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In-season temporal variability of soil carbon and nitrogen pools after half a century of a tillage and crop rotation gradient

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A B S T R A C T

Reduced soil disturbance and diversified crop rotations are practices that can enhance organic matter and soil health. Understanding how these practices influence seasonal soil carbon (C) and nitrogen (N) variability during the growing season is critical for agroecosystem sustainability. We assessed temporal dynamics of soil C and N pools at six sampling dates over a maize (*Zea mays L.*) growing season, in a 55-year tillage and crop rotation experiment on silt loam and clay loam alfisols. Crop rotation had a more consistent effect than tillage on soil C and N pools (0–20 cm depth), with the most diverse rotation increasing soil organic carbon (SOC), permanganate oxidizable carbon (POXC), mineralizable carbon (Min C), total nitrogen (TN), autoclaved-citrate extractable (ACE) protein, inorganic N at both sites. No-Till increased C and N pools in the clay loam, but not in the silt loam soil. In general, fractions of C (POXC and Min C) and N (ACE protein and inorganic N) were more seasonally variable than total pools (SOC and TN). Despite temporal variation, tillage and rotation effects remained mostly consistent throughout the growing season, except for Min C which values decreased, and treatment differences diminished as the season progressed. Our findings suggest that 1) crop diversification with perennials enhances soil C and N regardless of soil type or tillage; 2) long-term No-Till has stronger effects in clay loam than silt loam soils, and 3) although C and N pools vary seasonally, long-term management effects persist throughout the growing season.

1. Introduction

Assessing the impact of conservation management practices on soil health is essential for addressing challenges posed by climate change and building ecosystem resilience in agriculture. Practices such as long-term no-till (Nunes et al., 2018; Wulanningtyas et al., 2021) and crop diversification have shown to enhance carbon accrual in soils (Bowles et al., 2020; Smith et al., 2023; Sprunger et al., 2020; Wang et al., 2022; Zhang et al., 2020); thereby underscoring their crucial role in promoting beneficial soil function. Functions such as nutrient cycling, water cycling and retention, and soil protection from erosion are supported from the accumulation and protection of soil organic matter (Lehmann et al., 2020). These functions in turn can promote crop performance (Oldfield et al., 2022) to enhance agricultural sustainability.

Nutrient and SOM cycling in soils under no-till management are dependent on several factors including climate, soil properties, topography, and time, therefore have varied effects on crop productivity (Pittelkow et al., 2015). Sufficient time is needed for no-till or reduced tillage effects to be detected in soils (Cusser et al., 2020). In addition, increased crop diversification can be a key factor for improving and sustaining soil conditions and agronomic outcomes in no-till farming, due to increased complexity of soil carbon substrate entering the soil (Garland et al., 2021). Therefore, long-term no-till and crop diversification studies are needed to assess the long-term implications of these practices in a specific region and agroecosystem.

Most research on soil management practices draw inferences from only one or two sampling time points per year (e.g., Nunes et al., 2018). In doing so, assumptions must be made that soil indicators remain static,

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or trends do not change over the growing season. However, studies have demonstrated that there is in-season temporal variability of soil C and N pools fractions (i.e. labile and sensitive to management), and this variability likely influences soil processes and associated crop responses (Culman et al., 2013; Diederich et al., 2019). To our knowledge, no studies have evaluated total and labile C and N temporal variability in soils after long-term tillage and rotation gradients. In contrast, fewer studies have looked at short-term temporal dynamics in soil C and N pools (Culman et al., 2013; Diederich et al., 2019; Naasko et al., 2024). Evaluating in-season C and N temporal dynamics will help elucidate the long-term management effects of practices such as no-till and crop rotational diversity, and its implications for ecosystem services.

The Triplett-Van Doren long-term tillage experiments were established in Ohio in 1962 to evaluate a gradient of tillage and crop rotation diversity in two sites in Ohio with contrasting soil characteristics (Van Doren et al., 1976). These experimental trials have been maintained for more than 60 years, creating an optimal setting to evaluate in-season variation of soil C and N pools. Previous studies conducted in the Triplett and Van Doren No-Till Experiment focused on soil carbon dynamics, soil physical properties (Burgos Hernández et al., 2019; Kumar et al., 2014; Lal et al., 1994; Lal and Vandoren, 1990; Mahboubi et al., 1993), and crop performance (Dick et al., 1991; Van Doren et al., 1976; Van Doren and Triplett, 1973). Our study aims to evaluate in-season variability of soil C and N fractions over a corn (*Zea mays L.*) growing season in soils under varying tillage and crop rotation practices in one of the oldest no-till and crop rotation experiments in the world, the Triplett-Van Doren experiment. Specifically, our objectives were to:

1) Determine how long-term tillage intensity and crop rotational diversity impact soil C and N pools over a corn growing season.
2) Quantify the magnitude of temporal variability among soil C and N pools over two sites with contrasting soils.

We hypothesized that increasing crop rotation diversity with perennials and minimizing soil disturbance with reduced tillage will lead to increases in soil C and N pools across the entire growing season. We also hypothesized that labile C and N pools will have higher temporal variability compared to total pools. We thought greater temporal variability would especially be seen in soils with increased tillage intensity and decreased crop rotation diversity, given the expected lower soil C and N values in these treatments.

2. Materials and methods

2.1. Sites description

This study was conducted in the Ohio State University's Triplett-Van Doren long-term tillage and crop rotation experiment, one of the oldest no-till experiments in the world. The experiment consisted of two sites 1) Hoytville site at the Northwest Agricultural Research Station (41.222210, −83.761857, elevation 693 m), and 2) Wooster site at the Ohio Agricultural Research and Development Center (40.763588, −81.906316, elevation 1020 m), Ohio, United States (Dick et al., 2013).

The Hoytville site (established in 1963) is on a Hoytville clay loam (Fine, illitic, mesic Mollic Epiaqualfs with 350, 390, and 260 g kg^{-1} clay, silt, and sand, respectively), a very deep and poorly drained soil with a slope range of 0–1 percent and high shrink-swell potential (Soil Survey Staff, 2019a). The Hoytville site soil has a pH of 6.5, 21 g kg^{-1} soil organic carbon (SOC), 2.0 g kg^{-1} total nitrogen (TN), and a cation exchange capacity of 19.7 $cmol_c$ kg^{-1}. Subsurface tile drainage was installed in 1952 in Hoytville to improve the soil drainage. The Wooster site (established in 1962) is on a Wooster-Riddles silt loam (Fine-loamy, mixed, mesic Typic Fragiudalfs, with 140, 460, and 400 g kg^{-1} clay, silt, and sand, respectively), a well-drained deep soil with a slope range of 2–6 percent and a low to no shrink-swell potential (Soil Survey Staff, 2019b). There is no drainage system in Wooster (Dick et al., 1986). The Wooster site soil has a pH of 6.3, 18 g kg^{-1} SOC, 1.9 g kg^{-1} TN, and cation exchange capacity of 7.3 $cmol_c$ kg^{-1}. Both sites have humid continental climate with a mean annual air temperature of 10.6 °C (both sites) and cumulative precipitation of 826 mm and 1132 mm and in the Hoytville and Wooster sites, respectively, during 2018 (CFAES Weather System, 2025).

2.2. Experimental design and treatments

Both sites were arranged as full factorial, randomized complete block design with three tillage treatments and three crop rotation treatments replicated three times. The tillage treatments were 1) No-Till (lowest intensity), 2) Chisel Till (medium intensity), and 3) Moldboard Plow (highest intensity). The No-Till consists of zero soil disturbance where crop residues are continuously left undisturbed on the soil surface. Each year tillage in the Chisel Till and Moldboard Plow treatments is performed in the spring at Wooster and in the fall at Hoytville. The Chisel Till leaves approximately 30 percent of the residues from the previous crop on the soil surface. The Moldboard Plow treatment is a full inversion of the soil to a depth of 20 cm, incorporating most of the residues into the soil.

The crop rotations were 1) Continuous Corn, 2) Corn-Soybean, a two-year rotation of corn-soybean (*Glycine max*), and 3) Corn-Forage-Forage, a three-year rotation of oats (*Avena sativa*) as a nurse crop seeded historically with a perennial grass-legume meadow mix, including species such as orchard grass (*Dactylis glomerata*), tall fescue *(Festuca arundinacea)*, alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), and clover (*Trifolium repens* L.). Plots were 22.3 m by 4.3 m at the Wooster site and 30.5 m by 6.4 m at the Hoytville site. Corn was planted between mid to late May at both sites.

Starter urea fertilizer (46–0–0; 34–45 kg ha^{-1}) was banded at 5 cm by 5 cm at corn planting. For both sites, all plots were fertilized during the V5 corn stage with 202 kg ha^{-1} of nitrogen in plots where corn was planted. Phosphorus and potassium fertilizer were applied as necessary based on soil test results (Culman et al., 2020).

2.3. Soil sampling and analysis

For this study, the corn phase of all nine treatments (3 tillage x 3 crop rotations x 3 replicates) were sampled. Soils were sampled to a depth of 0–20 cm during the 2018 growing season using a soil probe (2.5 cm diameter core, 6 cores per plot) in the quarter row position, or halfway between the center of row and in-row position. Soils were sampled at key corn growth stages, at approximately one-month intervals, for a total of 6 sampling dates. The samplings occurred within the same week for both sites, to examine the temporal variability of C and N pools across the growing season: early May (pre-plant), mid June (V5 or fifth leaf), mid July (V10 or tenth leaf), early August (R1 or silking), early September (R4 or dough), and mid October (R6 or physiological maturity). For each site, 162 soil samples were collected by the end of the growing season (n = 27 per month sampled). A total of 324 soil samples were collected by the end of this study. Field-moist soil samples were sieved to 8 mm and mixed until homogeneous, dried in an oven at 38°C for 48 hours and ground to < 2 mm with a flail grinder to prepare for soils analyses.

Permanganate oxidizable carbon (POXC) was quantified following Weil et al. (2003) with modifications from Culman et al. (2012). Briefly, 2.5 g of soil was reacted with 20 mL of a 0.02 M potassium permanganate ($KMnO_4$) solution in 50 mL centrifuge tubes. The tube was shaken for two minutes (180 strokes per minute) using a reciprocal shaker and allowed to settle for 10 minutes. Then, 0.5 mL of supernatant was transferred and mixed with 49.5 mL of deionized water. Finally, the sample absorbance was read in a 96 well plate reader spectrophotometer at 550 nm.

Mineralizable carbon (Min C) was quantified upon rewetting soils up to 50 % water holding capacity (WHC) (Franzluebbers et al., 2000;

Haney et al., 2001). The 50 % WHC was determined empirically based on the mass difference between 10 g of dried and sieved (2 mm) soil samples that were saturated with deionized water and allowed to drain for 30 minutes and the mass after drying overnight at 105°C (Haney and Haney, 2010). To determine Min C, 10 g of 2 mm ground, dry soil was rewetted to 50 % WHC with deionized water in a 50 mL polypropylene centrifuge tube. After the rewetting of soils, tubes were capped tightly with lids containing rubber septum, sealed with parafilm, and incubated at 25°C for 24 hours. Proceeding incubation, 1 mL of headspace air was extracted using a syringe and injected into an LI-820 infrared gas analyzer (LI-COR, Biosciences, Lincoln, NE) to determine the concentration of CO_2. Finally, mineralizable C was calculated as the difference between a sample and a blank control using the ideal gas law and the headspace volume.

Autoclaved-citrate extractable soil protein (ACE protein) was quantified following (Hurisso, Moebius-Clune, et al., 2018). Organically bound soil nitrogen was extracted using 24 mL of 0.02 mol L^{-1} sodium citrate solution added to 3 g of soil in a 50 mL glass centrifuge tube. Samples were shaken for 5 minutes (180 strokes per minute) and autoclaved for 30 minutes at 121°C. Samples were left to cool and shaken again for 3 minutes. After shaking, 1.5 mL of the soil solution was transferred to 2 mL centrifuge tubes and centrifuged at 10,000 x g for 3 minutes. Quantification of protein was done by using colorimetric bicinchoninic-acid (BCA) assay (Thermo Scientific, Pierce, Rockford, IL) in a 96-well spectrophotometric plate reader at 562 nm.

Soil inorganic nitrogen (N), the sum of soil nitrate and ammonium (mg N kg $soil^{-1}$) was measured colorimetrically (Doane and Horwáth, 2003). Soil N was extracted using 2 mol L^{-1} KCl (30 mL per 3 g soil), shaken for 30 min and centrifuged (2000 RPM) for 10 min. Samples were read at 450 and 640 nm for NO_3^- and NH_4^+, respectively. Soil organic carbon (SOC) and total nitrogen (TN) were measured by direct combustion (Nelson and Sommers, 1982) with a Costech CHN analyzer (Valencia, CA) by a commercial laboratory (Spectrum Analytic Inc., Washington Court House, OH).

2.4. Statistical analyses

Data were analyzed for each site independently with analysis of variance using the PROC MIXED procedure in SAS v.9 (SAS Institute, Cary, NC) with sampling date as a repeated measure. Tillage and crop rotation were treated as fixed effects and block as a random effect with significant differences determined at $\alpha = 0.05$. Mean differences were performed for tillage treatments averaged over rotation treatments and vice versa with an adjusted Tukey's pairwise comparison ($\alpha = 0.05$) using SAS v.9, by monthly samplings and across all monthly samplings, by site. Data for soil inorganic N were log-transformed to satisfy assumptions of normality.

Coefficient of variation (CV) was used to assess temporal variability across the growing season. The CVs were calculated for each plot independently as a percentage of the standard deviation normalized by the mean across all six sampling dates. Plot-level CVs were then averaged across all 9 treatments and replications (n = 27) for each site, or by tillage across all crop rotation treatments, or by crop rotation across all tillage treatments. Analysis of variance was performed on plot-level CVs between tillage treatments and crop rotation treatments as described above. Graphs were created using the ggplot2 (Wickham, 2019) package in RStudio (RStudio Team, 2020).

3. Results

3.1. Tillage and rotation effects at hoytville

At the Hoytville site with clay loam soil, both tillage and crop rotation significantly impacted all measured soil C and N pools (Table 1). Across all six sampling dates, the No-Till and Corn-Forage-Forage treatments consistently returned the highest values for tillage and crop rotations, respectively (Table 2). Conversely, C and N pools were numerically lower in the Moldboard Plow treatment and Corn-Soybean rotation at Hoytville. The significant crop rotation by tillage system interaction in most measured variables was the result of increased values with the combination of Corn-Forage-Forage rotation and No-Till (Table 1, Figure S1). For SOC only, sampling date interacted with tillage, with most differences verified at the V10 sampling stage (July), where No-Till returned the lowest SOC values, followed by higher values under Chisel Till and Moldboard Plow (Fig. 1). There were no other significant sampling date interactions at the Hoytville site (Table 1).

Tillage impacted C and N pools at Hoytville, with No-Till returning the highest values for these pools, followed by Chisel Till and Moldboard Plow with the lowest values (Fig. 1, Table 2). Generally, the temporal dynamics were consistent across sampling dates with respect to differences across tillage treatments, except for the SOC in July, and inorganic N in June. In contrast to SOC, Min C, TN, and protein where differences between tillage gradients were reported for most months sampled, POXC and inorganic N only displayed differences in August and July, respectively. Likewise, crop rotation had relatively consistent temporal trends at Hoytville, with Corn-Forage-Forage returning the highest values, and Continuous Corn and Corn-Soybean intermediate to lowest (except for ACE protein) for most months sampled (Fig. 2, Table 2). Soil ACE protein

Table 1
Analysis of variance with sampling date as a repeated measure over the corn growing season. Values shown are F-statistics with statistical significance denoted with asterisks. F-statistics reported for inorganic N are based on log transformed values.

Effect	SOC		POXC		Min C		TN		ACE Protein		Inorganic N	
Hoytville clay loam												
Tillage (T)	49.0	***	5.3	* *	36.8	***	81.9	***	77.7	***	6.1	**
Rotation (R)	18.3	***	15.0	***	9.1	**	28.1	***	59.5	***	6.1	**
T x R	2.8	*	4.2	**	2.3		8.3	***	12.6	***	1.5	
Sampling Date (SD)	1.9		8.6	***	11.8	***	28.9	***	52.5	***	9.8	***
SD x T	2.4	*	0.9		1.6		0.6		1.3		0.9	
SD x R	1.2		1.1		0.4		0.5		0.8		0.5	
SD x T x R	1.3		1.1		0.6		0.6		0.4		0.6	
Wooster silt loam												
Tillage (T)	2.4		2.1		4.5	*	2.8		1.9		14.7	***
Rotation (R)	11.0	***	15.9	***	12.2	***	10.3	***	10.4	***	13.0	***
T x R	0.6		2.3		0.5		0.8		0.4		0.6	
Sampling Date (SD)	0.7		23.7	***	15.4	***	31.6	***	18.2	***	15.6	***
SD x T	1.1		1.3		1.8		0.8		0.7		1.6	
SD x R	0.8		0.5		2.4	*	1.0		0.6		1.4	
SD x T x R	0.4		0.9		0.8		0.6		0.6		0.7	

"*** " significance level: $p < 0.001$; "**" significance level: $p < 0.01$; "* " significance level: $p < 0.05$; soil organic carbon (SOC), permanganate oxidizable carbon (POXC), 24-hour incubation mineralizable carbon (Min C), total nitrogen (TN), autoclaved-citrate extractable (ACE) protein, and inorganic nitrogen (NO_3-N + NH_4^+-N).

Table 2
Means ± standard errors for soil C and N pools at both sites across all six sampling dates.

Site	Factor		SOC (g kg^{-1})	POXC (mg kg^{-1})	Min C (mg kg^{-1})	TN (g kg^{-1})	ACE Protein (g kg^{-1})	Inorganic N (mg kg^{-1})
Hoytville clay loam	Tillage	Moldboard Plow	18.0 ± 0.3 c	594.7 ± 7.6 b	36.7 ± 1.3 c	2.22 ± 0.05 b	3.8 ± 0.1 c	20.9 ± 2.5 b
		Chisel Till	19.2 ± 0.3 b	601.3 ± 8.0 b	44.5 ± 1.3 b	2.26 ± 0.04 b	4.1 ± 0.1 b	24.7 ± 2.5 ab
		No-Till	22.3 ± 0.4 a	635.2 ± 10.7 a	57.7 ± 2.2 a	2.62 ± 0.06 a	4.8 ± 0.1 a	32.4 ± 4.4 a
	Rotation	Continuous Corn	19.7 ± 0.4 b	605.9 ± 8.8 b	44.0 ± 1.9 b	2.36 ± 0.06 b	4.5 ± 0.1 a	23.0 ± 2.4 b
		Corn-Soybean	18.5 ± 0.4 b	576.3 ± 7.2 b	42.6 ± 1.9 b	2.24 ± 0.05 c	3.7 ± 0.1 b	23.5 ± 3.0 b
		Corn-Forage-Forage	21.2 ± 0.3 a	649.1 ± 8.7 a	52.3 ± 2.1 a	2.50 ± 0.06 a	4.5 ± 0.1 a	31.5 ± 4.2 a
Wooster silt loam	Tillage	Moldboard Plow	13.4 ± 0.3	444.3 ± 9.4	56.1 ± 2.0 b	1.40 ± 0.04	4.8 ± 0.1	27.3 ± 3.5 a
		Chisel Till	14.4 ± 0.2	468.5 ± 9.7	64.0 ± 1.8 a	1.53 ± 0.04	5.2 ± 0.1	23.9 ± 2.9 a
		No-Till	13.6 ± 0.2	445.9 ± 11.3	55.3 ± 1.7 b	1.42 ± 0.04	4.9 ± 0.1	11.5 ± 1.0 b
	Rotation	Continuous Corn	13.9 ± 0.2 b	445.4 ± 10.4 b	56.9 ± 1.9 b	1.40 ± 0.04	5.1 ± 0.1 a	12.9 ± 1.3 b
		Corn-Soybean	12.6 ± 0.2 b	420.2 ± 7.6 b	51.5 ± 1.3 b	1.35 ± 0.04	4.4 ± 0.1 b	18.5 ± 2.6 b
		Corn-Forage-Forage	14.9 ± 0.2 a	493.2 ± 9.9 a	67.1 ± 1.8 a	1.60 ± 0.04	5.3 ± 0.1 a	31.2 ± 3.6 a

Soil organic carbon (SOC), permanganate oxidizable carbon (POXC), 24-hour incubation mineralizable carbon (Min C), total nitrogen (TN), autoclaved-citrate extractable (ACE) protein, and inorganic nitrogen (NO_3^--N + NH_4^+-N).
Means are reported by tillage treatment averaged across all crop rotations, and conversely by crop rotations averaged across all tillage treatments (n = 6 sampling dates, 3 treatments, 3 replications = 54 observations). Values followed by different letters represent statistically different values between tillage treatments or between crop rotation treatments, with sampling date modeled as a repeated measure (α = 0.05).

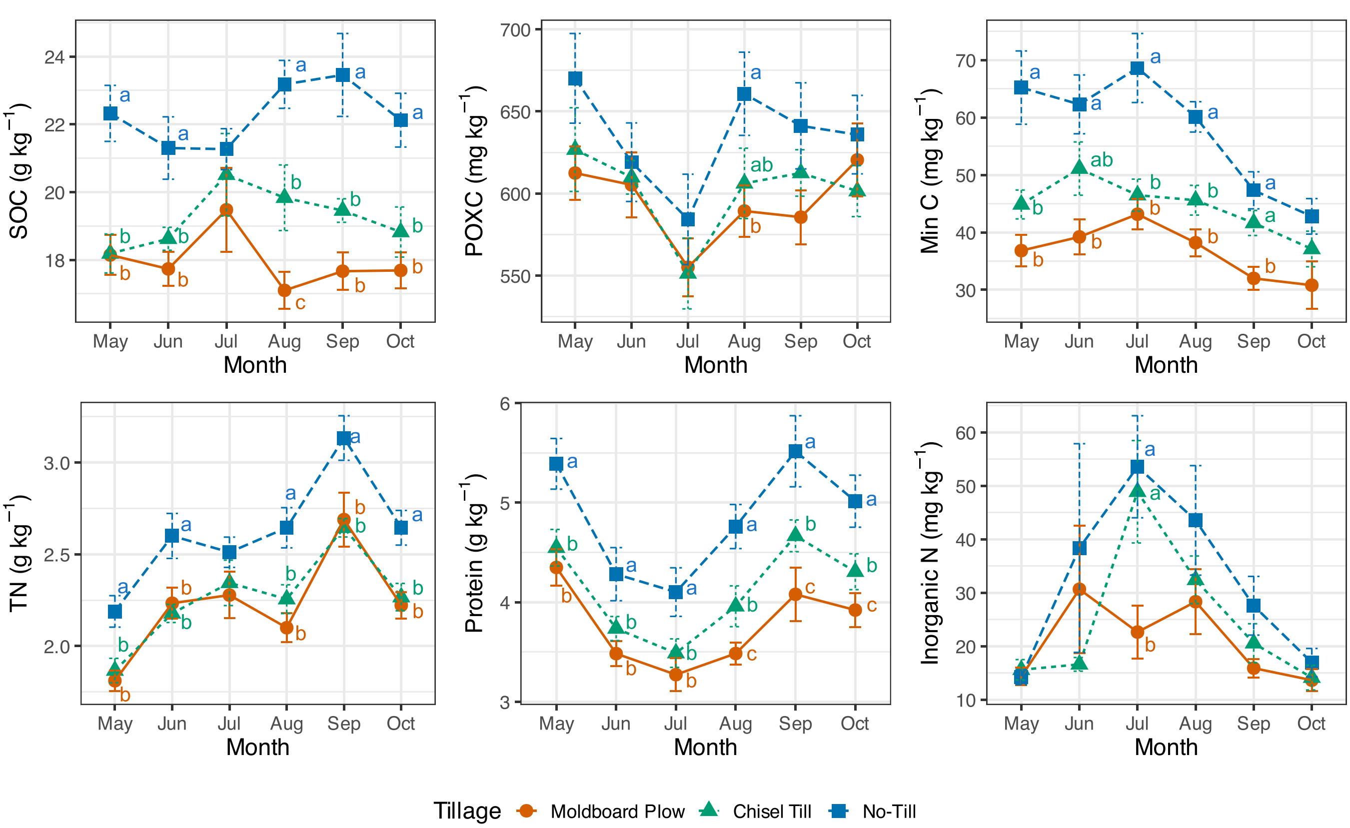

Fig. 1. Mean carbon and nitrogen pools at the Hoytville clay loam site for three tillage regimes averaged across crop rotation treatments. Pools include soil organic carbon (SOC), permanganate oxidizable C (POXC), 24-hour incubation mineralizable C (Min C), total nitrogen (TN), autoclaved-citrate extractable (ACE) protein, and inorganic N (NO_3^--N + NH_4^+-N). Tillage practices include Moldboard Plow (red circles, solid line), Chisel Till (green triangles, dotted line), and No-Till (blue squares, dashed line). Error bars represent one standard error of the mean. Inorganic N is presented as non-transformed data (n = 3 rotation treatments, 3 replications = 54 observations). Letters denote statistically different tillage treatment means at each individual sampling date (α = 0.05).

values had distinctive trends in terms of Continuous Corn having values as high as Corn-Forage-Forage. For inorganic N, differences between rotation gradients were only seen in July where the Corn-Forage-Forage had higher values than Continuous Corn.

3.2. Tillage and rotation effects at wooster

At the Wooster silt loam site, tillage only affected labile pools of mineralizable C and inorganic N, while crop rotation impacted all

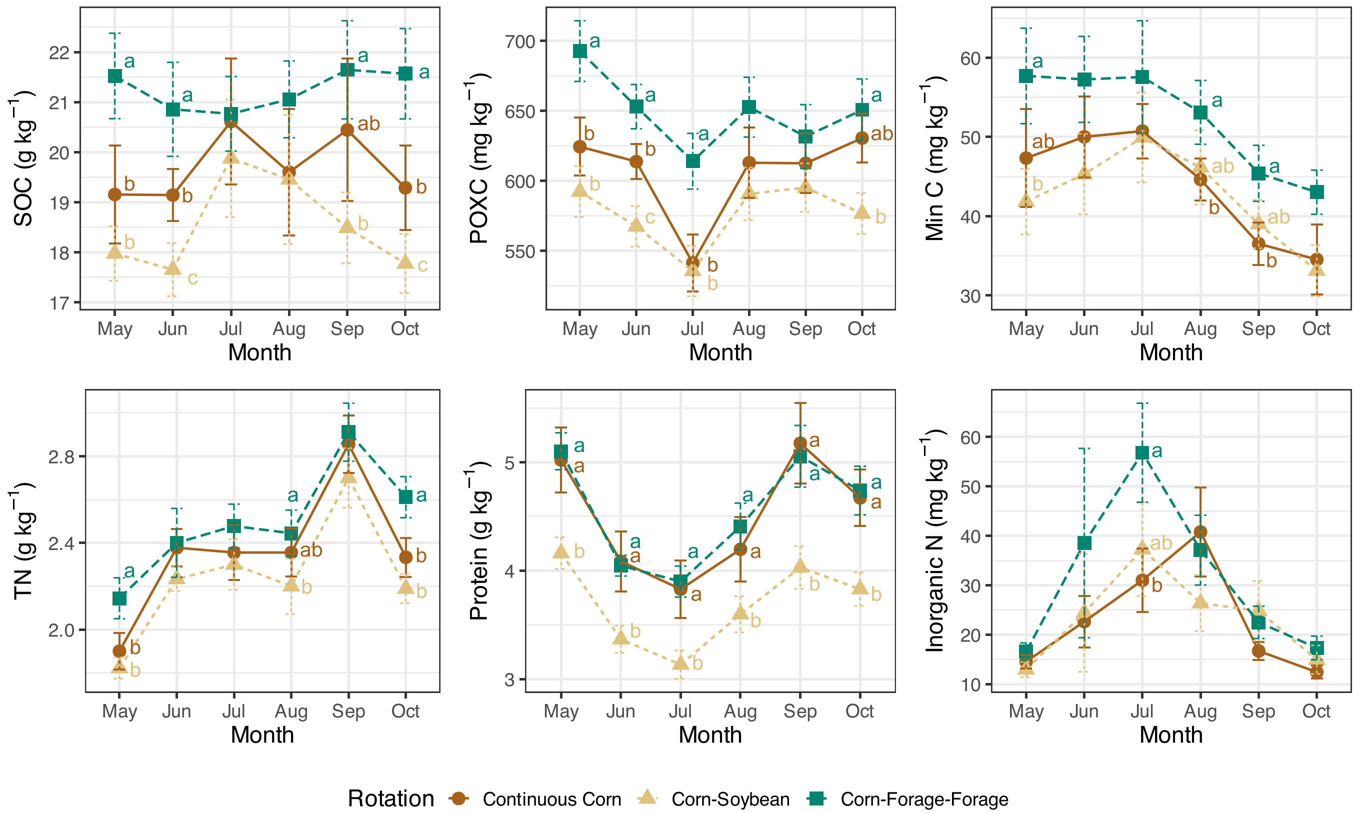

Fig. 2. Mean carbon and nitrogen pools at the Hoytville clay loam site for three crop rotation regimes averaged across tillage treatments. Pools include soil organic carbon (SOC), permanganate oxidizable C (POXC), 24-hour incubation mineralizable C (Min C), total nitrogen (TN), autoclaved-citrate extractable (ACE) protein, and inorganic N (NO_3^--N + NH_4^+-N). Crop rotational diversity treatments include Continuous Corn (brown circles, solid line), Corn-Soybean (sandy yellow triangles, dotted line), and Corn-Forage-Forage (dark green squares, dashed line). Error bars represent one standard error of the mean. Inorganic N is presented as non-transformed data (n = 3 tillage treatments, 3 replications = 54 observations). Letters denote statistically different crop rotation treatment means at each individual sampling date (α = 0.05).

measured C and N pools (Table 1). Across all sampling dates, Chisel Till had the highest mineralizable C values relative to Moldboard Plow and No-Till treatments, and No-Till had lower inorganic N values than Moldboard Plow and Chisel Till (Table 2). Crop rotation impacted C and N pools similarly at both sites, with No-Till having the highest values, Continuous Corn intermediate values, and Corn-Soybean the lowest values. At Wooster, there were no significant tillage by crop rotation interactions. However, the concentration of C and N pools under No-Till tended to increase under the Corn-Forage-Forage rotation, compared to the other rotations (Supplemental Figure 2). Similar to Hoytville, sampling date significantly affected all variables except for SOC, and sampling date interactions with till or crop rotation were minimal (Table 1).

Temporal trends of tillage treatments at Wooster were less consistent than at Hoytville (Fig. 3). Typically, the intermediate disturbance of Chisel Till returned consistently higher values of C and N pools relative to Moldboard Plow and No-Till, though the patterns differed by soil health indicator (Fig. 3, Table 2). Differences between months sampled were evident for Min C and inorganic N. For example, Chisel Till overall promoted the highest Min C during May, July, and August. In contrast, inorganic N levels between Chisel Till and Moldboard were similar within the growing season. Both Min C and inorganic N were lowest in the No-Till soils within the growing season. Chisel Till promoted higher values of SOC, POXC, TN, and protein compared to Moldboard Plow only the month of July. Unlike tillage, Wooster temporal trends based on crop rotation were generally consistent, with Corn-Forage-Forage returning higher values and Corn-Soybean returning lower values in most indicators (Fig. 4). Some indicators showing slightly different trends included ACE protein, and inorganic N. For instance, ACE protein values were similar between Corn-Forage-Forage and Continuous Corn during the growing season. For inorganic N, Continuous Corn and Corn-Soybean both had the overall lowest values particularly in the months of June and July.

3.3. Temporal variability between C and N indicators

Coefficient of variation (CV) revealed differences in temporal variability between measured indicators of C and N pools (Table 3). Across both sites, SOC had the lowest CVs and inorganic N had the highest CVs (Table 3). Although SOC tends to vary within the growing season, significant temporal effects were only seen for the Tillage and Sampling Date interaction in the Hoytville site (Table 1, Fig. 1). Also, although most variables fluctuated throughout the growing season across all sites and treatments, these ended up having generally similar values in the harvest stage (October) compared to the start of the season (May) except for Min C and TN. For instance, Min C mean values tended to be lower and TN tended to be higher in October compared to May (Figs. 1–4). In addition, ACE protein mean values tended to be lower in October compared to May in the Wooster silt loam (Figs. 3–4). Within the C pools across both sites, mineralizable C had the highest temporal variability, POXC intermediate and SOC the lowest. With respect to N pools, soil protein had the lowest temporal variability, total N intermediate, and inorganic N the highest across both sites (Table 3). Differences between soil C and N indicators coefficients of variation considering tillage and crop rotation treatments were minimal. In the Hoytville clay loam, seasonal variability of Min C was greater in the Continuous Corn compared to the Corn-Forage-Forage rotation, and soil protein variability was greater in the Continuous Corn compared to the Corn-Soybean rotation, with the Corn-Forage-Forage rotation falling in

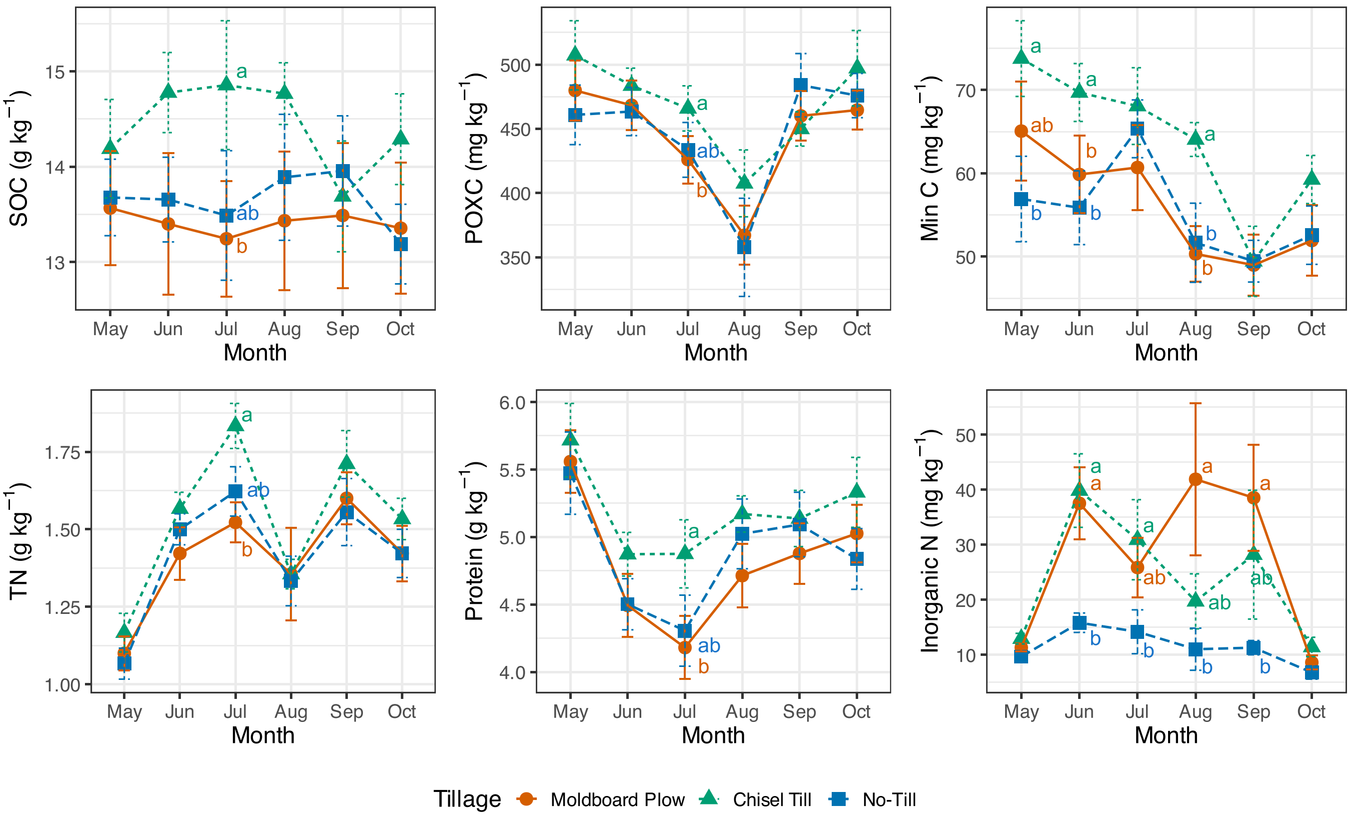

Fig. 3. Mean carbon and nitrogen pools at the Wooster silt loam site for three tillage regimes averaged across crop rotation treatments. Pools include soil organic carbon (SOC), permanganate oxidizable C (POXC), 24-hour incubation mineralizable C (Min C), total N (TN), autoclaved-citrate extractable (ACE) protein, and inorganic N (NO_3^--N + NH_4^+-N). Tillage practices include Moldboard Plow (red circles, solid line), Chisel Till (green triangles, dotted line), and No-Till (blue squares, dashed line). Error bars represent one standard error of the mean. Inorganic N is presented as non-transformed (n = 3 rotation treatments, 3 replications = 54 observations). Letters denote statistically different tillage treatment means at each individual sampling date (α = 0.05).

between (Table 3). In the Wooster silt loam, CV for inorganic nitrogen was higher in the Moldboard Plow and No-till soils compared to the Chisel tillage (Table 3).

4. Discussion

4.1. Tillage implications at Hoytville and Wooster

This study assessed the effects of more than half a century of tillage and crop rotation on the in-season temporal dynamics of soil C and N pools. No-till increased C and N pools concentrations across the entire growing season only in the Hoytville clay loam. In contrast, tillage effects were less evident in the Wooster silt loam, where Chisel Till increased Min C concentrations and No-Till decreased inorganic N concentrations, when averaged across the growing season. Other studies have evaluated the effects of long-term no tillage on soil C and N temporal dynamics (Burke et al., 2019; Diederich et al., 2019; Franzluebbers et al., 1995; Naasko et al., 2024). These studies report contrasting results; for example, one study found that no-till increased labile C and N pools across the entire growing season compared to tillage (Franzluebbers et al., 1995), as seen in the Hoytville clay loam site in this study. In contrast, other studies reported less differences between tillage treatments (Diederich et al., 2019; Naasko et al., 2024), as seen in our Wooster silt loam site.

Fewer studies, however, have evaluated in-season temporal dynamics of long-term no-till. Our results from the Hoytville clay loam soil follow trends from other non-temporal studies conducted in the same experimental area, where long-term no-till compared to more intensive tillage systems increased SOC and TN pools in the surface 20 cm of depth (Burgos Hernández et al., 2019). Similar studies in other regions have also reported an increase of total and labile C and N pools in soils with long-term no-till (Aziz et al., 2013; Nunes et al., 2018; Pecci Canisares et al., 2021; Van Eerd et al., 2014; Weidhuner et al., 2021; S. Zuber et al., 2018).

At the Wooster silt loam site, Chisel Till was the only tillage treatment that significantly impacted soil Min C at 0–20 cm depth, and this treatment along with Moldboard Plow also promoted higher plant-available nitrogen (inorganic N) compared to no-till. These trends might be explained by the Wooster no-till soils having higher compaction levels (from penetration resistance assessment) in the top 20 cm, compared to the other tillage gradients (Burgos Hernández et al., 2019). High soil compaction can limit additions and protection of soil organic matter by limiting and modifying root growth in the top soil layer (Chen and Weil, 2011). These results suggest that chisel tillage might favor soil carbon and nitrogen turnover in the Wooster silt loam, potentially due to enhanced breakdown, incorporation, and access for decomposition of organic matter compared to the more compacted no-till soils in this site (Fiedler et al., 2016; Kumar et al., 2014). Although Moldboard Plow promoted higher nitrogen levels compared to no-till in the silt loam soil, this practice has been known to be detrimental to soil health properties over the long term, causing lasting effects such as depletion of soil organic carbon (Kumar et al., 2012) and erosion (Klik and Rosner, 2020). Total pools of C and N at the Wooster silt loam tended to be enhanced by reduction on tillage intensity and inclusion of perennials in the rotation. A previous study conducted in the same experimental area also found higher contents of soil organic C in the no-till treatment compared to Moldboard plow, but only for the top 10 cm of depth in the continuous corn rotation (Nakajima et al., 2016). However, another study found non-significant impacts of no-till evaluating soil C and N stocks in the top 0–10, 10–20, and 20–30 cm of depth (Burgos

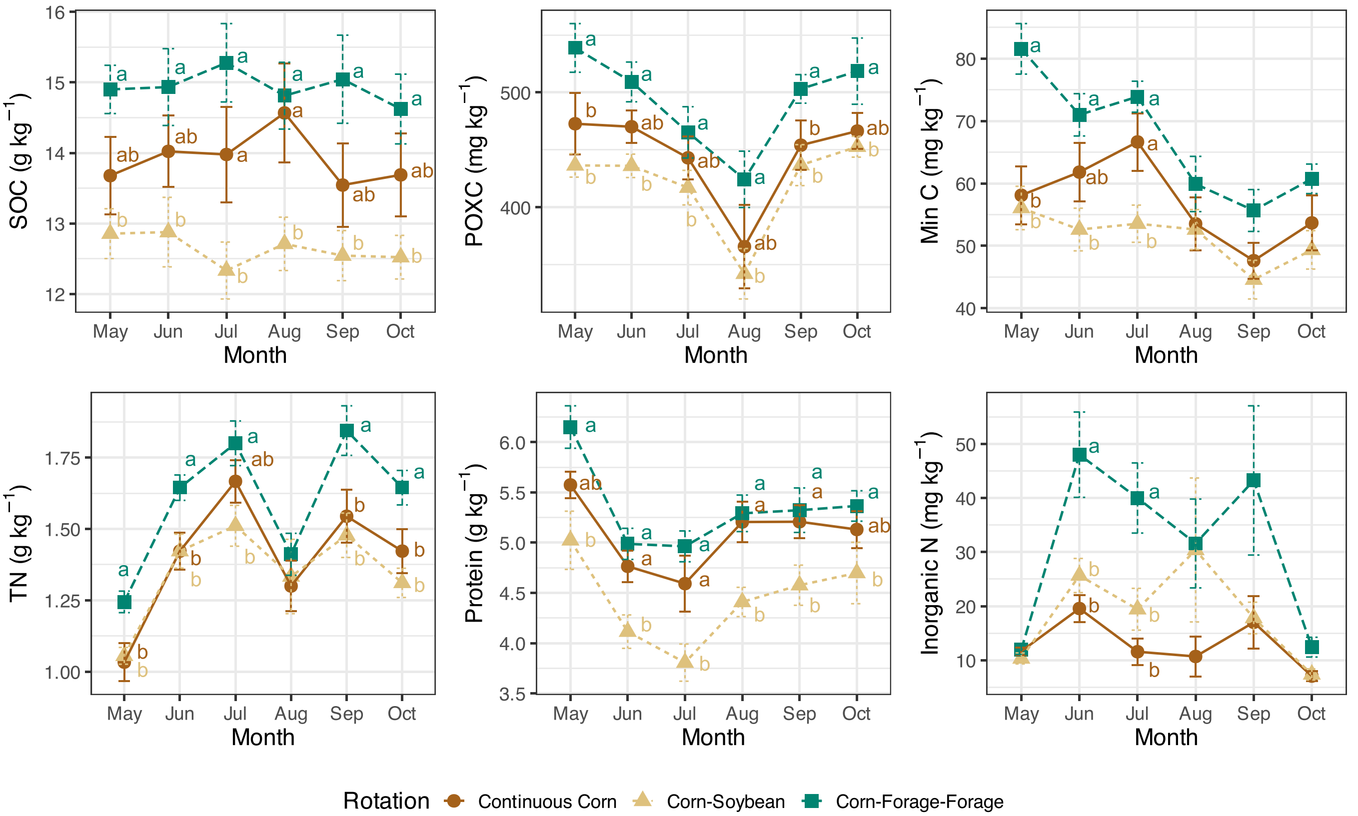

Fig. 4. Mean carbon (C) and nitrogen (N) pools at the Wooster silt loam site for three crop rotation regimes averaged across tillage treatments. Pools include soil organic carbon (SOC), permanganate oxidizable C (POXC), mineralizable C, total nitrogen (TN), autoclaved-citrate extractable (ACE) protein, and inorganic N (NO_3^--N + NH_4^+-N). Crop rotational diversity treatments include Continuous Corn (brown circles, solid line), Corn-Soybean (sandy yellow triangles, dotted line), and Corn-Forage-Forage (dark green squares, dashed line). Error bars represent one standard error of the mean. Inorganic N is presented as non-transformed data (n = 3 tillage treatments, 3 replications = 54 observations). Letters denote statistically different crop rotation treatment means at each individual sampling date (α = 0.05).

Table 3
Temporal variability of soil properties as measured by coefficient of variation over the growing season for Hoytville and Wooster sites.

Site	Factors	Treatment	SOC	POXC	Min C	TN	ACE Protein	Inorganic N
	All		7.4	7.7	20.6	14.7	13.0	21.1
	Tillage	Moldboard Plow	6.6	6.1	19.1	16.8	12.1	19.2
		Chisel	8.1	8.2	17.5	13.7	12.8	20.0
Hoytville clay loam CV (%)		No-Till	7.3	8.8	25.3	13.7	14.0	24.1
	Crop Rotation	CC	8.8	8.8	26.8 a	15.1	14.4 a	19.9
		CS	9.1	7.3	18.5 ab	16.1	11.9 b	22.6
		CFF	4.2	6.9	16.5 b	13.0	12.5 ab	20.8
	All		6.2	11.9	17.7	17.9	11.3	24.7
	Tillage	Moldboard Plow	5.3	11.5	17.9	17.8	12.1	27.1 a
		Chisel	5.8	11.1	17.1	18.3	9.0	20.0 b
Wooster silt loam CV (%)		No-Till	7.3	13.2	18.0	17.6	12.7	27.1 a
	Crop Rotation	CC	6.9	12.7	17.1	19.3	11.3	19.9
		CS	5.9	11.5	16.8	17.9	12.3	22.6
		CFF	5.7	11.6	19.1	16.6	10.2	20.8

Coefficients of variation values were calculated across 6 sampling stages for each plot. Factor values under 'All' represent the average of all plots across the 9 experimental treatments (n = 27), values in 'Tillage' represent the averaged tillage treatments across all crop rotations (n = 9), and conversely the values in 'Crop Rotations' represent the average by crop rotations across all tillage treatments (n = 9). Mean CV values for inorganic N are presented as transformed data. Values followed by different letters represent statistically different CVs between tillage treatments or between crop rotation treatments (α = 0.05). Soil organic carbon (SOC), permanganate oxidizable carbon (POXC), 24-hour incubation mineralizable carbon (Min C), total nitrogen (TN), autoclaved-citrate extractable (ACE) protein, and inorganic N (NO_3^--N + NH_4^+-N).

Hernández et al., 2019). Inconsistent findings between soil C and N pools stocks and concentrations suggest that soil bulk density plays an important role in SOC and TN storage.

Consistently higher concentrations of total C and N in the Hoytville clay loam under No-Till (Table 2) might be a result of higher clay content, aggregate stability, porosity, compaction, and moisture retention compared to the Wooster silt loam soil. Higher clay content can increase mineral association of soil organic matter with clays through the formation of organo-mineral complexes and stabilization from cation bridging (Feng et al., 2014; Galicia-Andrés et al., 2021; Sarkar et al., 2018; Singh et al., 2018). Soils with higher clay content can also have higher capacity for soil aggregation, which could enhance the physical protection of SOM from long-term no-till (Schweizer et al., 2019). In contrast, soils with lower clay content, like the silt loam, can have inherently lower aggregate stability and may be more susceptible to organic matter losses under no-till due to weaker microaggregate formation and lower mineral-association capacity (Krause et al., 2018). Although this study did not evaluate soil aggregate stability, differences in soil aggregation and organic matter stabilization between the two soil textures, along with higher compaction, and lower moisture retention and biological activity could explain the contrasting responses of C and N pools to no-till between sites (Burgos Hernández et al., 2019; Gupta and Germida, 2015; Kan et al., 2022). This observation that fine-textured soils exhibit greater organic matter accumulation under no-till, with higher concentrations of carbon and nitrogen pools compared to soils with lower clay content, is consistent with findings from Fiorini et al., (2020). These results suggest that soil texture plays a critical role in determining the effectiveness of no-till in enhancing soil C and N pools, with fine-textured soils showing greater potential for long-term organic matter accumulation.

4.2. Rotation implications at Hoytville and Wooster

Greater crop rotational diversity increased C pools in this study aligned with observations from other studies (Culman et al., 2013; Lehman et al., 2017) especially those that included perennial forages (Chahal et al., 2021; Deiss et al., 2021; Diederich et al., 2019; Naasko et al., 2024; Sprunger et al., 2020). Having perennial forages as part of rotations can increase labile and stable soil C and N pools through enhanced and undisturbed root system growth and diversity (Sprunger et al., 2024). For example, research has shown that increasing aboveground diversity with crop rotations can intensify belowground ecological interactions, including biological networks and rhizodeposition which promote soil C inputs and nutrient cycling compared to continuous crop monoculture (K. Zhang et al., 2021). A potential reason for the consistently low soil C and N in the Corn-Soybean rotation might be the quantity and quality of residues and increased decomposition rates from microbial activity that might promote C and N loss (Hall et al., 2019; McDaniel et al., 2014) compared to more complex root systems like in the Corn-Forage-Forage rotation.

Our results suggest that the integration of more diversified crop rotations, especially with perennial cover crops, should be considered to increase both carbon and nitrogen pools in soils. Benefits of diversified crop rotations that contributed to increases in soil C and N include functions like nutrient cycling, carbon sequestration, aggregate stability, and agroecosystem resilience (Blanco-Canqui et al., 2015; Chahal et al., 2021; Liu et al., 2022; Tiemann et al., 2015). Crop rotational diversity has also been shown to increase soil nitrogen pools and proposed as a strategy to reduce synthetic N fertilization needs in other studies (Breza et al., 2023). Therefore, increasing crop rotational diversity can be used as a practice for improving biologically based nitrogen cycling and reducing synthetic nitrogen fertilization needs in grain crops.

4.3. Temporal variability of indicators

In this study, in-season temporal variability was documented with all variables measured, with SOC generally showing no significant variability. Despite SOC showing a significant Tillage and Sampling Date interaction in the Hoytville site where Chisel tended to be more variable than the other tillage gradients, no significant differences were detected. This suggests that while reduced tillage may influence short-term fluctuation of SOC, it remained largely stable likely due to its inherently slow turnover. The temporal stability of SOC in soil with reduced tillage can be attributed to greater soil organic matter stabilization from chemical and physical protection (Kan et al., 2022). Seasonal variability of SOC has been assessed in other studies (Burke et al., 2019; S. B. Wuest and Durfee, 2024) particularly in no-till soils (Schiedung et al., 2017; S. Wuest, 2014).

Seasonal variability of all soil labile C and N indicators supports findings from other studies that have examined temporal dynamics (Burke et al., 2019; Culman et al., 2013; Diederich et al., 2019; Hurisso, Culman, et al., 2018). For example, Hurisso et al. (2018) reported Min C as being the most temporally variable among other C indicators, including POXC and total soil organic matter. The greater seasonal variability of Min C is partially explained by its sensitivity to co-varying factors such as precipitation and soil water content (Das et al., 2019), and to soil C and N inputs across the growing season, derived from sources such as root and microbial biomass deposition and fertilizer inputs (Franzluebbers et al., 1994). Burke et al. (2019) found that the temporal variability between SOC and POXC was similar in a sandy loam soil in semi-arid and irrigated cotton-growing system. This was consistent with our findings in the Hoytville clay loam, but inconsistent with the Wooster silt loam soil, where POXC CV was nearly double SOC CV. Contrasting results might be due to differences in soil types and weather.

High temporal variability (i.e., CV) of inorganic N was expected since this labile N pool can be rapidly used by crops, is more mobile in soil, and it is more susceptible to additions, transformations and losses, such as by leaching following a rainfall event (Hess et al., 2020). Contrary to our hypothesis, TN tended to have higher variability relative to soil protein which was not expected, since organic N pools tend to be more temporally variable due to biological activity (Haynes, 2005). These TN temporal variability might be due to strong influence of fluctuating inorganic N levels within TN. In contrast, less variation of soil ACE protein compared to TN were likely due to SOM physical protection within aggregates and association with minerals, which could confer greater stability for this organic nitrogen indicator (ACE protein). It is likely that mineral N fertilization side dressed immediately after the June (V5) sampling may have contributed to the variability of the N indicators, particularly inorganic N, and therefore total N. Moreover, previous research has shown that N fertilization can influence in-season microbial activity by shifting community composition and biomass (Garcia and Rice, 1994; Zeng et al., 2016), but that was not evident when looking at the soil ACE protein content.

It is also likely that maize phenology had an influence on soil C and N dynamics. The vegetative growth phase from June (V5) until tasseling (immediately before R1 in July) has higher demands for nutrient uptake, especially N (Bender et al., 2013). During the reproductive stages of corn, August (R1) until October (R6), large amounts of nutrients are translocated from vegetative to reproductive parts of the plant (Bender et al., 2013). Higher nutrient and water demand of corn during the vegetative stages could have increased root exudate production, which in turn promotes decomposition of soil organic matter as a physiological response to scavenge soil nutrients.

Significant variability of indicators resulting from tillage and rotation treatments, were only found for Min C and protein in Hoytville and inorganic N in Wooster. Higher variability of both Min C and protein in Continuous Corn compared to other rotations at the Hoytville silt loam soil, might be due to lack of diversity in root biomass and rhizodeposition that might cause a shift in microbial diversity and activity. For example, lack of diversity in organic matter inputs from continuous corn root deposition might reduce functional redundancy compared to more diversified inputs from root diversity (D'Acunto et al., 2018; King and Hofmockel, 2017; Town, 2022). Higher variability of inorganic N was found in the Moldboard Plow and No-till Wooster silt loam soils and might be due soil organic matter protection and breakdown of organic matter dynamics. Increased tillage intensity may contribute to the exposure of protected soil organic matter that is subsequently mineralized by soil organisms, absorbed by plants, or lost by leaching or volatilization (Balesdent et al., 2000; Ramesh et al., 2019), therefore increasing variability in inorganic N concentrations. On the other hand, accumulation of residues, lack of incorporation and homogenization of plant in no-till has been shown to increase biological activity and microbial hotspots that might lead to greater nitrogen variability (Schlatter et al., 2019).

The tendency of Min C (both sites) and ACE protein (in the Wooster silt loam) decreasing from May to October might be a result of continuous microbial decomposition of labile organic matter and changes in abiotic factors such as temperature and rainfall throughout the growing season (Brookes et al., 2017; Curtin et al., 2012). In contrast, the TN tendency to increase towards the end of the season could result from seasonal patterns in plant residue inputs, fertilization, and microbial turnover (Jagadamma et al., 2007; Malhi et al., 2006; Y. Wang et al., 2018). The tendency of ACE protein to decrease aligns with the observed increase in TN levels at the end of the growing season.

4.4. Overall implications of findings

Our findings suggest that increasing crop rotational diversity enhances the benefits of no-till farming by increasing the concentration of soil C and N pools, especially in the clay loam soil. Despite most tillage and crop rotation interactions not being significant, most soil C and N pools tended to increase in the no-till soils under the most diverse rotation (Corn-Forage-Forage) in the Wooster silt loam site. Significantly higher levels of SOC, POXC, TN, and ACE protein found in the Hoytville clay loam soils under No-Till may have been due to enhanced soil biological biomass, diversity and activity (Bonini Pires et al., 2020; Rodríguez et al., 2020) as well as improved soil physical properties (Iheshiulo et al., 2023). Similar results of increased soil C and N values in no-till soils under increased crop rotational diversity have been reported in other studies (Alhameid et al., 2017; Lehman et al., 2017; S.M. Zuber et al., 2015). Our results suggest that decreasing soil disturbance and increasing rotational diversity with perennial crops can enhance soil C and N pools, which are essential components of soil health.

The temporal variability observed in our study for most C and N pools emphasize the fact that the timing of soil sampling is crucial for accurate soil testing and decision-making. Land Grant Universities typically recommended that farmers consistently sample soils at the same time (e.g., the same month or season) across years (e.g., Culman et al., 2020). This consistency is essential for accurately tracking soil pH, extractable nutrients and C and N levels, particularly in nutrient-demanding agricultural systems like grain production. Fluctuations in labile C and N pools during critical nutrient-demanding crop stages can reflect the soil's ability to function as a nutrient source, and inform decisions regarding fertilization and amendment applications.

Finally, understanding the implications of these findings in relation to crop performance is particularly relevant to incentive adoption of conservation practices by farmers. A recent analysis of grain yields by de Camargo Santos et al. (in review) has revealed similar trends between tillage and rotation effects on grain yield response similar to our findings for soil C and N pools. Consistent with the findings reported here for soil C and N, corn grain yields were consistently impacted by crop rotation at both sites, with Corn-Forage-Forage having the highest yields in each tillage type (de Camargo Santos et al., in review). The effect of tillage on yields was site dependent. At Wooster, No-Till returned the highest grain yields among the tillage systems, which interestingly was poorly aligned in the soil data reported here, where most soil C and N pool values tended to be higher under Chisel Till. At Hoytville, No-Till yielded the most grain within the Corn-Forage-Forage crop rotation, while in the Continuous Corn rotation, Moldboard Plow had the greatest yields (de Camargo Santos et al., in review). Collectively these results suggest that long-term development of soil C and N pools are related to, but not perfectly aligned with long-term grain yields. Therefore, management goals intended to optimize both yields and critical soil functions (e.g., SOC accrual) may be soil-type dependent or even non-compatible in some cases. More research efforts are needed to identify and understand when building soil C and N pools do not follow similar trends with grain yield response.

5. Conclusions

This study assessed the effects of more than 55 years of tillage intensity and crop rotational diversity gradients on soil C and N dynamics in two Ohio (US) agricultural soils. After half century, No-Till consistently increased soil C and N pools in the Hoytville clay loam, but not in the Wooster silt loam. Crop rotational diversity had a large and consistent effect on C and N pools at both sites and across the growing season, with the most diversified rotation, Corn-Forage-Forage, promoting the highest concentrations of these indicators. The effects of No-Till on soil C accrual and N availability were enhanced under the most diversified crop rotation with perennials. Despite significant seasonal fluctuations in labile C and N pools and total N, the overall effects of tillage and crop rotation remained stable, suggesting that long-term management practices have a lasting impact regardless of short-term variability. SOC showed minimal seasonal variability, confirming its role as a stable soil C pool, while inorganic N showed high variability and expected increases following fertilization. Greater temporal variability over the growing season was observed for labile C and N pools and total N, reinforcing the importance of consistency in soil sampling timing for accurate soil testing and management practice monitoring. The overall trends for C and N pools in response to tillage and rotation remained consistent over time within a crop season, demonstrating the long-term benefits of diversified cropping systems and reduced tillage for building soil health.

The results of this study suggest that enhancing crop rotational diversity, especially with perennials, amplifies the benefits of No-Till for C accrual and N availability throughout the growing season, that are important for building soil health. This approach could offer farmers a viable strategy to improve soil C and N levels, which are critical for long-term soil productivity and resilience. However, while improving soil C and N pools generally correlates with better crop yields, this relationship is not always straightforward and may vary by soil type. Future efforts should focus on refining management practices to balance soil health and crop yield optimization, considering site-specific responses to tillage and crop rotation.

CRediT authorship contribution statement

Culman Steve W.: Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition, Formal analysis. **Ali Faheem:** Data curation. **Deiss Leonardo:** Writing – review & editing, Validation, Resources. **Gonzalez-Maldonado Noelymar:** Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Steve Culman reports financial support was provided by Ohio No-Till Council. 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. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.still.2025.106566.

Data availability

Data will be made available on request.

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