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# Microbial communities and enzyme activities in soils under alternative crop rotations compared to wheat–fallow for the Central Great Plains☆

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ABSTRACT

Winter wheat–fallow (W–F) rotation is the predominant cropping system in the Central Great Plains. However, other cropping systems are being suggested because reduced tillage and fallow can provide more residues that can increase soil organic carbon (SOC) content and other parameters related to soil quality and functioning. This study compared the microbial biomass and community composition and enzyme activities under native pasture and research plots under grass and different crop intensities (CI) established for 15 years in Akron, CO. The soil (Weld loam; fine, smectitic, mesic Aridic Paleustolls) was under alternative CI rotations (100 and 67%) of winter wheat (*Triticum aestivum* L.) (W), corn (*Zea mays* L.) (C), proso millet (*Panicum miliaceum* L.) (M), and fallow (F) under no-tillage (nt) compared to the typical 50% CI rotation (W–F) under either conventional tillage (ct) and nt. Relative to F–Wct, the 100% (C–M–W) and 67% (C–F–W) CI rotations increased soil microbial biomass C (MBC) and N (MBN) but only at the 0–5 cm depth. Native pasture and 15 years of undisturbed grass plots showed higher soil MBC up to 2–5-fold and 1.4–3-fold when compared to the cropping systems at 0–5 cm, respectively. Similar trends were found for MBN and several enzyme activities. Enzyme activities of C (β-glucosaminidase, β-glucosidase, and α-galactosidase) and P cycling (alkaline phosphatase, acid phosphatase and phosphodiesterase) as a group separated the 100 and 67% CI rotations from the 50% CI rotation (W–Fct) at 0–5 and 5–15 cm of soil. Separation in these enzyme activities was observed for rotations sampled under a crop (W–C–F) compared to when sampled under fallow (F–W–C). Principal component analyses (PCA) of fatty acids methyl esters (FAME) suggested a shift in the microbial community structure with greater fungal populations in pasture, grass, and CI rotations of 100 and 67% compared to W–Fct. The sum of fungal indicators (18:2ω6c, 18:3ω6c, 18:1ω9c, 16:1ω5c) was significantly correlated ($r > 0.60$; $P < 0.05$) to β-glucosaminidase, β-glucosidase, acid phosphatase and α-galactosidase activities. After 15 years, our results show that the combination of no-tillage and continuous cropping with reduced fallow frequency in two alternative (100 and 67% CI) rotations for the Central Great Plains have had a positive effect on soil quality parameters such as the microbial populations and community composition but only at 0–5 cm depth, and in several enzyme activities at both 0–5 and 5–15 cm.

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## 1. Introduction

Winter wheat (*Triticum aestivum* L.)–fallow using conventional tillage is the common cropping system in the Central Great Plains, comprising dryland farms in parts of Colorado, Wyoming, South Dakota, Nebraska, and Kansas in the United States. Fallow periods are included in the rotation to allow soil water to accumulate for winter wheat because water is a limiting factor for crop production in this region. However, government programs encourage the reduction or elimination of fallow land because of the increased potential for soil erosion and loss of soil organic matter (SOM). In 1986, the Conservation Reserve Program (CRP) was initiated in the Central Great Plains region by the conversion of thousands of acres to grass land to increase SOM accumulation and reduce soil erosion (personal communication, National Resources Conservation Service, NRCS). In addition, producers have incorporated increased cropping intensity and minimum tillage practices in search of more sustainable systems to replace winter wheat–fallow under conventional tillage (Bowman et al., 1999). According to Anderson et al. (1999), a rotation of winter wheat–summer crop–fallow has proven productive with summer crops such as proso millet [*Panicum miliaceum* L.] (Shanahan et al., 1988), corn [*Zea mays* L.] (Smika et al., 1986) or grain sorghum [*Sorghum bicolor* L.] planted after the winter wheat (Norwood et al., 1990). In addition, eliminating fallow in longer rotations such as winter wheat–corn–millet also has been successful, especially under no-till practices (Black et al., 1981). In comparison to the conventional winter wheat–fallow (W–F) rotation, the alternative rotations of winter wheat–summer crop–fallow and wheat–corn–millet can increase land productivity and reduce financial risk (Dhuyvetter et al., 1996). However, the productivity and sustainability of a system are also affected by numerous complex interactions related to soil quality and functioning (i.e., soil physical, microbial and biochemical properties), which have not been intensively investigated in this region.

Research plots were established in 1990 in the semiarid region near Akron, Colorado to compare the sustainability and productivity of different cropping intensities (CI) against the conventional winter wheat–fallow rotation. The CI are determined by the ratio of the number of crop(s) harvested divided by the number of years of the rotation with each entity assigned a value of one. Therefore, research plots in the Central Great Plains compare typical rotations such as conventional tilled or no-tilled wheat–fallow (W–Fct or W–Fnt, respectively), no-tilled winter wheat–corn–fallow (W–C–F), and no-tilled winter wheat–corn–proso millet (W–C–M) representing 50, 67 and 100% CI. Thus, the 100% CI means that there is no fallow in this rotation and there should be a crop harvested every growing season. Previous studies found significant increases in soil organic carbon (SOC) and microbial biomass C (MBC) in a 100% CI rotation (W–C–M) as compared to the 50% CI rotation (W–F) at the soil surface (0–7.5 cm) after 7–9 years of the establishment of these research plots (Bowman et al., 1999; Gajda et al., 2001). However, there were no differences in SOC between a 67% CI rotation (W–C–F) and 50% CI rotation (W–F) (Bowman et al., 1999). Similarly, no difference in SOC content was found in an early assessment (after 5 years) comparing continuous cotton (*Gossypium hirsutum*) to a crop rotation system that included fallow periods (rye (*Secale cereale*)–cotton–wheat–fallow) in semiarid soils from West Texas (Acosta-Martínez et al., 2004). However, differences were found in soil microbial biomass and enzyme activities between the crop rotation and continuous system depending on the crop when the sample was taken. Although 15 years have passed since the establishment of the research plots in the Central Great Plains semiarid region, there is not enough information about the soil microbial communities and processes in rotations with different crop and fallow intensities. Changes in soil microbial populations and enzyme activities may represent improvements or alterations in soil quality. This information may be used to compare management practices to improve soil quality and functioning in the Central Great Plains region.

The soil microbial community and functioning may respond to agricultural management and land use and any changes they experience are likely reflected in the functional integrity of soil (Insam, 2001). The microbial community plays a key role in soil aggregate stability, soil organic matter formation, and the potential for substrate metabolism from the degradation of plant residues, organic amendments, and xenobiotics. Thus, information of the soil microbial communities and processes can be related to soil functioning, and it can provide additional information helpful in determining the productivity and sustainability of a system. We hypothesize that increasing cropping intensity while reducing fallow frequency will modify the soil microbial community composition and increase the microbial biomass and enzyme activities. We believe this is especially true for the 100% CI rotation compared to the 50% CI rotation, due to the changes already found in soil organic C between these systems, but we are not certain if there will be differences in these soil properties between the 67% CI rotation compared to the 50% CI rotation. Therefore, the current study measured selected soil quality parameters such as the soil microbial biomass C and N, microbial community structure, and selected enzymatic activities involved in C and P cycling after 15 years in alternative no-tilled 100% CI rotation of winter wheat–corn–proso millet (W–C–M) and 67% CI rotation of winter wheat–corn–fallow (W–C–F) compared to a 50% CI rotation winter–fallow (W–F) under no-tillage and tillage practices at 0–5 and 5–15 cm depths. This study also compared the data generated from agricultural plots with long-term undisturbed soils under grass for 15 years and native pasture.

## 2. Material and methods

### 2.1. *Site description and management history*

The study was located at 40°N latitude and 103°W longitude on a Weld loam (fine, smectitic, mesic, Aridic Paleustolls) soil in Akron, CO (Anderson et al., 1999; Bowman et al., 1999). Site elevation is approximately 1400 m. The site's long-term annual average precipitation is 420 mm with 80% occurring between April and September. About 25% of the annual precipitation is received as snow; another 29% occurs as rain in July and August, a critical period of plant development for summer annual crops. Average daily temperature for the year

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**Table 1 – Description of cropping intensity and crop sequence**

| Cropping intensity[a] (CI)% | Crop sequence[b] |
|---|---|
| 50 | W–Fct; W–Fnt |
| 67 | W–C–F; C–F–W; F–C–W |
| 100 | W–C–M; C–M–W; M–W–C |

[a] The 100% CI means that there is no fallow in this rotation and there should be a crop harvested every growing season.
[b] W: wheat; F: fallow; C: corn; M: millet; ct: conventional tillage; nt: no-tillage. All rotations are under no-tillage except that wheat–fallow was studied both in no-tillage and conventional tillage practices.

is 9 °C, ranging from −2 °C in January to 23 °C in July. The research plots were established in 1990 under various CI that comprised of winter wheat (*Triticum aestivum L.*), corn (*Zea mays L.*), proso millet (*Panicum miliaceum L.*), and/or fallow. Previously, the land was cultivated with the common rotation for the region of wheat–fallow. The plots were established in a completely randomized block design with three field replicates of different cropping systems and an undisturbed grass system. The size of the plots was 9.1 m × 30.5 m with a 15.2 m alley between replicates. The different cropping sequences evaluated in our study are specified in Table 1.

The agricultural treatment plots were compared with undisturbed soils under pasture and the grass plots part of the study. The native pasture site has a mixture of bluegrama (*Bouteloua gracilis*) and buffalograss (*Buchleo dactyloides*), which was adjacent to the experiment before the initiation of the research plots. The grass plots were planted with smooth bromegrass (*Bromus inermis*). The pasture site and grass plots were not grazed or fertilized. All the crop rotations were conducted under NT practice except the traditional rotation (W–F) that was conducted in both NT and CT. Briefly, CT consist of mixing the top 10 cm of soil by three to six sweep plow operations as needed for weed control during fallow. The weeds were controlled during the fallow period for NT by repeated applications of glyphosate at 368 g ha$^{-1}$. Prior to corn planting, weeds were controlled by using a mixture of glyphosate at 552 g ha$^{-1}$ and Bicep, Lite II Magnum (Atrazine {6-chloro-N-ethyl-N′-(1-methylen)-1,3,5-triazine-2,4-diamine} + S-metolachlor {2-chloro-6′-ethyl-N-(2-methoxy-1-methylethyl) aceto-o-toluidide}) at 368 g ha$^{-1}$. Glyphosate at 368 g ha$^{-1}$ was used for weed control before millet and wheat planting. The combination of Dicamba (benzoic acid, 3,6-dichloro-2-metoxy) at 55 g ha$^{-1}$ and 2,4-D 74 g ha$^{-1}$ was applied at the two to three leaf stage of proso millet to control broadleaf weeds. Fertilizer N (ammonium nitrate, broadcast before planting) was applied to each plot according to soil tests obtained each year and projected crop yield for each individual crop in a rotation. For wheat only, inorganic fertilizer of 11-52-0 was banded with the seed at planting at 2.8 kg P ha$^{-1}$.

According to Gajda et al. (2001), the soil bulk density ranged in the plots from 1.35 to 1.67 g cm$^{-3}$, and soil texture ranged from 37 to 39% sand, 39–41% silt, and 22–23% clay at 0–7.5 cm. Bowman et al. (1999) reported bulk density was in average 1.35 g cm$^{-3}$ in the plots and 1.20 g cm$^{-3}$ in the pasture site at 0–15 cm. Soil water holding capacity of the top 152 cm is 24 cm with potential rooting depth of >152 cm (Anderson et al., 1999). Our current assessment, 15 years after the initiation of the plots, showed that soil pH ranged in the plots from 4.3 to 5.8 for 0–5 cm and from 5.1 to 6.1 for 5–15 cm depth (Table 2). Significant differences already exist for soil organic C content under the 100% CI rotation compared to the 50% CI rotation under conventional tillage (Table 2).

### 2.2. Soil sampling

Soil samples were taken in March 2004 and 2005 representing 14 and 15 years after the establishment of the research plots, respectively. For convenience, the first crop listed in the rotation specifies the crop under which the soil sample was taken. Samples were taken in 2004 from pasture (P) and from selected crops of the 100% CI rotation under NT (C–M–W or W–C–M), 67% CI rotation under NT (C–F–W or W–C–F), and 50% CI rotation under different tillage practices (W–Fnt or W–Fct). In 2005, the samples were taken under all crops (plots) from the 100% CI rotation under NT (C–M–W, W–C–M, and M–W–C), 67% CI rotation under NT (C–F–W, W–C–F, and F–W–C), and the conventional 50% CI rotation under NT (W–Fnt and F–Wnt) or CT (W–Fct and F–Wct). Samples were also taken from undisturbed systems such as pasture and plots planted to grass.

Each year a composite sample that comprised of 10 cores (2.54-cm diameter) (Oakfield soil probe, Forestry Supplies, Inc. Jackson, MS) was taken from each treatment replicate at 0–5 and 5–15-cm depth. Soil samples were stored in a sterile polypropylene bag kept cool using coolers during field sampling and stored at 4 °C in a walk-in cooler after collection. The field-moist soil samples were then sieved through a 5-mm mesh screen to remove stones and to homogenize the sample and stored at 4 °C until microbiological analysis were conducted the same month of sampling.

### 2.3. Soil analyses

Microbial biomass C (MBC) and N (MBN) contents were determined on field-moist soil equivalent to 15-g oven-dry weight by the chloroform-fumigation-extraction method using 0.5 M $K_2SO_4$ as an extractant (Vance et al., 1987). Briefly, organic C and N from the fumigated (24 h) and non-fumigated (control) soil were quantified by a CN analyzer (Shimadzu Model TOC-V/$_{CPH}$-TN). The non-fumigated control values were subtracted from the fumigated values. The MBC and MBN were calculated using a $k_{EC}$ factor of 0.45 (Wu et al., 1990) and $k_{EN}$ factor of 0.54 (Jenkinson, 1988), respectively. Each sample had duplicate analyses and results were expressed on an oven-dry basis. Soil moisture was determined after drying the sample at 105 °C for 48 h. Only the 2005 samples were analyzed for microbial biomass.

Fatty acids were extracted from the soils using the procedure described for pure culture isolates by the Microbial Identification System (MIS, Microbial ID, Inc., Newark, DE) as previously applied for soil analyses (Acosta-Martínez et al., 2004). Briefly, the method consists of four steps: (1) saponification of fatty acids of 3 g field-moist soil with 3 mL 3.75 M NaOH (methanol:water, 1:1) solution under heat (100 °C) for 30 min; (2) methylation of fatty acids by adding 6 mL of 6 M HCl in aqueous methanol (1:0.85) under heat (80 °C) for 10 min; (3) extraction of the FAMEs with 3 mL of 1:1 hexane:methyl-*tert*

**Table 2 – Soil chemical properties of the different winter wheat based rotations**

| Properties | 50% CI rotation[a] | | | | 67% CI rotation | | | 100% CI rotation | | | Undisturbed systems | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F–W | | W–F | | C–F–W | F–W–C | W–C–F | C–M–W | M–W–C | W–C–M | Pasture | Grass |
| | CT | NT | CT | NT | | | | | | | | |
| 2004 | | | | | | | | | | | | |
| Organic C (Mg ha$^{-1}$ soil) | | | | | | | | | | | | |
| 0–5 cm | NA[b] | NA | 4.9 e[c] | 6.8 d | 7.3 cd | NA | 6.8 d | 9.2 b | NA | 9.0 b | 13.1 a | 8.5 bc |
| 5–15 cm | NA | NA | 7.6 c | 8.6 b | 10.2 b | NA | 9.2 bc | 8.6 bc | NA | 10.4 b | 12.6 a | 10.1 b |
| Total N (Mg ha$^{-1}$ soil) | | | | | | | | | | | | |
| 0–5 cm | NA | NA | 0.49 e | 0.72 cd | 0.64 cd | NA | 0.62 de | 0.87 b | NA | 0.76 bc | 0.71 cd | 1.10 a |
| 5–15 cm | NA | NA | 0.74 c | 0.81 abc | 0.96 ab | NA | 0.85 abc | 0.71 c | NA | 0.98 ab | 1.03 a | 0.79 bc |
| pH | | | | | | | | | | | | |
| 0–5 cm | NA | NA | 5.5 | 5.1 | 4.6 | NA | 5.1 | 5.4 | NA | 4.9 | 5.5 | 5.3 |
| 5–15 cm | NA | NA | 5.7 | 5.2 | 5.6 | NA | 5.4 | 5.7 | NA | 5.4 | 5.9 | 5.6 |
| 2005 | | | | | | | | | | | | |
| Organic C (Mg ha$^{-1}$ soil) | | | | | | | | | | | | |
| 0–5 cm | 5.2 e | 6.8 de | 6.9 cde | 7.1 cde | 7.3 cde | 6.8 de | 7.1 cde | 10.6 ab | 10.4 ab | 9.0 bc | 12.6 a | 8.4 bcd |
| 5–15 cm | 8.9 d | 8.5 d | 9.4 bcd | 9.2 cd | 9.7 bcd | 8.5 d | 9.2 cd | 10.3 bc | 10.9 b | 10.1 b | 16.8 a | 10.4 bc |
| Total N (Mg ha$^{-1}$ soil) | | | | | | | | | | | | |
| 0–5 cm | 0.50 e | 0.55 de | 0.71 cde | 0.62 de | 0.59 de | 0.55 de | 0.62 de | 0.94 bc | 0.98 b | 0.93 b | 1.22 a | 0.77 bcd |
| 5–15 cm | 0.68 e | 0.74 de | 0.98 bc | 0.73 de | 0.81 cde | 0.74 de | 0.73 de | 0.86 cde | 1.1 b | 1.1 bc | 1.74 a | 0.93 bcd |
| pH | | | | | | | | | | | | |
| 0–5 cm | 5.8 | 5.5 | 5.1 | 5.3 | 4.8 | 4.8 | 5.0 | 4.5 | 5.3 | 4.3 | 5.5 | 5.9 |
| 5–15 cm | 6.1 | 5.7 | 5.3 | 6.0 | 5.2 | 5.8 | 5.3 | 5.5 | 5.5 | 5.1 | 6.0 | 6.0 |

[a] Different cropping intensities (CI) were studied with different fallow frequencies. The first crop in a rotation specifies the crop when the samples were taken. The 50% CI rotation was studied under conventional tillage (CT) and under no-tillage (NT). The other rotations were studied under no-tillage practices.

[b] Data not available.

[c] Lower case letter among different rotations within each row are significant different at $P < 0.05$.

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butyl-ether solution and rotating the samples end-over-end for 10 min, and (4) washing of the organic phases with 3 mL of 1.2% diluted NaOH by rotating the tubes end-over-end for 5 min. The FAMEs were analyzed using a 6890 GC Series II (Hewlett Packard, Wilmington, DE) equipped with a flame ionization detector and 25 m × 0.2 mm fused silica capillary column using ultra high purity hydrogen as the carrier gas. The temperature program was ramped from 170 to 250 °C at 5 °C $min^{-1}$. The FAME's were identified, and their relative peak areas determined by the MIS Aerobe method of the MIDI system. Each sample peak was compared to standard fatty acids (MIS, Microbial ID, Inc., Newark, DE) and interpolation of retention time was done using the equivalent chain length method by MIDI software. The FAME's are described by the number of C atoms, followed by a colon, the number of double bonds, and then by the position of the first double bond from the methyl(ω) end of molecules. Other notations are used for methyl, and for the *cis* and *trans* isomers indicated by Me, *c* or *t*, respectively. Branched fatty acids are indicated by the prefixes *i* and *a* for iso and anteiso, respectively.

Enzyme activities (β-glucosidase, α-galactosidase, acid phosphatase, and alkaline phosphatase) were assayed using 1 g of air-dried soil with their appropriate substrate and incubated for 1 h (37 °C) at their optimal pH as described in Tabatabai (1994). β-Glucosaminidase activity was determined similarly with the method of Parham and Deng (2000). The enzyme activities were assayed in duplicate with one control, to which substrate was added after incubation and subtracted from a sample value. The results were expressed in mg of *p*-nitrophenol (PN) released $kg^{-1}$ soil $h^{-1}$.

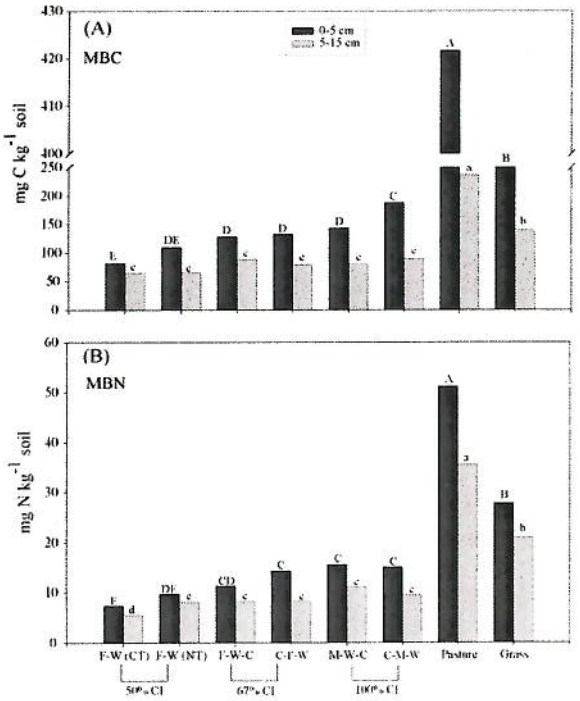


**Fig. 1 – Microbial biomass C (A) and microbial biomass N (B) as affected by different CI rotations at 0–5 and 5–15 cm depths after 15 years (2005 sampling).**

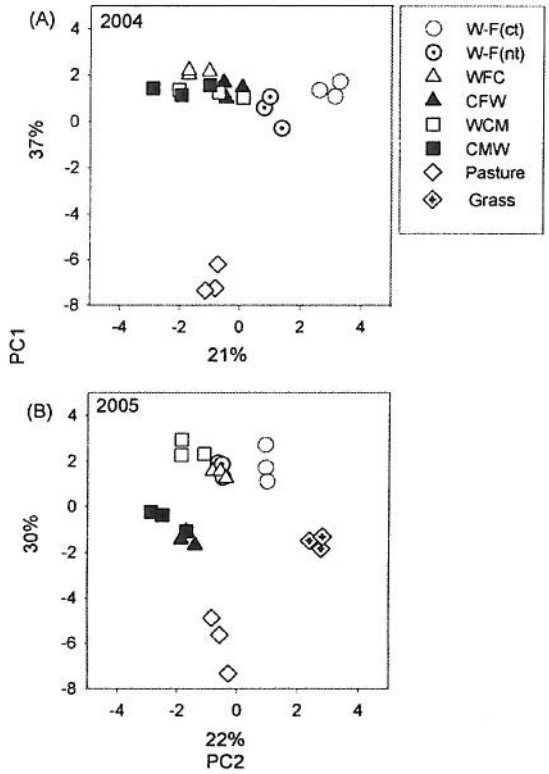


**Fig. 2 – Principal component analysis of soil FAMEs indicators of fungi (18:1ω9c, 18:2ω6c, 18:3ω6c and 16:1ω5c), and bacteria (15:0, *a*15:0, i15:0, *a*17:0, i17:0, 17:1ω9c, 10Me16:0, 10Me17:0, 10Me18:0) under different CI rotations at 0–5 cm depth after 14 (2004 sampling, A) and 15 years (2005 sampling, B).**

### 2.4. *Statistical analysis*

Statistical analyses, including ANOVA and mean separation by least significant differences (LSD), were performed to compare undisturbed systems (pasture and grass) and different rotations using the general linear model procedure of the SAS system (SAS Institute, 1999). All results were considered significantly different at $P < 0.05$ unless noted otherwise. Selected soil FAME markers (Frostegård and Bååth, 1996; Olsson, 1999; Madan et al., 2002) for fungal (18:1ω9c, 18:2ω6c, 18:3ω6c and 16:1ω5c) and bacterial (15:0, *a*15:0, i15:0, *a*17:0, i17:0, 17:1ω9c, 10Me16:0, 10Me17:0, 10Me18:0) populations were evaluated with principal component analysis (PCA) using the PRINCOMP procedure in SAS to compare the microbial community composition in undisturbed systems (pasture and grass) and different rotations studied.

## 3. Results

### 3.1. *Microbial biomass C (MBC) and N (MBN)*

Pasture plots showed between two- and five-fold greater MBC and between three- and seven-fold greater MBN compared to

Table 3 – Fatty acid composition in the soil studied as affected by different winter wheat based rotations after 15 years

| Fatty acids | 50% CI rotation[a] | | | | 67% CI rotation | | | 100% CI rotation | | | Undisturbed systems | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F–W | | W–F | | C–F–W | F–W–C | W–C–F | C–M–W | M–W–C | W–C–M | Pasture | Grass |
| | CT | NT | CT | NT | | | | | | | | |
| Area percent | | | | | | | | | | | | |
| Bacteria (B) | | | | | | | | | | | | |
| i15:0 | 5.03 bc[b] | 5.10 bc | 5.29 bc | 4.51 c | 7.17 abc | 5.90 abc | 5.19 bc | 8.48 a | 7.16 abc | 6.65 abc | 7.55 ab | 7.44 ab |
| a15:0 | 3.20 de | 2.91 e | 2.14 e | 2.84 e | 5.20 ab | 3.01 de | 4.11 bc | 6.31 a | 5.50 ab | 4.97 abc | 5.08 abc | 4.75 abc |
| i17:0 | 1.24 d | 1.55 bcd | 1.38 cd | 1.41 cd | 2.54 a | 1.73 abcd | 1.60 bcd | 2.37 ab | 1.95 abcd | 2.15 abc | 1.77 abcd | 1.83 abcd |
| a17:0 | 1.36 b | 1.48 b | 1.74 ab | 1.43 b | 2.84 a | 1.98 ab | 1.57 b | 2.82 a | 2.43 ab | 2.72 a | 2.30 ab | 2.41 ab |
| cy17:0 | 1.22 c | 1.33 bc | 1.83 abc | 1.42 bc | 2.15 abc | 1.88 abc | 1.44 bc | 2.91 a | 2.58 ab | 2.33 abc | 2.18 abc | 1.53 bc |
| cy19:0 | 0.68 b | 0.86 ab | 1.01 ab | 0.91 ab | 1.80 ab | 1.07 ab | 1.18 ab | 1.94 a | 1.79 ab | 1.45 ab | 1.47 ab | 1.14 ab |
| 17:1ω9c | 1.36 cdef | 1.08 def | 1.34 cdef | 0.77 f | 1.56 bcd | 1.35 cdef | 1.89 bc | 1.70 bc | 1.41 bcde | 1.36 cdef | 2.39 a | 2.03 ab |
| 10Me16:0 | 2.90 b | 2.81 b | 2.89 b | 2.85 b | 3.97 ab | 2.92 b | 3.10 b | 4.14 ab | 3.56 ab | 3.36 ab | 4.98 a | 4.40 a |
| 10Me17:0 | 1.12 cd | 1.02 d | 1.78 abcd | 1.27 bcd | 2.05 abc | 1.57 abcd | 1.39 abcd | 2.27 a | 2.14 ab | 2.00 abc | 1.42 abcd | 1.91 ab |
| 10Me18:0 | 0.66 a | 0.59 a | 0.83 a | 0.65 a | 1.08 a | 0.83 a | 0.88 a | 1.24 a | 1.21 a | 0.90 a | 0.94 a | 0.95 a |
| Sum (B) | 18.77 bc | 18.73 bc | 20.33 bc | 18.06 c | 30.36 ab | 22.24 abc | 22.35 abc | 34.18 a | 29.73 ab | 27.89 ab | 30.08 ab | 28.39 ab |
| Fungi (F) | | | | | | | | | | | | |
| 16:1ω5c | 1.93 d | 2.52 d | 1.51 d | 1.70 d | 6.43 bc | 2.60 d | 1.70 d | 1.92 d | 2.28 d | 2.77 d | 12.43 a | 9.57 ab |
| 18:2ω6c | 5.64 c | 6.20 c | 5.79 bc | 6.99 bc | 10.50 ab | 6.07 c | 5.62 c | 7.74 bc | 9.20 abc | 10.04 ab | 9.01 ab | 11.91 a |
| 18:3ω6c | 1.87 d | 2.14 cd | 2.73 cd | 2.13 cd | 3.97 bc | 2.66 cd | 2.30 cd | 4.73 ab | 3.22 bcd | 2.78 cd | 6.56 a | 2.84 bcd |
| 18:1ω9c | 9.00 b | 9.42 b | 9.73 b | 10.02 b | 15.27 ab | 9.21 b | 11.34 b | 16.85 a | 14.32 ab | 12.23 ab | 14.62 ab | 18.60 a |
| Sum[c] | 10.94 e | 11.94 de | 11.24 de | 11.72 de | 21.70 ab | 11.81 de | 13.04 de | 18.77 bc | 16.60 bcd | 15.00 bcd | 27.05 a | 28.17 a |
| Sum (F) | 18.44 d | 20.28 cd | 19.76 cd | 20.84 cd | 36.17 ab | 20.54 cd | 20.96 cd | 31.24 abc | 29.02 bc | 27.82 bc | 42.62 a | 42.92 a |
| Total (B + F) | 37.21 c | 39.01 c | 40.09 c | 38.90 c | 66.53 ab | 42.78 bc | 43.31 bc | 65.42 ab | 58.75 abc | 55.71 abc | 72.70 a | 71.31 a |

[a] Different cropping intensities (CI) were studied with different fallow frequencies. The first crop in a rotation specifies the crop when the samples were taken. The 50% CI rotation was studied under conventional tillage (CT) and under no-tillage (NT). The other rotations were studied under no-tillage practices.

[b] Different letters among rotations within each row are significantly different at $P < 0.05$.

[c] These values represent the sum of the mycorrhiza indicator FAMEs (16:1ω5c + 18:1ω9c).

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all the cropping systems plots at 0–5 cm soil depth (Fig. 1). Similarly, MBC was between 1.4- and 3-fold and MBN was between 1.8- and 4-fold greater in grassland compared to all the cropping systems at 0-5 cm soil depth. The soil MBC was also significantly ($P < 0.05$) higher in the 0–5 cm depth for the 100% CI rotation (C–M–W or M–W–C) and 67% CI rotation (C–F–W or F–W–C) compared to the 50% CI rotation under CT (F–Wct) (Fig. 1A). Generally, there was no differentiation between the 100% CI rotation and the 67% CI rotation, except in MBC when the 100% CI rotation was sampled under corn. Similar trends were found for MBN, and it was even greater in the undisturbed systems (pasture and grass) compared to the cropping systems at 5–15 cm depth (Fig. 1B). Although there was no significant difference in the soil MBC and MBN of F–Wnt compared to F–Wct at 0–5 cm depth, we still observed 24–25% higher MBC and MBN in F–Wnt compared to F–Wct. In addition, there was no significant difference in MBC and MBN among the rotations at 5–15 cm depth, except that MBN was the lowest in F–Wct compared to the other rotations.

### 3.2. *Microbial community composition*

Principal component analysis (PCA) for 2004 sampling (0–5 cm) showed a differentiation of FAME markers from soil microbial communities under pasture compared to all crop rotations (Fig. 2A). In addition, a separation of the soil microbial communities under the 50% CI rotation (W–Fct) from the rest

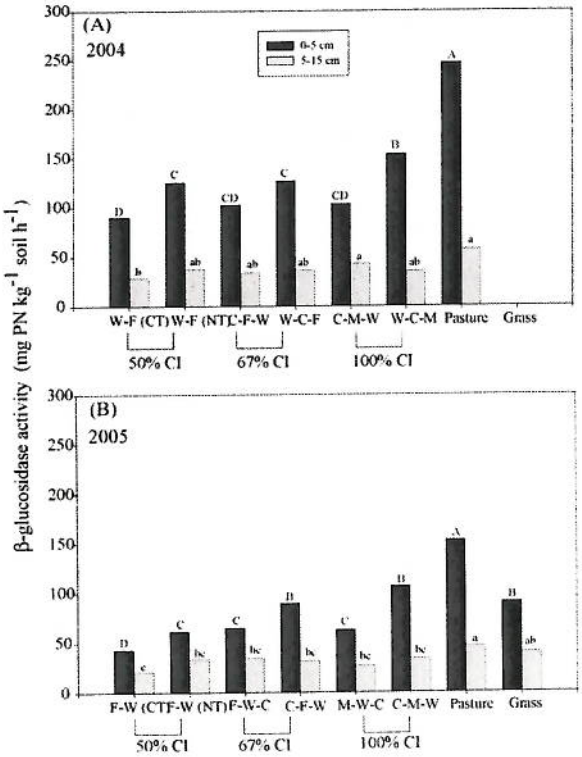


**Fig. 3 – β-Glucosidase activity as affected by different CI rotations at 0–5 and 5–15 cm depths after 14 (2004 sampling, A) and 15 years (2005 sampling, B). Grass systems were only sampled in 2005 (B).**

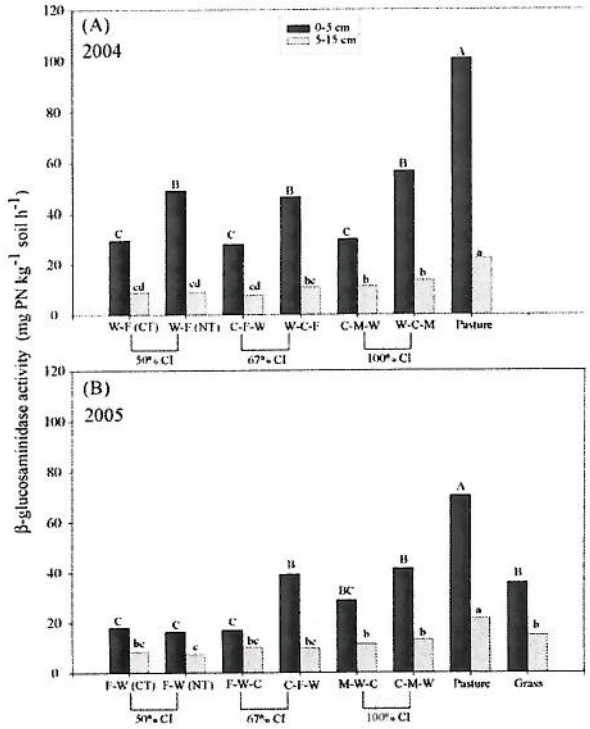


**Fig. 4 – β-Glucosaminidase activity as affected by different CI rotations at 0–5 and 5–15 cm depths after 14 (2004 sampling, A) and 15 years (2005 sampling, B). Grass systems were only sampled in 2005 (B).**

of the systems was observed. Similarly, the PCA for the 2005 samples (0–5 cm) showed a separation of the soil microbial communities under pasture and grass compared to crop rotations sampled under corn (C–F–W, C–M–W) and those sampled under wheat (W–Fct, W–Fnt, W–F–C, and W–C–M) (Fig. 2B). In addition, there was a separation of the soil microbial communities under the 50% CI rotation (W–Fct) from other rotations. Generally, there were no significant differences in the soil microbial communities among the cropping systems at 5–15 cm depth (data not shown).

There were no significant differences in the number of FAMEs extracted from the systems (ranged from 59 to 61 FAMEs), and the differences among systems were due to particular FAME markers. For the 2005 soil sampling, the sum of all FAME bacterial indicators were higher under pasture (up to 1.7-fold), grass (up to 1.5-fold), and the longer rotations without fallow period (C–M–W, M–W–C and W–M–C) (1.4–1.8-fold) compared with wheat–fallow under CT and NT practices at 0–5 cm (Table 3). Individual bacterial indicators such as a15:0, a17:0 and i17:0 were higher under the 67% CI rotation when sampled under corn (C–F–W) compared to the 50% CI rotation (F–W) under CT and NT practices.

The sum of four FAME fungal indicators (16:1ω5c, 18:2ω6c, 18:3ω6c and 18:1ω9c) was higher in soils (0–5 cm) under pasture and grass compared to the cropping systems, except when the samples were taken under corn, and up to 2.3-fold compared to the 50% CI rotation (Table 3). Although not always

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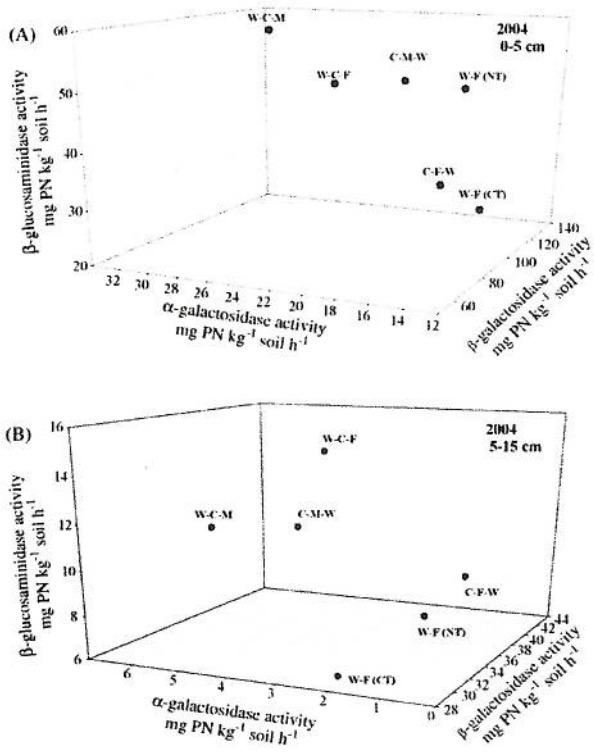


**Fig. 5 – Three-dimensional plots of C cycling enzyme activities (β-glucosidase, α-galactosidase and β-glucosaminidase) as affected by different CI rotations at 0–5 cm (A) and 5–15 cm (B) after 14 years. Pasture systems are not included.**

significant, the sum of the soil fungal FAMEs was higher in the 100% CI rotation compared to the 50% CI rotation, and in the 67% CI rotation under corn (C–F–W) than under fallow (F–W–C) or wheat (W–C–F). The sum of 18:1ω9c and 16:1ω5c was higher (up to 1.7-fold) in soils under the 100 and 67% CI rotations under corn compared to the 50% CI rotation.

The sum of bacteria (B) and fungal (F) indicators (14 FAMEs) was higher in soils under the undisturbed systems, pasture (72.7%) and grass (71.31%), the 100% CI rotation (55.71–65.42%), and in the 67% CI rotation sampled under corn (C–F–W = 66.53%) compared to the 50% CI rotation (37.21–40.09%).

### 3.3. *Enzyme activities*

The activities of soil β-glucosidase (Fig. 3) and β-glucosaminidase (Fig. 4) were higher in 2004 than in 2005, when lower precipitation and temperatures occurred. In 2004, the activity of β-glucosidase was higher under pasture compared to the agricultural rotations (37% for W–C–M to 63% for W–Fct) at 0–5 cm depth (Fig. 3A). β-Glucosidase activity at 0–5 cm depth tended to be higher under wheat residue in W–C–F (by 33%) and W–C–M (by 19%) than under corn residue in C–F–W and C–M–W, respectively. For 2005 sampling, there were no differences in β-glucosidase activity between grass and the rotations sampled under corn (C–M–W) at 0–5 cm depth (Fig. 3B). However, the activity of this enzyme was higher (40%) under corn (C–M–W) compared to when sampled under millet (M–W–C) in the 100% CI rotation, and higher (27%) under corn (C–F–W) than when sampled under fallow (F–W–C) for the 67% CI rotation in 2005. β-glucosidase activity was lower in the typical 50% CI rotation under conventional tillage (F–Wct) compared to the alternative rotations at 0–5 cm in 2005. Tillage significantly affected β-glucosidase activity, where it was lower with CT than NT at 50% CI rotation in both years of sampling but only at the 0–5 cm depth. There were no differences in β-glucosidase activity among the systems at 5–15 cm, except for 2005, where higher enzyme activity was found under pasture compared to the agricultural systems.

Soil β-glucosaminidase activity showed the same trends of β-glucosidase activity (Fig. 4A and B). This enzyme activity also showed increases with the decrease of fallow periods in the rotations for 2005 samples (Fig. 4B). The enzyme activities showed similar trends, and thus, three-dimensional plots were prepared for the three related enzyme activities as a group (Figs. 5–7).

Generally, the three-dimensional plots were presented without the pasture or grass systems because they always showed higher enzyme activities compared to the agricultural systems, which mask the effect of rotation. The soil C cycling enzyme activities (β-glucosaminidase, β-glucosidase and α-galactosidase) as a group showed a separation (lower activities) of W–Fct and C–F–W rotations from the other

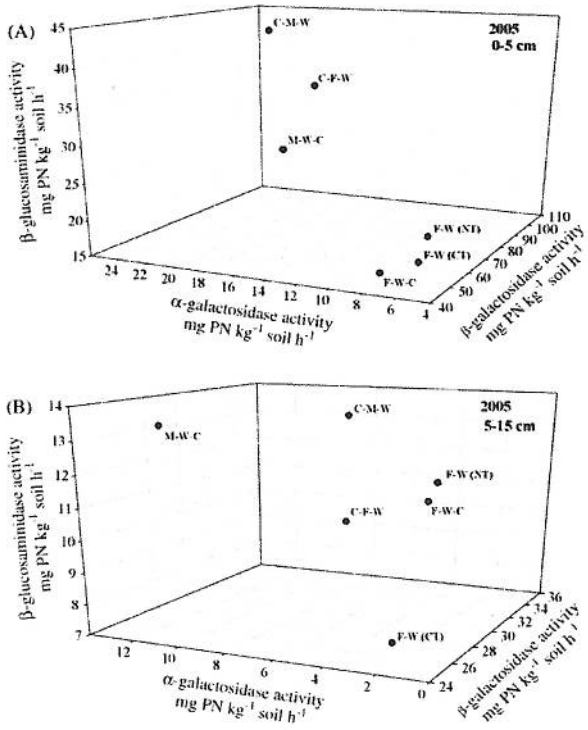


**Fig. 6 – Three-dimensional plots of C cycling enzyme activities (β-glucosidase, α-galactosidase and β-glucosaminidase) as affected by different CI rotations at 0–5 cm (A) and 5–15 cm (B) after 15 years. Pasture and grass systems are not included.**

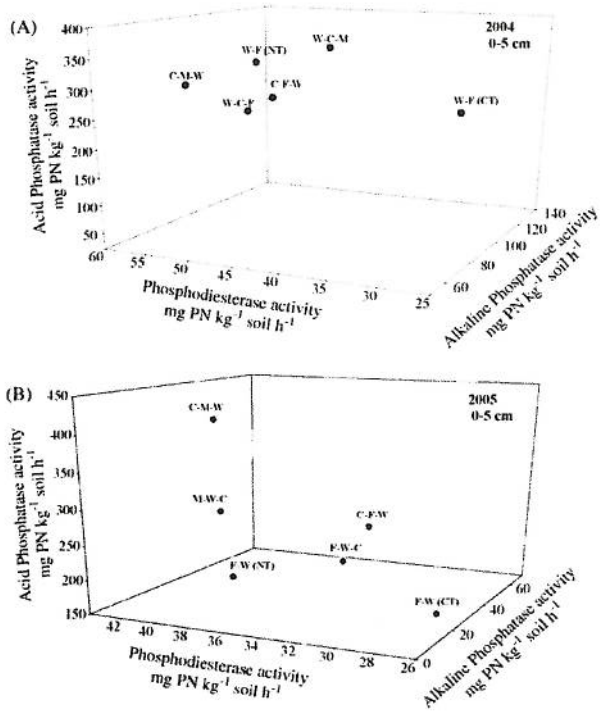


**Fig. 7 – Three-dimensional plots of P cycling enzyme activities (alkaline phosphatase, phosphodiesteras, and acid phosphatase) as affected by different CI rotations at 0–5 cm depth after 14 (2004 sampling, A) and 15 years (2005 sampling, B). Pasture and grass systems are not included.**

rotations at 0–5 and 5–15 cm depths (Fig. 5A and B). For the 2005 samples collected at 0–5 cm depth, there were higher activities in the 100% CI rotation (C–M–W, M–W–C) and 67% CI rotation when sampled under corn (C–F–W) compared to rotations sampled under fallow (F–Wnt, F–W–C, and F–Wct) (Fig. 6A). For samples collected at the 5–15 cm depth, the following separation of the enzyme activities was observed: C–M–W, M–W–C > C–F–W, F–W–C, F–Wnt > F–Wct (Fig. 6B).

The three-dimensional plots for the soil P cycling enzymes (acid phosphatase, alkaline phosphatase, and phosphodiesterase) showed the lowest activities in the 50% CI rotation (W–Fct) compared to the other rotations at 0–5 cm in 2004 (Fig. 7A). The same plots for samples taken in 2005 showed similar trends (Fig. 7B). The same trends were found for samples taken at 5–15 cm and thus, the data is not shown.

## 4. Discussion

In our study, the native pasture site represented the undisturbed system with the highest levels of microbial population size and enzyme activities for this soil when compared to the agricultural systems. Similarly, other studies have found higher enzyme activities, microbial biomass and fatty acid indicators of bacteria and fungal populations in pasture soils compared to the agricultural soils due to the positive impacts of the surface cover, vegetation, and lack of tillage of pasture on soil properties (Acosta-Martínez et al., 2004). Higher soil microbial populations and enzyme activities in native pasture observed in our study were due to the higher SOC and total nitrogen (TN) compared to the cropping systems. The grass plots were established at the same time as the agricultural plots, and thus, our assessments provided information of how the soil microbial properties of this undisturbed system diverged from agricultural systems after 15 years. The grass systems showed lower SOC, microbial biomass and enzyme activities than native pasture most likely due to differences in their root systems and root exudates (Fang et al., 2001) and to the short establishment period (15 years) compared to pasture. However, there were still significantly higher MBC, MBN, enzyme activities and microbial community shifts on the grass soils compared to the cropping systems while SOC was not always significantly different between these systems. Our findings demonstrate the significant impacts of soil organic matter quality differences between systems for sustaining different levels of soil microbial populations and enzyme activities. These findings are due to the beneficial effects of greater grass root biomass and surface cover on the soil microbial communities compared to the winter based rotations, especially those with fallow periods.

The combination of continuous cropping (100% CI) and NT practices in the W–C–M rotation changes water relations compared to wheat–fallow under CT (W–Fct) by maintaining more crop residue and mild temperatures on the soil surface (Nielsen et al., 2005). The combination of NT and CI greater than 50% could result in greater residues and root densities that promoted the higher soil microbial biomass and community shifts found in our study. Previous research reported that crop rotation systems provide greater amounts of plant residues of varying degrees of decomposability that would support greater microbial populations and diversity compared to monoculture continuous systems (Miller and Dick, 1995; Friedel et al., 1996; Robinson et al., 1996; Moore et al., 2000). Higher soil MBC and MBN associated with higher cropping intensity (100% CI) were also in agreement with greater particulate organic matter C (POM–C) at the same site after 7 years of the establishment of the plots (Bowman et al., 1999).

Compared to the 50% CI rotation (F–Wct), the higher soil MBC, MBN, and enzyme activities that we observed with intensive cropping (100 and 67% CI rotations) was probably due to increased SOC over the 15 years of the plot establishment. These findings are of ecological significance because no differences have been detected in soil organic C content under the 67% CI rotation (W–C–F) and 50% CI rotation (W–F) after 7 years (Bowman et al., 1999) and 15 years of the establishment of the rotations (Table 2). The increase in microbial populations over the 15 years with the intensive rotations have resulted in the synthesis of more enzymes (proteins) that became stabilized as the SOC content increased. The changes in several key enzymes in soil processes, which were evaluated in this study, demonstrate the higher nutrient input and nutrient cycling in soils under these rotations, and can be interpreted as changes in soil microbial functional diversity (Nannipieri et al., 2002).

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The separation observed in PCAs for undisturbed systems (pasture and grass) and alternative rotations (100 and 67% CI) from the 50% CI rotation (W–Fnt or W–Fct) was due to differences in FAME markers for fungal and bacterial populations. The greater enzyme activities and shifts in microbial community that we found in this study demonstrated beneficial effects in soil microbial functioning due to the combination of NT practices (Kennedy and Schillinger, 2006) and reduced fallow frequency (Ndiaye et al., 2000; Schutter et al., 2001). Similar to previous findings, significant correlations ($r > 0.60$; $P < 0.05$) were found between the sum of fungal indicators (18:2ω6c, 18:3ω6c, 18:1ω9c, 16:1ω5c) and the activities of β-glucosaminidase, β-glucosidase, acid phosphatase and α-galactosidase (Acosta-Martínez et al., 2004). Frey et al. (1999) reported that the greater amount of fungal hyphae in no-tilled agroecosystems could be because: (1) the reduced disturbance facilitates establishment and maintenance of extensive hyphal networks (Wardle, 1995), and (2) fungal mycelial growth can bridge the soil-residual interface and utilize the spatially separated C and N resources by the translocation of N from the soil inorganic N pool into the C-rich surface residues (Beare et al., 1992). The higher abundance of the FAMEs 18:1ω9c and 16:1ω5c in the undisturbed systems compared to the agricultural soils and in the alternative rotations compared to the 50% CI rotation is of ecological significance as they have been suggested as mycorrhizal indicators (Olsson, 1999; Madan et al., 2002). The fact that rotations sampled under corn, which were previously under wheat (i.e., C–M–W), showed higher abundance of these mycorrhizal FAMEs than when sampled under wheat (i.e., W–C–M) agree with Mäder et al. (2000), who detected maximum AM fungal structures in wheat roots at plant maturity. Generally, wheat is planted during September to establish a stand and begin tillering activity before winter causes dormancy, and thus, wheat was not mature enough to establish mycorrhizal associations that could be detected by our soil sampling in March (Stromberger et al., 2007). Nevertheless, our findings demonstrate that a management history of alternative continuous cropped wheat based rotations enhanced mycorrhizal associations. Improvement in plant–mycorrhizal associations is beneficial in semiarid and arid environments, such as the Central Great Plains, where water is a limitation for wheat production. Our FAME data suggest that plant–mycorrhizal associations could have contributed to the >65% annualized yield increase in the 100% CI rotation that was reported by Anderson et al. (1999). Similar to our findings, a study comparing winter fallow systems with cover-cropped systems, reported higher fungal and even protozoan FAME markers in the cover cropped soils due to an increase in the amount of residue incorporated and made available for fungal growth (Schutter et al., 2001).

The fact that microbial biomass was not significantly different in the 67% CI rotation (F–W–C or C–F–W) compared to the 50% CI rotation under NT (W–Fnt) suggest the beneficial impacts of no-tillage for a wheat–fallow rotation. However, microbial biomass and FAME markers did not differentiate between the wheat–fallow rotation under CT compared to that under NT, while studies by Mikha (personal communication) in the same plots showed higher aggregation in this rotation under NT compared to CT. Other studies have demonstrated the relationship between microorganisms and aggregation in soils (Miller and Dick, 1995; Schutter et al., 2001) because of their decomposition products and mechanical binding of soil particles by fungal growth (Lynch and Bragg, 1985). Our findings in the wheat–fallow rotation disagree with other studies showing that reduced or no-tillage management can increase fungal populations, a major component of the microbial biomass in arable soils (Jenkinson and Ladd, 1981; Kennedy and Schillinger, 2006). Therefore, we hypothesize that there may have been certain type of fungi not represented by the FAME markers, which contributed to aggregate formation and stabilization in wheat–fallow under NT compared to CT. However, in contrast to the soil microbial biomass and community composition data, higher enzyme activities were observed in winter–fallow under NT compared to CT. Generally, greater yield was previously observed with NT management practice in the same plots by Anderson et al. (1999). Increases in plant biomass return and in root density could have increased the microbial biomass temporarily in the no-tilled wheat–fallow rotations possibly increasing the total residue decomposition-associated enzymes in soil. Numerous studies have reported the beneficial impacts of conservative tillage management on soil organic C (Franzluebbers et al., 1995), enzyme activities (Acosta-Martínez and Tabatabai, 2001; Acosta-Martínez et al., 2003), and microbial biomass and community structure (Angers et al., 1993; Franzluebbers et al., 1994, 1995; Frey et al., 1999; Pankhurst et al., 2002). Thus, our findings suggest that the negative impacts of fallow periods on soil microbial communities under a wheat–fallow rotation cannot be overcome by the application of conservative tillage practices.

In our study, the soil enzyme activities and some of the FAMEs were significantly ($P < 0.05$) affected by the particular crop that was in rotation when the sample was taken. However, this trend was not observed for the soil microbial biomass. Both microbial biomass and enzyme activities were affected by the crop type in a related study in West Texas, but the soil had higher clay and SOC contents than the soils of our study (Acosta-Martínez et al., 2004). Other studies have reported differences in soil MBC and MBN as affected by the variations in the concentrations and types of organic compounds released by the roots of different plants (Lynch and Bragg, 1985; Moore et al., 2000). In addition, our study showed that the microbial biomass was not impacted by the CI at 5–15 cm depth after 15 years, but the enzyme activities related to C and P cycling still showed separation in three-dimensional plots similar to the trends at 0–5 cm depth. Reductions in microbial populations have been found due to the decreases in water availability, oxygen, and substrates with soil depth. However, it appears that the lack of response of the microbial biomass to the crop rotations studied at the lower soil depth is due to the lack of rhizosphere effect during the fallow periods in a rotation.

In general, we observed more significant detrimental impacts of the fallow periods on the microbial populations than on enzyme activities. The higher sensitivity of the enzyme activities to the management evaluated may occur because the assays currently available can detect not only the intracellular enzyme activity, but also the activity of enzymes already stabilized in soil organic matter and clay complexes (extracellular enzymes) (Tabatabai, 1994; Acosta-Martínez

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et al., 2004). The microbial populations in soils under fallow could have been more affected by sudden environmental parameters typical of semiarid regions (i.e., high temperatures and low precipitation with sudden rain events) than the enzyme activities (Acosta-Martínez et al., 2003). A previous study comparing mixed prairie sod soils with cropped to wheat or left fallow soils reported the microbial community was influenced by wheat inputs during the wheat cycle, whereas during fallow it was influenced by the physicochemical differences in the soil resulting from tillage (Drijber et al., 2000). Thus, our results revealed that the enzyme activities can be a good indicator of the soil changes associated to the management history studied as they are more persistent in different pools in soils than microorganisms.

## 5. Conclusions

The results from the soil microbial biomass, microbial community structure, and enzyme activities demonstrate that fallow periods and conventional tillage can negatively affect soil quality in the 50% CI rotation (W–F). These properties did not vary significantly between the alternative 100% (W–C–M) and 67% (W–C–F) CI rotations. Higher soil enzyme activities, microbial biomass and fungal populations were encouraged by the intensive cropping rotations (i.e., 100 and 67% CI) due to the higher crop residues maintained by NT practices and less fallow frequency compared to the 50% CI rotation. However, the soil microbial biomass was still lower under the continuously cropped 100% CI rotation compared to the grass plots even after 15 years of the initiation of the study, but the soil enzyme activities were similar under grass and the alternative rotations (100 and 67% CI) under corn. The microbial responses observed in the 100% CI rotations were due to the higher SOC compared to the 50% CI rotation. Improvements in the soil properties measured in the 67% CI rotation, even at shallow soil depths (0–5 cm) may indicate that they could lead to increases in other soil quality parameters such as organic matter content, aggregation and soil water infiltration, and perhaps increase water efficiency use of wheat impacting soil sustainability and productivity of the system.

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