Cropping system effects on soil biological characteristics in the Great Plains

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Abstract

Soil biological quality can affect key soil functions that support food production and environmental quality. The objective of this study was to determine the effects of management and time on soil biological quality in contrasting dryland cropping systems at eight locations in the North American Great Plains. Alternative (ALT) cropping systems were characterized by greater cropping intensity (less fallow), more diverse crop sequences, and/or reduced tillage than conventional (CON) cropping systems. Soil biological properties were assessed at depths of 0–7.5, 7.5–15, and 15–30 cm from 1999 to 2002 up to three times per year. Compared to CON, ALT cropping systems had greater microbial biomass and potentially mineralizable N. ALT cropping systems also had greater water stable aggregates in the surface 7.5 cm, but only at four locations. Total glomalin (TG), an organic fraction produced by fungi associated with aggregate stability, differed only at one location (Mandan), where the ALT cropping system had 27% more TG than the CON cropping system. Fatty acid methyl ester (FAME) profiles were highly location dependent, but total extracted FAME tended to be higher in ALT cropping systems. Soil biological properties fluctuated over time at all locations, possibly in response to weather, apparent changes in soil condition at sampling, and the presence or absence of fallow and/or legumes in rotation. Consequently, preplant and post-harvest sampling, when weather and soil conditions are most stable, is recommended for comparison of soil biological properties among management practices. Overall, ALT cropping systems enhanced soil function through: (1) improved retention and cycling of nutrients and (2) maintenance of biodiversity and habitat, implying improved agro-ecosystem performance over time.

Key words: cropping systems, soil biology, Great Plains, soil quality

Introduction

Soil biota mediate important ecosystem processes such as energy flow, nutrient cycling, and water infiltration and storage[1]. Improved soil management may enhance the activities of soil flora and fauna, including decomposition of organic residues, assimilation and release of plant nutrients, creation of biopores, and production of compounds in soil thought to enhance aggregate stability[2–5]. Collectively, soil biota affects both agricultural productivity and environmental quality and, therefore, warrants careful consideration when evaluating the sustainability of cropping systems.

Cropping systems influence soil biota predominately through the kind and quantity of plant residue food sources they provide, and their impacts on the soil physical and chemical environment[3]. Crop type and sequence, cropping intensity, tillage and residue management, and fertilization represent management components that shape the environment in which soil biological activity takes place. However, the impacts of cropping systems are greatly altered by soil type and climate. Furthermore, cropping system effects on soil biota may take a considerable time to accrue to the

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point that they become measurable. In a review of soil biological characteristics in conventional (CON) and alternative (ALT) agricultural systems, Ryan[6] found that management practices may need to be in place for more than 10 years before they have a consistent influence on the soil biological community. Such an influence may take even longer when systems have a relatively low level of production and few inputs, due to limiting factors such as low rainfall or extreme temperatures[6]. Interestingly, these limiting factors characterize the climate of the Great Plains[7,8].

Cropping systems with intensive crop sequences and/or reduced tillage in the Great Plains have been found to possess more soil microbial biomass[9–11], potentially mineralizable N (PMN)[11–14], and total glomalin (TG)[15]. Such trends in soil biological characteristics are attributed to greater crop residue, root mass, and soil organic matter (SOM) accumulation in the soil surface of these systems. Specific responses of soil microbial communities to management practices in the Great Plains indicate that no-till, relative to conventional tillage, results in increased fungal abundance[16], higher populations of denitrifying bacteria[17], and greater ester- and phospholipid-linked fatty acid methyl esters (FAME) under fallow conditions[18]. These results underscore the capacity of no-till to favor the growth and activity of soil micro-organisms that can improve soil structure but also increase gaseous N loss by denitrification.

In 1999, a multi-location study was initiated to evaluate a number of soil physical, chemical and biological properties proposed for assessing soil quality[19]. The objectives of this study were to (1) quantify temporal dynamics of soil quality attributes in established cropping systems; (2) assess impacts of contrasting management on soil quality attributes; and (3) evaluate recently developed methods for assessing soil quality (e.g., microbial biomass by microwave irradiation, FAME and TG). The study's objectives allowed for the evaluation of management impacts on a consistent set of soil biological properties across multiple locations over time, which, to our knowledge, has not been conducted in the Great Plains.

Materials and Methods

Description of locations and treatments

Contrasting management treatments within eight long-term cropping system experiments throughout the Great Plains were selected for the study (Table 1). Experiments were located near Akron, Colorado (CO); Brookings, South Dakota (SD); Bushland, Texas (TX); Fargo, North Dakota (ND); Mandan, ND; Mead, Nebraska (NE); Sidney, Montana (MT); and Swift Current, Saskatchewan (SK), Canada, and had been conducted from 9 to 32 years upon initiation of this study. Contrasting treatments were selected within each experiment representing CON and ALT cropping systems, with the latter characterized by reduced tillage, reduced occurrence of fallow, and increased crop diversity. In addition to the established treatments, relic areas under undisturbed native perennial vegetation were evaluated at four locations (Fargo, Mandan, Mead, and Sidney). A detailed description of locations and treatments is provided elsewhere[20].

Sampling method

Soil samples were collected up to three times per year over a period of 4 years at each location. Samples were collected

Table 1. Contrasting management treatments within eight long-term cropping systems. Treatments selected at each site differed in management intensity as characterized by either type or frequency of tillage, cropping intensity, and/or crop rotation diversity and are termed conventional (CON) or alternative (ALT).

Location/Soil series	Treatment	Crop sequence	Tillage	N rate[1]
Akron, CO	CON	WW–F[2]	Sweep (fallow)	Varied
Weld silt loam	ALT	WW–C–M	No tillage	Varied
Brookings, SD	CON	C–C	Chisel plow and disk	High
Barnes sandy clay loam	ALT	C–SB–SW–A	Chisel plow and disk	0
Bushland, TX	CON	WW–SO–F	No tillage	Varied
Pullman silty clay loam	ALT	WW–WW	No tillage	0
Fargo, ND	CON	DW–P	Fall plow	0
Fargo silty clay	ALT	DW–P	No tillage	0
Mandan, ND	CON	SW–F	Chisel plow and disk	Medium
Wilton silt loam	ALT	SW–WW–SU	No tillage	Medium
Mead, NE	CON	C–C	Tandem disk, 2×	High
Sharpsburg silty clay loam	ALT	C–SB–SO–OCL	Tandem disk, 2×	High
Sidney, MT	CON	SW–F	Tandem disk	45 kg ha^{-1}
Vida loam	ALT	SW–SW	No tillage	45 kg ha^{-1}
Swift Current, SK	CON	SW–F	Chisel plow and harrow	Varied
Swinton silt loam	ALT	SW–L	Chisel plow and harrow	Varied

[1] Varied, N-fertilizer application rate based on soil test results.
[2] Abbreviations: A, alfalfa; C, corn; DW, durum spring wheat; F, summer fallow; L, lentil; M, proso millet; OCL, oat + clover; P, field pea; SB, soybean; SO, sorghum; SU, sunflower; SW, spring wheat; WW, winter wheat.

prior to planting, at peak crop biomass, and after harvest in the same plots throughout the duration of the study. Two types of soil sample were collected from each plot at each sampling time. The first sample was collected to a depth of 30 cm in increments of 0–7.5, 7.5–15, and 15–30 cm with a step-down probe. The second soil sample was collected from the surface 0–7.5 cm for glomalin concentration, wet aggregate stability, and aggregate-size distribution, using a shovel or trowel in such a manner as to maintain aggregate integrity. Upon collection, the first set of samples was placed in cold storage at 4°C until processing, while the second set was immediately air-dried at <35°C for 3 or 4 days. A detailed description of the sampling and soil processing method can be found elsewhere[20].

Laboratory evaluations

Microbial biomass and PMN. Microbial biomass analyses were conducted within 1 week of receipt of samples to the laboratory. Microbial biomass C (MBC) was estimated by the microwave irradiation method using a 10-day incubation of irradiated and non-irradiated subsamples[21] with CO_2 production determined by gas chromatography[22]. Headspace of the non-irradiated samples was flushed with air, resealed, and incubated for an additional 10 days for estimates of mineralizable C and N. MBC was calculated from the difference between CO_2 released from irradiated and non-irradiated soils. Metabolic quotient (qCO_2) was calculated as mg of CO_2 respired per mg of total MBC[23]. Microbial biomass N (MBN) was estimated from the 10-day mineral N flush between irradiated and non-irradiated soils[24].

PMN was estimated from the NH_4-N accumulated after a 7-day anaerobic incubation at 40°C using 5 g oven-dried equivalent of field moist soil[25]. Ammonium before and after the incubation was estimated from $1:10$ soil:KCl ($2 M$) extracts by an indophenol blue reaction[26].

Aggregate stability and glomalin. Air-dried bulk soil was sieved to segregate the 1–2 mm size aggregates. Aggregates were premoistened with deionized water by capillary action for 10 min and then subjected to wet sieving for 5 min[27]. Soil particles passing through a 0.25 mm sieve were dried at 75°C and weighed. The material remaining on the 0.25 mm sieve was dispersed with 5% sodium hexametaphosphate and the coarse material was washed with deionized water, dried, and weighed. The initial and final weights of aggregates were corrected for the weight of coarse particles (>1 mm).

Glomalin was extracted from 1 g of aggregates using 50 mM citrate, pH 8.0, for 1 h cycles at 121°C until the supernatant was straw-colored, an indication that all of the glomalin had been removed. Supernatants for each soil sample were pooled, mixed, and an aliquot was centrifuged at 10,000 g for 3 min. Protein in the supernatant was detected by the Bradford dye-binding assay with bovine serum albumin as the standard[28]. Concentration of glomalin was extrapolated to mg g^{-1} of aggregated soil particles by correcting for the dry weight of coarse fragments >1 mm included in the weight of aggregates and for the volume of extractant. As a supplement to glomalin analysis, extractable Fe in bulk surface soil was estimated using inductively coupled plasma atomic emission spectroscopy after extraction by diethylenetriaminepentaacetic acid (DPTA)[29].

FAME extraction. Fatty acids were extracted from 5 g air-dried soil samples in acid-washed glassware using the Microbial Identification, Inc. standard protocol[30]. Samples were analyzed using a Varian 3800 gas chromatograph with a 30 m Rtx-2330 capillary column and a Saturn 2000 mass spectrometer (MS) as the detector (Varian, Inc., Palo Alto, CA, USA). Fatty acids were identified and quantified by comparison of retention times, MS fragments, and peak areas to components of the Supelco 37 Component FAME mix (#47885-U; Supelco, Inc., Bellefonte, PA, USA) and several individual standards (Sigma M-2799, H-3523, M-7656, M-6656, and M-3289; Sigma–Aldrich, St. Louis, MO, USA). Individual peak data for each fatty acid were converted to molar percentages by dividing peak area by the fatty acid molecular weight, then dividing by the total molar area of all fatty acids identified in the sample.

Statistical analyses

Microbial biomass, PMN, water-stable aggregates (WSAs), and glomalin were evaluated within locations using appropriate models in PROC MIXED[31]. Evaluations were conducted by depth using treatment and time as fixed effects and replication as a random effect. Time represented effects within years (preplant, peak biomass, and postharvest) and over years (1999–2002) and was assigned a dummy variable from 1 to 12 corresponding to the 12 separate sampling times. Additionally, biological properties were evaluated by grouping locations within their respective soil moisture regimes. For this evaluation, Brookings, Fargo, and Mead were grouped into an udic moisture regime, while Akron, Bushland, Mandan, Sidney, and Swift Current were grouped into an ustic moisture regime. Evaluations by soil moisture regime were conducted with PROC MIXED using data only from the preplant sampling time and 0–7.5 cm depth. Relationships between soil biological properties and relevant soil chemical and physical parameters as well as management, climatic, and sampling factors were identified by linear regression analysis using SAS[32] or Statistix (Analytical Software, Tallahassee, FL, USA).

Potential biomarker FAMEs[33,34] were analyzed individually or in small groups by depth using an appropriate model in PROC MIXED. Molar percentages of FAMEs were used in principal components analysis (PCA) across all locations, times, and depths using data from 2000 to compare primary factors affecting FAME profiles[35]. Factor

Table 2. Fatty acid methyl esters (FAMEs) used in principal components analysis (PCA) with eigenvector loadings >|0.20|. Positive or negative loadings farther from 0.0 have a greater relative effect on PC ratings.

PC 1		PC 2		PC 3	
FAME[1]	Loading	FAME	Loading	FAME	Loading
$12:0$[2]	0.34	$14:0$ *3OH*	0.29	$15:0$ *iso*	0.35
$13:0$ *iso*	0.31	$16:1$ ω*9c*	0.34	$15:0$ *anteiso*	0.39
$13:0$ *branched A*	0.22	$17:1$ *iso*	0.22	$16:0$ *iso*	0.33
$14:0$	0.28	$18:0$ *iso*	0.22	$16:0$ *2OH*	−0.28
$16:1$ ω*7c*	−0.21	$18:0$ *branched A*	−0.28	$17:0$ *branched C*	−0.22
$17:0$ *anteiso*	−0.21	$18:2$ ω*6c*	0.27	$17:1$ *branched A*	−0.27
$17:0$ *branched A*	−0.23	$19:0$ *cyclo*	−0.28	$18:1$ ω*9t*	−0.28
$17:0$ *branched A*	−0.23				
$17:0$ *cyclo*	0.21				
$18:0$ *branched A*	−0.26				

[1] Fatty acids are identified by the number of carbon atoms, the number of double bonds and the position of the first double bond from the methyl (ω) end of the molecule. Suffixes for fatty acids designate the existence of branching (*A*, *B*, *C*, *iso* and *anteiso*) or the presence of cyclopropane (*cyclo*) or hydroxy (*OH*) fatty acids. *Cis* and *trans* isomers are indicated by *c* and *t*, respectively[53].
[2] General characterization of fatty acids by organism type is as follows: hydroxy- and cyclo-FAMEs, Gram-negative bacteria; iso- and anteiso-FAMEs, Gram-positive bacteria; polyunsaturated C_{18} FAME, fungi; polyunsaturated C_{20} FAME, protozoa; other FAME, microeukaryote origin.

descriptions and eigenvector loadings for the PCA are presented in Table 2.

Results and Discussion

Microbial biomass C and N

Management affected MBC, MBN, and MBC:SOC, but not qCO_2 (Table 3). The ALT treatment at Fargo, Mandan, Mead, and Swift Current had a greater MBC and a higher ratio of MBC:SOC when compared to the CON treatment in at least part of the surface 30 cm (Table 3). At Brookings, Bushland, Fargo, Mandan, and Mead, the ALT treatment had greater MBN compared to the CON treatment. Increases in MBC, MBN, and MBC:SOC are indicative of improvements in SOM quality[36,37] and reflect an enhancement of a soil biological condition favoring internal recycling of nutrients[3]. Values of qCO_2 ranged from 0.02 to 0.16 mg CO_2 mg^{-1} MBC day^{-1}, with the highest values tending to occur at 0–7.5 cm.

Tillage, cropping frequency, and crop diversity were major drivers of microbial biomass levels within locations. At Fargo, where moldboard plow and no-till were contrasted within a spring wheat–field pea crop sequence, MBC, MBC:SOC, and MBN were 2-, 1.6-, and 1.5-fold greater, respectively, in the top 7.5 cm of the no-tilled plots compared to the moldboard plowed plots. Increased soil disturbance by tillage has been found to result in lower MBC and MBN[38–41], thereby reducing soil nutrient cycling potential. A combination of no-tillage and continuous cropping in the ALT treatment at Mandan contributed to 1.5- and 1.6-fold greater MBC and MBN than in the CON treatment (tandem disk/chisel plow, crop–fallow) at 0–7.5 cm. At Swift Current, where the tillage treatment was the same in CON (wheat–fallow) and ALT (wheat–lentil) treatments, there was 1.2–1.7-fold greater levels of MBC and MBC:SOC in the surface 30 cm of the wheat–lentil rotation compared to wheat–fallow. Elimination of fallow may have also contributed to differences in microbial biomass at Bushland, where the continuous wheat treatment had more MBN than a wheat–sorghum–fallow treatment at all three depth increments. MBC and MBN were greater in 4-year crop rotations (ALT) than continuous corn (CON) in the surface 15 cm at Brookings and Mead, underscoring the importance of crop diversity in enhancing the internal soil nutrient cycling potential in corn-based cropping systems.

The perennial vegetation areas at Fargo, Mandan, Mead, and Sidney generally had more MBC, MBN, and MBC:SOC compared to the cropped treatments (Table 3). Specifically, MBC, MBN and MBC:SOC were 2.3-, 2.1-, and 1.7-fold greater, respectively, under perennial vegetation compared to CON treatments when averaged across the four locations and three depth increments. Differences between perennial vegetation areas and ALT treatments were less pronounced (2.0-, 1.9-, and 1.5-fold difference for MBC, MBN and MBC:SOC, respectively).

Seasonal effects on microbial biomass were few and inconsistent across locations. At Fargo, MBC was greater ($P = 0.0303$) in summer than in spring at 7.5–15 cm, while MBC at Mandan was greatest ($P = 0.0394$) in fall compared to spring and summer at the same depth. Seasonal trends in MBN at Fargo and Mandan were similar to MBC. Other locations exhibiting seasonal effects on MBN included Brookings (0–7.5 cm; greatest in spring; $P = 0.0080$), Bushland (0–7.5 cm; greatest in spring; $P = 0.0181$), and Mead (15–30 cm; greatest in fall; $P = 0.0421$).

Table 3. Mean values for microbial biomass C (MBC), microbial biomass N (MBN), MBC : SOC, and qCO_2 within conventional (CON) and alternative (ALT) treatments and perennial vegetation areas (Grass) in eight long-term cropping system experiments.

| Location/Soil depth (cm) | Microbial biomass (kg ha^{-1}) | | | | | | MBC : SOC^1 (kg Mg^{-1}) | | | qCO_2^{-1} (mg CO$_2$ mg^{-1} MBC day^{-1}) | | |
| | Carbon | | | Nitrogen | | | | | | | | |
	CON^2	ALT	Grass	CON	ALT	Grass	CON	ALT	Grass	CON	ALT	Grass
Akron												
0–7.5	–^{3}	177	–	29.7	31.8	–	–	23.3	–	0.077	0.162	–
7.5–15	114	134	–	15.8	15.8	–	19.3	21.1	–	0.057	0.053	–
15–30	203	254	–	28.1	29.7	–	16.7	20.1	–	0.124	0.050	–
Brookings												
0–7.5	331	375	–	34.6	40.4**	–	17.9	20.8	–	0.061	0.048	–
7.5–15	366	380	–	32.3	31.0	–	18.4	20.0	–	0.026	0.027	–
15–30	548	544	–	57.8	51.4	–	15.5	17.0	–	0.020	0.021	–
Bushland												
0–7.5	209	331	–	26.3	37.1***	–	20.9	25.4	–	0.084	0.066	–
7.5–15	197	223	–	20.3	24.6**	–	22.3	25.1	–	0.025	0.027	–
15–30	305	384	–	35.5	42.3**	–	19.5	24.1	–	0.030	0.073	–
Fargo												
0–7.5	243	491***	645	42.0	62.6**	72.1	13.2	20.8***	21.8	0.050	0.025	0.039
7.5–15	259	334	634	42.9	42.3	77.5	13.6	14.2	28.8	0.023	0.019	0.014
15–30	391	401	787	64.9	52.0	92.8	11.1	11.0	19.0	0.021	0.024	0.016
Mandan												
0–7.5	297	444**	743	30.9	49.2**	90.6	15.3	19.7	24.1	0.045	0.048	0.054
7.5–15	213	227**	527	16.8	30.1***	45.1	11.5	15.8**	24.6	0.023	0.024	0.026
15–30	275	453	618	23.5	36.5	59.4	10.6	13.6**	21.0	0.023	0.020	0.030
Mead												
0–7.5	195	324**	812	29.6	36.7	100.9	13.1	22.3***	35.0	0.072	0.062	0.056
7.5–15	237	272*	352	28.1	32.2**	49.2	17.0	20.8	18.4	0.040	0.031	0.042
15–30	317	284	739	37.2	31.5	67.6	15.7	16.5	21.3	0.027	0.034	0.024
Sidney												
0–7.5	–	154	677	24.3	27.6	80.6	–	11.4	35.3	–	–	0.063
7.5–15	208	193	293	21.8	17.4	41.5	19.4	21.2	33.8	0.047	0.053	0.036
15–30	263	211	145	26.1	20.0	24.7	18.9	19.7	12.0	0.052	0.058	0.075
Swift Current												
0–7.5	288	491**	–	32.8	45.2	–	16.5	23.1*	–	0.057	0.087	–
7.5–15	227	352**	–	23.3	35.6	–	13.3	18.6**	–	0.029	0.023	–
15–30	239	320**	–	26.1	45.1	–	12.7	15.2	–	0.035	0.026	–

^{1} MBC : SOC, ratio of MBC to soil organic C; qCO_2, mg CO$_2$ respired mg^{-1} MBC day^{-1}.
^{2} Sampling times: Fargo—peak biomass, 2000; Mandan—preplant, 2000; Mead—preplant, peak biomass, and post-harvest, 2001 (average of three sampling times presented).
^{3} –, not estimated.
*, **, ***, values between CON and ALT treatments within a property and soil depth significantly different at $P \leq 0.1$, 0.05, and 0.01, respectively.

Table 4. Summary of regression analysis for microbial biomass carbon (MBC) and nitrogen (MBN), potentially mineralizable N (PMN), water-stable aggregates (WSAs), and total glomalin (TG) using adjusted r^2 of variables defining management, climatic, and sampling factors.

Parameter	Model with maximum adjusted r^2			Best two-component model	
	Adj r^2	Variables[1]	No. of variables	Adj r^2	Variables
0–7.5 cm					
MBC	0.161	D, F, L, R, S, T	6	0.10	D, T
MBN	0.146	D, F, N, R, S, T	6	0.08	F, T
PMN	0.107	F, L, N, R, T, S	6	0.06	F, S
WSA	0.59	D, F, L, N, R, S, T	7	0.40	F, S
TG	0.42	D, L, N, R, S, T	6	0.18	S, T
7.5–15 cm					
MBC	0.305	F, N, R, T	4	0.30	F, T
MBN	0.431	F, L, N, R, S, T	6	0.31	L, N
PMN	0.227	F, L, N, S, T	5	0.16	F, S
15–30 cm					
MBC	0.227	D, F, N, R, S	5	0.18	F, T
MBN	0.223	D, F, L, N, R, T	6	0.13	F, T
PMN	0.126	D, F, R, S	4	0.08	S, T

[1] D, maximum tillage depth; F, presence (1) or absence (0) of fallow in the rotation; L, presence (1) or absence (0) of legume in the rotation; N, total number of species included in rotation cycle; R, mean annual precipitation; S, sampling time and year of sampling; T, mean annual temperature.

Levels of MBC and MBN at 7.5–15 cm were more responsive to management, climatic, and sampling factors than at 0–7.5 and 15–30 cm (Table 4). Specific factors most closely associated with MBC and MBN in two-component models included the presence or absence of fallow and mean annual temperature. Both frequency of fallow and mean annual temperature were negatively correlated with MBC and MBN at all three depth increments (data not shown). Fallow represents an extreme condition for microorganisms, dramatically limiting the amount of available nutrients for growth and activity[42], while temperature greatly influences decomposition rates in soil[43], thereby altering levels of nutrients to support microbial activity.

Potentially mineralizable N

Management affected PMN at Brookings, Bushland, Fargo, Mandan, and Mead, where the ALT treatment had 8.0, 14.3, 6.8, 13.9, and 10.2 kg ha^{-1} more PMN, respectively, than the CON treatment in the surface 7.5 cm (Table 3). These results are supported by a previous evaluation[11], where treatments with intensive crop sequences and/or reduced tillage had higher levels of PMN than treatments with monoculture crop sequences, fallow periods, and/or significant tillage. At Brookings and Mead, increased crop rotation diversity in the ALT treatment likely contributed to greater PMN at 0–7.5 cm, as both locations contrasted 4-year rotations with continuous corn. Increased cropping intensity (continuous winter wheat) in the ALT treatment was responsible for greater PMN at Bushland for all three depth increments. A combination of increased cropping intensity and reduced tillage at Mandan resulted in greater

PMN in the surface 15 cm of the ALT treatment. Observations at Bushland and Mandan underscore the value of annual cropping and reduced tillage to enhance soil nutrient reserves in climatically extreme environments. At Fargo, surface accumulation of crop residue from the use of no-till resulted in greater PMN at 0–7.5 cm in the ALT treatment as compared to the CON treatment. An opposite trend was observed at 15–30 cm, where inversion of crop residue by moldboard plowing resulted in 2.3 kg ha^{-1} more PMN in the CON treatment.

There were two or three times more PMN in perennial vegetation areas at Fargo, Mandan, Mead, and Sidney than in the ALT treatment at 0–7.5 cm (Table 5). Differences in PMN between perennial vegetation areas and cropping systems can be a reflection of inherent soil fertility lost since conversion to production agriculture.

PMN did not differ between udic and ustic soil moisture regimes at 0–7.5 cm for the preplant sampling time ($P = 0.5658$; data not shown). Additionally, differences in PMN between ALT and CON treatments within moisture regimes were similar (10.9 and 9.8 kg ha^{-1} difference within udic and ustic moisture regimes, respectively). Results from regression analysis indicated management, climatic, and sampling factors were generally poor predictors of PMN (Table 4), as models with the maximum adjusted r^2 values were less than 0.25 at all three depth increments.

Sampling time had a significant effect on PMN at all locations, but only at Akron, Brookings, Bushland, and Mead was sampling time significant for more than one soil depth (data not shown). Trends in PMN over time were similar across all three depth increments at each location

Table 5. Mean values for potentially mineralizable N (PMN) within conventional (CON) and alternative (ALT) treatments and perennial vegetation areas in eight long-term cropping system experiments.

	PMN (kg ha^{-1})								
	0–7.5 cm			7.5–15 cm			15–30 cm		
Location	CON	ALT	Grass[1]	CON	ALT	Grass	CON	ALT	Grass
Akron	25.1	24.9	–[2]	9.1	10.0	–	23.0	14.6	–
Brookings	26.3	34.3**	–	17.8	17.4	–	21.5	21.5	–
Bushland	17.6	31.9***	–	8.0	10.4*	–	7.2	12.3**	–
Fargo	14.4	21.2**	*62.2*	11.7	10.1	*19.9*	12.9	10.6*	*15.2*
Mandan	21.7	35.6*	*67.1*	7.5	19.7***	*32.1*	9.0	15.7	*41.2*
Mead	19.3	29.5***	*70.9*	10.8	11.3	*23.6*	7.4	7.9	*19.8*
Sidney	20.3	20.4	*88.5*	8.5	13.0	*21.7*	6.3	10.8	*8.1*
Swift Current	23.7	45.8	–	14.2	22.6	–	12.5	15.1	–

[1] Grass, perennial vegetation area.
[2] –, not estimated.
*, **, ***, values between CON and ALT treatments within a property and soil depth are significantly different at $P \leqslant 0.1$, 0.05, and 0.01, respectively.

(data not shown), and were typically greatest within a year during the preplant sampling time, thereby providing an upper estimate of the capacity of the soil to supply plant-available N early in the growing season.

PMN and MBN are regarded as useful measures for assessing biologically active N reserves in soil. Assessment of PMN by anaerobic incubation, however, is considerably less involved and costly than MBN, and therefore the association between the two parameters is of interest to researchers. In this study, PMN was significantly correlated with MBN across locations and depths ($r = 0.31$; $P < 0.001$; $n = 1075$). A much stronger association between PMN and MBN was observed by Gajda et al.[11] at 0–7.5 cm ($r^2 = 0.841$). However, their evaluation was from one year (1998) with one sampling time (spring), which eliminated the temporal variability inherent to both parameters included in this study.

Aggregate stability and glomalin

Mean values for WSA and TG varied by location and treatment (Table 6). The lowest values for WSA were at Akron and Mandan (CON treatment) while the highest values occurred at Fargo (ALT treatment). TG ranged from about 1.7 to 5.5 mg g^{-1} across all locations, with lowest values at Akron and Sidney and highest values at Swift Current and Fargo. This range of values for TG was expected for cropped soils. TG values of 0.7–5.3 mg g^{-1} were found previously in experimental plots at Akron[15]. Soils at four sites in Corn Belt agro-ecosystems had TG from 0.7 to 3.8 mg g^{-1} and from 0.7 to 5.3 mg g^{-1} for CON tillage and no-tillage, respectively (S.F. Wright, unpublished data).

Management effects on WSA were observed at five of the eight locations (Table 6). The ALT treatment had greater WSA at four of the locations (Bushland, Fargo, Mandan, and Swift Current), with relative differences

between treatments ranging from 13 to 133%. The CON treatment at Sidney, however, had greater WSA than the ALT treatment. Possible differences in sampling protocol at

Table 6. Means for water-stable aggregates (WSA) and total glomalin (TG) at 0–7.5 cm for conventional (CON) and alternative (ALT) treatments in eight long-term cropping system experiments.

Location/Treatment	TG (mg g^{-1})	WSA (g kg^{-1})
Akron		
CON	1.75	110
ALT	1.83	124
Brookings		
CON	2.61	491
ALT	2.58	495
Bushland		
CON	2.76	377***
ALT	2.96	456
Fargo		
CON	4.45	739**
ALT	5.46	832
Mandan		
CON	2.81**	218***
ALT	3.57	507
Mead		
CON	3.28	584
ALT	2.65	622
Sidney		
CON	2.67*	486***
ALT	2.23	360
Swift Current		
CON	4.72	485***
ALT	5.12	609

*, **, ***, difference between CON and ALT treatments significant at $P \leqslant 0.1$, 0.05, and 0.01, respectively.

Sidney may be a contributing factor to the contrary trend in WSA between treatments (as discussed by Wienhold et al.[19]). In this study, TG differed between treatments only at Mandan, where the ALT treatment (continuous cropping with no-tillage) had 27% more TG than the CON treatment (crop–fallow with CON tillage) ($P < 0.05$).

Sampling time had a significant effect on WSA and TG at all locations, except for WSA at Swift Current (data not shown). Temporal fluctuations in WSA corresponded to those for TG except at Swift Current, where a precipitous decrease in TG during the 2000 growing season was not associated with a decrease in WSA. Locations with fallow in the rotation (Bushland and Mandan) had lower values for TG and WSA during the fallow period (data not shown).

WSA was affected more by management and sampling time than by climatic factors. The best two-component model for WSA (adjusted $r^2 = 0.40$) included presence or absence of fallow (F) and sampling time (S) (Table 4). The best model (adjusted $r^2 = 0.59$) included all variables. Pearson product moment correlations for WSA with the above variables showed a negative correlation with F and positive correlations with presence or absence of a legume (L), mean annual precipitation (R), S, and the number of species in the rotation (N) ($P < 0.001$; data not shown). The best two-component model for TG was S and the mean annual temperature (T) (adjusted $r^2 = 0.18$). The best model included all of the variables except F. Correlations for TG with the variables tested for regression models were positive for L and negative for R, S, T, and the maximum tillage depth (D) ($P \leqslant 0.05$; data not shown).

There were significant positive correlations between WSA and clay ($r = 0.61$, $P < 0.001$, and $n = 291$) and WSA and TG ($r = 0.43$, $P < 0.001$, and $n = 294$). Previous work by Kemper and Koch[44] has shown organic matter, clay, and iron oxide to account for 44% of the variance in WSA in western soils. More recent work indicates that glomalin, a fraction of SOM, is a major factor in aggregate stability[15,45]. Using the current data for the mean values for each plot, multiple regression of WSA with the variables SOM, clay, TG, and Fe resulted in the following equations ($n = 45$ and $P < 0.05$):

$$WSA = 6.39 + 14.65 \ SOM \ (r^2 = 0.56), \tag{1}$$

$$WSA = -1.69 + 0.80 \ clay + 9.66 \ SOM \ (r^2 = 0.66), \tag{2}$$

$$WSA = -8.12 + 0.84 \ clay + 4.48 \ SOM \\ + 6.22 \ TG \ (r^2 = 0.72), \tag{3}$$

$$WSA = -7.66 + 1.00 \ clay + 8.58 \ TG \ (r^2 = 0.70) \tag{4}$$

and the following stepwise model,

$$WSA = -17.1 + 1.35 \ clay + 0.23 \ Fe + 6.27 \ TG \\ (r^2 = 0.73, \ adjusted \ r^2 = 0.70). \tag{5}$$

These results indicated that measures of innate soil factors—clay and Fe—and glomalin accounted for 70% of the variability in WSA. Across locations, clay content

was highest at Fargo ($480 \ g \ kg^{-1}$), Mead ($375 \ g \ kg^{-1}$), and Bushland ($315 \ g \ kg^{-1}$), corresponding to three locations with high WSA. An exception to the positive association between clay and WSA was at Swift Current, where WSA was high for the ALT treatment, but clay content was relatively low for the soils examined (c. $235 \ g \ kg^{-1}$). However, TG at Swift Current was high, indicating that clay and TG may be important for aggregate stability in this soil.

FAME profiles

FAME profiles were primarily of bacterial origin (Table 2). Gram-positive (iso- and anteiso-FAMEs) and Gram-negative (hydroxyl and cyclo-FAMEs) bacteria were present in principal component (PC) 1, 2, and 3, while fungi (polyunsaturated C_{18} FAME) were present in PC 2 only. FAME profiles were highly dependent on location (Fig. 1a). Given the geographical breadth of the study, differences in climate, soil type, and management practices contributed to differences in the flora and fauna creating FAME profiles. Among factors contributing to FAME profiles, sampling depth had significant effects on PC 1, 2, and/or 3 at all locations (data not shown). The effect of depth is shown in Figure 1b, where distinctly different outcomes for PC 1 and 2 were observed across depths at Akron and Mead, locations representative of the ustic and udic soil moisture regimes, respectively. Locations where FAME profiles were analyzed for more than one sampling time yielded significant time effects (Fig. 1c), presumably due to shifts in populations of flora and fauna in response to fluctuations in weather. These findings corroborate with previous evaluations[46,47], where soil type, soil depth, and sampling time have been identified as having an overriding influence on FAME profiles.

Four locations—Brookings, Bushland, Mead, and Swift Current—had significant differences between treatments in PC 1 or 2 (Fig. 1d). At three of these sites—Bushland, Mead, and Swift Current—ALT treatments scored higher in PC 1 and/or 2 than CON treatments, indicating similar community shifts due to alternative management in these soils. At Akron (0–7.5 cm), Bushland (7.5–15 cm), Mandan (0–7.5 and 7.5–15 cm), and Swift Current (0–7.5 and 15–30 cm), the ALT treatment had greater total FAME than the CON treatment, indicating larger overall soil biomass (Table 7). Differences between treatments were most pronounced at Mandan, where total FAME in the ALT treatment was nearly double of that observed within the CON treatment. Unlike other locations, total extracted FAME at Sidney was greater within the CON treatment than the ALT treatment, corresponding to a similar trend between treatments observed for TG and aggregate stability.

Consistent trends between treatments in individual FAME biomarkers for bacteria, fungi, and protozoa were not common across locations, indicating a strong site-specific effect on soil biota composition. Only one location
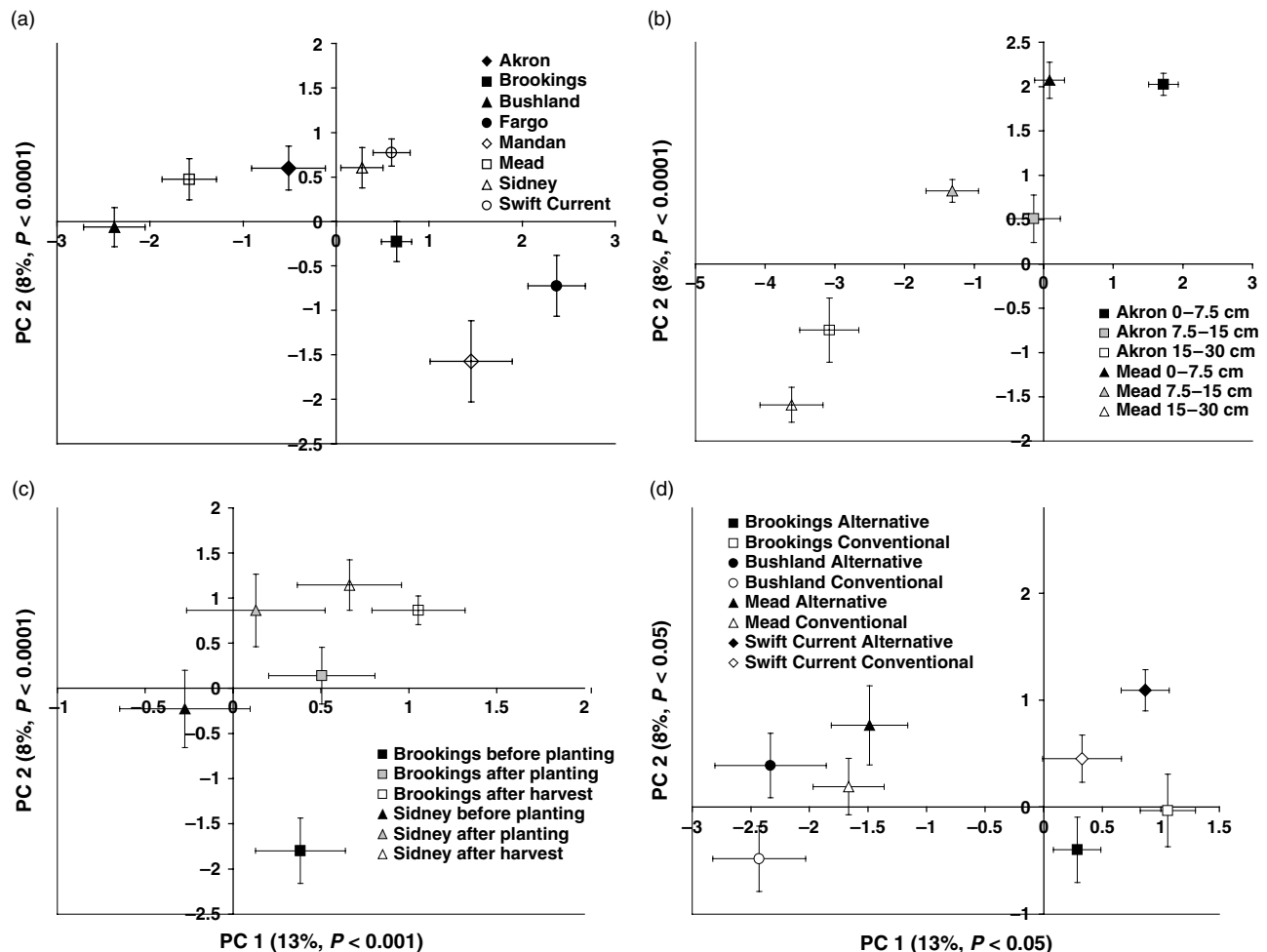
Figure 1. (a) Principal components analysis (PCA) of Fatty acid methyl ester (FAME) profiles across all locations in 2000. Data shown are the means by location, $\pm$ SEM. (b) PCA of FAME profiles across depths for Akron and Mead in 2000. Data shown are the means by location and depth, $\pm$ SEM. (c) PCA of FAME profiles across sampling times for Brookings and Sidney in 2000. Data shown are the means by location and sampling time, $\pm$ SEM. (d) PCA of FAME profiles of conventional and alternative treatments at several sites. Data shown are the means by location and treatment, $\pm$ SEM.

(Mandan) exhibited a significant treatment effect on Gram-negative bacteria, with a greater percentage observed in the ALT treatment at 0–7.5 cm. At Bushland and Swift Current, a greater proportion of Gram-positive bacteria was observed in CON treatment than ALT treatment at 7.5–15 or 15–30 cm, yet the opposite was observed at Sidney for the same depths (Table 7). Ratios of bacterial to fungal biomarkers increased with depth at all locations except Swift Current, indicating soils at most locations were increasingly bacteria-dominated at deeper soil depths. Within individual locations, the biomarker for fungal cells, namely polyunsaturated C_{18} FAME, was greater in ALT than CON treatments at Bushland (0–7.5 and 7.5–15 cm), Fargo (15–30 cm), and Sidney (0–7.5 cm) (Table 7).

The biomarker for protozoa, polyunsaturated C_{20} FAME[32], made up less than 1% of the proportion of total FAME at each location (Table 7). In spite of its relative scarcity, differences between treatments for the bio-marker were observed at Akron (0–7.5 cm), Brookings (7.5–15 cm), and Bushland (15–30 cm). In each case, the

ALT treatment had a higher percentage of protozoa biomarkers than the CON treatment, making it the only biomarker to respond consistently across locations with respect to trends between treatments. This finding is consistent with previous research by Schutter et al.[48], where greater amounts of fungal and protozoan FAME biomarkers were observed in alternatively managed soils (as reflected by cover crop usage) relative to soils fallowed over winter.

Summary and Conclusions

Cropping system effects on soil biological characteristics in this study generally followed expected trends based on results from previous evaluations in the Great Plains[11,14,15,49]. ALT cropping systems had greater MBC, MBN, and PMN than CON cropping systems in at least one-half of the locations included in this study. WSAs, an important biophysical indicator of soil condition, were greater in ALT than CON treatments at four locations.

Table 7. Mean values for FAME profile parameters at three depths for conventional (CON) and alternative (ALT) treatments in eight long-term cropping system experiments in 1999–2000.

Parameter	0–7.5 cm		7.5–15 cm		15–30 cm	
	CON	ALT	CON	ALT	CON	ALT
Akron						
Total FAME (nmol g^{-1})	21.8	34.4*	23.9	18.9	19.1	15.0
Hydroxy FAME[1] (%)	3.01	3.20	3.73	2.56	3.18	2.38
Iso-FAME + anteiso-FAME (%)	23.0	22.1	21.9	19.3	18.3	17.5
Polyunsat. C$_{18}$ FAME (%)	10.19	9.05	6.49	6.76	3.70	6.16
Bacterial/fungal markers	2.56	2.79	3.95	3.23	5.80	3.23
Polyunsat. C$_{20}$ FAME (%)	0.031	0.109*	0.025	0.112	0.032	0.102
Brookings						
Total FAME (nmol g^{-1})	63.6	75.8	55.5	58.7	38.9	46.1
Hydroxy FAME (%)	2.92	2.53	3.12	2.41	3.27	3.25
Iso-FAME + anteiso-FAME (%)	23.7	22.9	23.0	23.2	23.3	23.9
Polyunsat. C$_{18}$ FAME (%)	9.34	9.15	7.83	7.14	6.52	6.04
Bacterial/fungal markers	2.85	2.78	3.33	3.59	4.08	4.49
Polyunsat. C$_{20}$ FAME (%)	0.247	0.187	0.160	0.286*	0.125	0.198
Bushland						
Total FAME (nmol g^{-1})	55.0	64.0	36.2	65.4**	45.2	62.7
Hydroxy FAME (%)	2.35	2.74	1.84	1.50	1.21	1.38
Iso-FAME + anteiso-FAME (%)	21.0	22.0	19.9	16.8**	19.2	16.8*
Polyunsat. C$_{18}$ FAME (%)	6.77	7.71*	3.15	4.01*	2.69	1.96**
Bacterial/fungal markers	3.45	3.21	6.91	4.56**	7.60	9.27
Polyunsat. C$_{20}$ FAME (%)	0.028	0.049	0.076	0.107	0.018	0.151*
Fargo						
Total FAME (nmol g^{-1})	34.2	20.4	28.4	29.3	9.1	15.7
Hydroxy FAME (%)	2.33	1.70	5.13	2.31	2.68	3.14
Iso-FAME + anteiso-FAME (%)	28.3	25.4	27.9	24.7	30.7	26.2
Polyunsat. C$_{18}$ FAME (%)	12.47	4.56	5.10	9.41	1.32	4.56**
Bacterial/fungal markers	2.46	5.96	6.48	2.87	25.25	6.44
Polyunsat. C$_{20}$ FAME (%)	0	0.296	0	0.299	0.183	0
Mandan						
Total FAME (nmol g^{-1})	87.9	170.5**	53.0	104.1**	37.9	48.1
Hydroxy FAME (%)	2.65	3.82*	3.18	3.06	2.19	2.09
Iso-FAME + anteiso-FAME (%)	25.8	23.9	25.9	24.2	26.3	25.7
Polyunsat. C$_{18}$ FAME (%)	6.40	6.96	3.98	4.35	3.96	4.07
Bacterial/fungal markers	4.44	4.00	7.37	6.32	7.75	6.84
Polyunsat. C$_{20}$ FAME (%)	0.242	0.331	0.272	0.196	0.087	0.143
Mead						
Total FAME (nmol g^{-1})	57.9	68.4	30.6	34.0	31.0	24.6
Hydroxy FAME (%)	2.75	2.75	2.46	2.52	1.10	0.84
Iso-FAME + anteiso-FAME (%)	19.0	20.8	20.4	20.8	20.8	21.9
Polyunsat. C$_{18}$ FAME (%)	10.98	11.09	5.86	6.74	3.48	3.57
Bacterial/fungal markers	1.98	2.12	3.91	3.46	6.29	6.37
Polyunsat. C$_{20}$ FAME (%)	0.225	0.223	0.207	0.184	0.186	0.162
Sidney						
Total FAME (nmol g^{-1})	111.0	78.7**	46.8	37.1	30.3	23.8
Hydroxy FAME (%)	4.04	3.07	3.62	3.08	3.53	2.76
Iso-FAME + anteiso-FAME (%)	20.0	20.7	20.2	22.8*	18.5	23.5*
Polyunsat. C$_{18}$ FAME (%)	11.88	15.00**	6.13	7.43	5.36	5.88
Bacterial/fungal markers	2.02	1.58**	3.88	3.48	4.11	4.46
Polyunsat. C$_{20}$ FAME (%)	0.391	0.419	0.347	0.288	0.192	0.206
Swift Current						
Total FAME (nmol g^{-1})	40.2	73.7*	36.0	43.3	11.3	29.3**
Hydroxy FAME (%)	5.65	4.48	5.25	5.48	2.85	3.56
Iso-FAME + anteiso-FAME (%)	23.1	23.4	23.5	20.4*	21.8	21.8
Polyunsat. C$_{18}$ FAME (%)	7.83	7.91	6.83	6.93	7.83	9.11
Bacterial/fungal markers	3.67	3.52	4.21	3.73	3.15	2.78
Polyunsat. C$_{20}$ FAME (%)	0	0.074	0	0.077	0.054	0

[1] Biomarkers for microbial groups: hydroxy FAME, Gram-negative bacteria; iso-FAME + anteiso-FAME, Gram-positive bacteria; polyunsat. C$_{18}$ FAME, fungi; polyunsaturated C$_{20}$ FAME, protozoa[33,34].
*, **, Difference between CON and ALT treatments within a depth significant at $P \leqslant 0.05$ and 0.01, respectively.

However, differences in WSA between treatments did not necessarily translate to greater TG in ALT systems, as only one location (Mandan) had a significant difference in TG between treatments. FAME profiles varied greatly across locations, with few consistent differences between treatments. However, ALT treatments tended to have a more abundant soil biomass than CON treatments, as indicated by greater total extracted FAME.

Management factors controlling trends in soil biological properties between cropping systems included crop diversity, degree of soil disturbance by tillage, and presence/absence of fallow. Among the three factors, greater crop diversity—as shown through the continuous corn/4-year rotation contrasts at Brookings and Mead—resulted in greater levels of MBN and PMN in the surface 7.5 cm of the ALT treatment. Blocking locations by degree of soil disturbance or presence/absence of fallow did not yield consistent results across locations. Furthermore, even when two management factors were present at a location—such as at Akron, Mandan, and Sidney—the resulting trends in soil biological properties between cropping systems were not consistent. Such findings imply that generalizations regarding tillage and fallow effects on soil biological properties in the Great Plains cannot be made without considering site-specific attributes, such as spatial and temporal variability of soil properties and 'time in treatment' within a location.

All soil biological properties assessed in this study varied over time. This result was expected, as biological parameters are directly affected by weather-related factors such as temperature and moisture, and indirectly affected by plants through fluctuations in nutrients and carbon inputs. Consequently, no single sampling time can be recommended as being most appropriate for assessing the status of all soil biological properties. However, certain 'common sense' considerations apply, such as sampling when the climate is most stable and when there have been no recent soil disturbances[50]. When considering the three sampling times in this study (preplant, peak biomass, and post-harvest), the first and last times qualify by these criteria.

The amount of time necessary to detect changes in soil biological properties is a function of organic matter inputs to the soil, which, in turn, are largely dependent on climatic and edaphic factors dictating production potential[6]. The ability to detect changes, however, is also affected by the sampling scheme employed by the investigator. Selection of sampling depths, in particular, has a significant effect on whether changes in soil condition are observed. Sampling depths that are too large run the risk of diluting changes occurring at a particular depth increment in the soil profile. Conversely, smaller depth increments increase the potential for detecting change, but increase sampling and analysis demands, thereby rendering them impractical for most studies. In this study, most treatment effects were concentrated in the surface 0–15 cm, underscoring the importance of sampling to at least this depth in future studies. Partitioning the surface 15 cm into different depth increments (e.g., 0–5 and 5–15 cm) may be advisable in order to better quantify near-surface effects of management. Such a sampling scheme would offer advantages for detecting soil changes in regions where production levels are low or where management practices have been in place for less than 10 years.

In its simplest form, soil quality refers to the capacity of soil to function[51]. Soil functions vary by land use, but are generally regarded to include (1) water and solute retention and flow, (2) physical stability and support, (3) retention and cycling of nutrients, (4) buffering and filtering of potentially toxic materials, and (5) maintenance of biodiversity and habitat[52]. Based on the status of soil biological properties assessed in this study, it appears that ALT treatments at most of the locations enhanced functions 3 and 5, implying improved agro-ecosystem performance over time.

Temporal variation in soil biological properties in this study underscored the importance of interpreting results in the context of treatment and weather-related attributes unique to the time the soil samples are collected. Trends in soil biological properties over time occasionally could be explained based on knowledge of management impacts and weather conditions at a particular location. However, much variation defied a straightforward explanation. It is perhaps this issue that demands greater emphasis in future investigations of soil biological properties in Great Plains cropping systems. An improved understanding of seasonal patterns of microbial dynamics—along with effects on critical soil functions—could lead to greater production efficiencies, thereby enhancing agricultural sustainability within this climatically extreme region.

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