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Evaluation of microbial methods as potential indicators of soil quality in historical agricultural fields

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Abstract In agricultural ecosystems that have had consistent cropping histories, standard microbial methods may be used to evaluate past and present practices. Our objective was to evaluate several microbial methods that best indicate cropping histories and soil quality on long-term plots. We selected soil microbial carbon (C), phospholipid analyses, direct counts of total fungal and bacterial biomass, and soil enzymes (phosphatases) to measure direct and indirect microbial activity on the Sanborn Field and Tucker Prairie. The Sanborn Field has been under various cropping and management practices since 1888 and the Tucker Prairie is an uncultivated site. Seven different plots were chosen on the Sanborn Field and random samples were taken in the summit area on the Tucker Prairie, which represented a reference site. Soil microbial biomass C, phospholipids, and enzyme activity were reflective of the cropping and management histories observed on the Sanborn Field. Enzymatic activity was highly correlated to soil organic matter. The direct counts of fungal and bacterial biomass showed that fungal populations dominated these soils, which may be attributed to soil pH. Soil microbial biomass C and enzyme assays seemed to be better potential indicators of cropping histories than the other methods tested in the long-term plots.

Key words Phospholipids · Microbial biomass · Phosphatase · Cropping systems · Long-term experiments Prairie

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Introduction

According to Doran and Parkin (1994), soil quality may be defined as the capacity of soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health. An assessment of soil quality that includes soil biological, chemical and physical properties can provide valuable information for determining the sustainability of land management (Doran et al. 1994). There is a growing body of evidence that soil biological parameters may hold potential as early and sensitive indicators of soil stress or productivity (Dick 1992; Dick and Gupta 1994). In agricultural ecosystems that have maintained cropping and management practices for a long period of time (100 years), valuable information on soil physical and chemical properties relative to soil quality and land management is available. Although the role of microorganisms in soil fertility, plant nutrition, and soil organic matter transformations is well documented (Parkinson and Coleman 1991), standard and appropriate soil microbial methodology to assess the impact of these systems is lacking. The manner in which the microbial activity is determined is extremely important in natural environments, particularly soil systems. In the soil ecosystem, a diverse population of microorganisms exists coupled with varying environmental and climatic conditions. In agricultural ecosystems that have had consistent cropping histories one might be able to use standard microbial methodology to evaluate past and present practices.

Analyses measured included direct, indirect, and specific activity. For instance, enzymes are important

components involved in the dynamics of soil nutrient cycling. Enzyme activity in the soil environment is considered to be a major factor contributing to overall soil microbial activity (Frankenberger and Dick 1983) and, more recently, to soil quality (Visser and Parkinson 1992). Soil phosphatase activity was selected for study since it has been used often in attempts to describe soil microbial activity (Angers et al. 1993; Doran 1980). Other methods included were soil microbial biomass C, phospholipids, and direct estimates of total bacterial and fungal biomass. Our objective was to evaluate selected microbiological methods as potential biological indicators of soil quality in surface soils under long-term cropping and management systems.

Materials and methods

Several different methods were used to evaluate potential biological indicators of soil quality and the effect of cropping and management practices on the study sites. Each method is described below. Seven different plots were chosen on the Sanborn Field and one site on the summit area of the Tucker Prairie, which represented a nontilled, noncropped, and nonfertilized reference site (Table 1). The top-10-cm soil samples were collected in mid-November, 1992. The soils were sieved through a 0.75-mm sieve and the roots were handpicked from each sample as needed. Subsamples of each soil were taken to the Soil Testing Laboratory for pH and organic matter analyses. These plots were chosen to represent long-term cropping and management histories for Missouri soils (Table 1).

Study sites

The two study areas are located in the central section of Missouri, on a Putnam-Mexico soil association. Sanborn Field (SF) is located on the University of Missouri campus at Columbia. Individual field plots have been under different cropping and management practices established in 1888 (Table 1). The size of the individual plots was 31×10 m. Manure applied to selected plots was obtained from the University farm and added at 13.4 Mg ha^{-1}. Full fertility plots were fertilized based on soil test recommendations. The soil within this study area is classified as a Mexico silt loam (fine, montmorillonitic, mesic Udollic Ochraqualf), which is representative of the gently rolling, more erodible soils of the Midwest claypan area (Anderson et al. 1990). The crops within the selected plots were corn, soybean, and grasses (timothy).

The second study site is the Tucker Prairie (TP) Research Station located approximately 30 km east of the Columbia campus near Kingdom City, Mo. Tucker Prairie is a virgin site acquired by the University of Missouri in 1957. The native plant cover is characterized by several dominant warm season grasses including big bluestem (*Andropogon geradi* Vitman), little bluestem (*Schizachyrium scoparium* Nash), prairie dropseed (*Sporobolus heteropepis* Gray), and Indian grass (*Sorghastrum nutans* [L.] Nash) (Buyanovsky et al. 1987). These four species constitute about 75% of the total flora (more than 235 native plants) in this ecosystem. Both study sites are located in the same climatic region (Scrivner et al. 1972), with approximately 973 mm annual mean precipitation and 790 mm potential evapotranspiration. Specific soil data on the prairie are found in Table 1.

Acid and alkaline phosphatase analysis

Acid (EC#3.1.3.2) and alkaline (EC#3.1.3.1) phosphatase activities were determined using *p*-nitrophenol phosphate (PNP) as the substrate as described by Tabatabai (1982) with the following modifications. Half the volume of reagents and substrate was added to 0.5 g soil in 30-ml tubes. The tubes were capped, gently vortexed, and placed in a water bath at 37 °C for 1 h. After incubation and filtration, PNP presence in each sample was measured on a Pharmacia Ultrospec III spectrophotometer at 410 nm. Six samples of each soil were assayed twice for enzymatic activity. All values were reported on a dry soil weight basis. After drying soil at 105 °C for 24 h, soil moisture was determined on each sample.

Phospholipid analysis

Lipids were recovered from sieved 2-g soil samples by the chloroform-methanol extraction method (Findlay et al. 1989). All glassware was cleaned to reduce phosphorus residues. The cleaning procedure included (1) immersion in Versa Clean ^{TM} (Fisher) and (2) two distilled H_2O rinses, one rinse in 1 mol l^{-1} HCl, and a final five rinses in distilled water. Two grams fresh soil was mixed with chloroform : methanol : phosphate buffer (1 : 2 : 0.8), pH 7.4, 50 mol l^{-1} PO_4^-). Following vortexing, mixtures were allowed to stand for 2 h, after which the ratio was adjusted to 1 : 1 : 0.9 with additional chloroform and distilled water and re-vortexing. The soil was extracted in 50-ml test tubes (150×22 mm) with Teflon screw caps. After 24 h, the extraction mixture was separated into upper (aqueous) and lower (chloroform) layers. The aqueous layer was removed by aspiration and discarded. The soil chloroform mixture was filtered in a Büchner funnel through Whatman no. 2.0 filter paper. Filtrates were measured and quantitatively transferred to straight test tubes (200×25 mm). All chloroform was removed by evaporation from the tubes in a water bath at 55 °C with a stream of filtered (Whatman Carbon Cap) air. Phospholipids in the dried residues were re-solubilized in 2.5 ml fresh chloroform. Three 300-µl aliquots were removed from each sample and placed in a 5-ml ampoule. Chloroform was evaporated as described above and 1.35 ml potassium persulfate at 5% $K_2O_8S_2$ in 0.18 mol l^{-1} H_2SO_4 was added to the ampoules. The ampoules were heat-sealed and placed in an oven at 95 °C for 24 h for phosphorus hydrolysis. After hydrolysis, ampoule heat-seals were broken and ammonium molybdate VI tetrahydrate (ACS grade) (0.3 ml 2.5% solution in

Table 1 Characteristics of soil sampling sites (*FF* full fertility treatment based on soil test, *NF* no fertility treatment, *M* manure (13.4 Mg ha^{-1} applied annually), *CT* conventional tillage (moldboard plow, disk), *NT* reduced tillage, *OM* % organic matter)

Code	Description	Cropping history	pHs	OM
SF6	Sanborn Field plot 6	Continuous corn, FF, CT, since 1888	5.5	2.4
SF7	Sanborn Field plot 7	Continuous corn, FF, NT[a]	5.6	2.9
SF17	Sanborn Field plot 17	Continuous corn, NF, CT, since 1888	4.7	1.9
SF18	Sanborn Field plot 18	Continuous corn, FF, CT, since 1888	6.1	2.7
SF22	Sanborn Field plot 22	Continuous timothy, M, since 1888	6.1	4.0
SF23	Sanborn Field plot 23	Continuous timothy, NF, since 1888	5.1	3.1
SF39	Sanborn Field plot 39	Continuous soybeans, FF, CT, since 1990	6.8	2.5
TP	Tucker Prairie	Virgin prairie	4.4	5.2

[a] Continuous corn, FF since 1888; NT established in 1967

2.86 mol l^{-1} H_2SO_4) was added to each ampoule and allowed to stand for 10 min. Polyvinyl alcohol-malachite green [1.35 ml 0.111% polyvinyl alcohol (Sigma, 70000–100000 MW) with 0.011% malachite green] was added. After 45 min, absorbance at 610 nm of each sample was determined with a Pharmacia Ultrospec III spectrophotometer with the zero set with deionized water. Absorbance readings were compared with glycerol phosphate standards that were prepared from a 400-μmol l^{-1} stock solution (Findlay et al. 1989).

Soil microbial biomass C determinations

Soil microbial biomass C was determined by a modified chloroform fumigation and direct extraction method (Jordan and Beare 1991). Twenty grams moist soil was used and adjusted to 30% moisture basis on an oven dry weight basis. Samples were either fumigated with chloroform under vacuum or not fumigated for 24 h. Soil microbial biomass C from fumigated and unfumigated samples was extracted with 80 ml 0.5 mol l^{-1} K_2SO_4 after shaking for 30 min on a rotary shaker at 320 rpm. Biomass C of the extractant was evolved as CO_2 by the persulfate digestion procedure. Carbon dioxide was trapped in 1 ml 0.1 mol l^{-1} NaOH and titrated with 0.01 mol l^{-1} HCl. Soil microbial biomass C was calculated as the difference between duplicate fumigated and unfumigated samples and the mineralization constant ($k_c = 0.41$) was used as a conversion factor (Voroney and Paul 1984).

Direct estimates of total bacterial numbers

The total bacterial estimates were sent to the Soil Microbial Biomass Service at Oregon State University under the direction of Dr. Elaine Ingham in the Department of Botany and Plant Pathology. The total bacterial numbers were determined using the FITC (fluorescein isothiocyanate) method of Babiuk and Paul (1970). A 1 : 100 dilution was prepared and 1 ml of the dilution stained for 3 min with FITC. The total volume was filtered through a 0.2-μm pore-size, non-fluorescent (black-stained) Nucleopore filter and destained with sodium carbonate and pyrophosphate buffers. The filter was placed on a slide and the number of fluorescing bacteria in each of ten fields was counted. Background levels of contamination in each batch of buffer solutions was assessed and subtracted from sample estimates. These background counts were normally zero.

Diameters and numbers of bacteria were measured during observation of the ten filter fields and used to calculate biovolume. Bacterial biovolume (in cubic centimeters) was calculated by multiplying πr^2 for cocci and $\pi l r^2$ for bacterial rods. Biomass was calculated by converting direct estimates of bacterial biovolume to biomass using bacterial density averages of 0.33 g cm^{-3} (van Veen and Paul 1979). Biomass was expressed on a soil dry weight basis.

Direct estimates of total fungal biomass

Fungal biomass estimates were also conducted at the Soil Microbial Biomass Service at Oregon State University. The total fungal biomass was determined using a ten fold dilution of soil in phosphate buffer (0.2 *M*, pH 7.2) and shaken for approximately 5 min at 120 rpm. At 1- or 5-ml aliquot was removed from the 1 : 10 dilution and stained with fluorescein diacetate (FDA) for 3 min with appropriate buffers (Ingham and Klein 1984). An aliquot of the FDA suspension was mixed with 2% agar, and placed in a microscope slide well of known volume. Using epifluorescent microscopy, the length of the FDA-stained hyphae was determined. Using phase contrast microscopy, the length and diameter of all the hyphae was measured and recorded from three 18-mm length transects of each agar film. One agar film was observed per sample at X160 magnification. Background contamination for correction was determined by measuring hyphal lengths using sterile water samples instead of soil suspensions.

Total fungal length per volume of agar film was determined and average hyphal diameters were determined. Hyphal biovolume was calculated by multiplying $\pi r^2 l$; width was generally equal to 2.5 μm for fungi in soil. Fungal diameters can be as great as 10 μm for some Basidiomycetes and as narrow as 1 μm for Phycomycetes (Ingham, personal observation). Biovolumes are converted to biomass and expressed on a soil dry weight basis (Table 4).

Statistical analyses

Statistical analyses were performed on phospholipid, phosphatase, and microbial biomass assays by analysis of variance and standard deviations between replicates for each sample using the General Linear Models procedure for SAS programs (SAS 1988). We used six replicates for phospholipids and phosphatase and four replicates for soil microbial biomass C assays.

Results and discussion

Soil enzyme activity

Differences in phosphatase activity were detected among soils apparently related to crop management practices and/or soil properties. For example, acid phosphatase activity was 23% higher for continuous corn under no-till (SF7) than for continuous corn under conventional tillage (SF6) that had received full fertility treatment (Fig. 1). Acid phosphatase activity was also approximately 45% greater for full fertility continuous corn under no-tillage (SF7) than for no fertility continuous corn under conventional tillage (SF17) (Fig. 1). Phosphatase activities in no-tillage soils are generally higher than in the conventional soils (Doran 1980; Angers et al. 1993). Alkaline phosphatase activity was 50% higher for continuous corn with full fertility (SF18) than with no fertility treatment (SF17). Cropping systems on the soils selected from the

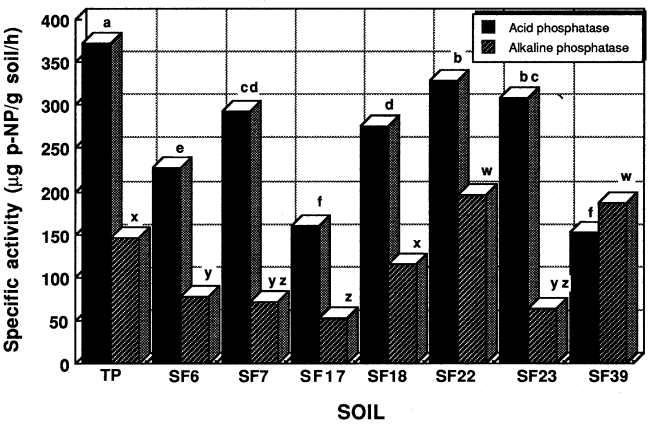

Fig. 1 Specific activity of soil phosphatase on Sanborn Field plots and Tucker Prairie. Within phosphatase activity (*a*–*f* acid, *w*–*z* alkaline) soils with the same letter are not significantly different ($P < 0.05$) according to the LSD test

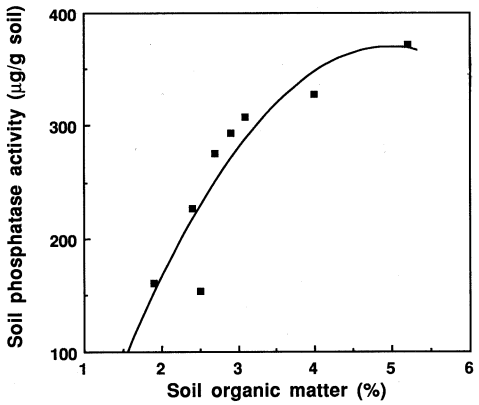

Fig. 2 Relationship between soil phosphatase activity and soil organic matter determined for Sanborn Field and Tucker Prairie sites. The regression equation is $Y = -204.3 + 230.8X - 23.2X^2$ ($r^2 = 0.82$)

Sanborn Field have affected soil properties over time. Enzymatic activity was highly correlated to soil organic matter with an $r^2 = 0.82$ (Fig. 2). In our study, examination of neighboring plots receiving different treatments might reveal important effects of soil properties on phosphatase activities. For example, in comparing SF22 and SF23, alkaline phosphatase activity was significantly higher for SF22, which also had higher pH and OM values. Dick (1984) similarly reported significant phosphatase activity in soils relative to soil pH and OM. Frankenberger and Dick (1983) also reported phosphatase activities which were significantly and positively correlated with organic C. Alkaline phosphatase showed a significant correlation with microbial activity as measured by O_2 uptake and CO_2 evolution, with respiration being indicative of an active microbial population (Frankenberger and Dick 1983).

Our results support previous suggestions that acid phosphatase activity may be an appropriate measurement as an indicator of relative soil microbial activity. This is especially important since most soil enzymes are believed to be entirely derived from the soil microbial population (Frankenberger and Dick 1983; Angers et al. 1993). Furthermore, enzymes catalyze many reactions in soils that are central to nutrient cycling, organic residue decomposition, etc.

Phospholipid analyses

The phospholipids reflected cropping practices in the Sanborn Field (Table 2). The treatments which received no fertility treatment for 100 years reflected the lowest quantity of extractable phospholipids with the exact opposite effect with plots that received full fertility treatment for 100 years. Tillage was an important factor in plots SF6 and SF7 under continuous corn with conventional tillage and no-tillage (Table 2). As expected, the uncultivated prairie plot (TP) yielded the highest phospholipid level, reflecting the higher organic matter content and associated active microbial populations.

Table 2 Phospholipids recovered from soils collected from the Sanborn Field and Tucker Prairie. Values are means of five replicate assays (values in parentheses are standard deviations of the means)

Plot	Phospholipids (nmol g soil^{-1})
SF6	46.6 (8.4)
SF7	67.5 (10.7)
SF17	21.5 (5.6)
SF18	57.8 (12.4)
SF22	65.4 (5.3)
SF23	37.1 (2.5)
SF39	77.2 (22.7)
TP	107.3 (7.8)

Soil microbial biomass C

Organic amendment, fertilizer source, cultivation, and crop species are important factors that influence soil microbial biomass C. Microbial biomass C was significantly higher in continuous corn, full fertility plots that were NT (SF7) compared with CT plots (SF6) (Table 3). Despite similar tillage in plots SF17 and SF18, microbial C was significantly greater in the latter due to full fertility treatment. Continuous grass plots with manure, SF22, yielded significantly higher microbial C than any of the Sanborn plots (Table 3). This was attributed to annual organic amendment under no tillage. Continuous grass receiving no organic amendment or inorganic fertilizers also exhibited a significantly higher biomass than other plots grown to corn and soybeans. The highest microbial C was measured from the Tucker Prairie site under continuous grass and other native species with no cultivation. These grass ecosystems are characterized by an active and larger microbial biomass and greater root turnover, as reflected in the total bacterial and fungal biomass (Table 4). Enzyme activity in SF22 and SF23 plots was also similar to that of the Tucker Prairie (Fig. 1).

Table 3 Microbial biomass C determined for soils collected from the Sanborn Field and Tucker Prairie (values are means of four replicate assays; standard deviations of the means are in parentheses)

Plot	Microbial C (µg g soil^{-1})
SF6	182 (7.9)
SF7	252 (22.6)
SF17	86 (20.7)
SF18	197 (41.9)
SF22	487 (67.8)
SF23	404 (67.4)
SF39	184 (15.8)
TP	564 (26.8)
LSD (0.05)	58.5

Total bacterial and fungal biomass

The greatest bacterial biomass was found in the Tucker Prairie plots, with continuous corn, no fertility, conventional tillage plots having the second greatest bacterial biomass (Table 4). Tillage did not seem to be a factor in the quantity of bacterial biomass present. Bacterial determinations either by plate or direct counts do not always reflect or correlate well with other methods that indicate soil microbial activity. Phosphatase activities did not correlate with microbial numbers determined by plate counts (Frankenberger et al. 1983). Enzymatic activities may be associated with active soil microorganisms and microbial numbers do not accurately reflect microbial activity (Frankenberger et al. 1983).

However, the fungal biomass seemed to better reflect cropping and management histories. Plots cultivated to corn under either no-tillage or conventional tillage and receiving full fertility treatment (SF6, SF7, SF17) reflected intermediate fungal biomass levels (Table 4). Plots SF18 and SF39 yielded the lowest fungal biomass values. We are uncertain of the reason for the lowest fungal biomass observed in plot SF18. Soybean residues decompose considerably faster than corn residues and support lower fungal populations (Broder and Wagner 1988), thus explaining the low fungal biomass detected on plot SF39. The greatest fungal biomass was shown in the grassland/prairie plots. Fungal biomass more clearly reflected the cropping histories than bacterial biomass.

Conclusions

Microbiological methods used to assess soil quality should reflect cropping and management histories in long-term studies and support soil chemical and physical properties. Of the methods evaluated, microbial biomass C and enzyme activity seemed to be the best indicators of the effects of cropping and management practices in long-term fields. Phosphatase activity significantly correlates with soil organic matter and soil microbial biomass (Frankenberger and Dick 1983).

Table 4 Total bacterial and fungal biomass determined for soils collected from the Sanborn Field and Tucker Prairie (values are means of three replicate assays; standard deviations of the means are in parentheses)

Plot	Bacterial biomass ($\mu g\ g\ soil^{-1}$)	Fungal biomass ($\mu g\ g\ soil^{-1}$)
SF6	6.7 (1.0)	29.4 (1.2)
SF7	6.5 (1.2)	30.2 (2.4)
SF17	7.4 (0.5)	33.3 (2.2)
SF18	6.2 (0.5)	9.9 (1.9)
SF22	4.7 (0.9)	39.1 (2.8)
SF23	4.3 (0.5)	42.0 (3.3)
SF39	2.4 (0.4)	17.4 (3.1)
TP	21.0 (1.4)	92.8 (12)
LSD (0.05)	1.5	8.4

Soil organic matter levels may provide an indication of the productivity or potential sustainability of a specific agricultural soil. With appropriate soil biological methods in combination with soil physical and chemical properties, early indicators of soil quality may be detected. Historical fields like Sanborn offer insightful information for evaluating both long- and short-term soil indicators.

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