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Long-term tillage, rotation and perennialization effects on particulate and aggregate soil organic matter

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ABSTRACT

Perennialization and reducing tillage have increased soil organic matter (SOM) in both aggregate and particulate organic matter (POM) in short-term and small scale experiments, but there is a need for investigations into the long-term effects of agroecosystems on these dynamic pools of SOM. The objectives of this study were to investigate how management varying in crop rotation, tillage intensity and organic management from 1990 to 2013 has affected POM and aggregate C and N, and assess the relationship between these SOM fractions and biomass C inputs. We hypothesized that tillage, low biomass inputs, and annual crops in rotation would be associated with decreased POM and aggregate C and N. Soil from six systems from the Wisconsin Integrated Cropping Systems Trial (WICST) on a Phaeozem, or Mollisol, was sampled in 2013: Continuous Maize (*Zea mays* L.), Maize–Soybean [*Glycine max* (L.) Merr.], Organic Grain (including maize, soybean, and wheat [*Triticum aestivum* L.] sequentially seeded with oats [*Avena sativa* L.] and berseem clover [*Trifolium alexandrinum* L.]), Conventional Forage (three years alfalfa [*Medicago sativa* L.] followed by maize), Organic Forage (two years' alfalfa with oats nurse crop followed by maize), and Pasture (rotationally grazed, seeded to a mixture of red clover [*Trifolium pratense* L.], timothy [*Phleum pretense* L.], smooth bromegrass [*Bromu sinermis* L.] and orchardgrass [*Dactylis glomerata* L.]). Among all systems at 0–25 cm depth, we found significantly greater concentrations of POM-C in the Pasture (4.4 g C kg^{-1} soil) and POM-N in Pasture (0.30 g N kg^{-1} soil) and Organic Forage (0.25 g N kg^{-1} soil). The Organic Grain system had lower concentrations of macroaggregates and lower stocks of C and N within macroaggregates. Across all systems, belowground biomass C input was significantly positively correlated with POM-C, POM-N, and aggregate C and N. The data supported our hypothesis in part, as results indicate that frequent cultivation in the form of tine weeding and rotary hoeing for weed control in Organic Grain rotation is likely disrupting formation of aggregates and storage of C and N therein. However, in systems that were chisel plowed every one to three years, high biomass C inputs maintain POM-C and POM-N and soil aggregation equivalent to the fully perennial system.

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1. Introduction

There is immense interest in adjusting agricultural management to increase concentrations of soil organic carbon (SOC) for environmental benefits including climate change mitigation, improved soil fertility, erosion prevention and improved infiltration (Lal, 1993, 2001; Sanchez et al., 2004). Since SOC may be preserved due to its chemical characteristics, physical protection in aggregates, or immobilization in microbial biomass, it is difficult to predict which agricultural practices may increase SOC over the long term. Many soil processes can take decades to reach equilibrium (Six et al., 2004a,b), and so consistent management over time is needed to explore the long-term implications of adoption of cropping systems (Dick, 1992; Drury et al., 1998). The Wisconsin Integrated Cropping Systems Trial (WICST), established in 1990, was designed with input from farmers, researchers and extension agents, and applies six sets of management practices as whole systems (Table 1,Posner et al., 1995). No one practice can be tested by itself, e.g. tillage intensity across a range of rotations, but the strength of WICST is that each treatment represents a management system (grain vs. forage based, conventional vs. organic) and the long-term effects of these systems can be

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Table 1
Wisconsin Integrated Cropping Systems Trial (WICST) details including crop rotations, yield, tillage regime, and biomass C input estimates, 1993–2013. (Adapted and updated from Sanford et al., 2012 and Jokela et al., 2011).

Type	System	Crop phase	Average yield[a]	Primary tillage equipment	Estimated annual C input[b] (kg ha^{-1})	
			Mg ha^{-1}		Belowground	Aboveground
Grain	Continuous Maize	Maize	10.7	Chisel plow	4030	2355
	Maize–soybean	Soybean	3.6	No till	2007	1061
		Maize	11.1	Strip-till	4192	2450
		System mean			3093	1751
	Organic Grain	Soybean	3.2	Chisel plow	1774	938
		Winter wheat/clover-oats mixture	3.9	Field cultivator	1742	840
		Maize	8.9	Chisel plow	3732	1944
		System mean			2416	1241
Forage	Conventional Forage	Alfalfa seeding	5.8	Chisel plow	3143	734
		Alfalfa	11.0	None	822	2644
		Alfalfa	9.8	None	1183	7456
		Maize	12.0	Chisel plow	6731	2603
		System mean			2976	3361
	Organic Forage	Oat–alfalfa seeding	8.2	Chisel plow	2773	1649
		Alfalfa	11.2	None	1291	6671
		Maize	10.2	Chisel plow	5601	2182
		System mean			3221	3501
	Pasture	Mixed pasture	8.3	None	1606	4437

[a] Forage yields are reported at 100% dry matter (DM), corn yields at 84.5% DM, soybean yields at 87% DM, and wheat yields at 86.5% DM.
[b] Biomass C inputs to soil were estimated based on 1993–2013 WICST yields and harvest indices and shoot/root ratios from Bolinder et al., 2007. Post-harvest residue, cover crop biomass, cow or chicken manure, roots and root exudates are included in estimates.

compared in the same environment. The WICST occurs on a Typic Argiudoll, a productive silt loam which covers over 12 million ha of the United States Corn Belt, where management may have an enormous impact on SOC stocks (Lal, 2001). Agricultural management, variation in crop physiology and yields at WICST create an ecological gradient of biomass C inputs, which may affect both free and protected SOC (Table 1, Culman et al., 2010; Kong and Six, 2010; Rasse et al., 2005). We measured particulate organic matter (POM) and aggregate C and N, which have been proposed as potential indicators of management effects on SOC storage (Denef et al., 2007; Marriott and Wander, 2006b), after 23 years of varying tillage, rotation and inputs at WICST.

The inclusion of forage perennials, grazed or harvested, in a cropping system has important effects on storage of SOC due to a shift in timing and type of organic inputs, a greater root/shoot ratio, and a reduction in disturbance (lack of tillage). Soil under perennial vegetation has been shown to store more C than under annual crops, as the increased root presence and associated microbial activity provides SOC around which aggregates are formed (Culman et al., 2010; DeLuca and Zabinski, 2011; O'Brien and Jastrow, 2013). DuPont et al. (2010) showed that root production was halved by the no-tillage conversion of perennial grassland to annual wheat and microbial biomass C was significantly reduced in the top 40 cm of soil; changes in SOC after conversion to annual crop production are due to crop physiology as well as lack of disturbance. Many field studies have shown that reducing tillage increases storage of SOC (Halvorson et al., 2002; Havlin et al., 1990; Lal, 1984; Six et al., 1999), but there is also evidence that tillage regime does not change total SOC in some conditions, and that tilled systems may redistribute SOC lower in the soil profile (Baker et al., 2007; Gál et al., 2007). The variation in SOC response to tillage may be due to the variation in mechanism of SOC storage. Tillage reduces soil aggregation, promoting decomposition of the SOM within aggregates which is essential for long-term SOC storage (Denef et al., 2004; Six et al., 2000a; Yoo et al., 2011). Given the equivocal conclusions of past studies, it is important to take into account soil type, tillage frequency and type, and both free and protected SOC pools when assessing the impact of tillage on SOC storage.

By separating the large-size, labile POM and associated C and N, researchers have been better able to track a fast-cycling pool of SOC, which is vulnerable to loss upon shifts in management. The POM-C has been shown to be easily decomposed (Mirsky et al., 2008) and reflective of inputs (Marriott and Wander, 2006a). The POM-C and POM-N were increased in continuous maize compared with maize–soybean rotation (Coulter et al., 2009), and decreased in tilled soils compared with native sod more drastically than did SOC or total nitrogen (TN) (Cambardella and Elliott, 1992). Rotations with livestock, prairie and manure treatment have also increased POM-C compared with maize fields (Hernandez-Ramirez et al., 2009; Maughan et al., 2009). The sensitivity to management shown in these studies suggests that POM is a biologically labile but somewhat physically protected pool of SOM, so when management disrupts soil structure, POM-C and POM-N are affected more dramatically than are SOC or TN (Marriott and Wander, 2006b).

Soil aggregates have proven to be a useful, measurable tool for investigating SOC in natural and agroecosystems (Six and Paustian, 2014). Aggregate hierarchy theory as defined by Tisdall and Oades (1982) maintains that aggregates form around particles of SOM, and that stability of aggregates increases as size decreases due to the nature of the SOM acting as aggregate binding agents. Work using physical fractionation and C isotope analysis has revealed that organo-mineral associations with silt and clay are the most stable, followed by binding into microaggregates (53–250 μm) by persistent binding agents such as mycorrhizal byproduct glomalin, and finally binding into macroaggregates (<250 μm) by fungal hyphae, roots, and more temporary organic compounds such as polysaccharides (Denef et al., 2001; Gunina and Kuzyakov, 2014; Jastrow, 1996; Kleber et al., 2007; Six et al., 2004a; Tisdall and Oades, 1982). Physical protection prevents decomposition of simple bioavailable C compounds like proteins and glucose for

decades or even centuries (Marschner et al., 2008; Schmidt et al., 2011). However, physically protected SOM is vulnerable to decomposition after disturbance. Specifically, C associated with occluded microaggregates within macroaggregates has been shown to account for reduced SOC in tilled farmland compared with no-tillage (Denef et al., 2004, 2007; Six et al., 1999).

In this study, our objectives were to investigate how aggregate and POM-C and N were affected by a diverse range of cropping systems and assess the relationships between POM or aggregate measurements and below or aboveground biomass C inputs. We hypothesize that high frequency of tillage, low biomass inputs, and annual crops in rotation would be associated with decreased POM and aggregate C and N. Since aggregate fractionation yields a coarse POM fraction via an intricate, multi-step process and the POM extraction method was designed with high-throughput practical application in mind, comparison of the two methods will also generate valuable information about the type of analysis necessary to determine differences among agroecosystems.

2. Materials and methods

A complete description of soil organic matter extraction procedures is available in Supplemental materials.

2.1. Site description

The study took place within the WICST, which was established at the University of Wisconsin Agricultural Research Station in Arlington, WI in 1990. The soil is Plano silt loam (fine-silty, mixed, superactive, mesic Typic Argiudoll in USDA Soil Taxonomy). The mean annual temperature at Arlington is 6.9 °C and mean annual precipitation is 898 mm (1981–2010, National Oceanic and Atmospheric Administration). In 2013, mean annual temperature was 7.6 °C and mean annual precipitation was 978 mm.

The WICST is a randomized complete block trial with four blocks, with all phases of the six cropping systems represented each year (details are shown in Table 1), and a full site and study description can be found in Posner et al. (1995). Plots are 0.3 ha and commercial farm-scale equipment is used for all field work. Management has changed slightly over the years to reflect best management practices and advances in technology. The six cropping systems are divided into grain (Continuous Maize, Maize–Soybean, and Organic Grain) and forage (Conventional Forage, Organic Forage, and Pasture). The Pasture is rotationally grazed by six heifers per plot. Cattle manure slurry is applied in the fall prior to maize and first year alfalfa seeding in Conventional and Organic Forage systems. In Organic Grain, pelletized composted poultry manure is applied prior to maize and wheat crops, and a green manure cover crop of oats and clover is sown after wheat harvest. Maize in Continuous Maize, Maize–Soybean, and Conventional Forage receives commercial fertilizer at recommended rates (Laboski and Peters, 2012). The Continuous Maize, Organic Grain, and Conventional and Organic Forage systems are chisel-plowed in the fall prior to maize planting and Maize–Soybean is strip-tilled in the fall prior to maize planting. A chisel plow is also used prior to soybean planting in Organic Grain and alfalfa seeding in Conventional and Organic Forage. Soybeans in Maize–Soybean are planted using a no-till drill, as was red clover in the pasture. Additional cultivation, including use of a tine weeder, a rotary hoe, and Danish tine and/or disk hiller row cultivator for weed control, is performed as needed in maize, soybean and wheat of Organic Grain and Forage systems, while in Continuous Maize, Maize–Soybean, Conventional Forage, and Pasture weeds are chemically controlled.

2.2. Soil sampling

Soils were sampled to 50 cm depth for POM in April of 2013 using a tractor-mounted probe. Six samples (2.4 cm diameter cores) were composited into one sample per plot, and only in the plots where maize was planted in 2013 and the Pasture treatment. In the three grain systems, samples were split into 0–5, 5–25 and 25–50 cm, and analyzed for POM separately. The 0–25 cm data presented here are a weighted average of 0–5 and 5–25 cm. In the three forage systems, samples were split into 0–25 and 25–50 cm.

In November 2013, nine undisturbed cores (5.2 cm diameter) were taken to a depth of 25 cm for aggregate fractionation. Samples were collected from the plots that would be planted with maize in 2014 and from the Pasture treatment. Cores were kept refrigerated until analysis. Three cores were dried and weighed to determine bulk density. For aggregate fractionation, soil from the remaining six cores was composited into a single sample for each treatment.

2.3. Particulate organic matter

Particulate organic matter was determined according to an updated version of the method in Marriott and Wander (2006b). Briefly, soil was shaken and dispersed using sodium hexametaphosphate (SHMP, 50 g per L solution). Material >53 μm, the POM, was retained and rinsed 12 times. This method has been proposed as a rapid assessment of free organic matter (Marriott and Wander, 2006b). The fraction captured here includes coarse sand >53 μm, but since SOM has been shown to associate only minimally with sand (Zinn et al., 2007) and soil at this site has only 6% (±2) sand (G. S. Sanford, personal communication) we assume that the contribution of sand-associated C to this fraction is minimal.

2.4. Aggregate fractionation

Aggregate fractionation was carried out according to the methods of Six et al. (1998) with 100 g of moist soil. A 5.0 g subsample was weighed and dried at 50–60°C to determine the water content of the soil at the start of the procedure. Briefly, soil was separated into macroaggregates (>250 μm), microaggregates (53–250 μm) and silt and clay (<53 μm) by wet-sieving. A 20.0 g subsample of oven-dried macroaggregates was separated into occluded coarse POM (cPOM, >250 μm), occluded microaggregates (53–250 μm), and occluded silt and clay (<53 μm) using a microaggregate isolator as described in Six et al. (2002). All material was dried at 50–60 °C for a minimum of 24 h. Since all aggregate fractions were determined using wet-sieving, "aggregates" in this study are water-stable aggregates.

2.5. Carbon and nitrogen analysis

A subsample (8–10 mg) of each aggregate fraction, whole soil and POM was ground to a fine powder, and packed into 5 mm × 9 mm tin capsules. Carbon and N were determined by dry combustion using a Flash EA 1112CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy). Since inorganic carbon is negligible in these soils (Paul et al., 2001), organic C was assumed to be the same as total C. The POM-N, g N in POM kg^{-1} soil, was determined by multiplying the concentration of N in POM by the concentration of POM in the bulk soil. The proportion of soil N found in POM was determined by dividing the POM-N, g kg^{-1} soil, by the total N, g kg^{-1} soil. The POM-C and proportion of soil C found in POM were calculated in the same manner.

Aggregate C and N concentration were determined by multiplying the concentration of C or N in an aggregate fraction by the mass of each aggregate fraction per kg bulk soil. Aggregate C and N concentrations are presented in Supplemental Fig. 1. These

concentrations were converted to mass C or N per unit area, or stock of C and N (Fig. 4), using bulk density measurements taken at the same time. When aggregate fractions C and N were summed, we found sum soil C concentrations to be 4.2% less on average than was soil C concentrations in bulk samples, and sum soil N 3.7% less than was bulk soil N on average. Summing total C and N from aggregate fractions was slightly less variable than was bulk C and N, (perhaps due to the small sample [5.0 g per soil core] representing each treatment and the inherent field variability of SOC) so we present the aggregate fraction C and N as a proportion of summed aggregate fraction C and N (Fig. 5). Soil recovery from the fractionation process averaged 98% in the Pasture system, 99% in Organic Forage, and 100% in all other systems (Fig. 3), so we are confident that all material is represented in the sum of fraction C and N.

2.6. Biomass C input estimates

The C input estimates were calculated based on the approach of Sanford et al. (2012) and Jokela et al. (2011) to quantify the aboveground C inputs based on established harvest indices for maize (0.50) and soybean (0.40) (Bolinder et al., 2007) and aboveground biomass measurements between 1993 and 2013 in all WICST treatments. Root/shoot ratios and rhizodeposition estimates from Bolinder et al. (2007) were used to calculate belowground inputs.

2.7. Statistical analysis

All soil data (bulk density, POM-C, POM-N, SOC, TN, aggregate weight, aggregate C and N, proportion of C and N in POM and all aggregate fractions, and C:N ratio of whole soil, POM, and aggregate fractions) were analyzed with ANOVA using PROC MIXED in SAS software version 9.2 (SAS Institute Inc, Version 9.3, Cary, NC). Depth (for POM data only) and system were treated as fixed effects while block and block by system interactions were treated as random effects. The C:N ratio of aggregate fractions was compared using PROC MIXED in SAS treating fraction as a fixed effect. Differences among means were determined using Tukey's adjusted least squares means. Where assumptions of equal variance appeared to be violated (proportion of soil C and N in POM), the logit transformation $[\log(x/(1-x))]$ was used (Warton and Hui, 2011). Linear regression models between SOM measurements and biomass estimates were created using the lm function in R 3.1.1 (R Development Core Team, 2014). Models were developed using total biomass C as well as above- and belowground portions, but since patterns were driven by differences in belowground biomass C we present regressions for above- and belowground biomass C separately. All significance was determined at $\alpha = 0.10$.

3. Results

3.1. Total carbon and nitrogen and particulate organic matter

The Pasture system had significantly greater SOC than did Organic Grain, and significantly greater TN than did Maize–Soybean and Organic Grain (Table 2). There was a significant main effect of system on concentrations of POM-N, POM-C, and the proportion of soil C in POM (Table 2). In the Pasture and Organic Forage systems, POM-C made up a greater proportion of the whole soil C than in Continuous Maize. No effect of system was seen on C:N ratio of POM or whole soil. There was a significant interaction between system and depth in POM-N and POM-C (Fig. 1). At 0–25 cm, the Pasture had significantly greater POM-C than all other systems, and significantly greater POM-N than all systems except Organic Forage. The Organic Grain and Maize–Soybean systems had significantly less POM-C and POM-N than did Organic Forage, Conventional Forage and Pasture at 0–25 cm. At 25–50 cm, all systems had statistically similar concentrations of POM-C and POM-N. There was a significant effect of depth on all POM measurements, with greater values observed at 0–25 cm than at 25–50 cm. Linear regressions between both above and belowground biomass C and POM-C and POM-N at 0–25 cm were significant at $P < 0.0001$ (Fig. 2, Table 3). Linear regression models show that belowground biomass C explains 57% of the variation in POM-C and 66% of the variation in POM-N among all systems (Fig. 2). Aboveground biomass C explains 31% of POM-C variation and 27% of POM-N variation.

3.2. Aggregate distribution, bulk density, and C and N

Significant differences were seen among systems in concentration of macroaggregates, free silt and clay, and occluded microaggregates (Table 4, Fig. 3). Soil under Organic Grain contained significantly less macroaggregate mass than did Conventional Forage, Organic Forage and Pasture, less occluded microaggregate mass and more free silt and clay than did Maize–Soybean, Conventional Forage, Organic Forage and Pasture.

Bulk density at 0–25 cm differed significantly among the six systems ($P < 0.035$), with Maize–Soybean and Organic Grain

Table 2
Treatment means and ANOVA *P*-values for particulate organic matter (POM) and whole soil C and N in six cropping systems at the Wisconsin Integrated Cropping Systems Trial (WICST).

Treatment		Soil fraction															
		g kg^{-1} soil															
		SOC		TN		POM-C		POM-N		POM C:N		POM-C: SOC		POM-N: TN		Whole C:N	
System	Continuous Maize	15.9	ab[a]	1.55	ab	1.42		0.0986		12.8		0.074	b	0.056		11.7	
	Maize–soybean	12.8	ab	1.33	b	1.28		0.0869		13.6		0.082	ab	0.057		11.2	
	Organic Grain	12.5	b	1.32	b	1.30		0.0890		14.4		0.087	ab	0.058		10.5	
	Conventional Forage	15.8	ab	1.74	ab	1.83		0.135		13.0		0.142	a	0.084		10.2	
	Organic Forage	16.2	ab	1.76	ab	2.01		0.149		12.4		0.109	ab	0.076		10.5	
	Pasture	19.0	a	1.94	a	2.57		0.173		14.4		0.115	a	0.077		11.0	
Depth	0–25 cm	20.8	a	2.09	a	3.00	a	0.205	a	14.7	a	0.152	a	0.099	a	11.5	a
	25–50 cm	10.2	b	1.15	b	0.502	b	0.0416	b	13.4	b	0.053	b	0.037	b	10.2	b
		P-value															
ANOVA	System	0.0811		0.0191		<0.0001		<0.0001		0.115		0.0217		0.150		0.390	
	Depth	<0.0001		<0.0001		<0.0001		<0.0001		0.0005		<0.0001		<0.0001		0.0021	
	System × depth	0.118		0.594		0.0005		0.0002		0.235		0.430		0.270		0.0915	

[a] Lowercase letters denote significant differences among systems or between depths, at $P < 0.10$. Where the interaction between depth and system was significant, see Fig. 1 for means separation among systems at each depth.

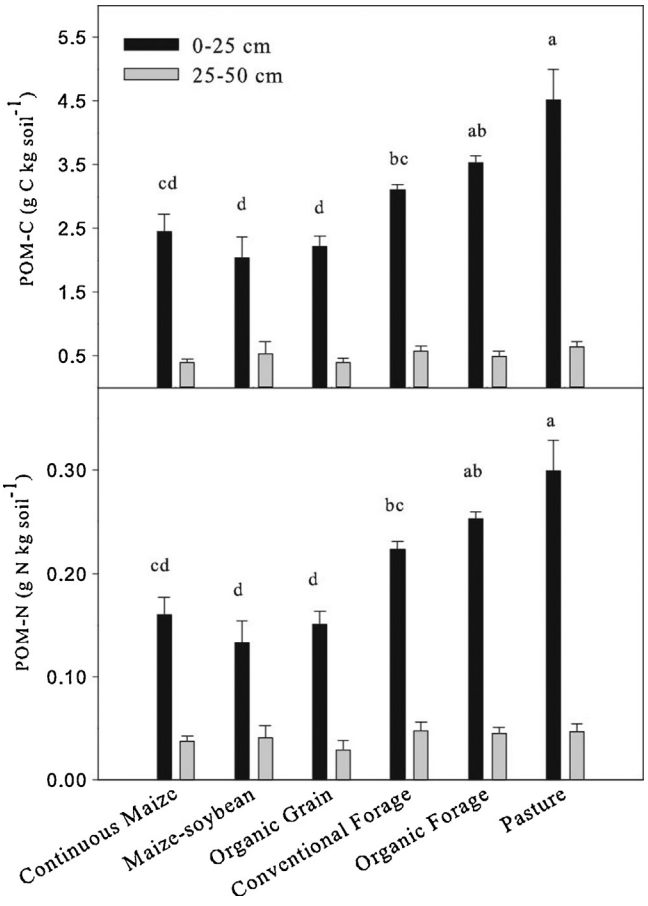


Fig. 1. Concentrations of particulate organic matter (POM) C and N across six Wisconsin Integrated Cropping Systems Trial (WICST) cropping systems at two soil depths. Bars represent standard error and lowercase letters denote significant differences ($P < 0.10$) among systems at a given depth.

having a significantly greater bulk density (1.38 and 1.36 g cm^{-3}, respectively) than did the Pasture system (1.23 g cm^{-3}). However, when bulk density was applied to soil aggregate C and N concentration data to calculate C and N stock, statistical models suggested similar conclusions whether C and N were analyzed on a concentration or stock basis. Thus only stock is presented (Fig. 5).

The macroaggregate-C stock was greater in Conventional Forage and Pasture than in Organic Grain, and macroaggregate-N stock was greater in Conventional Forage and Pasture than in Organic Grain and Continuous Maize (Fig. 5). However, only in the Pasture system did macroaggregates contain a greater proportion of TN and SOC than macroaggregates in Organic Grain (Fig. 4). The occluded microaggregate C and N stocks in Pasture were greater than in Organic Grain. The occluded silt and clay C and N stocks in Maize–Soybean, Conventional Forage and Pasture were greater than in Organic Grain. The proportion of C and N associated with free silt and clay was greater in Organic Grain than in Pasture system. The cPOM had an increased C:N ratio (average 18.4) compared with all other fractions (average 12.5, $P < 0.0001$), but C:N ratios did not differ among systems in any aggregate fraction or in whole soil (data not shown). The cPOM C and N in Organic Forage were greater than in Maize–Soybean (Fig. 5).

In models relating aggregate data to biomass C estimates, microaggregate C and N had weak negative relationships with aboveground biomass C (Table 4). Models of belowground biomass C revealed significant positive relationships with C and N in macroaggregates, microaggregates, occluded microaggregates, occluded silt and clay, and occluded coarse POM, and with weight of macroaggregates, occluded microaggregates, and occluded silt and clay. However, belowground biomass C was negatively correlated with free silt and clay C.

4. Discussion

4.1. Particulate organic matter

We hypothesized that inclusion of perennials in rotation and no-tillage would increase POM, but these management decisions are tightly coupled to biomass C inputs in this trial and it may be that the inputs are driving POM concentrations. For example, the Conventional Forage system, which rotates three years of alfalfa with one year of maize, did not significantly differ from Continuous Maize in POM-N or POM-C. In the Continuous Maize system, the flush of C mineralization associated with tillage may be offset by the large biomass C input (Table 1). The Conventional Forage system is tilled only every three years, and has comparable biomass C to Continuous Maize, but perhaps the low C:N ratio of alfalfa [approximately 15.1 according to Bruulsema and Christie (1987)] leads to more rapid biomass decomposition and less storage as POM than in Continuous Maize. Residue in Continuous Maize had a C:N ratio of 57:1 in 2013 (Cates et al., unpublished data). Lower C:N ratio material has a faster initial decomposition rate, which may reduce storage as POM (Enríquez et al., 1993; Nicolardot et al., 2001) but some models suggest that high-quality residue can more easily be incorporated into microbial biomass,

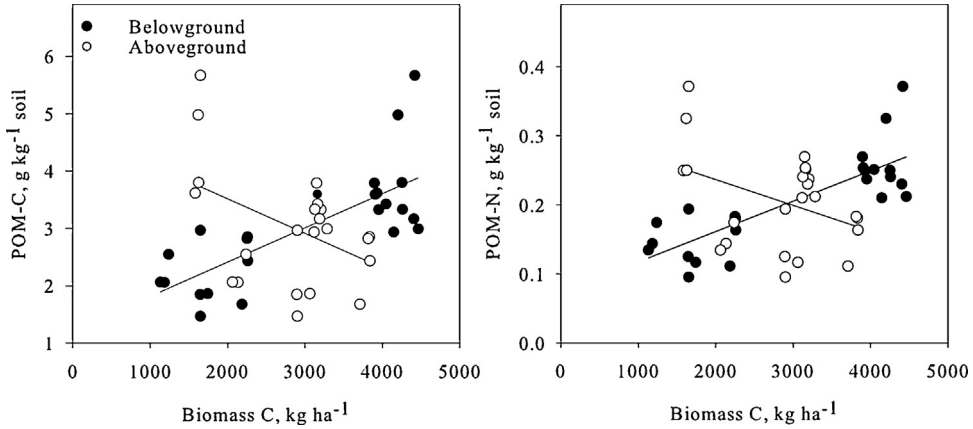


Fig. 2. Linear regression analysis of average biomass C inputs at WICST, 1993–2013, (updated from Sanford et al., 2012) and 2013 particulate organic matter (a), POM-C and (b), POM-N across six cropping systems. Filled circles represent belowground biomass and open circles represent aboveground biomass. See Table 4 for regression parameters.

Table 3
ANOVA *P*-values for analyses of effect of cropping system on soil aggregate distribution by mass, and proportion and stock of C and N found in aggregates (0–25 cm depth).

Measurement	Soil fraction					
	Macroaggregates, <250 μm	Microaggregates, 53–250 μm	Silt & clay, <53 μm	Occluded microaggregates, 53-250 μm	Occluded silt & clay, <53 μm	Occluded coarse POM, >250 μm
Proportion of total soil dry weight	0.0045	0.6115	0.0004	0.0084	0.1844	0.1785
Proportion of total soil C	0.2186	0.6707	0.0437	0.0383	0.4308	0.1301
Proportion of total soil N	0.2175	0.6713	0.0744	0.0716	0.579	0.0964
C in fraction, Mg ha^{-1}	0.0109	0.5824	0.7482	0.0070	0.0192	0.0775
N in fraction, Mg ha^{-1}	0.0055	0.2576	0.8802	0.0037	0.0309	0.0483

Table 4
Parameters of statistically significant regressions ($P < 0.10$) between POM or aggregate C and N, 0-25 cm, and aboveground or belowground biomass C input estimates. See table S1 for *P*-value and R^2 of non-significant regression tests.

	P-value	R^2	β_0	β_1
Soil organic matter fraction	Regression with aboveground biomass C inputs			
POM-C (g kg^{-1} soil)	0.0060	0.31	5.2	−0.00073
POM-N (g kg^{-1} soil)	0.010	0.27	0.35	−0.000072
Microaggregate C (Mg ha^{-1})	0.090	0.13	3.6	−0.00028
Microaggregate N (Mg ha^{-1})	0.043	0.17	0.36	−0.000033
	Regression with belowground biomass C inputs			
POM-C (g kg^{-1} soil)	<0.0001	0.54	0.78	0.00078
POM-N (g kg^{-1} soil)	<0.0001	0.71	0.045	0.000056
Macroaggregate, (g kg^{-1} soil)	0.00035	0.45	0.32	0.000058
Silt & clay (g kg^{-1} soil)	<0.0001	0.52	0.61	−0.000063
Occluded microaggregate (g kg^{-1} soil)	0.0031	0.33	0.18	0.000036
Occluded silt & clay (g kg^{-1} soil)	0.033	0.19	0.15	0.000014
Macroaggregate C (g kg^{-1} soil)	0.0021	0.36	6.33	0.0021
Macroaggregate N (Mg ha^{-1})	0.00037	0.44	0.49	0.00023
Microaggregate C (Mg ha^{-1})	0.043	0.17	2.16	0.00025
Microaggregate N (Mg ha^{-1})	0.008	0.28	0.18	0.000031
Occluded microaggregate C (Mg ha^{-1})	0.0033	0.33	3.11	0.0017
Occluded microaggregate N (Mg ha^{-1})	0.0013	0.38	0.26	0.00017
Occluded silt & clay C (Mg ha^{-1})	0.0027	0.34	2.08	0.00046
Occluded silt & clay N (Mg ha^{-1})	0.0016	0.37	0.19	0.00038
Occluded coarse POM C (Mg ha^{-1})	0.020	0.22	0.41	0.00016
Occluded coarse POM N (Mg ha^{-1})	0.0099	0.26	0.019	0.000014
SOC, sum (Mg ha^{-1})	0.040	0.18	17.7	0.0018
TN, sum (Mg ha^{-1})	0.012	0.25	1.54	0.00021

leading to more long-term SOM storage (Cotrufo et al., 2013). A low C:N ratio and high decomposition rate in soybean residue may also help explain why we found no difference in POM-C or POM-N concentrations among the three grain systems despite less tillage in strip-tilled Maize–Soybean. This is in contrast to results reported by Sindelar et al. (2015) who found a 36% increase in POM-C in strip-till compared with chisel tilled fields.

According to our analysis, belowground biomass C is a better predictor of POM-C and N enrichment than is aboveground biomass C. The Pasture best illustrates this phenomena, as it has

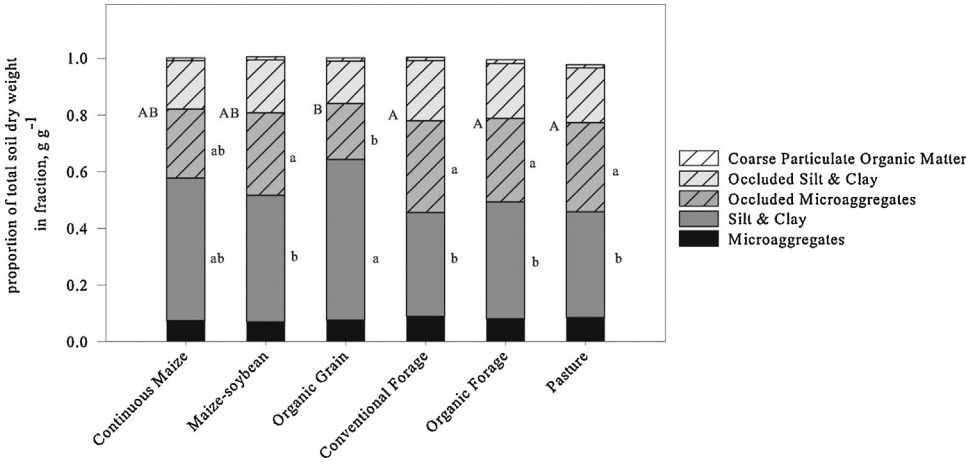


Fig. 3. Proportion of soil mass found in each soil fraction in six Wisconsin Integrated Cropping Systems Trial (WICST) cropping systems at 0–25 cm. Bars with diagonal lines represent fractions isolated from within macroaggregates. Uppercase letters to the left of bars represent significant differences among systems in macroaggregates. Lowercase letters to the right of bars represent significant differences among systems for all other soil fractions. $P < 0.10$.

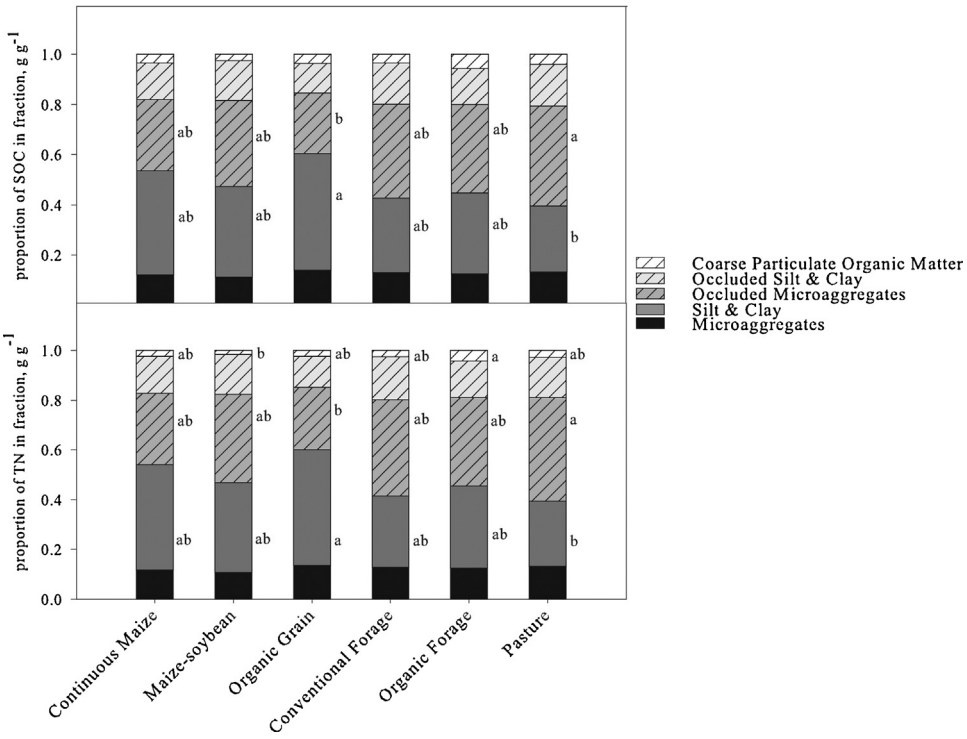


Fig. 4. Proportion of soil C and N in each soil fraction in six Wisconsin Integrated Cropping Systems Trial (WICST) cropping systems at 0–25 cm. Bars with diagonal lines represent fractions isolated from within macroaggregates. Lowercase letters to the right of bars represent significant differences among systems within soil fractions. $P < 0.10$.

relatively high belowground biomass C, relatively low aboveground biomass C, and high POM-C and N concentrations. Continuous Maize, in contrast, has low belowground biomass C, high aboveground biomass C, and low POM-C and N. This concurs with Kong and Six (2010) cropping systems study on a silt loam and silty clay loam, which found a mean root:shoot ratio of 3.8 for new C incorporated into coarse POM, a forest study which found twice as much incorporation of root C as needle C into particulate soil (Bird et al., 2008). This may be due to root effects on microbial activity, or interactions between root hairs and soil particles (Rasse et al., 2005)

4.2. Soil aggregates

Decreased soil aggregation in the Organic Grain system, as measured by proportion of soil mass in micro- or macroaggregates, may be driven by the restrictions of organic management. Biomass C inputs are decreased in Organic Grain because yields are lower without mineral fertilizer inputs, and increased rotational diversity increases frequency of lower-biomass crops like wheat and soybean. Due to mechanical weed control, Organic Grain had greater tillage intensity than did any other WICST system and our hypothesis that tillage would decrease aggregation and C and N in aggregates was supported in part. Tillage did not diminish silt and clay C and N in Organic Grain, as organo-mineral associations are normally unaffected by agricultural disturbance (Huggins et al., 1998; Kleber et al., 2007; Kögel-Knabner et al., 2008). Tillage has been shown to negatively affect aggregate formation and stability on a loess-derived soil in France (Oorts et al., 2007) and a Michigan Hapludalf (Grandy and Robertson, 2007). There was no significant difference in aggregation between the three grain rotations despite the varying tillage regimes (Table 1), but for occluded microaggregate C and N stocks and mass, Maize–Soybean > Continuous Maize > Organic Grain, a sequence which mirrors the intensity of tillage in these systems. Similarly, Sheehy et al. (2015) found that mean aggregate size decreased as tillage intensified on a Cambisol and a Regosol in the boreal region.

While comparing aggregate C and N in Pasture, Conventional Forage, and Organic Grain, the difference between highly perennialized and never or seldom tilled cropping systems (Pasture and Conventional Forage) and a tilled annual crop rotation (Organic Grain) is quite apparent: aggregates were more enriched in C and N in fully or mostly perennial systems. But contrary to our hypothesis, neither annual rotational diversity nor moderate perennialization in Organic Forage increased aggregate C and N relative to Continuous Maize and Maize–Soybean. Tillage every one to two years was sufficient to prevent aggregate enrichment in C and N, illustrating the vulnerability of aggregates to any tillage event. Evidence suggests that a single tillage event causes decreased aggregation for at least one year (Grandy and Robertson, 2006). After five years of perennial bioenergy crops, Tiemann and Grandy (2014) found increased (dry-sieved) aggregate stability and SOC protection at the same Arlington, WI research station where WICST is located. Our data concur with Tiemann and Grandy's (2014) evidence of C accrual primarily in stable microaggregates in perennial systems, as we found that the Pasture system has greater C and N in the occluded microaggregates and occluded silt and clay fractions than does the Continuous Maize system. There is potential for the development of very stable microaggregates within macroaggregates when macroaggregates are not disturbed by tillage (Six et al., 1999; Tisdall and Oades, 1982). This is only strictly true of the Pasture system at WICST, as the alfalfa-based systems are tilled every two or three years.

The relationships between belowground biomass C and aggregate C and N show much stronger positive relationships in macroaggregate components than do other soil fractions, supporting our hypothesis that high biomass inputs increase aggregate C and N (Table 4). This is a convincing demonstration that macroaggregates are held together by more recently added organic

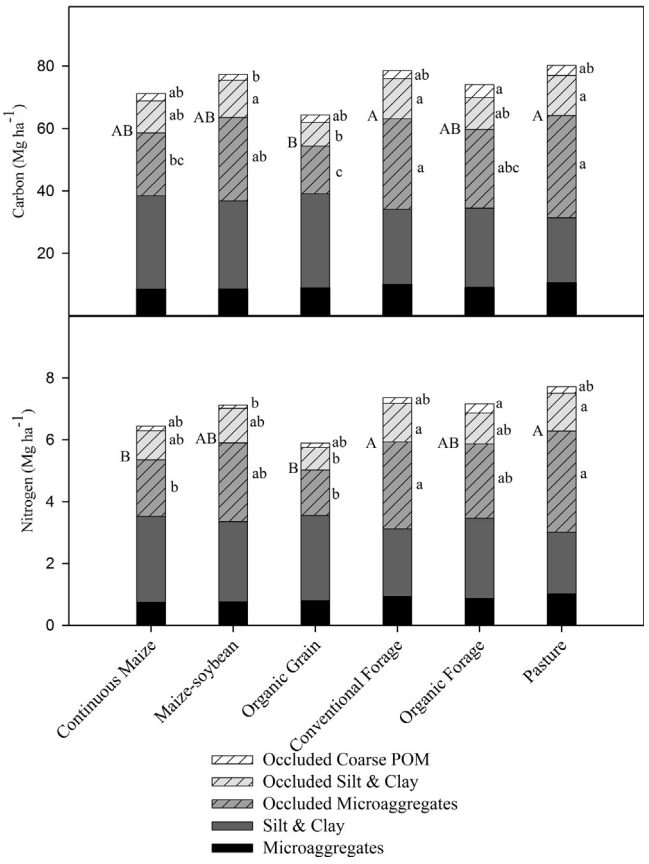


Fig. 5. Stock of carbon and nitrogen in each soil fraction in six Wisconsin Integrated Cropping Systems Trial (WICST) cropping systems at 0–25 cm. Bars with diagonal lines represent fractions isolated from within macroaggregates. Uppercase letters to the left of bars represent significant differences among systems in macroaggregates. Lowercase letters to the right of bars represent significant differences among systems for all other soil fractions. $P < 0.10$.

matter than in microaggregates. Microaggregates are held together by persistent binding agents such as microbial and root exudates, and thus, are less correlated with fresh biomass inputs (Six et al., 2000a; Tisdall and Oades, 1982). Further, quantity and quality of SOM associated with silt and clay is thought to be driven by mineralogy (Kögel-Knabner et al., 2008; Six et al., 2000b), which does not differ among systems, so no significant relationship between inputs and free silt and clay C and N was expected. In contrast, Singh et al. (2015) found no relationship between residue inputs and any aggregate fractions on a clay soil in Finland, which suggests that the impact of biomass inputs on aggregation can be modulated by the environment.

Assessing organic matter storage at WICST may be impeded by the fact that SOC in these systems is not in steady state, but declining. Between the initiation of WICST in 1990 and 2009, SOC in the top 90 cm of the soil profile decreased 12.0 Mg C ha^{-1}, and SOC has decreased in all six systems except in the top 15 cm of the Pasture system (Sanford et al., 2012). The stability in SOC under pasture may be due to larger concentrations of POM-C protected in the never-tilled pasture, and models predict that eventually this POM will be incorporated into protected aggregate fractions (Segoli et al., 2013). The trial-wide decrease in SOC over time indicates that the physical protection afforded by aggregates in non-Pasture systems is not sufficient to prevent ongoing mineralization of SOC. Evidence of interactions among precipitation, soil texture and C inputs in SOC accrual (McSherry and Ritchie, 2013) suggest that a C-rich silt loam like this Typic Argiudoll is more likely to lose SOC despite best management than is a coarser textured soil which begins with less SOC (Bellamy et al., 2005). Theory suggests that eventually SOC will reach a steady-state reflecting the mineralogy, C inputs and climate in a given system (Stewart et al., 2007; West and Six, 2006). Since C inputs and disturbance differ within the WICST, at this theoretical steady state it may be possible to differentiate all systems (instead of only the most extreme) using aggregate fractions and POM measurements. The C and N stocks (Fig. 5) in various aggregate fractions show that soil under these systems is not saturated, but could still potentially accumulate SOM through both aggregate and organo-mineral protection (Castellano et al., 2015).

4.3. Comparison of methods

The cPOM isolated by aggregate fractionation is larger (>250 μm) than is the POM separated by the Marriott and Wander (2006a) method (>53 μm) and is only macroaggregate protected material, so we do not expect >250 μm cPOM results to be equivalent to the >53 μm POM results. The two measurements do show similar trends, as the >53 μm POM and >250 μm cPOM were both enriched in C and N in Organic Forage compared with Continuous Maize, Maize–Soybean and Organic Grain. However, note that aggregate fractionation samples were taken in plots to be planted to maize in 2014, while POM samples were taken in plots to be planted to maize in 2013. Although WICST is blocked to account for field variation, spatial heterogeneity in soil is still too great to compare cPOM and POM concentrations directly (see Cambardella et al., 1994).

Since the >250 μm cPOM consists of large, relatively fresh material which has been physically protected within macroaggregates (Six et al., 2002), we expected this material to be increased in all systems with perennial root systems, but ANOVA did not detect differences between forage systems and Continuous Maize or Organic Grain, and according to regression analysis belowground biomass C inputs explained only 23% of cPOM-C variation and 27% of cPOM-N variation. The >53 μm POM, in contrast, was strongly correlated to belowground C. This is probably not due to the greater size of the >53 μm POM, as a Uruguayan Mollisol had more recent C in >200 μm POM than in >50 μm POM, implying that larger particles are broken into smaller particles through decomposition (Salvo et al., 2014). But the physical protection of the >250 μm cPOM within macroaggregates takes time, and so this fraction is not highly correlated to fresh biomass C inputs. Overall the >53 μm POM was more sensitive than was the >250 μm cPOM to the management gradient at WICST, which is consistent with expectations for this method. As the smallest and most variable aggregate fraction, >250 μm cPOM is not fit to detect differences among management regimes on this soil type.

5. Conclusions

The POM and aggregate C and N assessed at WICST illustrate how biomass C input quantity and quality, tillage intensity and perennialization interact in complex ways over decades to sustain SOM in a silt loam soil. We hypothesized that increased perennialization and biomass C inputs and reduced tillage would increase both POM and aggregate C and N. Our study provides strong evidence of that perennialization increases SOM in POM and aggregates but weaker support for reduced tillage. Free organic matter, measured as POM, was greater in systems with higher belowground biomass C inputs, and longer periods of perennials in rotation (which are themselves correlated). The stock of aggregate-protected C and N was less in an organic system with low biomass C inputs and tillage for weed control than in a diverse perennial pasture, but the aggregate distribution and C and N stock of

systems which were more moderate in tillage and perenniality were very similar. The primary differences due to management were found in the fractions isolated from within macroaggregates, concurring with previous work that macroaggregate weight, C and N concentrations decrease rapidly when disturbance increases or biomass inputs decrease. Our work suggests that management which boosts belowground biomass C inputs and minimizes tillage to any degree will increase POM, but increasing concentrations of physically protected SOM, and overall SOC, requires a more rigid adherence to these principals.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.still.2015.09.008.

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