Increasing Labile Soil Carbon and Nitrogen Fractions Require a Change in System, Rather Than Practice

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The influence of tillage, carbon (C) and nitrogen (N) inputs and perennialization on labile fractions of soil organic matter (SOM) has been widely investigated, but research on the temporal and depth variation of labile C and N fractions in long-term agroecosystems representative of the US Corn Belt is lacking. In this study, labile soil fractions as influenced by different agriculture management approaches common in the north-central United States were characterized through measurements of potentially mineralizable N (PMN), mineralizable C (C-min), and permanganate oxidizable C (POXC), which are known indicators of the biologically active pool of SOM. Soils were sampled five times in six different cropping systems at the Wisconsin Integrated Cropping Systems Trial: three annual cropping systems (continuous corn, strip-till corn–soybean, organic grain) and three forage cropping systems (conventional forage, organic forage, grazed pasture). Across all sampling dates (May–September) and soil depth (0–15 and 15–30 cm), forage-based cropping systems generally had greater PMN, C-min, and POXC, with C-min and PMN expressing the largest differences between forage and grain-based cropping systems. Labile soil C and N fractions across cropping systems were typically greatest in July or August and were ~50% greater in the top 0 to 15 cm of soil than the 15 to 30 cm. These results suggest sampling timing and depth for PMN, POXC, and C-min need to be standardized to optimize their use as soil health indicators and that shifts in the system toward perenniality will be required to increase labile C and N fractions on the highly fertile Mollisols of the US Corn Belt.

Abbreviations: CAAA, corn–alfalfa–alfalfa–alfalfa; CC, continuous corn; C-min, mineralizable carbon; COA, organic corn–oat/alfalfa–alfalfa; CS, strip-till corn–no-till soybean; CSW, organic corn–soybean–winter wheat–oat cover crop interseeded with berseem clover; PAS, pasture; PMN, potentially mineralizable nitrogen; POXC, permanganate oxidizable carbon; SOC, soil organic carbon; SOM, soil organic matter; TC, total carbon; TN, total nitrogen; WIST, Wisconsin Integrated Cropping Systems Trial.

Core Ideas

- **Soil sampling time and depth should be considered when analyzing soil health metrics.**
- **Soil health improvement may require large cropping system shifts in Midwest regions.**
- **Shift in system toward perenniality may be required to build labile soil fraction for WI conditions.**
- **C-min and PMN provided more unique responses to management, space, and time than TC and TN.**

Achieving long-term sustainability and productivity of agroecosystems through the management of soil organic matter (SOM) is of interest to the scientific and farming communities. Agricultural production systems rely heavily on SOM for ecosystem services such as nutrient cycling, water holding capacity, aggregation, and improved soil structure (Gregorich et al., 1997; Johnston et al., 2009; Morrow et al., 2016). Unfortunately, it can take years before improvement in SOM can be detected on a total carbon (TC) basis because it is relatively insensitive to management changes (Wander and Drinkwater, 2000).

To address these limitations, researchers have more recently included measurements of the relatively small but labile fraction of SOM (<20% of total SOM) to better understand how management practices affect short-term and long-term C and N dynamics in soil (Culman et al., 2013a). High absolute values of labile C and N fractions, which are sensitive to management changes, typically indicate higher levels of crop nutrient turnover and availability and have been correlated

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with agronomic yield (de Moraes Sá et al., 2014; Franzluebbers, 2018). Additionally, labile fractions have a relatively quick turnover time (days to years) (Wander, 2004) and have been shown to be more sensitive to changes in management practices than total soil organic C (SOC) or SOM (Haynes, 2005; Janzen et al., 1998). Agricultural stakeholders are becoming increasingly interested in tracking labile C and N fractions, particularly within the framework of soil health, to monitor how management changes may be improving their soil regarding agricultural sustainability and productivity.

Three measures of labile soil C and N fractions that are being increasingly used in the research setting and that rely on soil extractions or short-term incubations are potentially mineralizable nitrogen (PMN), mineralizable C (C-min), and permanganate oxidizable C (POXC). Potentially mineralizable N has been proposed as measurement of N availability in soil (Drinkwater et al., 1996; Gregorich et al., 1997; Haynes, 2005; Waring and Bremner, 1964) and is considered the fraction of N mineralized from plant, animal, and microbial residues as well as available N liberated from microbial necromass (Griffin, 2008) within a 7-d anaerobic incubation period. Given its dependence on N transformation via microbial processes, this biological incubation method can be used to estimate soil N availability primarily in soil research laboratories. Mineralizable C is comprised of labile C fraction substrates, such as plant root exudates and microbial necromass and biomass, as well as lightly decomposed plant residues and extracellular enzymes (Liang et al., 2017; Wander, 2004) and is broadly defined as the amount of organic C mineralized to CO_2 given a certain time, temperature, and moisture. Mineralizable C is typically measured by short-term aerobic incubation methods (Franzluebbers et al., 1996) and has been shown to measure SOM turnover (Gregorich et al., 1997), to serve as a proxy for N availability (Haney et al., 2008), to indicate potential microbial activity in the soil (Gregorich et al., 1997; Franzluebbers et al., 2000), and is sensitive to changes in agricultural management (Culman et al., 2013a, 2013b). The fraction of organic C that is susceptible to partial oxidation by a weak concentration of potassium permanganate ($KMnO_4$) is commonly referred to as POXC and has been suggested to reflect a biologically active soil C fractions across a wide range of diverse soils (Culman et al., 2012; Weil et al., 2003) and has been shown to be sensitive to recent management changes (Culman et al., 2012).

Soil depth has also been shown to be an important factor for labile soil C fraction measurements (Culman et al., 2012; Franzluebbers, 2016; Hurisso et al., 2016) because soil C resources are depth dependent (Franzluebbers, 2016). Considering soil C and N cycling are closely linked (Griffin, 2008), labile soil N fraction measurements presumably exhibit differences between soil depths as well. In addition to the effects of soil depth, temporal dynamics have also been observed in PMN, POXC, and C-min measurements at a long-term field trial in southwestern Michigan (Culman et al., 2013a), with the highest PMN, POXC, and C-min values observed during the middle of the growing season.

Labile soil metrics used in combination with total SOM, TN, and TC in long-term research trials such as the Wisconsin Integrated Cropping Systems Trial (WICST; https://wicst.wisc.edu/) in Arlington, WI, provide the unique opportunity to simultaneously evaluate long-term cropping system sustainability and short-term nutrient availability. Previous studies conducted at WICST have found that systems that differ in singular management practices (e.g., conventional vs. organic, conventional tillage vs. no tillage) within a given cropping system type did not determine short- and long-term soil C and N measurements as significantly as systems that differed in cropping system type (e.g., annual cash grain vs. forage). For example, forage cropping systems with the greatest degree of perennialization resulted in soils with greater particulate organic matter and aggregate C and N and showed decreased rates of SOC loss after nearly 25 yr of best management practices (Cates et al., 2016; Sanford et al., 2012). A study by Jokela et al. (2011) found that, across all six cropping systems, only the annual grain vs. alfalfa-based dairy forage systems and conventional vs. organic alfalfa–based dairy forage systems were significantly different in their POXC and PMN measurements at the 5- to 20-cm soil depth.

Although previous studies have provided a critical framework for understanding how long-term cropping system practices affect soil properties in representative regions of the Midwest, few have documented the temporal trends of chemical or microbially defined labile soil C and N fractions, especially with respect to how sampling time would affect treatment comparisons (Culman et al., 2013a; Hurisso et al., 2018). Given the use of these measures as soil health metrics (particularly POXC and C-min), further exploration of their depth and temporal trends is critical. We sought to address these knowledge gaps by assessing labile C and N fractions at various depths and at five different sampling dates within the 2016 production season at the long-term cropping systems trial known as WICST.

The objectives of this study were (i) to determine the impact of a singular change in practice within a forage or grain cropping system (e.g., conventional tillage vs. no-tillage, conventional vs. organic) vs. a more profound system change (e.g., a shift from cash grain to forage cropping system) on labile and TC and total N (TN) fractions after 25 yr of best management practices; (ii) to evaluate how labile soil C and N fractions, measured as PMN, POXC, and C-min, change with soil depth; and (iii) to assess how measures of PMN, POXC, and C-min change within the course of the production season. We hypothesized that forage-based cropping systems, which have the greatest perennialization, would have greater absolute labile soil C and N values than annual cash-grain cropping systems based on the aforementioned findings at WICST (Cates et al., 2016; Jokela et al., 2011). Furthermore, we hypothesized that these fractions would be greatest in the top 0 to 15 cm, with maximum values reached in the middle of the growing season and then steadily declining. We evaluated these hypotheses by measuring TC and TN and labile fractions via PMN, POXC, and C-min five times throughout the 2016 growing season.

MATERIALS AND METHODS

Site Description, Experimental Design, and Management

This study was conducted within the WICST, a long-term trial established in 1989 at the University of Wisconsin-Madison Arlington Agricultural Research Station in Arlington, WI (43°18′ N, 89°20′ W). According to USDA Soil Taxonomy, the soils at WICST are classified as Plano silt loam (fine-silty, mixed, superactive, Mesic Typic Argiudoll). Formed under tallgrass prairie vegetation in loess deposits over calcareous glacial till, these soils are relatively deep, highly fertile, and well-drained. The experimental design of the WICST trial is a randomized complete block design with four blocks. Each plot is 155.5 × 18.3 m (0.3 ha), which is large enough for commercial-scale farm equipment to be used for field applications, planting, cultivation, and harvest. Since establishment in 1989, field management has evolved to adequately reflect best management practices and advances in agricultural technology (e.g., hybrid cultivars, fertilizer rates, etc.). A full study and site description of WICST can be found in Posner et al. (1995).

There are six common Midwest cropping systems represented at WICST, with each phase of each cropping system rotation represented each year and replicated across four blocks. The three-annual cash-grain systems are: continuous corn (*Zea mays* L.) (CC), strip-till corn and no-till soybean [*Glycine max* (L.) Merr] (CS), and organic corn–soybean–winter wheat (*Triticum aestivum* L.)–oat (*Avena sativa* L.) cover crop interseeded with berseem clover (*Trifolium alexandrinum* L.) (CSW). The three dairy-forage systems are: corn–alfalfa–alfalfa–alfalfa (*Medicago sativa* L.) (CAAA), organic corn–oat/alfalfa–alfalfa (COA), and a rotationally grazed pasture (PAS). The pasture is seeded with a mixture of orchardgrass (*Dactylis glomerata* L.), tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass (*Lolium perenne* L.), gain festolium (*Schedonorus pratensis x Lolium multiflorum*), and red clover (*Trifolium pratense* L.). Liquid dairy manure (131,300 kg ha^{-1}) is applied in the fall to the CAAA before corn and to the CSW before corn and the oats/alfalfa planting. Since 2008, CSW has received composted poultry manure (4040 kg ha^{-1}) in the spring prior to corn planting. Organic corn–oat/alfalfa–alfalfa, CAAA, and CC are all disk-chiseled in fall prior to planting, whereas CS is strip tilled and CSW is disk-chiseled in the spring. Field cultivation occurs prior to planting in the CC, CSW, CAAA, and COA, with an additional four tillage and cultivations executed after planting in the CSW and COA. During the 2016 growing season, all conventional grain and forage corn plots were planted with starter fertilizer on 3 May with the corn variety 103d P0339 AMXT from Pioneer Seed Co. in Arlington, WI. The organic grain and forage plots were planted with the corn variety 96d 41R00 from Blue River Hybrids Organic Seed Co. in Ames, IA, on 20 May, and weeds were controlled mechanically with field cultivation and rotary hoeing on 2, 7, 14, and 22 June 2016. Synthetic N fertilizer (28% urea-ammonium nitrate) was applied to CC and CS cropping systems at a rate of 200 kg ha^{-1} on 17 June, and corn was harvested on 20 October for all plots in 2016.

Soil Sampling

Soils were sampled every month of the corn growing season in 2016 at depths 0 to 15 cm and 15 to 30 cm. The sampling dates were 19 May, 16 June, 14 July, 15 August, and 19 September. Soil was collected within the corn phase of each rotation, as well as the pasture. In the corn, nine soil cores (2.5 cm diameter) were collected between rows and composited into a single sample for each depth per plot. As with the corn plots, nine soil cores from the pasture were collected from middle of the plot and composited into a single sample for each depth. Samples were dried at 32°C in a forced-air drier for 1 wk and ground to pass through a 2-mm sieve with a hammer mill.

Potentially Mineralizable Nitrogen

Potentially mineralizable N was measured following the method of Ugarte and Wander (2012), an adaptation of Drinkwater et al. (1996). Briefly, 5 g of each soil was extracted with 50 mL 2 M potassium chloride (KCl), shaken for 1 h, centrifuged for 5 min at 1320 rcf, decanted, and filtered through a 2.7-μm filter paper. A 5-g sample of each soil was also combined with 10 mL H_2O in 50-mL centrifuge tubes to achieve anaerobic conditions and incubated for 1 wk at 40°C in duplicate. After the 1-wk incubation period, incubated tubes were extracted with 40 mL of 2.5 M KCl to bring volume of extract to a 2 M concentration, centrifuged at 1320 rcf, and filtered through a 2.7-μm filter paper. Filtered extracts were frozen until analysis. The concentration of NH_4–N for all soils was determined colorimetrically following the modified Berthelot method as proposed by Hood-Nowotny et al. (2010). The 7-d PMN was determined by calculating the difference between the initial NH_4–N concentration and the average NH_4–N concentration after 7 d.

Carbon Mineralization

Ten grams of air-dried soil was weighed, placed into a beaker, and then placed into a 1-L canning jar and moistened to 50% water-filled pore space with deionized water dispensed onto the soil surface with a micropipette. An average bulk density of 1.15 was used for all soil samples when calculating water-filled pore space, considering that dry bulk density for these soils range from 1 to 1.25 g cm^{-3}. The flush of CO_2 after the 24-hour incubation was determined with a closed loop system that circulated respired CO_2 through a Li-Cor LI-820 infrared gas analyzer. One-day CO_2–C was calculated as the difference between time zero and 1-d CO_2 concentration. The CO_2 concentration at time zero was calculated by taking the average CO_2 ppm reading of three blanks at the end of the incubation and subtracting it from the average ppm of each sample for each run.

$$\text{C-min} = [(\text{Cv} \times \text{M} \times \text{P})/(\text{R} \times \text{T})(\text{H}/\text{W})]0.001/0.0001$$

where Cv is the average ppm of CO_2, M is the molecular weight of C (12 mg $mumol^{-1}$), P is the barometric pressure (1 atm), R is universal gas constant (0.0820575 L × atm × °K × mole), T is the incubation temp in K (295.15), H is the volume of the incu-

bation chamber (0.932 L), and W is the mass of the soil used in the incubation (10 g). The volume of the tubing connecting the chamber to the gas analyzer was considered negligible.

Permanganate Oxidizable Carbon

Permanganate oxidizable C was analyzed and calculated according to the modified procedure of Culman et al. (2012) that was derived from the procedure of Weil et al. (2003). Air-dried and ground (2 mm) soil was weighed at 2.50 g and combined with 18 mL H_2O and 2 mL of 0.2 mol $KMnO_4$ (equivalent to 0.02 mol L^{-1} $KMnO_4$) in a 50-mL centrifuge tube. Tubes were shaken for exactly 2 min at ~240 oscillations per minute and allowed to settle for exactly 10 min in a dark place. After settling, 0.5 mL of supernatant from the tube was transferred to another centrifuge tube containing 49.5 mL of deionized water and immediately shaken. Once thoroughly mixed, 275 mL of diluted supernatant was transferred to a 96-well plate, and sample absorbance was read using a spectrophotometer at 550 nm.

Table 1. Averages and ANOVA results for total carbon (TC), total nitrogen (TN), NH_4–N concentration, NO_3–N concentration, and PAN (NH_4–N + NO_3–N) as affected by six cropping systems [continuous corn (CC), strip till corn–soybean (CS), organic corn–soybean–winter wheat–oat/berseem clover (CSW), corn–alfalfa–alfalfa–alfalfa (CAAA), organic corn-oats/alfalfa-alfalfa (COA), and rotationally grazed pasture (PAS), two depths, and sampling date and their interactions.

Treatment	TC	TN	NH_4–N	NO_3–N	PAN
	g kg^{-1}		mg kg^{-1}		
Cropping system (C)					
CC	21.4bc†	2.18bc	6.54b	28.4a	35.0a
CS	23.0bc	2.27bc	9.14a	18.7b	28.0b
CSW	19.3c	2.01c	3.20c	8.26d	11.5cd
CAAA	22.6bc	2.33b	2.98c	13.2c	16.1c
COA	24.2ab	2.36ab	3.55c	19.8b	23.3b
PAS	27.7a	2.63a	4.37bc	3.4e	7.70d
Depth (D), cm					
0–15	26.7a	2.63a	6.70a	21.6a	28.3a
15–30	19.4b	1.96b	3.23b	9.00b	12.2b
Time (T)					
19 May 2016	22.4c	1.99c	3.53b	7.63c	11.0d
16 June 2016	22.7bc	1.97c	3.57b	14.0b	17.5c
14 July 2016	23.6a	2.27b	9.93a	14.9b	24.8b
15 Aug. 2016	23.2ab	2.66a	4.02b	27.2a	31.2a
19 Sept. 2016	23.2ab	2.59a	3.78b	12.8b	16.6c
			p Value		
Sources of variation					
C	0.055	0.049	0.004	<0.0001	0.0002
D	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
C × D	0.218	0.183	0.0005	0.175	0.040
T	0.002	<0.0001	<0.0001	<0.0001	<0.0001
C × T	0.308	0.993	<0.0001	<0.0001	<0.0001
D × T	0.227	0.059	<0.0001	0.044	0.020
C × D × T	0.530	0.917	<0.0001	0.047	0.0007

† Lowercase letters denote significant differences within a column among cropping systems, between sampling depths, or among sampling times ($P \leq 0.10$).

Total Soil Carbon and Nitrogen

For total organic C and TN analysis, 2 mm whole soil subsamples were ground to a fine powder from each cropping system, depth, and time of collection at WICST, weighed, and packed into a 5 mm × 9 mm tin capsule. Total N and TC were determined by the dry combustion method using a Flash EA 1112CN Automatic Elemental Analyzer (Thermo Finnigan). Inorganic C has been found to be insignificant in these soils (Paul et al., 2001); thus, organic C was assumed to be the same as TC and SOC.

Statistical Analysis

Analysis of variance was performed using the PROC MIXED procedure of the Statistical Analysis System (SAS version 9.4; SAS Institute [2107]) for testing the effects of cropping system, sampling date, and depth of soil sampling, using $\alpha = 0.1$ for determination of significance. Cropping system, soil depth, and sampling time were considered fixed effects. Block; block × cropping system interaction; and the interaction of block, cropping system, and depth were considered random effects. For each soil measurement, covariance structures such as ante-dependence, compound symmetry, autoregressive, and heterogeneous compound symmetry were tested, and the structure that provided the lowest Akaike information criterion was selected. Time was considered a repeated measure (REPEATED) to account for autocorrelation between sampling dates within a plot. An autoregressive (TYPE = ar) covariance structure was used for C-min, POXC, and TC measures; an ante-dependence structure was used for plant available N and NO_3–N; and a heterogeneous compound symmetry (TYPE = csh) covariance structure was used for NH_4–N, PMN, and TN. Normality and homogeneity of variance assumptions were met for these statistical analyses, so data transformation was unnecessary. Within a given soil measurement, if significant three-way interaction effects were found after running an overall ANOVA on the factors of interest, SLICE statements were used to partition Fisher's LSD (LSMEANS) for a given interaction among cropping system, sampling date, and time. All significant fixed effects and two and three-way interactions were graphed.

RESULTS

Weather

The second, third, and fourth sampling dates had similar maximum and minimum daily air temperatures, and the first and last sampling dates had the lowest air temperatures during the 2016 growing season (Supplemental Fig. S1). Cumulative precipitation between sampling dates fluctuated throughout the growing season, with a substantial rainfall event (>40 mm) occurring just prior to sampling in June. The August sampling date had the greatest maximum and minimum air temperatures and cumulative precipitation between sampling dates.

Total Nitrogen, Total Carbon, and Inorganic Nitrogen

The organic forage system (COA) and pasture (PAS) had higher TC and TN contents compared with the cash-grain systems and CAAA across depths and sampling times (Table 1).

Cropping system, depth, and sampling time were all significant factors of TC and TN content, but the interactions between depth and time were only significant for TN content (Fig. 1). The CC and CS systems did not have significantly different TC or TN, with CS having slightly higher TC and TN contents than CC. The CC, CS, and COA systems had greater TC and TN than the organic grain cropping system, whereas the organic forage system (COA) had significantly greater TC and TN than the organic annual cash grain system (CSW) across sampling times and depths (20 and 15%, respectively).

Across depth and cropping systems, all inorganic N concentrations were lowest in May; NO_3–N and plant available N concentrations peaked in mid-August, whereas NH_4–N concentrations reached their maximum in mid-July (Table 1). Like TC and TN, inorganic N concentrations decreased with depth by 50% or more. Additional data regarding inorganic N concentrations can be found in Supplemental Fig. S2, S3, and S4.

Labile Carbon and Nitrogen Fractions

Cropping system, sampling depth, sampling time, and their three-way interactions were significant for PMN and C-min, whereas only main effects were significant for POXC (Table 2). Apart from PAS, PMN, POXC, and C-min did not significantly increase within grain or forage cropping systems when averaged across sampling depth and sampling time; however, forage cropping systems generally had greater labile soil C and N concentrations than the annual cash-grain systems. Although these labile C and N fraction measurements decreased with depth and were greater in the dairy forage systems, labile C and N seasonal dynamics differed between cropping systems within given sampling dates and depths.

The three dairy forage cropping systems generally had higher PMN than the cash-grain systems in the top 0 to 15 cm of soil for the May, July, and September sampling dates, with PAS and CAAA having the greatest PMN across depth and time (Table 2; Fig. 2). Potentially mineralizable N generally followed the same trend as TN, with the highest values observed in PAS and the lowest values observed in the three-annual cash-grain cropping systems. The two cropping systems that received synthetic N fertilizer in June (CC and CS) were not significantly different across depth and time but did show significant differences in their temporal PMN trends.

The three dairy forage cropping systems had significantly greater C-min values than all annual cash grain cropping systems, with COA and PAS having significantly higher C-min and POXC. In particular, PAS had significantly higher C-min values in the top 0- to 15-cm soil depth for every sampling date (Fig. 3). Like PMN, the two cropping systems that received synthetic N fertilizer in June (CC and CS) had significant differences in their temporal trends, and some cropping systems that did not receive synthetic N (CSW, CAAA, and COA) had significant C-min temporal dynamics. The CS and CC systems did not significantly differ in their C-min values across depth and time (Table 2).

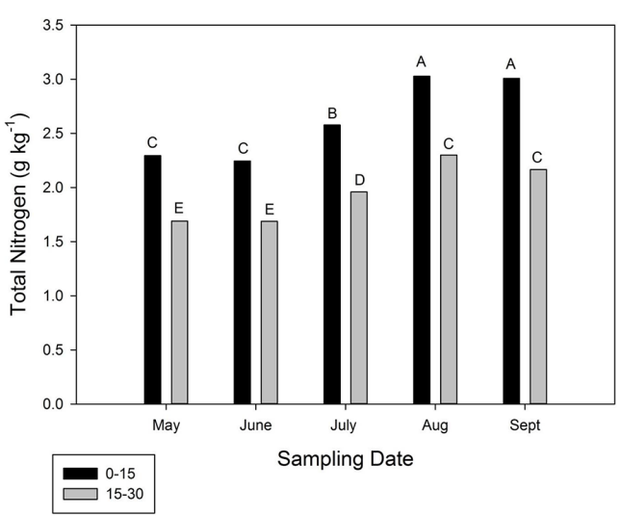


Fig. 1. Total nitrogen content of soils averaged across all cropping systems as affected by five sampling dates (19 May, 16 June, 14 July, 15 Aug., and 19 Sept. 2016) and two soil depths (0–15 cm, 15–30 cm). Letters indicate significant differences between the two sampling depths across sampling dates ($P \leq 0.10$).

Table 2. Averages and ANOVA results of potentially mineralizable nitrogen (PMN), carbon mineralization (C-min), and permanganate oxidizable carbon (POXC) by six cropping systems [continuous corn (CC), strip till corn–soybean (CS), organic corn–soybean–winter wheat–oat/berseem clover (CSW), corn–alfalfa–alfalfa–alfalfa (CAAA), organic corn–oats/alfalfa–alfalfa (COA), and rotationally grazed pastured (PAS)], two depths, and sampling date and their interactions.

Treatment	PMN	C-min	POXC
		mg kg^{-1}	
Cropping system (C)			
CC	40.5c†	67.9c	501bc
CS	43.3bc	73.5c	504bc
CSW	40.2c	73.8c	494c
CAAA	49.9b	82.6b	553ab
COA	48.5bc	83.8b	561a
PAS	62.3a	119a	597a
Depth (D), cm			
0–15	65.8a	109a	687a
15–30	29.1b	57.9b	382b
Time (T)			
19 May 2016	40.8d	82.3bc	499c
16 June 2016	45.2c	79.2c	537b
14 July 2016	47.8bc	88.8a	563a
15 Aug. 2016	55.2a	85.5ab	542ab
19 Sept. 2016	48.2b	81.5c	532b
		p Value	
Sources of variation			
C	0.003	<0.0001	0.029
D	<0.0001	<0.0001	<0.0001
C × D	0.143	0.0001	0.933
T	<0.0001	0.0005	0.0002
C × T	<0.0001	0.002	0.213
D × T	0.168	0.487	0.823
C × D × T	0.070	0.038	0.784

† Lowercase letters denote significant differences within a column among cropping systems, between sampling depths, or among sampling times, at $P \leq 0.10$.

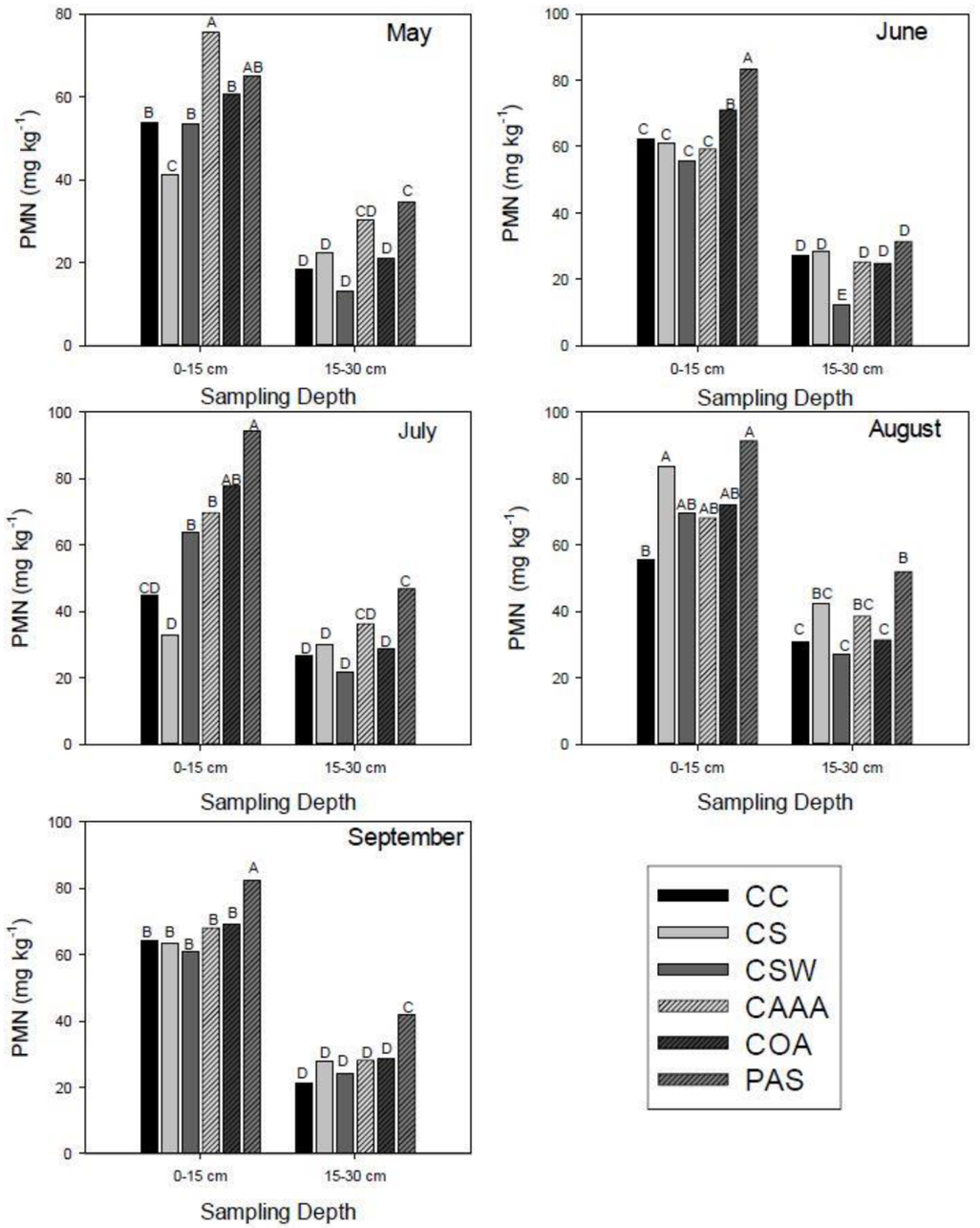


Fig. 2. Potentially mineralizable nitrogen (PMN) content of soils as affected by six cropping systems (continuous corn [CC], strip till corn–soybean [CS], organic corn–soybean–winter wheat–oat/berseem clover [CSW], corn–alfalfa–alfalfa–alfalfa [CAAA], organic corn–oats/alfalfa–alfalfa [COA], and rotationally grazed pastured [PAS]) at two depths (0–15 cm, 15–30 cm) for each of the five sampling dates (19 May, 16 June, 14 July, 15 Aug., and 19 Sept. 2016). Letters indicate significant differences across cropping systems and depths within each sampling date ($P \leq 0.10$).

Broadly, the POXC values followed TC content trends regarding cropping systems, depth, and temporal variability (Tables 1 and 2), with POXC values being significantly greater in the top 0 to 15 cm as compared with the bottom 15 to 30 cm in cropping systems irrespective of sampling date. Permanganate oxidizable C values of CC and CSW were not significantly different in each sampling date and depth; nor were the POXC values between CC and CS across sampling date and depth (Table 2; Fig. 4). The CSW had the lowest POXC across depth and time, as well as early, mid-, and end of the growing season in the lower 15- to 30-cm soil depth.

There is an overall temporal trend for C-min and PMN across cropping systems and depth; most cropping systems reached their maximum values by July or August and then stabilized or decreased. However, temporal trends for C-min and PMN values differed within a given cropping system across depths and within a given depth between cropping systems (Fig. 5 and 6). Moreover, C-min and PMN temporal trends differed for a given cropping system and depth. The cropping systems that did not receive synthetic N (i.e., CSW, CAAA, and COA) had significant PMN temporal trends throughout the growing season. The three forage systems generally had similar temporal PMN trends throughout the growing season,

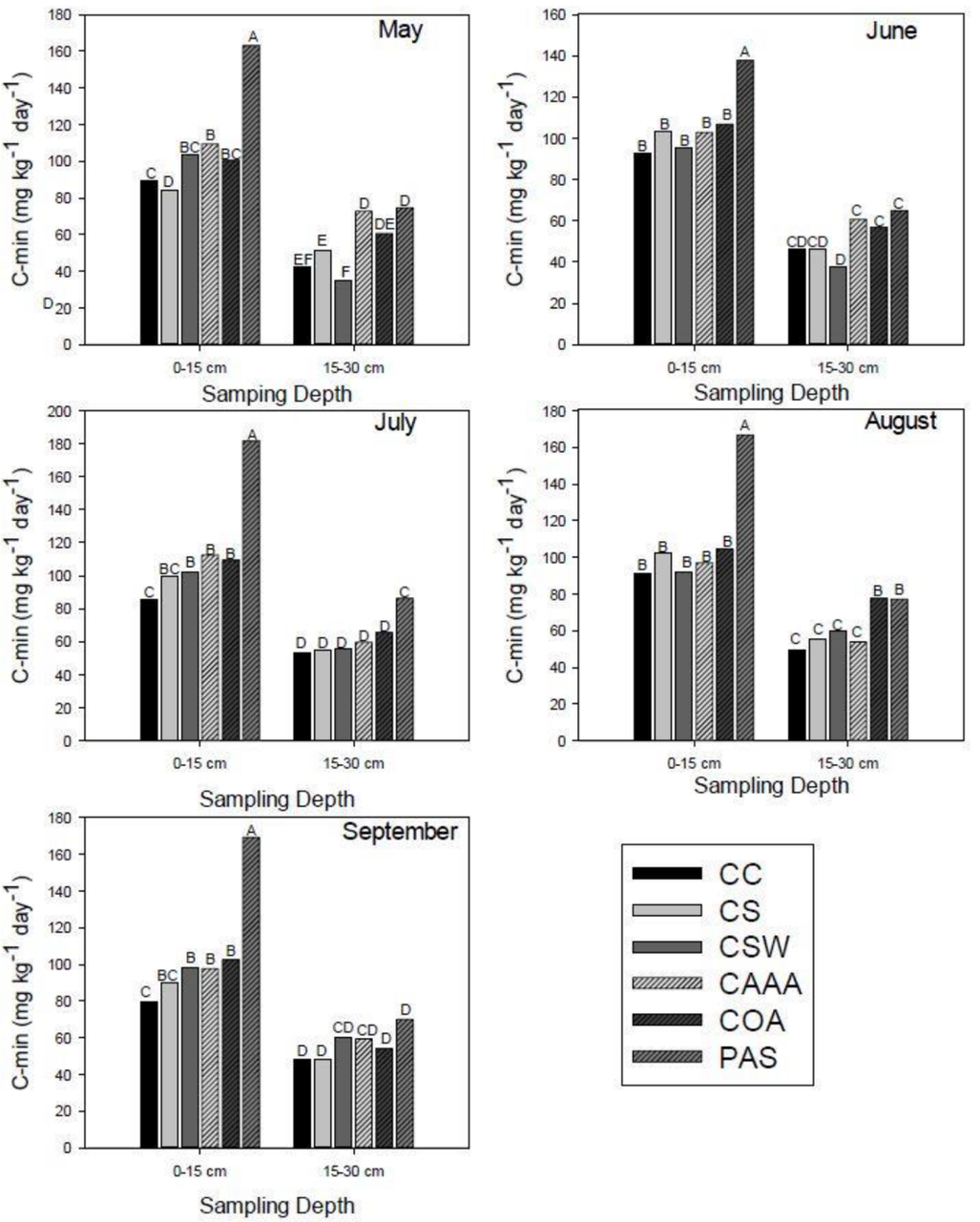


Fig. 3. Carbon mineralization (C-min) content of soils as affected by six cropping systems (continuous corn [CC], strip till corn–soybean [CS], organic corn–soybean–winter wheat–oat/berseem clover [CSW], corn–alfalfa–alfalfa–alfalfa [CAAA], organic corn–oats/alfalfa–alfalfa [COA], and rotationally grazed pastured [PAS]) at two depths (0–15 cm, 15–30 cm) for each of the five sampling dates in 2016 (19 May, 16 June, 14 July, 15 Aug., and 19 Sept. 2016). Letters indicate significant differences across cropping systems and depths within each sampling date ($P \leq 0.10$).

except for CAAA in the top 0 to 15 cm, whereas only the conventionally managed cropping systems (CC and CS) followed similar PMN temporal trends in the cash grain systems (Fig. 5).

Temporal trend similarities between cropping systems were more apparent at 15 to 30 cm than at 0 to 15 cm within the forage and cash-grain cropping systems. The PAS and COA had significantly different C-min values between sampling dates within forage-based cropping systems, whereas CSW and CS have significantly different C-min values between sampling dates within the annual cash grain cropping systems (Fig. 6). In the top 0 to 15 cm, C-min values of PAS fluctuate throughout the growing season, with minimum values occurring in June and maximum values in July. In the lower 15 to 30 cm, the temporal trend in PAS is similar to that in the top 0 to 15 cm, with COA following a very similar trend but with maximum values occurring in August rather than July. As with PMN, CC and CS appear to follow similar trends as one another in both the 0- to 15-cm and 15- to 30-cm soil depths.

DISCUSSION

Soil Carbon and Nitrogen Fraction Replenishment

Maximum C-min and PMN values were consistently observed during mid-growing season in most cropping systems

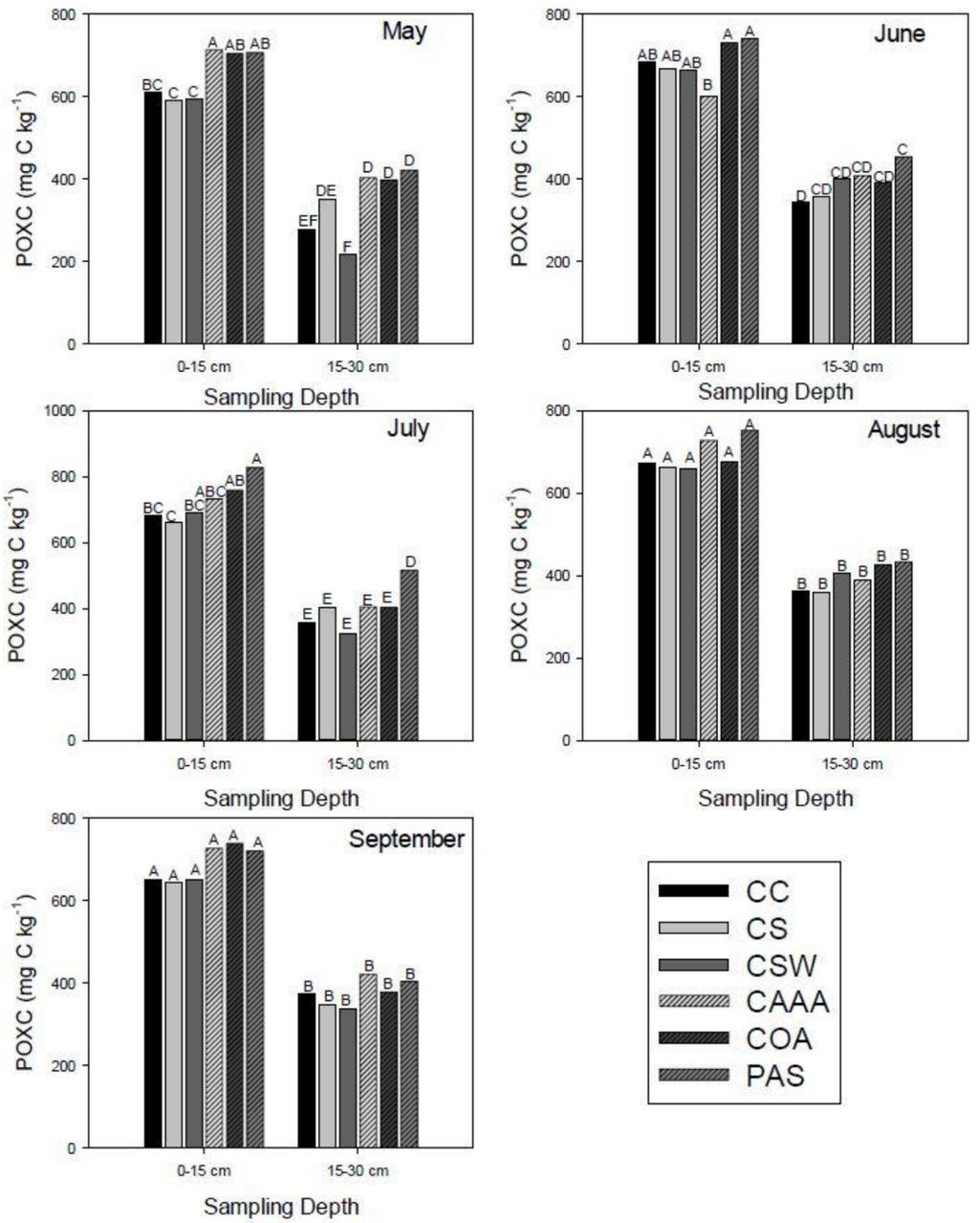


Fig. 4. Permanganate oxidizable carbon (POXC) content of soils as affected by six cropping systems (continuous corn [CC], strip till corn–soybean [CS], organic corn–soybean–winter wheat–oat/berseem clover [CSW], corn–alfalfa–alfalfa–alfalfa [CAAA], organic corn–oats/alfalfa–alfalfa [COA], and rotationally grazed pastured [PAS]) at two depths (0–15 cm, 15–30 cm) for each of the five sampling dates (19 May, 16 June, 14 July, 15 Aug., and 19 Sept. 2016). Letters indicate significant differences across cropping systems and depths within each sampling date ($P \leq 0.10$).

studied, which is comparable to the findings of Culman et al. (2013a) on a sandy soil in Michigan. Although seasonal trends in potential N mineralization have been found to be less pronounced than C mineralization (Campbell, 1999a, 1999b), our results indicate otherwise, particularly for C-min values in the grain and forage systems in the top 0 of 15 cm of soil (Fig. 6). Fluctuations in C-min and PMN as seen in our study differ from those found in Campbell et al. (1999a) on a Swinton silt loam in Saskatchewan, where potential N mineralization remained constant or steadily decreased throughout the wheat growing season, whereas C mineralization increased until autumn harvest in wheat cropping systems. This difference likely stems from dissimilar climate and crop type because biomass inputs with large C/N ratios (e.g., wheat residue) likely determined seasonal soil C and N mineralization dynamics. Additionally, manure applications to the dairy forage systems in the study presented here may contribute to the difference in trends from other published literature, including the study by Bonde and Rosswall (1987) in Sweden, which also included alfalfa but received no additional N inputs. In a study by Franzluebbers et al. (1994), mineralizable N followed a similar temporal trend throughout the growing season as mineralizable C in the top 0 to 5 cm but only if the wheat

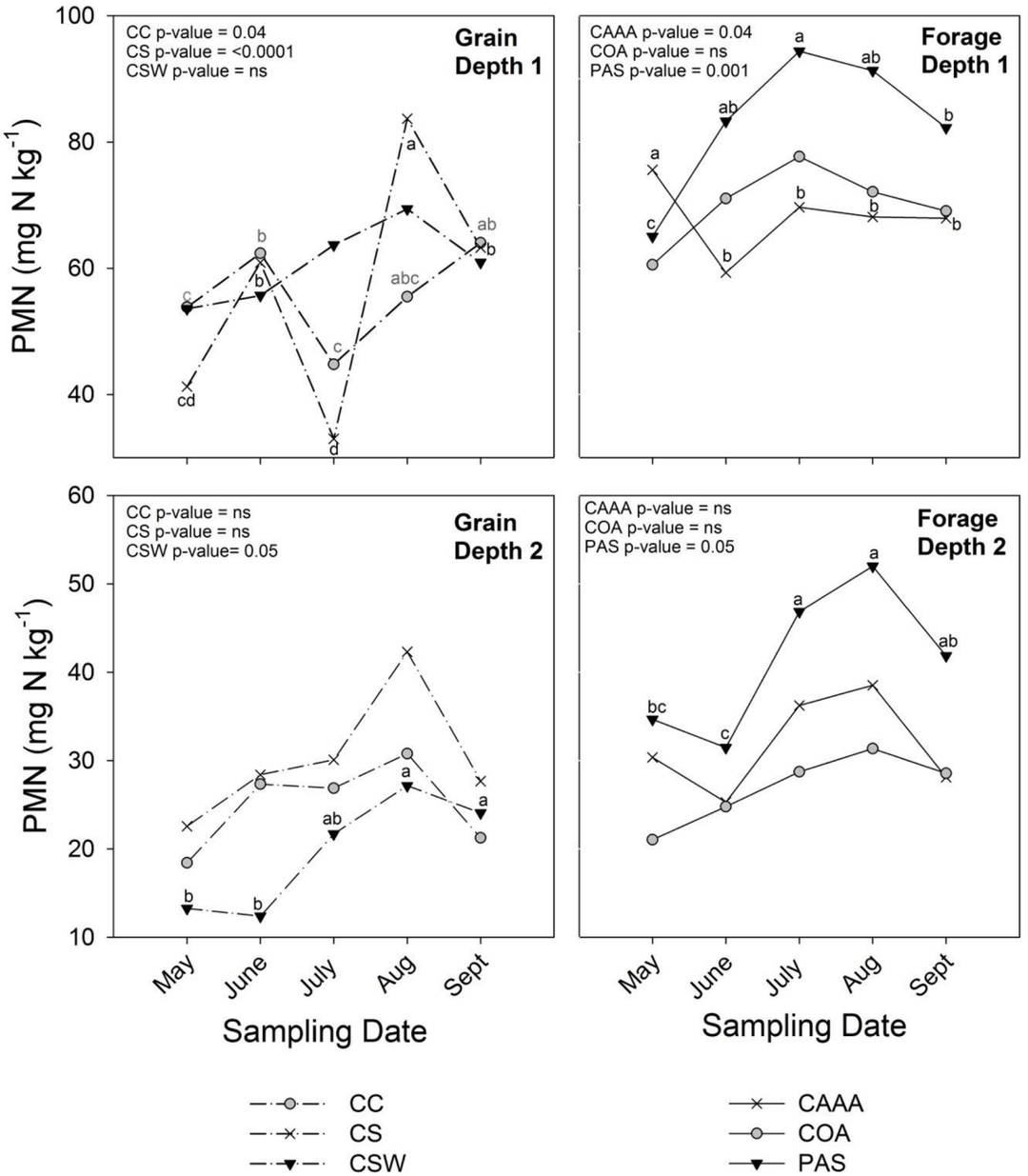


Fig. 5. Within-season soil sampling time effect on potentially mineralizable nitrogen (PMN) content (19 May, 16 June, 14 July, 15 Aug., and 19 Sept. 2016), six cropping systems (continuous corn [CC], strip till corn–soybean [CS], organic corn–soybean–winter wheat–oat/berseem clover [CSW], corn–alfalfa–alfalfa–alfalfa [CAAA], organic corn–oats/alfalfa–alfalfa [COA], and rotationally grazed pastured [PAS]) at Depth 1 (0–15 cm) and Depth 2 (15–30 cm). Letters indicate significant differences within a given cropping system across sampling dates and sampling depth ($P \leq 0.10$).

cropping system received synthetic fertilizer N inputs. Potential N and C mineralization trends observed in Franzluebbers et al. (1994) deviated at lower soil depths (5–20 cm) if fertilized and at all depths if the wheat cropping system was not fertilized. This is not the case for the cropping systems at WICST: systems that received synthetic fertilizer N mid-season (i.e., CC and CS) did not have synchronized PMN and C-min trends at either depth, and systems that did not receive synthetic N mid-season (i.e., CSW, CAAA, COA) had significant temporal trends throughout the growing season. The differences among these studies and the study at WICST implies that PMN and C-min temporal dynamics are likely driven by a complex suite of factors, such as soil moisture and temperature, quality and quantity of organic C and N inputs, seasonal rhizodeposition, synthetic N fertilization, tillage depth and frequency, and depth of observation (Campbell et al., 1999a; Franzluebbers et al., 1994).

CS and CC appear to follow similar PMN and C-min temporal trends at both depths (Fig. 5 and 6), similar to the results Franzluebbers et al. (1994) obtained on a silty clay loam in south-central Texas. PAS had significantly greater PMN values in July as compared with May at both soil depths, and greater C-min values in July as compared with June at both soil depths. The potential implication of these results are threefold: (i) synthetic N fertilizer application may influence temporal dynamics in C mineralization

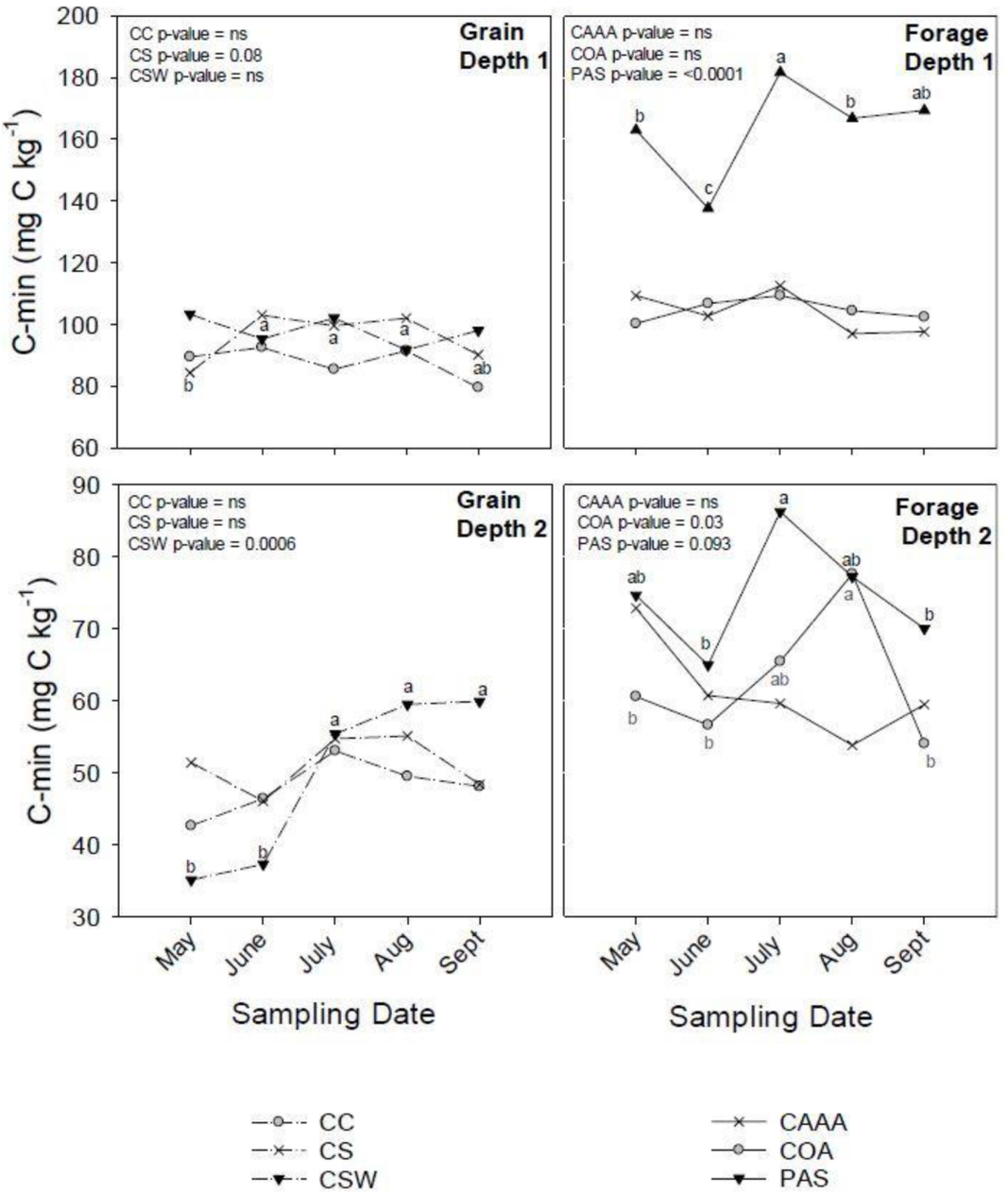


Fig. 6. Within-season soil sampling time effect on carbon mineralization (C-min) content (19 May, 16 June, 14 July, 15 Aug., and 19 Sept. 2016), six cropping systems (continuous corn [CC], strip till corn–soybean [CS], organic corn–soybean–winter wheat–oat/berseem clover [CSW], corn–alfalfa–alfalfa–alfalfa [CAAA], organic corn–oats/alfalfa–alfalfa [COA], and rotationally grazed pastured [PAS]) at Depth 1 (0–15 cm) and Depth 2 (15–30 cm). Letters indicate significant differences within a given cropping system across sampling dates and sampling depth ($P \leq 0.10$).

trends to a greater degree than crop rotation and tillage frequency (ii) mid-season soil C and N fraction replenishment occurs in a wide range of cropping systems, and (iii) the impact of sampling time is significant when comparing across agroecosystems, which corroborates recent findings by Hurisso et al. (2018) that sampling at the same time each year will be necessary to adequately compare cropping system's labile C and N fractions.

Long-term Cropping System Effects on Total Soil Carbon and Nitrogen Contents

After 28 yr at WICST, CC and CSW did not have significantly different TN or TC content when averaged across depth and time, with CC having greater TN and TC contents than the CSW (Table 1). Similar SOC trends were found at the same study site for these cropping systems (Cates et al., 2016; Sanford et al., 2012), although they contrast with other literature that found that organic cropping systems have greater SOC concentrations (Gattinger et al., 2012; Marriott and Wander, 2006; Pimentel et al., 2005; Teasdale et al., 2007) and TN (Marriott and Wander, 2006; Pimentel et al., 2005) as compared with conventional cropping systems. Two factors may contribute to the conventional grain cropping system at WICST having similar TN and TC contents to the organic grain system despite the increased plant diversity and cover cropping in the organic grain system. First, multiple studies that have been con-

ducted at WICST (Cates et al., 2016; Jokela et al., 2011; Sanford et al., 2012) have reported that CC had greater below- and aboveground C inputs as compared with CSW. Jokela et al. (2011) reported that CC had an estimated 75% greater above- and belowground inputs as compared with CSW, whereas Cates et al. (2016) reported that CC had an estimated 90% greater annual belowground C inputs and 66% greater annual aboveground C inputs as compared with CSW. Second, CSW undergoes more frequent tillage and cultivation events than CC, especially within the corn phase of CSW. The combined factors of reduced annual C inputs and intensive tillage likely result in the similar TC and TN of CC and CSW, despite manure and cover crop inputs.

The organic grain system (CSW) had less TC and TN across sampling depth and time than CS and COA, although the differences were only significant between CSW and COA (Table 1). Teasdale et al. (2007) found that organic management practices that included additions of manure (similar to the approach with CSW) increased SOC to a greater extent than no-tillage systems receiving synthetic fertilizer. Although our study yielded different results, the differences between CS and CSW may be driven by the same agronomic factors controlling the difference between CC and CSW: tillage frequency within the corn phase and annual C inputs. The CS system receives an estimated 41% greater belowground and 28% greater aboveground annual C inputs as compared with CSW (Cates et al., 2016). Given the inherent reduction in tillage in a no-till cropping system, CS also had significantly fewer soil disturbance events than CSW. The differences between COA and CSW likely come from similar factors, although the difference between the two systems is probably amplified by the incorporation of a perennial legume in COA. Additionally, the COA has an estimated 33% greater aboveground and 182% greater belowground annual C inputs as compared with CSW (Cates et al., 2016). Although both systems receive manure and undergo intensive tillage during the corn phase of their rotation, COA has less soil disturbance in its overall rotation. Thus, reduction in tillage both within and outside of the corn phase in addition to increased annual C inputs appear to be driving differences between cropping systems in TN and TC after 25 yr of management.

Long-Term Cropping System Effects on Labile Carbon and Nitrogen

Our results indicate that, across sampling depth and dates, shifts within a cropping system (grain or forage) in the context of this study were not enough to significantly affect PMN, POXC, and C-min measurements, with the exception of PAS (Table 2). There was no significant difference in PMN, POXC, or C-min values within the annual cash-grain systems. The perennial forage cropping systems with the greatest plant diversity (COA and PAS) had significantly greater C-min and POXC than all cash-grain cropping systems, whereas the perennial forage cropping systems with the greatest perenniality and legume incorporation (PAS and CAAA) had the greatest PMN across all cropping systems (Table 2). This suggests that, rather than a singular change in practice within a grain system (e.g., conventional tillage vs. no-tillage, conventional vs. organic), a more profound system change must occur to see increased labile C and N fractions on Mollisols in the Midwest region of the United States.

Averaged across cropping systems and sampling time, all labile C and N measures significantly decreased with soil depth (Table 2), and relative differences in labile C and N measures between cropping systems varied with soil depth (Fig. 2–4). Other studies conducted in Michigan (Culman et al., 2013b), Texas (Dou et al., 2008), and New Brunswick (Dessureault-Rompré et al., 2013) found similar trends when evaluating labile C and N fractions. Permanganate oxidizable C was the only labile soil measure in this study that was significantly greater in the upper 0 to 15 cm as compared with the lower 15 to 30 cm for every cropping system (Fig. 4). A study by Plaza-Bonilla et al. (2014) found that relative differences in POXC between a tilled and a no-till cropping system varied with depth, with the no-till system having significantly more POXC only in the top 0 to 5 cm of soil. Similarly, Hurisso et al. (2014) found that relative differences in C-min and PMN values between a native prairie, CRP, no-till, conventional till, and historic wheat fallow varied significantly with depth. Hurisso et al. (2016) and Culman et al. (2012) call for standardizing depth of soil sampling when it comes to measurements of C-min and POXC; our results provide further support for these measures as well as PMN.

The CC and CS systems did not significantly differ in TC, TN, PMN, POXC, or C-min (Tables 1 and 2), suggesting that the shifts in management practices characteristic of the annual cash-grain cropping systems at WICST were not significant enough to shift labile or TC and TN contents in the context of this study. These results are not particularly surprising, considering Sanford et al. (2012) reported that both the CC and CS cropping systems at WICST resulted in soil C stock losses since WICST establishment in 1989. A meta-analysis conducted by Powlson et al. (2014) argues that no-till cropping systems show only a small increase in organic C as compared with conventionally tilled cropping systems; the majority of this C in labile forms would be easily decomposed if the soil were disturbed. In agreement with this argument, our results indicate a slight (but nonsignificant) increase in labile soil C in the CS cropping system as compared with the CC cropping system (Table 2; Fig. 3). Consistent with our findings, both long-term (Hurisso et al., 2014) and short-term (Bhowmik et al., 2017; Wang and Sainju, 2014) studies across a range of soil types and climates found that certain labile C or N concentrations were not significantly greater in no-till versus conventionally tilled systems. For this study, we observed no significant difference in TC or labile soil C and N content likely because CS receives less annual C above- and belowground inputs than CC (Cates et al., 2016; Jokela et al., 2011; Sanford et al., 2012) and because significant sources of N are removed from CS though the removal of soybean grain and biomass.

CONCLUSION

Our study provides evidence that, across two different depths (0–15 cm and 15–30 cm) and time within season (May–September), the different long-term management practices of

annual cash-grain systems represented at WICST do not significantly increase labile C and N fractions in Mollisols of the Midwest. This study suggests that a more substantial system shift, in this case from cash grain to forage-based cropping systems, is required to see significant increases in labile soil C and N fractions as opposed to singular changes in practice. More specifically, regardless of C or N measurement, pasture resulted in the greatest value. Differences among other cropping systems varied by measurement, with C-min and PMN expressing the clearest and largest differences between forage-based and grain-based cropping systems. In addition to cropping system effects, depth and sampling time interaction effects with cropping systems did not affect TC and TN, whereas C-min and PMN were both affected. Thus, C-min and PMN, which can be viewed as labile fractions of C and N, as well as biochemical indicators of soil health, provide unique responses to management, space, and time relative to TC and TN content alone. Although these labile soil C and N fraction measurements could provide a useful framework for agricultural stakeholders interested in tracking and predicting how certain management practices are affecting soil health and thus long-term sustainability, our study demonstrates and corroborates other recent studies that call for temporal and depth variation of these measurements to be considered when sampling.

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SUPPLEMENTARY MATERIAL

The following figures are supplementary data to this article.

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