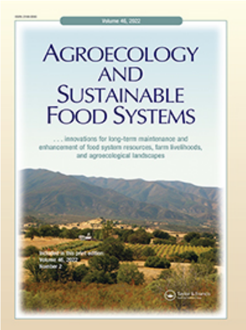

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Comprehensive impacts of diversified cropping on soil health and sustainability

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ABSTRACT

Conventional agriculture in the Midwest US lacks diversity, relies heavily on external inputs to maintain crop yields, and contributes to soil and water quality degradation. Using diverse crop rotations and incorporating livestock are promising solutions to these and other problems linked to current cropping systems dominated by maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.). To better understand how agricultural diversification comprehensively affects soil health and function, we compared 20 soil health parameters linked to critical soil ecosystem services in 1) a conventional 2-year maize-soybean rotation, and 2) a diverse 4-year maize-soybean-oat (*Avena sativa* L.)+alfalfa (*Medicago sativa* L.)-alfalfa rotation that periodically received cattle manure. The strongest and most salient improvements in soil health from the diversified, 4-year cropping system included: 8% reduction in soil resistance to root growth ($p = .006$), 16% increase in cation exchange capacity ($p = .001$), 157% increase in salt-extractable soil carbon ($p = .024$), and 62% increase in soil microbial biomass ($p = .017$). These comprehensive improvements in general soil functioning coincided with enhanced crop yields, reduced requirement for agricultural inputs, and decreased environmental impacts – all while maintaining profitability. Despite declines in cropping system diversity globally, but especially in the Midwest US, these results provide strong evidence for the benefits of diversification.

KEYWORDS

Animal manure; climate resilience; crop diversity; multi-functionality; regenerative agriculture; soil quality

Introduction

As agriculture has modernized and industrialized, it has also become more simplified and less diverse. Prior to the 1950s, Midwest US farms were smaller, regularly included small grains and perennial forages for livestock production, and were less specialized in cash grain production (Hatfield, McMullen, and Jones 2009; MacDonald and McBride 2009). Today, this region specializes almost

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exclusively in maize (*Zea mays L.*) and soybean (*Glycine max* (L.) Merr.), and contributes to over one-third of global maize and soybean production (FAO 2015; Ort and Long 2014). Maize and soybean are intensively grown on 80 million ha (10% of the contiguous US; Green et al. 2018; NASS 2016). This region accounts for 41% of annual US agricultural receipts (USDA ERS (United States Department of Agriculture, E.R.S 2018), and contributes to 8% of US agriculture's global warming potential (Hockstad and Hanel 2018). Negative environmental impacts associated with intensive maize-soybean production include: soil erosion rates that have far outpaced soil generation (Montgomery 2007), 18–60% declines in soil organic matter (De et al. 2020; Guo and Gifford 2002), and impaired water quality at both local and continental scales (Rabalais, Turner, and Wiseman 2001; Sharpley 1999). Thus, understanding the impact of this cropping system on soil and ecosystem services is of national and global importance (Liu et al. 2012).

Thousands of years prior to agricultural intensification, diverse crop rotations and livestock integration were commonplace practices used to maintain crop productivity and enhance soil health (Karlen et al. 1994; Smith and Nesbitt 1995). Currently referred to as regenerative farming (Pearson 2007; Sherwood and Uphoff 2000), these practices are now marginally used in agriculture, and have been replaced with synthetic fertilizers and pesticides that focus on 'plant nutrition' and 'crop protection,' rather than soil ecosystem services (i.e. soil health). Modern conventional agriculture has created a path dependency for farmers (Roesch-mcnally, Arbuckle, and Tyndall 2018) and is perpetuated by the notion that simplified, conventional systems are the most productive and profitable (Lin 2011). However, crop yields from diversified rotations are typically higher than those in mono- or bi-cultural rotations, a phenomenon called 'the rotation effect' (Bowles et al. 2020; Stanger and Lauer 2008). Additionally, economic analyses show diversified rotations can be as profitable as conventional systems due in part to the 'rotation effect,' but also from reductions in input cost (Davis et al. 2012; Hunt, Hill, and Liebman 2017; Poffenbarger et al. 2017).

The 'rotation effect' is not well understood but may be linked to improved soil health resulting from cropping system diversification (Karlen et al. 2006). Soil health is defined here as "the capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health" (Doran 1996). If enhanced soil health is responsible for enhanced provision of food, fiber, and fuel, then it may also confer additional soil ecosystem services (SES) including enhanced carbon (C) sequestration, water purification, nutrient cycling, habitat, and resource provision, and flood regulation (FAO 2015) . Using practices that enhance these SESs is critical to the future of farming, environmental quality, and human well-being.

To understand the effects of simple and more diverse cropping systems on soil health and , we leveraged new and preexisting data from a 15-year agroecosystem experiment in Iowa, US. This experiment compares a conventional 2-year to a diversified 4-year cropping system. Our primary objective was to evaluate the comprehensive impact of cropping system diversification on soil health. Secondarily, we contextualize our results by summarizing and synthesizing prior findings from this long-term experiment to identify broad-scale implications of diversified agroecosystems on agronomic and environmental performance (or sustainability).

Materials & methods

Experimental site and management

The Marsden Farm Experiment (MFE) is located near Boone, IA, USA (42.013628 °N, −93.784149 °W). Established in 2002, the MFE is a randomized complete block design of three cropping system treatments. Here, we compare two of the treatments: 1) maize-soybean rotation with synthetic N fertilizer only (2-year cropping system or 2YCS) and 2) maize-soybean-oat (*Avena sativa* L.) + alfalfa (*Medicago sativa* L.)-alfalfa rotation primarily receiving composted cattle manure with reduced rates of synthetic N (4-year cropping system or 4YCS). The MFE is a randomized complete block design experiment in which each rotation has all crop phases represented each year in four replicated blocks and each plot is 18 × 84 m. Fifty-year mean (± standard deviation) annual temperature and precipitation is 8.7 ± 0.9°C and 956 ± 211 mm, respectively. Soils at the site are classified under the USDA system as an order of Mollisols and range from 0% to 5% slopes. Further soil and climate information are provided in Table S1, and detailed management descriptions beyond those given below can be found in Liebman et al. (2008), Davis et al. (2012), and Hunt, Hill, and Liebman (2017).

The N fertilizer rates for all treatments were guided by local late spring soil nitrate or pre-sidedress nitrate tests (Blackmer et al. 1989). From 2003 to 2017, the 2YCS received total annual rates of 169 ± 36 kg N ha^{-1} as broadcast urea ammonium nitrate (UAN) in the maize year only. This UAN rate was split applied with 3/4 applied in early-to-mid May and remaining UAN was side-dressed in June when maize was V5/V6. The 4YCS received most of its nutrients from composted cattle manure (83 ± 19% of N) that was applied once during the 4YCS in the November preceding maize (Davis et al. 2012). The remainder of the N for the 4YCS was applied in June when maize was at V5/V6. Other essential plant nutrients were added according to local soil fertility test recommendations. For instance, on 11 April 2017 – the year of this study, all crops (except alfalfa) received 37 kg P, 83 kg K, and 28 kg S ha^{-1}. Alfalfa received 54 kg P, 222 kg K, and 28 kg S ha^{-1}.

Tillage differed between the two cropping systems (Liebman et al. 2008). The 2YCS was spring cultivated before both maize and soybean and chisel plowed in fall after maize (33 cm deep). The 4YCS was fall chisel plowed and spring cultivated between maize harvest and soybean planting, was no-till or spring disked between soybean and oats, moldboard plowed (23 cm deep) to terminate alfalfa and incorporate manure in the fall before maize, and was sometimes spring disked before the oat+alfalfa planting.

Soil sampling, lysimeter sampling, and analyses

All soils were collected from the maize phase of both rotations from the MFE in 2017 between Apr. and Nov. Soils were stored in coolers with icepacks, transported to a lab, and refrigerated until processing. Analyses were performed either eight times (monthly over the growing season), once during the growing season as part of a long-term monitoring effort, or three times (seasonally) as part of a sampling campaign targeted at crop diversity effects on rhizosphere soil nutrient dynamics. See Table 1 for abbreviations, sample numbers and methodological details for each soil health indicator. We selected these indicators because of either their direct or indirect links to SESs (Table 2, FAO 2015).

Monthly soil collections occurred on 27 Apr., 12 May, 6 Jun., 7 Jul., 8 Aug., 20 Sep., 8 Oct., and 5 Nov. These samples consisted of five randomly located soil cores in each of four experimental replicates, at 0–15 and 15–30 cm depths, with replicate subsamples at each depth within plots homogenized. GWC, SEOC and SEON, NO_3^-, MBC, MBN were all determined monthly. Seasonal soil samples were collected at three proximities from the maize root due to impact of growing crop and rhizodeposits on soil microbial activity at the rhizosphere, in the row, and in the interrow on 20 Jun., 14 Jul., and 20 Sept. for biological activity (BGase, and CO_2).

PAW, WSA, pH, STK, STP, CEC, TN, and SOC were each determined once during the season using soils from various monthly sampling dates collected at 0–15 and 15–30 cm depths. Soil collected 7 Jul. and 8 Aug. was used for PAW analysis using 2 mm sieved and dried soil. Soil collected on 12 May and 8 Oct. was 8 mm sieved, dried, and composited for WSA analysis. Soil collected on 20 Sept. was used to measure pH. Soil collected on 5 Nov. was used for STK, STP, CEC, TN, and SOC analyses.

BD, mesofauna, hardness, and earthworms were all measured once during the season. BD was measured on 7 Jun. at three depths (0–5, 15–20, and 25–30 cm) using a 4 cm diameter by 5 cm deep stainless steel, beveled metal cylinder. Mesofauna samples were taken on 7 Jul. using a modified Berlese funnel approach (Hessel 2014). Briefly, seven intact cylindrical cores were taken from each experimental replicate and inserted into a Berlese funnel apparatus consisting of a single greenhouse light creating a temperature and moisture gradient to drive the mobile mesofauna out through the bottom of the soil core

Table 1. Abbreviation, description and method, sample size (n), and method reference(s) for soil health indicators.

Category	Indicator	Abbreviation	Description and Method	n	Method Reference
Physical					
	Water-stable aggregates	WSA	Ability of aggregates to resist disruption from wetting. 100 g of 2–8 mm sieved, air dried soil was placed atop a series of sieve sizes: 4, 2, 1, 0.5, 0.25 mm. Sieves were lowered and raised 6 cm in water using a mechanical device at 80 rpm for 5 minutes. Mean weighted diameter (mm) reported.	8	Nimmo and Perkins (2002)
	Hardness	hardness	Ease of root growth and nutrient foraging, infiltration. Used a FieldScout SC900 Compaction Meter (Spectrum Technologies, Aurora, IL) affixed with a 1.27 cm cone tip, at 2.5 cm intervals to a depth of 43.2 cm at random locations.	8	Lowery and Morrison (2002)
	Gravimetric water content	GWC	*In situ* soil water supply. Moisture in soil after oven drying 105°C for 24 hr.	64	Topp and Ferre (2002)
	Bulk density	BD	Mass of soil per unit volume. Core method using 250 mL ring with a height of 5 cm.	8	Grossman and Reinsch (2002)
	Plant-available water	PAW	Potential plant available water held by the soil. Soil at equilibrium with pressure plates at field capacity and permanent wilting point (−33kPa and 1500kPa), and weighed before and after oven drying at 105°C for 24 hr.	8	Dane and Hopmans (2002)
Chemical					
	Salt Extractable Organic Carbon	SEOC	Labile, extractable C, microbial energy resource. Fresh soil sieved at 2 mm, extracted with 25 mL of 0.5 M K_2SO_4, shaken for 1 hour, let to settle for 30 minutes, and filtered through an 11 µm filter. Sample extractions stored in the freezer at −20°C until analysis on the Shimadzu TOC-L/TNM-1 Analyzer.	64	Chan and Heenan (1999)
	Salt Extractable Organic NItrogen	SEON	Labile, extractable N – easily mineralized by microbes. Same method as SEOC but combusted as NO_2 on TNM-1 unit.	64	NA
	Soil Organic Carbon	SOC	Quantity of C in organic matter (not $CaCO_3$). Soil sieved at 2 mm, air dried, ball milled, and oven dried at 105°C for two days. Soil was combined with equal or larger parts tungsten oxide catalyst and combusted in the presence of oxygen in an Elementar vario MACRO.	8	Nelson and Sommers (1996)
	Total nitrogen	TN	Quantity of N in organic matter. Same method as Total C.	8	Bremner (1996)
	Cation exchange capacity	CEC	Soil storage capacity of essential cations. Ammonium acetate replacement of cations for K+, Ca_2^+, Mg_2^+, and Na^+ at pH 7 using inductively coupled plasma mass spectrometry.	8	Sumner and Miller (1996)
	Nitrate	NO_3^-	N form most available to plants, and susceptible to leaching. Field-moist soil sieved at 2 mm, extracted with a 2 M KCl solution, filtered with an 11 µm filter. Sample extractions stored at −20°C until analysis with spectroscopy on the Biotek Synergy HTX Multi-Mode Microplate Reader.	64	Doane and Horwath (2003)

(*Continued*)

Table 1. (Continued).

Category	Indicator	Abbreviation	Description and Method	n	Method Reference
	pH_{CaCl2}	pH	Activity of $[H^+]$, 'master variable' controlling nutrient availability. Measured with HQ430D Laboratory Single Input pH glass electrode probe in 0.01 M $CaCl_2$ solution.	8	Thomas (1996)
	Soil Test Potassium	STK	An essential plant nutrient. Mehlich III extraction in the Iowa State Soil and Plant Analysis Lab.	8	Mehlich (1984)
	Soil Test Phosphorus	STP	An essential plant nutrient. Mehlich III extraction in the Iowa State Soil and Plant Analysis Lab.	8	Mehlich (1984)
Biological					
	Earthworms	earthworm	Phylum Annelida, soil megafauna, active in decomposition and nutrient cycling. In autumn, 25x25x25 cm pits excavated, hand-sorted in the field. Mustard solution poured to get any remaining earthworms burrowed below pits. Worms and eggs were collected and counted.	4	Čoja et al. (2008)
	Microbial biomass nitrogen	MBN	Biomass of multiple microscopic organisms, an indicator of N supply. 2 mm-sieved, field-moist soils fumigated with ethanol-free chloroform for 24 h. Fumigated and unfumigated samples were extracted with 25 mL of 0.5 M K_2SO_4, shaken for 1 hour, filtered through an 11 µm filter. Sample extractions were then stored in the freezer at −20°C until analysis using the Shimadzu TOC-L TNM Analyzer.	64	Horwath and Paul (1994), Brookes et al. (1985)
	Microbial biomass carbon	MBC	Biomass of multiple microscopic organisms, an indicator of active C. Same procedure as for microbial biomass nitrogen.	64	Horwath and Paul (1994), Joergensen (1996)
	Mesofauna	mesofauna	Soil biota (0.1–2 mm), mostly mites and collembola, active in residue decomposition. 15 cm diameter, 13 cm deep cores were collected in early summer and extracted in a Berlese funnel apparatus with overhead lamp. Mesofauna were collected in ethanol, stored for 6 months and identified.	4	Hessel (2014)
	β-glucosidase	BGase	Extracellular enzyme reflecting microbial activity. Methylumbelliferyl-linked substrate (4-Methylumbelliferyl β-D-glucopyranoside) incubated for 1 h. Assay read with spectroscopy on the Biotek Synergy HTX Multi-Mode Microplate Reader.	36	Deng, Kang, and Freeman (2011)
	Potentially mineralizable carbon	CO_2	Active C – available to microbes, a proxy for activity and N mineralization. 2 mm-sieved, air-dry soils brought to 50% water holding capacity, incubated in the dark at 25°C. Change in headspace CO_2 sampled at intervals between 2–8 hrs. Final value cumulative CO_2 produced in 3 days.	36	Franzluebbers et al. (2018), McDaniel et al. and Grandy (2016)

Table 2. Selected soil ecosystem services directly applicable to agriculture according to the food and agriculture organization of the United Nations (FAO 2015), and associated soil health indicators.

		Soil Health Indicator†																			
		Physical					Chemical									Biological					
Soil Ecosystem Service	**Description**	WSA	hardness	GWC	BD	PAW	SEOC	SEON	SOC	TN	CEC	NO_3^-	pH	STK	STP	earthworm	MBN	MBC	mesofauna	BGase	CO_2
Habitat/resources for organisms	Structure, water and nutrient resources for soil biota	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Nutrient cycling	Decomposition, nutrient transformation, storage, and availability			×		×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Provision of food, fiber, and fuel	Agricultural productivity			×	×	×			×	×	×	×		×	×						
Flood regulation	Precipitation infiltration and storage, reduced runoff	×	×		×				×							×					
Water purification	Removal of biological and chemical water contaminants	×							×		×						×	×			
Carbon sequestration	Accrual or storage of atmospheric carbon dioxide (CO_2)	×							×							×		×	×	×	×

†: See Table 1 for soil health indicator definitions.

into ethanol for preservation. Samples were stored in ethanol and later identified to order level; Acari were classified into morphogroups, with total abundance of mesofauna reported here.

Soil hardness was measured in-field on 13 Nov. using a digital cone penetrometer (FieldScout SC900; Table 1). Measurements were taken in each experimental replicate from eight non-trafficked interrow locations and two wheel-trafficked interrow locations to provide weighted sampling by the proportion of plot area affected by machinery traffic. In this analysis, hardness values within 0–30 cm depths were combined to give an average value for each plot from those depths. Earthworm abundances were measured on 8 Oct. using the pit excavation method following rains that activated estivating earthworms (Čoja et al. 2008).

Soil water was collected biweekly from suction cup lysimeters in 2017 installed 1.2 m below the surface of three of four treatment replicates. Adequate sub-soil moisture allowed for only seven lysimeter water samples on 27 Apr., 11 May, 22 May, 6 June, 20 June, 10 Oct., and 5 Nov. Soil lysimeter water NO_3^- was analyzed colorimetrically using the same method as soil-extractable NO_3^- (Table 1).

Data synthesis from previously published studies

Previously published data on productivity, economic, and environmental impact for 2YCS and 4YCS were synthesized to provide greater context to changes in soil health. These data include: herbicide rates, fossil fuel energy consumption, crop yield, net profitability, synthetic N-fertilizer inputs, and soil erosion in these systems reported between 2008 and 2016. (Hunt, Hill, and Liebman 2017, 2019; Hunt et al. 2020). We used farm records for costs of synthetic N-fertilizer and herbicide applications. Fossil fuel energy consumption model estimates included both upstream and on-site fossil fuel consumption for: production of N fertilizer, herbicide, diesel, seed, grain drying, and all field operations including manure composting and application. Not included in the analyses were farm machinery and grain drying equipment, and the manufacturing and use of P and K fertilizers. Net profitability was calculated using field operation logs for labor, input costs, and crop yields. Agricultural economics databases used year- and product-specific information, and Iowa-based estimates were used for machinery operations, seeds, labor, and crop insurance. Manure was assumed to have been acquired as a waste product without cost on-farm or on a neighboring farm and included only labor and machinery costs for application (Hunt, Hill, and Liebman 2017). Erosion model estimates were generated using ArcSWAT calibration to a Hamilton County watershed, followed by a parameterization of the model using site-specific values (Hunt, Hill, and Liebman 2019). We also gathered prior water quality data, as NO_3^--N, collected from the same buried soil lysimeters, but from a previously published seven-year study (2004 to 2011) across all phases of the rotation (Tomer and Liebman 2014).

Data analyses

All new data were checked for normality and heterogeneity of variances. Transformations were applied when raw data did not satisfy tests for normality or homogeneity – some data required log or exponential transformations. For ANOVA, we used linear mixed-models. For singular sampling events, block, treatment, soil depth, and treatment × depth interaction were fixed effects, and the block × treatment interaction was used as a random effect. For response variables tested at multiple dates or discrete soil depths or root proximities, the same linear mixed-model as shown above was applied, except with date and depth included as interacting and main fixed effects. The model for response variables tested at multiple dates and distances from the rhizosphere was the same as that for discrete depths, except that root proximity replaced depth in all factors including depth. The R package *lme4* (Bates et al. 2014) was used to fit each model, and *lmerTest* (Kuznetsova, Brockhoff, and Christensen 2017) was used to generate an ANOVA using Satterthwaite's degrees of freedom and Type III sums of squares. The R package *emmeans* (Lenth 2019) was used for pairwise comparisons and *p*-value corrections. Although some measurements were performed at multiple soil depths or time points, the *p*-values represent the main treatment effect unless there were significant interactions, and the percent differences were calculated using the overall treatment means. For temporal dynamics, we conducted comparisons for each date when there was a significant interaction with date. Significance was set at $\alpha = 0.05$, and marginal significance at $\alpha = 0.1$.

We normalized data to visualize and quantitatively compare soil health indicators on a spider (or radar) plot. Normalization calculation divided each respective experimental unit by the maximum value assuming greater-is-better or minimum, where less-is-better (e.g. BD, hardness and NO_3^-). Measurements at more than one date or depth were first divided by that date or depth maximum/minimum to incorporate into the overall calculation. Then, all normalized values were averaged, and a standard deviation created across depth and/or dates. A value closer to 1 indicates better soil health.

Results and discussion

Diversified cropping effects on physical soil health

Bulk Density (BD) and Hardness. Soil BD and hardness were significantly lower in the 4YCS compared to the 2YCS by 26% and 8%, respectively (Figure 1; Table 3). Compacted soils can decrease maize root growth, but this depends on the level of compaction and soil type (Panayiotopoulos, Papadopoulou, and Hatjiioannidou 1994). Our results point to superior conditions for root growth in the 4YCS compared with the 2YCS, which is consistent with a previous study that found a 17% increase in maize root length per unit soil volume (Lazicki, Liebman, and Wander 2016). Differences in inputs (like manure and alfalfa root residue) and diverse root architectures (and depth) from extended rotations with alfalfa may contribute to reduced BD, soil hardness (Bowen 1981; Elkins, Haaland, and Hoveland 1977), and improved aggregation throughout the soil profile (Degens 1997; Lafond, Angers, and Laverdière 1993). While we cannot distinguish whether crop diversity or manure enhanced the ease of soil penetration – e.g., moldboard plowing in prior autumn may have also decreased soil strength and BD – a diversified system effect (rotation + manure) was clear here and confirmed by other crop rotation and manure addition studies (Celik et al. 2010; Munkholm, Heck, and Deen 2013; Ozlu, Kumar, and Arriaga 2019). A further consideration is that these strong positive effects on BD and hardness coincide with other physical parameters despite one intensive tillage event in the 4YCS (moldboard plow after alfalfa); it should also be noted that there are fewer tillage passes over the length of the rotation in the 4YCS compared to the 2YCS.

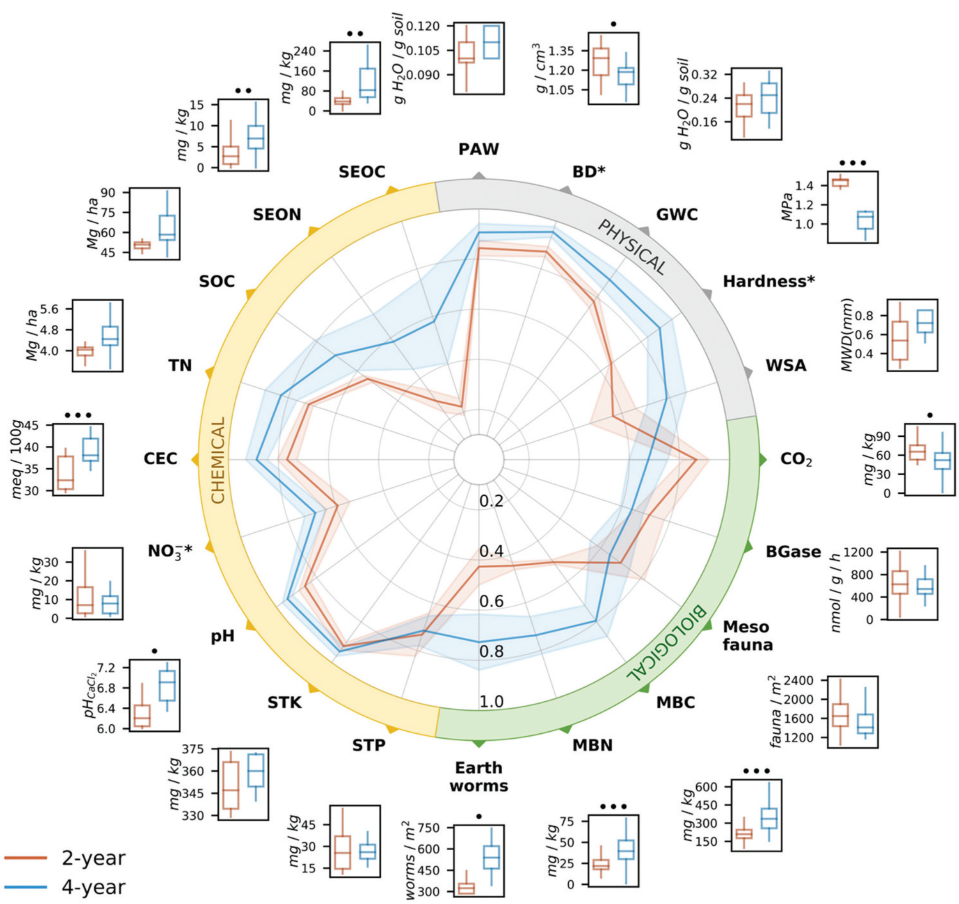

Figure 1. Normalized value (central radar plot) and raw data (periphery box plots) for conventional (2-year) and diversified (4-year) cropping systems. Solid lines in the radar chart indicate the mean normalized value, while shaded areas indicate the associated standard error. The middle bars in the box and whisker plots represent the median of the raw data for each treatment. For definition of soil health indicator abbreviations see companion Table 1. Significance at · $p < .1$, · · $p < .05$, · · · $p < .01$. *Parameters where smaller raw values indicate greater soil health (e.g. bulk density and hardness), and normalized values reflect this 'less is better.'

Table 3. Soil health indicators, respective soil ecosystem services, and effect of diversifying cropping systems (Companion Table to Figure 1)†.

Indicator	Units	2-Year Cropping System (2YCS)	4-Year Cropping System (4YCS)	Mean Treatment Effect (difference from 2YCS)	p-values
		Mean (standard error)			
Water-stable aggregates	mm	0.55 (0.11)	0.78 (0.17)	+40%	ns
Hardness	MPa	1452 (26)	1072 (82)	−8%	**0.0059**
Gravimetric water content	g H_2 O g dry soil^{-1}	0.21 (0.01)	0.24 (0.01)	+14%	ns
Bulk density	g cm^{-3}	1.26 (0.03)	1.11 (0.03)	−26%	0.0596
Plant-available water	g H_2O g dry soil^{-1}	0.10 (0.01)	0.12 (0.00)	+10%	ns
Salt extractable organic carbon	mg C kg^{-1}	44.2 (4.9)	113.6 (11.9)	+157%	**0.0237**
Salt extractable organic nitrogen	mg N kg^{-1}	4.0 (1.0)	8.1 (0.9)	+101%	**0.0178**
Soil Organic Carbon	Mg C ha^{-1}	51.5 (1.6)	64.5 (6.3)	+29%	ns
Total nitrogen	Mg N ha^{-1}	4.2 (0.1)	4.8 (0.3)	+17%	ns
Cation exchange capacity	meq 100 g^{-1}	33.8 (2.4)	39.2 (2.1)	+16%	**0.0017**
Nitrate-N	mg N kg^{-1}	13.27 (3.02)	8.64 (1.41)	−35%	ns
pH_{CaCl2}	Unitless	6.46 (0.23)	6.90 (0.19)	+10%	0.0532
Soil Test Potassium	mg kg^{-1}	348 (6)	363 (13)	+7%	ns
Soil Test Phosphorus	mg kg^{-1}	28 (5)	27 (3)	−2%	ns
Earthworms	count m^{-2}	318 (54)	542 (83)	+71%	0.0636
Microbial biomass nitrogen	mg N kg^{-1}	24 (2)	44 (3)	+80%	**0.0068**
Microbial biomass carbon	mg C kg^{-1}	219 (14)	348 (21)	+62%	**0.0196**
Mesofauna	count m^{-2}	1689 (282)	1559 (235)	−7%	ns
β-glucosidase	nmol g^{-1} h^{-1}	650 (172)	602 (224)	−7%	ns
Potentially mineralizable carbon	mg C kg^{-1}	69 (9)	55 (16)	−20%	0.0879

†2YCS = two-year cropping system; ns = no significance.

Gravimetric Water Content (GWC), Plant Available Water (PAW), and Water Stable Aggregates (WSA). GWC, PAW, and WSA all pointed the same direction – i.e., 4YCS greater compared to 2YCS – but non-significantly so (Figure 1, Table 3). GWC was 14% greater under the 4YCS compared to the 2YCS (Figure 1, Table 3), a consistent trend found throughout the growing season (Fig. S2). Studies on similar cropping system level diversification and soil water availability are scarce, however, one study from Midwest US showed 71 years of cattle manure increased water retention at −0.033 and −1.5 MPa respectively by 18% and 21% (Blanco-Canqui, Hergert, and Nielsen 2015). Using the monthly-measured GWC and BD values in each rotation, we calculated the difference in water storage in the top 30 cm of soil. On average, the 4YCS consistently stored 8 ± 2 mm more rain over the entire growing season (Fig. S2), during what was the 7th driest growing season in 50 years (IA Mesonet 2020). More specifically, June and July 50-year average rainfall is 127 and 110 mm, but in 2017 these months were 44 and 25 mm respectively. This translated to 11 and 32% more water in the 4YCS in the months of June and July in 2017.

Diversified cropping effects on chemical soil health

Cation Exchange Capacity (CEC) and pH. Diversification increased the CEC by 16% and pH by 10% in the 4YCS compared to 2YCS (Table 3), indicating a greater storage capacity for essential cationic plant nutrients in the diversified rotation. CEC increases with clay content, organic matter and pH (Helling, Chesters, and Corey 1964), but management on the decadal scale generally only influences the latter two (Thomas, Dalal, and Standley 2007). Increased CEC does align with average soil organic carbon (SOC) increases discussed later (Table 3), as soil organic matter can be an important source of CEC (Thompson et al. 1989). Furthermore, CEC increases are also related to soil pH increases. CEC buffers soil against acidification, and near-neutral pH enhances effective CEC, because fewer acid-forming cations are present on exchange sites. The increased pH and CEC in the 4YCS likely resulted from lower synthetic N fertilizer rates, and proportionally greater manure application. Synthetic ammonium-based fertilizer induces nitrification, an acidifying soil process (Aula et al. 2016; Edwards et al. 1992). The 4YCS received N primarily from manure (81% of total N inputs), and unlike the 2YCS, received low synthetic N fertilizer (8 vs. 89 kg N ha^{-1} y^{-1}; Hunt, Hill, and Liebman 2019). Additionally, composted cattle manure applied in the 4YCS may buffer soil pH through organic acids and/or bicarbonates in the manure, even when mineral N-fertilization is moderate (Whalen et al. 2000).

Salt-extractable Organic C (SEOC) and N (SEON). The SEOC and SEON indicate the presence of low-molecular weight, dissolved and exchangeable organic matter in soil, and are proxies for microbial-labile sources of C and N (Haynes 2005; Sun et al. 2015). The 4YCS strongly increased SEOC (+157%) and SEON (+101%) compared to the 2YCS (Table 3). Manure application is likely the most parsimonious explanation for these increases (Haynes 2005; Jensen et al. 1997); however, crop rotations absent of manure have increased these salt-extractable pools (McDaniel and Grandy 2016). Consistent with our findings, other studies have found manure generally increases SEOC and SEON compared to synthetic N-fertilizer alone (Long, Jiang, and Sun 2015; McDowell et al. 1998), although exceptions for SEOC exist (Sun et al. 2015). Additionally, the effect whereby 4YCS increased SEOC may also be due in part to change in pH, which increases dissolution of low-molecular weight forms of organic matter (i.e., DOC; You, Yin, and Allen 1999). Combined manure and synthetic N-fertilizer has increased SEON by 143%, compared to systems fertilized with mineral N alone, and related to microbial biomass and crop yield increases among treatments (Sun et al. 2015).

Soil Organic Carbon (SOC). The 4YCS increased SOC by 29%, but not significantly (Figure 1, Table 3). In general, greater C inputs to soil should lead to greater increases in SOC with all other factors regulating SOC loss kept the same (Kong et al. 2005; Larson et al. 1972; Liu et al. 2014). We have two major sources of C in these agroecosystems: those from plant inputs of both cropping systems and manure inputs only in the 4YCS. Past C input calculations for this long-term experiment accounted for aboveground biomass, root biomass, manure inputs (Poffenbarger et al. 2020), and estimates of root exudates (Lazicki, Liebman, and Wander 2016), and estimated that the 4YCS received 8–26% less C than did the 2YCS (mostly due to lower aboveground inputs after hay harvest during alfalfa years). Greater diversity of plant inputs from crop rotation, legumes, and animal manure application have also been shown to increase SOC (Kallenbach and Grandy 2011; McDaniel, Tiemann, and Grandy 2014b), and may explain the slow trend toward greater SOC, but this is much more difficult to quantify. However, it is more likely that SOC changes slowly, and even moderate changes can be difficult to detect with statistical certainty in Iowa soils (De et al. 2020; Lazicki, Liebman, and Wander 2016; Poffenbarger et al. 2020).

Even though SOM is the cornerstone of soil health and influences almost all other soil health indicators, quantifying changes in SOC after management intervention is difficult. Emergence of private (or voluntary) C markets has rapidly expanded and intensified interest in quantifying changes in SOC after a switch in soil management (Vermeulen et al. 2019). However, detecting SOC changes in Midwest US, and regions around the world dominated by Mollisols, is especially difficult due to large preexisting SOC concentrations (> 2%; Franzmeier, Lemme, and Miles 1985). Tracking a relatively small 'signal' within large 'noise' can be challenging due to slow rates of SOC change and high spatial variability (De et al. 2020; Goidts, Van Wesemael, and Crucifix 2009; Poffenbarger et al. 2020), or even impossible due to potential C saturation (Stewart et al. 2007). For instance, deep soil cores (0–90 cm) taken from this same MFE but in 2014 were analyzed for SOC stocks and found no difference between 4YCS and 2YCS, 146 ± 11 vs. 156 ± 16 Mg C ha^{-1} (Poffenbarger et al. 2020). These results stress that with soil health evaluations, we should not solely focus on changes in SOC. While SOC is central to healthy soil function and SESs, changes are slow and difficult to measure with current methods. Our data illustrate the importance of using more sensitive indicators that are more directly linked to other critical SESs of interest for more rapid indication of progress in soil health and SES provisioning.

Nitrogen Dynamics. N is arguably among the most important macronutrients for crop growth (Schlegel, Dhuyvetter, and Havlin 1996; Smil 2004), and is a key limiting nutrient in most agricultural soils. Total N (TN) was on average 17% greater, but like SOC, the difference was not significant (Table 3). TN is partially composed of what is thought to be bioavailable, or total extractable N forms, which consist of organic extractable N (i.e., SEON) and inorganic N (mostly NH_4^+ and NO_3^-). Combined, both organic and inorganic extractable N forms only amount to less than 5% of total N in these soils. Inorganic N was comprised of >90% NO_3^- in both cropping systems. Seasonal soil NO_3^- was more stable and 35% lower in the 4YCS, but not significantly different (Figure 1, S3, Table 3). Moreover, SEON accounted for 48% of total extractable N forms in the 4YCS versus 23% in the 2YCS. Alfalfa roots and composted cattle manure were likely contributors to SEON and TN increases in the 4YCS. Kelner, Vessey, and Entz (1997) found that the abilities of alfalfa to both fix atmospheric N_2 and reach deep soil NO_3^- reserves allowed for 2nd year alfalfa stands to add greater amounts of N than soybeans. Although N_2 fixation is variable for both legumes, on average, alfalfa is estimated to fix 150 kg N ha^{-1} y^{-1}, compared to 84 kg N ha^{-1} y^{-1} by soybeans, with fixation for both generally diminishing with increased inorganic N (Kelner, Vessey, and Entz 1997; Russelle and Birr 2004). Additionally, the use of animal manure combined with synthetic fertilizer N was found to be more effective at increasing TN than using synthetic N fertilizer alone (McGill et al. 1986; Sun et al. 2015).

Plant Available P and K (STP and STK). Essential plant macronutrients, STP and STK, measured with Mehlich III extractions were not significantly different between treatments. This was an expected result, since recommended rates of these nutrients are applied based on annual soil fertility tests. This result demonstrates that when planning manure application rates, soil and manure testing in combination with small supplemental synthetic N applications were effective strategies for avoiding excess STP and STK (Sadeghpour et al. 2016), a common issue associated with manure application (Hooda et al. 2001). Phosphorus inputs were comparable between both systems. Composted cattle manure is high in P, and the 4YCS received 11 kg P ha^{-1} y^{-1} (supplemented with 9 kg P ha^{-1} y^{-1} from synthetic fertilizer), compared to 15 kg P ha^{-1} y^{-1} synthetic P in the 2YCS (Hunt, Hill, and Liebman 2019).

Diversified cropping effects on biological soil health

Earthworms. While most earthworms in the Midwest US are non-native, they are still considered an important soil ecological engineer, nutrient cycler, and indicator of general soil health in agroecosystems (de Bruyn 1997; Hendrix 2006). In our study, diversification increased earthworm abundance in the 4YCS by 71% compared to the 2YCS (Figure 1, Table 3). Most likely, the alfalfa residues and composted cattle manure increased earthworm abundance and activity as shown in other studies (Dick 1992; Sauvadet et al. 2016; Shipitalo, Protz, and Tomlin 1988). Compared to maize residues, alfalfa residues can increase earthworm biomass and casting by 5% and 43%, respectively (Shipitalo, Protz, and Tomlin 1988). Reduced herbicide use in the 4YCS also may have played a role in increasing earthworm abundance (Gaupp-Berghausen et al. 2015; Zaller et al. 2014), but others have demonstrated that crop rotations have a far greater influence on earthworms than herbicide use (Tomlin, Tu, and Miller 1995).

Mesofauna. Soil mesofauna comprise key predators in soil food webs and are critical for recycling nutrients. They are generally thought to be more sensitive to agroecosystem management practices than soil microorganisms (Behan-Pelletier 1999; Wardle et al. 1995). In our study, however, soil mesofauna abundances (mites and collembola) and community composition were rather insensitive to cropping system diversification and there was no significant difference between the 2YCS and 4YCS (Figure 1, Table 3). Combined abundances of mites and collembola were 1–2 orders of magnitude lower than those obtained in other agricultural systems in the region (50,000–100,000 individuals m^{-2}; Crossley, Mueller, and Perdue 1992; Culik, de Souza, and Ventura 2002; Edwards and Lofty 1969; Reddy 1984). This discrepancy could be attributed to 2017 being one of the driest growing seasons on record (IA Mesonet 2020). Mesofaunal communities in both systems were predominantly comprised of mites and collembola, which comprised about 61% and 20% of the total community, respectively. The 4YCS did have greater mite species richness, containing 11 Acari morphogroups, while the 2YCS only had 8 present, which is consistent with studies comparing similar cropping practices (Kruczynska and Seniczak 2011; Osler et al. 2008).

Soil Microbial Biomass (MB). Microbial biomass is a relatively small but critical pool of total soil organic matter (1–5%). Average soil microbial biomass carbon and nitrogen (MBC and MBN) were both significantly greater in the 4YCS versus 2YCS, by 62% and 80%, respectively (Figure 1, Table 3). These increases in MBC and MBN persisted across eight sampling dates during 2017 (*data not shown*); and were consistent with other studies showing responses to manure amendments (Kallenbach and Grandy 2011), diversified crop rotations (McDaniel et al., 2014a; McDaniel and Grandy 2016), and a previous report from this experiment (King and Hofmockel 2017). Since C is thought to be limiting for soil microorganisms (Ekblad and Nordgren 2002; Soong et al. 2020), quality of C additions through manure and plant inputs with narrow C-to-N ratio likely increased microbial biomass (Campbell et al. 1991; Lynch 1984; McDaniel and Grandy 2016). Other mechanisms explaining the large increase in MB under diversified cropping systems include greater soil water content (Skopp, Jawson, and Doran 1990), circumneutral pH (Aciego Pietri and Brookes 2009; Geisseler and Scow 2014), and increases in SEOC (Sun et al. 2015). Improvements in the soil environment and in available resources should not only increase microbial abundance but also alter microbial community composition (Drenovsky et al. 2004; Kravchenko et al. 2019; Zhalnina et al. 2015) and enhance microbial metabolism (Lupwayi, Rice, and Clayton 1999; McGill et al. 1986; Zhalnina et al. 2015).

Microbial Activity: Beta-glucosidase (BGase) and potentially mineralizable C (CO_2). The extracellular hydrolytic enzyme BGase and PMC measured via incubation are both indicators of soil microbial activity. BGase activity approximates both activity and microbial C demand, while soil respiration reflects microbial C consumption under optimal conditions. Both C demand (BGase) and consumption (PMC) commonly increase with increases in MB (Franzluebbers et al. 1996; Turner et al. 2002); and more specifically, when MB increases with organic inputs from diverse rotations and manure (McDaniel and Grandy 2016; Rochette and Gregorich 1998). Thus, it was unexpected that BGase and PMC did not coincide with increases in MBC and were lower on average in the 4YCS than the 2YCS, albeit statistically insignificant (Table 3). A similar finding of greater MBC, but lower BGase activity, was previously reported from this experiment at 0–10 cm depth (King and Hofmockel 2017). At this depth, the authors reported an 18% increase in MBC but a 46% decrease in BGase in the 4YCS compared to 2YCS. However, at 10–20 cm depth the 4YCS increased both MBC and BGase by 97% and 124%, respectively. The reason for these differences with depth is unknown, but may be due to changes in labile C supply with depth – C becomes more limiting with depth and requires greater BGase production in 4YCS compared to the 2YCS. One possible explanation for these divergent findings is that the 4YCS soil microbial community was more efficient at processing C inputs (Anderson and Domsch 1990; Kallenbach et al. 2019; Wardle and Ghani 1995). Soils from the 4YCS respired 20% less in response to having 8–26% less estimated C inputs than the 2YCS (Poffenbarger et al. 2020) but resulted in 62% more microbial biomass. Greater MB with less C inputs from plants combined with more efficient C use could help maintain, or even slightly increase, SOC compared to the 2YCS (Manzoni et al. 2012; Sinsabaugh et al. 2013). Furthermore, increased availability of soil N to MB, here provided through organic forms of N (manure and alfalfa), has been shown to enhance microbial C use efficiency (Li et al., 2018a; Wild et al. 2019). This also supports the notion that SOC may be slowly increasing in the 4YCS compared to the 2YCS, despite the lack of statistical significance observed here and in other studies (Lazicki, Liebman, and Wander 2016; Poffenbarger et al. 2020).

Potentially mineralizable C is one of the most commonly used biological soil health tests (Bünemann et al. 2018), and correlates with labile resources available to microorganisms, the size and activity of the MB, and even crop yield and N requirements (Allen, Singh, and Dalal 2011; Franzluebbers 2018). However, our finding of the 4YCS decreasing PMC (and BGase) compared to the 2YCS was incongruent with the plethora of published studies reporting positive soil health changes resulting from agroecosystem diversification. We conclude that short-term PMC measurements and enzyme activities should not be evaluated alone, but interpreted with additional biological soil health indicators like MB, and potentially other physical and chemical parameters.

Consequences of increased soil health for soil ecosystem services

SESs are interdependent and can be associated with multiple different soil health indicators (Table 2). Here, we assess and highlight the impact of the most salient soil health responses to cropping system diversity on six related SESs.

Flood Regulation. The near unanimous improvements in physical soil health indicators (Figure 1) point to more rapid water infiltration (Angers and Caron 1998; Ehlers 1975), and reduced runoff and erosion (Zhang et al. 2007), thereby enhancing flood regulation. The Midwest US is increasingly susceptible to flooding due in part to both increased total precipitation and event intensity (Schoof, Pryor, and Surprenant 2010; Villarini et al. 2011). The 4YCS decreased soil hardness and reduced BD by an average of 0.1 g cm^{-3} compared to the 2YCS (Figure 1). Reducing BD by 0.2 g cm^{-3} can double soil hydraulic conductivity in a coarse-textured soil (Patel and Singh 1981). At a regional scale, these physical improvements to soil could help retain water from intense precipitation events and mitigate flood damage, such as those in the Midwest US in 2008 that cost $15B (Budikova et al. 2010). $10B was from Iowa alone, including $4B in crop damage (U.S. Department of Commerce 2009).

In Midwest US agroecosystems, producers struggle with both extremes of too much and too little soil water – even within the same location but at different times of the year (Hatfield, Wright-Morton, and Hall 2018). The structural soil properties that increase the capacity to reduce flood damage also improves the capacity of the soil to store water in times of scarcity. As the Midwest US springs become wetter and summers drier (Kunkel et al. 2013; Pryor, Barthelmie, and Schoof 2013), managing soil to promote soil structure that supports infiltration and water storage will be even more integral to enhancing agricultural system resilience (Table 2). Between 1989 and 2009, drought accounted for 30% of insured crop losses in the Midwest US (Hayes 2013). In rainfed systems, crops like maize are increasingly susceptible to heat stress due to rising temperatures and vapor pressure deficits associated with climate change (Lobell et al. 2014; Ort and Long 2014). Crop stress and climate models suggest that maize yield losses from heat stress could approach 40% by 2060 (Ort and Long 2014). Increased crop diversity can reduce yield variability that results from climate extremes (Bowles et al. 2020; Gaudin et al. 2015).

Water Purification. Conventional agriculture affects water quality via what are probably the most pressing environmental issues for the Midwest US: erosion and nutrient leaching. In conventional systems, erosion can occur when fields are fallowed seven months of the year. Both erosion and timing of nutrient applications before crops can take them up contribute to the release of both N and P from farm fields that affects local and regional water quality (Matson, Naylor, and Ortiz-Monasterio 1998; Rabalais, Turner, and Wiseman

2002). Models have shown the 4YCS reduces erosion by over 60% (Table 4). Differences in erosion may be attributable to increased duration of crop cover in the 4YCS alfalfa years. Additionally, during 2017, the 4YCS reduced soil lysimeter water NO_3^- concentrations in early spring by 57% and generally decreased soil NO_3^- in the top 0–15 cm compared to the conventional, 2YCS (Figure 2, S3). This window between April and June is when most nutrient losses occur in the Midwest US. For example, during this period 71% of annual tile drainage and 75% of annual NO_3^- losses occurred (Randall and Vetsch 2005). A longer study of NO_3^- in groundwater showed this 4YCS reduced concentrations by 22% throughout the entire rotation, compared to a 2YCS (Tomer and Liebman 2014; Table 4).

Improved physical soil properties help regulate water purification by reducing erosion and slowing and storing water and increasing the availability of NO_3^--N to crops during the growing season (Basche and DeLonge 2017; Hatfield, Sauer, and Prueger 2001). Chemical and biological aspects of soil health also play a role in reducing N leaching (Figure 2; Tomer and Liebman 2014). The greater than two-fold proportion of extractable N in organic N compounds, rather than NO_3^--N, reflects greater labile N storage in forms less likely to leach and impair water quality (Christou et al. 2005). Additionally, microbial N immobilization may play a major role in water purification by reducing the average seasonal NO_3^- pool, thereby reducing leaching susceptibility (Evans et al. 2006; Partey, Preziosi, and Robson 2014), as also seen in other diversified agroecosystems (Figs. 3, S4; Table 3; Huhta, Setälä, and Haimi 1988; Partey, Preziosi, and Robson 2014; Setälä et al. 1990).

Provisioning of food/fiber/fuel & nutrient cycling

The 15-year record of maize and soybean production shows that the 4YCS increased maize and soybean yields by 5% and 21%, respectively, compared to the 2YCS (Table 3). This was likely a combined effect of multiple soil health

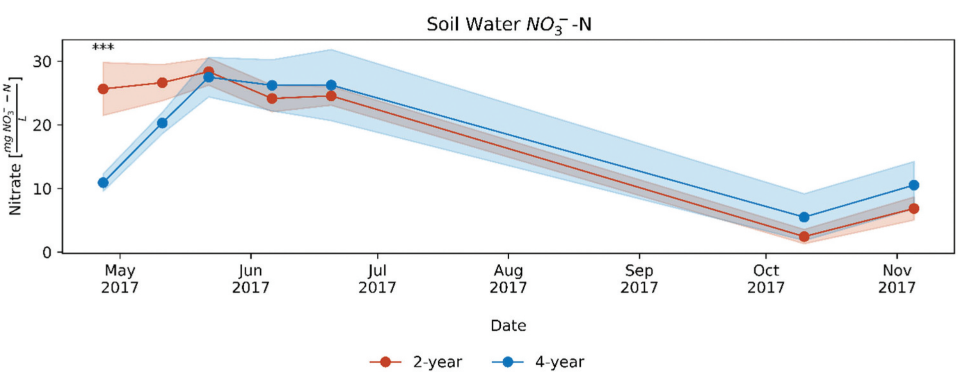

Figure 2. Nitrate concentration in soil water at 120 cm depth under the maize year of both crop rotations in 2017. Means shown with standard error in shaded area. Significance at * $p < .1$, ** $p < .05$, *** $p < .01$.

improvements in the diversified system, relative to the conventional system (Figure 1, Table 2). The same physical aspects of soil health that regulate soil water movement, storage, and purification are also important to improving soil as a medium for plant growth. A less compact soil with greater pore space and connectivity allows greater root growth, access to more water (Angers and Caron 1998; Basche and DeLonge 2017; Hatfield, Sauer, and Prueger 2001), more nutrients (Rosolem, Foloni, and Tiritan 2002), ultimately improving yields (Ball et al. 2005; Calonego and Rosolem 2010).

We show corroborating evidence with changes in chemical aspects of soil health. More specifically, SEON showed one of the greatest positive differences between the 4YCS and 2YCS, illustrating the importance of N being in a less mobile, yet labile form. Also, CEC, a measure of ability of soil to store essential cationic plant nutrients, was greater in the 4YCS compared to 2YCS. Soil organic matter can be a catalyst for crop capture of soil resources (King et al. 2020) but may not necessarily change the quantity of resources present. Furthermore, the 4YCS presumably increased chemical availability of nutrients at a near-neutral pH (e.g., N, S, K, and Mo) compared to the 2YCS. Collectively, the 4YCS had greater nutrient storage capacity, availability and abundance of soil C and plant-available nutrients - most likely due to the addition of composted cattle manure. Finally, there is supporting evidence in the literature for both macro and microbiota contributing to enhanced nutrient cycling. For example, one meta-analysis attributed a 25% increase in crop yield due to the presence of earthworms (Van Groenigen et al. 2014) and another meta-analysis showed net N mineralization was regulated by microbial biomass stoichiometry (Li et al. 2020b). Observed pathogen and weed suppression at this site may also have contributed to enhanced yields (Westerman et al. 2005).

Habitat/Resources for Organisms & C Sequestration. Abundance and activity of soil biota are thought to be highly sensitive and respond more rapidly to management practices than other soil properties and also are critically linked to many SESs (Table 1; Doran and Zeiss 2000; Lehman et al. 2015). The soil health movement has responded with recent emphases on soil microbial biomass, activity, and community composition (Karlen et al. 2021). Diversifying cropping systems had some of the strongest effects on earthworm and microbial biomass abundances (Figure 1, Table 3), indicating improved soil habitat for these biota in the 4YCS compared to the 2YCS. Microbial biomass is very dynamic and linked to many SESs including nutrient supplying power (Li et al., 2018b) and formation of persistent soil organic matter (Grandy and Neff 2008; Liang et al. 2011; Liang, Schimel, and Jastrow 2017). There is likely a positive feedback between the physical soil properties (or the 'habitat') and biological activity. A recent study describes this process as increasing the 'microbial spatial footprint' (Kravchenko et al. 2019), which,

Table 4. Productivity, external inputs, and environmental impacts for 2-year and 4-year cropping systems (2YCS and 4YCS, data span 2003–2016)[†].

Agronomic and Environmental Benefits	Units	Rotation: 2-Year Cropping System (2YCS)	Rotation: 4-Year Cropping System (4YCS)	Effect Size (% Difference from 2YCS)	p-value[‡]	Reference
		Mean (standard error)				
Productivity						
Corn Yield	Mg ha^{-1}	10.2 (0.2)	10.6 (0.2)	5%	< 0.01	Hunt et al. (2020)
Soybean Yield	Mg ha^{-1}	2.8 (0.1)	3.4 (0.1)	21%	< 0.001	Hunt et al. (2020)
Whole Rotation Net Profitability	$ ha^{-1}	833 (71)	872 (60)	5%	NS	Hunt, Hill, and Liebman (2019)
System External Inputs						
Synthetic N Fertilizer	kg N ha^{-1}	89 (6)	8 (3)	−86%	< 0.0001	Hunt, Hill, and Liebman (2019)
Herbicides	kg active ingredient ha^{-1}	1.4 (0.15)	0.7 (0.07)	−89%	< 0.0001	Hunt, Hill, and Liebman (2017)
Fossil Fuel Energy	GJ ha^{-1} y^{-1}	9.5 (0.35)	3.4 (0.14)	−65%	< 0.05	Hunt et al. (2020)
Environmental Impact						
Nitrate-N in Soil Water (at 1.2 m depth)	mg L^{-1}	12.6 (0.5)	9.8 (0.6)	−22%	< 0.05	Tomer and Liebman (2014)
Soil Erosion	Mg ha^{-1}	2.6 (0.5)	1.0 (0.2)	−62%	< 0.001	Hunt, Hill, and Liebman (2019)

[†]More information on methods found in Supplemental Materials.
[‡]NS indicates no significance.

through a positive feedback could increase SOC in the long term and further improve structure and pore space. Despite this connection, we did not observe any statistical difference in SOC between the 2YCS and 4YCS.

Economic feasibility of diversified cropping systems

Fifteen years of the diversified, 4YCS has enhanced SESs that will help society address water quality impairment (Boesch et al. 2009; Turner and Rabalais 2003), restore degraded soils (Liu et al. 2012; Montgomery 2007), and provide resilience to climate extremes – all of which will be vital in feeding 10 billion people by 2100 (Bengtsson, Shen, and Oki 2006). This diversified cropping system mitigates these most pressing environmental issues in the region, without increasing costs to producers (Table 4).

Additional economic benefits for producers extend beyond enhanced yields and include cost reductions from reduced need for external inputs. For example, diversification practices in the 4YCS reduced energy use by half and both herbicide and N-fertilizer use were reduced by nearly 90% (Table 4). These input cost reductions offset the additional labor requirements involved in the 4YCS, which made the 2YCS and 4YCS economically comparable (Table 4; Davis et al. 2012; Hunt, Hill, and Liebman 2017; Poffenbarger et al. 2017). Increased labor demands, however, could also improve job prospects in rural America, which suffers high poverty rates, and a shrinking job market (Thiede et al. 2017).

Conclusion

We showed that diversified agroecosystems can improve soil health, mitigate environmental impacts, and be an economically viable alternative to the business-as-usual maize-soybean cropping system. Our soil health results, combined with previous work at this long-term experiment, point to comprehensive positive environmental outcomes and climate resilience under the 4YCS compared to the conventional 2YCS. Despite the potential benefits of integrated, diversified systems, there are many barriers to adoption. These include: farmer 'path dependency' due to government policy, a lack of markets for diverse agricultural products, and sunken farmer investments in specialized equipment and infrastructure (Boody et al. 2005; Bowman and Zilberman 2013; Roesch-mcnally, Arbuckle, and Tyndall 2018; Weisberger et al. 2021). Policymakers, food corporations, landowners, and consumers are called to consider creative methods of encouraging the reintegration of forage perennials, diverse grains, and livestock into agroecosystems of the Midwest US as a means of regenerating soil health, harnessing greater production in healthy soils, and reducing agricultural environmental impact, all while maintaining farm profitability.

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Author contributions

RB and MM wrote the paper and conceived of the study. RB, MD, and MDL collected and analyzed samples. RB conducted statistical analyses, RB and JM developed tools to analyze and visualize data. ML and NL provided access to long-term experiment, guidance on experimental protocol, and/or contributed data. All authors edited the manuscript.

Additional information

Supplementary Information accompanies this paper at the link.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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