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BIOLOGICAL PROPERTIES OF SOIL AND SUBSURFACE SEDIMENTS UNDER ABANDONED PASTURE AND CROPLAND

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Summary—Little is known about the effects of most surface land-use practices on shallow subsurface microbial communities. We analyzed duplicate cores taken aseptically from up to 10 m depth from unconsolidated valley sediments (soils) beneath an abandoned pasture reverting to tall grass prairie and cropland. Both profiles had similar soil texture, with moderately higher silt under cropland and a slight decrease in clay with depth. Soluble organic C was about two times higher in the grassland site and dissolved O_2 was about 8% lower compared with the cropland site. Water content and C-to-N ratios were greatest at the grassland surface but were less in the grassland than the cropland site within 2 m depth. In general, numbers of aerobic heterotrophic bacteria and protozoa decreased with depth until the saturated zone (4.3 m in grassland and 5.3 m in the cropland site). Bacterial numbers as determined by plate counts were about 10-fold less at the groundwater interface than in the surface soils at both sites. Direct microscopic counts of total bacteria were approximately the same in the surface soil and the sediments at the top of the water table at both sites. The top of the water table generally did not exhibit elevated microbial biomass or activity relative to deeper sediments. There was no significant relationship between protozoan numbers and microbial thymidine uptake at the cropland site, but a negative relationship was observed at the grassland site. The data suggest that cultivation may affect microbial biomass and activity in the subsurface, as well as community interactions between protozoa and bacteria.

INTRODUCTION

Groundwater quality is inextricably linked to subsurface microbial activity. Groundwater is frequently used for drinking in rural areas and the quality of groundwater is likely a reflection of surface land use. Thus, understanding how surface activities influence subsurface microbial communities will enhance our ultimate understanding of anthropogenic effects on water quality. Groundwater contamination with xenobiotic compounds can significantly alter communities of groundwater microbes (Dobbins *et al.*, 1992), but less is known about the effects of agricultural practices on subsurface microbial communities (Hall, 1992; Madsen, 1995).

The microbial ecology of the subsurface is only beginning to be understood. All common types of microbes found in soil can be isolated from material collected from great depths, except where rocks or sediments do not contain interstices large enough for microbes to move through (Ghiorse and Wilson, 1988; Madsen and Ghiorse, 1993). Total numbers of microorganisms typically decrease within several meters below the surface. After this initial decline, numbers usually do not continue to decrease further with depth (Ghiorse and Wilson, 1988; Madsen and Ghiorse, 1993), and numbers within the saturated zone occasionally can be similar to those observed at the surface (Beloin *et al.*, 1988).

We report here soil and sediment characteristics and microbial numbers and activities from below an old field-grassland and a cultivated field. A similar analysis of unconsolidated river valley sediments had been performed at a nearby site (Sinclair *et al.*, 1990), but microbial distributions were not linked to surface land-use practices in that study. Because of the lack of agreement concerning methods to estimate microbial numbers and activity, we used several techniques to measure each of these variables. Bacterial numbers were estimated by plate counts, direct counts, counts of active bacteria and bacterial C by fumigation extraction. Microbial activity was determined by thymidine incorporation and CO_2 evolution. Correlation analysis was used to establish patterns among variables.

MATERIALS AND METHODS

Study sites and sampling

All wells were drilled at the Konza Prairie Research Natural Area, a 3500-ha area dedicated to tallgrass

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prairie ecological research. The area lies within the Flint Hills of northeast Kansas, and the underlying geology consists of alternating layers of limestone and shale. Samples were collected from two boreholes each that were drilled to bedrock in a grassland and cropland area in the Kings Creek valley, where clay-based unconsolidated sediments overlie the limestone bedrock.

The grassland site has been in pasture since 1939 and was used for livestock grazing as recently as 1980. At one point before 1976, the area was planted with brome (*Bromus* sp.); it probably has never been fertilized, and was likely plowed when planted with brome. The site has not been grazed for 14 years, is burned every year or two and is reverting to tallgrass prairie. About 50% of the grass is big bluestem (*Andropogon gerardii*), a dominant grass in the tallgrass prairie. We will refer to the site as 'grassland'. The cores were collected during drilling from wells approximately 100 m up-slope from the stream bed. Cores from duplicate boreholes were taken in 60 cm lengths to limestone bedrock (8 m) on 19 April 1993. The two boreholes were approximately 7 m apart. At the time of drilling, the capillary fringe occurred at a depth of 4 m, and the top of the water table was found at approximately 4.3 m.

The cropland area is 1 km downstream in an area that is geologically similar to the grassland site (J. Oviatt, *pers. commun.*). The soil at both sites is mapped as Reading silt loam (fine, mixed, mesic Typic Argiudolls). The agricultural area was under cultivation for soyication bean (*Glycine max*) and wheat (*Triticum aestivum*) (including routine addition of N and P fertilizer) and was under continuous cultivation between 1939 and 1950 as evidenced by areal photography. Wells in this area also were drilled about 100 m from the stream. Duplicate cores were collected on 25 April 1993 down to bedrock (10.3 m), with the capillary fringe starting at 5.1 m and the water table at 5.3 m. The two boreholes were approximately 9 m apart.

Wells were drilled with a hollow core auger with a split barrel in the stem. The center of the barrel was lined with 5.1 cm dia polycarbonate sleeves. No drilling fluids or muds were used and all equipment was pressure washed with water before drilling at each site. Cores were collected in lengths of 60 cm. The split core and the sleeves were sanitized with 90% ethanol–10% H_2O and air-dried immediately before use. When cores were removed from the borehole, a subcore (2 cm long in a 1.4 cm dia syringe base with plunger intact) was taken immediately for O_2 analysis. Temperature of the cores was taken, and each core was sealed immediately with O_2 diffusion-resistant plastic wrap and stored on ice. The cores in their sleeves were stored at 4°C until they were partitioned on 29 April 1994. Sanitized tools (dipped in 95% ethanol–5% H_2O and flamed) were used to remove the material from the core sleeves into four sterile Whirl-Pak bags, where they were stored at 4°C until analysis. Results are presented g^{-1} dry soil.

Chemical and physical methods

Soluble organic C was extracted from 10 g moist soil with addition of 50 ml of 0.5 M K_2SO_4, shaking for 1 h at 200 rev min^{-1}, and centrifugation for 10 min at 18,000 × *g*. The supernatant was analyzed for dissolved organic C using a Dohrman DC180 carbon autoanalyzer (Rosemount Analytical, Santa Clara, CA) by ultraviolet-promoted persulfate oxidation and infrared detection of the CO_2. A significant C contribution from extractable inorganic C occurs in these soils so separate injections were performed to quantify inorganic and total C. Organic C was obtained by difference and coefficient of variation within standards was estimated at $< 5\%$ with a lower limit of detection at 1 mg C l^{-1}. Dissolved O_2 was measured within 6 h after removal from the site. Measurements were taken with a cathode type microelectrode (Revsbech and Jørgensen, 1986) with a 30 μm dia sensing tip imbedded in an 18 gauge stainless steel needle.

Gravimetric soil water content was determined by oven-drying duplicate subsamples from each depth-interval at 105°C for 24 h and was calculated as g H_2O g^{-1} dry soil × 100. Total organic C and N were determined on air-dried soils with a Carlo Erba CNS analyzer (Carlo Erba Strumentazione Rodana, Milan, Italy). Total organic C samples were acidified to remove carbonates prior to C analysis. Soil texture was analyzed by the hydrometer method (Forth *et al.*, 1982).

Microorganisms

Viable aerobic bacteria were enumerated by standard plate count techniques (Wollum, 1982). Bacteria were separated by aseptically blending 10 g soil or sediments with 100 ml of 0.1% sodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$; pH 7) for 1 min in two, 30 s intervals. Serial 10-fold dilutions were then prepared with sterile sodium pyrophosphate, and fractions (0.1 ml) of the resulting dilutions were spread-plated in triplicate on 5% peptone–tryptone–yeast–glucose (PTYG) extract agar media (Balkwill, 1990). Plates were incubated at 25°C for approximately 7 days (until a significant change in bacterial colonies was not observed). Actinomycetes and fungi were not counted. The values obtained from replicate determinations within the same core at each depth were averaged.

Direct counts of total bacteria and active bacteria were performed with 5-cyano-2, 3-ditolyl tetrazolium chloride (CTC), 4′,6-diamdino-2-phenyl-indole (DAPI) and epifluorescence microscopy (Rodriguez *et al.*, 1992; Yu *et al.*, 1995). Active bacteria were stained with the redox dye CTC (2.0 mM final concentration; Polyscience Inc., Warrington, PA) for 4 h with shaking at 160 rev min^{-1}. These counts did not include addition of organic C. The samples were

counterstained with DAPI (final concentration of 10 $\mu g\ ml^{-1}$) for 10 min to stain all DNA-containing cells.

Microbial biomass C was estimated using the fumigation extraction method (Sparling and West, 1988). Field-moist soil (20 g dry wt) from each depth interval was added to two 125 ml Erlenmeyer flasks. All flasks were covered with parafilm and conditioned for 5 days at 25°C. The control flasks were then promptly extracted with 0.5 M K_2SO_4 at a soil to extractant ratio of 5 to 1, as described previously. Soil moisture for fumigated samples that contained less gravimetric water than occurred at field capacity (− 33 kPa) was adjusted by adding distilled water to assure maximum extractable C flush values (Ross, 1989). Samples with moisture in excess of this value (saturated zone) were not adjusted or dried. The samples were fumigated with ethanol-free $CHCl_3$. The soil organic C flush after fumigation was extracted and analyzed as described above. Estimation of microbial biomass C was obtained using the equations of Voroney *et al.* (1993), and using an extraction efficiency factor (K_c) of 0.34.

Soil microbial activity was estimated from CO_2 evolution. Microbial activity was determined from aerobic incubation of field-moist soil (20 g) in a 160 ml vial with a butyl rubber stopper over 48 h at 15°C. Headspace CO_2 was quantified using a Schimadzu gas chromatograph (Schimadzu Scientific Instruments, Inc., Columbia, MD) equipped with a thermal conductivity detector (TCD) and a Porapak Q column (0.318 cm × 2 m, 80–100 mesh). The carrier gas was He at a flow rate of 14 ml min^{-1} and column, injector, and detector temperatures were set at 70°C.

Thymidine incorporation also was used to estimate bacterial activity (Kaplan *et al.*, 1992). Approximately 20 g wet weight samples of sediments were made into 80 ml slurries with filter sterilized (0.2 μm) well water from the site. These slurries were used to dispense approximately 1 g of sediment into sterile 20 ml borosilicate glass vials. Filter sterilized well water (10 ml) was added to each vial. Unlabeled thymidine was added at concentrations of 0, 1, 2, 5 and 10 μM to sediments from each depth. Twenty μCi of [methyl-^{3}H] thymidine were added to each sample. Samples for blank corrections were kept for 30 min in the dark at 15°C, and incubations were stopped before label addition with 0.5 ml 30% formalin. Incubations were terminated similarly with formalin at 30 min after label addition. DNA was extracted by precipitation and centrifugation (Kaplan *et al.*, 1992) and a scintillation counter was used to quantify incorporated ^{3}H. Samples from both sites at surface, middle and maximum depths were used to determine the time course of uptake from 0 to 60 min with 2 μM unlabeled thymidine added. These time courses established linearity over the 30 min incubation (data not shown).

Protozoan populations were estimated using a modification of the MPN techniques described by Singh (1946) and Sinclair and Ghiorse (1987). Soil or sediment samples (10 g) were weighed and transferred aseptically to a sterile blender containing 90 ml sterile phosphate buffer (2.2 mM KH_2PO_4, 4.02 mM K_2HPO_4, pH 7) and blended for 30 s. This mixture was used to make four additional 10-fold serial dilutions (10^{-1} to 10^{-5}). Aliquots (200 μl) from each serial dilution were placed in six wells of a sterile Corning 96-well tissue culture plate to which 50 μl of sterile 1.5% agar had already been added. Ten μl of a non-growing (stationary phase) *Enterobacter aerogenes*-phosphate buffer slurry (spectrophotometer absorbance of 0.680 at 750 nm) also were added to each well to provide a bacterial food source for the protozoa. Plates were kept for 30 days in the dark at 25°C. After incubation, a sample from each well was examined under a microscope (400 × power) for the presence or absence of protozoa. A computer program (Hurley and Roscoe, 1983) was used to calculate protozoan MPN values.

Results are presented with standard deviation to represent variance, except in the microbial abundance graphs. In these graphs minimum and maximum values were used to clarify presentation. Correlation analysis was used for exploratory statistical description of relationships among the variables. Because many of the variances were not equal and not normal, the nonparametric Spearman's Rank Order Correlation method was used for all variables. The correlations should be viewed with some caution because of possible problems with spatial autocorrelation. For comparisons between sites by depth, a paired difference *t*-test was used with mean values at each depth paired with the corresponding value from each site. Bonferroni's correction was used to account for the fact that numerous *t*-tests were made.

RESULTS

Soil texture was similar at both sites (Fig. 1). Clay decreased slightly with depth in the grassland site but not in the cropland site (Table 1). There was a somewhat greater (marginally significant) proportion of silt in the cropland site than the grassland site (Table 2). The general pattern was no strong physical discontinuity with depth until bedrock was reached. At the time of sampling, soil water content was high at the surface of the grassland due to exceptionally high precipitation in the two preceding months, decreased in the unsaturated zone, and increased in the water table [Fig. 2(A)]. Soil water content increased consistently with depth in the profile of the cropland site at the time of sampling [Fig. 2(A)]. Soluble organic C was higher in the grassland soil than in the cropland soil throughout the soil profile [Fig. 2(B)]. Oxygen content was lower in the grassland than the cropland site [Fig. 2(C)]; O_2 and soluble organic C exhibited a significant negative

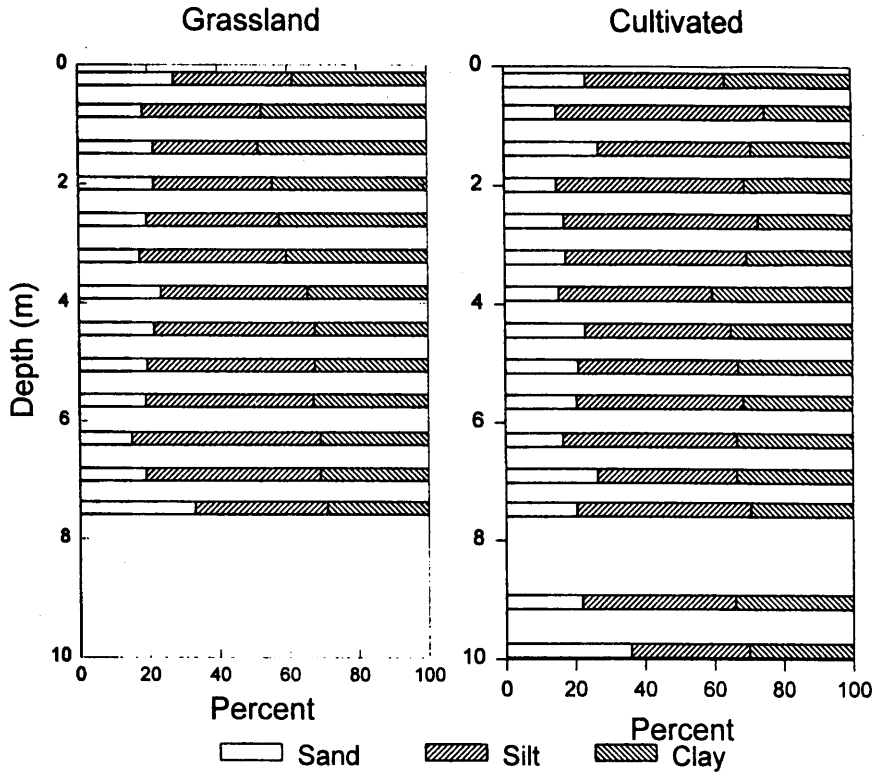


Fig. 1. Soil texture characteristics for the grassland and the cropland sites.

correlation (Table 1). Oxygen increased with depth in the cropland but not in the grassland site.

Soil organic C and N contents in both ecosystems were significantly greater ($P < 0.001$) in the surface (< 1 m) depth intervals than at the other depths [Fig. 2(D) and (E)]. Surface organic C and N values were significantly higher ($P < 0.001$) in the grassland site relative to the cropland site. A rapid decrease in organic C and N concentrations was observed beneath the active root zone in both systems. Throughout the profiles in the vadose and saturated zones there was a trend for slightly greater organic C and N values in the cropland site relative to the grassland site. The C-to-N ratios were generally higher in the cropland site relative to the grassland site [Fig. 2(F)].

Numbers of aerobic heterotrophic bacteria established by plate counts were greater in surface soils than at the water table at both sites [Fig. 3(A)]. Paired t-tests suggested higher plate counts on average at the cropland site compared to the grassland site (Table 2). Direct counts of total bacterial cells varied little with depth or between sites [Fig. 3(B), Table 2]. Counts of active bacteria were extremely variable and did not exhibit consistent trends with depth or site [Fig. 3(C), Table 2]. Microbial biomass C by fumigation–extraction was high at the surface of the grassland, but dropped close to zero by 2 m [Fig. 3(D)]. Microbial biomass C increased slightly with depth for the cropland site, and exhibited a subsurface peak at the water table in the grassland site.

Table 1. Correlations among depth, O_2 content, soluble organic carbon (SOC), bacterial plate counts (Plate), DAPI total bacterial counts, CTC active bacterial counts, most probable number of protozoa (Prot), CO_2 evolution (Resp), thymidine uptake (Thym), microbial biomass (Biom), C-to-N ratio (CN) and % clay (Clay) over all sites. Only significant relationships are presented ($P < 0.05$); values in upper right corner of the diagonal are the correlation coefficients, those in the lower left corner are the P values

Variable	Depth	O_2	SOC	Plate	DAPI	CTC	Prot	Resp	Thym	Biom	CN	Clay
Depth	1			−0.50			−0.80		0.27		0.26	−0.39
O_2		1	−0.48			−0.34			0.57			
SOC		0.0003	1						−0.35		−0.35	
Plate	0.0001			1			0.45				0.406	
DAPI					1	0.60	0.21			0.23		
CTC		0.0241			0.0001	1			−0.32			
Prot	0.0001			0.0004	0.032		1					
Resp								1				
Thym	0.0430	0.0001	0.0071			0.0321			1			−0.35
Biom					0.039					1	0.35	
CN	0.0229		0.0005	0.0001			0.0084			0.013	1	
Clay	0.0001								0.0005			1

Table 2. Paired difference *t*-tesis (Koopmans, 1981) for comparison of measured values between grassland and cropland sites. Values were paired by depth. A positive mean and *t* value between sites indicates that the values for the parameter were greater overall at the grassland site. Two-tailed probability is given. Because seven tests were made, a significance level of 0.05/7 = 0.007 should be used to indicate a significant difference

Parameter	Average difference (standard deviation)	*t* value	*P*
% Silt	−6.76 (10.87) %	−2.24	<0.05
Soluble organic C	1.45 (0.39) mg C g^{-1} soil	48.86	<0.001
O_2	−0.08 (0.07) % saturation O_2	−12.80	<0.001
Plate count	−0.463 (0.72) 10^5 CFU g^{-1} soil	−8.27	<0.001
DAPI direct count	−8.61 (70.6) 10^6 cells g^{-1} soil	0.52	>0.5
CTC direct count	1.34 (1.92) 10^5 cells g^{-1} soil	0.19	>0.5
Protozoa	−1.14 (4.6) 10^4 g^{-1} h^{-1}	−3.17	<0.01
Respiration	0.020 (0.311) μg CO_2-C g^{-1} h^{-1}	0.867	>0.2
Thymidine activity	−42.7 (37.1) DPM g^{-1} h^{-1}	−14.97	<0.001

The plate count method of determining bacterial numbers did not correlate with the direct counts by the DAPI or CTC methods. However, there was a significant correlation between the DAPI and the CTC methods (Table 1). Counts of active bacteria determined with CTC (9.81×10^5 cells g^{-1}) averaged 1.2% of those obtained with DAPI (8.40×10^7 total cells g^{-1}), and plate counts(3.66×10^6 CFU g^{-1}) were 4.4% of the DAPI numbers. The overall mean plate counts were 2.2 times greater than active (CTC) counts.

Protozoan numbers decreased by four orders of magnitude between the top and bottom of the profiles [Fig. 3(E)]. Protozoan numbers were two to seven orders of magnitude less than bacterial numbers determined by plate counts or direct counting techniques. As with the other measures of microbial abundance, variance within each site was high for the protozoa.

Respiration rates were not correlated significantly with depth [Table 1, Fig. 4(A)] but did decrease sharply in the surface 1 m of the grassland. Thymidine activity increased with depth [Table 1, Fig. 4(B)], but the correlation coefficient was weak. Microbial activity methods also did not correlate with each other (Table 1). The only significant correlation between the activity measures and the estimate of bacterial numbers was a negative relationship between thymidine activity and the number of active bacteria (CTC). Thymidine activity was negatively correlated ($P < 0.05$) with sand and clay content, and positively with silt ($P < 0.0001$).

Protozoan numbers correlated positively with bacterial numbers as determined by plate counts (Table 1), and both numbers decreased with depth. Protozoan numbers correlated positively with DAPI (total) bacterial counts and microbial biomass. There was a significant negative correlation ($P < 0.05$, linear regression) between protozoa and thymidine incorporation at the grassland site [Fig. 5(A)], but no significant interaction between the two at the cropland site [Fig. 5(B)].

DISCUSSION

The lower organic C at the cropland site could be the result of several factors. Disturbance by tillage and cultivation of the soil could increase oxidation rates of organic C near the surface (Tate, 1987). Removal of aboveground biomass and lower root biomass of winter wheat and row crops compared to native grasses could lower C inputs as well. Repeated N addition also may allow microbes to oxidize organic C more thoroughly. The higher organic C content in the subsoil of the cropland site relative to the grassland site suggests movement of partially-oxidized soil organic C from the surface to the lower depths of the profile. Other studies have suggested transport of C from the surface to the subsurface (McGarity and Myers, 1968; Myers and McGarity, 1971; Spalding *et al.*, 1978). Although there was a slight increase in organic N in the lower depths of the cropland site relative to the grassland site, the higher C-to-N ratio in lower depths of the cropland site suggests greater movement of organic C relative to organic N with cultivation.

The lower concentrations of O_2 in the grassland site could indicate higher metabolic rates of microbes than in the cropland site, but this conclusion is not supported by either method of estimating total microbial activity. Thus, lower O_2 in the grassland is more likely related to external factors such as surface land use. Previous work has verified that oxic groundwaters predominate in the limestone aquifers adjacent to the unconsolidated sediments (Edler and Dodds, 1992; Eichem *et al.*, 1993). Monitoring of O_2 concentrations in ground water at the two sites (unpubl. data) verifies the microelectrode measurements showing lower O_2 in the grassland.

There was a lack of correlation among methods for enumerating bacteria with depth and between sites. This may not be surprising since DAPI measures all cells with DNA, living or dead, CTC all actively respiring cells, and plate counts all cells able to grow on a defined laboratory medium. Our research supports results for groundwater noted by others (e.g. Albrechtsen and Winding, 1992) in that direct counts of total bacteria were two to three orders of magnitude higher than plate counts and direct counts of active bacteria. Plate counts are known to suffer from problems of selective media and probably only represent a fraction of the viable bacteria (Atlas and Bartha, 1993; Zuberer, 1994). Suzuki *et al.* (1993)

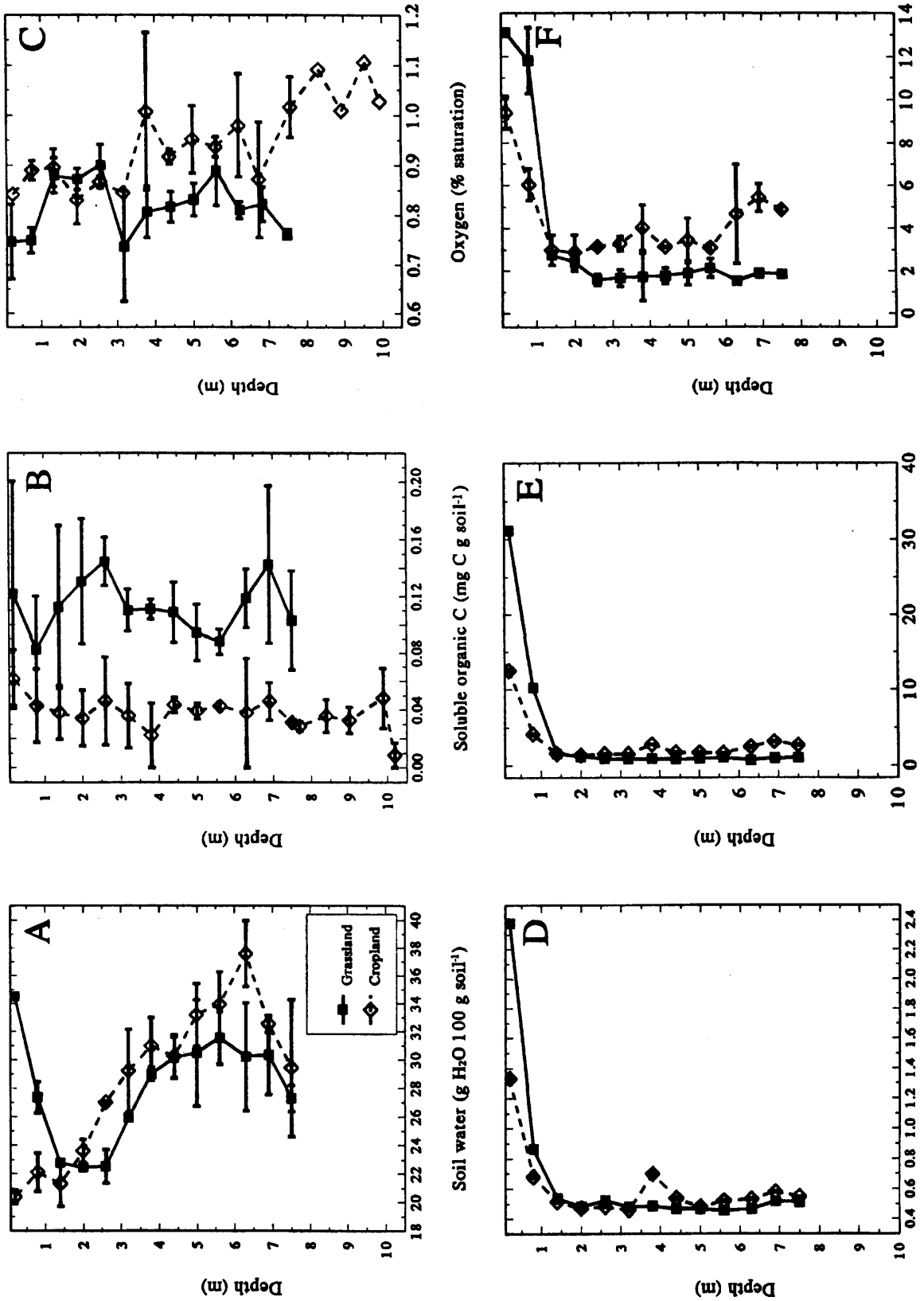


Fig. 2. Water content (A), soluble organic C (B), O_2 concentrations (C), total organic N (D), total organic C (E), and C-to-N ratios by weight (F) as a function of depth. Error bars = 1 SD. The water table was 5.3 m at the cropland and 4.3 m at the grassland site.

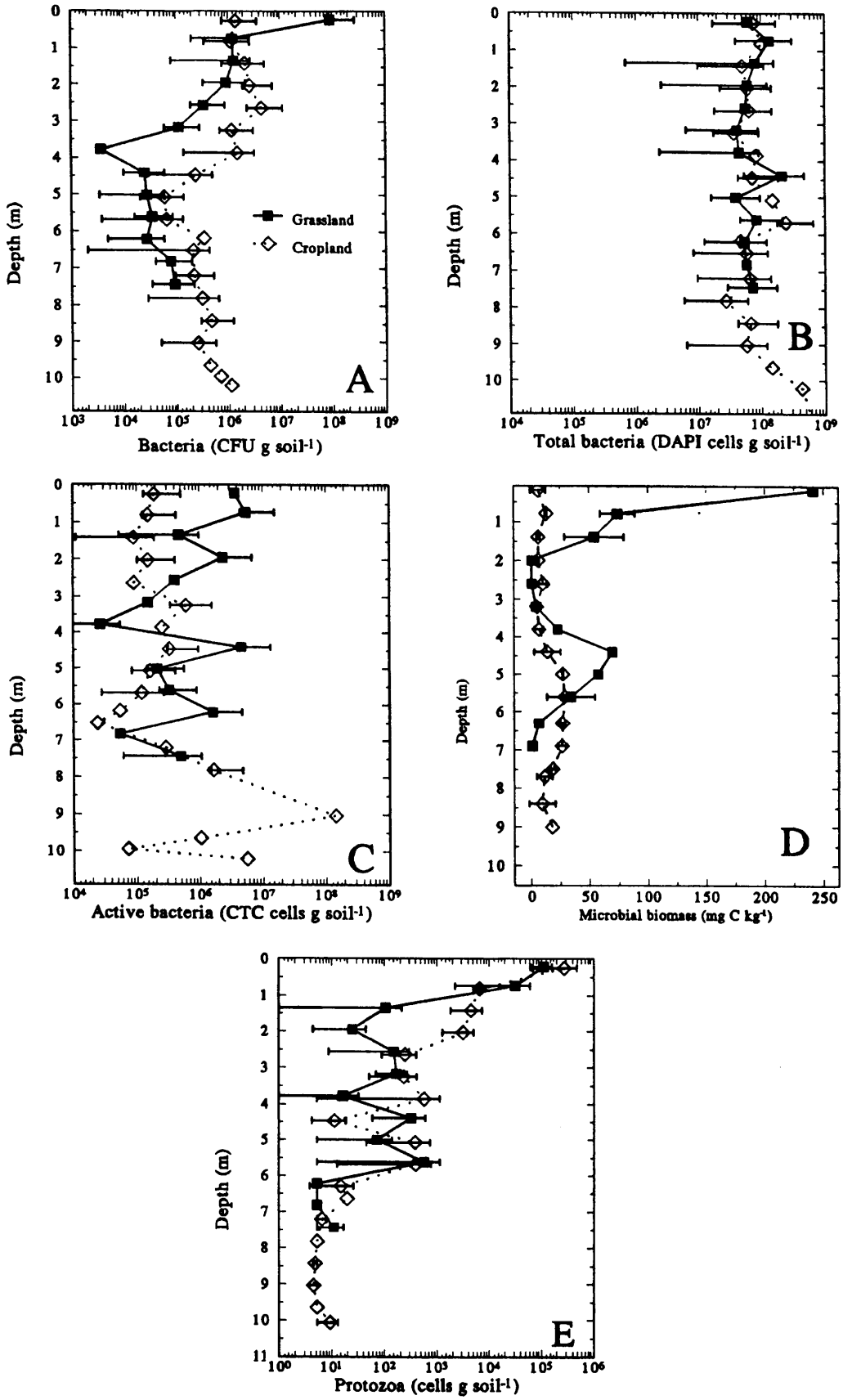
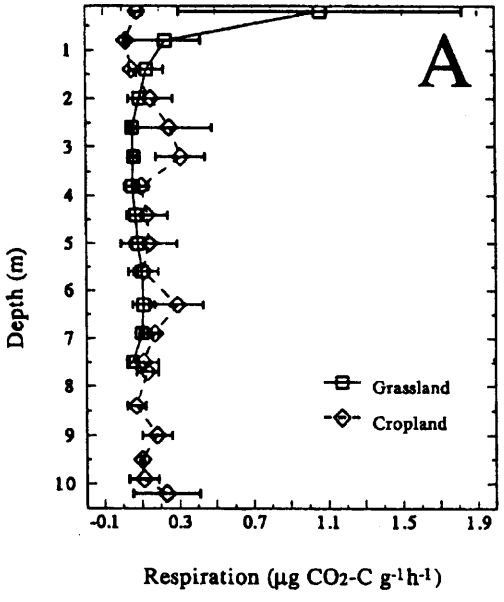


Fig. 3. Microbial counts by depth including bacterial plate count (A), direct count total (DAPI) bacteria (B), direct count total viable (CTC) bacteria (C), microbial biomass (D), and most probable number of protozoa (E). Error bar = min and max values. The water table was 5.3 m at the cropland and 4.3 m at the grassland site.

showed that DAPI counts can underestimate microbial numbers in planktonic samples relative to acridine orange methods, but acridine orange stains organic detritus indiscriminately in sediments, making reliable estimation of bacterial numbers difficult. Plate counts and those of active (CTC) bacteria do not differ greatly from each other. However, given the variance we observed, differences among depths and between sites were difficult to establish.

Bacterial numbers for the soil and near the top of the water table, as determined with direct and plate count methods, were similar to those reported in other studies for soil and groundwater respectively



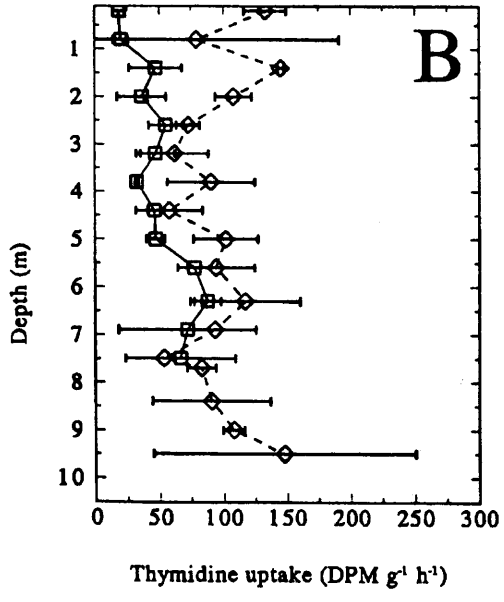


Fig. 4. Bacterial activity as a function of depth as measured by CO_2 evolution (A) and thymidine uptake (B). Error bar = 1 SD.

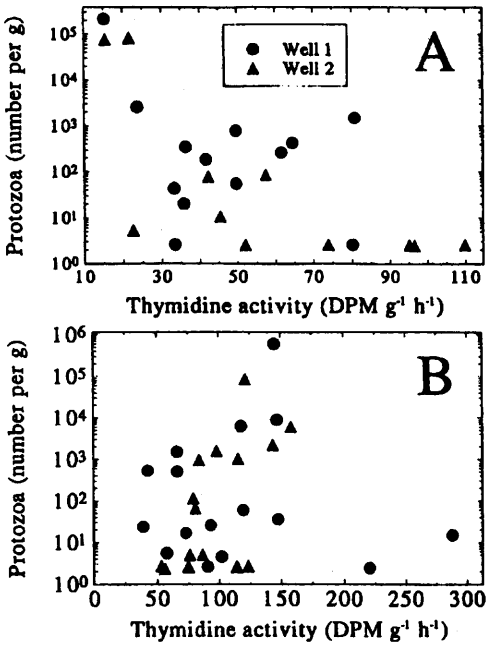


Fig. 5. Relationship between numbers of protozoa and bacterial activity at the grassland (A) and cropland (B) sites. Different symbols represent replicate wells at the same site.

(Table 3). The general trends were that numbers were greater in the surface soil than at the groundwater interface and that direct counts exceeded plate counts. There was a greater discrepancy between direct counts and plate counts at the groundwater interface than at the soil surface. This trend holds for three of the four studies allowing this comparison (Table 3). In four out of five studies, the plate counts and direct counts were more similar at twice the water table depth than at the interface. These data suggest that there may be a lower proportion of viable bacteria, or a different bacterial community, at the groundwater interface than at the soil–air interface.

Subsurface bacterial communities often vary greatly with depth, but such patterns were not evident in our data. Reports of increased populations within the subsurface sediments are often related to physical factors. Sinclair and Ghiorse (1989) reported a positive correlation between bacteria and sand content and a negative correlation with clay content. An increase in denitrifier populations above a layer with increased clay content in nearby layered subsoils has also been shown (Rice and Rogers, 1993). The data reviewed in Table 3 argue against sediments near the top of the water table always being sites where microbial numbers are greatly enhanced. Plate counts and direct counts at twice the water table depth equaled or exceeded those at the interface in most studies. We found no subsurface zones of greatly increased bacterial communities by all measures; however, sharp textural discontinuities were not observed in the profiles at our sites.

Our data suggest that one or few physical or chemical factors are unlikely to control microbial

Table 3. Total bacterial direct counts and plate counts at the soil surface and near the top of the water table for a variety of studies. All numbers presented g^{-1} dry weight of sediment

	Soil		Interface		2 × Interface depth		
Site	Total	Plate	Total	Plate	Total	Plate	Reference
Near landfill, Vejen, Denmark	ND	ND	3.16×10^7	6.31×10^4	7.94×10^7	3.16×10^5	Albrechtsen and Winding, 1992
Flood plain, field, Lola Oklahoma	1.0×10^9	1.25×10^7	1.25×10^7	BLD	6.31×10^7	1.0×10^6	Beloin *et al.*, 1988
Grassland, Kansas	1.17×10^8	4.26×10^6	1.37×10^8	4.90×10^4	7.28×10^7	1.82×10^4	This study
Cropland, Kansas	1.83×10^6	1.26×10^6	2.85×10^8	7.24×10^4	2.88×10^3	6.92×10^5	This study
River valley sediments, Kansas	6.31×10^9	1.0×10^8	1.26×10^7	3.16×10^5	$1.58 \times 10^{7*}$	$5.01 \times 10^{5*}$	Sinclair *et al.*, 1990

*Values taken from approximately 4.5 m below water table; BLD = below limit of detection; ND = not determined.

abundance and activity in shallow subsurface environments. Position relative to the water table, amounts and rates of downward movement of organic C and N, soil texture and other factors all may have influences. It is apparent, however, that surface land use can alter some or all of these controlling factors.

Relationships among consumption of bacteria, bacterial numbers, and bacterial activity are not easy to predict, given our scant knowledge of subsurface microbial food webs. General ecological studies have shown that there can be top-down control of primary production in lakes (Carpenter and Kitchell, 1988) and rivers (Power, 1990). Protozoa may control bacterial numbers and production in soil (Coleman, 1994; Pussard *et al.*, 1994). Madsen *et al.* (1991) showed that numbers of protozoa in groundwater increased with organic C enrichment with concomitant increases in bacterial numbers. In our study, protozoan numbers correlated positively with numbers of bacteria determined by plate counts, direct DAPI counts and fumigation, but not direct counts of active bacteria. The protozoan numbers may be underestimated because the blending required to obtain homogeneous samples may injure amoebae, but culture techniques also tend to yield greater numbers than direct counts (Cowling, 1994; Foissner, 1994).

Protozoa likely are more influenced by bacterial numbers (density of consumable food) than number of active cells or microbial activity. Bacterial and protozoan numbers have been correlated in deep subsurface studies (Sinclair and Ghiorse, 1989). It is well established that increased consumption of bacteria by protozoa is observed when bacterial numbers are elevated (Fenchel, 1980), and a number of investigators have demonstrated stimulation of microbial activity by protozoa (Pussard *et al.*, 1994). The fact that microbial activity is not related overall to protozoan numbers and that bacterial numbers are not inversely related to protozoa in our study suggests that, at the time of sampling, protozoa were not controlling bacterial activity.

Some of the differences between sites in the microbial communities were probably related to surface land-use practices and it is clear that surface conditions can be coupled to subsurface microbial biomass and activity. These differences could be attributed to alterations of conditions in the subsurface associated with cultivation. The influence of such changes on the microbial biomass and activity rarely has been established by other investigators (Madsen, 1995). Carbon and nitrogen flux is clearly altered by cultivation, and other effects are possible as well. Further research is needed, however, to determine how different types of surface management alter the subsurface ecology.

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