Soil Enzyme Activities Associated with Differential Outcomes of Contrasting Approaches to Soil Fertility Management in Corn and Soybean Fields

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Abstract Sustainable agricultural practices such as reduced tillage and optimized fertilization may have potential to improve soil health and increase availability of plant nutrients and yields. However, there is very little information relating soil quality or health to crop productivity, particularly under farmer management. Therefore, the objective was to investigate the effects of two contrasting approaches to crop fertility management on crop productivity, soil test measurements, and soil enzyme activity as integrative measures of soil health. A three year study was conducted at on-farm sites in Ohio, Illinois, and Iowa where topsoil (0-15 cm) and crop yield of *Zea mays* L. (corn) and *Glycine max.* (L.) Merr. (soybean) rotations were collected from two contrasting fertility management systems. The two contrasting approaches tested were the Maximum Farming System (MFSyst) and a more Conventional system (Conv) that differ in approaches to tillage and the frequency of fertilizer applications during the growing season. The MFSyst approach resulted in significantly higher yields, soil nutrient test levels along with β-glucosidase (GLU) and arylsulfatase (ARYL) which are sensitive soil health indicators. Nitrogen use efficiency (NUE) of corn was significantly elevated by nearly 18%, corn yield correlated with GLU activities, and soil test phosphorous (P) levels were reduced by over 50% using the MFSyst approach. These results indicate that improvements in soil health detected by soil GLU and ARYL enzyme activities are associated with significant improvements in soil quality and crop productivity.

Keywords: soil fertility management, soil health, soil enzyme activities, soil organic matter, corn nitrogen use efficiency, soybean

Cite This Article: Nicola Lorenz, Brian B. McSpadden Gardener, Nathan R. Lee, Cliff Ramsier, and Richard P. Dick, "Soil Enzyme Activities Associated with Differential Outcomes of Contrasting Approaches to Soil Fertility Management in Corn and Soybean Fields." *Applied Ecology and Environmental Sciences*, vol. 8, no. 6 (2020): 517-525. doi: 10.12691/aees-8-6-26.

1. Introduction

Soil is a vital ecosystem and, on a human time scale, a nonrenewable natural resource [1]. Protecting soil health is a national priority, and an integral part of protecting the environment and ensuring food security and thus, promoting human well-being [2], but quantification of dynamic soil properties remains challenging. In addition, soil health studies have not always shown that supposedly better soil management (e.g. organic farming) results in healthier soils which are more productive [3,4]. However, studies have shown that certain soil properties can detect effects of diverse crop and soil management systems and putative indicators of soil quality or health [5,6,7,8,9]. Based on 192 soil health studies across the USA, Stewart et al. [10] pointed out that some of the most responsive soil health indicators were biological indicators such as β-glucosidase (GLU) and phenol oxidase activities, fungal biomass, and soil microbial biomass. Research has shown that hydrolytic soil enzyme activities are well suited as soil health indicators because certain ones are sensitive for detecting soil management, disturbance or contamination [11]. In particular β-glucosidase (GLU) and arylsulfatase (ARYL) have been shown to be sensitive for detecting changes in soils due to a variety of management systems [6,12-21]. In fact, soil enzyme activities have shown to be sensitive to changes in land management within 1 to 3 years, long before SOM increased significantly [6,19].

An important property of a soil health indicator is that it can be calibrated. A specific requirement for this is low seasonal variability. This is a likely problem with most soil biological measures where recent/short term conditions such as moisture fluctuation, disturbance (e.g. tillage) or a high-quality organic input (e.g.

incorporating rich green manure) causes a large but short-term response and not show the true status of the soil. Certain enzymes have an advantage to overcome this because they remain relatively stable on a seasonal basis [6,19] which can, in part, be attributed to them being extracellular enzymes stabilized in the soil matrix. This has been referred to as "abiontic", a term coined by Skujinš [22] to describe extracellular enzymes that are of biological origin but no longer under the control or associated with viable cells. These extracellular enzymes can be stabilized on clays, silt, or humic colloids and remain catalytic, with typically 40-80% of the activity associated with abiontic fraction [17,23,24]. This abiontic factor provides another mechanistic property for using soil enzyme activities as a soil health indicator because it is related to soil organic matter accumulation, evidenced by correlation of enzyme activities with soil organic matter content [14,25]. Thus, conditions that promote stabilization of organic matter and associated structural properties (e.g., aggregation and porosity) would also promote stabilization of enzymes in the soil matrix. But since enzyme assays are much more sensitive to changes in activity than most measures of soil organic matter, it can be viewed as an early and sensitive indicator of soil management. Soils that have been managed to promote soil quality (e.g., minimum tillage, organic amendments, crop rotations, etc.) should have higher biological activity, which would be reflected in greater enzyme production and potential to stabilize and protect enzymes complexed in the soil matrix (i.e., through increased production of organic colloids and aggregation there is increased complexation and protection of enzymes). Indeed, soil aggregation has been shown to be correlated with the activity of certain enzymes [26,27,28,29] and negatively correlated with soil compaction at forests or agricultural sites [30,31,32] and aggregation. Thus, soil enzyme activities may be a more practical pedo-transfer indicator for reflecting changes in soil structure because enzyme assays are considerably simpler than the labor-intensive soil structural methods. A further advantage for practical applications is that certain enzyme assays can be measured using air dried soils because the ability to detect soil management effects are maintained [6,18] which is superior to most biological assays that must be run immediately after sampling or the soil sample be frozen. This property and that enzyme assays have low labor costs makes them attractive to commercial soil testing labs for high throughput analyses. Mechanistically GLU is important because of its role in the carbon cycle as the final degradation step for cellulose, and the subsequent release of glucose, a critical energy source for microorganisms [33,34]. Secondly, the abiontic GLU fraction contributes a significant amount of its total activity [17], providing an index of the ability of a soil to stabilize and protect GLU. ARYL is important in the sulfur (S) cycle because it releases plant available SO_4^{2-} [15,34]. It may also be an indirect indicator of fungal biomass as only fungi contain ester sulfate, the substrate for ARYL. Therefore, fungal biomass is highly correlated with ester sulfate and so as fungal biomass changes there is a corresponding response of the microbial to increase or decrease sulfatase enzymes depending on the size of the fungal ester sulfate pool.

Although GLU and ARLY assays have been shown to be sensitive for detecting soil management effects; much of this data was generated on research plots with relatively few studies on farmers' fields. Furthermore, there is very little information relating enzyme activities or other soil health indicators to crop productivity. This is an important consideration as it would be expected that healthier soils should increase yields. However, it is difficult to make comparisons on soil health in farmers' fields because each farm tends to be unique and may not have consistent management over time or provide replication of a management system across farms. In particular nutrient management can vary widely and have direct effects on yields that might obscure the overall relationship of soil health to crop yield. Thus, the research was undertaken to address this gap in soil health development with soil enzyme by comparing two management systems, side by side across the Midwest with the dominant corn-soybean rotation. The Maximum Farming systems (MFSyst) provides an opportunity for such a study as it is a prescribed set of practices that are uniformly practiced by participating farmers - this includes focus on no-tillage, banding and split application of N, use of micronutrients, addition of Ca sulfate (gypsum), and strict use of soil testing for fertilizer rates of application. The MFSsyt was then compared to an adjacent field at each site where conventional soil management was being practiced which included more disruptive tillage practices paired with pre-plant fertilizer management. The objectives of this study were to determine the ability of GLU and ARYL activities to detect the effects of contrasting crop fertility management practices on soil fertility and crop productivity, and, thereby, be used as indexes of soil health in relation to corn and soybean production in the Midwest USA.

2. Materials and Methods

2.1. Experimental Design and Sample Collection

Nine pairs of fields across three US states, at three sites in Iowa (near DeWitt), three sites in Illinois (near Prairie City), and three sites in Ohio (near Circleville) were tested in this study. All sites are located in a humid continental climate, with warm summers and cold winters. Mean annual temperature at DeWitt (Iowa) is 9.4°C, with average temperatures of 18.4°C in the main crop growth period from April to September and -6.5°C from October to March. Mean annual precipitation at DeWitt (Iowa) is 936 mm, with 618 mm rainfall occurring from April to September. Mean annual temperature at Prairie City (Illinois) is 10.9°C, with average temperatures of 19.6°C in the main crop growth period from April to September and -4.2°C from October to March. Mean annual precipitation at Prairie City (Illinois) is 980 mm, with 618 mm rainfall occurring from April to September. Mean annual temperature at Circleville (Ohio) is 11.4°C, with average temperatures of 19°C in the main crop growth period from April to September and 3.8°C from October to March. Mean annual precipitation at Circleville is 988 mm, with 564

mm rainfall occurring from April to September (data from NOAA, https://www.ncdc.noaa.gov/cdo-web/).

All soils tested are silt loams and silty clay loams; soil types at sites in Iowa and Illinois are luvic Phaeozems, and Ohio (Circleville) soils are orthic Luvisols. Particle-size distribution is presented in the results section below. The sites in Illinois (near Prairie City) are located at 204 m above sea level (a.s.l.) and situated on ground moraines (Loess) with 0-5 % slopes. The sites at DeWitt (Iowa) are at 216 m a.s.l. and situated on ridges with 2-9 % slopes. The sites at Circleville (Ohio) are at 212 m a.s.l. and located on recessional moraines and ground moraines with 0-5 % slopes.

Each site had the following two crop management systems that were managed by farmers: 1) Maximum Farming System (MFSyst) developed by Ag Spectrum, DeWitt, IA, USA, and 2) a conventional crop management system (Conv). The contrasting management practices (Table 1) were implemented for at least 15 years.

Major management differences are fertilizer placement at planting and side-dressing for MFSyst, whereas Conv fertilization occurred at pre-planting. Micronutrients are applied annually within the furrow as a starter or with foliar sprays at MFSyst, while conventional management reduces micronutrient application and does so only after soil or plant tests show deficiency. Further, MFSyst promotes conservation tillage with no-tillage (eight out of nine fields) or vertical tillage (one out of nine fields). Vertical tillage is a form of conservation tillage which was done with a combination of disk and inline rippers resulting in cutting through the topsoil in rows in a depth of 0-20 cm (no inversion), and slicing plant residue, which remained mostly on the soil surface. Conv management used relatively more disruptive methods such as chisel tillage where equipment is used to cut and mix residue into the soil in 0-15 cm depth (six out of nine fields), and moldboard plow tillage inverted/mixed the topsoil in 0-30 cm depth (three out of nine fields).

Soils were sampled in spring of 2015, 2016, and 2017 and each field was sampled at three or four individual sampling points (pseudo-replications). Field subsampling points were defined after using USDA-NRCS web soil survey information such as soil series, soil texture, elevation, exposition, and slope to identify matching sampling spots at adjoining fields. Soil sampling points with similar features present in both adjoining fields were selected, GPS marked, and soil was sampled with a soil corer by taking randomly 15 soil cores (2.5-cm diameter) in 0-15 cm depth at each sampling point, soil was mixed, bagged and brought back to the lab in a cooler. In total, 192 soil samples were collected, resulting in $N = 32$ per

management system per year. Soils were passed through a 2-mm sieve, homogenized and kept at 4°C until enzyme analyses. Soil samples for soil chemistry were air-dried at room temperature for three days and subsequently stored at 4°C.

Corn and soybean yields were measured by hand harvests which were performed prior harvest in 2015, 2016, and 2017, as described below. Additional data on nitrogen fertilizer applications on the fields tested were collected in 2019 by surveying the farmers involved.

2.2. Soil Analyses

Soil chemical analyses (pH, soil organic matter (SOM), cation exchange capacity (CEC), nitrate (NO_3^-), potassium (K), phosphorus (P), sulfur (S), magnesium (Mg), calcium (Ca), iron (Fe), manganese (Mn), copper (Cu), boron (B), and zink (Zn)) were conducted by Midwest Laboratories (Omaha, NE, USA) using the S1A (2015), S3C (2016, 2017) testing packages. Briefly, soil pH was determined in water, SOM was determined by weight loss-on-ignition [35], plant available P was extracted using weak Bray, containing 0.025 M HCl and 0.03 M NH_4F [36], plant available NO_3^- was extracted with water. Soil K, Mg, Ca, and S were extracted with neutral, 1M NH_4-acetate [37] and analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Soil Fe, Mn, Cu, B, and Zn were extracted with 0.005 M Diethylenetriamine pentaacetate (DTPA) as described in Lindsay & Norvell [38] and detected via ICP-AES. Soil texture (% sand, % silt, % clay) analyses was also conducted at Midwest Laboratories (Omaha, NE, USA) using the hydrometer method [39].

Analyses of arylsulfatase (EC 3.1.6.1 arylsulfate sulfohyrolase) activity (ARYL) and β-glucosidase (EC 3.2.1.21 β-d-glucoside glucohydrolase) activity (GLU) were performed as described by Tabatabai [34] except toluene was not used due to the short incubation time. Briefly, for each sample, two 1 g- samples were weighed each in two Erlenmeyer flasks and incubated in buffered substrate solution at 37°C. Substrate solutions were prepared using *p*-nitrophenyl sulfate (Sigma N3877; St. Louis, MO) and ρ-nitrophenyl-β-D-glucoside (Sigma N7006), respectively. A third assay with substrate-free buffer was incubated for each sample and served as a control. To each assay 0.5 M $CaCl_2$ and 0.1 M $C_4H_{11}NO_3$ (tris(hydroxymethyl)aminomethane) pH 12 were added after incubation, suspensions were filtered and absorbance of the ρ-nitrophenol (PNP) product was measured at 415 nm and calibrated against a PNP standard. ARYL and GLU activities are expressed as µmol PNP g^{-1} h^{-1}.

Table 1. Key differences in the Maximum Farming Systems-based and Conventional approaches to crop nutrient management

Management	Maximum Farming System (MFSyst) Approach	Conventional (Conv) Agronomic Approach
Soil Amendments	Manage soil pH Regular gypsum use	Manage soil pH only
Tillage	Focus on no-tillage and vertical tillage to conserve soil structure	More intensive tillage using chisel and moldboard plowing
Macronutrient management	Use of starter fertilizer and banded N at planting Use of side dress N Minimize/eliminate dry box P and K additions	Emphasize preplant applications of N, P, and K
Micronutrient management	Apply with in furrow starter Apply with foliar sprays	Apply only when plant or soil test deficiency is noted

2.3. Yields

Corn yields were estimated at around 2-3 weeks pre-harvest by using the hand-harvest field method [40] with slight modifications. Corn yield was measured at each of the soil sampling points (GPS marked) by counting the number of stalks and the number of ears per plant in 5.3 m of a row. Then, five ears per 5.3 m of row were randomly collected and brought back to the lab. The corn ears were dried at 30 ºC for three days and the dry corn kernels were weighed. Subsequently, corn yield was calculated in kg ha^{-1} by considering the number of corn stalks present in 5.3 m of a row, the row width, and the weight of the corn kernels of five corn plants.

Soybean yields were estimated at 2-3 weeks pre-harvest by using the hand-harvest field method [41] with slight modifications. Soybean yield was measured at each of the soil sampling points (GPS marked) by counting the number of soybean plants in 5.3 m of a row. Then, ten soybean plants per 5.3 m row section were collected and brought back to the lab. Soybean pods were separated from the plants and the pods were dried at 30 ºC for three days and then soybean seeds were separated from the pods and weighed. Soybean yield was calculated in kg ha^{-1} by considering the number of soybean plants present in 5.3 m row section, the row width, and the weight of the soybean kernels of ten soybean plants.

2.4. Corn Nitrogen Use Efficiency

Nitrogen use efficacy for corn (NUE) was calculated using the following equation:

$$NUE = corn\ yield \left[kg\ ha^{-1} \right] / N\ fertilizer\ input \left[kg\ ha^{-1} \right]. (1)$$

2.5. Statistical Analyses

Exploration of the data generated in this study revealed that several soil test variables were not-normally distributed by treatment using the Kolmogorov-Smirnov test and extensive attempts with a variety of transformations did not produce not produce normalized data. Hence, data were analyzed using non-parametric tests. Furthermore, an imbalance in cropping occurred because some sites did not have the same crop every year for the paired treatments and therefore an ANOVA could not be performed on yield data or NUE data. After careful evaluation of all data in this study it was concluded that data should be analyzed by year and across years when feasible to determine differences in sample measurements due to the two contrasting management regimes. Medians and interquartile ranges (IQR) were calculated and Mann Whitney U-tests were used for pair-wise comparisons and Spearman's correlation analyses were conducted using Minitab (v 19) and the level of significance achieved was noted for each test conducted.

3. Results

Soils tested in this study under different management had a similar physical structure when comparing the field pairs. The data presented in Table 2 indicates that all soils tested were silt loams, silty clay loams or loams. Silt content in soils tested ranged from 40 - 69 %, and clay content varied between 14 - 32 %. Sand content is lowest in Illinois soils (7 - 11 %), varying in Iowa (9 - 26 %) and highest in Ohio (28 - 42 %). It is important to note that there were no significant textural differences within a given site when comparing the two management treatments MFSyst and Conv except for site IL-1, where silt and clay contents were noted to be marginally, but significantly different when comparing MFSyst and Conv.; cation exchange capacity (CEC) was not significantly different overall for the two management treatments MFSyst and Conv, however, MFSyst trends to show slightly higher CEC compared to Conv in 7 of the 9 pairings (Table 2).

Table 2. Texture of soils sampled from test sites in Iowa (IA), Illinois (IL), and Ohio (OH). Data on soil series, soil texture (% sand, % silt, % clay), and soil cation exchange capacity (CEC) in 0 - 15 cm soil depth are shown for adjacent fields differing in management. Values in bold are significantly higher when comparing Maximum Farming System (MFSyst) and Conventional (Conv) management at the same site ($N = 32$, $p < 0.01$)

| Site	USDA-NRCS soil series	Management	sand	silt	clay	CEC
			---------------- % -----------------			meq 100g^{-1}
IA-1	Colo silty clay loam, Klinger silt loam, Kenyon loam	MFSyst	26	53	21	20.4
		Conv	25	53	22	20.4
IA-2	Tama and Atterberry silt loam	MFSyst	9	68	23	18.5
		Conv	9	69	22	14.3
IA-3	Tama silt loam	MFSyst	10	69	21	16.2
		Conv	11	67	22	15.3
IL-1	Ipava and Osco silt loam, Sable silty clay loam	MFSyst	7	62	**32**	19.7
		Conv	8	**67**	25	18.3
IL-2	Ipava and Osco silt loam, Sable silty clay loam	MFSyst	11	64	25	21.7
		Conv	10	60	30	22.2
IL-3	Ipava and Osco silt loam, Sable silty clay loam	MFSyst	11	63	26	23.6
		Conv	10	60	30	20.5
OH-1	Crosby and Miamian-Kendallville silt loam, Kokomo silty clay loam	MFSyst	38	42	20	13.3
		Conv	28	49	23	11.8
OH-2	Crosby and Miamian-Kendallville silt loam	MFSyst	42	41	17	9.8
		Conv	42	40	18	9.5
OH-3	Crosby and Corwin silt loam, Eldean-Kendallville loam	MFSyst	37	46	17	11.3
		Conv	39	47	14	9.9

A

B
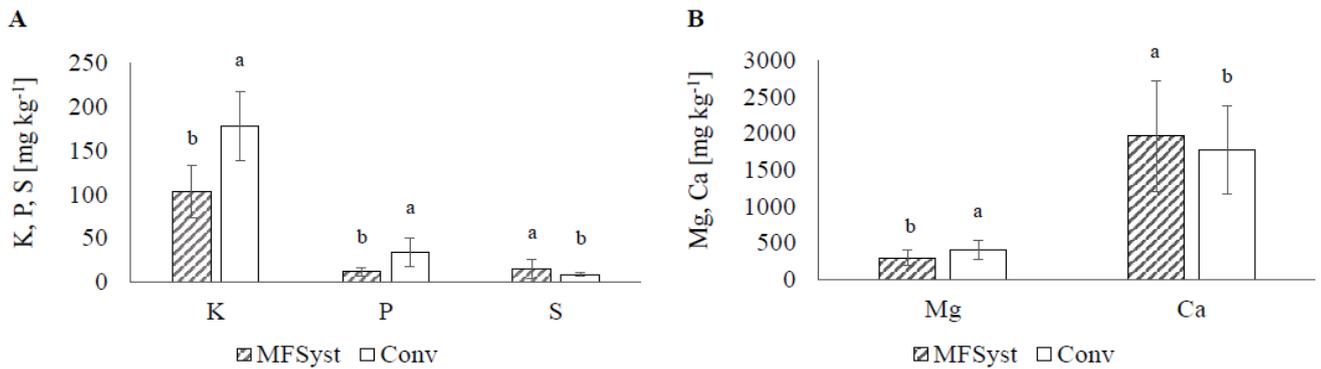
Figure 1. Soil macronutrient concentrations under Maximum Farming System (MFSyst) management and Conventional (Conv) management practices. Data displayed as medians with interquartile ranges over three states, 3 sites, and 3 years ($N = 96$). **A**: Exchangeable potassium (K), available phosphorus (P), and sulfur (S). **B**: Magnesium (Mg) and Calcium (Ca). Bars within Mg or Ca having the same letter are not significantly different at $p < 0.01$

Interesting, this trend was associated with significantly more readily extractable Ca ($p < 0.1$) and S ($p < 0.01$) as well as lower base saturations for both K ($p < 0.01$) and Mg ($p < 0.01$) in MFSyst when compared to Conv (Figure 1A and Figure 1B).

In addition, MFSyst soils had lower available P concentrations overall ($p < 0.01$) when compared to Conv soils (Figure 1A).

Soil pH presented in Table 3 indicates that most soils measured around pH 6, but MFSyst sites were slightly more acidic when compared to Conv ($p < 0.01$). Soluble salts (SS), nitrate-N (NO_3^-), iron (Fe), copper (Cu), and boron (B) were significantly higher in soils receiving MFSyst management when compared to Conv (Table 3).

ARYL and GLU activities and SOM in 0-15 cm depth were significantly ($p < 0.05$) higher in the MFSyst-managed fields as compared to Conventional (Conv) fields in all three test years (Figure 2A, 2B, and 2C). Specifically, median GLU activity was higher ($p < 0.02$) by 13% and ARYL activity was higher ($p < 0.001$) by 34%, and the median SOM content was elevated ($p < 0.01$) by 9% overall when comparing MFSyst with Conv.

Table 3. Median soil pH, soluble salts (SS), NO_3^- test values and micronutrient (Fe, Mn, Cu, B, and Zn) concentrations on fields of contrasting approaches to fertility management in 0 - 15 cm soil depth ($N = 32$ per year). Significant differences between MFSyst and Conv over three years are given as Overall p

Year	Management	pH	SS	NO_3^-	Fe	Mn	Cu	B	Zn
			mmhos cm^{-1}		---------------------- mg kg^{-1} ----------------------				
2015	MFSyst	6.0	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
	Conv	6.1	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
2016	MFSyst	5.7	0.48	71	52	16	1.6	0.3	2.0
	Conv	5.8	0.40	52	44	14	1.0	0.2	1.7
2017	MFSyst	6.0	0.28	22	51	24	1.6	0.2	1.9
	Conv	6.2	0.20	11	49	27	1.4	0.2	2.0
Overall p		**	*	**	**	*NS*	***	*	*NS*

ND Not detected, *NS* Not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
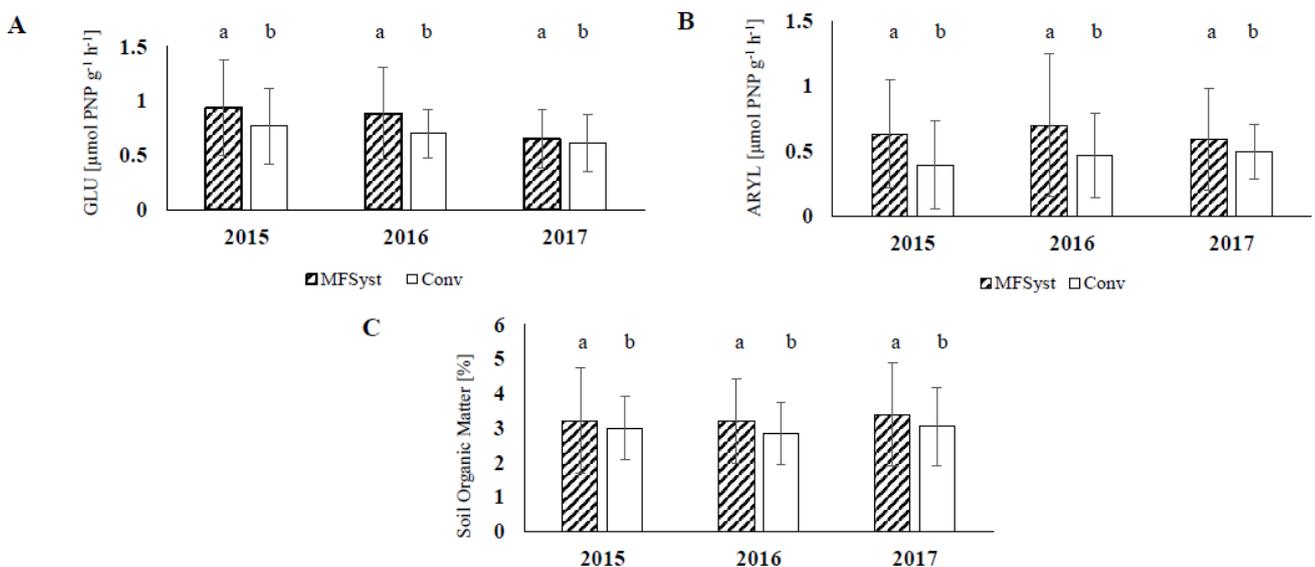
A

B

C

Figure 2. Medians and interquartile ranges of soil enzyme activities (A, B) and SOM (C) in 0-15 cm depth under maximum farming system (MFSyst) and conventional (Conv) management in years 2015 ($N = 32$), 2016 ($N = 32$), and 2017 ($N = 32$). Bars within a year having the same letter are not significantly different at $p < 0.05$
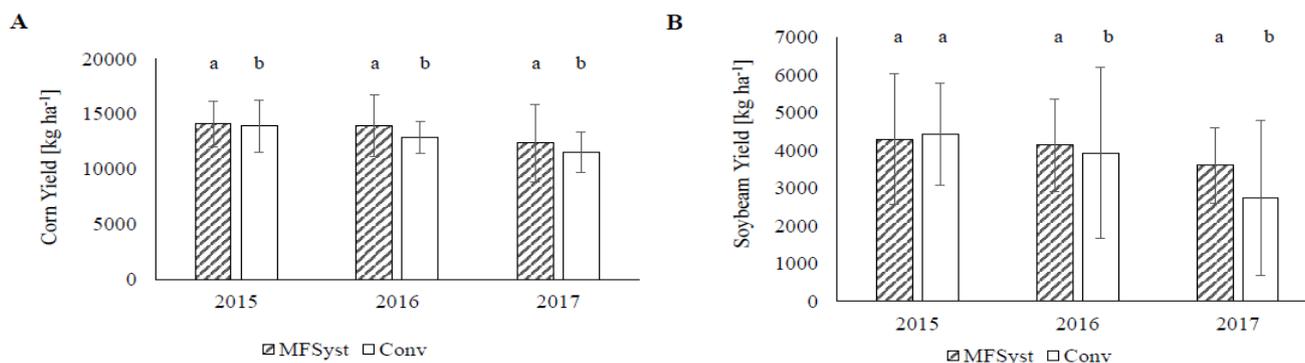
A

B

Figure 3. Medians and interquartile ranges of corn (A) and soybean (B) yields at maximum farming system (MFSyst) and conventional (Conv) management in years 2015, 2016, and 2017 (Corn yield at MFSyst $N = 54$, Conv $N = 48$; Soybean yield at MFSyst $N = 42$, Conv $N = 37$). Significant differences between MFSyst and Conv are indicated by different letters

Median corn yields of the MFSyst-managed plots were higher than those of the Conv-managed fields in all three test years, ($p < 0.08$) by about 7.2% overall (Figure 3A). In contrast to corn, median soybean yields were higher in just two of the three test years, however they trended 2.5% lower overall in MFSyst ($p = 0.48$; Figure 3B).

Correlation analysis revealed that GLU activities were significantly positively correlated with corn yield and SOM, but not to soybean yield (Table 4). ARYL activities were solely correlated to GLU and SOM, not to any yield data. SOM was not associated with corn or soybean yields (Table 4).

Table 4. Correlation coefficients (r-values) of soil β-glucosidase (GLU) and arylsulfatase (ARYL) activities [µmol PNP g^{-1}h^{-1}], soil organic matter (SOM [%]) with corn and soybean yields [kg ha^{-1}] (Corn yield N= 102, Soybean yield N = 79)

	GLU	ARYL	SOM
		Corn Fields	
ARYL	0.49**		
SOM	0.70**	0.44**	
Corn yield	0.38**	0.04*NS*	0.172*NS*
		Soybean Fields	
ARYL	0.40**		
SOM	0.69**	0.344**	
Soybean yield	0.10*NS*	0.10*NS*	-0.05*NS*

** Significant at $p < 0.01$, *NS* Not significant.

The highly significant differences in soil health measures, such as GLU, ARYL, and SOM and soil test nutrient levels noted above were reflected in differences in corn and its agronomic nitrogen use efficiency when comparing MFSyst to Conv management (Table 5).

When compared to Conv, nitrogen fertilizer input was lower at MFSyst fields (211 kg ha^{-1}), but median corn yield was higher which resulted in a higher agronomic N use efficiency ($p < 0.01$) in MFSyst-managed fields by about 18% when compared to Conv (64 *vs.* 54 kg harvested grain per kg N fertilizer applied).

Table 5. Nitrogen input, corn yield, and nitrogen use efficiency (NUE) at fields under Maximum Farming System (MFSyst) and conventional (Conv) management ($N = 15$ for MFSyst, $N = 12$ for Conv)

Management	N input [kg ha^{-1}]	Corn Yield [kg ha^{-1}]	NUE
MFSyst	211 ± 29	13,063 ± 2363	64 ± 15
Conv	233 ± 64	12,354 ± 1572	54 ± 13
p	NS	*	***

NS = not significant, * = $p < 0.1$, *** = $p < 0.01$.

4. Discussion

4.1. Soil Nutrients

Optimum soil nutrient management is critical for economic returns, reducing environmental impacts and crop productivity; and as such nutrients are an important component of soil health assessments [42,43,44]. Soil nutrient availability in agricultural systems should ideally be balanced, offering sufficient nutrients for plant nutrition and reducing the potential of nutrient loss. Optimized N and P management is of particular concern because the application of excessive fertilizer is an unnecessary expense and leads to avoidable non-point pollution.

Median corn yields were significantly higher for MFSyst over Conv by 7.2 % over the three years. This can likely be attributed to the optimized nutrient management and in particular split applications of N fertilizer. Additionally, the higher quality of soil as evidenced by increased SOM and enzyme activities could contribute the greater productivity of corn with the MFSyst management.

In the current study there was a less significant management effect on soybean yield in contrast to corn. This would suggest that corn is sensitive to soil health [45], in our study more than soybeans. A possible explanation is that corn in comparison to soybean has a higher nutrient demand and corn is a non-legume so it is more sensitive to management and thus more sensitive for optimal soil conditions. Second, nodulation of soybean might have been sub-optimal in our study since the pH in some soils tested was below 5.8 and this might have suppressed soybean nodulation which in turn might have prevented soybeans from fully profiting from atmospheric N fixation [46]. In soils with pH 5.8 - 7 soybeans are in an optimal environment for nodulation to fix atmospheric nitrogen and steadily supply N during critical growth stages.

It is well established that applications of excess N fertilizer can leach from soils and pollute groundwater [47] and P can be lost from soils by surface water runoff and pollute streams, rivers, lakes, and oceans [48]. Thus, the goal of agricultural fertilization should be a reduction of N and P fertilization while maintaining profitable yields; second, similarly micronutrient nutrition should be optimized as well [43]. After assessing soil nutrient test values (Figure 1, Table 3) and N input (Table 5), it

became clear that an overall optimized fertilization path was followed by the MFSyst-managed farming system. Minimizing and eliminating dry box P and K additions at the MFSyst-managed fields resulted in lower P and K soil test values at MFSyst- compared to the Conv system.

Regular application of micronutrients resulted in higher soil test values for Fe, Cu, and B at MFSyst-managed fields, which would be expected to reduce plant stresses and increase higher yields [43]. In addition, MFSyst-managed plots had significantly more readily extractable Ca and S in topsoil (0 - 15 cm) reflecting additions of gypsum, which has shown to be effective in stimulating soil aggregation and promoting P stabilization [49]. Such changes may lead to increased microbial activity [50]. Surprisingly, the significantly lower soil test nutrient levels for P, K, and Mg at MFSyst noted here were accompanied by higher corn yields, indicating that there was adequate and more efficient P or K utilization.

These results above that suggest greater nutrient efficiency are supported by higher NUE on corn in MFSyst-managed plots (by about 18%) when compared to Conv (64 *vs.* 54 kg harvested grain kg^{-1} fertilizer N, respectively). This was inspite of a lower average application N rate of 211 for MFSyst compared to 233 kg N ha^{-1} for Conv. The MFSyst had higher levels of soil nitrate (NO$_3^-$) in MFSyst in years 2016 and 2017. Those higher NO$_3^-$ levels could be due to split application of N and the higher SOM that could drive microbial activity and N mineralization at MFSyst fields [16,51].

4.2. Soil Enzyme Activities and SOM

There was a statistically significant increase in SOM for MFSyst over Conv. However, on a relative basis the measured differences between the two management systems was greater for soil enzyme activities than SOM. Since enzymes are largely if not entirely produced by microorganisms in soils, measuring enzyme activities provides an integrative index of the overall microbial community. Likely in this study it was the difference in tillage intensity that most affected the microbial community, and not differences in nutrient management. In particular saprophytic fungi are known to be more affected by tillage than bacteria, by disrupting hyphal networks [26].

These enzyme results are consistent with other studies on tillage [52,53,54,55] where conservation tillage increased the activity of a variety of enzymes. This can be attributed to reducing or eliminating disturbance and maintaining crop residues on the soil surface that can increase both content and quality of SOM [7], β-glucosidase has consistently been able to detect tillage effects [20] finding no-till increased activity over conventional tillage by 54.5% in a sandy clay loam soil and [21] demonstrating 19% greater activity in a silt loam. Arylsulfatase has also been sensitive for having significantly higher activities in soil managed under no-till [12,13,14].

It would be expected that higher quality soils should be more productive [56]. In the case of corn, higher yields were associated with elevated SOM and enzyme activities. There have been relatively few studies relating soil health

indicators with crop productivity. Interestingly, a study at three long-term experiments that had diverse crop management treatments found virtually no correlation of yields with the Cornel Comprehensive Assessment of Soil Health (CASH) and Haney Soil Health (HSH) indicators despite there being crop management effects on yields [4]. The CASH test integrates a number of soil chemical, physical, and biochemical measurements whereas HSH measures respiration of air-dried soils; however, none of these include enzyme assays. In contrast, our study found a significant correlation of GLU activities with corn yield, In addition, SOM was closely correlated to GLU activities which points towards stabilization and protection of enzymes by SOM [24].

This shows the potential for enzyme activities to be a precursor for soil organic matter accumulation. Indeed, previous research [19] showed that enzyme activities could detect cover cropping effects within 1 to 3 years but at the same time found no detectable changes in SOM. This is consistent with another study where GLU and ARYL activities detected management effects but none due to SOM [6]. In that regard our current study would indicate that GLU activity reflects the potential for SOM accumulation as it correlated closely with SOM but was more highly correlated with corn yield than SOM and on a relative basis had a greater increase in activity than SOM. Since there were no significant treatment effects on soybean yields it would follow that no there was no significant correlation of enzyme activities with soybean yields.

5. Conclusions

The optimized crop nutrient management combined with no- or reduced-tillage of the MFSyst resulted in elevated soil enzyme activities that would suggest greater soil health over a more tillage-intensive conventional (Conv) management approach. This was supported by greater SOM with the elimination or reduction of tillage with MFSyst. Several of the differences between MFSyst and Conv observed in soil chemical properties were expected given the contrasting nature of systems-focused and conventional management fertilization regimes. For example, the application of gypsum at MFSyst is likely to have led to the noted increases in soil Ca and S concentrations and the repeated application of micronutrients could have contributed to the differences in micronutrient concentrations such as Zn, Cu, and B. Perhaps most significantly, the decreased reliance on supplemental P and K in MFSyst-managed fields is noteworthy, which resulted in and 100% higher P and 50% higher K test values in adjacent conventionally managed fields. Yields did not apparently suffer at the MFSyst-managed fields, in fact corn yield and corn NUE were significantly higher at MFSyst.

Corn yields and SOM were correlated with higher GLU activities which indicated that GLU activity was the most suitable integrative indicator for soil health. Overall, it can be concluded that optimized soil and nutrient management can have positive effects on soil health that was related to corn yield.

Acknowledgements

Abbreviations

ARYL, arylsulfatase activity; Conv, Conventional management; GLU, β-glucosidase activity; MFSyst, Maximun Farming System; NUE, nitrogen use efficiency; SOM, soil organic matter

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