at planting (Week 4), and at peak strawberry growth (37 or 30 wk). For each sampling date, means followed by a different letter are significantly different at $P < 0.05$. Lowercase letters are used to separate basal nitrification means, while uppercase letters are used to separate potential nitrification means. Absence of letters indicates no significant differences. Error bars represent the standard deviation, $n = 4$. CP chloropicrin; EC, emulsifiable concentrate; PrBr, propargyl bromide.

from lysed cells, which may act as a competitive inhibitor of acid phosphatase in soils (Tabatabai, 1994). The decrease in arylsulfatase activity in fumigated soils may affect S metabolism as it participates in the mineralization of organic sulfate esters. The recovery of β-glucosidase activity after its initial decrease in all fumigant treatments suggests that the effects of soil fumigation on cellobiose degradation are not persistent. Using chloroform as a fumigant, Zelles et al. (1997) and Klose and Tabatabai (2002b) reported that β-glucosidase activity was not sensitive to fumigation. Our findings indicate that dehydrogenase activity, an indicator of microbial activity, recovered within the growing season. Dehydrogenase measurements may be influenced not only by enzyme concentration, but also by the nature and concentration of organic C substrates and of alternative electron acceptors such as NO_3^- or SO_4^{2-} (Ladd, 1978). Our field experiments suggest that P and S metabolism is reduced in the short term in soils fumigated with CP-EC, InLine, Midas, and PrBr, whereas microbial biomass and activity and processes involved in cellobiose degradation are less sensitive.

In this study, soil fumigation had a strong initial depressive effect on potential nitrification and the adverse effect was higher for the MeBr alternatives than the MeBr treatment. The initial loss of potential nitrification in fumigated soils ranged between 75% (MeBr + CP plots) and 99% (Midas and PrBr plots). Generally, higher potential nitrification rates in MeBr-fumigated soils relative to the other fumigants may be due to (i) variations in the sensitivity of different microbial groups to the various fumigants or (ii) stimulation of MeBr degradation in soil during oxidation of an ammonia fertilizer by nitrifiers as suggested by several authors (Ou et al., 1997; Rasche et al., 1990). Rasche et al. (1990) reported that the two ammonia-oxidizing nitrifiers *Nitrosomonas europaea* and *Nitrosolobus multiformis* degraded MeBr in the presence of an NH_4^+–N source. The

half-life of MeBr and the tested alternatives was reported to range between 1 d for CP-EC and 22 d for MeBr (Ajwa et al., 2003). Toyota et al. (1999) reported that the size of ammonium and nitrite oxidizer populations was reduced up to four-fold by fumigation with metam sodium and populations showed only a slight recovery 15 wk later. Nitrification was shown to be sensitive to chloroform fumigation of soils (Zelles et al., 1997).

CONCLUSIONS

Short-term soil fumigation with MeBr alternatives reduced the activities of acid phosphatase, arylsulfatase, β-glucosidase, and dehydrogenase enzymes, and had significant effects on microbial respiration and nitrification. All fumigants studied affected total oxidative activities of microorganisms and biochemical reactions involved in organic matter degradation and C, P and S mineralization. Consequently, the turnover of nutrient elements from organic into mineral forms for plant uptake is likely to be reduced in fumigated soils. Results of this study also showed that the effect of soil fumigation on microbial populations and enzyme activities may depend on the soil properties, climatic conditions, soil management practices, and activity of the fumigants. Long-term evaluation of the effect of multiple soil fumigation with PrBr, InLine, Midas, and CP on other functional and structural properties of soil microbial communities and the interactions between fungal pathogens and beneficial rhizobacteria should be considered in future research.

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