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Microbial Dynamics in Oil-Impacted Prairie Soil

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Abstract

A remote site in the Tallgrass Prairie Preserve (Osage County, OK) was contaminated with crude oil by a pipeline break in 1992. In 1996, the contaminated soil was bioremediated by blending with uncontaminated soil, prairie hay, buffalo manure, and commercial fertilizers, and spreading in a shallow layer over uncontaminated soil to create a landfarm. The landfarm was monitored for two years for aerobic and anaerobic bacteria, soil gases indicative of microbial activity, and for changes in the concentration of total petroleum hydrocarbons (TPH). Levels of hydrocarbon degraders and soil gas indicators of aerobic degradation were stimulated in the landfarm during the first warm season relative to uncontaminated prairie soil. However, these same indicators were less conclusive during the second warm season, indicating depletion of the more easily degradable hydrocarbons, although the landfarm still contained 6,800 mg/kg TPH on the average at the beginning of the second warm season. Methane formation and methanogen counts were clearly stimulated in the first warm season relative to uncontaminated prairie soil, indicating that methanogenesis plays an important role in the mineralization of hydrocarbons even in these shallow soils.

Index Entries: Crude oil; bioremediation; TPH; methanogenesis; hydrocarbon-degrading bacteria.

Introduction

Environmental factors such as temperature, moisture level, aeration, pH, nitrogen, and metabolizable carbon are considered to be major controllers

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of microbial numbers and activity in soil *(1)*. As soil is known to be quite heterogeneous with regard to most parameters influencing microbes *(2)*, it is likely that microbial populations fluctuate over time in numbers and level of activity, rather than maintaining a stable equilibrium. Equilibrium conditions are especially unlikely during landfarming of petroleum-contaminated soil, when it is common to add various chemicals and manipulate the soil in order to increase the rate of degradation *(3)*. The petroleum represents an influx of at least partially metabolizable carbon and the fertilizer is intended to offset any deficiency in major nutrients, often assumed to be nitrogen *(3)*. The combination of increased carbon and nitrogen potentially has a cascade of effects on various microbial groups, through mechanisms such as an alteration in oxygen level during rapid aerobic respiration, and changes in redox potentials, among others. The object of the current study was to elucidate some of the alterations of vital physical and chemical parameters after petroleum and nitrogen addition, and how these anthropogenic disturbances promulgate through the microbial community. Specifically, we first documented how microbial numbers and respiration were affected by crude-oil contamination and the addition of nitrogen-containing fertilizer, focusing on certain groups of bacteria known to be important in carbon cycling; then determined the relevant time scale of this disturbance; and finally examined the dynamic effects of changes in temperature and soil moisture as they occurred seasonally. Although it is known that microbial densities do change during bioremediation, most field studies do not sample frequently enough to track these changes, nor do they have the correlated temperature and moisture measurements available to help interpret these dynamics, as we do. The effects of specific fertilizers on nitrogen cycling and the release of nitrous oxide during the first season were presented previously *(4)*.

Methods

Site History

The study site is located in the Tallgrass Prairie Preserve (Osage County, OK, The Nature Conservancy). This region in northern Oklahoma has historically been the locale of oil production. In 1992 an accidental pipeline break contaminated an area approximately 13 m (at widest) by 35 m by 1 m deep with crude oil. In 1995, contamination levels ranged from 6,000–33,000 mg/kg (3 samples, total petroleum hydrocarbons by EPA Method 418.1, Southwest Laboratory of Oklahoma, Inc.).

Treatments

Bioremediation of the site began in May 1996. The contaminated soil was mechanically moved to a treatment site (approximately $27 \times 24 \times 0.3$ m) adjacent to the west and mixed with uncontaminated soil (free of petroleum and brine) at a 5:1 ratio (uncontaminated: contaminated soil), bring-

ing the level of total petroleum hydrocarbons (TPH) to less than 3% (wt %). The amount of N, P, and K added were chosen to give the recommended ratio of 100 C:10 N: 1 P: 1 K *(3)*, but with 1/3 of the amount of N applied initially. 159 kg P_2O_5, 113 kg K_2O, 75 bales of prairie hay (bulking agent, also provides seeds of native grasses for revegetation) and approximately 363 kg (dry weight) bison manure (used for a bulking agent, seed, and micronutrient source, ~3% *N* or 11 kg *N*) were then applied evenly across the treatment site. The treatment site was divided into four contiguous strips and nitrogenous fertilizer applied evenly to the individual treatment sections as follows: "control," no nitrogen fertilizer added; urea; ammonium sulfate; and ammonium nitrate to give on the average 1000 mg-N/kg soil. All additions were mechanically tilled to a depth of 20–30 cm. The treatment site was retilled July 1997 during the second year. Soil moisture was measured but not controlled by adding water; rainfall provided the only input of water to the site.

Soil Sampling and Compositing

Wooden stakes were permanently installed to mark the outside vertices of each of the four treatment sections and to mark five ordination points for soil sampling. The ordination stakes were placed a minimum of 1 m from the edge of the treatment section, with the five stakes forming the pattern of a flattened "M." Soil gas surveys and collection of soil for chemical analysis and for bacterial enumeration (*see* Microbial Enumeration) were made at the same defined distances and orientation from the ordination stakes during any one collection date, giving a total of five sites sampled within each plot. The survey and collection sites were at a different distance and orientation for each different collection date so that the same areas of soil were not resampled. Sampling dates were ("days" refers to number of days after treatment began): May 21-d 5; May 29-d 13; June 12-d 27; July 1-d 46; and August 1-d 77 in 1996; June 4-d 383; July 8-d 418; and October 17-d 519 in 1997. Surveys and soil samples were taken at two different depths ("Levels") in the soil in 1996: Level 1 was 2–16 cm below the soil surface, Level 2 was 18–30 cm below the soil surface. Soil was collected from Level 1 only in 1997. Soil from the same level and treatment was composited, giving a total of eight composited samples. Soil was collected from one to three sites in the prairie several hundred yards to the west of the treatment area and/or across a gravel road to serve as an uncontaminated control. The June 4, 1997 sample was collected before retilling, the July 8, 1997 sample was taken less than 7 d after tilling. Soil cores were extracted for microbial enumeration by carefully pushing a sterile polyethylene centrifuge tube (50 mL size, approximately 5 cm diameter × 18 cm long) into the soil at the desired depth and sampling location. Soil was stored in the collection centrifuge tube at 4°C until subsampled for bacterial enumeration. Soil moisture was determined by gravimetric measurement after oven drying at 50°C to a constant weight (minimum of 1 wk). All bacterial counts are expressed as per gram of soil (dry weight).

Soil Gas Survey

Soil gases (O_2, CO_2, VOCs, i.e., volatile organic compounds; VOCs minus methane, values for methane obtained by subtraction) were monitored on each collection date using a GasTech O_2/CO_2 Monitor and a GasTech TraceTechtor hydrocarbon analyzer. Atmospheric standards of O_2 at 20.9% and CO_2 at 0.05% were used for calibration. Hexane was used as the VOC standard. Level 1 gases were sampled 7–16 cm below the surface, level 2 at 18–30 cm below the surface, using a gas-sampling probe inserted to the indicated depth.

TPH and Soil Chemical Analyses

TPH in composited soil samples were determined in-house using infrared analysis (TPH-IR; EPA Method 418.1) and gas chromatography (TPH-GC; EPA Method 8270) and also on two occasions (5/21/96, 11/11/97) by Soil Analytical Services, Inc. (SASI) College Station, TX. Samples collected 5/21/96 were analyzed by IR alone, and those from 11/11/97 were analyzed by both IR and GC. Samples to SASI were shipped by overnight delivery in completely filled glass jars with Teflon™-lined lids. Samples collected 11/11/97 were also analyzed by SASI for pH, percent moisture, sodium adsorption ratio (SAR), cation-exchange capacity (CEC), total Kjeldahl nitrogen (TKN), nitrate nitrogen (NO_3-N), ammonia nitrogen (NH_4-N), total phosphorus, available phosphorus (PO_4-P), and total organic carbon (TOC, %).

Microbial Enumeration

Aerobic Heterotrophic Bacteria and Hydrocarbon-Degraders

A soil suspension of composited soil was made for a series of 10-fold dilutions by removing 2.5 g of soil from each 50-mL collection tube into a new sterile 50-mL tube containing 25 mL of sterile isotonic saline (0.85% NaCl, pH 7.0) containing 100 µg/mL cycloheximide (cxy, Sigma Chemical Co., St. Louis, MO) as an anti-fungal agent, mixed thoroughly by vortexing at highest speed for 1 min, then diluted and spread as described below for aerobic heterotrophic bacteria and hydrocarbon-degraders. Dilutions of the soil suspension were spread-plated (three replicates/dilution) on the following types of media: PCA (Plate Count Agar-Difco) with cxy for total aerobic heterotrophs; mineral salts (MS) agar with trace metals (per L: 0.95 g NH_4Cl, 2.03 g $(NH_4)_2HPO_4 \cdot 2H_2O$, 1.05 g KH_2PO4, 0.2 g $MgSO_4 \cdot 7H_2O$, 15 g Difco Bacto Agar, 3 mg $FeSO_4 \cdot 7H_2O$, 18 mg $MnCl_2 \cdot 4H_2O$, 13 mg $Co(NO_3)_2 \cdot 6H_2O$, 0.4 mg $ZnSO_4 \cdot 7H_2O$, 0.022 mg Na_2MoO_4, 0.01 mg $CuSO_4 \cdot 5H_2O$, 1 g $CaCl_2$) and cxy containing one of the following aromatic hydrocarbons: naphthalene (NAP) added as crystals on the bottom of the plate lid for naphthalene-degraders; MS agar containing 0.8% phenanthrene (PHE) for phenanthrene-degraders. All plates were incubated at room temperature; colonies on PCA plates were counted after 48 h and

again at 1 wk, while colonies on NAP and PHE plates were counted after 1 wk and again at 2 wk. Individual colonies on NAP and PHE plates were transferred with a sterile toothpick from their original plates to two different sets of plates, one containing the same medium as the original plate, the other consisting of MS agar without any added hydrocarbon, in order to confirm the ability of these isolates to utilize hydrocarbons by their abundant growth on the former, and their lack of growth on the latter.

Sulfate-Reducing Bacteria (SRB) and Methanogens

Manipulations of these bacteria were performed in an anaerobic glove box. Ten g of composited soil was added to a serum bottle containing 90 mL of pre-reduced methanogenic mineral salts medium *(5)*, and carefully mixed. One-mL aliquots were withdrawn from the inoculated serum bottle with a sterile needle and syringe and used to inoculate each of three 9-mL bottles of SRB medium *(6)*. The procedure was repeated for a total of 10 serial dilutions. The tubes were incubated for 21 d at room temperature, then scored for growth and the formation of a black precipitate (iron sulfide) indicating the presence of SRBs. The most probable number (MPN) of bacteria in the original soil samples was estimated from the characteristic number for a three-tube MPN *(7)*. One-milliliter aliquots were also withdrawn from the inoculated serum bottle with a sterile needle and syringe and used to inoculate 20 mL anaerobic culture tubes with Balch stoppers and aluminum crimp seals containing 9 mL pre-reduced methanogenic mineral salts medium with the potential electron donors sodium acetate (2 g/L) and sodium formate (1 g/L) added *(5)*. The procedure was repeated for a total of 10 serial dilutions. The headspace of the tubes had been flushed repeatedly with nitrogen (N_2:CO_2, 80:20%, 10 psi or 0.68046 atmos) to maintain anaerobic conditions. The tubes were incubated at least 90 days before being scored for methane production by withdrawing 0.2 mL from the headspace and analyzing with a Varian 3300 gas chromatograph equipped with a Porapak Q column and a flame ionization detector with nitrogen as the carrier gas. The headspace gas analysis was performed at the University of Oklahoma (Norman, OK courtesy of Dr. M. McInerney, Dept. of Botany and Microbiology). Tubes were scored as positive for the presence of methanogens when methane production exceeded 2.12%, a percentage 10% greater than that which could be produced stoichiometrically from formate and acetate together.

Weather Data

Soil temperature and rainfall data was obtained from the Foraker Mesonet station, located within the Tallgrass Prairie Preserve. Soil temperatures were taken 5 cm below the surface, either under bare sod or under vegetation. Bare-sod values were used for the landfarm, under vegetation for the prairie. The Oklahoma Mesonet (Oklahoma Climatological Survey, Norman, OK) is a network of stations throughout the state that provides automated, real-time measurements of many different weather parameters.

Results

Soil Chemistry and TPH

At the end of two warm seasons of remediation, soil from all plots contaminated with crude oil had higher levels than the uncontaminated prairie for some parameters associated with brine (sodium, chloride, SAR), and with fertilizer (nitrate-nitrogen, available phosphate, potassium), as well as a higher CEC, higher levels of calcium, and somewhat higher levels of magnesium (Table 1). Higher levels of soluble cations would be expected as sodium replaces them in the clay matrix. The percent TOC in the prairie was similar to that in most of the contaminated plots, in spite of measurable TPH found in the latter. This may have been a consequence of the high-root density in the prairie soil. The contaminated plots had few plants, in contrast to almost complete vegetative coverage in the prairie. The contaminated plots differed somewhat in the relative proportion of nitrogen as nitrate vs ammonium and in the sum of nitrate-N plus ammonium-N, with the ammonium sulfate and ammonium nitrate-treated sites having a greater proportion as nitrate, and a higher total sum of nitrate and ammonium than the urea treatment or control; however, all contaminated sites had higher nitrogen levels than did the prairie. The higher N levels in the control contaminated plot than in the prairie could be owing to the following: leaching of nitrate from the N-fertilized plots (the other plots were slightly elevated in comparison to the control), and/or ammonification of the N-containing components of the prairie hay and bison manure added initially.

The oil in the contaminated soil showed evidence of partial degradation even before treatment. Samples taken on 5/21/96 had diminished amounts of light ends (C12 and below, data not shown) compared to crude oil sampled from the pipeline, reflecting the 4-yr interval before treatment. Samples taken 5 d after the start of landfarming had TPH levels ranging from 11,000–21,000 mg/kg (avg. 15,800 mg/kg, TPH-IR). All the contaminated plots had a similar time course and total percentage of TPH lost: 2 mo after treatment TPH-IR levels ranged from 4000–9000 mg/kg (avg., 6400 mg/kg, or 60% loss compared to the start of landfarming), these values were little changed at a year after the start of remediation. Total losses (calculated from initial TPH-IR values from samples collected 5/21/96 and compared to TPH-IR values from samples collected 11/11/97; *see* Table 1) averaged 64% and ranged from 50% (ammonium nitrate plot) to 70% (ammonium sulfate plot), with 60% lost from the control and the urea-treated plot. Chromatograms (not shown) of the 11/11/97 sample showed a highly degraded crude oil with few individual peaks and a large unresolved mass.

Microbial Numbers

Aerobic heterotrophic bacteria and the naphthalene- and phenanthrene-degrading bacteria all showed similar responses: densities were

Table 1
Soil Chemistry Post-Remediation

Treatments	pH	Moisture (%)	Soluble cations and anions (meq/L) CO_3^{-2}	HCO_3^-	Cl^-	SO_4^{-2}	Na^+	Ca^{+2}	Mg^{+2}	K^+
Control	6.60	17.3	<0.1	0.9	6.7	23.0	1.9	21.1	6.6	0.3
Urea	6.30	20.8	<0.1	0.8	6.5	28.1	3.0	29.8	8.6	0.6
$(NH_4)_2SO_4$	6.10	19.5	<0.1	0.8	4.6	27.0	1.3	27.7	9.1	0.6
$(NH_4)NO_3$	5.00	21.1	<0.1	0.9	14.7	4.7	0.9	16.7	5.5	0.3
Prairie	5.85	29.2	<0.1	0.8	0.4	2.1	0.0	4.8	2.0	0.1

Treatments	SAR	CEC eq/100	TKN[a] (%)	NO_3-N (mg/kg)	NH_4-N (mg/kg)	Total P (mg/kg)	PO_4-P (mg/kg)	TOC[b] (%)	TPH[c] (ppm)	TPH[d] (ppm)
Control	0.5	3.1	1870	21.6	23.0	734.9	42.9	2.77	3,803	4,820
Urea	0.7	4.2	2540	47.5	11.6	735.2	46.4	2.61	4,416	5,770
$(NH_4)_2SO_4$	0.3	3.5	1980	140.3	14.2	793.2	58.7	2.70	4,559	5,800
$(NH_4)NO_3$	0.3	2.5	2340	93.0	8.3	890.4	99.9	3.81	4,206	6,530
Prairie	0.0	0.6	2430	1.2	7.9	716.8	1.1	2.75	<100	<50

[a]TKN: Total Kjeldahl Nitrogen.
[b]TOC: Total organic carbon by dry combustion (LECO).
[c]EPA Method 8270 (GC).
[d]EPA Method 418.1 (IR).

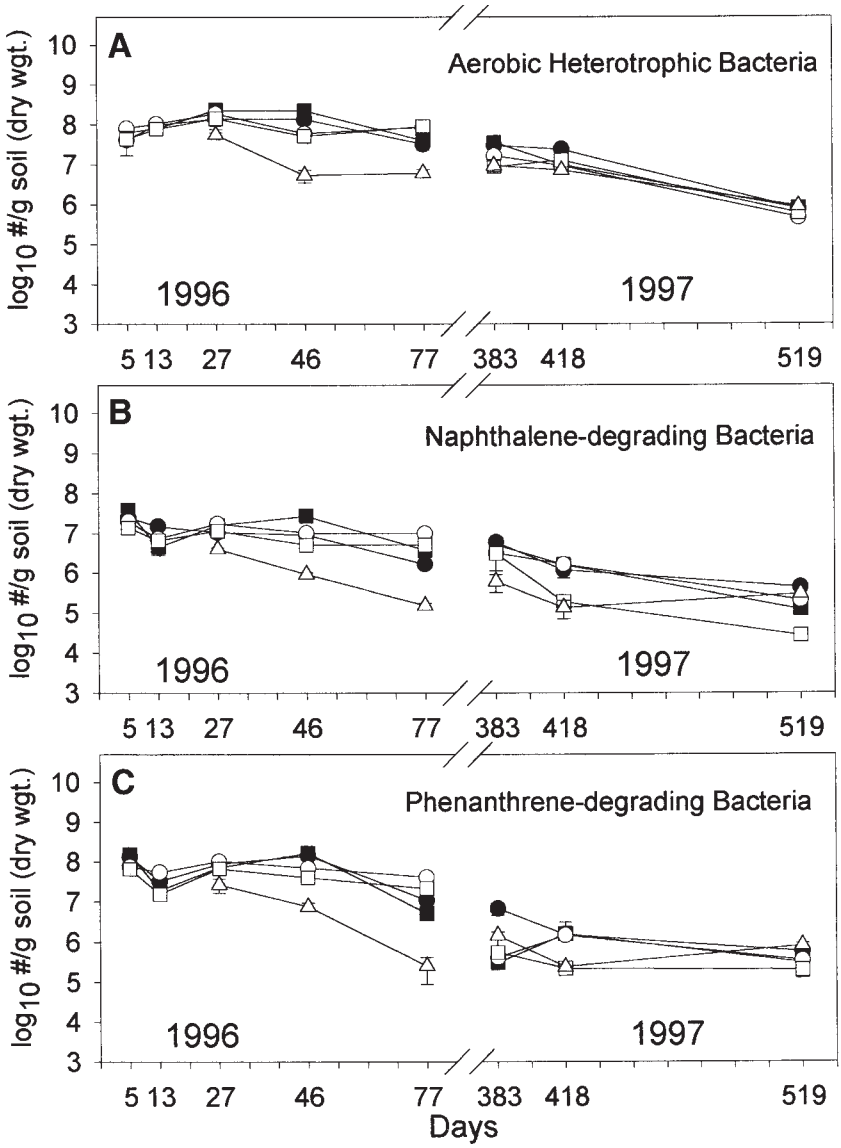

Fig. 1. Time course of population density of various aerobic bacteria (per g soil, dry weight) in the four treatment plots and uncontaminated prairie soil. Soil samples were from Level 1 (2–16 cm below the surface). Values shown are the means of three replicates; error bars are ± 1 SD. Filled circles, samples from the control treatment plot (no nitrogen added); filled squares, urea-treatment plot; open circles, ammonium sulfate treatment plot; open squares, ammonium nitrate treatment plot; and open triangles, prairie. **(A)** Aerobic heterotrophic bacteria. **(B)** Naphthalene-degrading bacteria. **(C)** Phenanthrene-degrading bacteria.

elevated in all contaminated plots relative to the uncontaminated prairie during the 1996 season, and dropped in 1997 to levels indistinguishable from the prairie (Fig. 1). The addition of sufficient nitrogenous fertilizer to

give a 10:3.3 C:N ratio apparently did not measurably stimulate the growth of these types of bacteria: densities were as high in the contaminated control plot as in the other three contaminated plots. It may be possible that either factors other than levels of nitrogen limited their growth, or they may have been outcompeted by other types of bacteria (such as nitrifying and/or denitrifying bacteria) for the added nitrogen. We had previously found relatively high densities of denitrifying bacteria in the plots during the first season, and that exceptionally high levels of NO_X were emitted from the soil *(4)*.

Sulfate-reducing bacteria were found in all plots as well as the uncontaminated prairie during the 1996 season (Fig. 2, densities not assessed in 1997). Although estimated numbers varied somewhat over the season in the various plots, there was no clear stimulation by oil, nitrogenous fertilizer, or even by the addition of sulfate in the form of ammonium sulfate. In a previous study, high numbers of sulfate-reducing bacteria had been associated with remediation of oil-contaminated soil *(8)*.

Evidence of methanogens were found in the contaminated plots during the 1996 season (Fig. 2, densities not assayed in 1997). They were much more abundant in the contaminated plots than in the uncontaminated prairie, suggesting that the crude oil fueled an increase in carbon flow through the soil microbial community including the anaerobic decomposers. The high methane levels seen in 1996 and intermittently in 1997 (Fig. 3, *see also* Microbial Activity) also attest to significant levels of anaerobic carbon degradation, even at relatively shallow depths. Although methanogens were usually below the level of detection in the uncontaminated prairie soil during sampling in 1996, the production of methane from such soils, seen especially in 1997 (Fig. 3), demonstrate that methane-producing microbes were present and active in prairie soil.

Microbial Activity

Soil Gases

During 1996, oxygen levels were lower, whereas carbon dioxide and methane levels were higher in the contaminated sites than in the prairie, indicating more microbial activity, both aerobic and anaerobic, in the petroleum-contaminated areas (Fig. 3). Prairie values were well outside the range of those in the control plot in 1996. In 1997, levels of all three monitored gases were similar for the contaminated sites and the prairie. This difference between years was owing to a greater rise in activity in prairie soil in 1997 (perhaps owing to increased soil moisture levels; *see* Fig. 4) rather than less activity in the contaminated sites, as measured by oxygen and carbon dioxide levels. Methane levels in the contaminated sites dropped substantially in 1997, whereas methane levels rose in the prairie. The soil gases showed more dramatic fluctuations over a single season than did the microbial numbers, but both numbers and activity indicated a convergence between the prairie and landfarm occurred beginning 1 yr after remediation.

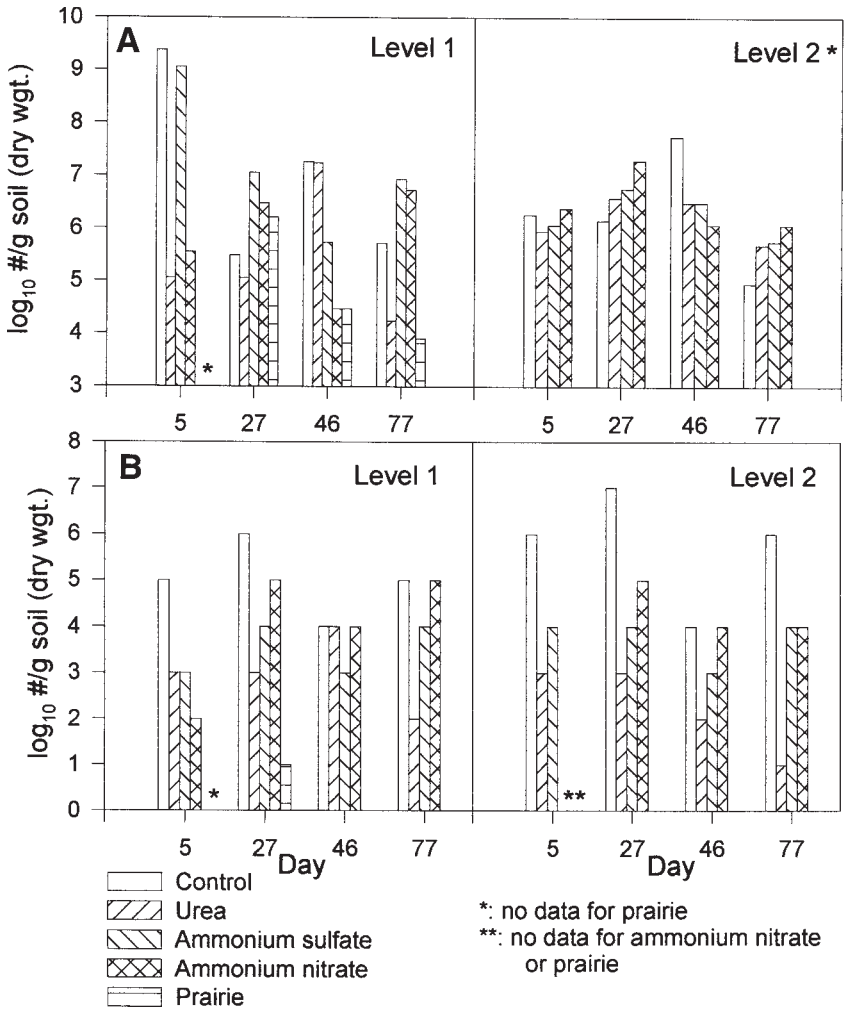

Fig. 2. **(A)** Time course of population density of sulfate-reducing bacteria (per g soil, dry weight) in the four treatment plots and uncontaminated prairie soil. Densities were derived from the most probable number (MPN) as estimated from the characteristic number for a three-tube MPN. Left figure, Level 1 (2–16 cm below the surface), right figure, Level 2 (18–30 cm below the surface). No Level 2 samples were taken in the prairie. **(B)** Time course of population density of methanogenic bacteria (per g soil, dry weight) in the four treatment plots and uncontaminated prairie soil. Densities were derived from measurements of methane in the headspace after inoculation and incubation of anaerobic culture tubes containing methanogenic media. Tubes were scored as positive for methanogens when methane production exceeded 2.12%. Left figure, (2–16 cm below the surface), right figure, Level 2 (18–30 cm below the surface). No Level 2 samples were taken in the prairie.

Rainfall

Average yearly rainfall in Osage County is 942 mm/yr (Oklahoma Climatological Survey). Rainfall was well below average during the first year of treatment (703 mm), with especially low levels of precipitation

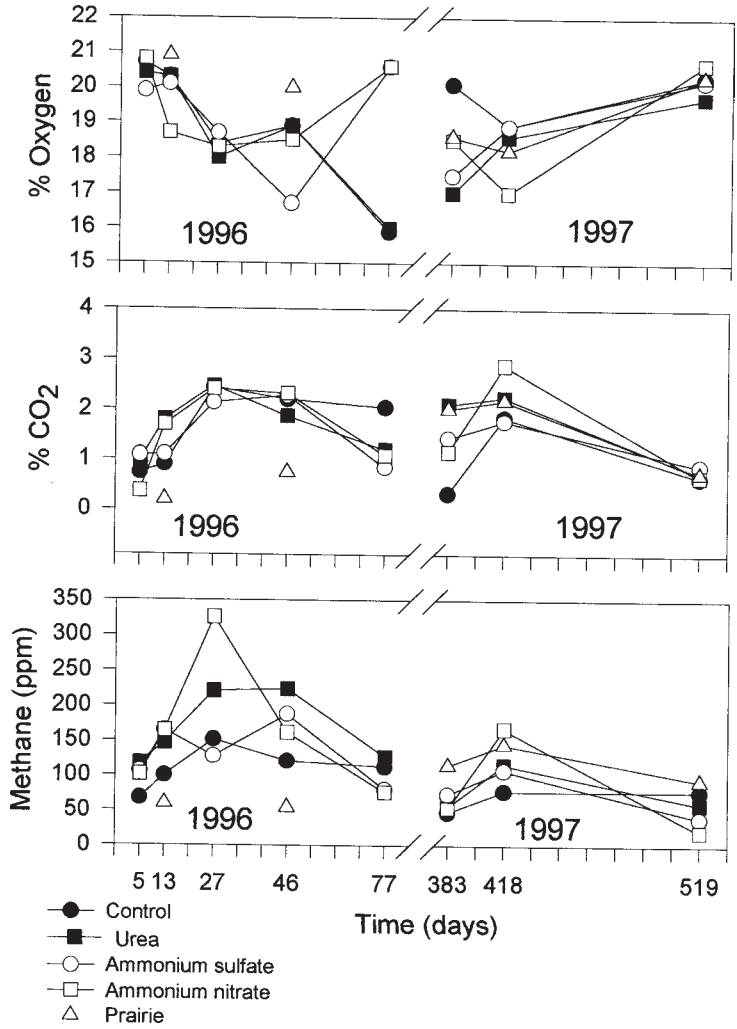

Fig. 3. Soil gases taken in the field. Measurements were made at five separate locations when possible, spatially distributed across the treatment plot. Mean values are shown. Y axis: "Days" are the number of days from start of remediation. X axis: Top figure, oxygen (%); Middle, carbon dioxide (%); Bottom, methane (ppmv, parts/million volume). Filled circles, samples from the control-treatment plot (no nitrogen added); filled squares, urea-treatment plot; open circles, ammonium sulfate treatment plot; open squares, ammonium nitrate treatment plot; and open triangles, prairie. Measurements were made 2–16 cm below the surface. No measurements were taken in the prairie on d 5, 27, or 77.

during critical summer months. In contrast, the total rainfall during 1997 was 1037 mm, with abundant rain during the summer. Measurements of soil samples confirmed that soil moisture levels were as high or somewhat higher on comparable dates in 1997 as in 1996 (Fig. 4; compare d 27 to d 318, d 46 to d 418), yet microbial numbers tended to be lower in 1997 than in 1996

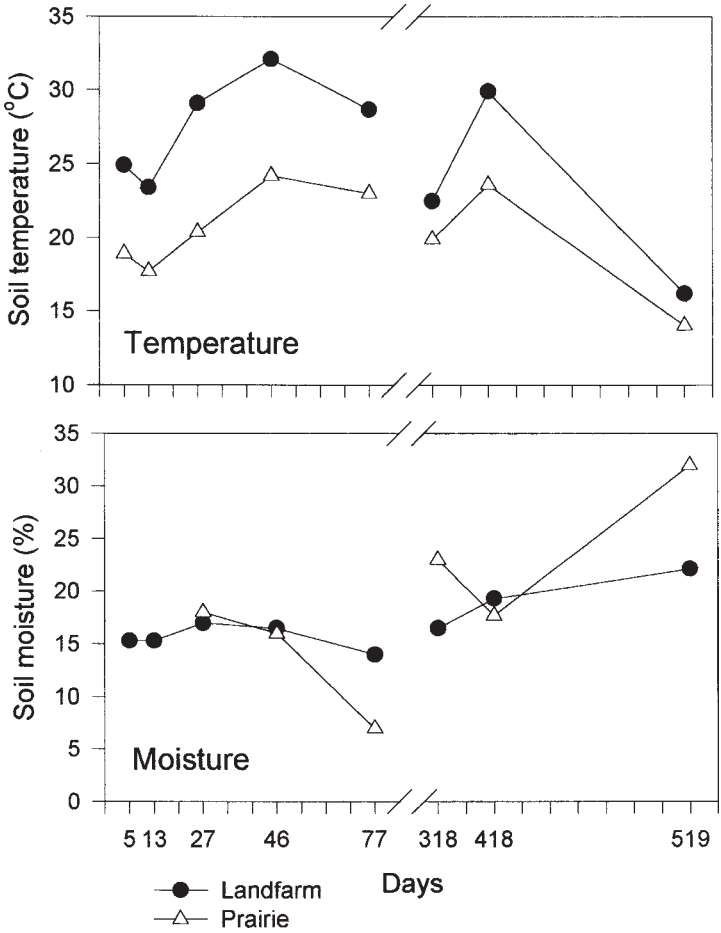

Fig. 4. Time course of soil temperature and % soil moisture (gravimetric) in the four treatment plots and uncontaminated prairie soil. Upper figure: Soil temperatures were obtained from the Foraker Mesonet station, located within the Tallgrass Prairie Preserve. Soil temperatures were taken 5 cm below the surface, either under bare sod or under vegetation. Bare-sod values were used for the landfarm, under vegetation for the prairie. Lower figure: % soil moisture was obtained after drying a portion of the soil used for the microbial enumeration. Values shown are averages for the four landfarm treatments, and for 1–3 samples from the prairie. Filled circles, mean values from the landfarm; open triangles, mean values from the prairie.

(Fig. 1), making it seem unlikely that limited soil moisture was responsible for the low bacterial counts in 1997.

Temperature

Soil temperatures were higher in 1996 in the landfarm than in 1997 on comparable dates (Fig. 4). The higher temperature in 1997 is unlikely to be the sole cause of the difference between years, as there was at least as much of a temperature differential between the landfarm and prairie in 1997, when microbial numbers (Fig. 1) and activity (Fig. 3) were similar. The

higher numbers of aerobic and hydrocarbon-degrading bacteria seen in 1996 in the landfarm might therefore be attributed to a combination of higher temperatures and abundant degradable hydrocarbons, with moisture levels in 1996 being sufficient for microbial growth.

The higher numbers (Fig. 1) but lower activity (Fig. 3) of bacteria seen in the prairie in 1996 vs 1997 were apparently not owing to higher temperatures in 1996, as temperatures were comparable both years (Fig. 4), although soil moisture was lower in 1996. In addition, unlike the landfarm, there was no man-made addition of a carbon source to the prairie.

Discussion

The landfarm still contained elevated levels, relative to the prairie, of nitrogen, phosphorus, and potassium at the beginning of the second warm season, as well as an average of 6,800 mg/kg TPH (TPH-IR), yet the numbers of aerobic heterotrophic bacteria and hydrocarbon-degrading bacteria were higher in the landfarm than in the prairie only during the first warm season. This, together with the pattern of soil-gas levels, suggests that the most easily degraded material was nearly exhausted in the first season. Soil-gas levels indicate that both aerobic and anaerobic carbon decomposition were stimulated by the hydrocarbons even within the relatively shallow confines of the landfarm.

Several different groups of microbes are potentially able to provide a link between aerobic carbon decomposition and anaerobic decomposition resulting in the production of methane *(9,10)*. Our previous work had found an association between elevated numbers of sulfate-reducing bacteria (SRB) and bioremediation of crude oil in field lysimeters *(8)*. SRBs in lake sediments were thought under some circumstances to produce H_2 as syntrophic partners of methanogens (reviewed in *9*). However, in the current study, the number of SRBs was not elevated in the landfarm, even though levels of sulfate were as much as 10-fold greater than in the prairie. An alternative linkage could be fermentative bacteria, which degrade carbon-containing polymers to low-weight carboxylic acids, including acetate and formate, hydrogen, and CO_2; and acetogenic bacteria, which produce acetate, formate, and hydrogen from higher-weight carboxylic acids *(10)*. Although we did not assay fermentative or acetogenic bacteria directly, it has been shown that acetate and CO_2 are the major carbon compounds produced initially upon anaerobic incubation of Kansas prairie soils, followed by consumption of acetate and the production of methane *(11)*. We did provide acetate as one of the potential electron donors in the methanogenic media and detected methanogens from soil samples collected throughout the landfarm.

Soil-gas levels, and by inference, microbial activity, correlated with soil temperatures; this was especially the case with methane production. Higher temperatures are associated with more methanogenesis, as most methanogens prefer temperatures 30°C or higher *(12)*. Acetate-production

and acetoclastic methanogens typically predominate at lower temperatures, whereas fermentation and production of H_2 by syntrophic bacteria occur at higher temperatures and favor H_2-consuming methanogens, as fatty-acid degrading syntrophs are inhibited by colder temperatures *(9)*. Methanogenesis in a landfarm could also be stimulated by the production of more anaerobic microsites through the increased activity of aerobic hydrocarbon-degraders depleting oxygen levels, as well as by slower rates of oxygen diffusion in very moist soils.

Measurements of methane reflect both the production of methane and its consumption by aerobic methanotrophic bacteria *(9)*. Grassland soils are usually sinks for methane *(11)* and the elevated levels of methane in the uncontaminated prairie in 1997 may have depended on a fortuitous combination of higher temperatures and sufficient moisture to produce a higher than average proportion of anaerobic microsites where methane production was favored over its consumption. Fertilization with nitrogen in the landfarm may be an additional factor that shifts the balance towards methane production, as nitrogen addition has been found to inhibit methane oxidation *(9)*.

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