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Supplementary Information for

Persistent soil carbon enhanced in Mollisols by well-managed grasslands but not annual grain or dairy forage cropping systems

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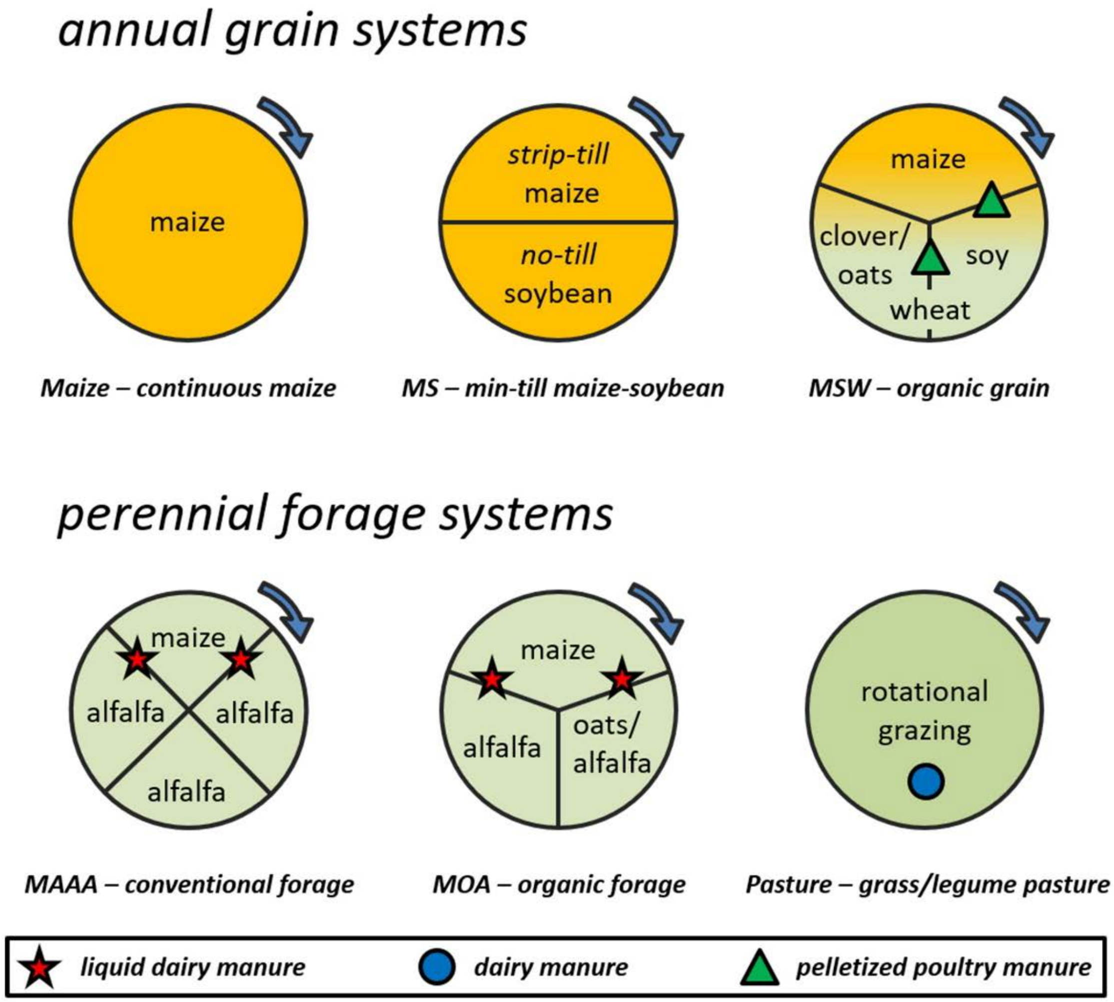

Fig. S1. The six cropping systems at the Wisconsin Integrated Cropping Systems Trial (WICST). These systems are broadly representative of the major agricultural land use on the Mollisols of the Midwestern US. The three annual grain systems include continuous maize (Maize), a maize-soybean rotation (MS), and an organically managed maize-soybean-wheat (MSW) rotation; the perennial forage systems include a maize-alfalfa-alfalfa-alfalfa rotation (MAAA), and organically managed maize-oats/alfalfa-alfalfa rotation (MOA), and a well-managed grazed pasture (Pasture).

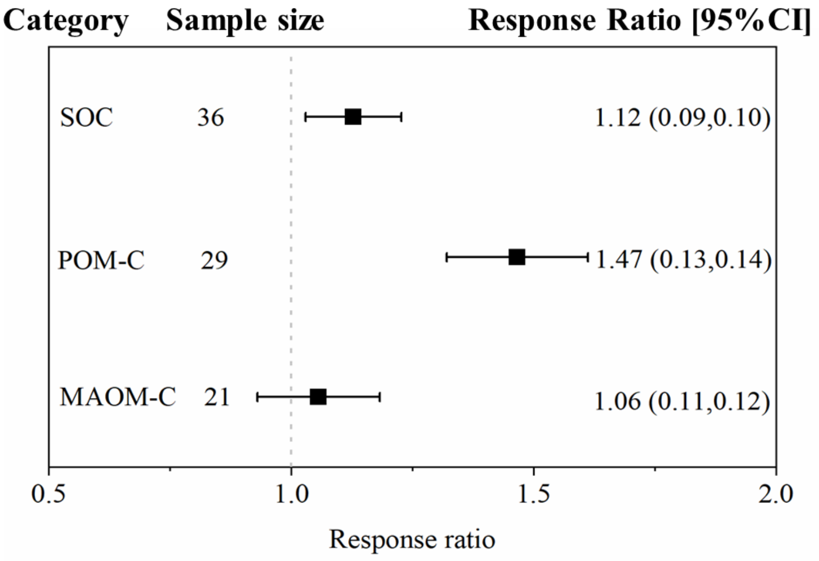

Fig. S2. Effects of alternative agricultural practices versus conventional practices on soil organic carbon (SOC), particulate organic matter-carbon (POM-C) and mineral-associated organic matter-carbon (MAOM-C) based on a meta-analysis. We reviewed and extracted data from 17 published studies in world's Mollisols. Comparisons of practices in these studies included no-till vs. conventional tillage, diversify crop rotation with legumes vs. monoculture, manure vs. synthetic nutrient additions, cover crops vs. no cover crops, or combinations of these practices. The length of experiments ranged from 4 to 60 years. Effect sizes of response ratio are mean and 95% confidence interval.

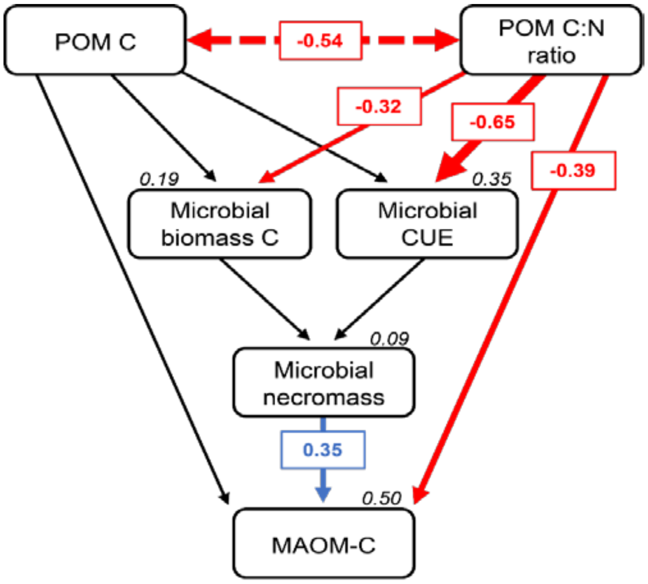

Fig. S3. Piecewise structural equation modeling (SEM) diagram showing the relationships among particulate organic matter (POM), the soil microbial traits, and mineral-associated organic matter (MAOM). Large boxes indicate variable names. Solid arrows indicate direct effects between variables. The dashed double-headed arrows represent correlations. Blue indicates a significant positive effect, red negative, and black indicates a non-significant effect. Values over arrows indicate the strength of standardized path coefficients or correlation coefficient. Values above boxes in italics indicate the R^2 for endogenous variables.

Table S1. **Estimated carbon inputs, N fertilizer application, and tillage intensity of systems at Wisconsin Integrated Cropping Systems Trial (WICST).** Maize: continuous maize, MS: maize-soybean with minimum tillage, MSW: organic maize-soybean-winter wheat, MAAA: maize followed by three years of alfalfa, MOA: organic maize-oat/alfalfa-alfalfa, and Pasture: well-managed grazed cool-season pasture. Numbers are averaged over crop rotation phases of each of the systems.

	Estimated annual C input (kg C ha^{-1})				**Mineral fertilizer N (Kg N ha^{-1})**	**Tillage score[2]**	**Relative tillage intensity**
	Aboveground plant input	**Belowground plant input**	**Manure input**	**Total**			
Maize	4154	2448	0	6601	162	3.00	0.53
MS	3185	1814	0	4999	74	0.15	0.03
MSW	2323	1290	243	3857	0	5.67	1.00
MAAA	1807	5465	1144	8416	2	1.75	0.31
MOA	1964	5071	1294	8329	0	3.67	0.65
Pasture	620	11369	904	12894	32	0.00	0.00

[1] Carbon input estimates based on annual yield and green manure data coupled with harvest index and root shoot coefficients from Allmaras et al. 2004 (1), Johnson et al. 2006 (2), and Bolinder et al. 2007 (3).

[2] System tillage score based on number of tillage passes per cropping system phase averaged across all phases.

Table S2. Soil organic carbon (C) and nitrogen (N) content, and C:N ratio of bulk soil, particulate organic matter (POM) and mineral associated organic matter (MAOM) by management treatment for the 0-30cm depth, and across management treatments for the 0-15 and 15-30 cm depths in the Wisconsin Integrated Cropping Systems Trial (WICST). Different letters indicate significant differences at $\alpha = 0.05$ among management treatments, or depths.

	Bulk Soil			**POM**			**MAOM**		
	C (mg g^{-1} soil)	**N (mg g^{-1} soil)**	**C:N ratio**	**C (mg g^{-1} soil)**	**N (mg g^{-1} soil)**	**C:N ratio**	**C (mg g^{-1} soil)**	**N (mg g^{-1} soil)**	**C:N ratio**
Management treatment									
Maize	21.3 b	1.78 c	11.8 a	2.62 b	0.16 c	17.4 a	17.4 b	1.62 c	10.7 a
MS	21.3 bc	1.81 c	11.7 ab	2.54 b	0.15 c	17.1 a	16.8 b	1.60 c	10.5 ab
MSW	20.1 c	1.82 c	11.0 c	2.94 ab	0.19 bc	15.7 b	17.0 b	1.67 bc	10.2 c
MAAA	22.5 bc	2.01 bc	11.2 bc	2.90 ab	0.21 ab	14.1 c	19.2 b	1.88 b	10.2 bc
MOA	23.7 ab	2.15 b	10.9 c	3.31 a	0.23 ab	15.0 bc	17.8 b	1.80 bc	9.8 d
Pasture	29.0 a	2.46 a	11.7 ab	3.48 a	0.25 a	14.7 c	23.5 a	2.27 a	10.4 bc
Depth									
0-15cm	26.6 a	2.28 a	11.7 a	4.26 a	0.29 a	16.3 a	20.1 a	1.98 a	10.1 b
15-30cm	19.4 b	1.73 b	11.1 b	1.68 b	0.11 b	15.0 b	17.2 b	1.64 b	10.5 a
***P*-value**									
Treatment (T)	<0.001	<0.001	0.009	0.024	<0.001	<0.001	<0.001	<0.001	<0.001
Depth (D)	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	0.001	<0.001	0.002
T × D	0.985	0.991	0.354	0.639	0.257	0.869	0.376	0.309	0.41

Table S3. The responses of soil organic carbon (SOC), particulate organic matter-carbon (POM-C), or mineral-associated organic matter-carbon (MAOM-C) to alternative practices on the world's Mollisols reported by 17 publications. "v"means significant treatment effects compared with control (conventional practice); "ns" means no significant difference between treatment and control. Bold values indicated data reported as Mg C ha^{-1}.

Study #	Country	Crop	Duration of trial (yrs)	Treatments	Soil depth (cm)	Treatment effects			SOC (%)		POM-C (%)		MAOM-C (%)		Reference
						SOC	POM-C	MAOM-C	Control	Alternative	Control	Alternative	Control	Alternative	
1	Argentina	Sunflower-wheat-maize-soybean	22	No-till vs. Till	0-10	√	√	ns	1.92	2.61	0.19	0.30	0.90	0.92	(4)
					10-20	ns	ns	ns	1.68	1.61	0.13	0.14	0.93	1.06	
2	Uruguay	Continuous cropping	10	No-till vs. Till	0-6	ns	√	ns	2.20	2.30	**1.92**	**2.42**	**14.5**	**15.0**	(5, 6)
					6-12	ns	ns	ns	2.00	1.90	**0.82**	**0.60**	**14.5**	**13.8**	
					12-18	ns	ns	ns	1.70	1.80	**0.38**	**0.48**	**12.8**	**13.0**	
3	Argentina	Maize-soybean-wheat	15	No-till vs. Till	0-5	ns	ns	ns	3.30	3.56	0.30	0.54	2.90	3.04	(7)
					5-20	√	ns	ns	3.02	3.29	0.29	0.49	2.74	2.80	
4	Argentina	Maize-sunflower-wheat	4	No-till vs. Till	0-5	ns	√	ns	3.29	3.47	0.61	0.82	2.65	2.69	(8)
					5-20	ns	ns	ns	3.15	3.08	0.52	0.59	2.59	2.62	
5	Argentina	Wheat-soybean-wheat-maize-maize-sunflower	4	No-till vs. Till	0-7.5	√	√	√	2.35	2.89	0.19	0.39	2.16	2.50	(9)
					7.5-15	ns	√	ns	2.39	2.50	0.22	0.15	2.17	2.35	
6	Argentina	Maize-soybean-wheat	20	No-till vs. Till	0-5	√	√	ns	**11.9**	**15.4**	**1.30**	**6.20**	**10.6**	**9.20**	(10)
					5-20	ns	ns	ns	**46.5**	**47.2**	**6.80**	**8.60**	**39.7**	**38.6**	
7	United States	Maize-soybean	24	Diversify rotation with legumes; Organic vs. conventional	0-25		√				0.25	0.35			(11)
8	United States	Maize-soybean	8	Diversify rotation with legumes	0-10	ns	ns		**28.3**	**27.0**	**2.91**	**2.35**			(12)
					10-20	ns	√		**31.0**	**31.6**	**0.89**	**2.03**			
9	United States	Maize-soybean	10	Diversify rotation with legumes	0-20	ns	ns		2.65	2.69	0.22	0.26	2.43	2.43	(13)
10	United States	Maize-soybean	22	Diversify rotation with legumes	0-15	ns	√		3.28	3.85	0.42	0.52			(14)
11	United States	Site 1 Maize-soybean	60		0-15	√	√	ns	**45.4**	**52.7**					(15)

						15-30	ns	ns	ns	**40.3**	**47.1**					
		Site 2	Maize-soybean	35	Diversify rotation with legumes	0-15	ns	ns	ns	**31.9**	**36.3**					
						15-30	ns	ns	ns	**27.6**	**29.0**					
		Site 3	Maize-soybean	12		0-15	ns	ns	ns	**47.7**	**43.8**					
						15-30	ns	ns	ns	**42.7**	**40.6**					
12	Argentina	Continuous soybean		8	Oat CC vs. No CC	0-5	√	√	ns	2.35	2.79	0.59	0.90	1.76	1.89	(16)
						5-10	ns	ns	ns	2.23	2.29					
						10-20	ns	ns	ns	2.07	2.01					
13	China	Maize-soybean		14	Manure vs. NPK	0-20	√	√	ns	2.77	3.40	0.26	0.52	2.51	2.88	(17)
14	India	Rice-Wheat		42	NPK+Manure vs.NPK	0-15	√	√		1.81	2.68	0.41	0.75			(18)
						15-30	√	√		1.14	1.56	0.25	0.45			
15	China	Maize		21	NPK+Manure vs.NPK	0-10	√	√		1.50	2.36	0.28	0.68			(19)
						10-20	√	√		1.32	2.06	0.22	0.48			
16	China	Maize		24	Natural fallow vs. Continuous cropping	0-20	√	√	ns	2.82	3.21	1.03	1.21	1.79	2.00	(20)
17	Argentina	Site 1	Maize-soybean	13	Cover crop, diverse rotation, minimal use of agrochemicals vs. no CC, monoculture, heavy use of chemicals	0-20	√	√	ns	1.05	1.29	0.32	0.49	0.73	0.80	(21)
		Site 2		28		0-20	√	√	ns	1.57	1.79	0.28	0.40	1.29	1.39	
		Site 3		6		0-20	N	N	ns	1.46	1.54	0.27	0.30	1.19	1.24	
		Site 4		13		0-20	√	ns	√	2.15	3.00	0.29	0.43	1.86	2.57	

Table S4. Microbial biomass C (MBC), microbial C-use efficiency (CUE), concentrations of amino sugars including glucosamine (GluN), Muramic Acid (MurA), Galactosamine (GalN), and Mannose (ManM) and the total of the above four amino sugars, and the ratio of Glucosamine/Muramic Acid. Different letters show significant differences at $\alpha = 0.05$.

	MBC (mg kg^{-1})	**Microbial CUE**	**GluN (μg g^{-1})**	**MurA (μg g^{-1})**	**GalN (μg g^{-1})**	**ManM (μg g^{-1})**	**Total amino sugars (μg g^{-1})**	**GluN: MurA**
Management Treatment								
Maize	321 c	0.20 d	767	95.8	258 b	19.8 bc	1140 b	7.99 c
MS	309 c	0.20 d	882	86.6	316 ab	23.8 bc	1308 ab	9.99 ab
MSW	591 b	0.22 cd	793	77.2	257 b	19.0 c	1146 b	10.3 a
MAAA	543 b	0.36 b	964	110	332 ab	26.9 b	1433 ab	8.93 bc
MOA	842 a	0.27 c	910	90.1	310 b	26.8 b	1336 ab	10.3 a
Pasture	642 b	0.45 a	1095	100	396 a	40.8 a	1632 a	10.9 a
Depth								
0-15cm	577	0.29	909	95.7	334	23.1 b	1362	9.43
15-30cm	505	0.27	894	91.1	289	29.2 a	1303	10.0
***P*-value**								
Treatment (T)	<0.001	<0.001	0.071	0.084	0.020	<0.001	0.05	<0.001
Depth (D)	0.115	0.430	0.818	0.465	0.069	0.006	0.545	0.069
T × D	0.011	0.071	0.045	0.085	0.092	0.001	0.05	0.018

Table S5. Pearson correlation coefficients of measured variables. Variables include particulate organic matter-carbon (POM-C), C:N ratio of POM, mineral-associated organic matter-carbon (MAOM-C), microbial C-use efficiency (CUE), total amino sugars (AS) content, relative proportion of aliphatic and aromatic C functional groups contained in MAOM, and activity of polyphenol oxidase.

Variables	POM-C	POM C:N ratio	MAOM-C	MBC	CUE	Total AS	GluN:MurA	Aliphatic C	Aromatic C	PPO
POM-C	1									
POM C:N ratio	-0.54***	1								
MAOM-C	0.48***	-0.59***	1							
MBC	0.34*	-0.41**	0.25	1						
CUE	0.23	-0.58***	0.48***	0.24	1					
Total AS	0.25	-0.29*	0.51***	0.17	0.28	1				
GluN:MurA	-0.03	-0.09	0.00	0.24	0.15	0.34*	1			
Aliphatic C	0.62***	-0.64***	0.94***	0.35*	0.44**	0.47**	0.02	1		
Aromatic C	-0.72***	0.68***	-0.88***	-0.39**	-0.41**	-0.47**	-0.01	-0.98***	1	
PPO	0.55***	-0.21	0.11	0.57***	-0.15	0.00	0.09	0.25	-0.33	1

*$p<0.05$, **$p<0.01$, ***$p<0.001$.

Table S6. C functional groups of mineral associated organic matter (MAOM) at 0-15 and 15-30 cm depths in the Wisconsin Integrated Cropping Systems Trial (WICST). Aliphatic, aromatic, and C-O functional groups were determined from relative peak areas using diffuse reflectance infrared Fourier transform spectroscopy in the mid–infrared region (mid–DRIFTS). Different letters suggest significant differences at α= 0.05.

	Aliphatic C-H (2930 cm^{-1})	**Aromatic C=C (1620 cm^{-1})**	**Aromatic C=C (1530 cm^{-1})**	**C-O (1159 cm^{-1})**
Management Treatment				
Maize	24.7 bc	55.0 ab	11.9 a	8.43 ab
MS	23.2 c	55.7 a	12.3 a	8.83 a
MSW	24.9 bc	54.8 ab	11.6 a	8.68 a
MAAA	26.9 b	53.1 b	11.3 a	8.63 a
MOA	26.0 bc	53.3 b	12.1 a	8.56 a
Pasture	31.9 a	50.4 c	9.62 b	8.05 b
Depth				
0-15cm	28.7 a	51.6 b	11.4	8.23 b
15-30cm	23.8 b	55.8 a	11.6	8.83 a
***P*-value**				
Treatment (T)	<0.001	<0.001	0.002	0.033
Depth (D)	<0.001	<0.001	0.647	<0.001
T × D	0.270	0.216	0.020	0.092

Table S7. The activities of oxidative enzymes including polyphenol oxidase (PPO) and peroxidase (PER) and hydrolytic enzymes including α-glucosidase (AG), β-glucosidase (BG), β-cellobiohydrolase (CBH), and N-acetylglucosaminidase (NAG). Different letters show significant differences at α = 0.05.

	PPO (μmol h^{-1} g^{-1})	**PER (μmol h^{-1} g^{-1})**	**AG (μmol h^{-1} g^{-1})**	**BG (μmol h^{-1} g^{-1})**	**CBH (μmol h^{-1} g^{-1})**	**NAG (μmol h^{-1} g^{-1})**
Management Treatment						
Maize	1.98 b	4.09 b	5.33 c	272 ab	82 ab	84 ab
MS	1.92 b	4.47 b	7.56 c	232 ab	68 b	60 bc
MSW	3.65 a	3.69 b	17.3 b	292 a	78 ab	80 ab
MAAA	1.34 b	4.56 b	5.41 c	192 b	65 b	41 c
MOA	5.16 a	7.69 a	25.4 a	330 a	96 a	103 a
Pasture	1.74 b	3.55 b	8.34 c	259 ab	79 ab	71 abc
Depth						
0-15cm	3.85 a	4.90	16.7 a	329 a	106 a	76
15-30cm	1.41 b	4.45	6.45 b	196 b	50 b	70
***P*-value**						
Treatment (T)	<0.001	0.007	<0.001	0.117	0.234	0.015
Depth (D)	<0.001	0.487	<0.001	<0.001	<0.001	0.523
T × D	0.008	0.815	0.223	0.804	0.400	0.479

Table S8. **Cropping system details between 1993 and 2018 at the Wisconsin Integrated Cropping Systems Trial (WICST), Arlington, WI, USA.**

System code [1]	Crop species	Crop phase	Dry matter yield (Mg ha^{-1}) [2]	Tillage equipment [3]	Tillage passes per year	Annual N-P-K inputs [4] (kg ha^{-1})	Input Source [5]
Maize	1	maize	9.8	CP, FC	3	160-8-35	F
MS	2	maize	10.1	ST	0.3	148-11-42	F
		soybean	3.3	NT	0	1-2-22	
MSW	5	maize	8.0	CP, FC, RH/TW	7	0-0-9 / 138-51-66 [6]	F, CPM
		soybean	2.9	CP, FC, RH/TW	6	0-0-4 / 0-0-55	
		wheat / cover crop [7]	3.6 (2.5)	CP, FC	3	0-0-0 / 55-19-63	
MAAA	2	maize	11.0	CP, FC	3	88-31-160	F, M1
		alfalfa	5.8	CP, FC	3	100-29-155	
		alfalfa	11.4	--	0	0-1-96	
		alfalfa	10.1	--	0	0-0-108	
MOA	3	maize	9.0	CP	7	71-20-121	F, M1
		oat-alfalfa	8.5	CP, FC	3	85-24-135	
		alfalfa	11.4	--	0	0-0-128	
Pasture	5+	pasture	8.4	--	0	32-1-30	F, M2

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[1] Maize: continuous maize, MS: maize-soybean with minimum tillage, MSW: organic maize-soybean-winter wheat, MAAA: maize followed by three years of alfalfa, MOA: organic maize-oat/alfalfa-alfalfa, Pasture: rotationally grazed cool season pasture.

[2] Both grain and straw yields (in parentheses) are reported for wheat.

[3] CP = chisel plow, FC = field cultivator, NT=no-till, RC=row cultivator, RH/TW = rotary hoe or tine-weeder, ST = strip-tillage.

[4] First year availability accounts only for the nutrients released to a growing crop during the same year it is applied. Manure and other organic forms of nutrients contain more total nutrients than are available to the crop in any given year. Legume N credits are not included.

[5] F = fertilizer (conventional or organic according to system management); CPM = composted poultry manure; M1 = applied manure; M2 = manure deposited by grazing livestock.

[6] Between 1993 and 2007 all nutrients for M-S-W were provided by organically approved fertilizers (e.g. 0-0-50) or N fixed by the green manure crop. Starting in 2008 composted pelletized poultry manure was added to the maize and wheat phases of the rotation to supply N, P, K, and micronutrients.

[7] Between 1993 and 2005 the cover crop was red clover (*Trifolium pratense* L.) frost seeded or drilled into winter wheat in early spring; beginning in 2006 this changed to a berseem clover (*Trifolium alexandrinum* L.)/oat (*Avena sativa* L.) mixture planted after wheat harvest.

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