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Integrated soil health management influences soil properties: Insights from a US Midwest study

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ABSTRACT

This study evaluated the effects of integrated soil health management in the US Upper Midwest over three years (2021–2023), under diverse cropping systems and soil textures. We assessed 15 field pairs, each consisting of one conventional (CV) and one soil health (SH) site, implementing contrasting management. Our analysis focused on four soil organic matter pools, six microbial indicators derived from phospholipid fatty acids (PLFA) and one physical indicator. Log response ratios (LRR) were calculated to compare pair-wise responses between medium and moderately fine-textured soils. Wet aggregate stability (WAS) showed consistent improvement; within each pair, more soil health based principles (reduced tillage, more cover crops and crop diversity) led to greater aggregate stability compared to the paired CV site. Medium-textured soils responded more strongly to soil health management than moderately fine-textured soils. To assess the effects of specific management practices, we built a mixed-effects model with practices and their interactions as fixed effects and soil health indicators as response variables. Results showed that most soil properties were significantly responsive to two management combinations, 1) tillage x cover crops, and 2) tillage x cover crops x crop diversity. Microbial indicators along with potentially mineralizable nitrogen (PMN) exhibited the strongest increases with integrated soil health management ($p < 0.05$), followed by permanganate oxidizable carbon (POXC) and total N ($p < 0.1$). Cover cropping alone moderately increased PMN ($p < 0.1$). While site-specific behavior varied based on texture and management intensities, our overall results supported the adoption of integrated soil health practices for healthier agricultural soils.

1. Introduction

Agricultural soils are overexploited to support about 95 % of global food production resulting in accelerated degradation (Hurni et al., 2015; Rinot et al., 2019; Rojas et al., 2016). Healthy soils are essential for sustainable land resource management (World Bank, 2008) and are defined as those continuing to function as a critical living ecosystem, sustaining plants, animals, and humans (Karlen et al., 2019; USDA NRCS, 2024). Recognizing the alarming issue of soil degradation, research over the past two decades has focused on improving agricultural soil health by adopting management principles developed by the USDA Natural Resources Conservation Services, specifically maximizing soil cover, maintaining living roots, increasing crop diversity, minimizing soil disturbance, and integrating livestock into cropping systems (Miner et al., 2020; Ye et al., 2021). Farmers across the US are slowly adopting these practices by reducing the intensity of tillage operations on their farms, planting more cover crops, and diversifying crop rotations (Islam & Reeder, 2014; Thierfelder & Wall, 2009), which is generating a need to monitor their long-term impact on soil health. Generally, healthy soils have high organic matter and stable aggregates, are well-drained, and have abundant microbial population and diversity. Soil assessment is critical for farmers to quantify their progress, for investors to economically evaluate their costs and benefits, and for governments to demonstrate the impact of their policies and facilitate their wider adoption (Bagnall et al., 2023).

Soil health assessments rely on measuring dynamic soil properties,

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known as soil health indicators, which are attributed to management practices and are least affected by inherent factors like soil texture and mineralogy (Amsili et al., 2021; Lehmann et al., 2020a,b; Nunes et al., 2020). The selected indicators should 1) represent soil health rather than inherent properties or fertility, (2) be sensitive to soil health principles, (3) economically accessible, and (4) uniquely link soil functions to ecosystem services (Bagnall et al., 2023; Lehmann et al., 2020a, b; Rinot et al., 2019; Williams et al., 2020). The popular set of indicators developed by the Comprehensive Assessment of Soil Health focused on identifying constraints in bio-physical soil functioning and guiding land managers in decision-making related to practices promoting healthier soils (Moebius-Clune et al., 2016). Bagnall et al. (2023) recommended a minimum suite of indicators to evaluate soil health at a continent scale based on the North American Project to Evaluate Soil Health Measurements (Norris et al., 2020). They suggest that by focusing on three key indicators, (organic C, aggregate stability, and C mineralization potential), stakeholders can effectively monitor soil with fewer economic constraints, increasing the possibility of further implementing soil health based practices.

However, specific objectives may require monitoring a different and wider range of indicators (Meena et al., 2024). For instance, soil health assessment for crop production often includes measuring organic C, nutrient availability, pH, microbial biomass, and penetration resistance (Andrews & Carroll, 2001; Cherubin et al., 2016; Idowu et al., 2009; Veerman et al., 2020). In contrast, climate change mitigation assessments would also require measuring greenhouse emissions, while those concerning water quality would include hydrological assessments of sediments and nutrient loss, as well as heavy metal analysis (Lehmann et al., 2020a,b). Therefore, identifying a suitable set of indicators is challenging for each study, as measuring all possible indicators is not cost-effective and no single indicator can capture all aspects of soil health (Takoutsing et al., 2016). Additionally, management-driven changes in soil properties may manifest over varying time scales with some appearing soon after implementing these practices, while others take several years to emerge (Strudley et al., 2008; Thierfelder & Wall, 2009). Stewart et al. (2018) reviewed 192 studies across the US and observed that properties like aggregate stability, infiltration, and microbial biomass can respond relatively quickly, within 3 years of cover cropping and reduced tillage operations. On the other hand, Angers & Eriksen-Hamel (2008) found that soil organic C takes longer, often >5 years to show positive changes. A small number of studies have developed or implemented soil health indices by weighting multiple indicators to quantify these responses to a single score (Maaz et al., 2023; Williams et al., 2020; Xue et al., 2019). However, decisions about the weight of different indicators may alter interpretations of soil health (Fine et al., 2017; Hussain et al., 1999). While these indices may be useful for large-scale estimations, they may be inaccurate while comparing inherently different soils (Lehmann et al., 2020a,b).

Previous studies have employed various methods to reduce the effects of soil parent material, topography, texture, and local climatic effects when analyzing the response attributed to management. One approach involved conducting investigations on research plots using randomized block design to maintain consistency across climate and pedological factors (Congreves et al., 2015; De Notaris et al., 2021; Krupek et al., 2022; Nunes et al., 2018; Pearsons et al., 2023; Ye et al., 2021; Zuber et al., 2017). On-farm studies have compared adjacent or paired sites with similar cropping systems and landscape positions to ensure that only management practices vary and other factors remain the same (Blair et al., 2024; Marinari et al., 2006; Sihi et al., 2017; Van Diepeningen et al., 2006; Williams et al., 2020). While research plots offer higher accuracy, on-farm investigations provide the benefits of studying real farm systems, reflecting the scale, management, and complex challenges faced by stakeholders (Drinkwater, 2002). For example, controlled plot systems comparing no-till versus tilled systems can oversimplify the complex agrosystems, as most farmers adjust their tillage strategies based on local factors like crop types, residue, weed pressure, and soil and weather conditions (Krupek et al., 2022; Williams et al., 2020). Even on-farm studies focusing on a single practice such as tillage, cover cropping, or crop diversity, limit the scope of studying comprehensive management as they are not designed to analyze multiple factors simultaneously (Krupek et al., 2022). A gap in the literature also exists for farm studies investigating management practices over the years for a more robust comparison aligning with the concept of the soil's "continued capacity" to function effectively (Lehmann et al., 2020a,b). Research studies have shown that soil properties gradually change with the long-term adoption of targeted management practices (Büchi et al., 2017), and also vary throughout the growing season (Diederich et al., 2019; Martin & Sprunger, 2022). Therefore, agronomic studies with samples over multiple years help in constraining variability more than single time points, and thus, are needed to advance current conservation strategies for sustainable land management.

The objective of our research was to address these gaps by sampling soils on working farms for three consecutive years to offer deeper insights than most existing studies. We investigated 30 participating farms in pairs (15 pairs) across diverse cropping systems and soil texture groups, implementing combined and varying management practices. This allowed us to reduce the effects of soil's inherent properties within a pair but also compare the effects of texture across farms with similar management. We selected 11 properties as the potential indicators of management-driven changes. We hypothesized that 1) continuous management practices incorporating reduced tillage, cover cropping, and crop diversity will increase the levels of all selected indicators in SH sites compared to CV sites, and 2) combining two or more of these practices would lead to better health than implementing any one practice alone.

2. Methodology

2.1. Study sites

We carried out this study for three years (2021–2023) on 30 farms in the Upper Mississippi River Basin of the US Midwest region in Minnesota and Wisconsin to represent a diversity of production systems in varying soil and crop types. Eighteen farm sites across seven counties were selected in Minnesota contributing to the Minnesota River, Des Moines River, Lower Mississippi River, Cedar River, and Upper Mississippi River Basins. Twelve sites across two counties in Wisconsin belonged to the Southeast Wisconsin-Fox River and Upper Rock Basins (Fig. 1). Minnesota sites have a 30-year (1991–2020) mean annual temperature (MAT) between 6.1–7.4 °C and mean annual precipitation (MAP) between 745–925 mm, while Wisconsin sites are slightly warmer and wetter with 7.1–8.5 °C MAT and 920–940 mm MAP (Table 1) (NOAA, 2024; Wisconsin State Climatology Office, 2024). All farms were recruited in pairs of two sites with similar soil and landscape positions, where one site was identified as the CV and the other was the SH site. Soil texture was categorized into three groups – coarse (loamy fine sand), medium (loam and silt loam), and moderately fine (clay loam and silty clay loam), based on the soil map unit components derived from USDA Web Soil Survey (Soil Survey Staff, 2023). We conducted a farmers' survey before the first sampling year (2021) to collect information on farm management including cash crops, cover crops, tillage operations, fertilizer and manure application, and animal grazing. All paired sites had a history of at least six years of employing contrasting management principles including the three study years, i.e., 2018–2023. Corn Grain (*Zea maize*) and Soybean (*Glycine* max) were the main cash crops across most of the study sites. Winter rye (*Secale cereale*) and a mixture of three or more crops were the most popular choices for cover cropping (Table 1).

2.2. Management practices and index

We classified the study sites as either CV or SH based on three out of four USDA-NRCS soil health principles – minimum tillage and more

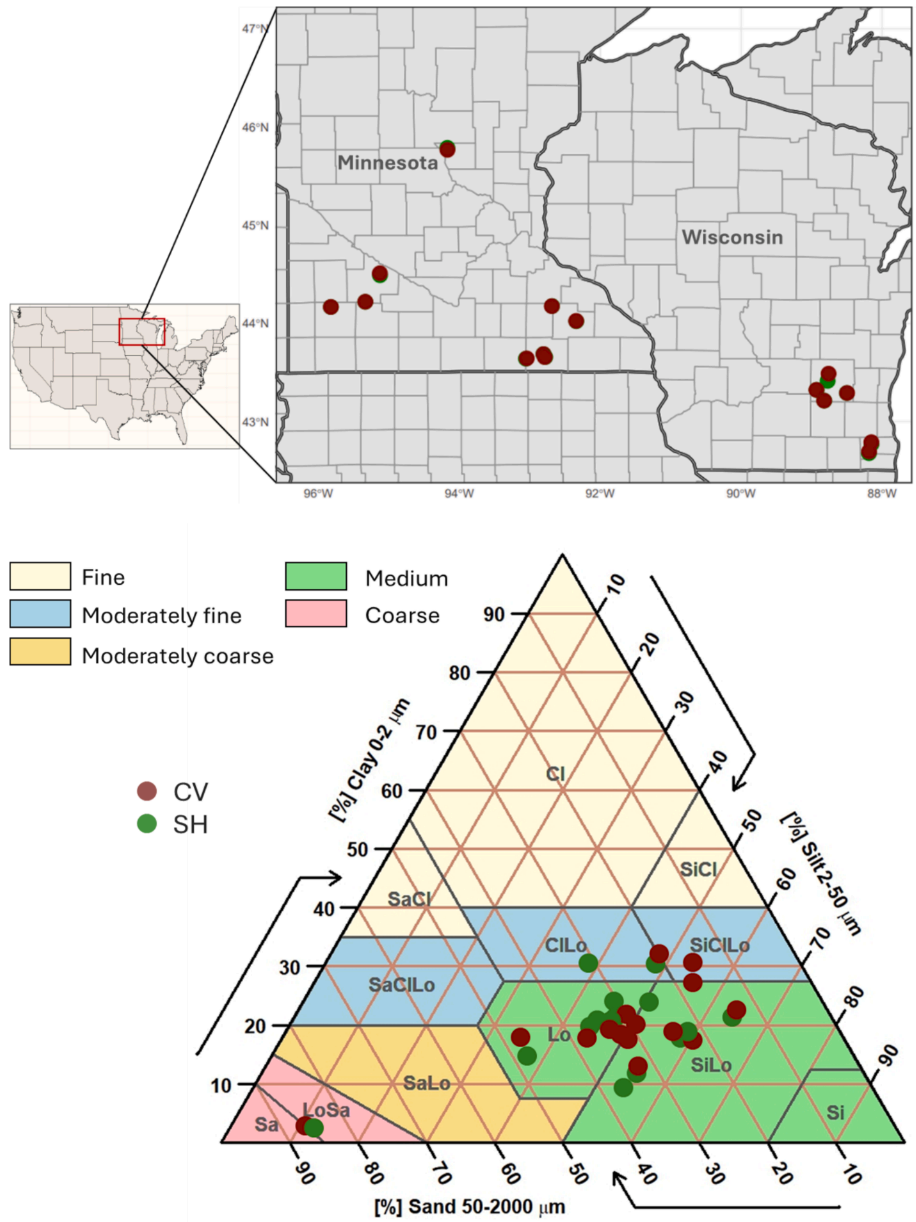

Fig. 1. All study sites location and texture categories (as per USDA classification). "CV" stands for conventional, and "SH" defines soil health sites.

cover crops and crop diversity (Chessman et al., 2019; Guo, 2021; Moebius-Clune et al., 2016). Fields employing full-width tillage operations like chisel plow, moldboard plow, or disk ripper, had fewer cover crops, and shorter crop rotations, were characterized as CV, while those incorporating reduced or no-till practices, planting cover crops, and had longer crop rotations, were characterized as SH sites. The significant variation in management practices across participating farms means that no meaningful management recommendations could be derived from analyzing the data categorically. To better represent the management practices, we quantified tillage, cover crops, and crop diversity as indices for each site (Blair et al., 2024). Tillage score was assigned based on the type of disturbance – full-width primary tillage as 1, partial width or secondary tillage/mechanical weeding as 0.5, and no tillage as 0. These scores were assigned for all annual tillage passes and summed up for the six years of recorded management data, thus ranging between 0–12. The scores were converted into indices by dividing with the maximum obtained score (i.e., 12), see eq (1). Cover crops or CC scores were attributed to the number of shoulder seasons (spring/fall) with a living root in the ground. The score varied from 0 (no shoulder season cover crop in any of the six years) to 12 (cover crops in both spring and fall, all years). Alfalfa and winter wheat were scored 2. CC scores were also normalized by dividing by 12, the highest obtained score across all sites. A crop diversity (CD) index was estimated by dividing the total number of different crop species (including both cash and cover crops) planted during the six years by the maximum number of crop species planted across all field sites (Krupek et al., 2022). A mixture of cover crops was counted as three different species.

$$\text{Tillage or CC or CD Index}_i = \frac{i^{th}\ \text{site score}}{\text{Maximum obtained score}} \tag{1}$$

2.3. Soil sampling and measurement

All fields were sampled during fall (October-November) in 2021–2023, after harvesting the cash crops (except perennial or cover crops). At the beginning of the project, two soil moisture sensor probes

Table 1
Study sites' descriptions (M1-9 from Minnesota and W1-6 from Wisconsin), farm management practices (2018–2023), and indices calculated based on these practices. Details about indices calculation are given in section 2.2. MAT, MAP, CC, CD, SH and CV refer to mean annual temperature, mean annual precipitation, cover crops, crop diversity, soil health and conventional.

Site	County	Texture	MAT (C)	MAP (mm)	Soil Types	Management	Cash crops	Cover crops	CC Index	CD Index	Tillage Practices	Tillage Index	Manure application method and time
M1	Benton	Coarse	6.13	753	Kost loamy fine sand, 0 to 2 % slopes	SH	Corn Silage, Potato, Kidney bean	Winter Rye	0.33	0.57	Field cultivator, disk ripper, chisel plow, disk harrow	0.67	Surface/ Knifed, Fall
					Kost loamy fine sand, 2 to 6 % slopes	CV	Soybean, Corn Grain	None	0.00	0.29	Chisel plow, mostly no-till	0.33	Surface, Fall/ Spring
M2	Redwood	Mod fine	7.31	746	Canisteo clay loam, 0 to 2 % slopes	SH	Soybean, Corn Grain	Winter Rye, Cover crops mix	0.33	0.86	No-till, vertical tillage 1″	0.04	NA
						CV	Soybean, Corn Grain	None	0.00	0.29	Chisel plow, field cultivator	0.75	NA
M3	Murray	Medium	6.82	757	Svea loam, 1 to 3 % slopes	SH	Soybean, Corn Grain, Alfalfa	Cover crops mix, Alfalfa	0.67	0.86	No-till	0.00	Surface, Fall
						CV	Soybean, Corn Grain	None	0.00	0.29	Chisel plow, field cultivator, disk ripper	0.75	Knifed, Fall
M4	Redwood	Medium	7.31	746	Storden-Ves complex, 6 to 10 % slopes	SH	Soybean, Corn Grain	Winter Rye	0.33	0.43	No-till	0.00	NA
						CV	Soybean, Corn Grain	Winter Rye	0.08	0.43	Chisel plow	0.50	NA
M5	Dodge	Medium	6.77	907	Spillville loam, 0 to 2 % slopes	SH	Soybean, Corn Grain	Winter Rye	0.50	0.43	No-till	0.00	Surface, Fall
						CV	Soybean, Corn Grain	None	0.00	0.29	Disk harrow	0.13	Surface, Fall
M6	Olmsted	Medium	6.95	909	Tama silt loam, 2 to 6 % slopes	SH	Soybean, Corn Grain	Cover crops mix	0.50	0.71	No-till	0.00	Surface, Fall
						CV	Soybean, Corn Grain	None	0.00	0.29	Field cultivator, chisel plow, disk harrow	0.54	NA
M7	Mower	Medium	6.85	925	Skyberg silt loam, 0 to 3 % slopes	SH	Soybean, Corn Grain	None	0.00	0.29	Ridge till	0.13	NA
						CV	Soybean, Corn Grain	None	0.00	0.29	Field cultivator, chisel plow, disk ripper	0.75	NA
M8	Freeborn	Medium	7.13	908	Newry silt loam, 1 to 3 % slopes	SH	Soybean, Corn Grain	Cover crops mix	1.00	0.71	Strip till	0.50	NA
						CV	Corn Grain	None	0.00	0.14	Field cultivator, disk riper	0.75	Surface, Fall
M9	Mower	Medium	6.85	925	Havana silt loam	SH	Cannabis (2019–2023), Corn Grain (2018)	Cover crops mix	1.00	0.71	No-till	0.00	Surface, Fall/ Spring
						CV	Soybean, Corn Grain	None	0.00	0.29	Field cultivator, disk ripper	0.75	NA
W1	Dodge	Medium	7.19	921	Mendota silt loam, 2 to 6 % slopes	SH	Soybean, Corn Grain, Winter Wheat	Winter Rye, Winter Wheat, Cover crops mix	0.67	1.00	Strip till, mostly no-till	0.08	Surface, Fall/ Summer
						CV	Soybean, Corn Grain	None	0.00	0.29	Disk ripper, chisel plow	0.42	NA

(continued on next page)

Table 1 (*continued*)

Site	County	Texture	MAT (C)	MAP (mm)	Soil Types	Management	Cash crops	Cover crops	CC Index	CD Index	Tillage Practices	Tillage Index	Manure application method and time
W2	Dodge	Medium	7.19	921	St. Charles silt loam, 0 to 2 % slopes	SH	Soybean, Corn Grain, Winter Wheat	Winter Rye	0.83	0.43	Disk ripper, mostly no-till	0.08	Surface, Fall
						CV	Soybean, Corn Grain	None	0.00	0.29	Disk ripper	0.92	NA
W3	Dodge	Mod fine	7.19	921	Pella silty clay loam, cool, 0 to 2 % slopes	SH	Soybean, Corn Grain	Winter Rye	1.00	0.43	No-till	0.00	Surface, Fall
						CV	Soybean, Corn Grain	None	0.00	0.29	Disk ripper, chisel plow, field cultivator	0.38	Integrated, Fall
W4	Dodge	Medium	7.19	921	Juneau silt loam, 2 to 6 % slopes	SH	Corn Silage (2018–2022), Alfalfa (2023)	Winter Rye, Alfalfa	1.00	0.43	No-till	0.00	Surface, Fall/ Spring
						CV	Soybean, Corn Grain, Winter Wheat	Cover crops mix	0.58	0.86	Disk ripper	0.33	NA
W5	Racine	Medium	8.36	939	Ozaukee silt loam, 2 to 6 % slopes	SH	Soybean, Corn Grain, Winter Wheat	Winter Rye, Cover crops mix	0.75	1.00	No-till	0.00	NA
						CV	Soybean, Corn Grain	None	0.00	0.29	Field cultivator, chisel plow	0.50	NA
W6	Racine	Medium	8.36	939	Aztalan loam, 2 to 6 % slopes	SH	Soybean, Corn Grain	Winter Rye	0.50	0.43	No-till	0.00	Surface, Fall/ Spring
					Ozaukee silt loam, 2 to 6 % slopes	CV	Soybean, Corn Grain	None	0.00	0.29	Disk ripper, chisel plow	1.00	NA

(AquaCheck Sub-Surface, Cape Town, South Africa) were installed at all farm sites to acquire real-time daily soil moisture data (Garg et al., 2025). Methods and data from the moisture monitoring effort are reported in detail in Kwakye et al. (in prep). Three soil samples were collected from within a 3 m radius of these sensors (Fig. 2). We sampled each location as a mixture of three 0–15 cm deep, soil sub-sample blocks collected using a sharpshooter shovel within a 0.5 m radius circle around a central point. Sampling was performed in two parts – a large undisturbed sample for aggregate stability and nutrient measurement, and a small hand-homogenized sample for microbial analysis, which was immediately stored in a cooler on dry ice before transferring to the laboratory refrigerator. Samples designated for nutrient analysis were air-dried before packaging. All samples were sent to the Soil Health Assessment Center, University of Missouri for analysis.

The soil indicators in this study (Table 2) were selected based on their ability to represent the physical, chemical, and biological health of the sampled soils, as well as their potential to respond to changes in management practices. Though different studies have identified different minimum data sets (e.g. Bagnall et al., 2023; Stott, 2019), this set of widely used indicators continues to build the literature available for comparing across studies and research sites, as detecting changes in soil health remains a challenge for soil scientists (Wander et al., 2019). Effective cation exchange capacity was estimated by measuring soil cations, including Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}, as the number of cations exchanged by unbuffered NH_4OAc from the soil exchange sites, replacing them with NH_4^+. After the cations were extracted, the soil samples were extracted with ethanol to remove excess NH_4^+, leaving only NH_4^+ ions on the exchange complex. The soil samples were then taken to a Foss Kjeldahl analyzer (Kjeldahl, 1883) and the amount of NH_4^+ ions was determined to directly measure the exchangeable cations (Supplementary material S1) (Soil Survey Staff, 2022). Soil available P (Supplementary material S1) was extracted in Bray no. 1 solution (NH_4F

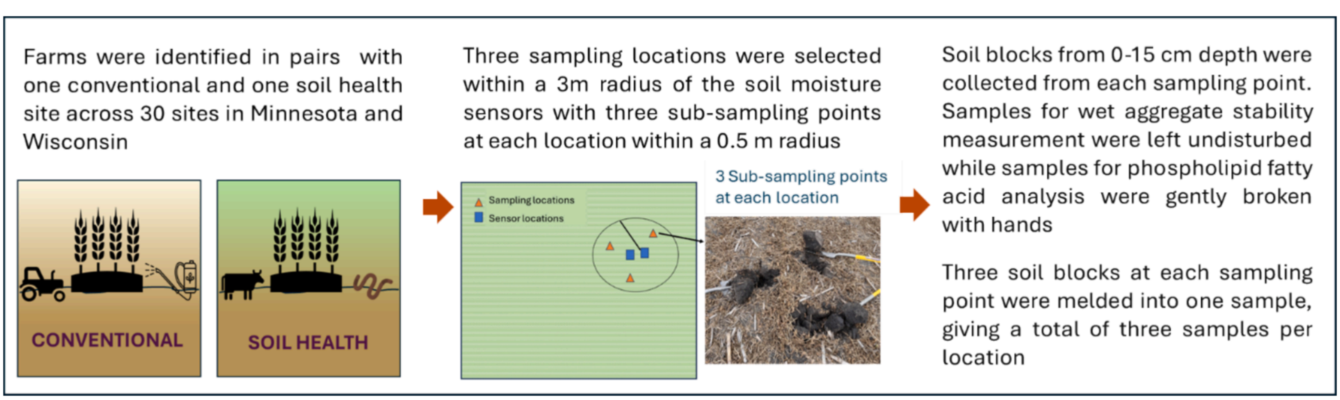

Fig. 2. Soil sampling procedure for the study.

Table 2
Methods of soil properties measurement used by the Soil Health Assessment Center at the University of Missouri.

Soil property	Units	Method of analysis	Reference
Calcium (Ca)	meq/100 g	Unbuffered ammonium acetate method, NH_4^+ was measured using the Kjeldahl method	(Kjeldahl, 1883; Soil Survey Staff, 2022)
Magnesium (Mg)	meq/100 g		
Potassium (K)	meq/100 g		
Sodium (Na)	meq/100 g		
Phosphorus (P)	ppm	Bray-1P method	(Bray and Kurtz, 1945)
pH	–	1:2 soil and 0.01 M Calcium chloride suspension	(Thomas, 1996)
Organic carbon (C)	%	Combustion analyzer	(Nelson & Sommers, 1996)
Permanganate oxidizable carbon (POXC)	mg C/kg	Weil method	(Weil et al., 2003)
Total nitrogen (N)	%	Combustion analyzer	(Bremner, 1996)
Potentially mineralizable nitrogen (PMN)	ppm	7-days anaerobic incubation	(Anderson et al., 2010)
Wet aggregate stability (WAS)	%	Sieve dipping method	(Soil Survey Staff, 2022)
Gram negative bacteria	nmol/g	Gas Chromatography	(Buyer & Sasser, 2012)
Gram positive bacteria	nmol/g		
Actinomycetes	nmol/g		
Arbuscular Mycorrhizal Fungi (AM Fungi)	nmol/g		
Fungi	nmol/g		
Total phospholipid fatty acids (PLFA)	nmol/g		

and HCl solution) and analyzed in a spectrophotometer at 882 nm wavelength (Bray and Kurtz, 1945). The sample pH (Supplementary material S1) was measured as the pH of the soil suspension in the presence of 0.01 M $CaCl_2$ (Thomas, 1996). The amount of organic C and total N were measured in a combustion analyzer. Permanganate oxidizable carbon was estimated using a $KMnO_4$ solution to oxidize the carbon present in the soil and measuring the change in color through spectrophotometric methods (Weil et al., 2003). Potentially mineralizable nitrogen was estimated by a 7-day anaerobic incubation method where it was quantified by subtracting the initial amount of NH_4^+ from the amount of NH_4^+ released during incubation, and NH_4^+ was extracted with 2 M KCl (Anderson et al., 2010). Soil's aggregate stability was determined by the wet sieving method, a protocol developed by the USDA-NRCS (Soil Survey Staff, 2022). It measures the retention of air-dry aggregates (1–2 mm) on a 0.5-mm sieve after submerging the sample in reverse osmosis water and then agitating.

Microbial biomass and community composition in soil samples were characterized by PLFA extractions (Buyer & Sasser, 2012). Sieved soil samples were dried *in vacuo* overnight in the centrifugal evaporator and extracted using Bligh–Dyer (500 ml methanol, 250 ml chloroform, and 200 ml 50 mM K_2HPO_4 in H_2O) extractant under a stream of nitrogen. The extract was dried, dissolved in chloroform, and added to a 96-well solid phase extraction plate containing 50 mg of silica per well. Phospholipids were eluted with 0.5 ml of 5:5:1 methanol: chloroform: H_2O into glass vials, dried, and transesterified using 0.561 g KOH dissolved in 75 ml methanol to which 25 ml toluene was added. The resulting fatty acid methyl esters were analyzed by Gas Chromatography and PLFA peaks were assigned to microbial groups by Sherlock Software (version 6.0, MIDI Corp, Newark, NJ). We present here Actinomycetes, Gram positive bacteria, Gram negative bacteria, Fungi, and AM Fungi as the PLFA bioindicators (Mann et al., 2019). The software assigned mono-unsaturated fatty acids (e.g., 16:1ω7c) and cyclopropanes to Gram negative bacteria, saturated branched chain PLFAs (e.g., 15:0iso and 15:0anteiso) to Gram positive bacteria, 10-methyl fatty acids (such as 10Me16:0 and 10Me18:0) to Actinomycetes, and 16:1ω5c to AM Fungi. For the Fungi population, the MIDI software only assigned 18:2ω6,9 peak but we added 18:1ω9 manually during analysis due to its significant presence in our dataset (Frostegård et al., 2011). Total PLFA represents the total microbial mass present in the soil, calculated by summing all the PLFAs identified in each sample.

2.4. Data analysis and statistics

We statistically analyzed all data in RStudio [2023.06.1 (R Core Team, 2024)]. The data were analyzed in categorical groups of SH vs CV by calculating natural logarithm of the response ratio, LRR, for each soil property using CV sites as the reference values as given in eq (2). The LRR is a valuable metric for soil studies as it quantifies proportional changes in response variables when comparing different management conditions. Previous studies like Aranguren & Cañón (2023), Bagnall et al. (2022), Xue et al. (2019), and Zuber & Villamil (2016) have effectively employed LRR to evaluate responses between management systems, such as organic vs conventional management, and tillage vs non-tillage systems, etc. In this study, site M1 was excluded from LRR analysis due to more intense tillage operations performed in the SH site compared to its CV counterpart, leading to misrepresentation of the management category.

$$LRR = \ln\left(\frac{Y_{SH} - Y_{CV}}{Y_{CV}}\right) \tag{2}$$

where Y_{SH} refers to the average value (across sites) of a soil property corresponding to the SH site and Y_{CV} value corresponds to the CV site, with a 90 % confidence interval. An LRR > 1 indicates positive changes in the respective soil property due to soil health management. The ratios were calculated separately and compared for medium-textured and moderately fine-textured soils.

In the next step, a mixed-effects model (lme4 package) was fitted with the three management variables (tillage, cover crops, and crop diversity) as fixed effects along with their interactions, and soil texture categories (coarse, medium, and moderately fine) as the random effect (Bates et al., 2015; Schielzeth & Nakagawa, 2013; Slaets et al., 2021; Wood & Bowman, 2021). The data were log-transformed to meet the assumptions of normality and homoscedasticity (Williams et al., 2020; Witzgall et al., 2024). We used type III ANOVA using Satterthwaite's method at an alpha level of 0.1 to account for the high data variability anticipated in on-farm studies. Results obtained from the mixed-effects model are shown by plotting a heatmap of *p*-values and interaction plots of fixed effects. A correlation matrix was additionally generated to visualize the relationships among soil response variables through the Pearson correlation coefficient (*r*). Spider diagrams were plotted using Origin Pro 2023b (10.0.5.157).

3. Results

3.1. Correlation between soil properties

We averaged soil data across the three years (data by year presented in Supplementary material S1) to generate a correlation matrix highlighting the linear relationships among various soil properties, as illustrated in Fig. 3. The analysis revealed strong positive linear correlations among all microbial groups (r = 0.75–0.99) and soil organic matter pool indicators (r = 0.74–0.99). The linear associations between microbial

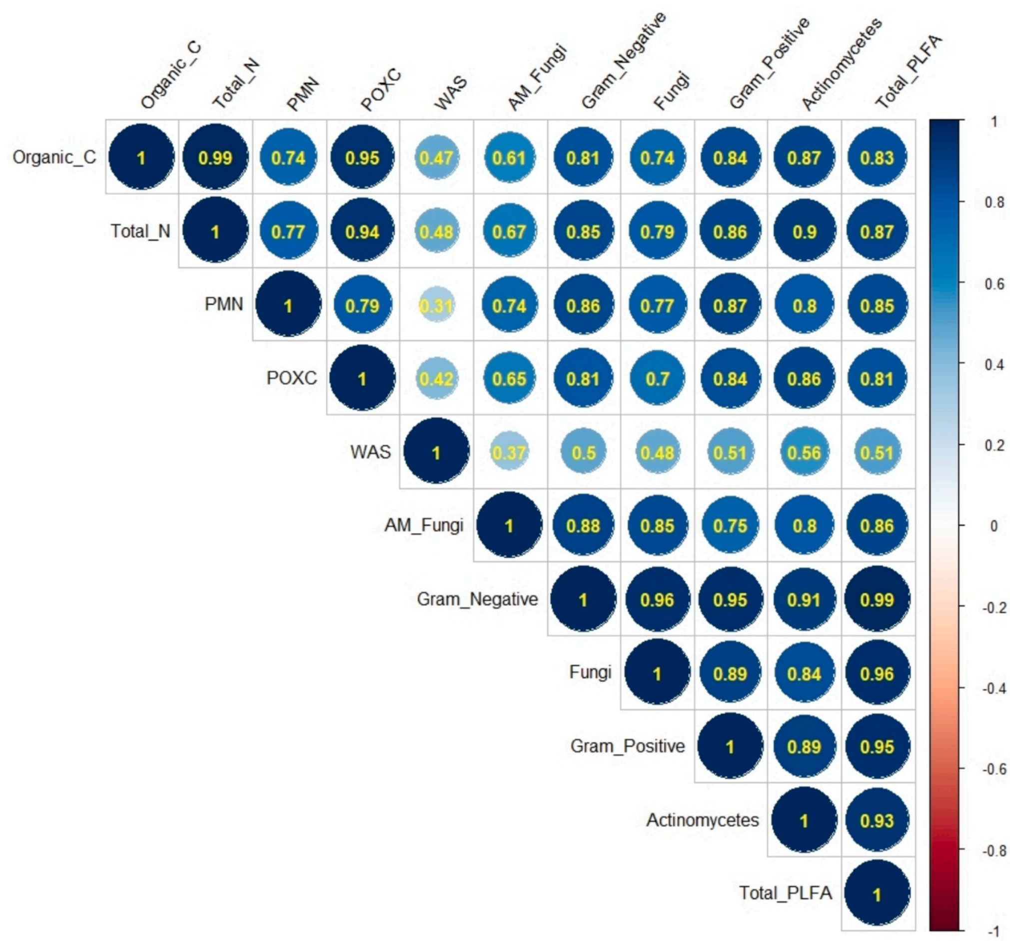

Fig. 3. Matrix representing correlation coefficients (r) among all soil properties averaged across the three years of study. Here, PMN, POXC, WAS, and Total PLFA refer to concentrations of potentially mineralizable nitrogen, permanganate oxidizable carbon, wet aggregate stability, and total phospholipid fatty acids in the soil. AM_Fungi, Gram_Negative, Fungi, Gram_Positive, and Actinomycetes are relative abundance of various microbial guilds within phospholipid extractions.

indicators and nutrients were also strong, with r ranging between 0.61–0.90. The bacterial groups (Gram negative, Gram positive, and Actinomycetes) exhibited a greater association with C and N availability in soil ($r > 0.8$) compared to the fungal groups (Fungi and AM Fungi), where $0.6 < r < 0.8$. Wet aggregate stability showed a moderately positive correlation with both microbial ($r = 0.37$–0.56) and organic matter indicators ($r = 0.31$–0.48).

3.2. Pair-wise response to management

We found that the mean LRR for medium-textured soils were weakly positive, representing higher levels of estimated soil properties in the SH sites than in their paired CV sites (Fig. 4). The only exceptions to this trend were Fungi in 2022 (LRR = −0.035), and PMN and POXC in 2023 (LRR = −0.002 and −0.001, respectively), where slight negative LRR were observed. The highest mean LRR was observed for wet aggregate stability (0.49–0.79). Other moderate to strong positive responses were observed in AM Fungi (0.07–0.31), Actinomycetes (0.04–0.20), and Total PLFA (0.02–0.19). Conversely, moderately fine-textured soils exhibited more variability in their responses with most properties fluctuating between positive and negative ratios across the years. The only exception to this variability was AM Fungi, which showed a consistently positive response (0.16–0.53) in all three years. It is important to note that the higher variability and larger confidence intervals may be attributed to the limited number of sites representing moderately fine-textured soils (two pairs) compared to medium-textured soils (twelve pairs).

To understand how various indicators respond at specific locations, we present the normalized differences in soil indicators at M2 and M9 (Fig. 5), where the calculated management indices for CV sites are identical (Table 1). Practices at SH sites are slightly different, with M2 SH incorporating more diversity in cash and cover crops (CD index is 0.86 for M2 and 0.71 for M9), while M9 SH incorporated cover crops at every available point in rotation (CC index is 0.33 for M2 and 1.00 for M9). At the M2 SH site, only AM Fungi and wet aggregate stability values were greater than CV, whereas M9 SH demonstrated consistently greater values of all soil indicators. The variability in response may be attributed to differences in soil texture (M2 has moderately fine texture, M9 has medium texture), the intensity of practices (more cover cropping in M9), and crop variety. Notably, the M9 SH site has been growing Cannabis as its cash crop for the past five years (2019–2023), which may partially have improved soil properties through the decomposition of Cannabis roots, known to be a rich source of organic matter (Asiimwe et al., 2022), while corn or corn/soybean have a limited positive impact, acting either as a small C source or a sink (Gamble et al., 2021; Suyker & Verma, 2012). The variability specific to these two sites exemplifies the challenge of generalizing soil health results across working farms.

3.3. Soil properties' response to management indices

Using the indexed management variables, we found that combined management practices more strongly influenced soil properties than individual management practices (Fig. 6). All microbial indicators were significantly influenced by the interaction between tillage x cover crops

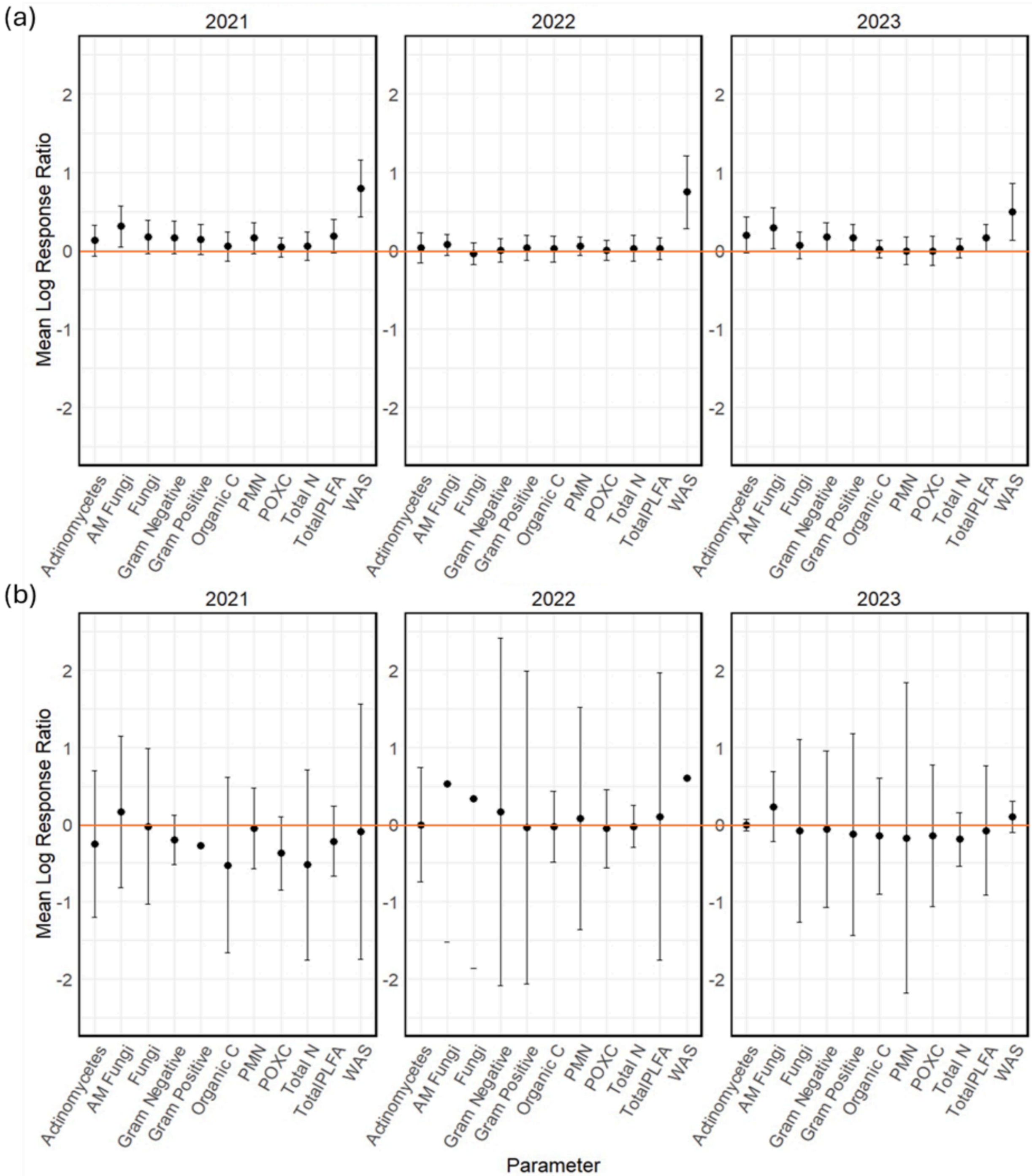

Fig. 4. LRR with 90% confidence intervals for soil properties averaged across sites by texture: (a) medium-textured soils and (b) moderately fine-textured soils. Here, PMN, POXC, WAS, and Total PLFA refer to concentrations of potentially mineralizable nitrogen, permanganate oxidizable carbon, wet aggregate stability, and total phospholipid fatty acids in soil. AM Fungi, Gram Negative, Fungi, Gram Positive, and Actinomycetes are relative abundance of various microbial guilds within phospholipid fatty acid extractions. A positive LRR indicates a higher value in the SH site compared to its CV pair, and negative value indicates higher amounts in the CV site. For a clear comparison between the two texture classes, we kept the y-axis scale the same for both graphs, so confidence intervals for AM Fungi, Fungi, and wet aggregate stability are not visible for moderately fine-textured soils in 2022.

with p values ≤ 0.01. Similarly, the three-way interaction among tillage x cover crops x crop diversity significantly increased the microbial abundance in soil with $p < 0.05$ for all identified groups. Among the organic matter indicators, PMN showed the most significant response to management. The tillage x cover crop and tillage x cover crop x crop diversity factors were significantly associated with increased PMN, while cover crops alone had only a moderately significant effect on PMN ($p < 0.1$). Total N also increased with tillage x cover crop ($p < 0.1$), while POXC was equally influenced by tillage x cover crop and tillage x cover crop x crop diversity ($p < 0.1$). The responses observed in wet aggregate stability and organic C were statistically insignificant; however, they followed the same trend as other indicators with the lowest observed p-values among all practices for tillage x cover crop and tillage x cover crop x crop diversity.

To explore the nature of the two- and three-way interactions in the mixed-effects models, we present the example of total PLFA, which

Fig. 5. Spider diagrams comparing soil properties within a pair for sites M2 (moderately fine texture) and M9 (medium texture). All values were normalized by dividing with the maximum value specific to each parameter across all sites. Here, PMN, POXC, WAS, and Total PLFA refer to concentrations of potentially mineralizable nitrogen, permanganate oxidizable carbon, wet aggregate stability, and total phospholipid fatty acids in soil. AM Fungi, Gram negative, Fungi, Gram positive, and Actinomycetes are relative abundance of various microbial guilds within phospholipid fatty acid extractions.

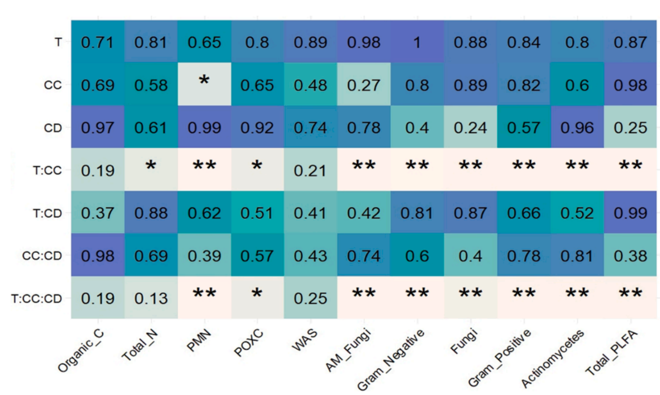

Fig. 6. Mixed-effects model results shown in a heatmap of *p*-values with lighter color representing more significant responses. Here T, CC, CD, T:CC, T:CD, CC:CD, and T:CC:CD refer to tillage, cover crops, crop diversity, tillage x cover crops, tillage x crop diversity, cover crops x crop diversity, and tillage x cover crops x crop diversity, respectively. The parameters PMN, POXC, WAS, and Total PLFA refer to potentially mineralizable nitrogen, permanganate oxidizable carbon, wet aggregate stability, and total phospholipid fatty acids in soil, respectively. AM Fungi, Gram negative, Fungi, Gram positive, and Actinomycetes are relative abundance of various microbial guilds within phospholipid fatty acid extractions. * and ** indicate $p < 0.1$ and < 0.05.

appears to decline with tillage (Fig. 7a). However, tillage reduction has a stronger impact when combined with more frequent cover crops (Fig. 7b), represented by steeper plot slopes for farms with cover crops (CC indices = 0.3–1.0) compared to reduced-tillage sites without cover crops (CC index = 0). Similarly, more crop diversity is associated with higher PLFA levels under reduced tillage conditions (Fig. 7c). Therefore, reduced tillage combined with more cover crops and higher crop diversity appears to be the best practice for attaining higher microbial biomass. The pattern exhibited by the interaction plots for total PLFA data was similarly observed in other soil properties as well. This is consistent with our earlier analysis, where most of these properties were found to be highly correlated, indicating a likely similar influence of management practices across multiple soil health indicators.

4. Discussion

Indexing sites based on the intensity of their management practices allowed us to assess the responses specific to individual practices and their combinations in our mixed-effects model. This approach allowed us to assess whether the soil response was proportional to the intensity of practice application, which varied widely within and beyond the SH and CV categories. For example, the CV site in W4 has a Tillage Index = 0.33, CC Index = 0.58, and CD Index = 0.86, scoring similarly to M2 and M5 SH sites (Tillage, CC, and CD indices are 0.04, 0.33, 0.86 and 0.00, 0.50, 0.43, respectively). In another case, the M7 sites differed only in their tillage practices (0.75 for CV and 0.13 for SH) while the CC (0.00) and CD (0.29) indices were the same. Indexing enabled us to center management practices in our analysis, interrogating whether similar practices resulted in similar soil response, while still controlling for texture. We acknowledge the breadth of practices covered by this study as both a weakness and a strength, as we lack the power to pinpoint specific soil impacts of practices applied consistently but can confidently report soil dynamics in realistic farm management scenarios.

The mixed-effects model and LRR results together provided complementary insights into how management practices affected soil properties. By accounting for the varying intensities of tillage, cover crops, and crop diversity, the mixed-effects model captured the combined effects of these practices on soil microbial properties and soil organic matter pools through PMN and POXC. However, the model did

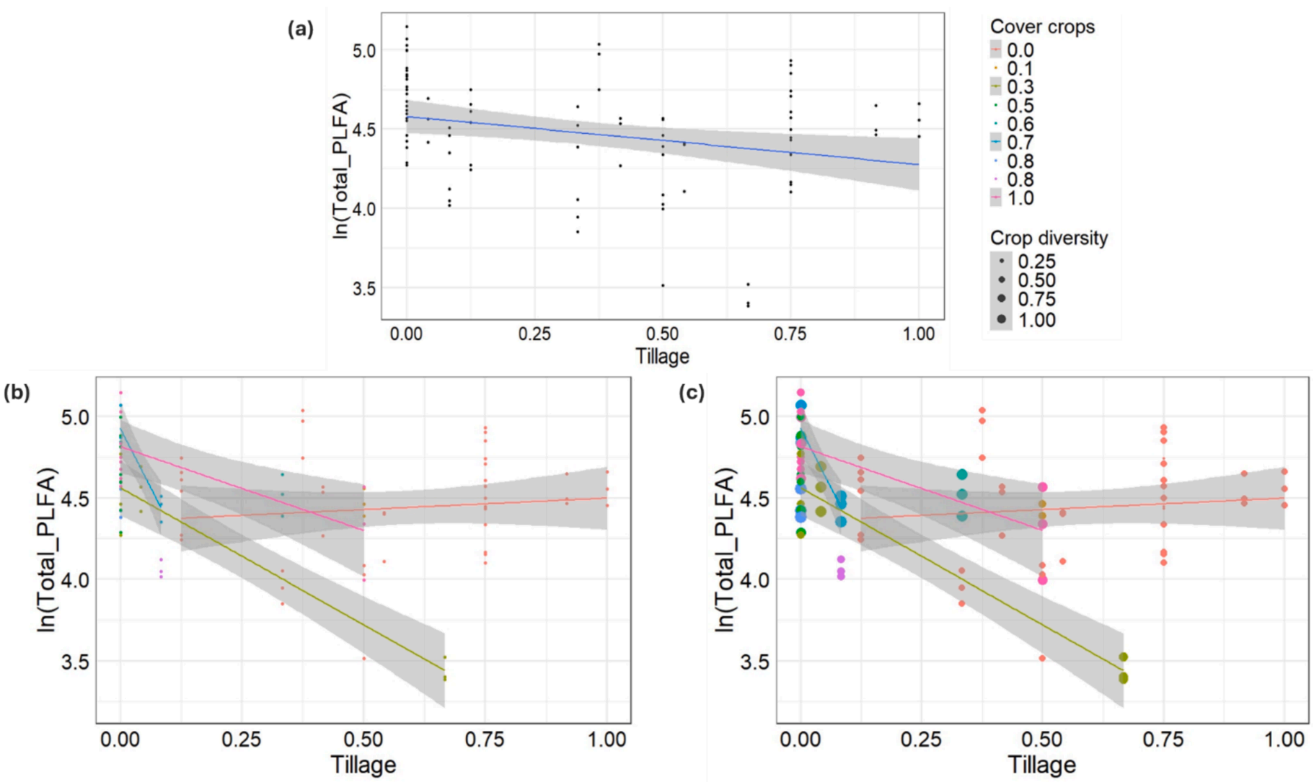

Fig. 7. Interaction plots showing natural log values of (a) Total PLFA vs tillage, (b) Total PLFA vs tillage for different cover crop levels (represented by color), and (c) Total PLFA vs tillage for different levels of cover crops and crop diversity (represented by color and size, respectively). The grey area shows the 95% confidence intervals around the regression lines. Total PLFA indicates the total phospholipid fatty acids in soil.

not detect a significant effect on wet aggregate stability, which showed a positive response in the LRR analysis. This could be explained by the fact that LRR is more sensitive to localized changes because it compares SH and CV sites within each pair, demonstrating the beneficial effects of management on wet aggregate stability. This difference emphasized the usefulness of LRR in detecting site-specific or localized effects that may not be visible in a more complex, site-wide model like the mixed-effects model.

4.1. SH versus CV fields

We categorized the data by texture to calculate the mean LRR specific to each soil texture group. The LRR values associated with wet aggregate stability and AM Fungi were consistently positive across both groups, highlighting their sensitivity to soil health practices. Previous studies have revealed that AM Fungi strongly respond to soil health-based management (Aranguren & Cañón, 2023; Muhammad et al., 2021; Njeru et al., 2014). They promote soil health development by enhancing plant nutrient availability, ecological interactions, and soil aggregation through the secretion of soil proteins and exudates (Giri & Varma, 2020; Lehmann et al., 2020a,b; Parihar et al., 2019). Therefore, higher levels of AM Fungi are typically associated with strong soil aggregation and ecological functions (Giri & Varma, 2020; Rillig et al., 2015). Our findings support this idea as SH sites demonstrated higher levels of both AM Fungi and wet aggregate stability (Blair et al., 2024). Williams et al. (2020) reported that wet aggregate stability exhibited the most sensitivity to management while comparing soil properties between managed and unmanaged soils. Although our study did not include non-degraded or unmanaged reference sites (Aranguren & Cañón, 2023; Williams et al., 2020), each paired field in our study served as a reference for its counterpart, to identify the direction of changes in soil health. While wet aggregate stability did not depend on the intensity of management practices, as suggested by our mixed-effects model results, the positive LRR indicates that wet aggregate stability consistently improved in many local contexts when soil health principles were applied, regardless of the specific intensity levels of these practices. Among all the complexity surrounding soil health assessment and ecosystem service provision, the consistent response of this property may provide a way forward for land managers and policy makers to assess and document improvements in soil health, which may provide climate adaptation and water quality benefits (Lewandowski & Cates, 2023).

The response to soil health management showed more profound effects in medium-textured soils than in moderately fine-textured soils. Similar results have been reported in literature while comparing texture-wise response of soil properties to cover crops (Blanco-Canqui et al., 2015; Blanco-Canqui & Jasa, 2019; Muhammad et al., 2021; Salazar et al., 2022) and tillage (Lozano et al., 2013; Taboada et al., 1998). Muhammad et al. (2021) outlined that medium-textured soils support microbial growth due to their ability to provide optimal levels of soil aeration and moisture whereas finer soils, due to anaerobic conditions, restrict the growth of aerobic microorganisms. A similar set of paired on-farm observations noted lower PMN and extracellular enzyme activities in sites with the highest clay content (Blair et al. 2024). Brennan and Acosta-Martinez (2017), argued that decomposition of organic material is faster in coarse or medium-textured soils compared to fine-textured soils, making changes in microbial biomass harder to detect in medium-textured soils, but we did not have sufficient coarse-textured soils data to effectively test this hypothesis.

4.2. Impact of management practices

Our first hypothesis was partially confirmed as practices based on soil health principles resulted in healthier soils, reflected by increased microbial population and organic matter pools. However, the response was inconsistent. We also found that soil properties were most responsive to integrated management practices, supporting our second hypothesis. This aligns with the findings from a long-term field-plot experiment conducted at Cornell University spanning over 20 years (1992–2016, sampled in 2017) focused on soil health practices (Nunes et al., 2018). While long-term no-till significantly improved soil health, the benefits were further amplified by the addition of cover crops over 4 years and perennial grass rotations for 12 years (Nunes et al., 2018). This highlights how crop rotation and cover crops enhance soil health in ways that complement tillage practices, as no-till is less effective without the soil organic matter-building impact of these practices (Mitchell et al., 2017). Similarly, Williams et al., (2020) found that a combination of crop diversity, tillage, and organic amendments improved soil properties on 20 farms in Sweden.

There are several potential reasons why a combination of no tillage, more cover crops and crop rotations work best together. One explanation is that when tillage is combined with cover crops, it accelerates the decomposition of labile organic matter inputs from the cover crops, reducing their potential to build long-term soil C. Tillage exposes more soil surface to oxygen and nutrients, increasing microbial activity and leading to a faster breakdown of cover crop biomass, potentially reducing the amount of biomass remaining in fall soil (Six et al., 2002). In contrast, a no-till environment is relatively stable, providing some protected soil environments where organic matter decomposes at a slower rate. This allows cover crop C inputs to persist for a longer period and provides sustained benefits such as improved soil structure, moisture retention, and nutrient availability. Cover crops and crop rotations may also improve the agronomic outcomes of a no-till system, as diverse rotations have been shown to increase yields (Smith et al., 2008; Zhao et al., 2020), and cover crops may help suppress weeds (Osipitan et al., 2018; Scopel et al., 2013). Higher yields add more organic matter to the system in the form of crop residues. Crop rotations also improve soil health by promoting biodiversity, disrupting disease cycles, and preventing the accumulation of pests commonly found in monoculture systems.

We observed that microbial groups were more sensitive to soil management than organic matter pools, which is consistent with the findings of a *meta*-analysis conducted by Stewart et al. (2018). Balota et al. (2014) observed a similar trend where microbial parameters responded more strongly than organic C to 23 years of winter cover cropping and varying tillage systems in an Oxisol from Paraná State, Southern Brazil. In contrast, a 31-year study showed comparable improvements in both microbial and nutrient properties of the soil (Mbuthia et al., 2015). This may suggest that microbial parameters are quicker to react to environmental changes including in-season fluctuations (Leitner et al., 2021; Muhlbachova et al., 2015), whereas organic C changes are relatively slow to manifest (Angers & Eriksen-Hamel, 2008; Stott, 2019). Although a few studies documented positive changes in organic C within 5 years of improved management (Bai et al., 2019; McCarty et al., 1998), others showed that changes may take several decades to occur (De et al., 2020). Significant soil C losses are common in corn-dominated cropping systems, even when cover crops are used, suggesting that management practices may be only marginally effective in mitigating huge C losses associated with harvest activities (Cates & Jackson, 2019; Gamble et al., 2021). In addition, cooler climates and shorter growing seasons make establishing of winter cover crops difficult, and low establishment provides only marginal benefits (Strock et al., 2004; Wilson et al., 2014).

The only indicator reflecting the soil's capacity to supply nutrients to crops that showed a significant relationship with individual cover cropping practice ($p < 0.1$) is PMN, which estimates N supply to plants through microbial processes (Mahal et al., 2018). The use of cover crops prolongs the period of primary productivity, decreasing N losses from the system through leaching or runoff by transforming inorganic N into organic N, and contributes extra residue, which after decomposition, may enhance labile organic matter pools and PMN levels. In addition to retaining N within the system, legume cover crops also introduce N into the system by fixing atmospheric N, which eventually contributes to plant-available N forms through the process of mineralization, as reflected by increased PMN (Sanchez et al., 2001; Tonitto et al., 2006). While cover crops can increase the total C and N in the longer term, increases in labile fractions such as PMN or POXC may be quicker (Chahal & Van Eerd, 2020). In a *meta*-analysis of 43 published studies, Mahal et al. (2018) found a substantial 211 % increase in PMN levels in studies planting legume crops and a 77 % increase with a mix of legume and non-legume crops, while non-legume cover crops showed little to no effect. In contrast, Moore et al. (2014) reported a 38 % increase in PMN under non-legume cereal rye (*Secale cereale*) cover when planted after corn or corn-soybean rotation. Peregrina et al. (2012) examined the effects of resident vegetation cover in a vineyard in Northern Spain, dominated by annual grass and forbs (such as *Bromus hordeaceus L. ssp. hordeaceus*, *Hordeum murinum L.*, *Diplotaxis erucoides (L.) DC.*, *Sonchus asper (L.) Hill*, etc.). Their findings demonstrated improvements in PMN levels and reduction in soil N-NO_3^- pools, indicating a more active microbial biomass promoting N immobilization and recycling under cover crop treatments. In our study, most fields used grasses and brassicas as cover crops, while others planted a mixture of legumes and non-legumes (*Secale cereale*, *Camelina sativa*, *Triticum secale*, *Trifolium pratense*, etc.), making it challenging to identify whether the difference in cover crop variety had any noticeable effect.

5. Conclusion

In this large-scale on-farm study, we assessed the impact of diverse management practices on soil health. Our statistical findings highlight the benefits of adopting integrated management approaches (reduced tillage, increased use of cover crops, and greater crop diversity) to promote healthier farm soils, lending support to some policy initiatives that promote bundling of practices for higher cost-share payment rates. Among all the parameters investigated, PLFA microbial biomass and PMN emerged as highly sensitive indicators in comparison to organic C, total N, and POXC. We noticed significant improvements in wet aggregate stability and AM Fungi levels when fields employing integrated soil health management were directly compared with paired conventionally managed fields, regardless of soil texture. However, sites with medium-textured soil were more responsive to soil health management than moderately fine-textured soils. We observed that both analytical approaches, mixed-effect modeling and LRR comparison between pairs, revealed interesting differences in soil health. While the former gave us a picture of indicator responses across sites and textures, LRR was useful in detecting localized responses to management systems.

Our results showed that integrated soil health management is more effective at increasing common soil health indicators' levels but also reveal the relatively weak response of many common indicators when examined independently. Researchers must continue to search for indicators that are not only responsive to management (such as microbial populations, PMN, POXC, and wet aggregate stability in this study) but are also useful for making on-farm decisions and predicting environmental outcomes. Our dataset illustrated the moderate effectiveness of a paired survey approach, including a replicable approach for parsing complicated agronomic management into continuous indices of soil health intensity.

CRediT authorship contribution statement

Anuradha Garg: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Samuel Kwakye:** Writing – review & editing, Supervision, Methodology, Data curation. **Anna Cates:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Heidi Peterson:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Kathryn LaBine:** Writing – review &

editing, Investigation, Data curation. **Greg Olson:** Writing – review & editing, Investigation, Data curation. **Vasudha Sharma:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Heidi Peterson reports financial support for the project was provided by United States Environmental Protection Agency. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2025.117214.

Data availability

Data will be made available on request.

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