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Influence of fumigants on soil microbial diversity and survival of *E. coli* O157:H7

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The aim of this study was to assess the effects of soil fumigation with methyl bromide (MeBr; CH_3Br) and methyl iodide (MeI, iodomethane; CH_3I) on the microbial community structure and diversity in two soils and determine the effects of microbial diversity on the survival of *Escherichia coli* O157:H7 from contaminated irrigation water. Polymerase chain reaction (PCR) was used to amplify 16S rRNA from total bacterial community composition and the products were subjected to denaturing gradient gel electrophoresis (DGGE). The Shannon-Weaver index of diversity (H') was used to determine the effects of both fumigants on soil microbial diversity. The effect was more severe in sandy soil than in clay soil at the normal application rate of MeBr and MeI. Our results showed that MeBr and MeI have about the same effects on soil microbial diversity. The two fumigants had greater impact on microbial diversity in sandy soil than in clay soil and this resulted in higher survival of *E. coli* O157:H7 in sandy soil than in clay soil during the 50 days that the study was conducted. MeBr has been used as soil fumigant for >40 years with no serious detrimental effects on agricultural production and our research also suggests that the use of MeI may also produce no long-term detrimental effects on agricultural production since both fumigants had about the same effects on soil microbial communities. Therefore, soil systems with reduced microbial diversity may offer greater opportunities for the survival of pathogenic bacteria such as *E. coli* O157:H7.

Keywords: Methyl bromide; methyl iodide; fumigant; *Escherichia coli* O157:H7; denaturing gradient gel electrophoresis; microbial diversity; survival.

Introduction

Methyl bromide (MeBr; CH_3Br) is a versatile and highly effective fumigant used for pre-plant soil fumigation. It has been used extensively to control pests and plant pathogens such as nematodes, soil-borne diseases, and weeds in economically important crops such as strawberries and nursery stock throughout the world.[1] Methyl bromide was phased out in 2005 in the United States and other developed countries[2] and it will be phased out in developing countries by 2015 because of its stratospheric ozone depletion potential and subsequent contribution to global warming. However, critical use exemptions have been granted for the production of high value-cash crops such as strawberries, tomatoes, and peppers.[3]

The MeBr phase-out resulted in an intensive search for alternative fumigants and the development of other integrated pest management strategies to replace MeBr. Methyl iodide (MeI, iodomethane) is another fumigant, yet to be registered, that is considered a promising alternative to MeBr for soil-borne pest control in high value-cash crops. Methyl iodide's fate, transport characteristics, and effectiveness as a biocide are similar to those properties of MeBr.[4] Methyl iodide has a distinct advantage over MeBr in that its atmospheric lifetime is only 4–8 days, compared to 0.4–0.9 years for MeBr.[5] Therefore, it is unlikely that MeI will reach the stratosphere and contribute to ozone depletion,[6,7] although the volatilization of MeI may be similar to that of MeBr.[8] MeI is not considered an ozone depleter, but excessive MeI emission into the ambient air can cause moderate toxicity. Massive exposure to MeI can lead to pulmonary edema. Although the toxicity of fumigants to target pests has been evaluated, there is limited information available regarding the impact of MeI on soil microbial communities. Changes in the soil microbial population can be observed following fumigation with MeBr and other fumigants.[9] Ibekwe *et al.*[10] observed that the Shannon-Weaver index of diversity determined 1 week after fumigant application was lower for MeBr than for chloropicrin, 1,3-D, and metam sodium. This index remained lower for MeBr than for the other fumigants even after 12 weeks

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of incubation. Changes in the microbial community composition as a result of fumigant application may lead to changes that affect microbial diversity of the affected soil.

Soil fumigation is a common practice used for baby greens production in the Salinas valley of California,[11] but this is not a common practice in the desert region of southern California and Arizona. Soil fumigation reduces the survival of many soil borne pathogens, but little research has been done on the survival of *E. coli* O157:H7 in fumigated soils.[12] Furthermore, risks of *E. coli* O157:H7 contamination during post-fumigation could increase because of reduced competition from antagonistic bacteria in the soil. After fumigation, soils are commonly irrigated before planting. Contaminated irrigation water may be one of the main sources of *E. coli* O157:H7 outbreaks linked to the consumption of leafy vegetables. At least half of the outbreaks involving *E. coli* O157:H7 during the last decade have been associated with the central coastal region of California. Soil characteristics and environmental conditions in an area with no history of *E. coli* O157:H7 outbreak, such as the Yuma area of Arizona, may differ greatly from those prevalent area such as Salinas, where the bacterium has been found on multiple occasions.[13] Ibekwe *et al*.[14] inoculated two different soil types with *E. coli* O157:H7 strain isolated from irrigation water, and concluded that the bacterium survived for more than 45 days. Islam *et al*.[15] reported that *E. coli* O157:H7 survived in soil for much longer periods than corresponding lettuce plants when soil was amended with manure. Moreover, practices that affect characteristics of the soil could affect the persistence of the pathogen within the total microbial population. This study focused on the effects of the fumigants on changes in microbial diversity in the presence of *E. coli* O157:H7 as a contaminant. In some cases in the Salinas Valley region of California, soils are fumigated before strawberry planting, and the same soil may be used in a rotation for the planting of some leafy greens. The two soils were selected because these are the dominant soil types in the San Bernardino, CA area used for rearing over 250,000 cattle in about 30,000 km2. The study area used to have the highest concentration of cattle in the United States. Cattle are the main source of *E. coli* O157:H7 in the environment and it is necessary to determine their survival where they may have the greatest impact on the environment. Our main objectives were to determine the effects of fumigants on microbial diversity as well as the effects of soil microbial diversity on the survival of *E. coli* O157:H7 in the two major soils in the area.

Materials and methods

Soils and chemicals

Clay soil (Willows silty clay, saline-alkaline) and sandy soil (Dello sand) were collected from Mystic Lake dry bed and the Santa Ana River bed, respectively, in Riverside County, California. The clay soil has a bulk density of 1.51 Mg m-3 with 3.7 % sand, 49.1 % silt, and 47.2 % clay. The sandy soil has a bulk density of 1.67 Mg m-3 with 99.1 % sand, 0.20 % silt, and 0.70 % clay. The soils were sieved through a 4 mm sieve before planting, and the high salt content (electrical conductivity = 15 ds m^{-1}) in the clay soil was reclaimed as described by Ibekwe and Grieve.[16] These soils were chosen because of the reasons stated above.

Methyl iodide (>99% purity) was purchased from Chem Service (West Chester, PA) and methyl bromide (>99% purity) was obtained from Great Lakes Chemical Company (West Lafayette, IN).

Bacterial strain and growth conditions

E. coli O157:H7 strain 72 pGFP was kindly provided by Dr. Pina Fratamico.[17] This strain contains Shiga-like toxin genes 1 and 11 (*stx*1, *stx*2) and the pGFP cDNA vector expressing green fluorescent protein (GFP) containing an ampicillin resistance gene and was used for pathogen enumeration from soil. *E. coli* O157:H7 was cultured at 37°C overnight in modified Tryptic Soy broth (mTSB)(Difco Laboratories Inc., Cockeysville, MD) supplemented with 100 μg of ampicillin ml^{-1} (Sigma, St Louis, MO). Cells were harvested by centrifugation at 5000 *g* for 10 min and resuspended in phosphate buffered saline (PBS) (Fisher Scientific, Pittsburgh, PA) to a concentration of $\sim 10^8$ CFU mL^{-1}.

Growth Chamber experiment

Plastic trays (58.2 × 43.2 × 18.5 cm) were filled with approximately 40 kg of soil. The soils were irrigated and bacteria inoculated with approximately 4.2×10^8 *E. coli* O157:H7 to bring soil moisture to about 12 %. The 12 % was used instead of water holding capacity of the soil because movement of a volatile chemical in soil is controlled by its distribution behavior over the soil–water–air phases. If the soil is too wet, distribution of MeBr will be reduced, and if the soil is too dry it may result in increased volatilization. Bacteria were inoculated (10^8 CFU mL^{-1}) into the irrigation lines with Cole-Parmer high performance liquid chromatography (HPLC) pump (Cole- Parmer, Chicago, Illinois) and delivered through PVC pipes to each tray with five surface drip lines two days before fumigation. Soil samples were collected immediately after inoculation for community analysis, heterotrophic plate counts, and *E. coli* O157:H7 concentration. E. coli O157:H7 concentrations at the surface of the soils (0–3 cm depth) immediately after inoculation were approximately 10^6 by plate count. After the initial sample collection, trays were manually tarped with a virtually impermeable plastic film; 0.038 mm thick Hytibar® (Klerk Plastics, Belgium) and fumigants were applied in triplicates per treatment for each soil. Fumigant application rates were selected according to the

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recommendations for each chemical, which were reduced by a factor of six to account for the smaller depth of soil in the trays treated in these studies (≤18 cm) compared to the depth of soil treated in typical soil fumigation (60 cm). A total of 30 trays were used for each soil with three trays per fumigant treatment rate or control. Application rates for MeBr and MeI were approximately 48 Kg ha^{-1} and 40 Kg ha^{-1}, respectively. These rates were designated 1X to indicate approximate field application rates. Methyl bromide and MeI were also applied at half this rate (0.5X) to examine the effect of lower fumigant concentrations on the soil microbial diversity, and non-fumigated controls (0X) were included. To avoid the emission of fumigants from the trays into the growth chamber, syringes were used to inject MeBr (gas) and MeI (liquid) into the soil through the tarp, and the puncture hole was covered immediately with duct tape. Soil trays were left in the growth chamber for 10 d. After 10 d, trays were moved outside and the plastic film was removed. Trays remained open outside in an enclosed area, covered with standard galvanized chain-link fence and aerated for 2 d before they were moved back to the growth chamber for the continuation of the experiment. The growth chambers were kept under the same environmental conditions at 20°C and 70 % relative humidity. Soil (0-3 cm deep) samples were collected on the day of inoculation with *E. coli* O157:H7, and prior to fumigation and on 10, 21, 28, 35, and 50 days after fumigant application. The samples were collected in separate sterile petri dishes or collection bags. Soil was transferred to ziploc bags and 10 g sample was used for serial dilution. Total bacterial community DNA, *E. coli* O157:H7, and heterotrophic bacteria were determined on the samples. *E. coli* O157:H7 population was determined by plating soil on modified tryptic soy agar (TSA; Becton Dickinson) plates containing 100 μg of ampicillin mL^{-1} (TSA-A) and the gfp expressing *E. coli* O157:H7 were enumerated and the result expressed as CFU g^{-1} soil. The GFP-labeled *E. coli* O157:H7 colonies were counted under an UV light. Heterotrophic bacteria were enumerated using tryptic soy agar and the results were expressed as CFU g^{-1} soil.

DNA extraction, PCR amplification, and DGGE analysis

Community DNA was extracted from soil samples (0.5 g) with the Ultra Clean Soil DNA Kit (MoBio Laboratories, Solana Beach, CA) and stored at −20°C. A 236-bp DNA fragment in the V3 region of the small subunit ribosomal RNA genes of eubacteria was amplified by using primer set PRBA338f and PRUN518r.[18] Ready-To-Go PCR beads (Amersham Pharmacia Biotech, Piscataway, NJ) and 5 pmol of primers in a total volume of 25 μL were used in the PCR reaction. PCR amplifications were done under the following conditions: 92°C for 2 min; 30 cycles of 92°C for 1 min, 55°C for 30 s, 72°C for 1 min followed by a final extension at 72°C for 6 min.

Denaturing gradient gel electrophoresis (DGGE) was performed with 8 % (wt/vol) acrylamide gels containing a linear chemical gradient ranging from 30 to 70 % denaturant with 100 % defined as 7 M urea and 40 % formamide. Gels were run for 3.5 h at 200 V with the Dcode™ Universal Mutation System (Bio-Rad Laboratories, Hercules, CA). DNA was visualized after ethidium bromide staining by UV transillumination and photographed with a Polaroid camera. Major bands were excised for identification of bacterial species. Bands were placed into sterilized vials with 20 mL of sterilized, distilled water and stored overnight at 4°C to allow the DNA to passively diffuse out of the gel strips. Ten mL of eluted DNA was used as the DNA template with the bacteria primers above but without the GC-clamp. DNA was cloned into the TOPO TA cloning kit (Invitrogen, Carlbad, CA). Isolation of plasmids from *E. coli* was performed using the Qiagen plasmid mini kit (Valencia, CA). Four plasmids from each band were sequenced to check for purity of clones. The purified plasmids were sequenced with the ABI PRISM Dye Terminator Cycle Sequencing Kit with AmpliTaq DNA Polymerase, FS (Applied Biosystems, Foster City, CA) with forward and reversed primer M13.

Statistical analysis

DNA fingerprints obtained from the 16S rRNA banding patterns on the DGGE gels were photographed and digitized using ImageMaster Labscan (Amersham-Pharmacia Biotech, Uppsala, Sweden) and analyzed.[10] Band positions as determined by ImageMaster fingerprinting software were converted to Rf values between 0 and 1, and profile similarity was calculated by non-metric multidimensional scaling (NMS),[19] Non-metric multidimensional scaling is an iterative ordination method that is well–suited to data that are non-normal or are on arbitrary, discontinuous, or otherwise questionable scales. The main advantages of NMS are that it avoids the assumption of linear relationship. Its use of ranked distances tends to linearize the relationship between distances measured in species space and distances in environmental space. Unlike principal correspondence analysis (PCA) and correspondence analysis (CA) family of ordinations that rely on linear relationships NMS can use data that are not normally distributed which are typical of most ecological data.

The comparison of the different measured richness, evenness, and diversity was done by using a one-way analysis of variance, and Tukey's studentized range test for post hoc analysis.[20] Richness (S) refers to the number of bands detected in a given soil sample. The DGGE evenness (E), a measure of how evenly DGGE bands were distributed in a given soil sample, was calculated as $E = H'/\ln(S)$. Diversity was calculated by using the Shannon index of diversity (H') to compare changes in diversity of microbial communities within all treatments at each time[21] by using the following

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function:

$$H' = -\sum P_i \log P_i H' = -\sum P_i \log P_i$$

when $P_i = n_i/N$, n_i is the height of peak, and N is the sum of all peak heights in the curve. Simpson's diversity index (D) for infinite population = 1 − sum (Pi × Pi) where Pi = importance probability in element i (element i relativized by row total). Statistical analysis using general linear model (GLM)[20] was used to compare the indices of diversity (H') within weeks of obtaining the indices from different treatments. Sequence identification was performed by using the BLAST database (National Center for Biotechnology Information: www.ncbi.nlm.nih.gov) to identify the major bands excised from DGGE.

E. coli O157:H7 strain 72 pGFP survival (CFU g^{-1} soil) were fitted to a biphasic model as proposed by Geeraerd *et al.*[22] with the Geeraerd and Van Impe inactivation model-fitting tool (GInaFiT) and as described by Franz *et al.*[23] In the model plate counts of zero were replaced by 10 CFU gdw^{-1}, which was the calculated detection limit of the dilution plating procedure. Most of the survival curves showed shoulders, tails, and a biphasic pattern; hence first-order kinetics could not explain the model. Therefore, the log-transformed survival data were fitted into the model:

$$\log CFU(t) = \log(N_0) + \log \times \left(\begin{array}{l} \left(f \cdot e^{-k} \max 1^{\cdot t} \cdot \dfrac{e^{k} \max 1 \cdot s1}{1 + (e^{k} \max 1^{\cdot S} 1) \cdot e^{-k} \max 1^{\cdot t}} \right. \\ \quad \left. +(1-f) \cdot e^{-k} \max 2^{\cdot t} \right). \\ \left(\dfrac{e^{k} \max 1 \cdot s1}{1 + (e^{k} \max 1^{\cdot S} 1) \cdot e^{-k} \max 1^{\cdot t}} \right)^{\frac{k_{\max 2}}{k_{\max 1}}} \end{array} \right)$$

where t is time in days, N_0 is the number of cells present at t_0, f is the fraction of the initial population in a major less-resistant subpopulation, ($1 - f$) is the fraction of the initial population in a minor more-resistant subpopulation (at t_0), $k_{\max 1}$ and $k_{\max 2}$ (day^{-1}) are the specific inactivation rates of the two subpopulations, and sl is the initial shoulder length.

Results

Changes in soil microbial community diversity following fumigation

DGGE analysis of 16S rRNA fragments was used to examine the effects of MeBr and MeI on soil microbial communities. (Fig. 1a and b are examples from clay soil 1 and 7 weeks after fumigation). The most drastic effect occurred on the first week of the experiment as most bands did not appear during the first week (Figure 1a) and higher number of bands re-appeared during week 7 (S1-10 are bands cut from DGGE for bacterial sequence analysis-see Fig. 1 a & b).

The diversity of bacterial communities were examined in detail by the use of analysis of variance to determine differences in species richness, evenness, Shannon, and the Simpson diversity indices between the two soils (Fig 2a). Richness or the number of bands were significantly higher in clay soil ($P < 0.0001$) than in sandy soil, and evenness, Shannon and Simpson indices were also significantly higher in clay soil than sandy ($P = 0.05$) before the application of fumigants. Analysis of variance was done on the total data set to determine the effects of soils, time in weeks, fumigants, and fumigant treatment rates on total microbial community diversity in the two soils. Soil, time ($P < 0.0001$), fumigant ($P = 0.05$) were major factors resulting in significant decrease in richness, Shannon, and the Simpson indices in both soils (Table 1). Fumigant application significantly resulted in decrease in bacterial evenness. As a result of these findings in Table 1, data were analyzed to see the weekly effects in both soils (Fig. 2b & c).

In the clay soil there was a significant ($P \leq 0.05$) decrease in diversity in MeBr treated soil at 1X (B1X-E) compared to the control and MeI treatments during the first 10 days of the study (Fig. 2b). By days 21, 28, and 36, H' did not show drastic changes from day 10. By day 50 (week 7), diversity indices had increased, in some cases by a factor of two (Fig. 2b). In the sandy soil (Fig. 2c), and at day 10 of the study, two treatments with MeBr and MeI at normal fumigant application rates without *E. coli* O157:H7 inoculation produced significantly lower diversity indices with less than two detectable bands. By day 21 the diversity indices from these two treatments has increased but were still significantly lower than the control with pathogen inoculation. At days 28 and 36 the overall structure of microbial community did not show a drastic change from week 3. However, 50 days after fumigation, microbial communities from sandy soil have recovered and between 9 and 12 bands were detected from the treatments compared to week 1.

To compare fumigant effects on microbial community, DGGE patterns were analyzed by NMS (Fig. 3a & b) for the study period. Clay soil was separated into three main clusters (Fig. 3a) by time. This is in agreement with the diversity data. Most of the samples from day 10 (w1) clustered together (C1), while samples from day 50 (w7) were together (C2) and days 21, 28, and 36 (w3, w4, and w5) formed a different cluster (C3). About 67 % of day 10 (w1) samples were grouped together in group 1 while about 95 % of day 50 (w7) samples were similar indicating that microbial community in day 50 were more closely related than the structure in day 10. The same pattern of separation was observed for sandy soil except that day 50 (w7) in sandy soil was a subset of weeks 3–5 (Fig. 3b).

Major bands from growth chamber study were excised, cloned, and sequenced. Most of the bacteria recovered from the growth chamber soils were dominated by *Proteobacteria* and uncultured bacterial phyla (see figure 1 for clay soil and Table 2). New bands in the growth chamber clay

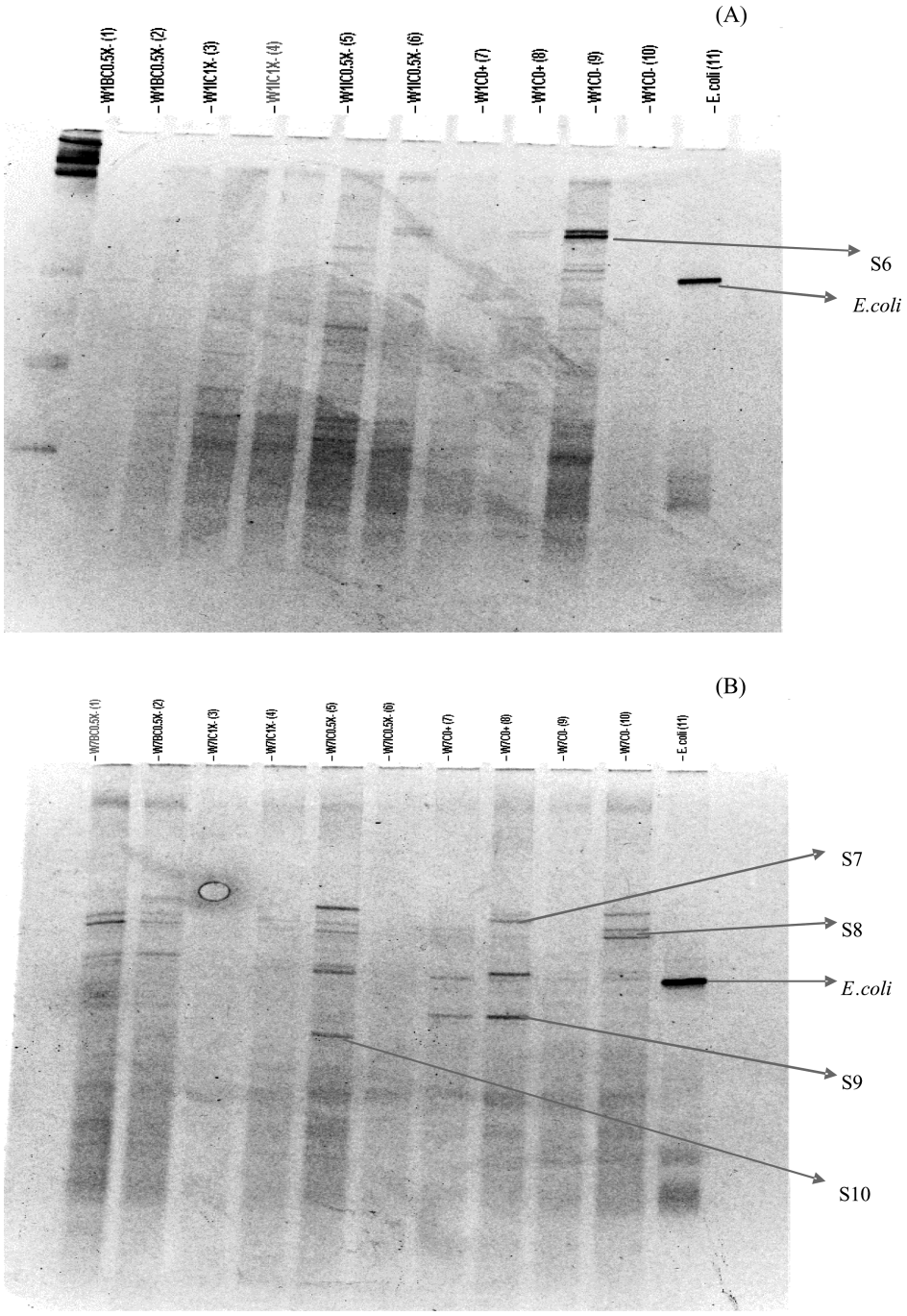

Fig. 1. DGGE analysis of 16S rRNA fragments of total bacterial population extracted from soil during the growth chamber study (a) clay soil one week after fumigation and (b) clay soil seven weeks after fumigation. Symbols in (a) indicate week 1 (W1), methyl bromide or methyl iodide treatment (B or I), clay soil (C). Symbols in (b) are similar for week seven (W7). The numbers 0X, 0.5X and 1X represent no fumigant application, half the normal application rate, and normal fumigant application rate, respectively. The sign '-' and '+' at the end indicate treatments without and with contamination with *E. coli* O157:H7. The numbers in parenthesis represent gel lanes. S1-S10 indicates bands cut from DGGE for bacterial sequence analysis (see Table 2).

soil appeared 50 days (w7) after fumigation treatment and this was an indication of the emergence of new microbial species after the initial fumigation effects on soil microbial composition.

Microbial diversity influence on survival of E. coli *O157:H7*

Mean comparison by days and methods was used to determine the impact of fumigants on the survival of *E. coli* O157:H7 in the two soils after fumigation. Since

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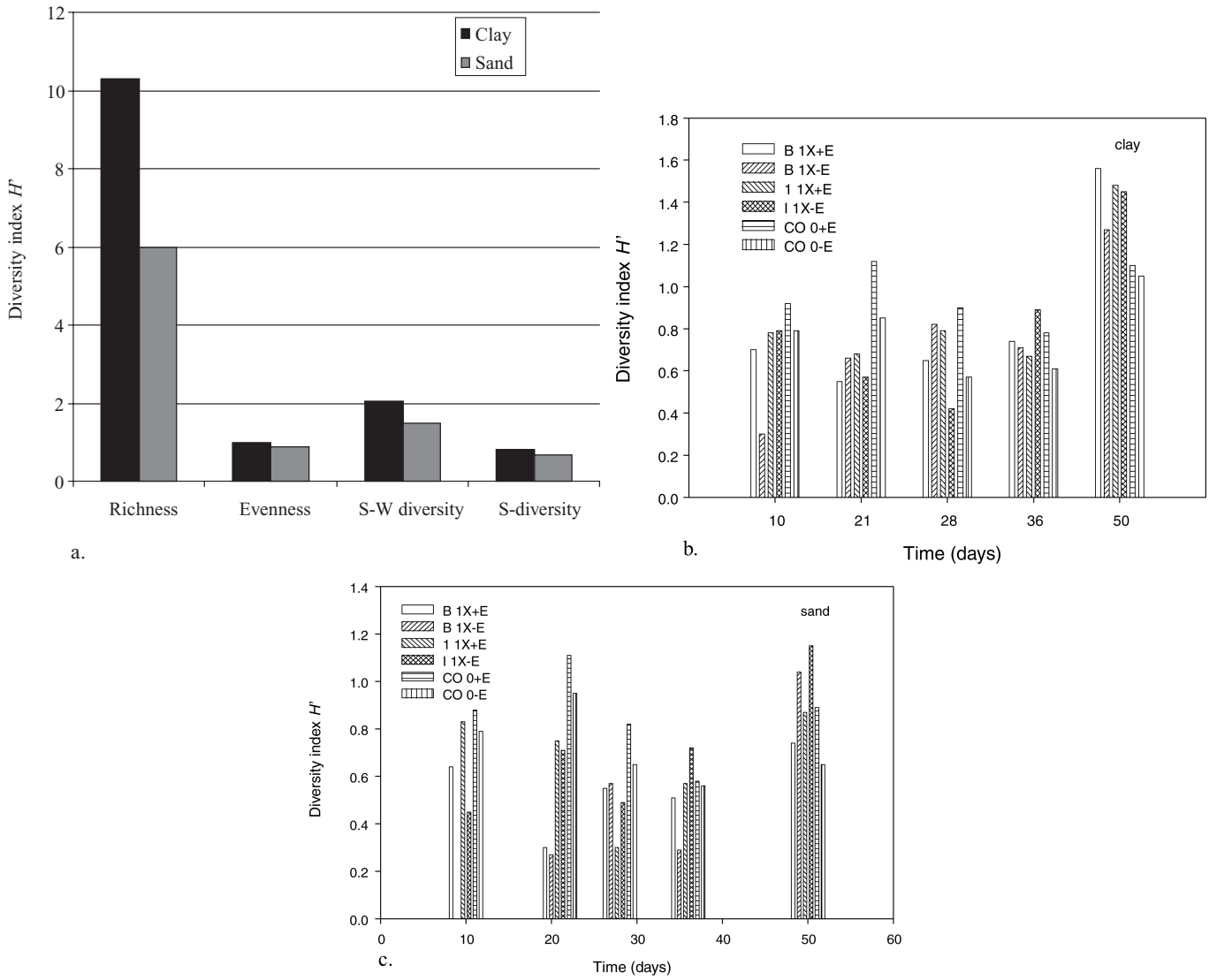

Fig. 2. Numerical analysis of DGGE bands by Shannon index of diversity (H) from growth chamber soil: (2a: total diversity before fumigant application). Numerical analysis of DGGE bands by Shannon-Weaver index of diversity (H) from growth chamber soil (Fig 2b: clay soil; 2c: sandy soil). Means with the same letter are not significantly different at $P \leq 0.05$ using Tukey's Studentized Range Test. B1X+E indicates treatment with methyl bromide at normal field application with the inoculation of *E. coli* O157:H7 to the soil, B1X-E indicates treatment with methyl bromide at normal field application without the inoculation of *E. coli* O157:H7 to the soil, 1 1X+E indicates treatment with methyl Iodide at normal field application with the inoculation of *E. coli* O157:H7 to the soil, I 1X-E indicates treatment with methyl iodide at normal field application without the inoculation of *E. coli* O157:H7 to the soil, CO 0+E indicates control soil without fumigation but inoculated with *E. coli* O157:H7, CO 0-E indicates control soil without fumigation and no inoculated with *E. coli* O157:H7.

one of our objectives was to determine the effects of fumigants on *E. coli* O157:H7, direct comparison of the two fumigants and the control was done using plate count (Fig. 4a & b). Ten days after fumigation, *E. coli* O157:H7 was significantly lower ($P = 0.0001$) in fumigated sandy soil than the control at the normal application rate (Fig. 4a). During the rest of the study, there were no significant differences on the effect of the two fumigants on the pathogen, except on day 36 ($P = 0.046$) and 50 ($P = 0.0001$). The behavior of the pathogen in clay soil was very different from sandy soil. Pathogen concentrations were significantly lower ($P = 0.023$) in fumigated samples than in the control. The population decline in the growth chamber study was further analyzed using the biphasic model. The fitting curves showed an initial linear decline in all the treatments during the first 10 days with a significant difference between the control and fumigated samples as the initial shoulder length (sl) was 0 for both soils. However, for both control treatments the population size did not reach the detection limit (ttd) of 10 CFU g^{-1}

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Table 1. Significant differences in bacterial diversity colonizing growth chamber clay and sandy soils after 50 days by denaturing gradient gel electrophoresis (DGGE).

Source	*Richness (S)*	*Evenness (E)*	*Shannon-index (H′)*	*Simpson index (D)*
Soil	***	*	***	***
Time-weeks	***	NS	***	***
Soil*time	***	NS	***	***
Fumigant	*	***	***	***
Soil*fumigant	NS	NS	NS	NS
Time*fumigant	NS	NS	NS	NS
Soil*time*fumigant	NS	*	NS	NS
TRT-rate	NS	NS	NS	NS
Soil*TRT	NS	NS	NS	NS
Time*TRT	NS	**	*	**
Soil*time*TRT	NS	NS	*	*
Fumigant*TRT	*	NS	NS	*
Soil*fumigant*TRT	***	**	NS	*
Time*fumigant*TRT	NS	***	**	*
Soil*time*fumigant	NS	NS	NS	**
Soil*time* fumigant* TRT	NS	NS	NS	*

S = Richness (number of non-zero elements in row) E = Evenness, H = Shannon's diversity index and D = Simpson's diversity index for infinite population. * = significant at (P = 0.05), ** (P = 0.01), *** (P ≤ 0.0001), NS = not significant.

during the 50 days of the experiment due to earlier onset of tailing at about 35 days. Overall, in fumigated soils, it took longer to reach the detection limit in sandy soil than in clay soil, indicating higher survival in sandy soil than clay soil.

Discussion

Effects of fumigants on microbial diversity

Fumigation of soil with MeBr is known to result in the temporary decline of bacterial species in soil,[10] and enhanced availability of resources from lysed cells, upon which the fumigant survivors can recolonize rapidly and in high numbers after fumigation.[24] Rapid decolonization was demonstrated by Xio and Duniway[24] in a strawberry field after fumigation. Our study is in agreement with the above authors. As shown in Figures 2 b&c, 50 days after fumigant application, microbial diversity is significantly higher in fumigated soils than the control. Other studies with strawberry soil colonizers in fumigated in comparison to native soils suggest that there may be differences in deleterious and beneficial colonizers following soil fumigation.[9] The modified bacterial community structures revealed higher diversity indices in clay soil than in sandy soil and subsequent clustering by time of treatment (Figs. 2a and 3). This suggests that microbial communities in clay and sandy soils were differently impacted by fumigants. The Shannon-Weaver index of diversity (H), calculated from the number and relative intensity of DGGE bands, was consistently higher in clay soil than in sandy soil (Fig. 2a). Gel analysis of DNA from fumigated soil in the growth chamber revealed that at these application rates, MeBr exerted stronger effects than MeI on the diversity of the microbial community in sandy soil. No significant differences were observed between MeBr and MeI effects in clay soil. The impact of both fumigants was more severe in sandy soil than in clay soil. Soil properties, including texture, moisture, porosity, and organic matter content, have long been recognized to affect pest control achieved by soil fumigation.[25]

By using container trials and small field plots, Becker *et al.*[26] showed that MeI was significantly more effective than MeBr against the plant parasitic nematodes *Meloidogyne incognita*, *Heterodera schachtii* and *Tylenchulus semipenetrans* and the plant pathogenic fungus *Rhizoctonia solani* when these fumigants were applied at equimolar rates. Since MeBr has been used as a soil fumigant for >40 years with no serious detrimental effects on agricultural production, this research suggests that the use of MeI may also produce no long-term adverse effects since both fumigants had about the same effects on soil microbial communities. Soil fumigation generally increases root health, growth, and fruit yields in strawberries even when major pathogens are not present in soil.[27,28]

Table 2. Sequence analysis of bands excised from denaturing gradient gel electrophoresis (DGGE) gels derived from bacterial 16S rRNA extracted from soil (S1–S10).

Bands	*Related bacterial sequences*	*Phyla*	*Sequence similarity*	*Accession no.*
S1	uncultured soil bacterium	uncultured	95	AY242672
S2	uncultured bacterium	uncultured	100	AY36199
S3	uncultured *Gamma proteobacterium*	*Proteobacteria*	99	AJ853877
S4	uncultured Antarctic soil bacterium	uncultured	94	AF419205
S5	uncultured organism	uncultured	89	AY707533
S6	uncultured bacterium	uncultured	98	AY546500
S7	uncultured organism	uncultured	98	AY707537
S8	unidentified bacterium	uncultured	90	AJ518278
S9	Uncultured bacterium clone A0Z/14	uncultured	95	AY465146
S10	*Leptolyngbya* sp. PCC 73110	*Cyanobacteria*	100	AF132785

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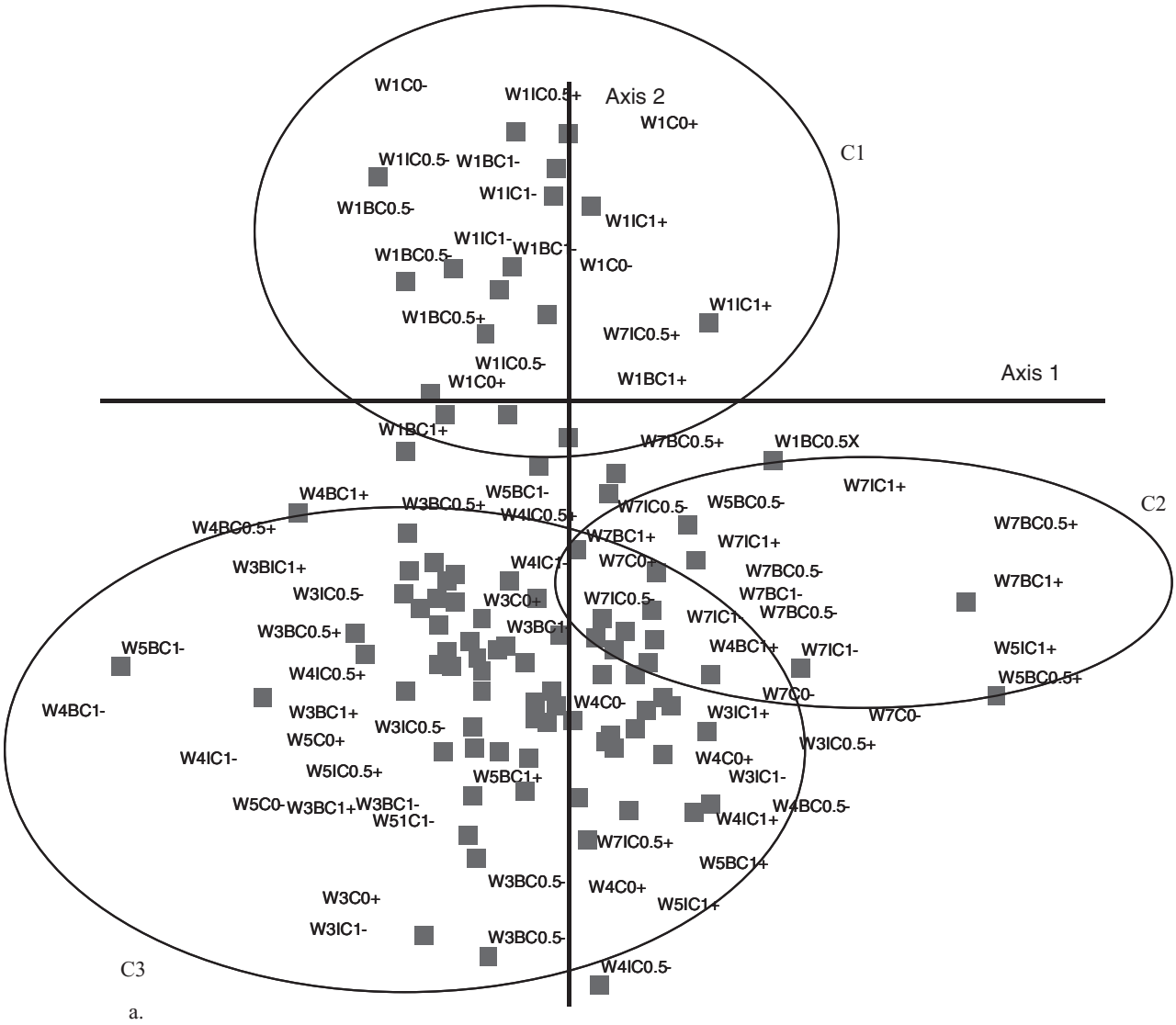

Fig. 3. Cluster analysis of microbial communities generated by the analysis of DGGE 16S rRNA PCR patterns from both clay (a) and sandy soil (b) with all the treatments. Symbols in (a) indicate week 1 (w1), methyl bromide or methyl iodide treatment (B or I). The numbers 0X, 0.5X and 1X represent no fumigant application, half the normal application rate, and normal fumigant application rate, respectively. The sign '-' and '+' at the end indicate treatments without and with contamination with *E. coli* O157:H7. The numbers in parenthesis represent gel lanes.

Effects of microbial diversity on the survival of E. coli *O157:H7*

In a recent study, the effects of *E. coli* O157:H7 was assessed in a loamy sand soil obtained from species-rich grassland, in which the microbial community composition had been modified by progressively enhanced fumigation depths.[29] The authors showed that *E. coli* O157:H7 in the soils with modified community structures was clearly consistent with the hypothesis that within the single selected habitat (soil), which was relatively unaffected in respect to abiotic conditions like pH, moisture and soil chemical conditions, microbial diversity was the main determinant of the survival of the pathogen. This was in a total agreement with our study which shows that survival of the pathogen was higher in sandy soil with lesser microbial diversity than clay soil during the short period of our study. However, longer survival of *E. coli* O157 in clay soil may have been influenced by interaction between soil particles in the clay particle sizes that provided niches for the pathogen and moisture/nutrients within the niches. An other factor that may contribute to the survival of pathogens in the soil was soil microbial diversity. This might have increased the activities of the native microbial population, which decreased the competitive success of the introduced pathogen. The current assessment of the survival of *E. coli* O157:H7, in relation to soil microbial diversity and community composition, is important, as this organism has been shown to be able to colonize edible plants

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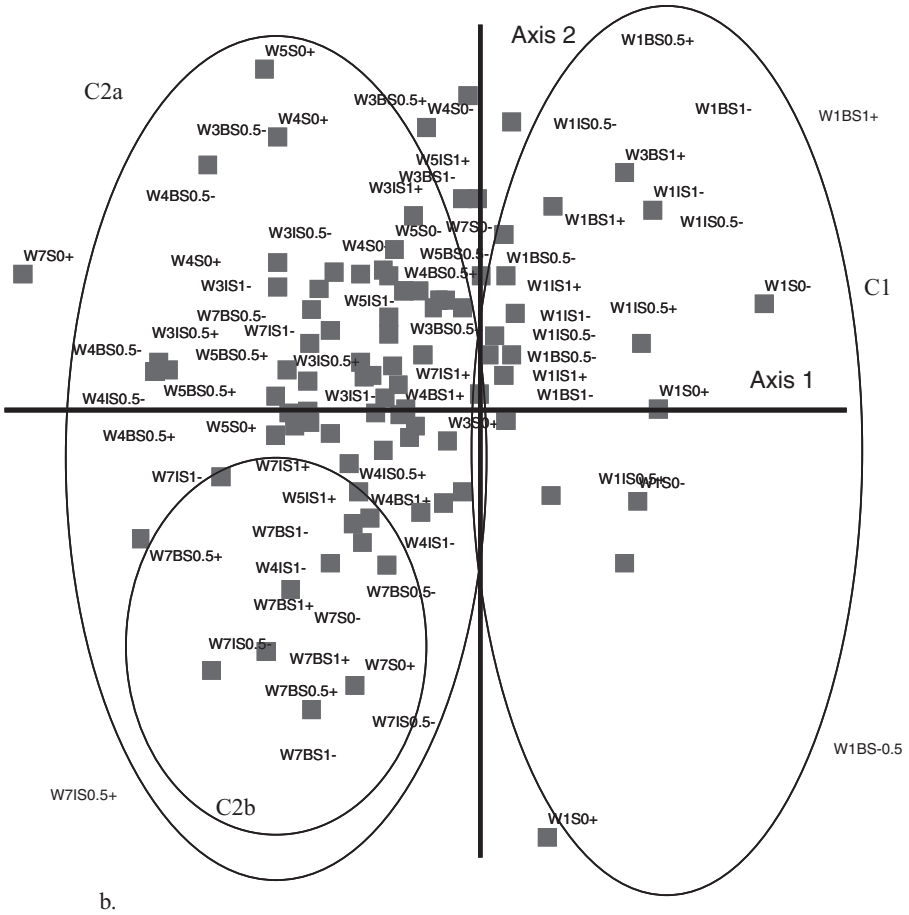

Fig. 3. (Continued)

such as lettuce from manure and irrigation water.[30] Our results showed that *E. coli* O157:H7 can survive longer than 50 d in both soils. Others have reported survival between 2 and 56 days in manure amended soil (Franz *et al.*,[31]) and 34 d or more in sandy loam soil amended with cow manure.[15] Others reported longer *E. coli* O157:H7 survival times of between 154 and 217 d in soils mended with inoculated compost (Islam *et al.*,[15]). The results from our study are in agreement with the previous studies because they relied on inoculating the substrate with relatively high densities of the pathogen ($>10^6$ CFU g^{-1}).

The long term survival of *E. coli* O157:H7 in our study followed a non-log-linear model. Therefore, the biphasic Geeraerd model in which the initial rate of decline is followed by a slower second phase (tailing) was used to accurately describe *E. coli* O157:H7 survival behavior. This model has been successfully used in food microbiology[30] and in soil and manure.[23,31] Klerks *et al.*[32] has also demonstrated that the number of *S. enterica* colony forming units (CFU) per g of lettuce rhizosphere was negatively correlated to the species richness of the surface sterilized lettuce cultivars. It has been shown that in environmental substrates *E. coli* O157:H7 die-off occurs often in two stages.[33,34] Therefore, the use of first-order kinetics and log-linear modeling of survival of *E. coli* O157:H7 in soil is clearly not appropriate. Fumigants are known to kill bacteria in the soil at the initial contact, and there after, the population may recover.

In conclusion, our study showed that the effects of MeBr and MeI on soil microbial communities were most severe during the first week after application. After this period, stable microbial communities were obtained until week 7 of the experiment. Comparative efforts should be made to study the impact of these fumigants on soil microbial community structures in the field with special attention to the spatial and temporal distribution of fumigants in soil. Soil microbial community changes are expected to depend on application technique, soil properties, and environmental conditions, including wind direction, rain, and temperature. Therefore, in the dry arid region with limited rainfall, microbial community will re-establish after the initial effects due to fumigation and the use of MeBr and MeI as fumigants may not have the long-term effects on the overall soil quality.

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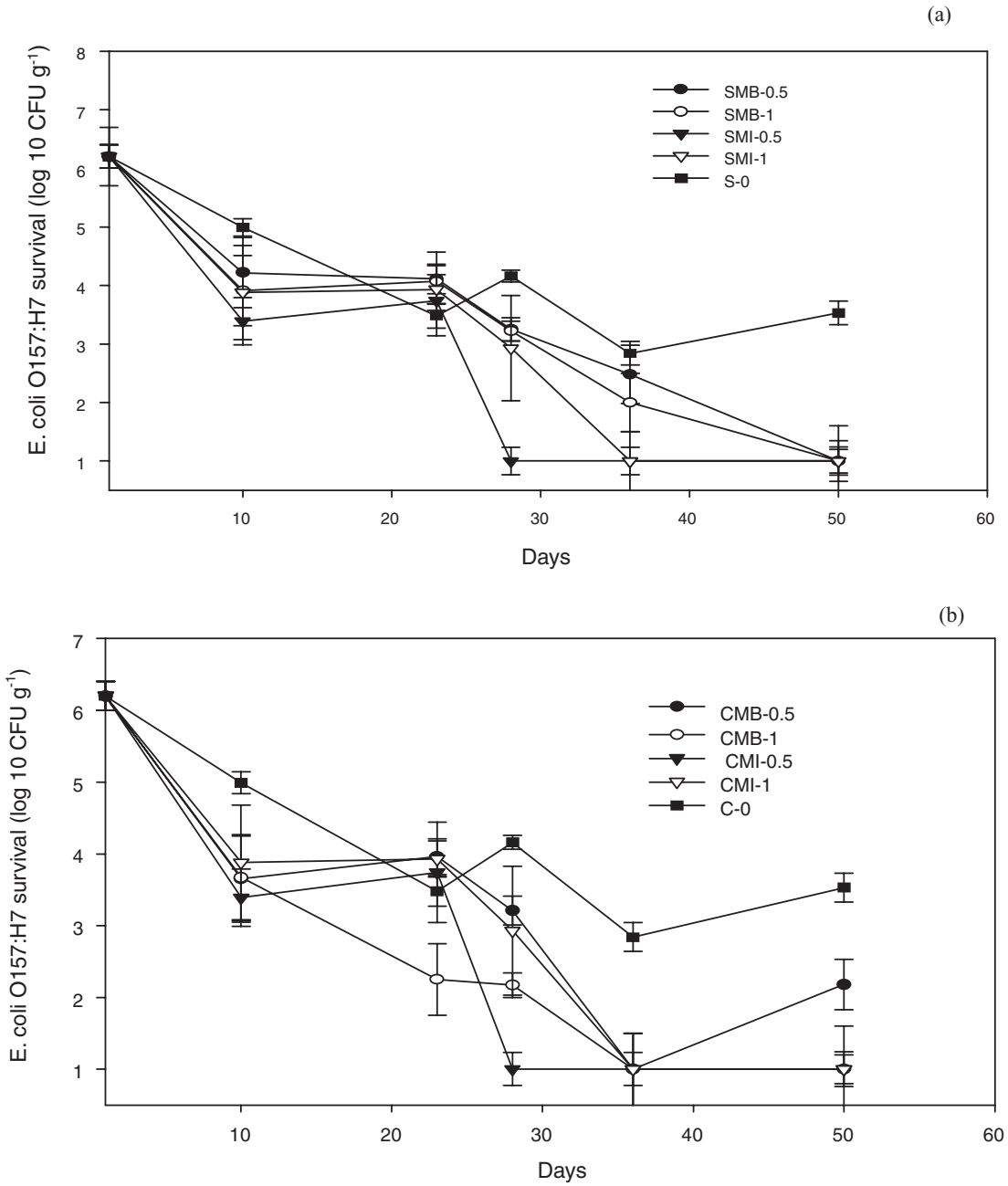

Fig. 4. Survival of *E. coli* O157:H7 in sandy (A) and clay (B) soils in the growth chamber soils. Plate counts of zero were replaced with 10 CFU g dw^{-1}, which was the calculated detection limit of the dilution plating procedure.

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