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ORIGINAL PAPER

Soil organic carbon, aggregation and fungi community after 44 years of no-till and cropping systems in the Central Great Plains, USA

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Abstract

Implementing sustainable agricultural land management practices such as no-till (NT) and diversified crops are important for maintaining soil health properties. This study focuses on the soil health of three long-term (44 years) tillage systems, NT, reduced tillage (RT), and conventional tillage (CT), in monoculture winter wheat–fallow (W-F) (*Triticum aestivum* L.) and wheat–soybean (W-S) (*Glycine max* (L.) *Merrill*) rotation. Soil organic carbon (C) was higher in NT than CT in the surface 0–5 cm, but not different in the 5–15 cm, demonstrating SOC stratification on the soil profile. The soil water content was higher in NT followed by RT and CT in the top 0–5 cm. We found an association between increased carbon, aggregation, and AMF biomass. Greater soil aggregation, carbon and AMF were observed in NT at 0–5 cm soil depth. The W-S cropping system had greater soil microbial community composition based on fungi biomass, AMF and fungal to bacteria ratio from phospholipid fatty acid analysis (PLFA). Large macroaggregates were positively correlated with total C and N, microbial biomass, Gram+, and AMF. Soil water content was positively correlated with macroaggregates, total C and N, and AC. No-till increased soil carbon content even after 44 years of cultivation. By implementing conservation tillage systems and diversified crop rotation, soil quality can be improved through greater soil organic C, water content, greater soil structure, and higher AMF biomass than CT practice in the Central Great Plains.

Keywords Soil health · Crop diversification · PLFA · Tillage · Monoculture

Introduction

Soil health is defined as the ability of a specific soil to function in a natural or controlled ecosystem to nourish plants and animal production, preserve water and air quality, and maintain human well-being (Bünemann et al. 2018; Karlen et al. 1997). The future of food production, carbon (C) sequestration, and resistance to extreme weather patterns partially depend on the health of soils, which are affected by agricultural cultivation in soil. Soil health, crop management, selection, and rotation are critical to climate-resilient agricultural production (Sarto et al. 2020a; Tubiello et al. 2002). The goal of research in agricultural soil practices is to identify areas of C preservation or the prevention of accelerated organic C oxidation while sustaining soil health and crop yields (Sarto et al. 2020b; Loveland and Webb 2003). Soil health of the US central Great Plains are especially critical as these soils are vulnerable with wind and water erosion and soil moisture retention is critical to sustain crop productivity (Rice et al. 2022).

Land management practices, such as tillage, fertilizer, crop rotation, integrated systems (Sarto et al. 2020a), and herbicides affect the soil quality, specifically structure, moisture, nutrients, and biota (Paul 2014). There are various advantages and disadvantages with no-till (NT), reduced

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tillage (RT), and conventional tillage (CT). Generally, NT practices are widely performed to maintain soil moisture, control erosion, sequester organic C, and preserve soil fertility (Pires et al. 2020; Izumi et al. 2004; Baker et al. 2007). Crop residue under NT conditions decomposes slower due to the thermostability of organic compounds, less microbial accessibility, and less favorable temperature and moisture conditions (Cooper et al. 2021; Franzluebbers et al. 1995; Loveland and Webb 2003). Crop rotation can also, potentially increase P and K contents in the topsoil layer (Bassegio et al. 2015; Sarto et al. 2021). Reducing soil disturbance through RT reduces the negative impact on soil quality (Reicosky 2003). Intensive crop cultivation is also known to unsettle soil aggregates, reduce water infiltration, increase wind and water erosion, and reduce soil organic matter (Rice et al. 2022; Sarto et al. 2020c; Pires et al. 2020; Beare et al. 1997; Roger-Estrade et al. 2010).

Biologically, soil organic carbon is the source of carbon and energy for most soil microorganisms and fauna. Soil organic carbon accumulation is the net effect of increasing C inputs and decreasing C losses in agricultural soils (Rice et al 2022). Increased soil organic carbon enhances the biomass and diversity of the soil biota (Sarto et al. 2020a, b, c; Rice et al. 2022). Increasing crop frequency and species diversity in agricultural systems promote nutrient cycling, microbial biomass and activity, aggregate stability, and soil protection. Crop diversification may increase the soil microbial community composition (Pires et al. 2020).

The cropping system and tillage practices implemented have been studied to explore the change in soil water content, soil pH, plant-available nutrient, water-stable aggregates, total C and nitrogen (N), and soil microbial community composition as indicators of soil health. The objectives of this study were to: (i) evaluate differences in soil pH, aggregate size distribution, extractable soil nutrients, and phospholipid fatty acid (PLFA); and (ii) distinguish different soil properties under different tillage management systems for long-term (44 years) continuous wheat–fallow (W-F) and wheat–soybean (W-S) rotations in the Central Great Plains of the U.S. This study has three main points, the first is the long-term tillage and crop rotation interactions as an asset for soil quality assessment, the second is the PLFA analysis for microbial community composition, and the third is the evidence of carbon retention in the 0–5 cm surface with time for no-till in the Central Great Plains.

Materials and methods

Site description

The field experiment was conducted at Ashland Bottoms, Kansas (Riley County: 39°07′ N. 96°36′ W), located approximately 14.5 km south of Manhattan, Kansas (Doyle et al. 2004). The agriculture site started in 1974 and was previously a flood plain of the Kansas River, now made up of 20% clay, 71% silt, and 9% sand in the 0–5 cm depth (McVay et al. 2006). The soil type of the field is characterized as a Muir silt loam (*fine-silty, mixed, mesic Cumulic Haplustolls*). The site received 746 mm of precipitation in 2017 and 820 mm of precipitation in 2018 with an average of about 870 mm per year since the start of the experiment. Before the initiation of the study, the Ashland Bottoms comprised of continuous moldboard plow or CT wheat production for 60 years (Doyle et al. 2004). Selected soil chemical and physical characteristics were determined (0–5 and 5–15 cm) 44 years after the beginning of the experiment (Table 1).

Experimental design and treatments

The treatments consisted of three tillage (NT, RT, and CT) methods and two cropping systems (winter Wheat–Fallow, W-F and Wheat–Soybean, W-S) from 1975 to 2018. Three tillage treatments with four replications were applied to the cropping systems in a split-plot randomized block design with study plots of 6.1 × 18.3 m (Doyle et al. 2004). Disk or chisel operations were avoided in no-till plots before planting the crops and after harvesting. The crops were planted directly into the plant residues, and fertilizer was broadcast before planting without soil integration. The field annually received 100 kg N ha^{-1} and 10 kg P ha^{-1}. Herbicide was used for weed control when needed. The CT plots were similar to local tillage practices. Soybean plots were disked (15 cm) and chiseled (25 cm) once a year after harvesting in the summer and left unplanted until the spring. Wheat was planted in October by drilling and harvested in the summer. A disk or rotary tiller (5–10 cm) incorporated broadcast fertilizer, followed by planting. A combination of mechanical and chemical herbicides were used for weed control but varied by weed emergence. Reduced tillage plots were not tilled in the fall after harvesting but tilled before planting, similar to CT plots using a disk-field cultivator. Herbicide

Table 1 Soil properties measured in 2018 after long-term (44 years) tillage and crop rotation

Soil properties	0–5 cm	5–15 cm
pH	5.2	5.0
P Mehlich^{-3}	150	98
Ca^{2+} (mg kg^{-1})	3016	2085
Mg^{2+} (mg kg^{-1})	217	245
K^{+} (mg kg^{-1})	461	312
Sand (%)	9	10
Silt (%)	71	70
Clay (%)	20	20

was broadcasted onto the soil surface and not incorporated with tillage.

Soil sampling

Composite soil core samples (300–600 g) were taken with an inside diameter of 18 mm after plant harvesting on August 30, 2018, from all tillage systems and crop rotations divided into depth increments of 0–5 and 5–15 cm for phospholipid fatty acid analysis. Phospholipid fatty acid soil samples were placed in polyethylene Ziplock® Brand Freezer Bags (S. C. Johnson & Son Inc., Racine, WI) and kept in a cooler with ice for approximately 2 h before being stored in a laboratory freezer held at − 4 °C until analysis. Intact soil samples for gravimetric soil water content, soil pH, soil extractable cations, and water-stable aggregate analysis were taken from 0–5 to 5–15 cm soil horizons with a spade and stored in at room temperature as described by Mikha and Rice (2004). Phospholipid fatty acid soil samples were taken with multiple core samples to get a representative homogeneous mixture for biological analysis.

Gravimetric soil water content

Moist samples from the field were passed through a 4 mm sieve to remove large roots, rocks, and organic matter. The water content of the soil samples was determined by weighing and oven drying 10 g of the sieved soil at 105 °C for at least 48 h until constant weight. After 48 h of drying, the weight of the oven-dry soil was used with the weight of the tin and moist soil. The gravimetric soil water content percent was determined for each soil using the equation:

$$\% \text{ Soil water} = \frac{\text{Moist Soil Sample (g)} - \text{Oven Dry Soil (g)}}{\text{Oven Dry Soil (g)}} x100$$

Soil pH, nutrients, and bulk density

The soil samples from each depth and treatment were homogenized, air dried, roots removed, passed through 2 mm sieves, and then sent to the Kansas State University Soil Testing Laboratory for soil pH and chemical analysis on plant-available micro- and macro-nutrients in soils. The Mehlich-3 extractable phosphorus was determined by using Lachat Quickchem 8000 to perform colorimetric assays as described by Frank et al. (1998). The cations, specifically calcium, potassium, and magnesium, were analyzed by an inductively coupled plasma (ICP) spectrometer after being extracted with ammonium acetate (1 M, pH 7.0) and low-sodium filter paper (Warncke and Brown 1998). Bulk density was determined using a stainless-steel core (50 mm internal diameter and 50 mm length) that was introduced into the soil at 0–5 and 5–15 cm depths, removed, and oven-dried (105 °C, 48 h) (Sarto et al. 2020a).

Water-stable aggregates

The fresh soil samples were separated along natural breaks, air-dried for 24 h, and pre-sieved with a 4 mm diameter sieve to remove large stones and organic matter for aggregate analysis. Fifty grams of soil was weighed and placed in a Yoder wet-sieving apparatus modified for recovery of all particle fractions as described by Mikha and Rice (2004). Each soil sample was separated into four aggregate size classes (4000–2000 µm, 250–2000 µm, 53–250 µm, and 20–53 µm diameter). The air-dried soil was placed on the top sieve of the 2000 µm, above the 250–2000 µm, and 1 L of distilled water was added to submerge the soil in water for 10 min before the 10 min wet-sieving action. The oscillation time at 10 min, stroke length at 4 cm, and frequency 30 cycles min^{-1} were held steady. After the soil was wet-sieved, the oscillation container was poured into the finer sieves of 53 and 20 µm diameter. Floating organic matter was removed in the 2000 µm fraction sieve. The individual particle fractions were dried out at 55 °C for over 24 h until the water completely evaporated out. Each dried fraction was then weighted to determine the percent aggregated aggregate size fraction within the soil. Aggregates from each tillage treatment were fractionated into macroaggregate (4000–2000 and 250–2000 µm) and microaggregate (53–250 and 20–53 µm) size classes.

Total carbon and nitrogen

To determine SOC and TN, four soil cores per plot were taken at depths of 0–5 and 5–15 cm to account for within-plot variability in October 2018. Air-dried soil samples with roots removed were ground into a fine powder with a mortar and pestle. Plant material was removed during the process. The powder was sieved through a 53 µm diameter mesh sieve and analyzed by dry combustion using a C/N Elemental Analyzer gas chromatograph with a thermal conductivity detector (Flash EA 1112 Series Thermo Finnigan Italia S.p.A., MI, Italy) (Mikha and Rice 2004; Rice et al. 2021). Stocks of SOC were calculated using measured bulk density, SOC, depth of the soil layer and compared in equivalent soil masses following the method described by Wendt and Hauser (2013). We analyzed SOC from the beginning of the experiment in 1974, 2002, and 2018.

Phospholipid fatty acid analysis

The total lipids were extracted from 5 g of soil using a modification of the Bligh and Dyer (1959) extraction (White and Rice 2009). Soil cores were divided into 0–5 and 5–15 cm depths and combined by depth to make a composite sample per plot. Samples were frozen, lyophilized, and ground with a mortar and pestle. Soils were incubated in 2 methanol:1 chloroform:0.8 phosphate buffer to extract the phospholipid fatty acids (PLFA) from the neutral and glycol lipid fatty acid. Using silicic acid chromatography, phospholipid fatty acids were separated from the total lipid extract. The fatty acids were cleaved from the glycerol backbone, and the harvested fatty acids were methylated to form fatty acid methyl esters (FAME). The FAMEs were analyzed using a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) equipped with a DB5-MS column (30 m × 250 µm i.d. × 0.25 µm film thickness; Agilent Technologies, Santa Clara, California, USA). FAME peaks were identified by comparison with the bacterial acid methyl esters mix (BAMEs; Matreya LLC, USA). Tentative assignments of FAME peaks that were not present in the BAMEs mix were made by mass spectral interpretation. Peak concentration was quantified using the internal standard methyl nonadecanoate (19:0). Fatty acids were grouped into gram-positive bacteria (i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-negative bacteria (2-OH 10:0, 2-OH 12:0, 3-OH 12:0, 2-OH 14:0, 3-OH 14:0, 16:1ω7c, cy17:0, cy19:0), actinomycetes (10-Me 16:0 and 10-Me 18:0), Arbuscular mycorrhizal fungi (AMF) (16:1ω5), and fungi (18:2ω6,9c). The fungal to bacterial (F:B) ratio is determined by dividing the sum of AMF and saprophytic fungi PLFA by the sum of actinomycetes, gram-positive, and gram-negative bacteria PLFA. Total PLFA biomass is the sum of the microbial groups and common fatty acids measured (11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0) (Pires et al. 2020; Sarto et al. 2020b,c). The fungal to bacterial (F:B) ratio is determined by dividing the sum of AMF and saprophytic fungi PLFA by the sum of actinomycetes, gram-positive, and gram-negative bacteria PLFA.

Statistical analysis

The experimental design was completely randomized with four replications and analyzed as a 3 × 2 factorial. The effects were three tillage practices and two cropping systems. The effects from tillage intensity and cropping systems were analyzed using analysis of variance (ANOVA). If significant differences were observed, individual comparisons were made using Tukey test ($p < 0.05$), with the statistical software Sisvar 5.6 (Ferreira 2010). Spearman correlation matrix with significance was calculated to determine the correlation for all possible variables.

Results

Soil water content, soil pH, bulk density, and nutrients

In the topsoil (0–5 cm), NT had significantly ($p < 0.05$) higher soil water content (24.2%) than RT and CT, while CT was not significantly different from RT (Table 2). In 5–15 cm soil depth, NT had significantly greater soil water content (22.7%) than RT, but CT was not statistically different from NT and RT. Monoculture wheat (W-F) had significantly greater percent soil water than the wheat–soybean (W-S) rotation in the 0–5 cm soil depth (Table 2). There was no difference in soil water between the cropping systems at 5–15 cm.

There was no significant difference in soil pH from tillage practices in both soil depths after 44 years of cultivation. However, monoculture wheat (W-F) had significantly lower soil pH in both 0–5 cm (pH = 5.0) and 5–15 cm (pH = 5.3) compared to W-S crop rotation (Table 2). Soil bulk density was different between tillage treatments, but similar between rotations (Table 2). No-till had higher bulk density than RT

Table 2 Effect of tillage and cropping system on soil water content, soil pH and bulk density

	Soil Water (%)	Soil pH	Bulk density (g cm^{-3})
Tillage	0–5 cm		
CT	19.9 b	5.6 a	1.36 b
RT	17.3 b	5.6 a	1.36 b
NT	24.2 a	5.3 a	1.4 a
Rotation			
W-S	18.0 b	6.0 a	1.38 a
W-F	22.9 a	5.0 b	1.37 a
Tillage	5–15 cm		
CT	21.6 ab	5.6 a	1.37 b
RT	20.5 b	5.6 a	1.38 b
NT	22.7 a	5.5 a	1.42 a
Rotation			
W-S	21.4 a	5.9 a	1.38 a
W-F	21.9 a	5.3 b	1.40 a

Different letters in the same column indicate significant differences (Tukey's test, $p < 0.05$)

CT conventional tillage, *RT* reduced tillage, *NT* no-till, *W-S* wheat–soybean, *W-F* wheat–wheat.

and CT at 0–5 and 5–15 cm. The bulk density was not significantly different by the cropping system.

There was significantly greater phosphorus content in the 0–5 cm NT compared to both RT and CT. As soil depth increased from 0–5 to 5–15 cm, the phosphorus content in soils decreased as expected. Soil phosphorus content was higher in the monoculture W-F than the W-S rotation of both 0–5 cm (189 mg kg^{-1}) and 5–15 cm (125 mg kg^{-1}) depths (Table 3). There was no significant difference in the soil cations (Ca, K, Mg, Na) among the tillage types in both soil depths. The calcium content was significantly higher in W-S rotation in both 0–5 cm (2370 mg kg^{-1} of soil) and 5–15 cm (2538 mg kg^{-1} of soil) compared to the W–F monoculture. The magnesium content was also significantly greater in W-S rotation in both 0–5 cm (272 mg kg^{-1} of soil) and 5–15 cm (278 mg kg^{-1} of soil). While the potassium content was significantly greater in monoculture W–F in both 0–5 (508 mg kg^{-1} of soil) and 5–15 cm (361 mg kg^{-1} of soil).

Aggregate-size distribution

No-till had significantly greater soil macroaggregates (53.4%) followed by RT (44.2%) and CT (31.1%) at the soil surface (0–5 cm) (Table 4 and Fig. 1). Cropping systems had no significant effect on macroaggregate in the 0–5 cm soil depth. The CT at 0–5 cm had the greatest microaggregates (59.2%), followed by RT (45.7%) and CT (35.7%). The W-S crop rotation also had a significantly greater microaggregate (53.3%) than W–F monoculture (40.4%) in the 0–5 cm soil depth. There was no significant difference in macro- and microaggregate size distribution in both crop rotations and tillage types for soil depth 5–15 cm.

Table 3 Effect of tillage and cropping systems on soil chemical properties

	P Mehlich-3	Ca	K	Mg
mg kg^{-1} soil				
Tillage	0–5 cm			
CT	138 b	1838 a	460 a	230 a
RT	156 b	1813 a	471 a	220 a
NT	195 a	1842 a	454 a	205 a
Rotation				
W-S	137 b	2370 a	415 b	272 a
W-F	189 a	1292 b	508 a	164 b
Tillage	5–15 cm			
CT	101 a	2153 a	306 a	229 a
RT	118 a	2083 a	301 a	245 a
NT	101 a	2020 a	330 a	260 a
Rotation				
W-S	88.6 b	2538 a	263 b	278 a
W-F	125 a	1632 b	361 a	212 b

Different letters in the same column indicate significant differences (Tukey's test, $p < 0.05$)

CT conventional tillage, *RT* reduced tillage, *NT* no-till, *W-S* wheat–soybean, *W-F* wheat–wheat, *Mehlich-3 P* extractable soil phosphorus, *Ca* extractable soil calcium, *K* extractable soil potassium, *Mg* extractable soil magnesium.

Table 4 Soil aggregate size distribution for macroaggregates (> 250 µm) and microaggregates (20–250 µm) effect by tillage systems and cropping systems

	Aggregate size distribution (%)	
	> 250 µm	20–250 µm
Tillage	0–5 cm	
CT	31.1 b	59.2 a
RT	44.2 ab	45.7 ab
NT	53.4 a	35.7 b
Rotation		
W-S	38.5 a	53.3 a
W-F	47.4 a	40.4 b
Tillage	5–15 cm	
CT	33.6 a	58.3 a
RT	39.9 a	50.1 a
NT	39.5 a	45.4 a
Rotation		
W-S	34.3 a	52.7 a
W-F	41.0 a	49.8 a

Different letters indicate significant differences (Tukey's test, $p < 0.05$)

CT conventional tillage, *RT* reduced tillage, *NT* no-till, *W-S* wheat–soybean, *W-F* wheat–wheat, *> 250 µm* percent of aggregates less than 4000 µm and larger than > 250 µm diameter, *20–250 µm* percent of aggregates less than 250 µm and larger than 20 µm.

Total carbon and nitrogen

Total C content was significantly higher in the NT (18.9 g kg^{-1} soil) at 0–5 cm topsoil compared with CT (12.5 g kg^{-1} soil) (Table 5). In NT, total C and N stratification was indicated by a decline from 18.9 g C kg^{-1} soil and 1.96 mg N kg^{-1} soil in the 0–5 cm depth to 16.5 g C kg^{-1} soil and 1.88 mg N kg^{-1} soil in the 5–15 cm depth.

Soil C stocks were significantly affected by tillage practices over time at 0–5 cm depth, but not at 5–15 cm (Fig. 2). Initially, C stocks were not different with tillage. However, in 2002, C stock was significantly higher in NT and RT compared to CT (Doyle et al. 2004). In 2018, C stock had further increased under NT resulting it being significantly higher than CT and RT in the topsoil layer (0–5 cm). Carbon stock was not different between tillage practices in the 5–15 cm soil layer during the assessment period (44 years). During the 44 years, carbon sequestration into the soils from NT increases by 49%

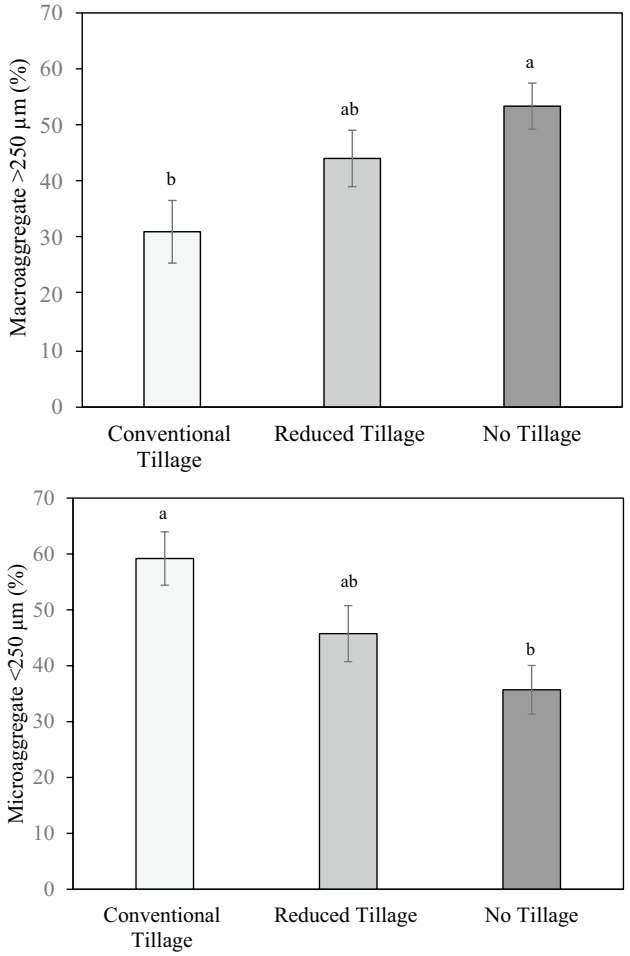

Fig. 1 Soil macroaggregate (> 250 µm diameter) and microaggregate (< 250 µm diameter) in 0–5 cm soil depth on different tillage systems ($p < 0.05$). Different letters indicate significant differences (Tukey's test, $p < 0.05$)

Soil microbial community composition

As soil depth increased from 0–5 to 5–15 cm, soil microbial biomass of the soil microbial communities decreased. No significant difference was observed in total microbial biomass, gram +, and gram- bacteria due to tillage and cropping system in both soil depths (Table 6). However, there was a significantly greater actinomycetes in W–F monoculture (2.33 nmol g^{-1} soil) compared to W-S crop rotation (1.42 nmol g^{-1} soil) in the 0–5 cm soil. Arbuscular mycorrhizal fungi (AMF) were higher in NT (2.12 nmol g^{-1} soil) compared to CT (1.41 nmol g^{-1} soil) in 0–5 cm soil depth, while RT (1.72 nmol g^{-1} soil) was not significant different among tillage treatments. Arbuscular mycorrhizal fungi were significantly greater in monoculture W–F (0.89 nmol g^{-1} soil) compared to W-S rotations (0.63 nmol g^{-1} soil) in the 5–15 cm depth (Fig. 3). Fungi were significantly greater in W-S rotation (0.66 nmol g^{-1}

Table 5 Changes in soil total carbon and total N by tillage and cropping system

Tillage & Cropping system	Total carbon	Total nitrogen
	g kg^{-1} soil	g kg^{-1} soil
0–5 cm		
CT	12.5 b	1.46 b
RT	14.9 ab	1.59 b
NT	18.9 a	1.96 a
W-S	15.2 a	1.56 a
W-F	15.7 a	1.78 a
5–15 cm		
CT	13.3 a	1.62 a
RT	14.1 a	1.64 a
NT	16.5 a	1.88 a
W-S	15.4 a	1.68 a
W-F	13.9 a	1.75 a

Different letters in the same column indicate significant differences (Tukey's test, $p < 0.05$)

CT conventional tillage, *RT* reduced tillage, *NT* no-till, *W-S* wheat–soybean, *W-F* wheat–wheat, *Total Carbon* total combustible organic carbon, *Total Nitrogen* organic nitrogen.

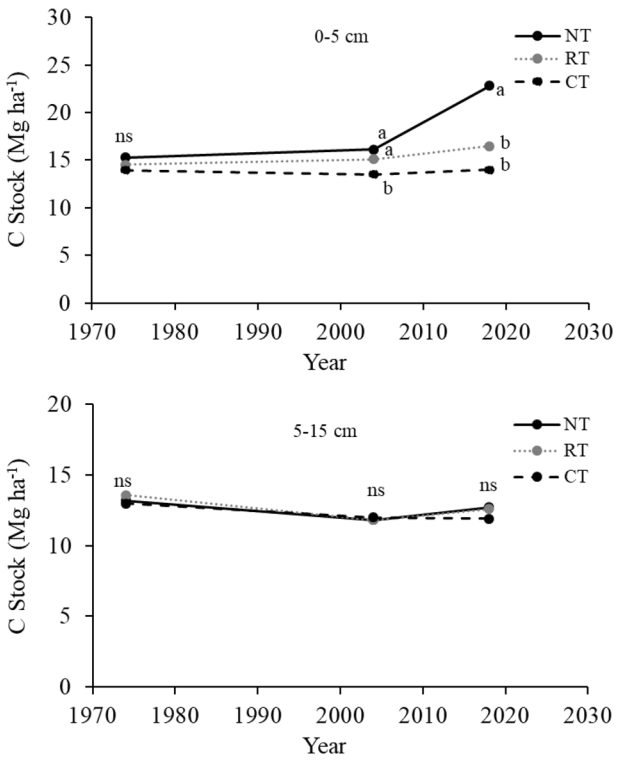

Fig. 2 Effects of tillage practices (conventional [CT], reduced [RT], and no-tillage [NT]) on soil C stock over 44 years in the central Great Plains, USA

Table 6 Changes in soil microbial community by different tillage and cropping systems

Tillage & Rotation	Microbial biomass	Gram +	Gram –	Actinomycetes	AMF	Fungi	Fungal/bacteria
nmol PLFA g^{-1} soil							
0–5 cm							
CT	42.9 ns	13.1 ns	4.3 ns	1.8 ns	1.4 b	1.7 ns	0.17 ns
RT	46.8 ns	13.7 ns	6.0 ns	1.8 ns	1.7 ab	1.8 ns	0.17 ns
NT	47.2 ns	14.6 ns	6.1 ns	2.0 ns	2.1 a	1.4 ns	0.16 ns
W-S	42.9 ns	13.1 ns	5.0 ns	1.4 b	1.6 ns	1.6 ns	0.17 ns
W-F	48.4 ns	14.6 ns	5.8 ns	2.3 a	1.8 ns	1.7 ns	0.16 ns
5–15 cm							
CT	29.2 ns	10.3 ns	3.8 ns	1.5 ns	0.8 ns	0.7 ns	0.10 a
RT	30.2 ns	10.4 ns	4.4 ns	1.8 ns	0.9 ns	0.6 ns	0.09 ab
NT	25.6 ns	9.1 ns	4.8 ns	1.7 ns	0.6 ns	0.3 ns	0.07 b
W-S	29.8 ns	10.3 ns	3.6 ns	1.8 ns	0.9 a	0.7 a	0.10 a
W-F	26.9 ns	9.6 ns	5.1 ns	1.5 ns	0.6 b	0.4 b	0.07 b

Different letters in the same column indicate significant differences (Tukey's test, $p < 0.05$)

CT conventional tillage, *RT* reduced tillage, *NT* no-till; *W-S* wheat–soybean, *W-F* wheat–wheat, *Gram +* gram-positive bacteria, *Gram –* gram-negative bacteria, *AMF* arbuscular mycorrhizal fungi, *Fungi* Saprophytic fungi; *Fungal/bacteria* [(Gram +) + (Gram–) + (Actinomycetes)]/(AMF + Fungi);, *ns* not statistically significant.

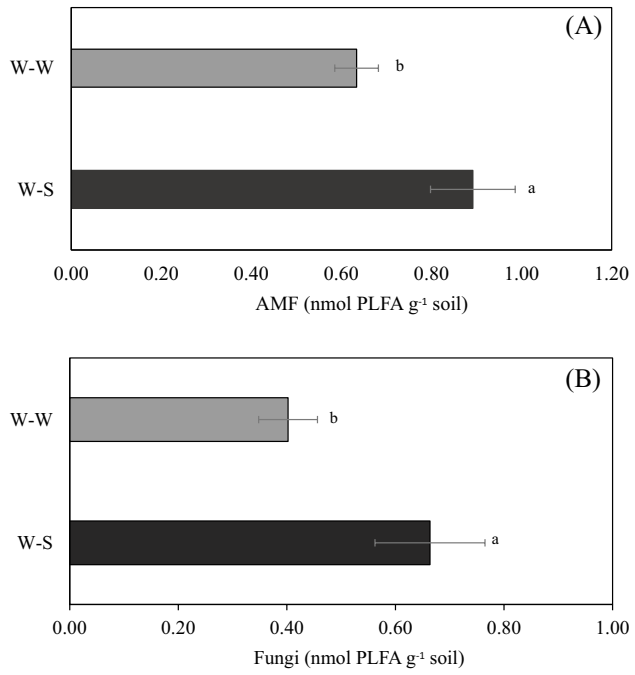

Fig. 3 Arbuscular mycorrhizal fungi **A** and fungi **B** ($n = 12$) affected by cropping system in 5–15 cm soil depth. W-S: wheat–soybean; W-F: wheat–wheat; AMF: arbuscular mycorrhizal fungi. Different letters indicate significant differences (Tukey's test, $p < 0.05$)

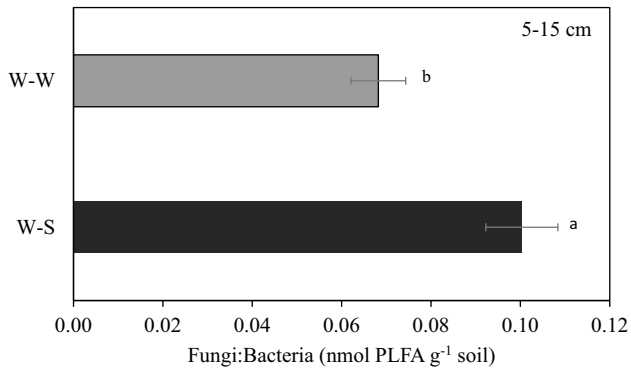

Fig. 4 Fungi:bacteria affected by cropping system at 5–15 cm. W-S: wheat–soybean; W–F: wheat–wheat; Fungi:bacteria: [(Gram +) + (Gram–) + (Actinomycetes)]/(AMF + Fungi). Different letters indicate significant differences (Tukey's test, $p < 0.05$)

soil) compared to monoculture W–F (0.40 nmol g^{-1} soil) in the 5–15 cm depth (Fig. 3). No fungal biomass differences were observed for tillage type for both soil depths and 0–5 cropping system. Fungi to bacteria ratios in the 5–15 cm soil depth were significantly greater in CT (0.10 nmol g^{-1} soil) compared to NT (0.7 nmol g^{-1} soil) but not for RT (0.09 nmol g^{-1} soil). In comparison, W-S rotation (0.10 nmol g^{-1} soil) had a greater F:B ratio than the W–F monoculture (0.07 nmol g^{-1} soil) in the 5–15 cm soil depth (Fig. 4).

Spearman correlations

Measurements with very high positive Spearman correlations coefficients (r) and significant p-values ($\alpha = 0.05$) included Mg and Ca (0.93), pH and Ca (0.9), pH and Mg (0.82), total carbon and total nitrogen (0.92), microbial biomass and gram + (0.92), microbial biomass and AMF

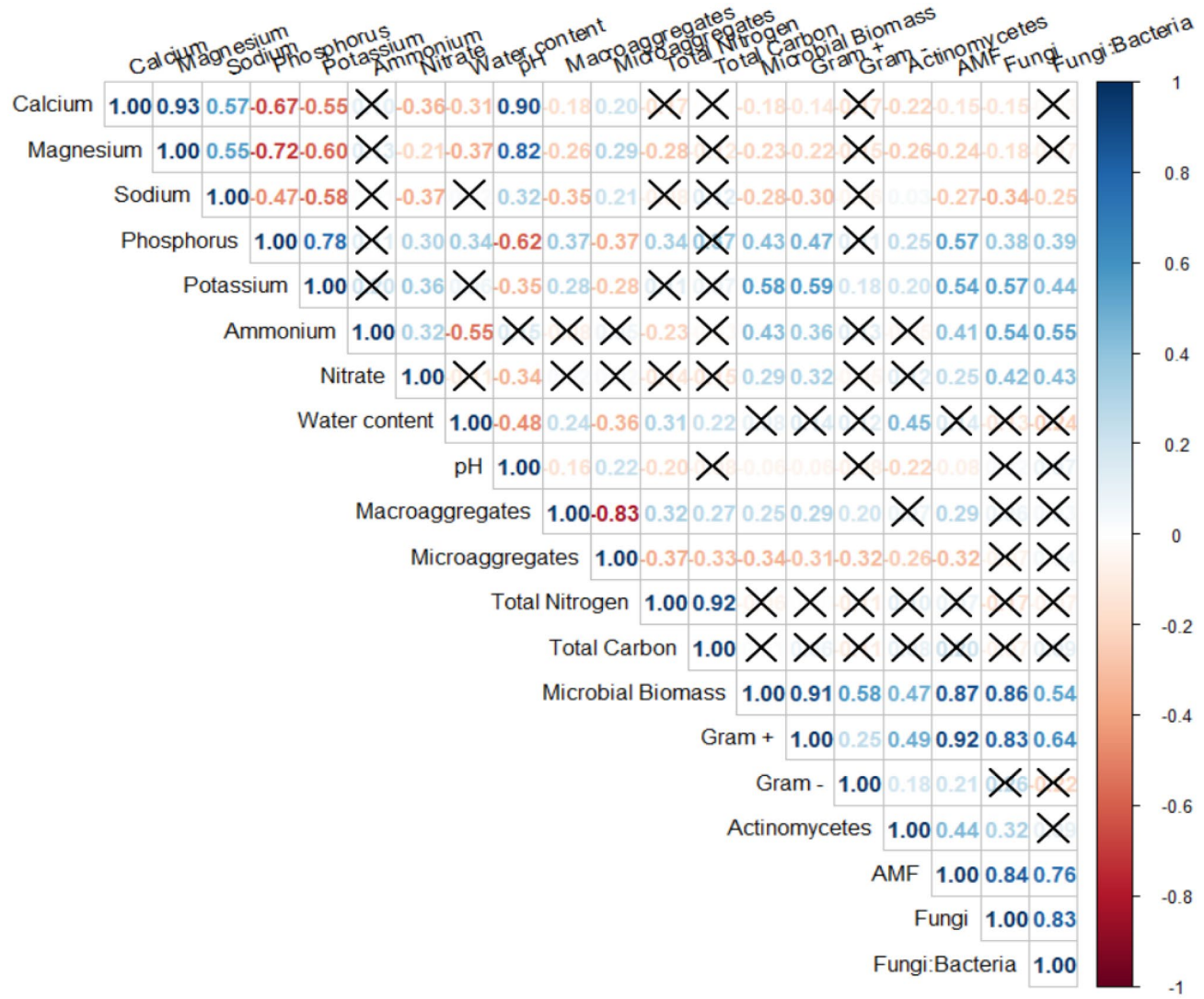

Fig. 5 Spearman correlation of soil chemical, physical and microbial community composition

(0.87), gram + bacteria and AMF (0.92), microbial biomass and fungi (0.86), AMF and fungi (0.84), gram + and fungi (0.83), and fungi and F:B ratio (0.83) (Fig. 5). Measurement with very high negative correlation coefficients and significant p-values included macroaggregates and microaggregates (− 0.83).

Large macroaggregates were positively correlated with total C and N, microbial biomass, Gram +, and AMF. Soil water content was positively correlated with macroaggregates, total C and N, and AC. Microaggregates were negatively correlated with total C and N, Microbial biomass, Gram +, Gram –, AC, and AMF.

Discussion

Soil water content, macroaggregates, and total carbon and nitrogen affected by tillage and crop rotation

In our study, NT had higher soil water content at the 0–5 cm depth compared to RT and CT. Sauer et al. (1996) stated that the presence of residue reduced evaporation as much as 34 to 50%. No-till had significantly greater soil water in the 5–15 cm layer followed by CT and RT. Greater coverage plant residue of the NT soil surface and greater soil C provides an effective barrier to reduce evaporation than bare soil (Van Donk et al. 2010; Klocke et al. 2009). Pikul and Aase (1995) found infiltration rates were greater under NT because of the soil surface protection. The impact of rainfall on a bare soil surface can result in a substantial decrease in

infiltration over very short periods of time, as illustrated by Ben-Hur et al. (1998). The end result on lands with any slope is runoff and less water stored in the soil profile for later use by a crop (McVay et al. 2006). The results of this study support this idea since NT had greater amounts of soil water followed by RT and then CT. The NT system was able to retain the soil water because of superior soil structure and infiltration from greater soil macroaggregates and, greater soil residue on the soil surface (Alvarez and Steinbach 2009; Klocke et al. 2009). Positive correlation of soil water content, macroaggregates, and total C and N were found in this study (Fig. 5) similar to other studies with long-term tillage and cropping systems that suggest greater organic matter storage and water retention in systems with higher macroaggregates formation (Al-Kaisi et al. 2014; Sekaran et al. 2021; Datta et al. 2022).

Soil pH, nutrients and bulk density affected by tillage and crop rotation

Soil pH was lower in W-F in both soil depths partially attributed to the N fertilization (100 kg N ha^{-1}). The decomposition of organic matter can contribute to a decrease in soil pH (Jat et al. 2018; Hong et al. 2019). The pH in topsoil, generally richer in organic matter, produces greater soluble organic acids from decomposition that lowers soil pH (Jat et al. 2018; Hong et al. 2019). Hydrogen ions are produced from the fertilizer oxidation of NH_4^+ to NO_3^-, which increases soil acidity after long-term use (Bouman et al. 1995; Schroder et al. 2011). The soybean crop did not have N fertilizer applied, so hydrogen ions were produced by the ammonia-based fertilizer on wheat crops from the earlier cycles of wheat production. Thus, the W-F rotation had twice the amount of N applied to the field than the W-S. Although not statistically significant, the NT treatment had the greatest soil acidity in the 0–5 cm horizon (pH = 5.3) because the N fertilizer was surface applied and not incorporated into the soil to allow for buffering of soil pH. The soil pH of CT (5.6) and RT (5.6) was slightly higher. Similar corn and wheat studies by Blevins et al. (1978) showed the application of N fertilizers on NT systems acidified the topsoil in a short amount of time. Increased soil acidification from N fertilizer produces protons displacing exchangeable bases, specifically Ca and Mg, and lowers effective cation exchange capacity (CEC) (Bouman et al. 1995). However, the neutralization of the acidic soil condition is easier to manage with lime additives directly applied to the surface soil (Blevins et al. 1978).

Soil phosphorus content was greatest in monoculture (W-F) NT at 0–5 cm, most likely due to surface buildup of phosphorus fertilizer. Soils that have undergone tillage and mixing allowed for the incorporation of phosphorus that leads to uptake through plant growth. Water contributes to the eluviation or the transport of soluble inorganic and organic colloidal particles into lower depths (Paul 2014). The Ca and Mg content was significantly lower in the W-F compared to the W-S for both sampling depths, which could be due to lower CEC from greater soil acidification in W-F plots. Increasing soil acidification (pH 6.5–3.8) leads to higher amounts of extractable cation dissolving and leaching out from exchange sites (Haynes and Swift 1986). Leaching of exchangeable Ca and Mg is evident by the prominent decrease in Ca and Mg for W-F compared to W-S, while all tillage treatments had similar Ca and Mg content. Soil pH below 5.5 has been reported to greatly increase cation leaching (Haynes and Swift 1986). Even though NT had a lower pH of 5.3 at 0–5 cm than both tilled treatments (pH 5.6), NT had higher organic matter content that contributed towards a cation exchange source for accumulating a larger capacity in adsorbing cations (Jiang et al. 2018). The K content was significantly higher in W-S rotation compared to W-F in both sampling depths. Cropping sequences with soybean have a greater positive effect on soil potassium accumulation than grain-based cropping sequences (Singh and Shivakumar 2010).

Aggregate-size distribution affected by tillage and crop rotation

No-tillage had significantly greater quantity of macroaggregates than CT because there was less soil disturbance, and greater soil organic carbon at 0–5 cm. Greater soil organic matter, evident by the greater total carbon in NT 0–5 cm depth, promotes macroaggregate formation. Macroaggregates are resistant to slaking, resilient to compaction, increases water storage, and reduce bulk density (Oldfield et al. 2018; Blanco-Canqui et al. 2009; Nielsen et al. 2005). In this study, large macroaggregates were positively correlated with total C and N, microbial biomass, Gram+, and AMF (Fig. 5). The RT also allowed for greater macroaggregates formation and stabilization in the soil. Mikha et al. (2013) reported that CT managed soils had proportionally more microaggregates and prevented macroaggregate formation from tillage in the top 0–20 cm soil depth. Our study found negative correlation between microaggregates and total C and N (Fig. 8). As a result of long-term tillage, soils are more susceptible to wind and water erosion, greater soil organic carbon decomposition, loss of soil nutrients, and reduced soil structure (Blanco-Canqui et al. 2009; Six et al. 2000; Mikha and Rice 2004). Soil macroaggregate stability (> 250 µm) is highly influenced by soil management practices (Sarto et al. 2020a; Mikha et al. 2013). Mechanical tillage disrupts these large aggregates and releases confined organic substrates for nutrient availability (Maron et al. 2018).

Total carbon and nitrogen affected by tillage and crop rotation

The long-term tillage of crop residues into the lower 15 cm contributed to a more uniform distribution of C and N. Long-term no-till promoted greater C and N stratification from gradual residue additions to the surface profile (Hernanz et al. 2002; Franzluebbers 2002). The RT treatments also contributed to greater crop residue in the surface leading to C stratification, but not to a higher degree as NT soils. In contrast, the tillage effect and subsurface residue incorporation in the CT may have increased soil organic C and N within the 5–15 cm compared to the 0–5 cm soil depth. Based on total C, greater soil C accrual in NT followed by RT and then CT treatments may be due to tillage intensities increasing affecting residue incorporation in the top 0–15 cm soil depth. Tillage disturbs the soil, causes reduced macroaggregates, promotes microbial decomposition of soil organic C and N, generates subsurface compaction, and increases the number of pores but decreases connectivity along pores (Lipiec and Hatano 2003; Galdos et al. 2019; Lienhard et al. 2013; Mikha and Rice 2004). Another factor influencing high total C in NT is soil water content or water-filled pore space (Galdos et al. 2019; Linn and Doran 1984). Soil water content affects the aeration and diffusion of oxygen in the soil pore networks. In turn, water and oxygen levels impact nitrification, denitrification, and microbial respiration in the soil (Linn and Doran 1984). Long-term NT improves physical properties of soil structure thru greater soil pore connectivity, decreases pore tortuosity, and increases macroporosity, which all aid in soil nutrient cycling, water dynamics, gas diffusion, and root growth (Galdos et al. 2019; Pires et al. 2017; Alhameid et al. 2017). Greater soil structure in NT, in turn, increases OM accrual from crop growth and OM protection in aggregates (Alhameid et al. 2017; McCarthy et al. 2008). No-till crops with surface residue contribute to the development of fields with higher water infiltration/drainage and retention (Abid and Lal 2009) and greater undisturbed root development and stabilization (Lynch and Wojciechowski 2015). This influences higher nutrient retention and OM in soil aggregates (Gupta and Germida 2015; McCarthy et al. 2008) since rates of microbial mineralization of crop residue in soil can supply greater plant nutrition during critical plant growth stages (Grzyb et al. 2020).

Common N fertilizers (NH_4^+, NO_3^-, urea, and anhydrous NH_3) applied to fields promote nitrification through microbial oxidation to form nitrite and nitrate in the soil. Even though there was twice as much N fertilizer applied to the W-F rotation, high nitrate concentrations were not evident in W-F fields versus W-S fields. However, total inorganic N was greatest in CT, while total N was greatest in NT. Lower inorganic N concentrations in NT are attributed to multiple factors affect the N cycle; greater potential ammonia volatilization, decreased N mineralization and greater denitrification. Furthermore, total N was significantly greater in NT systems, which indicates organic N protection, specifically in macroaggregates. Significantly higher total C occurred in 0–5 cm of NT systems compared to CT soils. However, total soil C and N measurements may not completely detect changes in nutrient content by tillage. Measurements of biologically active fractions, specifically microbial biomass C and N, could better evaluate nutrient dynamics (Drury et al. 1991; Franzlubbers et al. 1995).

Soil fungal community composition affected by tillage and crop rotation

The soil diversity as measured by soil microbial community composition, changes due to different agricultural management practices, such as tillage, fertilization, and crop rotation (Sarto et al. 2020a; Maron et al. 2018). Changes in soil microbial community composition can predict changes in soil organic matter, specifically plant residue decomposition, energy flows, nutrient cycling, soil aggregation, and soil C sequestration, as affected by different cropping systems (Acosta-Martínez et al. 2011; Lynch and Bragg 1985). Greater microbial diversity is linked to increased nutrient availability and enhanced ecosystem stability and productivity, but functional redundancy from soil microorganisms is unknown (Maron et al. 2018). This study found significantly greater AMF composition in NT compared to CT in the 0–5 cm depth mainly because NT had less soil disturbance, greater soil moisture, and higher soil organic C content. Other studies have exhibited mycorrhizae and filamentous fungi predominating in NT; whereas bacteria dominates in soils that are frequently disturbed with high fertilizer inputs and more available organic matter to decompose (Guggenberger et al. 1999; Frey et al. 1999). Greater bacterial composition was not seen in CT. However, actinomycetes had a significantly higher composition in 0–5 cm of the W-F rotation. Most actinomycetes are strict saprophytes that recycle nutrients and support organic degradation (Goodfellow and Williams 1983). There were no other statistically significant results in microbial community and tillage or crop rotation in the 0–5 cm depth. In general, total microbial biomass was highest in 0–5 cm NT but not statistically significant. White and Rice (2009) concluded NT has the highest total PLFA reflecting the built-up of substrates and recalcitrant metabolites, which leads to C sequestration and soil aggregation (Six et al. 2006).

The soil microbial biomass (total PLFA) decreased as soil depth increased in all treatments, most likely in response to increased bulk density, reduced soil organic matter, and less soil water infiltration. The lack of soil disturbance in

NT may also have contributed to a stratified microbial composition due to stratified soil organic C and N sources. The F:B ratios were unexpectedly higher in CT soils compared to NT soils at 5–15 cm depth. Pires et al. 2020 highlighted that the residue incorporation under CT increased microbial biomass, and total bacteria in deeper layers (10–30 cm), reinforcing that the NT management results in a strong nutrient stratification. Soils with greater disturbance (CT) were expected to have lower fungal biomass and greater bacterial biomass. Overall, the fungal communities were more abundant in CT compared to NT, while bacterial communities were dominant over fungal communities in all treatments.

The diversified cropping rotation (W-S) had significantly greater AMF, fungi, and F:B ratios in the 5–15 cm depth compared with W-F rotation. More diverse crop rotations contribute to higher substrate richness, specifically different crop residues between wheat and soybean. Lupwayi et al. (1999) concluded that legume-based crop rotations increased soil microbial biomass more than rotations without legumes. Long-term studies by Bardgett and McAlister (1999) found that crops that depend more on fertilizer amendments have lower fungal biomass since fungi are negatively affected by high mineral N. The difference in microbial biomass indicates a larger proportion of fungi was active in the decomposition process of diverse crop residue. Microbial communities with greater AMF promote C sequestration, protection, and aggregation (Six et al. 2006). Filamentous growing fungi create an exploratory network supporting nutrient redistribution, space colonization, particulate realignment, and soil entanglement (Ritz and Young 2004).

Conclusions

The 44-year long-term NT soil management promoted AMF, improved aggregation, and contributed to C and N sequestration. Macroaggregate, AMF biomass and total C and N decreased with increased tillage disturbances at the soil surface.

Greater AMF in NT compared to CT reflects a management system that promotes hyphae growth, contributing to greater soil C and N retention and macroaggregate stability at 0–5 cm soil depth.

Higher AMF, saprophytic fungi, and F:B ratios in 5–15 cm in the W-S rotation indicate greater microbial community biomass associated with diverse crop rotations versus monoculture (wheat) system.

No-till increased soil carbon content even after 44 years of cultivation. The results of this study imply long-term conservation practices in higher intense cropping system improve soil quality, which increase crop resiliency and potentially mitigate future weather volatilities.

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Declarations

Conflict of interests The authors have no relevant financial or non-financial interests to disclose.

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