



TRICHLOROETHYLENE (TCE) AND TOLUENE EFFECTS ON THE STRUCTURE AND FUNCTION OF THE SOIL COMMUNITY

MARK E. FULLER,^{*,1,2†} KATE M. SCOW,¹ SEAN LAU³ and HOWARD FERRIS³

¹Department of Land, Air and Water Resources, University of California, Davis, CA, U.S.A.,

²Ecotoxicology Program, University of California, Davis, CA, U.S.A. and ³Department of Nematology, University of California, Davis, California 95616, U.S.A.

(Accepted 14 August 1996)

Summary—The effects of trichloroethylene (TCE) and toluene on indigenous soil communities were examined in soil microcosms, with special emphasis on community structure and function. Soil communities were exposed to TCE (1, 30 and 60 $\mu\text{g ml}^{-1}$) plus toluene (20 $\mu\text{g ml}^{-1}$), TCE only (1, 30 and 60 $\mu\text{g ml}^{-1}$), and toluene only (20, 60, 100 and 200 $\mu\text{g ml}^{-1}$) (all concentrations given as $\mu\text{g ml}^{-1}$ soil solution after taking into account sorption and volatilization). Soil microbial populations exposed to 30 or 60 $\mu\text{g TCE ml}^{-1}$ plus 20 $\mu\text{g toluene ml}^{-1}$, or to toluene concentrations greater than 60 $\mu\text{g toluene ml}^{-1}$, exhibited different sole carbon source utilization patterns than control soils, as revealed by Biolog Gram negative (GN) plates. These differences remained even 30 days after all the TCE and toluene had been removed from the system. Exposure to TCE alone did not alter Biolog patterns to the same degree as TCE combined with toluene. Thirty and 60 $\mu\text{g TCE ml}^{-1}$, either in the presence or absence of 20 $\mu\text{g toluene ml}^{-1}$, slightly reduced basal respiration after 28 days of incubation, whereas substrate induced respiration was not affected. Total culturable heterotrophs were not sensitive to TCE and toluene, and were only slightly reduced after exposure to 200 $\mu\text{g ml}^{-1}$ toluene for 45 days. Most protozoa were insensitive to TCE and toluene, but TCE was toxic to flagellates. Significant decreases in numbers of nematode types occurred after exposure to 30 or 60 $\mu\text{g TCE ml}^{-1}$. The nematode maturity index (MI), an index of diversity based on trophic groupings, was not sensitive to TCE or to TCE combined with toluene in an agricultural soil, whereas the MI in a riparian soil was reduced by concentrations of 30 $\mu\text{g TCE ml}^{-1}$. These results indicate that TCE and toluene adversely affect the biota of the soil ecosystem, sometimes for prolonged periods. © 1997 Elsevier Science Ltd

INTRODUCTION

There is a growing interest in understanding how the different members of a soil community interact (Hunt *et al.*, 1987; Ingham *et al.*, 1991; Beare *et al.*, 1992), and what role different classes of biota have in nutrient cycling and aboveground ecology (Ingham *et al.*, 1985; Crossley *et al.*, 1989; Huhta *et al.*, 1989; Wright *et al.*, 1989; Carey *et al.*, 1992; Lavelle, 1994). At the same time, there has been a realization that only limited data are currently available on how anthropogenic inputs to the environment may affect the soil biocenosis (Coleman *et al.*, 1992; Esher *et al.*, 1992; Curtis *et al.*, 1994).

Microorganisms are responsible for a large proportion of the decomposition and nutrient cycling that occurs in soil. Microbial activity is also the primary mechanism by which organic pollutants are removed from soil. While microorganisms represent one of the largest reservoirs for essential soil nutri-

ents, predators such as amoebae, nematodes and micro-invertebrates, are essential for the efficient release of these nutrients for plant use. Larger soil fauna also contribute to nutrient turnover through physical mixing, maintenance of soil aeration and redistribution of microflora (Crossley *et al.*, 1989; Wright *et al.*, 1989; Lavelle, 1994).

The effects of pesticides on soil microorganisms and their functions has been more intensely studied than the effects of industrial pollutants, and some generalizations about these effects have been summarized by Domsch *et al.* (1983). For instance, nitrification and organic matter decomposition are among the processes most sensitive to agrochemicals, while total bacterial numbers and biomass are quite insensitive. The authors concluded that adverse effects that lasted less than 60 days were tolerable when viewed in light of the fluctuations in these organisms and their activities brought about by natural environmental stresses, such as water potential or temperature. Hicks *et al.* (1989) have proposed applying the same principles used for pesticide testing to the assessment of effects of all classes of xenobiotic compounds found in the en-

*Current address: Environmental Research Division, ER/203-J070, Argonne National Laboratory, Argonne, IL 60439.

†Author for correspondence.

vironment. In Germany, studies on the effects of pentachlorophenol, 2,4-D and aldicarb at the ecosystem level demonstrated shifts in plant and animal community structure, as well as changes in nutrient cycling (Mathes and Weidemann, 1990). The exposed areas remained distinct from their respective controls even 1–4 years after the last exposure to the chemical, thus reinforcing the idea that altered ecosystems may only slowly (or perhaps never) return to their native states.

A greater understanding of the effects of pollutants on soil biota can be achieved by simultaneously studying the effects of these compounds on several trophic levels. The absence of effects at one level does not preclude effects at other levels due to the highly complex and integrated nature of the soil community.

The effects of trichloroethylene (TCE) and toluene, both of which are widespread environmental pollutants (Westrick *et al.*, 1984), on humans are well documented (World Health Organization, 1985; Hughes *et al.*, 1994; Meek and Chan, 1994), while their potential environmental effects have only been investigated in a cursory fashion (Kanazawa and Filip, 1986; Elnabarawy *et al.*, 1988; Ferrando and Andreu-Moliner, 1992). TCE is degraded under aerobic conditions by cometabolism, and phenol, toluene, propylene, natural gas methane and ammonia have been shown to be suitable cosubstrates to support this process (Fliermans *et al.*, 1988; Nelson *et al.*, 1988; Vannelli *et al.*, 1990; Ensley, 1991; Ensign *et al.*, 1992). Previous work in this laboratory has shown that the concentrations of TCE and toluene, singly and in combination, determine both the degradation kinetics for these compounds and the population dynamics of the organisms responsible for their biodegradation (Mu and Scow, 1994). In addition, it has been shown that a metabolite of TCE degradation, presumably TCE epoxide, is more toxic to the organisms that degrade TCE than is TCE itself (Wackett and Householder, 1989). To further our understanding of the interactions of these two chemicals with other soil biota, we conducted experiments to determine the effects of different concentrations of TCE and toluene on the microbial and faunal communities of soil, and to test the hypothesis that the effects caused by TCE alone would be less than those caused by the combination of TCE and toluene. The relative reversibility of observed effects in closed systems was also examined to assess the resiliency of the soil biota after exposure to anthropogenic stresses.

MATERIALS AND METHODS

Yolo silt loam (typic xerorthents, 1.2% OC, pH 6.8) was collected from an agricultural field at the University of California, Davis in Yolo County.

Riparian soil was collected along Putah Creek, adjacent to the agricultural fields. All soils were dried enough to pass through either a 2 or 4 mm sieve and stored in plastic bags at 4°C until use. Prior to starting experiments, soil was incubated at 25°C for 18–24 h. In all experiments, soil was maintained at a moisture content of 16% (w/w), which is equivalent to approximately 70% of the moisture in the soil at 33 k Pa (0.33 bar).

Soils were weighed into 254 ml Qorpak Boston Rounds (Fisher Scientific, Fair Lawn, NJ) equipped with 24 mm Mininert caps (Dynatech Precision Sampling Corp., Baton Rouge, LA). Soils were incubated with initial TCE concentrations of 0, 1, 30 and 60 $\mu\text{g TCE ml}^{-1}$ in the presence or absence of 20 $\mu\text{g toluene ml}^{-1}$, or with 0, 20, 60, 100 and 200 $\mu\text{g toluene ml}^{-1}$ alone. These concentrations were chosen based on previous experiments which demonstrated that initial TCE and toluene concentrations lead to differential patterns of biodegradation (Mu and Scow, 1994), which in these experiments would lead to different overall exposures to the test compounds. Sorption and Henry's partition coefficients were used when calculating TCE and toluene additions; all concentrations are reported as $\mu\text{g ml}^{-1}$ soil solution (Fan and Scow, 1993). TCE and toluene were added as small volumes of pure liquid or saturated gas as needed, and bottles were rotated 20 times immediately after adding the compounds to facilitate mixing of the compounds throughout the soil. Bottles were kept in an environmental chamber maintained at $25 \pm 2^\circ\text{C}$. Biodegradation of TCE and toluene was measured by disappearance of the chemicals from the headspace using gas chromatography. The oxygen concentration in each sealed bottle was measured immediately before terminating each incubation. TCE or toluene were removed from the soil by purging with moist air for 20 min, while shaking each bottle every 4 min. GC analysis showed that this purging reduced the highest TCE concentrations to below 1 $\mu\text{g ml}^{-1}$, and the highest toluene concentrations to below 5 $\mu\text{g ml}^{-1}$, with further manipulation reducing the residual to below detection limits. The soil pH was measured in a 2:1 soil paste in distilled water.

Determination of biologic sole carbon source utilization patterns and calculations of functional diversity indices

Three replicate bottles of 50 g (dry wt) of Yolo soil were incubated with 0, 1, 30 or 60 $\mu\text{g TCE ml}^{-1}$ with or without 20 $\mu\text{g toluene ml}^{-1}$, and with 0, 20, 60, 100 or 200 $\mu\text{g toluene ml}^{-1}$. After incubation and removal of remaining TCE and toluene, 1 g soil samples from each bottle were placed in 9 ml sterile soil extraction buffer (SEB, 50 mM, .694 mg l^{-1} KH_2PO_4 , .854 mg l^{-1} K_2HPO_4 , pH 6.8) in screw cap tubes and shaken at 0.3 m arc s^{-1} for

5 min. The suspension was diluted 1:10 with SEB twice more, the final dilution being 2 ml into 18 ml of SEB, contained in a 40 ml polycarbonate Oak Ridge tube (Fisher Scientific, Fair Lawn, NJ). The tubes were centrifuged at $500 \times g$ for 2 min to pellet clay particles, after which 140 μl of the cleared supernatant was added to each well of a Biolog Gram negative (GN) plate (Biolog Inc., Hayward, CA). Each plate has 96 wells, each with a single carbon compound and one well with no added substrate. The inoculated plates were placed in a sealed container along with a small vial of distilled water to maintain a moist environment and incubated at $25 \pm 2^\circ\text{C}$. Optical densities (595 nm) of the microtitre wells were measured after 50, 66 and 90 h using a Molecular Devices Corporation microplate reader (Menlo Park, CA) connected to an IBM computer equipped with SOFTmax(v. 2.01) software.

The functional diversity of the microbial community, defined as utilization of Biolog substrates, was calculated based on Zak *et al.* (1994). The specific indices calculated were chosen to give a broad picture of several aspects of carbon source utilization. The control well OD_{595} was subtracted from each well before making the calculations. Richness, S , defined here as the total number of substrates utilized, was calculated by adding up all the wells in the Biolog plate with an OD_{595} at least two times that of the control well. Diversity, H' , was calculated using the equation

$$H' = \sum_{i=1}^{C^*} p_i \ln p_i, \quad (1)$$

where C^* is the total number of substrates ($=95$) and p_i is the ratio of the i th substrate's OD_{595} to the total OD_{595} of the plate. Higher values of H' denote greater diversity. Evenness, E , is a measure of the equability of substrate utilization between all utilized substrates. E was calculated two different ways, firstly as the modified Hill's ratio

$$E1 = \frac{1/\lambda - 1}{e^{H'} - 1}, \quad (2)$$

where λ is the sum of the squares of p_i ; and secondly by the method of Peilou (Ludwig and Reynolds, 1988)

$$E2 = \frac{H'}{\log(S)}. \quad (3)$$

The closer $E1$ and $E2$ are to unity, the more equal the distribution of activities. Ludwig and Reynolds (1988) have commented that $E2$ is more sensitive to species richness than $E1$.

Additionally, soil was incubated with 60 μg TCE ml^{-1} in the presence of 20 μg toluene ml^{-1} and 100 and 200 μg toluene ml^{-1} for 28 days, vented and allowed to incubate further in order to assess the reversibility of effects on Biolog patterns. After 28

days, three replicate bottles of each treated soil were assayed for the desired activities, while the remaining bottles were resealed and re-incubated. After an additional 7 and 30 days, the remaining exposed and control soils were assayed.

Determination of nematode abundance and calculation of maturity index

Three replicate bottles of 100 g (dry wt) of Yolo soil were incubated with 0, 1, 30 or 60 μg TCE ml^{-1} with or without 20 μg toluene ml^{-1} . Additionally, four replicate bottles of 50 g (wet wt) riparian soil were incubated with 0, 1 or 30 μg TCE ml^{-1} . After incubation and removal of remaining TCE and toluene, nematodes were extracted by a wet-sieving-sugar-centrifugation method (Jaffee *et al.*, 1988), and members of each family were quantified. The maturity index (MI) was calculated as described by Bongers (1990). The MI is based on grouping of nematode taxa into five categories ranging from "colonizers" characterized by rapidly reproducing opportunists (r-strategists), to "persisters" characterized by slower reproductive rates (K-strategists). Colonizers are assigned a value of 1 and persisters a value of 5, while families which have characteristics of both categories are assigned intermediate values on the colonizer-persister (c-p) scale. The MI value is calculated as the weighted mean of the individual c-p values:

$$MI = \sum_1^i v(i) * f(i), \quad (4)$$

where $v(i)$ is the c-p value of taxon i and $f(i)$ is the proportion of taxon i in a sample (Bongers, 1990). Nematodes belonging to families with a c-p value of 1 were ignored in all MI calculations because their presence is indicative of substrate availability rather than chemical stress (Bongers and Korthals, 1993). Abundance results are presented as number of nematodes 100 g soil $^{-1}$ (dry wt).

Enumeration of protozoa

The serial dilutions above for the soil exposed to TCE and toluene were used for determining protozoa by the microplate most probable number (MPN) procedure of Ingham (personal communication). Plates were incubated for 7 days, after which the presence of amoebae, ciliates and flagellates in each well was determined using a microscope equipped with Nomarsky imaging optics. Presence or absence data were used as input for a BASIC computer program designed to calculate the MPN (Clarke and Owens, 1983). Results are presented as the MPN of protozoa g soil $^{-1}$ (dry wt).

Soil arthropod activity and plant germination

Three replicate bottles of 100 g (dry wt) of Yolo soil were incubated with 0, 1, 30 or 60 μg TCE

ml⁻¹ with or without 20 µg toluene ml⁻¹. Bottles were inspected periodically for evidence of soil arthropod activity, by the presence of visible burrows in the soil. The number of plant seedlings in each bottle emerging from indigenous seeds was also counted and recorded over the course of the incubation.

Determination of microbial densities and activities

The effects of TCE and toluene on the number of culturable heterotrophs in soil was determined by incubating three replicate bottles of 100 g (dry wt) of Yolo soil with 0, 1, 30 or 60 µg TCE ml⁻¹ with or without 20 µg toluene ml⁻¹, and with 0, 20, 60, 100, or 200 µg toluene ml⁻¹. After incubation and removal of remaining TCE and toluene, soil samples (1 g) from each bottle were placed in 9 ml of sterile SEB in screw cap tubes and shaken at 0.3 m arc s⁻¹ for 5 min. Serial dilutions in SEB were made and plated in triplicate using the spread plate method on 0.1 strength trypticase soy agar. Plates were incubated in plastic bags at 25°C for 2–4 weeks, after which colonies on plates having 20 to 200 colonies were counted. Data are presented as colony forming units (CFU) g soil⁻¹ (dry wt).

The effects of TCE and toluene on respiration were determined by incubating three replicate bottles of 50 g (dry wt) of Yolo soil with 0, 1, 30 or 60 µg TCE ml⁻¹ with or without 20 µg toluene ml⁻¹. After incubation and removal of remaining TCE and toluene, substrate induced respiration (SIR) was carried out as described by Smith *et al.* (1985), except that 3 g soil was used and incubations were performed in 25 ml serum bottles. SIR is calculated by subtracting the CO₂ produced by unamended soil from the value obtained from glucose amended soil and is expressed as µg CO₂ produced g soil⁻¹ h⁻¹. Basal respiration rates were obtained from the unamended bottles. Additionally, samples of unexposed soil and soil incubated with 60 µg TCE ml⁻¹ and 20 µg toluene ml⁻¹ were also tested for their ability to mineralize [¹⁴C]citrate (S. A. 60 mCi mmol⁻¹, Sigma, St Louis, MO) and [¹⁴C]phenol (S. A. 130 mCi mmol⁻¹, Arlington Heights, IL) using biometer flasks designed in our laboratory. Citrate and phenol were added to the soil in 0.1 ml sterile water to achieve final concentrations of 273 and 64 ng ml⁻¹, respectively, with total activities of approximately 19,000 dpm for both compounds. The biometers consisted of 285 ml Mason jars (Ball Corporation, Muncie, IN) which held two 25 ml glass scintillation vials. One vial contained the soil and the radiolabelled chemical, and the other contained 1.0 ml of 0.5 N NaOH to trap carbon dioxide. Two holes drilled in the lid allowed the base to be sampled with 1.2 mm (o.d.) stainless steel cannulae (Thomas Scientific, Swedesboro, NJ). Base from the traps was mixed with 5 ml of UniverSol biodegradable scintillation

cocktail (ICN Biomedicals Inc., Irvine, CA) in 7.0 ml polypropylene scintillation vials (Fisher Scientific, Fair Lawn, NJ), capped, mixed and counted for 5.0 minutes in a Beckman LS 6000IC liquid scintillation counter (Beckman Instruments, Fullerton, CA).

The effects of TCE and toluene on soil dehydrogenase activity was determined by adaptation of the methods of Jeffrey and Paul (1986) and von Mersi and Schinner (1991). Three replicate 2 g (dry wt) samples of Yolo soil in 25 ml vials were incubated with 0, 1 or 60 µg TCE ml⁻¹. After 8 days, soil was vented and extracted with 18 ml Tris-NaCl buffer (6.35 g TRIZMA[®] hydrochloride, 1.18 g TRIZMA[®] base and 9.0 g NaCl l⁻¹ of NANOpure water, 50 mM, pH 7.5). Three 1 ml aliquots of each extract were placed in 25 ml vials, amended with 0.2 ml of INT (2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride, 0.4%), mixed and incubated at 25°C for 21 h. INT-formazan was extracted from each solution by adding 4.8 ml methanol and measuring the absorbance at 470 nm of the solution after sonicating for 5 min followed by 10 min of horizontal shaking. Results were calculated as ng INT-formazan produced ml⁻¹ soil extract.

Analytical methods

TCE and toluene were quantified by injecting 50 µl of headspace into a GC-14A gas chromatograph (Shimadzu, Columbia, MD) using a gastight syringe (Hamilton, 1705RN). The GC was equipped with a 30 m, RTX502.2 megabore column (Restek Corp., Bellefonte, PA) and a photon ionization detector. The injector temperature was 200°C. Column temperature was maintained at 90 or 200°C for 6 min (isothermal measurement). The carrier gas was ultrapure He (99.995%) at a flow rate of 15 ml min⁻¹ having a linear velocity of 30 cm s⁻¹. The makeup gas was ultrapure He with a flowrate of 30 ml min⁻¹.

Carbon dioxide and oxygen concentrations in sealed bottles were measured by injecting 1.0 ml headspace samples into an Horiba PIR-2000 CO₂ analyzer (Horiba Ltd, Kyoto, Japan) connected serially to an S-3A O₂ analyzer (Applied Electrochemistry Inc., Sunnyvale, CA). Both devices sent output to Omniscrite chart recorders (Houston Instrument, Austin, TX). The carrier gas was N₂ flowing at 150 ml min⁻¹.

Data analysis

Microbial, protozoa and seedling abundance and soil respiration data were analyzed by ANOVA using StatView (v. 4.0, Abacus Concepts Inc., Berkeley, CA). Treatment means were tested for significant differences at the 5% level using the Bonferroni-Dunn *post hoc* procedure. Protozoa MPN data were transformed by taking the reciprocal to make the standard deviations approximately

equal before statistical tests were done. Means of nematode counts and maturity indices were compared using Duncan's multiple range test.

Biolog data were analyzed by principal component analysis (PCA) and canonical correspondence analysis (CCA) using CANOCO (v. 3.1, Microcomputer Power, Ithaca, NY). Principal components analysis is an ordination method designed to reduce a large number of variables to a small number of uncorrelated indices, or axes, that are linear combinations of the original variables (Manly, 1986). Canonical correspondence analysis allows a direct analysis of the effect of specific environmental variables because the ordination axes are constrained to be linear combinations of the environmental variables (ter Braak, 1987). For our results, the initial concentration of TCE and toluene were used as forwardly selected environmental variables while running CANOCO. On CCA biplots, the influence of environmental variables is indicated by arrows whose lengths are proportional to their importance. The statistical association between TCE and toluene concentrations and the observed Biolog patterns were tested using the Monte Carlo permutation test (Collins, 1987). Polygons were drawn to delineate data points from each treatment to facilitate interpretation.

RESULTS

Effects of TCE and toluene concentrations on biodegradation, oxygen concentrations and pH

The degradation of TCE and toluene was inversely proportional to the initial TCE concentration in Yolo soil. Toluene readily degraded within 10 days in soil exposed to 0 or 1 $\mu\text{g TCE ml}^{-1}$. In soil exposed to 30 $\mu\text{g TCE ml}^{-1}$, toluene degradation did not commence for 20 days, and 10% still

remained when the experiment was terminated after 30 days. No toluene degradation occurred when the initial TCE concentration was 60 $\mu\text{g TCE ml}^{-1}$. Degradation of TCE did not occur in the absence of toluene, or when initial TCE concentrations were greater than 1 $\mu\text{g TCE ml}^{-1}$. Approximately 40% of TCE was cometabolized after 10 days in soil exposed to 1 $\mu\text{g TCE ml}^{-1}$ and 20 $\mu\text{g toluene ml}^{-1}$, and only decreased another 10% over the duration of the incubation. Final O_2 concentrations in the bottles after 30 days were not appreciably different among treatments (12–18%, v/v) and no change in soil pH was observed.

Increasing the initial toluene concentrations in Yolo soil resulted in longer lag periods before the onset of toluene degradation. Twenty $\mu\text{g toluene ml}^{-1}$ was completely degraded in soil after 10 days of incubation, whereas soil exposed to 60 and 100 $\mu\text{g toluene ml}^{-1}$ did not commence degradation for 10 and 20 days, respectively. Degradation of 60 $\mu\text{g toluene ml}^{-1}$ was completed by 18 days, while the soil required 45 days to consume 95% of an initial 100 $\mu\text{g toluene ml}^{-1}$. Initial concentrations of 200 $\mu\text{g toluene ml}^{-1}$ were never observed to be degraded over the course of the 45 day experiment. Degradation of 60 and 100 $\mu\text{g toluene ml}^{-1}$ reduced O_2 concentrations to 8 and 2%, respectively. These levels were significantly lower than those in bottles containing unexposed soil and soil exposed to 20 and 200 $\mu\text{g toluene ml}^{-1}$, which possessed 14, 11 and 13% O_2 , respectively. Incubation for 28 days with 100 and 200 $\mu\text{g toluene ml}^{-1}$ only reduced the O_2 concentration to about 8 and 13%, respectively. No changes in soil pH were observed for any of the treatments.

Effects of TCE and toluene on Biolog patterns and functional diversity indices

Exposure of Yolo soil to 30 and 60 $\mu\text{g TCE ml}^{-1}$ in the presence and absence of 20 $\mu\text{g toluene ml}^{-1}$

Table 1. Functional diversity parameters based on Biolog data for Yolo soil exposed to different concentrations of TCE and toluene (66 h incubation of Biolog GN plates)[†]

	Richness (<i>S</i>) ^a	Diversity (<i>H'</i>) ^b	Evenness ^b	
			E1	E2
Control soil	45–53	3.90–4.06	0.82–0.86	2.35–2.56
TCE only				
1	49	3.99	0.83	2.37
30	42	3.89	0.80	2.40
60	38	3.89	0.87	2.39
TCE + 20 $\mu\text{g ml}^{-1}$ toluene				
1	46–52	3.95–4.01	0.83–0.85	2.33–2.38
30	38–41	3.90–3.93	0.76–0.78	2.47–2.52
60	19–27	3.45–3.49	0.68–0.75	2.44–2.69
Toluene only				
20	35–50	3.76–4.02	0.77–0.83	2.35–2.44
60	35	3.80	0.77	2.47
100	9	3.58	0.57	3.85
200	0	3.11	0.54	NA ^c

[†] Ranges indicate that data was drawn from two separate experimental trials.

^a Total substrates demonstrating an $\text{OD}_{595} \geq 3 \times$ control well OD_{595} .

^b See Methods section for equations employed.

^c NA, not applicable.

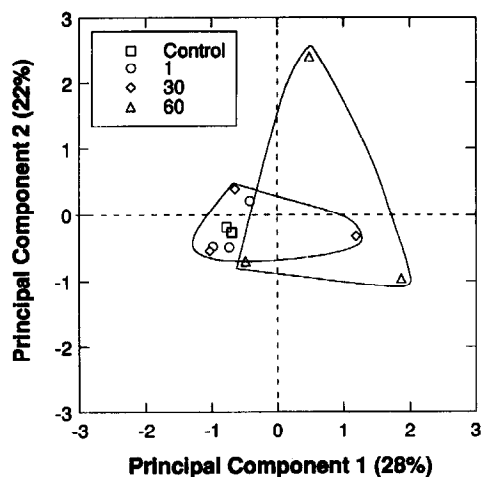


Fig. 1. Principal component analysis for Biolog patterns of agricultural soil exposed to different amounts of TCE (all concentrations are in $\mu\text{g ml}^{-1}$). Numbers in parentheses indicate the percentage of the variance accounted for by a particular axis.

for 28 days resulted in decreases in substrate utilization (S), diversity (H') and evenness ($E1$, $E2$) as determined by sole carbon source utilization patterns of the soil microbial community (Table 1). The TCE concentration was not significant in determining the pattern of carbon source utilization when data from soil exposed to TCE alone was analyzed using principal component analysis (PCA), although the treatments were visually separated (Fig. 1). However, CCA of data combined from three separate experiments in which soil was exposed to TCE and toluene revealed shifts in carbon source utilization (Fig. 2). The observed separ-

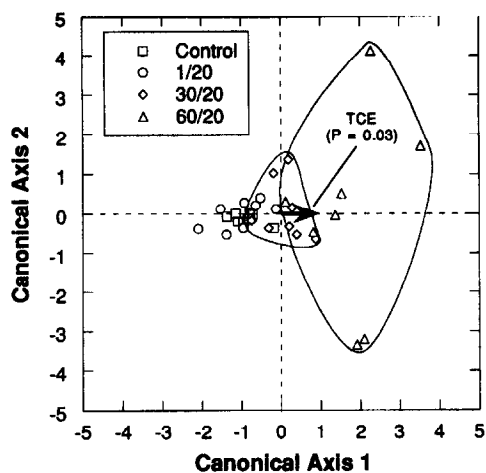


Fig. 2. Canonical correspondence analysis for Biolog patterns of agricultural soil exposed to different amounts of TCE and toluene (all concentrations are in $\mu\text{g ml}^{-1}$). \square , control; \circ , 1 TCE + 20 toluene; \diamond , 30 TCE + 20 toluene; \triangle , 60 TCE + 20 toluene.

ation of the treatments along the x -axis was significantly dependent upon the initial TCE concentration ($P = 0.03$). Treatment of soil with 60 $\mu\text{g TCE ml}^{-1}$ resulted in substantial differences between replicates, while the patterns for the other treatments were usually reproducible, especially for the unexposed soil. The substrates most responsible for the separation observed in the graph are given in Table 2. PCA of Biolog patterns was performed for soil exposed to 60 $\mu\text{g TCE ml}^{-1}$ and 20 $\mu\text{g toluene ml}^{-1}$ for 28 days, and then vented and re-incubated. Some recovery of initial Biolog patterns was observed, as evidenced by the overlapping of treat-

Table 2. Substrates positively and negatively associated with exposure to high concentrations of TCE or toluene, allowing exposed and unexposed soils to be separated by multivariate analysis of Biolog patterns

	Positive association	Negative association
High TCE		
5 Experiments	NONE	α -ketovaleric acid (AC)
4 Experiments	gentiobiose (C)	D-galacturonic acid (AC)
	thymidine (N)	<i>p</i> -hydroxyphenylacetic acid (AC)
	D-alanine (AA)	γ -hydroxybutyric acid (AC)
	itaconic acid (AC)	
	glycyl-L-aspartic acid (AA)	
	L-threonine (AA)	
	glucose-6-phosphate (C)	
3 Experiments	L-ornithine (AA)	phenylethylamine (AM)
	formic acid (AC)	cellobiose (C)
	D-serine (AA)	D,L-carnitine (AA)
	α -ketobutyric acid (AC)	
High Toluene		
2 Experiments	formic acid (AC)	2-aminoethanol (AM)
1 Experiment	β -methyl-D-glucoside (C)	D-galacturonic acid (AC)
	acetic acid (AC)	glucose-6-phosphate (C)
	Tween 40 (P)	putrescine (AM)
	α -ketovaleric acid (AC)	D-glucuronic acid (AC)
		Tween 80 (P)
		D-glucosaminic acid (AC)
		mannitol (C)
		γ -hydroxybutyric acid (AC)
		D-alanine (AA)

AA, amino acid; AC, acid; AM, amine; C, carbohydrate; N, nucleotide; P, polymer.

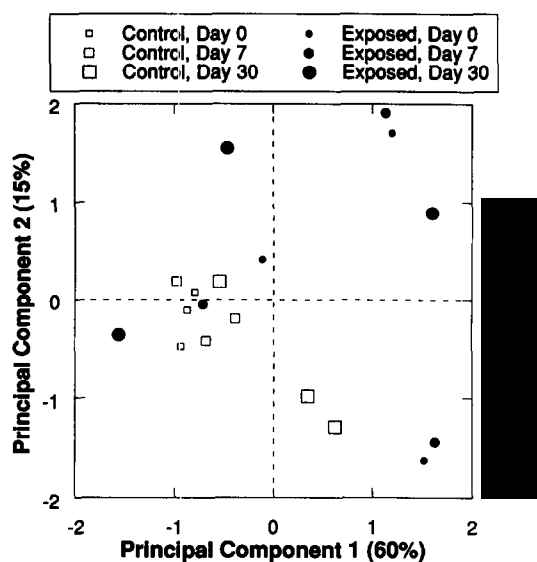


Fig. 3. Recovery of Biolog patterns in Yolo soil exposed to $60 \mu\text{g TCE ml}^{-1}$ and $20 \mu\text{g toluene ml}^{-1}$ for 28 days, vented and incubated for an additional 7 or 30 days as revealed by principal component analysis. Squares, control soil; circles, exposed soil. Numbers in parentheses indicate the percentage of the variance accounted for by a particular axis.

ments along the x-axis (Fig. 3). Diversity indices for exposed soil, however, did not return to those seen in the unexposed soil. Diversity indices tended to decrease over time for all treatments including unexposed soil (Table 3).

The higher toluene concentrations also resulted in marked reductions in all functional diversity indices (Table 1). CCA detected differences between treatments with respect to carbon source utilization patterns (Fig. 4), with treatments distributed along the x-axis which was significantly related to initial toluene concentrations ($P = 0.01$). Those carbon substrates that were responsible for differences among

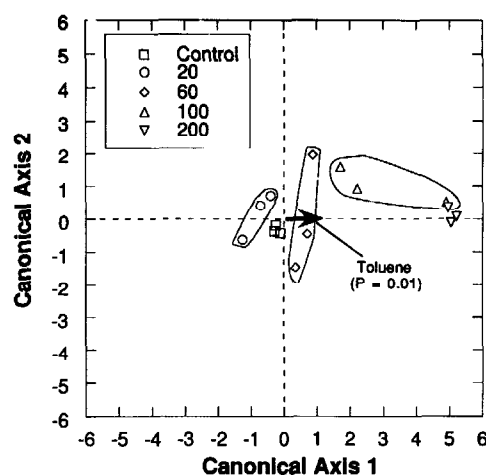


Fig. 4. Canonical correspondence analysis for Biolog patterns of agricultural soil exposed to different amounts of toluene (all concentrations are in $\mu\text{g ml}^{-1}$).

treatments are listed in Table 2. Exposure to $200 \mu\text{g toluene ml}^{-1}$ was very toxic to soil microorganisms, as evidenced by the absence of utilization of any of the Biolog substrates. No significant recovery of the indices was observed in soil that had been exposed for 28 days to 100 or $200 \mu\text{g toluene ml}^{-1}$, vented and allowed to incubate for an additional 7 or 30 days (Table 3). The patterns of the exposed ($100 \mu\text{g toluene ml}^{-1}$) and unexposed soils did not overlap even after 30 days of incubation without toluene (Fig. 5).

Effects of TCE and toluene on microbial populations and activities

No significant changes in total heterotrophs were observed between treatments after 30 days exposure to TCE with or without toluene, with 0.1 strength TSA plate counts of approximately $1.4 \pm 0.2 \times 10^8 \text{ CFU g}^{-1}$ soil. Yolo soil exposed for

Table 3. Functional diversity parameters based on Biolog data for Yolo soil exposed to different concentrations of TCE and toluene for 28 days, vented, and allowed to incubate for an additional 7 or 30 days

	Richness (S) ^a	Diversity (H) ^b	Evenness ^b	
			E1	E2
0 Days post-vent				
Control	51	4.02	0.83	2.36
60 TCE + 20 toluene	22	3.61	0.74	2.58
100 toluene	3	3.33	0.40	9.66
200 toluene	ND ^c	ND	ND	ND
7 Days post-vent				
Control	48	3.97	0.83	2.37
60 TCE + 20 toluene	19	3.49	0.74	2.51
100 toluene	5	3.07	0.50	4.80
200 toluene	ND	ND	ND	ND
30 Days post-vent				
Control	37	3.77	0.84	2.41
60 TCE + 20 toluene	23	3.49	0.73	2.59
100 toluene	3	2.68	0.48	3.85
200 toluene	1	3.19	0.34	NA ^d

^a Total substrates demonstrating an $\text{OD}_{595} \geq 3 \times$ control well OD_{595} .

^b See Methods section for equations employed.

^c ND, not determined.

^d NA, not applicable.

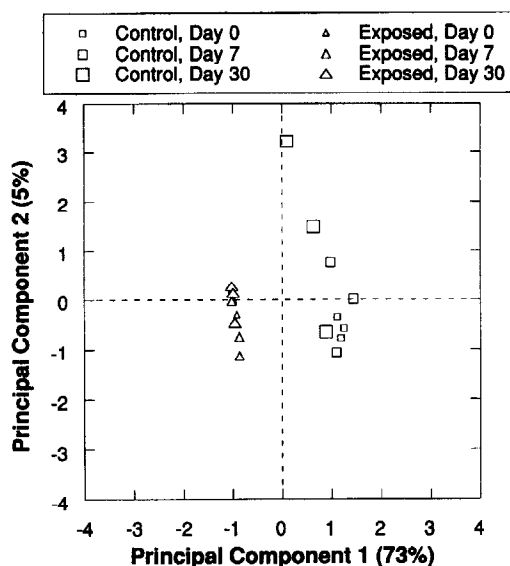


Fig. 5. Lack of recovery of Biolog patterns of agricultural soil exposed to $100 \mu\text{g}$ toluene ml^{-1} for 28 days, vented and incubated for an additional 7 or 30 days as revealed by principal components analysis. Squares, control soil; triangles exposed soil. Numbers in parentheses indicate the percentage of the variance accounted for by a particular axis.

45 days to different toluene concentrations also exhibited no changes in the number of colonies appearing on 0.1 strength TSA plates ($3.5 \pm 0.3 \times 10^8$ CFU g^{-1} soil), except for a 6-fold reduction in CFUs after incubation with $200 \mu\text{g}$ toluene ml^{-1} ($5.6 \pm 2.7 \times 10^7$ CFU g^{-1} soil). During the first week of incubation, total plate counts in soil exposed to $20 \mu\text{g}$ toluene ml^{-1} , in both the presence and absence of $1 \mu\text{g}$ TCE ml^{-1} , increased approximately 2-fold.

SIR was not affected by different concentrations of TCE regardless of the presence of toluene, but the average response significantly decreased from 20.8 ± 2.1 to $13.8 \pm 1.1 \mu\text{g}$ $\text{CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ after 9 and 28 days of incubation, respectively ($P < 0.001$). Basal respiration in the absence of added glucose

was significantly lower in soil exposed to 1, 30 and $60 \mu\text{g}$ TCE ml^{-1} in the presence of toluene, and 30 and $60 \mu\text{g}$ TCE ml^{-1} in the absence of toluene, than in unexposed soil ($P \leq 0.05$). Over the duration of the experiment, the average basal respiration exhibited a significant decrease, falling from $39.5 \pm 2.4 \mu\text{g}$ $\text{CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ on day 9 to $28.8 \pm 2.1 \mu\text{g}$ $\text{CO}_2 \text{ g}^{-1} \text{ h}^{-1}$ on day 28 ($P < 0.0001$).

Exposed and unexposed soil utilized citrate and phenol virtually identically; both soils mineralized 75% of the citrate and 33% of the phenol in 250 h. Neither TCE nor toluene, alone or in combination, affected soil dehydrogenase activity, which ranged from 4.6 to $5.9 \text{ ng INT-formazan produced ml}^{-1}$ soil extract.

Effects of TCE and toluene on nematodes, protozoa, arthropods and seedlings

A list of nematode families identified, their corresponding c-p values and initial abundances in the two soils are given in Table 4. Total nematode numbers (across all families and trophic groups) were higher in Yolo soil than in the riparian soil (Table 5, Experiment 1). In the absence of TCE and toluene, nematode abundance appeared to increase in both soils after 28 days of incubation. While the abundance of nematodes in Yolo soil increased in the presence of $1 \mu\text{g}$ TCE ml^{-1} , an increase was not seen in the riparian soil (Table 5). After 28 days, nematode abundance in both soils was lowest after exposure to $30 \mu\text{g}$ TCE ml^{-1} (Table 5).

In Yolo soil amended with 0 and $1 \mu\text{g}$ TCE ml^{-1} , total nematode numbers approximately doubled in 28 days in the presence of $20 \mu\text{g}$ toluene ml^{-1} , but did not change when toluene was absent. Nematode abundance decreased after exposure to 30 and $60 \mu\text{g}$ TCE ml^{-1} , but was not further affected by the presence of toluene [Fig. 6(A)] ($P < 0.05$). Nematodes in different trophic classifications differed in their sensitivity to TCE. At concentrations of 0 and $1 \mu\text{g}$ TCE ml^{-1} , bacterial-feeding nematode populations were significantly increased when toluene was also present. The number of bacterial-feeders decreased

Table 4. Trophic groupings, colonizer-persister (c-p) values and percent representation in the community for nematode families in Yolo and riparian soils prior to the addition of TCE and toluene

Trophic group or family	Bongers c-p value	Percent of community in	
		Yolo	Riparian
Bacterial feeders			
<i>Cephalobidae</i>	2	25	14
<i>Prismatolaimidae</i>	3	3	10
<i>Rhabditidae</i>	(1)	3	33
Fungal feeders			
<i>Aphelenchoididae</i>	2	6	10
<i>Aphelenchidae</i>	2	31	0
Herbivores			
<i>Pratylenchidae</i>	(3)	11	5
<i>Tylenchidae</i>	(2)	14	10
Predators			
<i>Dorylamidae</i>	4	8	19

c-p values in parentheses were not used to calculate the maturity index.

Table 5. Effect of TCE concentration on nematode abundance and MI values in Yolo and riparian soil after 28 days of incubation

TCE ²	Toluene ³	Abundance ¹		MI	
		Yolo	Riparian	Yolo	Riparian
<i>Experiment 1</i>					
Initial		1504	288	2.2	2.9
0	-	2165 (290)a	956 (198)a	2.2 (0.1)a	2.7 (0.2)a
1	-	2827 (528)a	708 (119)a	2.1 (0.1)a	2.6 (0.3)a
30	-	1771 (412)a	628 (234)a	2.2 (0.1)a	2.3 (0.1)b
<i>Experiment 2</i>					
Initial				2.27	
0	-			2.19 (0.05)abc	
	+			2.09 (0.01)c	
1	-			2.14 (0.03)bc	
	+			2.10 (0.03)c	
30	-			2.34 (0.05)ab	
	+			2.28 (0.15)abc	
60	-			2.39 (0.19)a	
	+			2.10 (0.07)c	

¹ Nematodes 100 g⁻¹ soil.

² μg ml⁻¹ soil solution.

³ 20 μg toluene ml⁻¹ soil solution.

Data are presented as mean (±standard deviation). Numbers followed by different letters denote significant differences ($P \leq 0.05$). Each soil type was analyzed separately.

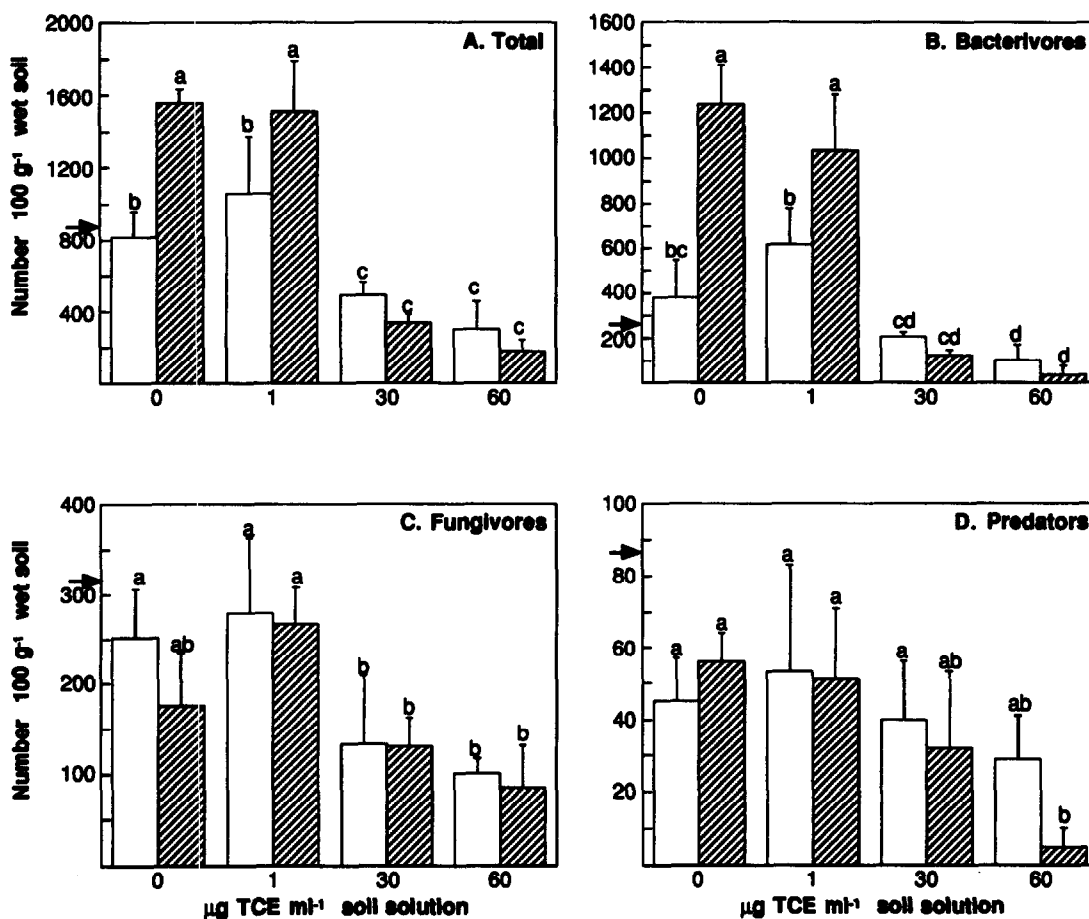


Fig. 6. Nematode abundance levels in agricultural soil exposed to different levels of TCE. Plain bars, without toluene; striped bars with toluene (20 μg ml⁻¹). (a) total nematodes (including plant parasites); (b) bacterivorous nematodes; (c) fungivorous nematodes; (d) predatory nematodes. Error bars denote 1 standard deviation. Bars marked with the same letters are not significantly different ($P \leq 0.05$) as determined by Duncan's multiple range test. Arrow indicates the initial nematode abundance before soil was incubated.

Table 6. Effect of TCE concentration and/or toluene ($20 \mu\text{g ml}^{-1}$) on seed germination and arthropod activity in Yolo soil after 10 days

TCE	Toluene	Seedlings	Burrows [†]
0	–	15 (4)a	++
	+	13 (3)a	++
1	–	16 (3)a	+ ¹
	+	12 (3)a	–
30	–	1 (1)b	–
	+	0 (0)b	–
60	–	0 (0)b	–
	+	0 (1)b	–

[†] Burrows were scored as to their apparent abundance along the glass-soil interface.

¹ Burrows were much smaller diameter.

Data are presented as mean (\pm standard deviation). Numbers followed by different letters denote significant differences ($P \leq 0.05$).

after exposure to 30 and $60 \mu\text{g TCE ml}^{-1}$, and no stimulatory effect of toluene was observed [Fig. 6(B)] ($P < 0.05$). Fungivorous nematodes were not stimulated by the presence of toluene at low TCE concentrations, and were decreased after exposure to 30 and $60 \mu\text{g TCE ml}^{-1}$ [Fig. 6(C)] ($P < 0.05$). The low numbers of predatory nematodes were only reduced at the highest TCE exposure when toluene was also present [Fig. 6(D)] ($P < 0.05$).

In the absence of TCE and toluene, the MI of the riparian soil was higher than that of Yolo soil (Table 5, Experiment 1). This was primarily the result of the greater diversity of nematodes in the riparian soil compared to Yolo soil, including a greater proportion of representatives with c-p values of 3 and 4 (Table 4). The MI of the riparian soil declined slightly after incubation with 0 and $1 \mu\text{g TCE ml}^{-1}$, and was significantly lower after exposure to $30 \mu\text{g TCE ml}^{-1}$ ($P < 0.05$). Conversely, the MI values for Yolo soil were not affected by exposure to any concentration of TCE (Table 5, Experiment 1). Since Yolo soil possessed a greater proportion of nematodes with c-p values of 1 and 2 (Table 4), the effects of TCE on the more sensitive representatives of the higher maturity classes were not easily discerned.

In Experiment 2, the effect of TCE concentration on the MI values of Yolo soil showed no pattern. However, at all concentrations of TCE, the MI value was numerically lower after incubation in the presence of toluene than in its absence (Table 5, Experiment 2). While the majority of nematodes in Yolo soil belonged to c-p classes 1 and 2, only c-p classes 2–5 were included when calculating the MI. Of the taxa used, nematodes in class 2 represented the majority and constituted a near constant proportion of the total community, even when the total number of nematodes declined after incubation with higher TCE concentrations.

The MPN estimates of total protozoa, as well as ciliates and amoebae, were not affected by incubation with any concentration of TCE or toluene. Flagellate numbers were significantly reduced by exposure to $60 \mu\text{g TCE ml}^{-1}$ in the presence, but not

absence, of $20 \mu\text{g toluene ml}^{-1}$ ($P < 0.05$). None of the lower concentrations, with or without toluene, had any effect on the abundance of flagellates.

Soil arthropod activity, as evidenced by burrows along the soil-glass interface, was evident in the control bottles. However, no burrowing was obvious in soil exposed to $1 \mu\text{g TCE ml}^{-1}$ in the presence of toluene nor in any soils incubated with 30 or $60 \mu\text{g TCE ml}^{-1}$ in the presence or absence of toluene (Table 6). Germination of indigenous seeds was very rarely observed in Yolo soil exposed to 30 or $60 \mu\text{g TCE ml}^{-1}$ for 10 days, regardless of the presence of toluene, whereas the remaining treatments supported many seedlings (Table 6).

DISCUSSION

Effects on TCE and toluene biodegradation

Biodegradation data of TCE and toluene agreed with the results of Mu and Scow (1994) and showed clearly the dependence of kinetics on initial TCE and toluene concentrations. Other researchers have reported similar substrate interactions while working with TCE cometabolic systems (Wackett and Householder, 1989; Folsom *et al.*, 1990; Alvarez-Cohen and McCarty, 1991; Henry and Grbic-Galic, 1991; Heald and Jenkins, 1994). The mechanism underlying these results is believed to be competitive inhibition of TCE and toluene for the required enzymes, however TCE toxicity, leading to enzyme inactivation, may also involved (Fan and Scow, unpublished data).

Effects on the microbial community

Since organic matter degradation is one of the microbial activities considered by Domsch *et al.* (1983) to be most sensitive to agrochemicals at recommended application rates, we felt it was relevant to assess the effects of TCE and toluene on carbon source utilization and respiration of exposed soil communities.

To screen the use of a wide range of carbon sources easily, we decided to employ Biolog plates. Garland and Mills (1991) were the first to attempt

to distinguish microbial communities in natural systems on the basis of their Biolog patterns. Since then, Garland and Mills (1994) have found that microbial communities present in hydroponic solutions used to grow dwarf wheat (*Triticum aestivum* Cv *Yecora roja*) possessed Biolog patterns indicating high utilization of carboxylic acids and amino acids. Conversely, communities taken from a wheat waste bioreactor demonstrated high carbohydrate degradation abilities. Zak *et al.* (1994) used Biolog analysis to compare microbial communities in soils from different plant communities and analyzed the data by both univariate (functional diversity indices) and multivariate analyses (cluster and principle component analyses). They postulated that the soil microbial communities reflected the current vegetation growing in the soil, and that the communities grouped along a natural moisture gradient. Winding (1994) was not only able to distinguish between different soils based on cluster analysis of Biolog data, but was also able to show differential metabolic diversity of the microbial community in different sized fractions of the same soil. Using constructed microbial communities, Haack *et al.* (1995) demonstrated that different mixtures of organisms exhibited reproducible carbon source utilization patterns in Biolog plates. Interpreting the patterns with regard to the numbers or activities of specific microbial types was not found to be a simple task, and their results with actual soil communities lead them to conclude that microscale heterogeneity in soil further complicated direct interpretation of Biolog data. Bossio and Scow (1995) have been able to demonstrate that agricultural management practices applied to rice fields reproducibly altered soil Biolog patterns, and multivariate analysis of the data were interpretable within an ecological framework.

Our results indicate a consistently lower functional diversity in soil, as defined by utilization of Biolog substrates, exposed to either 30 or 60 $\mu\text{g TCE ml}^{-1}$ in combination with 20 $\mu\text{g toluene ml}^{-1}$, or exposed to 100 and 200 $\mu\text{g toluene ml}^{-1}$ only. Exposure to TCE and toluene caused larger reductions in the richness and diversity of carbon source utilization than did exposure to TCE alone. Exposed soils were also not observed to recover after TCE and toluene had been removed from the system. One explanation for this, considering the similarity in total plate counts among treatments, is that only specific guilds or groups of microbes were negatively affected by TCE and toluene. Diltz *et al.* (1992) found that surface soil exposed to unleaded gasoline under field conditions harbored a less diverse microbial community than unexposed soil (as determined by physiological testing of soil isolates) even 15 weeks after the gasoline application, whereas initially diminished microbial numbers quickly returned to densities which equaled those of

the control. In Antarctic soils, Kerry (1990) found that petroleum contamination resulted in a shift from fungal-dominated to bacterial-dominated microbial communities, and Cox *et al.* (1994) have shown that TCE contamination resulted in a greater proportion of Gram negative biomass in subsurface sediments. These results indicate the potential for TCE and toluene to alter both the structure and function of the soil microbial community.

The toxicity of TCE and toluene to microorganisms has been demonstrated under both laboratory and field conditions. For instance, Blum and Speece (1991) and Volskay and Grady (1988) demonstrated that methane production by methanogens, oxygen consumption by aerobic heterotrophs and respiration by activated sludge organisms were reduced by 50% in liquid cultures spiked with 13, 130 and 260 $\mu\text{g TCE ml}^{-1}$, respectively. The concentrations of toluene necessary to cause the same degree of inhibition of these activities were 580, 110 and 500 $\mu\text{g toluene ml}^{-1}$, respectively.

In TCE contaminated soil and sediment, Phelps *et al.* (1988) found that [^{14}C] thymidine was not incorporated into DNA, nor was [^{14}C] acetate utilized for lipid synthesis in samples containing greater than 300 $\mu\text{g TCE ml}^{-1}$, while Cox *et al.* (1994) reported reduced phospholipid fatty acid biomass measurements in sediments containing as little as 6–9 $\mu\text{g TCE ml}^{-1}$. The relative sensitivities of different strains of the important soil bacterium *Pseudomonas* to toluene and other aromatics has also been examined, and results indicate that very high concentrations (> 5%, v/v) reduce the integrity of the cell membrane, which may ultimately lead to cell lysis (Cruden *et al.*, 1992). Most of these effects were observed at concentrations much greater than those used in our experiments. The effect measured was based on the total activity of the whole community, and, therefore, does not address the effects of lower TCE and toluene concentrations on individual species present in the soil. TCE and toluene may directly kill more sensitive species, perhaps by membrane disruption, or may inactivate crucial enzymes needed to maintain viability. These compounds may act, directly or indirectly, to suppress the specific enzymatic activities needed to degrade the Biolog substrates. This seems unlikely, however, since the extended incubation of Biolog plates (sometimes for more than 10 days) should have been sufficient time for any suppressed enzymes to be induced.

Another possible reason for suppression is that phenolic intermediates of toluene degradation, such as catechol, cresol, benzoic acid and benzaldehyde (Barker *et al.*, 1987) may have accumulated in the soils exposed to high initial toluene concentrations. Toxic amounts of these metabolites may have been added to Biolog plates with the inoculum, leading to inhibition of carbon source utilization. Again,

this is probably not correct since soil exposed to 200 μg toluene ml^{-1} , which did not degrade and would therefore not contain these compounds, was the most severely affected with respect to Biolog patterns and functional diversity indices.

The carbon sources positively and negatively associated with exposure to high concentrations of either TCE or toluene were different, indicating that TCE and toluene affected the soil microbial community in different ways (Table 3). Though the identity or role of the microbial populations affected by TCE are not possible to discern in a Biolog analysis of an entire community, patterns in the type of substrate affected by the pollutants were apparent. One organic acid, α -ketovaleric acid, was negatively associated with high TCE concentrations in all experiments, while three other acids were negatively associated in all but one experiment. Three acids were positively associated with high concentrations of TCE in three out of five experiments, while [^{14}C] citrate was identically mineralized by unexposed and TCE treated soil. Organic acid utilization ability is common among environmental bacteria, most notably by *Pseudomonas* spp. (Kreig and Holt, 1984), indicating that this important group of soil organisms may be negatively affected by TCE. Of particular interest was the observation that phenyl compounds (*p*-hydroxyphenylacetic acid and phenylethylamine) were often negatively associated with TCE treatment, indicating TCE may adversely affect those organisms capable of degrading phenyl compounds. Thus, high concentrations of TCE may interfere with the decomposition of lignin and lignocellulose in soil. However, control and TCE-exposed soil showed no differences in their abilities to mineralize [^{14}C] phenol. Furthermore, Fig. 6 indicates that the recovery of the soil community after exposure to high TCE concentrations is neither rapid nor consistent, although the control and treated soils eventually do converge.

Neither TCE nor toluene affected the overall bacterial activity in soil, as indicated by little, if any, effect on SIR, basal respiration and dehydrogenase activity in Yolo soil. These results agree with those of Blair *et al.* (1989), who detected no change in soil respiration after an initial application of naphthalene to soil. In another study, Wang and Bartha (1990) found that diesel oil in concentrations of up to 70 mg total hydrocarbons g^{-1} soil (including a high percentage of aromatic and polycyclic aromatic hydrocarbons) did not significantly decrease fluorescein diacetate (FDA) hydrolysis, another measure of general activity, relative to uncontaminated soil. A concentration of 150 μg toluene ml^{-1} caused a 50% reduction in the Polytox[™] respiration assay which represents the activity of a mixture of different bacteria (Elnabarawy *et al.*, 1988). This further indicates the relative

insensitivity of microbial respiration to the presence of this compound.

The number of culturable bacteria in both agricultural and riparian soils were not substantially altered after incubation with TCE or toluene for approximately 1 month, regardless of concentration. The only exception to this was a 6-fold decrease in culturable heterotrophs after exposure to 200 μg toluene ml^{-1} . It might be expected that bacterial numbers would increase in treatments in which toluene was completely degraded, but the increase that would be expected to be supported by 20–100 μg toluene ml^{-1} would be likely to cause a net increase in the total heterotroph counts. Another possibility is that the populations initially rose, but then fell by the end of the experiment (see below).

Effects on the nematode community

In the absence of toluene, TCE concentrations greater than 30 μg TCE ml^{-1} were toxic to nematodes in both Yolo and riparian soil. Bacterivorous nematodes were stimulated by the presence of 20 μg toluene ml^{-1} alone or 1 μg TCE ml^{-1} plus toluene, indicating that prey densities may have been increased by toluene. Total culturable heterotrophs were not different in any of the treatments after 28 days, but the preliminary experiments which showed a 2-fold increase in heterotrophs after 7 days indicate that the populations of bacteria and bacterivorous nematodes may reach their maximum densities at different times. Mu and Scow (1994) demonstrated that toluene degraders increased by 5 orders of magnitude after soil was amended with 20 μg toluene ml^{-1} and 1 μg TCE ml^{-1} , while numbers in soil receiving 20 μg toluene ml^{-1} but 60 μg TCE ml^{-1} showed no net increase in toluene degrader numbers. It is possible that the microorganisms recently grown on toluene served as prey for the bacterivores as long as the TCE concentration was not too high. Initial work suggests that TCE is directly toxic to individual species of nematodes (S. Lau and H. Ferris, unpublished data).

The effects of TCE toxicity on MI values were more evident in the riparian soil where there was a higher initial nematode diversity, including representatives of higher maturity classes. Presumably, those higher classes are more sensitive to disturbances, including chemical exposures, than are the lower classes (Bongers and Korthals, 1994). Data from the riparian soil supported this idea, since the rates of population decline were higher with increasing c-p value ($P < 0.01$). In the absence of toluene, the MI for Yolo soil was not informative since the community was predominantly bacterial-feeding nematodes, which represented two disturbance-resistant c-p classes. The lower MI values in Yolo soil exposed to 30 μg TCE ml^{-1} and 20 μg toluene ml^{-1} suggest that the sensitivity of species with higher c-p values to TCE is heightened in the pre-

sence of toluene, lending support to the proposed synergistic effects of these two chemicals.

Effects on protozoa

Total protozoan populations in agricultural soil were generally insensitive to TCE and toluene exposure, with the exception of flagellates. The MPN method used in our research, however, enumerates both active and encysted protozoa (Foissner, 1994), thus interpretations of the effects of these compounds needs to be made with caution. Ferrando and Andreu-Moliner (1992) found that it took relatively high concentrations of toluene (ca. 100–500 μg toluene ml^{-1}) to kill 50% of the marine rotifers used in their liquid culture assays. Sinclair *et al.* (1993) enumerated total protozoa in subsurface soils contaminated by aviation gasoline and jet fuel (up to 2500 μg fuel carbon g^{-1}) and concluded that protozoa were not adversely affected provided that bacterial numbers were sufficient enough to support their nutritional requirements. Crude oil (up to 40 kg oil m^{-2}) was shown to have no effect on the protozoan fauna of soil by both Borisovich (1985) and Foissner (1994). Since crude oil contains a fair percentage of aromatics, including toluene, these earlier findings lend support to the present results indicating no substantial effects of TCE and toluene on soil protozoa.

General conclusions

This is the first comprehensive assessment of the effects of organic pollutants on multiple trophic levels of soil biota. The viability and activity of the general microbial community, based on plate counts, SIR and INT reduction, was not adversely affected by TCE and toluene. However, subpopulations within the community able to degrade specific carbon compounds were affected in a significant and long term manner, by these compounds, and such effects may correspond to changes in carbon cycling even after a contaminated site is remediated. Furthermore, the hypotheses that TCE alone causes less changes in the microbial community that TCE in combination with toluene was shown to be correct when looking at the richness and diversity of sole carbon source utilization by the soil microbial community. Considering such effects in combination with obvious negative effects of TCE and toluene on soil nitrogen cycling processes reported by Fuller and Scow (1996), it is apparent that TCE, and to a greater degree, toluene, have the potential to affect microbially-mediated nutrient cycling in terrestrial ecosystems.

Numerous researchers have demonstrated the role of protozoa, microbivorous nematodes, and other soil fauna in regulating microbial populations and releasing immobilized nutrients in microbial biomass for uptake by plants (Hunt *et al.*, 1987; Sohlenius *et al.*, 1988; Crossley *et al.*, 1989; Beare *et*

al., 1992; Lavelle, 1994). While significant effects of TCE and toluene on protozoa were not observed in our experiments, the treatments causing reductions in nematode abundance and soil arthropod activity corresponded with those showing a lack of seed germination. Further work, including quantitative analyses of soil arthropods and more controlled plant studies, is needed to better understand the potential for long term effects of environmental pollutants on terrestrial ecosystems.

Acknowledgements—We wish to thank Andy Tran, undergraduate laboratory assistant, for his aid in conducting this research, and Rob Venette for his excellent comments and suggestions during manuscript preparation. This research was funded in part by the Ecotoxicology Program of the University of California Toxic Substances Research and Teaching Program. Further support came from the National Institute of Environmental Health Science's Superfund Basic Research Program; Hatch Experiment Station Project 5108-H; the Kearney Foundation of Soil Science; U.S. EPA Center for Ecological Health Research at UC Davis; and the USDA Cooperative State Research Service jointly funded by the U.S. EPA for the conduct of the Agriculture in Concert with the Environment Program. Although the information in this document has been funded in part by the United States Environmental Protection Agency, it may not necessarily reflect the views of the Agency and no official endorsement should be inferred.

REFERENCES

- Alvarez-Cohen L. and McCarty P. L. (1991) Effects of toxicity, aeration, and reductant supply on trichloroethylene transformation by a mixed methanotrophic culture. *Applied and Environmental Microbiology* **57**, 228–235.
- Barker J. F., Patrick G. C. and Major D. (1987) Natural attenuation of aromatic hydrocarbons in a shallow sand aquifer. *Ground Water Monitoring Review* **7**, 64–71.
- Beare M. H., Parmelee R. W., Hendrix P. F. and Cheng W. (1992) Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. *Ecological Monographs* **62**, 569–591.
- Blair J. M., Crossley D. A.J. and Rider S. (1989) Effects of naphthalene on microbial activity and nitrogen pools in soil-litter microcosms. *Soil Biology & Biochemistry* **21**, 507–510.
- Blum D. J. W. and Speece R. E. (1991) A database of chemical toxicity to environmental bacteria and its use in interspecies comparisons and correlations. *Research Journal of the Water Pollution Control Federation* **63**, 198–207.
- Bongers T. (1990) The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia* **83**, 14–19.
- Bongers T. and Korthals G. (1993) The maturity index, an instrument to monitor changes in the nematode community structure. In *Proceedings of the International Symposium on Crop Protection*, p.80, Gent, Belgium.
- Bongers T. and Korthals G. (1994) The behavior of maturity index and plant parasite index under enriched conditions. In *Proceedings of the 22nd International Nematology Symposium*, p.39 Gent, Belgium.
- Borisovich T. M. (1985) The effect of oil pollution on soil protozoa. In *Soil Fauna and Soil Fertility* (B. R. Striganova, Ed.), pp. 282–284. Nauka, Moscow.

- Bossio D. A. and Scow K. M. (1995) Impact of carbon and flooding on the metabolic diversity of microbial communities in soils. *Applied and Environmental Microbiology* **61**, 4043–4050.
- Carey P. D., Fitter A. H. and Watkinson A. R. (1992) A field study using the fungicide benomyl to investigate the effect of mycorrhizal fungi on plant fitness. *Oecologia* **90**, 550–555.
- Clarke K. R. and Owens N. J.P. (1983) A simple and versatile micro-computer program for the determination of most probable number. *Journal of Microbiological Methods* **1**, 133–137.
- Coleman D. C., Odum E. P. and Crossley J. D. A. (1992) Soil biology, soil ecology, and global change. *Biology and Fertility of Soils* **14**, 104–111.
- Collins M. F. (1987) A permutation test for planar regression. *Australian Journal of Statistics* **29**, 303–308.
- Cox E. E., Major D. W., Acton D. W., Phelps T. J. and White D. C. (1994) Evaluating trichloroethene biodegradation by measuring the *in situ* status and activities of microbial populations. In *Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbon Compounds* (R. E. Hinchee, A. Leeson, L. Semprini and S. K. Ong, Eds), pp. 37–49. Lewis, Ann Arbor.
- Crossley D. A. Jr., Coleman D. C. and Hendrix P. F. (1989) The importance of the fauna in agricultural soils: Research approaches and perspectives. *Agriculture, Ecosystems and Environment* **27**, 47–55.
- Cruden D. L., Wolfram J. H., Rogers R. D. and Gibson D. T. (1992) Physiological properties of a *Pseudomonas* strain which grows with *p*-xylene in a two phase (organic-aqueous) medium. *Applied and Environmental Microbiology* **58**, 2723–2729.
- Curtis P. S., O'Neill E. G., Teeri J. A., Zak D. A. and Pregitzer K. S. (1994) Belowground responses to rising atmospheric CO₂: Implications for plants, soil biota and ecosystem processes. *Plant and Soil* **165**, 1–6.
- Diltz M. S., Hepfer C. E., Hartz E. and Baker K. H. (1992) Recovery of heterotrophic soil bacterial guilds from transient gasoline pollution. *Hazardous Waste and Hazardous Material* **9**, 267–273.
- Domsch K. H., Jagnow G. and Anderson T.-H. (1983) An ecological concept for the assessment of side-effects of agrochemicals on soil microorganisms. *Residue Reviews* **86**, 65–105.
- Elnabarawy M. T., Robideau R. R. and Beach S. A. (1988) Comparison of three rapid toxicity test procedures: Microtox[®], Polytox[®], and activated sludge respiration inhibition. *Toxicity Assessment* **3**, 361–370.
- Ensign S. A., Hyman M. R. and Arp D. J. (1992) Cometabolic degradation of chlorinated alkenes by alkene monooxygenase in a propylene-grown *Xanthobacter* strain. *Applied and Environmental Microbiology* **58**, 3038–3046.
- Ensley B. D. (1991) Biochemical diversity of trichloroethylene metabolism. *Annual Review of Microbiology* **45**, 283–299.
- Esher R. J., Marx D. H., Ursic S. J., Baker R. L., Brown L. R. and Coleman D. C. (1992) Simulated acid rain effects on fine roots, ectomycorrhizae, microorganisms, and invertebrates in pine forests of the southern United States. *Water, Air, and Soil Pollution* **61**, 269–278.
- Fan S. and Scow K. M. (1993) Biodegradation of trichloroethylene and toluene by indigenous microbial populations in soil. *Applied and Environmental Microbiology* **59**, 1911–1918.
- Ferrando M. D. and Andreu-Moliner E. (1992) Acute toxicity of toluene, hexane, xylene and benzene to the rotifers *Brachionus calyciflorus* and *Brachionus plicatilis*. *Bulletin of Environmental Contamination and Toxicology* **49**, 266–271.
- Fliermans C. B., Phelps T. J., Ringelberg D., Mikell A. T. White D. C. (1988) Mineralization of trichloroethylene by heterotrophic enrichment cultures. *Applied and Environmental Microbiology* **54**, 1709–1714.
- Foissner W. (1994) Soil protozoa as bioindicators in ecosystems under human influence. In *Soil Protozoa* (J. F. Darbyshire, Ed.), pp. 147–193. CAB International, Wallingford.
- Folsom B. R., Chapman P. J. and Pritchard P. H. (1990) Phenol and trichloroethylene degradation by *Pseudomonas cepacia* G4: kinetics and interactions between substrates. *Applied and Environmental Microbiology* **56**, 1279–1285.
- Fuller M. E. and Scow K. M. (1996) Effects of toluene on microbial-mediated processes involved in the soil nitrogen cycle. *Microbial Ecology* **32**, 171–184.
- Garland J. and Mills A. L. (1994) A community-level physiological approach for studying microbial communities. In *Beyond the Biomass: Compositional and Functional Analysis of Soil Microbial Communities* (K. Ritz, J. Dighton and K. E. Giller, Eds), pp. 77–83. Wiley, Chichester.
- Garland J. L. and Mills A. L. (1991) Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Applied and Environmental Microbiology* **57**, 2351–2359.
- Haack S. K., Garchow H., Klug M. J. and Forney L. J. (1995) Analysis of factors affecting the accuracy, reproducibility, and interpretation of microbial community carbon source utilization patterns. *Applied and Environmental Microbiology* **61**, 1458–1468.
- Heald S. and Jenkins R. O. (1994) Trichloroethylene removal and oxidation toxicity mediated by toluene dioxygenase of *Pseudomonas putida*. *Applied and Environmental Microbiology* **60**, 4634–4637.
- Henry S. M. and Grbic-Galic D. (1991) Influence of endogenous and exogenous electron donors and trichloroethylene oxidation toxicity on trichloroethylene oxidation by methanotrophic cultures from a groundwater aquifer. *Applied and Environmental Microbiology* **57**, 236–244.
- Hicks R. J., Stotzky G. and van Voris P. (1989) Review and evaluation of the effects of xenobiotic chemicals on microorganisms in soil. *Advances in Applied Microbiology* **35**, 195–253.
- Hughes K., Meek M. E. and Windle W. (1994) Trichloroethylene: Evaluation of risks to human health from environmental exposure in Canada. *Environmental Carcinogenicity and Ecotoxicology Reviews: Part C of The Journal of Environmental Science and Health* **12**, 527–543.
- Huhta V., Wright D. H. and Coleman D. C. (1989) Characteristics of defaunated soil: I. A comparison of three techniques applied to two different forest soils. *Pedobiologia* **33**, 417–426.
- Hunt H. W., Coleman D. C., Ingham E. R., Ingham R. E., Elliott E. T., Moore J. C., Rose S. L., Reid C. P.P. Morley C. R. (1987) The detrital food web in a short-grass prairie. *Biology and Fertility of Soils* **3**, 57–68.
- Ingham E. R., Parmelee R., Coleman D. C. and Crossley D. A. Jr. (1991) Reduction of microbial and faunal groups following application of streptomycin and captan in Georgia no tillage agroecosystems. *Pedobiologia* **35**, 297–304.
- Ingham R. E., Trofymow J. A., Ingham E. R. and Coleman D. C. (1985) Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. *Ecological Monographs* **55**, 119–140.
- Jaffee B. A., Gaspard J. T., Ferris H. and Muldoon A. E. (1988) Quantification of parasitism of the soil-borne nematode *Criconebella xenoplax* by the nematophagous

- fungus *Hirsutella rhossiliensis*. *Soil Biology & Biochemistry* **20**, 631–636.
- Jeffrey W. H. and Paul J. H. (1986) Activity measurements of planktonic microbial and microfouling communities in a eutrophic estuary. *Applied and Environmental Microbiology* **51**, 157–162.
- Kanazawa S. and Filip Z. (1986) Effects of trichloroethylene, tetrachloroethylene and dibromomethane on enzymatic activities in soil. *Applied Microbiology and Biotechnology* **25**, 76–81.
- Kerry E. (1990) Microorganisms colonizing plants and soils subject to different degrees of human activity, including petroleum contamination, in the Vestfold Hills and MacRobertson land. *Antarctica. Polar Biology* **10**, 423–430.
- Kreig N. R. and Holt J. G. (1984) *Bergey's Manual of Systematic Bacteriology*, pp. 964. Williams and Wilkins, Baltimore.
- Lavelle P. (1994) Faunal activities and soil processes: adaptive strategies that determine ecosystem function. In *15th World Congress of Soil Science, Acapulco, Mexico*, vol. 1, pp. 189–220. International Society of Soil Science and Mexican Society of Soil Science.
- Ludwig J. A. and Reynolds J. F. (1988) *Statistical Ecology: A Primer on Methods and Computing*. Wiley, New York.
- Manly B. F. J. (1986) *Multivariate Statistical Methods: A Primer*. Chapman and Hall, New York.
- Mathes K. and Weidemann G. (1990) A baseline-ecosystem approach to the analysis of ecotoxicological effects. *Ecotoxicology and Environmental Safety* **20**, 197–202.
- Meek M. E. and Chan P. K. L. (1994) Toluene: Evaluation of risks to human health from environmental exposure in Canada. *Environmental Carcinogenicity and Ecotoxicology Reviews: Part C of The Journal of Environmental Science and Health* **12**, 507–515.
- Mu D. Y. and Scow K. M. (1994) Effect of trichloroethylene (TCE) and toluene concentrations on TCE and toluene biodegradation and the population density of TCE and toluene degraders in soil. *Applied and Environmental Microbiology* **60**, 2661–2665.
- Nelson M. J.K., Montgomery S. O. and Pritchard P. H. (1988) Trichloroethylene metabolism by microorganisms that degrade aromatic compounds. *Applied and Environmental Microbiology* **54**, 604–606.
- Phelps T. J., Ringleberg D., Hedrick D., Davis J., Fliermans C. B. and White D. C. (1988) Microbial biomass and activities associated with subsurface environments contaminated with chlorinated hydrocarbons. *Geomicrobiology Journal* **6**, 157–170.
- Sinclair J. L., Campbell D. H., Cook M. L. and Wilson J. T. (1993) Protozoa in subsurface sediments from sites contaminated with aviation gasoline or jet fuel. *Applied and Environmental Microbiology* **59**, 467–472.
- Smith J., McNeal B. and Cheng H. (1985) Estimation of soil microbial biomass: an analysis of the respiratory response of soils. *Soil Biology & Biochemistry* **17**, 11–16.
- Sohlenius B., Boström S. and Sandor A. (1988) Carbon and nitrogen budgets of nematodes in arable soil. *Biology and Fertility of Soils* **6**, 1–8.
- ter Braak C. J. F. (1987) Ordination. In *Data Analysis in Community and Landscape Ecology* (R. H. G. Jongman, C. J. F. ter Braak and O. F. R. van Tongeren, Eds), pp. 91–169. Centre for Agricultural Publishing and Documentation (Pudoc), Wageningen.
- Vannelli T., Logan M., Arciero D. M. and Hooper A. B. (1990) Degradation of halogenated aliphatic compounds by the ammonia-oxidizing bacterium *Nitrosomonas europaea*. *Applied and Environmental Microbiology* **56**, 1169–1171.
- Volskay J., V. T. and Grady J., C. P. L. (1988) Toxicity of selected RCRA compounds to activated sludge microorganisms. *Journal of the Water Pollution Control Federation* **60**, 1850–1856.
- von Mersi W. and Schinner F. (1991) An improved and accurate method for determining the dehydrogenase activity of soils with iodinitrotetrazolium chloride. *Biology and Fertility of Soils* **11**, 216–220.
- Wackett L. P. and Householder S. R. (1989) Toxicity of trichloroethylene to *Pseudomonas putida* F1 is mediated by toluene dioxygenase. *Applied and Environmental Microbiology* **55**, 2723–2725.
- Wang X. and Bartha R. (1990) Effects of bioremediation on residues, activity and toxicity in soil contaminated by fuel spills. *Soil Biology & Biochemistry* **22**, 501–505.
- Westrick J. J., Mello J. W. and Thomas R. F. (1984) The groundwater supply survey. *Journal of the American Water Works Association* **76**, 52–59.
- Winding A. (1994) Fingerprinting bacterial soil communities using Biolog microtitre plates. In *Beyond the Biomass: Compositional and Functional Analysis of Soil Microbial Communities* (K. Ritz, J. Dighton and K. E. Giller, Eds), pp. 85–94. Wiley, Chichester.
- World Health Organization (1985) *Trichloroethylene*. Environmental Health Criteria 50, World Health Organization, Geneva.
- Wright D. H., Huhta V. and Coleman D. C. (1989) Characteristic of defaunated soil: II. Effects of reinoculation and the role of the mineral component. *Pedobiologia* **33**, 427–435.
- Zak J. C., Willig M. R., Moorhead D. L. and Wildman H. G. (1994) Functional diversity of microbial communities: a quantitative approach. *Soil Biology & Biochemistry* **26**, 1101–1108.