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Sheep Grazing Enhances Coarse Relative to Microbial Organic Carbon in Dryland Cropping Systems

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Abstract

Sheep (*Ovis aries* L.) grazing, a cost-effective method of weed control compared with herbicide application and tillage, may influence soil C fractions by consuming crop residue and weeds and returning C through feces and urine to the soil. We examined the effect of three weed management practices (sheep grazing, herbicide application, and tillage) and two cropping sequences (continuous spring wheat [*Triticum aestivum* L.] [CSW] and spring wheat-pea [*Pisum sativum* L.]/barley [*Hordeum vulgare* L.] mixture hay-fallow [W-P/B-F]) on soil microbial biomass C (MBC), potential C mineralization (PCM), and particulate organic C (POC) in relation to soil organic C (SOC) at the 0- to 30-cm depth from 2009 to 2011 in southwestern Montana. The MBC at 0 to 5 cm was greater with tillage on CSW than tillage on W-P/B-F in 2009 and 2011, but was greater with herbicide application on CSW than tillage on CSW in 2010. The POC at 0 to 5 cm and 15 to 30 cm was greater with sheep grazing than herbicide application on CSW and W-P/B-F, but at 5 to 15 cm was greater with grazing on CSW. The MBC, PCM, and POC at all depths decreased from 2009 to 2011. Crop residue incorporation into the soil increased MBC with tillage on CSW. Lower proportions of labile than nonlabile organic matter through feces and urine probably reduced MBC at the soil surface, but increased POC with sheep grazing compared with herbicide application on CSW and W-P/B-F. Sheep grazing may increase coarse soil organic matter compared with microbial biomass in dryland cropping systems.

Keywords: Carbon mineralization, herbicide application, microbial biomass carbon, particulate organic carbon, sheep grazing, tillage

1. Introduction

Sheep grazing is an increasingly popular and cost-effective method of controlling weeds in dryland cropping systems in the northern Great Plains (Johnson et al., 1997; Hatfield et al., 2007; Miller et al., 2015). Sheep are often allowed to graze during fallow periods (e.g. before crop planting, after grain harvest, and during summer fallow) to control weeds and pests, reduce feed cost, and increase nutrient cycling (Johnson et al., 1997; Hatfield et al., 2007). Summer fallow is typically practiced in a dryland traditional farming system, such as in the northern Great Plains, USA, that includes conventional till with two-year rotation of crop-fallow to conserve soil water, release plant nutrients, control weeds, increase succeeding crop yields, and reduce the risk of crop failure (Aase & Pikul, 1995). Tillage and herbicide application can be effective in controlling weeds, but are also expensive, resulting in some of the highest variable costs for small grain production in dryland cropping systems (Johnson et al., 1997). Tillage and fallow can expose soil to erosion and herbicide application can contaminate soil, water, and air, all of which can increase risks to human and animal health (Fenster, 1997).

Some of the other negative effects of tillage and fallow are reductions in soil organic matter and water storage

efficiency and increase in saline seeps development that degrade soil health, quality, and productivity (Tanaka & Aase, 1987; Black & Bauer, 1988). Intensive tillage disrupts soil aggregation, incorporates crop residue into the soil, and increases the oxidation of soil organic matter (Schomberg & Jones, 1999; Sainju et al., 2007). Fallowing reduces the amount of crop residue returned to the soil, increases soil temperature and water content, and enhances microbial activity which increases the mineralization of organic matter (Aase & Pikul, 1995; Halvorson et al., 2002). As a result, the traditional dryland farming practices have reduced soil organic matter by 30-50% in the last several decades and also annualized crop yields in the northern Great Plains (Aase & Pikul, 1995; Halvorson et al., 2002; Sainju et al., 2007).

Animal grazing on crop residues and weeds can increase soil C sequestration and microbial biomass and activity compared with nongrazing (Franzluebbbers & Stuedemann, 2003, 2008; Maughan et al., 2009). Carbon returned to the soil through feces and urine during animal grazing can increase C cycling, improve soil water-nutrient-crop yield relationships, and sustain soil quality and productivity (Abaye et al., 1997; Franzluebbbers & Stuedemann, 2003; Sainju et al., 2010). Some of the other benefits of the integrated crop-livestock system are meat, milk, wool, and manure production (Franzluebbbers & Stuedemann, 2003; Hatfield et al., 2007).

Soil organic C (SOC) changes slowly due to management practices as a result of large pool size and inherent spatial variability (Franzluebbbers et al., 1995). Variations in SOC alone do not adequately reflect changes in soil health and quality (Franzluebbbers et al., 1995; Bezdicsek et al., 1996). Measurements of biologically active fractions of SOC, such as microbial biomass C (MBC or C in microbial cell) and potential C mineralization (PCM or microbial activity) that change rapidly with time (e.g. within a growing season), can better reflect changes in soil health and quality as well as nutrient dynamics due to immobilization-mineralization (Saffigna et al., 1989; Bremner & van Kessel, 1992). Particulate organic C (POC or coarse organic matter fraction), an intermediate C fraction between active and slow fractions and an important C substrate for microorganisms that bind soil particles together to form aggregates (Six et al., 2000), also changes rapidly due to management practices (Cambardella & Elliott, 1992). As a result, POC can also be a sensitive indicator of changes in soil organic matter (Franzluebbbers & Stuedemann, 2003). Conservation management practices, such as no-till, can increase soil C fractions, especially active and intermediate fractions, compared with traditional management practices, such as conventional till (Franzluebbbers et al., 1995; Sainju et al., 2007). Animal grazing can increase MBC and POC in perennial cropping systems (Frank & Goffman, 1998; Franzluebbbers & Stuedemann, 2003), but the effect can be variable in annual cropping systems (Franzluebbbers & Stuedemann, 2008).

Little is known about the effect of sheep grazing on active and intermediate soil C fractions relative to SOC in dryland cropping systems. Our objectives were to: (1) quantify the effects of weed management practices (sheep grazing, tillage, and herbicide application) and cropping sequences (continuous spring wheat [CSW] and spring wheat-pea/barley mixture hay-fallow [W-P/B-F]) on MBC, PCM, and POC relative to SOC from 2009 to 2011 in southwestern Montana, USA and (2) identify if sheep grazing for weed control can be a viable option for improving soil health and quality compared with tillage and herbicide application in dryland cropping systems. We hypothesized that sheep grazing would increase MBC, PCM, and POC on CSW compared with tillage and herbicide application on W-P/B-F.

2. Materials and Methods

2.1 Experimental Site, Treatments, and Crop Management

The study was described in detail by Barsotti et al. (2013). Briefly, the study was conducted from 2009 to 2011 at the Fort Ellis Research Center, Montana State University (45°40'N, 111° 2'W, altitude 1468 m), Bozeman, Montana, USA. Mean monthly air temperature at the site ranges from -5.6 °C in January to 19 °C in July and the total annual precipitation (113-yr average) is 465 mm. The soil is a Blackmore silt loam (fine-silty, mixed, superactive, frigid Typic Argiustolls) with 250 g kg⁻¹ sand, 500 g kg⁻¹ silt, 250 g kg⁻¹ clay, and 7.2 pH at the 0- to 30-cm depth. Previous treatments (2004-2008) at the site included three weed management practices (sheep grazing, tillage, and herbicide application) as the main plot and three cropping sequences (CSW, spring wheat-fallow [SW-F], and winter wheat-fallow [WW-F]) as the split-plot variable arranged in a completely randomized block design with three replications. The CSW was one-year crop rotation, but SW-F and WW-F were two-year rotations where each crop phase (fallow and spring wheat in SW-F and fallow and winter wheat in WW-F) occurred every year (Table 1). In two-year rotations, fallow occurred on one plot in the first year and spring wheat or winter wheat occurred on second plot in the second year. In the subsequent year, the crop phases were switched in each plot, thereby completing the two-year cycle.

Table 1. Crop allocation in cropping sequence treatments within each weed management practice from 2004 to 2011. Cropping sequences are CSW, continuous spring wheat; SW-F, spring wheat-fallow; W-P/B-F, spring wheat-pea/barley mixture hay-fallow; and WW-F, winter wheat-fallow

Year	Cropping sequence				
	CSW		SW-F		WW-F
	Plot 1	Plot 1	Plot 2	Plot 1	Plot 2
2004	Spring wheat	Fallow	Spring wheat	Winter wheat	Fallow
2005	Spring wheat	Spring wheat	Fallow	Fallow	Winter wheat
2006	Spring wheat	Fallow	Spring wheat	Winter wheat	Fallow
2007	Spring wheat	Spring wheat	Fallow	Fallow	Winter wheat
2008	Spring wheat	Fallow	Spring wheat	Winter wheat	Fallow
Cropping sequence change	CSW		W-P/B-F		Alfalfa
	Plot 1	Plot 1	Plot 2	Plot 3	Plot 1
2009	Spring wheat	Spring wheat	Pea/barley hay	Fallow	Alfalfa
2010	Spring wheat	Pea/barley hay	Fallow	Spring wheat	Alfalfa
2011	Spring wheat	Fallow	Spring-wheat	Pea/barley hay	Alfalfa

From 2009 to 2011, the same weed management practices were continued as the main plot treatment, but cropping sequences were changed in the split-plot treatment (Table 1). While CSW was continued as one of the cropping sequences as usual, two phases (spring wheat and fallow) of SW-F and one phase (winter wheat) of WW-F were replaced by three phases (spring wheat, pea/barley mixture hay, and fallow) of a three-year rotation of W-P/B-F, where each phase was present on three separate plots in every year. As with two-year rotations, these phases were rotated on each plot in every year, thereby completing the three-year cycle in W-P/B-F. The fallow phase in WW-F was replaced by alfalfa (*Medicago sativa* L.) in 2009. For this study, cropping sequences with only CSW and W-P/B-F were selected. As a result of the treatment history, SOC stock at 0 to 30 cm varied from 89 Mg C ha⁻¹ with tillage on W-P/B-F to 100 Mg C ha⁻¹ with grazing on CSW in 2008 (Sainju et al., 2010). These values were used as covariates for data analysis to eliminate prior year's SOC levels on soil C fractions (Barsotti et al., 2013; Sainju et al., 2014).

The grazing treatment consisted of grazing with a group of western white-faced sheep at a stocking rate of 29 to 153 sheep d⁻¹ ha⁻¹. Sheep were grazed day and night before crop planting in the early spring (February-April) and after harvest in the autumn (October-December) on CSW. In addition to these periods, sheep were also grazed during the summer fallow period (May-September) on W-P/B-F. Grazing ended when about ≤ 47 kg ha⁻¹ of crop residue and weeds remained in the plot. The herbicide application treatment included application of post emergence herbicide before planting, after crop harvest, and during summer fallow for weed control. The tillage treatment consisted of tilling the plots before planting, after crop harvest, and during summer fallow with a Flexi-coil harrow (John Deere 100, Kennedy, MN) to a depth of 15 cm to control weeds and for seedbed preparation. The size of the main plot was 91.4 m \times 76.0 m and split plot 91.4 m \times 15.2 m.

Nitrogen fertilizer as urea (45% N) was broadcast at 202 and 252 kg N ha⁻¹ to spring wheat in CSW and W-P/B-F, respectively, before planting in mid-May, 2009 to 2011. For pea/barley mixture hay, N fertilizer was broadcast at 134 kg N ha⁻¹. Nitrogen fertilizer was left at the soil surface in the grazing and herbicide application treatments and incorporated into the soil to a depth of 15 cm using Flexi-coil harrow in the tillage treatment. Nitrogen rates were adjusted to soil NO₃-N content to a depth of 60 cm measured after grain and hay harvest in the autumn of the previous year. Because the soil contained high levels of extractable P and K (Sainju et al., 2011), no P and K fertilizers were applied.

Immediately after fertilization, spring wheat was planted in CSW and W-P/B-F using a drill equipped with double disc openers spaced 30 cm apart. Similarly, barley hay and Austrian winter pea hay were planted in W-P/B-F at the same time. In August and September, total crop biomass (grains, stems, and leaves in spring wheat and stems and leaves in pea/barley mixture hay) was determined by collecting biomass samples and oven drying at 60°C for 3 d. Spring wheat grain yield (oven-dried basis) was determined using a combine harvester after oven-drying a subsample at 60°C for 3 d. Spring wheat biomass (stems and leaves) was determined by

deducting grain yield from total biomass. Biomass of spring wheat after grain harvest and pea/barley were removed from the soil for hay with a self-propelled mower-conditioner and square baler in the herbicide application and tillage treatments. In the grazing treatment, sheep were grazed over swathed spring wheat biomass and pea/barley mixture forage.

2.2 Soil Sampling and Analysis

Soil samples were collected at the 0- to 30- cm depth from five locations in the central rows of each split plot (30 cores per split-plot or 90 cores per main plot) using a hydraulic probe (4 cm i.d.) in October (one month after crop harvest), 2009 to 2011. Soil cores were divided into 0 to 5, 5 to 15, and 15 to 30 cm depth increments. A portion (≈ 10 g) of the sample from each depth increment was oven-dried at 105°C for 24 h, from which dry weight of the soil core was determined and divided by the volume of the core to determine the bulk density. Remaining soil samples were air-dried, composited by depth, and ground to pass a 2-mm sieve. The SOC concentration in soil samples was determined by using a high combustion C and N analyzer (LECO Corp., St. Joseph, MI) after grinding the sample to 0.5 mm and pretreating with 0.6 mole L^{-1} HCl to remove inorganic C. For determining POC concentration, 10 g soil was dispersed with 30 mL of 5 g L^{-1} sodium hexametaphosphate for 16 h and the solution was poured through a 0.053 mm sieve (Cambardella & Elliott, 1992). The solution that passed through the sieve and contained mineral-associated and water-soluble C was oven dried at 50°C for 3 to 4 d and SOC concentration was determined by using the analyzer as described above. The POC concentration was determined by the difference between SOC in the whole-soil and that in the particles that passed through the sieve after correcting for the sand content.

The PCM concentration in air-dried soils was determined by the method described by Haney et al. (2004). Ten grams soil was moistened with water at 50% field capacity [0.25 m³ m⁻³ (Aase & Pikul, 2000; Pikul & Aase, 2003)] and placed in a 1 L jar containing beakers with 2 mL of 0.5 mole L^{-1} NaOH to trap evolved CO_2 and 20 mL of water to maintain high humidity. Soil was incubated in the jar at 21°C for 10 d. At 10 d, the beaker containing NaOH was removed from the jar and PCM concentration was determined by measuring CO_2 absorbed in NaOH, which was back-titrated with 1.5 mole L^{-1} BaCl_2 and 0.1 mole L^{-1} HCl. The moist soil used for determining PCM was subsequently reused for determining MBC concentration by the modified fumigation-incubation method for air-dried soils (Franzluibbers et al., 1996). The moist soil was fumigated with ethanol-free chloroform for 24 h and placed in a 1 L jar containing beakers with 2 mL of 0.5 mole L^{-1} NaOH and 20 mL water. As with PCM, fumigated moist soil was incubated at 21°C for 10 d and CO_2 absorbed in NaOH was back-titrated with BaCl_2 and HCl. The MBC concentration was calculated by dividing the amount of CO_2 -C absorbed in NaOH by a factor of 0.41 (Voroney & Paul, 1984) without subtracting the values from the nonfumigated control (Franzluibbers et al., 1996).

The contents (Mg C ha^{-1} or kg C ha^{-1}) of SOC, POC, PCM, and MBC at individual depth increments were calculated by multiplying their concentrations (g C kg^{-1} or mg C kg^{-1}) by the appropriate bulk density and thickness of the soil layer for each treatment and soil depth using the equivalent soil mass method as described by Ellert and Bettany (1995). Using this method, corrections for soil C contents were made for sheep grazing and herbicide application (no-till) treatments relative to tillage (conventional till) treatment. Total contents at 0 to 30 cm were determined by summing the contents from individual depths.

2.3 Data Analysis

Data for soil C fractions at a depth were analyzed using the Analysis of Covariance in the SAS-MIXED model after considering SOC in 2008 as a covariate (Littell et al., 2006). The covariance model eliminates the effect of SOC variability due to prior years' treatments so that the effect of treatments during the experimental years can be measured (Littell et al., 2006; Barsotti et al., 2013; Sainju et al., 2014). Weed management practice was considered as the main plot treatment and the fixed effect, cropping sequence as the split plot treatment and another fixed effect, and year as the repeated measure variable. Replication and replication \times weed management practice interaction were considered as random effects. As W-P/B-F had three cropping phases with each phase of the cropping sequence occurring every year, data for soil C fractions for this sequence were averaged across phases and the average value was used for the analysis. Means were separated by using the least square means test when treatments and interactions were significant (Littell et al., 2006). Linear regression analysis was used to determine the rate of change of soil C fractions when year and treatment \times year interaction were significant. The timings of 0.5, 1.5, and 2.5 yr used in the regression analysis represented the actual periods of soil sampling in October 2009, 2010, and 2011, respectively, since the project was initiated in April 2009. Statistical significance was evaluated at $P \leq 0.05$, unless otherwise stated.

3. Results and Discussion

3.1 Microbial Biomass Carbon

Using SOC in 2008 as a covariate, MBC varied significantly among years at all depths (Table 2). Differences in weed management practices, cropping sequences, and C returned to soil through feces and urine through sheep grazing among treatments from year to year resulted in significant weed management \times cropping sequence \times year interaction for MBC at 0 to 5 cm. The MBC at 0 to 5 cm was 17% greater with tillage on CSW than tillage on W-P/B-F in 2009 (Table 3). In 2010, MBC was 40% greater with herbicide application and tillage on CSW than herbicide application on W-P/B-F. In 2011, MBC was 21-22% greater with tillage on CSW than grazing on CSW and tillage on W-P/B-F.

Table 2. Analysis of variance for soil microbial biomass C (MBC), potential C mineralization (PCM), particulate organic C (POC) contents, MBC/soil organic C (SOC) ratio, PCM/SOC ratio, and POC/SOC ratio at the 0- to 30-cm depth. * Significant at $P \leq 0.05$, ** Significant at $P \leq 0.01$, *** Significant at $P \leq 0.001$, NS not significant; ^a SOC content in 2008 used as covariate for data analysis

Source	Soil depth, cm					Soil depth, cm				
	0-5	5-15	15-30	0-15	0-30	0-5	5-15	15-30	0-15	0-30
	MBC					MBC/SOC				
Weed management (W)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Cropping sequence (S)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
W \times S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Year (Y)	***	***	**	***	***	***	**	NS	***	**
W \times Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
S \times Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
W \times S \times Y	**	NS	NS	NS	NS	**	NS	NS	NS	NS
SOC08 ^a	NS	NS	NS	*	NS	**	NS	**	*	***
	PCM					PCM/SOC				
Weed management (W)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Cropping sequence (S)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
W \times S	NS	NS	NS	NS	NS	NS	NS	*	NS	*
Year (Y)	***	***	**	***	***	***	***	NS	***	**
W \times Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
S \times Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
W \times S \times Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SOC08 ^a	NS	NS	NS	*	NS	**	NS	*	NS	*
	POC					POC/SOC				
Weed management (W)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Cropping sequence (S)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
W \times S	*	*	*	**	NS	*	*	NS	**	NS
Year (Y)	NS	***	*	***	***	*	***	NS	***	**
W \times Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
S \times Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
W \times S \times Y	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SOC08 ^a	***	*	NS	***	***	***	NS	NS	***	*

Incorporation of left over crop residue after aboveground biomass harvest with higher C/N ratio of spring wheat than pea/barley mixture (Barsotti et al., 2013) into the soil due to tillage appeared to increase MBC at the surface layer with tillage on CSW from 2009 to 2011 (Table 3). Although spring wheat biomass during the wheat phase was greater in W-P/B-F, annualized biomass (average biomass across cropping phases in a year) was higher in CSW due to continuous cropping than W-P/B-F where absence of crops during the fallow period reduced biomass (Barsotti et al., 2013). The C/N ratio of spring wheat was 41 compared with 29 for pea/barley mixture (Barsotti et al., 2013). Similarly, the C/N ratio of sheep feces as measured in this experiment was 17. Although aboveground biomass was harvested for hay, it could be possible that the incorporation of greater amount of left over crop residue with higher C/N ratio into the soil after harvest increased MBC due to increased substrate availability and slower decomposition of the residue. Residues with higher C/N ratio decompose more slowly than residues with lower C/N ratio (Kuo et al., 1997). Sainju et al. (2007) also reported greater MBC in conventional till than no-till due to residue incorporation into the soil to a greater depth. Non-disturbance of soil and/or placement of residue at soil surface increased MBC at the surface layer with herbicide application on CSW only in 2010.

Table 3. Effects of weed management practice and cropping sequence on soil microbial biomass C (MBC) and MBC/soil organic C (SOC) ratio at the 0- to 5-cm depth from 2009 to 2011. Values are mean (\pm standard error). Weed management practices are Herbicide, weed control by herbicide application; Grazing, weed control by sheep grazing; and Tillage, weed control by tillage. Cropping sequences are CSW, continuous spring wheat; and W-P/B-F, spring wheat-pea/barley mixture hay-fallow. LSD (0.05) is the least significant difference between treatments at $P = 0.05$

Weed management practice	Cropping sequence	MBC (kg C ha ⁻¹)			MBC/SOC ratio (kg Mg ⁻¹)		
		2009	2010	2011	2009	2010	2011
Herbicide	CSW	190 \pm 14	180 \pm 17	183 \pm 19	10.4 \pm 0.7	9.8 \pm 1.1	10.3 \pm 1.1
	W-P/B-F	196 \pm 11	140 \pm 12	184 \pm 16	10.9 \pm 0.5	8.1 \pm 0.8	10.5 \pm 1.0
Grazing	CSW	184 \pm 14	155 \pm 18	167 \pm 19	11.2 \pm 0.7	8.8 \pm 1.1	9.9 \pm 1.1
	W-P/B-F	183 \pm 10	160 \pm 13	187 \pm 15	10.7 \pm 0.5	9.3 \pm 0.8	10.7 \pm 1.0
Tillage	CSW	202 \pm 14	180 \pm 18	211 \pm 21	12.2 \pm 0.7	7.2 \pm 1.1	12.3 \pm 1.3
	W-P/B-F	167 \pm 10	163 \pm 13	165 \pm 15	9.4 \pm 0.5	9.2 \pm 0.8	9.8 \pm 1.0
LSD (0.05)		29	35	40	1.5	2.1	2.5

Reduction in MBC with sheep grazing, regardless of cropping sequences, was a surprise, although grazing returned C through feces and urine to the soil compared with herbicide and tillage treatments where aboveground biomass was harvested for hay. Consumption of most of the labile portion of crop residue by sheep and return of higher proportion of nonlabile than labile organic matter in the digested material through feces and urine can be a possible reason for reduced MBC in the grazing treatment. Another possible reason for lower MBC with sheep grazing could be a result of rapid decomposition of feces in the soil due to its lower C/N ratio than crop residues. Because sheep grazed on crop residue and weeds in annual cropping systems in our experiment, it appeared that additional C inputs in feces and urine were not enough to increase MBC with sheep grazing. Although moderate cattle grazing in perennial cropping systems can increase soil microbial biomass compared with non-grazing or hayed system due to enhanced plant growth (Franzluebbbers & Stuedemann, 2003), our results showed the reverse trend of grazing on MBC in annual cropping systems, a case similar to that obtained for lower microbial biomass due to cattle grazing than no grazing in annual cropping systems (Franzluebbbers & Stuedemann, 2008). It may be possible that sheep grazed heavily in our experiment, since the amount of residue left in the soil after grazing was ≤ 47 kg ha⁻¹. Heavy grazing, however, can reduce soil microbial biomass (Holt, 1977). Reduced crop residue, followed by rapid decomposition of residue with lower C/N ratio of pea/barley mixture than spring wheat may have reduced MBC with tillage on W-P/B-F.

The MBC at all depths declined with year for all depths, regardless of treatments (Table 4). The MBC declined from 3 kg C ha⁻¹ yr⁻¹ at 0 to 5 cm to 35 kg C ha⁻¹ yr⁻¹ at 0 to 30 cm. Removal of aboveground biomass for hay in tillage and herbicide treatments and consumption of residue by sheep in the grazing treatment may have reduced MBC in all treatments from 2009 to 2011.

Table 4. Regression coefficients for the relationships between soil microbial biomass C (MBC), potential C mineralization (PCM), particulate organic C (POC), MBC/soil organic C (SOC), PCM/SOC, and POC/SOC ratios at various depths with year for Figures 1, 2, and 3. Units in intercept are kg C ha⁻¹ for MBC and PCM, Mg C ha⁻¹ for POC, kg Mg⁻¹ for MBC/SOC and PCM/SOC ratios, and Mg Mg⁻¹ for the POC/SOC ratio. Units in slope are kg C ha⁻¹ yr⁻¹ for MBC and PCM, Mg C ha⁻¹ yr⁻¹ for POC, kg Mg⁻¹ yr⁻¹ for MBC/SOC and PCM/SOC ratios, and Mg Mg⁻¹ yr⁻¹ for the POC/SOC ratio

Parameter	Soil depth	Intercept	Slope	R ²	P
MBC (kg C ha ⁻¹)	0-5 cm	177	-3	0.01	0.92
	5-15 cm	326	-20	0.27	0.65
	15-30 cm	375	-16	0.20	0.70
	0-15 cm	502	-22	0.16	0.73
	0-30 cm	940	-35	0.29	0.74
MBC/SOC (kg Mg ⁻¹)	0-5 cm	10.5	-0.12	0.03	0.94
	5-15 cm	8.7	-0.12	0.02	0.91
	15-30 cm	8.5	-0.19	0.27	0.65
	0-15 cm	9.1	-0.11	0.01	0.92
	0-30 cm	8.8	0.05	0.01	0.94
PCM (kg C ha ⁻¹)	0-5 cm	82	-11	0.99	0.02
	5-15 cm	142	-21	0.93	0.06
	15-30 cm	156	-13	0.72	0.35
	0-15 cm	223	-31	0.97	0.04
	0-30 cm	377	-43	0.90	0.11
PCM/SOC (kg Mg ⁻¹)	0-5 cm	4.7	-0.64	0.98	0.04
	5-15 cm	3.8	-0.45	0.96	0.07
	15-30 cm	3.5	-0.10	0.75	0.30
	0-15 cm	4.1	-0.49	0.97	0.03
	0-30 cm	3.8	-0.32	0.99	0.02
POC (Mg C ha ⁻¹)	0-5 cm	5.8	-0.3	0.99	0.01
	5-15 cm	11.3	-1.7	0.84	0.26
	15-30 cm	9.8	-0.9	0.36	0.59
	0-15 cm	17.0	-1.9	0.88	0.24
	0-30 cm	26.9	-2.9	0.69	0.30
POC/SOC (Mg Mg ⁻¹)	0-5 cm	0.32	-0.02	0.96	0.07
	5-15 cm	0.30	-0.04	0.85	0.25
	15-30 cm	0.21	-0.02	0.16	0.73
	0-15 cm	0.30	-0.03	0.75	0.33
	0-30 cm	0.27	-0.05	0.57	0.45

The MBC/SOC ratio also varied with year at all depths, except at 15 to 30 cm, with a significant weed management × cropping sequence × year interaction at 0 to 5 cm (Table 2). The trend for the MBC/SOC ratio among treatments was also similar to MBC (Table 3). The MBC/SOC ratio at 0 to 5 cm was greater with tillage on CSW than herbicide application on CSW, and grazing and tillage on W-P/B-F in 2009. In 2010, the MBC/SOC ratio was greater with herbicide application than tillage on CSW. In 2011, the MBC/SOC ratio was greater with tillage on CSW than tillage on W-P/B-F. Regardless of treatments, the MBC/SOC ratio at all depths declined from 2009 to 2010, except at 0 to 30 cm (Figure 1, Table 5).

Similar trends for MBC and the MBC/SOC ratio with treatments and years indicate that MBC is a sensitive indicator for rapid changes due to management practices compared with SOC. Residue incorporation into the soil due to tillage or surface placement of residue of higher C/N ratio of spring wheat than pea/barley mixture may have increased the MBC/SOC ratio with tillage or herbicide application with CSW. The proportion of SOC in MBC obtained in this experiment was within or slightly greater than the previously reported values of 4.1 to 7.9 g kg⁻¹ SOC in the northern Great Plains (Sainju et al., 2007, Sainju & Lenssen, 2011).

3.2 Potential Carbon Mineralization

With the elimination of the effect of 2008 SOC, PCM varied among years at all depths (Table 2). From 2009 to 2011, average PCM across treatments declined from 11 kg C ha⁻¹ yr⁻¹ at 0 to 5 cm to 43 kg C ha⁻¹ yr⁻¹ at 0 to 30 cm (Table 4, Figure 2). Removal of aboveground biomass for hay or through sheep grazing likely reduced PCM at all depths from 2009 to 2011, regardless of treatments. Addition of C inputs through feces and urine through sheep grazing had little impact on PCM.

The PCM/SOC ratio also varied among years at all depths, except at 15 to 30 cm (Table 2). Interaction was significant for weed management × cropping sequence at 15 to 30 and 0 to 30 cm. At 15 to 30 cm, the PCM/SOC ratio, averaged across years, was greater with tillage than grazing on CSW (Table 5). The PCM/SOC ratio was greater in W-P/B-F than CSW with grazing, but the trend reversed with tillage. At 0 to 30 cm, the PCM/SOC ratio was greater with tillage than herbicide application and grazing on CSW. The PCM/SOC ratio was also greater in W-P/B-F than CSW with grazing. The PCM/SOC ratio, averaged across treatments, declined from 0.10 kg Mg⁻¹ SOC yr⁻¹ at 15 to 30 cm to 0.64 kg Mg⁻¹ SOC yr⁻¹ at 0 to 5 cm from 2009 to 2011 (Figure 2, Table 4).

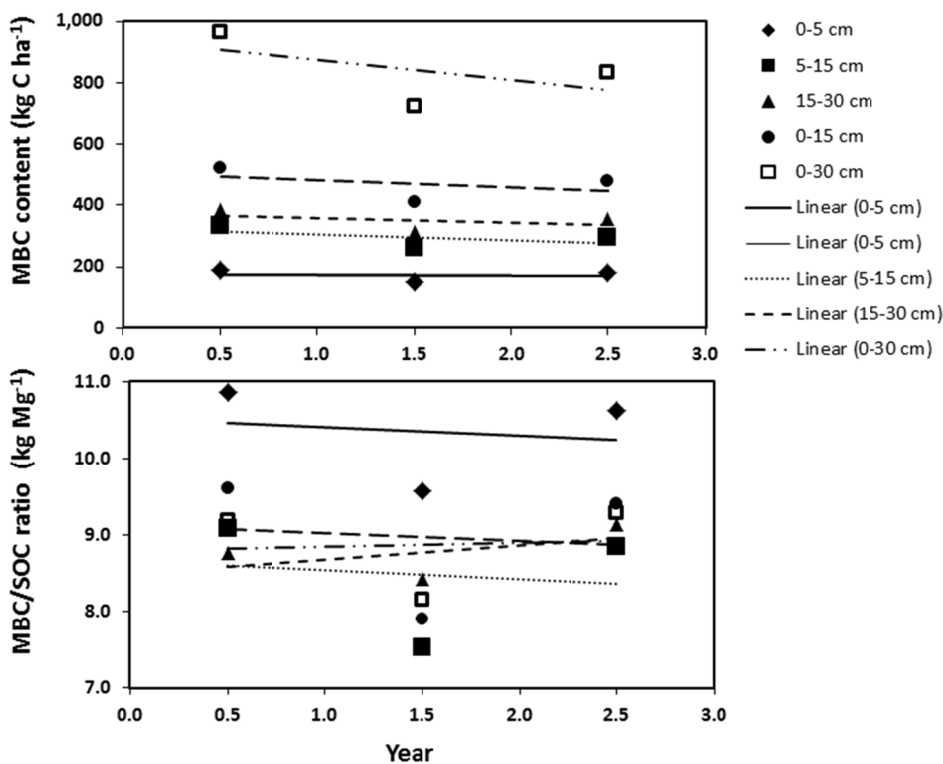


Figure 1. Relationships between soil microbial biomass C (MBC) and MBC/soil organic C (SOC) ratio at various soil depths averaged across treatments with year. Coefficients of linear regression analysis are shown in Table 4. The timings of 0.5, 1.5, and 2.5 yr used in the regression analysis represent the actual periods of soil sampling in October 2009, 2010, and 2011, respectively, since the project was initiated in April 2009

Table 5. Effects of weed management practice and cropping sequence on soil potential C mineralization (PCM)/soil organic C (SOC) ratio, particulate organic C (POC), and POC/SOC ratio at various depths. Values are mean (\pm standard error) averaged across years. Cropping sequences are CSW, continuous spring wheat; and W-P/B-F, spring wheat-pea/barley mixture hay-fallow. Weed management practices are Herbicide, weed control by herbicide application; Grazing, weed control by sheep grazing; and Tillage, weed control by tillage. Numbers followed by different lowercase letters within a column in a set are significantly different between cropping sequences within a weed management practice at $P = 0.05$ by the least square means test. Numbers followed by different uppercase letters within a row in a set are significantly different between weed management practices within a cropping sequences at $P = 0.05$ by the least square means test

Parameter	Soil depth	Cropping sequence	Weed management practice‡		
			Herbicide	Grazing	Tillage
PCM/SOC ratio (kg Mg ⁻¹)	15-30 cm	CSW	3.31(\pm 0.44)aAB	2.92 (\pm 0.45)bB	4.11 (\pm 0.45)aA
		W-P/B-F	3.12 (\pm 0.38)aA	3.51 (\pm 0.38)aA	3.48 (\pm 0.38)bA
	0-30 cm	CSW	3.18 (\pm 0.34)aB	3.13 (\pm 0.34)bB	3.78 (\pm 0.30)aA
		W-P/B-F	3.16 (\pm 0.30)a A	3.51 (\pm 0.30)aA	3.42 (\pm 0.30)aA
POC (Mg C ha ⁻¹)	0-5 cm	CSW	5.60 (\pm 0.51)aA	5.11 (\pm 0.49)aA	5.62 (\pm 0.51)aA
		W-P/B-F	4.71 (\pm 0.40)bB	5.93 (\pm 0.43)aA	4.43 (\pm 0.40)aAB
	5-15 cm	CSW	10.83 (\pm 1.41)aA	7.32 (\pm 1.39)aB	8.64 (\pm 1.41)aAB
		W-P/B-F	7.80 (\pm 1.12)bA	9.40 (\pm 0.98)aA	8.95 (\pm 0.99)aA
	15-30 cm	CSW	7.81 (\pm 1.60)aB	11.32 (\pm 1.62)aA	7.40 (\pm 1.61)aB
		W-P/B-F	8.35 (\pm 1.09)aA	7.63 (\pm 1.07))bA	7.95 (\pm 1.07)aA
	0-15 cm	CSW	16.36 (\pm 1.83)aA	12.71 (\pm 1.82)bB	14.02 (\pm 1.82)aAB
		W-P/B-F	11.80 (\pm 1.51)bB	15.61 (\pm 1.52)aA	14.85 (\pm 1.50)aA
POC/SOC ratio (Mg Mg ⁻¹)	0-5 cm	CSW	0.31 (\pm 0.03)aA	0.30 (\pm 0.03)aA	0.33(\pm 0.02)aA
		W-P/B-F	0.27 (\pm 0.02)bB	0.35 (\pm 0.02)aA	0.30 (\pm 0.02)aAB
	5-15 cm	CSW	0.28 (\pm 0.03)aA	0.23 (\pm 0.03)aB	0.25 (\pm 0.03)aAB
		W-P/B-F	0.22 (\pm 0.03)bA	0.25 (\pm 0.02)aA	0.25 (\pm 0.02)aA
	0-15 cm	CSW	0.29 (\pm 0.02)aA	0.25 (\pm 0.03)aB	0.27 (\pm 0.03)aAB
		W-P/B-F	0.23 (\pm 0.02)bB	0.28 (\pm 0.02)aA	0.27 (\pm 0.02)aA

Greater PCM/SOC ratio at 15 to 30 and 0 to 30 cm with tillage than herbicide application and grazing on CSW suggests that residue incorporation into the soil due to tillage increased C mineralization relative to C storage in the continuous cropping system, a case similar to that observed for MBC and the MBC/SOC ratio. Our results were similar to those observed for reduced C mineralization relative to C storage by cattle grazing compared to no grazing in annual cropping systems (Franzleubbers & Stuedemann, 2008). The trend was, however, opposite in perennial cropping systems (Frank and Goffman, 1998). The type of the animal (e.g. cattle vs. sheep) grazing reflecting the amount of C inputs in feces and urine, their distribution in the soil, and cropping system (perennial vs. annual cropping system) probably influenced C mineralization. The distribution of feces and urine by animals during grazing at the soil surface can be uneven; however, distribution can be more uniform with sheep than with cattle grazing (Abaye et al., 1997). Greater PCM/SOC ratio with W-P/B-F than CSW with grazing, but the reverse trend with tillage, can be related to differences in the duration of grazing and the amount of crop residue returned to the soil. It may be possible that increased feces and urine returned to the soil due to longer period of grazing during summer fallow increased PCM relative to SOC in W-P/B-F with grazing. In contrast, increased amount of crop residue and its incorporation into the soil increased PCM relative to SOC in CSW with tillage. Our PCM/SOC ratio values were greater than the reported values of 1.5 to 2.1 kg Mg⁻¹ SOC for dryland cropping systems in eastern Montana (Sainju & Lenssen, 2011). Differences in soil and climatic conditions likely resulted in variations in the PCM/SOC ratio among locations. Our site receives 115 mm more annual precipitation and the soil is silt loam compared with loam in eastern Montana.

As with PCM, removal of aboveground biomass for hay or through sheep grazing probably reduced the PCM/SOC ratio at all depths from 2009 to 2011, regardless of treatments. The rate of loss for the PCM/SOC ratio decreased with depth (Table 4, Figure 2), indicating that increased C substrate availability can increase C mineralization relative to C storage more in the surface than subsurface layers. The surface soil layer receives more C substrate through above- and belowground crop residue (roots) compared to the limited substrate availability through roots in the subsurface soil layers. Changes in the availability and decomposition of substrates through management practices can have a large impact on soil microbial activity compared with soil organic C which changes slowly (Franzluebbers et al., 1955; Sainju et al., 2007, Sainju & Lenssen, 2011).

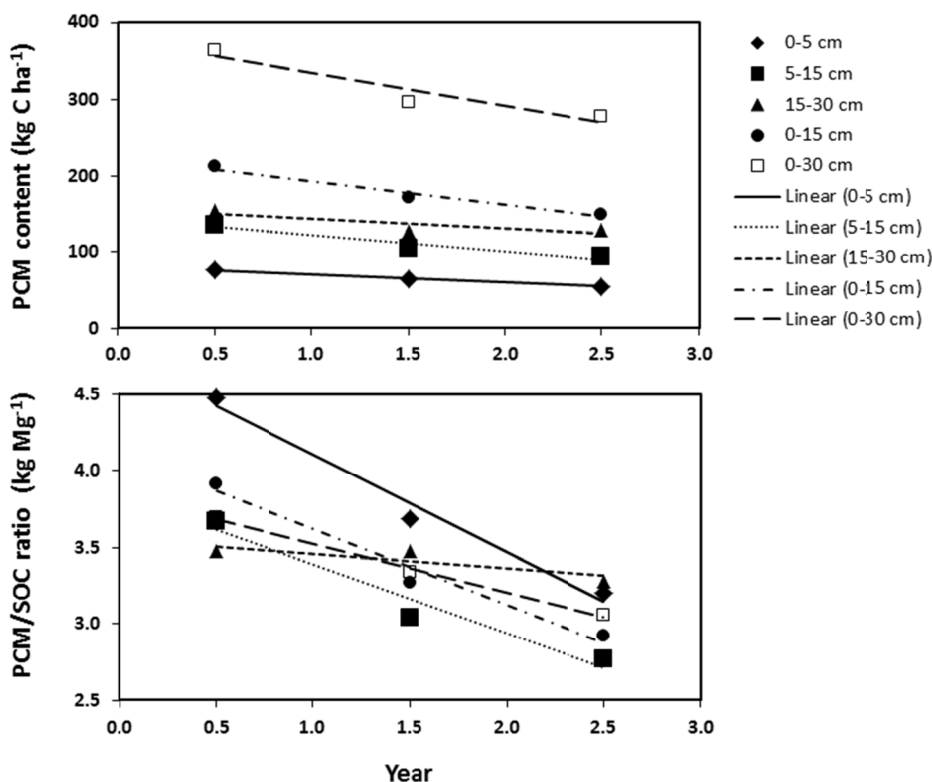


Figure 2. Relationships between soil potential C mineralization (PCM) and PCM/soil organic C (SOC) ratio at various soil depths averaged across treatments with year. Coefficients of linear regression analysis are shown in Table 4. The timings of 0.5, 1.5, and 2.5 yr used in the regression analysis represent the actual periods of soil sampling in October 2009, 2010, and 2011, respectively, since the project was initiated in April 2009

3.3 Particulate Organic Carbon

Differences in weed management practices and cropping sequences affected POC more than MBC and PCM (Table 2). As with MBC and PCM, POC varied among years at all depths, except at 0 to 5 cm. Significant interactions occurred for weed management \times cropping sequence at 0 to 5, 5 to 15, 15 to 30, and 0 to 15 cm.

Averaged across years, POC at 0 to 5 and 0 to 15 cm was 21-24% greater with sheep grazing than herbicide application on W-P/B-F (Table 5). At 5 to 15 and 0 to 15 cm, POC was 22 to 32% greater with herbicide application than grazing on CSW. At 15 to 30 cm, POC was 31 to 35% greater with grazing than herbicide application and tillage on CSW. The POC was also greater in CSW than W-P/B-F with herbicide application at 0 to 5, 5 to 15, and 0 to 15 cm and with grazing at 15 to 30 cm, but was greater in W-P/B-F than CSW with grazing at 0 to 15 cm. Averaged across treatments, POC at 0 to 5 cm declined at the rate of $0.3 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ from 2009 to 2011 (Table 4, Figure 3).

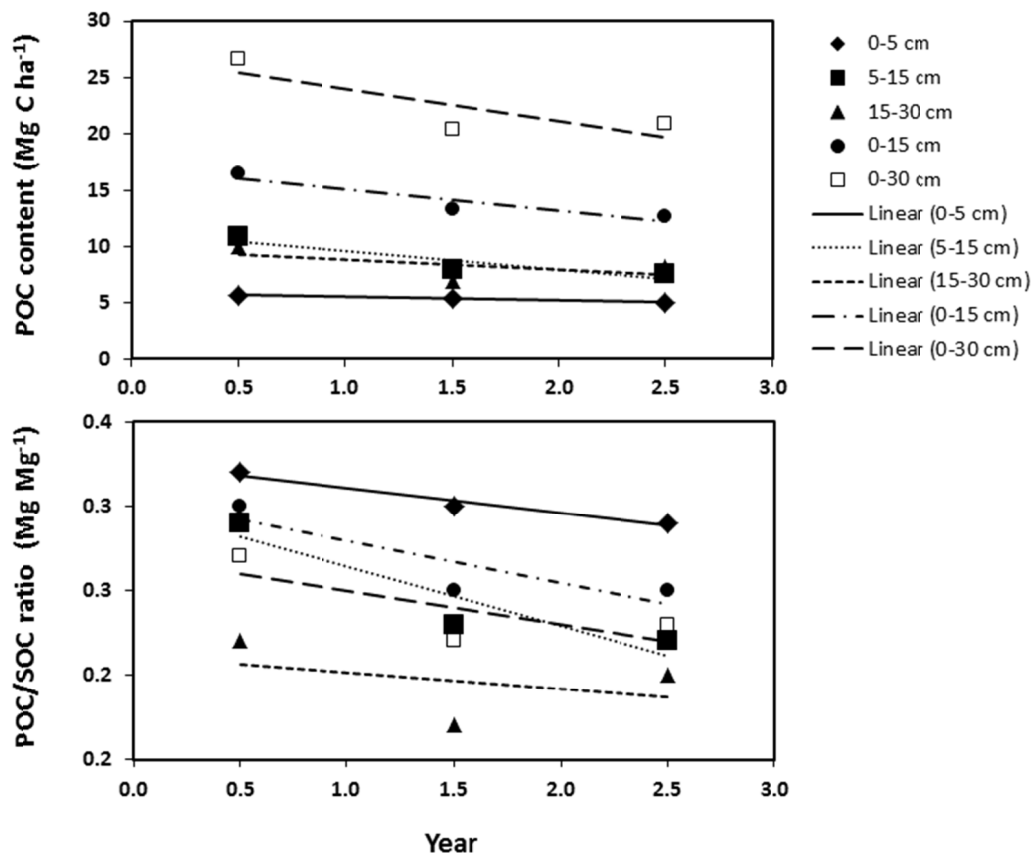


Figure 3. Relationships between soil particulate organic C (POC) and POC/soil organic C (SOC) ratio at various soil depths averaged across treatments with year. Coefficients of linear regression analysis are shown in Table 4. The timings of 0.5, 1.5, and 2.5 yr used in the regression analysis represent the actual periods of soil sampling in October 2009, 2010, and 2011, respectively, since the project was initiated in April 2009

Greater POC with grazing than herbicide application and tillage on W-P/B-F and CSW at 0 to 5, 15 to 30, and 0 to 15 cm indicate that increased return of nonlabile compared with labile portion of organic matter through feces and urine to the soil during sheep grazing likely increased C in coarse organic matter fraction. Our results are similar to those reported for increased POC with cattle grazing than nongrazing on annual and perennial cropping systems (Franzluebbers & Stuedemann, 2003, 2008). Reduced soil disturbance and mineralization of residue probably increased POC at 5 to 15 and 0 to 15 cm with herbicide application than grazing on CSW. Increased above- and belowground biomass residue returned to soil (Barsotti et al., 2013) may have enhanced POC in CSW compared to W-P/B-F with herbicide application at 0 to 5, 5 to 15, and 0 to 15 cm and with grazing at 15 to 30 cm. Although we did not measure belowground biomass, it was assumed that belowground biomass will behave similar to aboveground biomass, because above- and belowground biomass are positively correlated (Sainju and Lenssen, 2011). In contrast, longer duration of sheep grazing resulting in increased feces and urine returned to the soil surface possibly increased POC in W-P/B-F compared to CSW with grazing at 0 to 15 cm. Increased crop residue returned to the soil increased POC in eastern Montana (Sainju et al., 2007) and no-till increased POC compared to conventional till in Texas (Franzluebbers et al., 1995).

The POC/SOC ratio also varied among years at all depths, except at 15 to 30 cm (Table 2). Interaction was significant for weed management \times cropping sequence at 0 to 5, 5 to 15, and 0 to 15 cm. The POC/SOC ratio at 0 to 5 cm, averaged across years, was greater with grazing than herbicide application and at 5 to 15 cm was greater with grazing and tillage than herbicide application on W-P/B-F (Table 5). In contrast, the POC/SOC ratio at 5 to 15 and 0 to 15 cm was greater with herbicide application than grazing on CSW. The POC/SOC ratio at 0 to 5, 5 to 15, and 0 to 15 cm was greater in CSW than W-P/B-F with herbicide application. Averaged across treatments, the POC/SOC ratio at 0 to 5 cm declined at the rate of $0.02 \text{ Mg Mg}^{-1} \text{ SOC yr}^{-1}$ from 2009 to 2011 (Table 4, Figure 3).

Increased POC/SOC ratio at 0 to 5 and 0 to 15 cm with grazing and tillage compared with herbicide application on W-P/B-F indicates that feces and urine returned to soil through sheep grazing or residue incorporation into the soil due to tillage increased coarse organic matter fraction relative to total organic matter when the duration of sheep grazing was longer during the fallow period (Table 5). When the grazing duration was shorter in CSW, the POC/SOC ratio at 5 to 15 and 0 to 15 cm was enhanced with herbicide application compared to sheep grazing on CSW, likely a result of reduced organic matter mineralization due to undisturbed soil condition. Increased crop residue returned to the soil (Barsotti et al., 2013) probably increased POC relative to SOC at 0 to 5, 5 to 15, and 0 to 15 cm in CSW compared to W-P/B-F with herbicide application. This suggests that management practices can change POC more readily than SOC. The POC/SOC ratios obtained in this study were within the ranges of 0.13-0.60 Mg Mg⁻¹ SOC as reported by several researchers in the northern Great Plains, USA (Sainju et al., 2007, 2011).

Continuous removal of aboveground biomass for hay and through sheep grazing likely reduced POC and the POC/SOC ratio, especially at the surface layer, from 2009 to 2011, a case similar to those observed for other C fractions. Crop residue removal can reduce POC compared to non-removal (Sainju and Lenssen, 2011; Barsotti et al., 2013). The rate of decline at 0 to 5 cm was greater for POC than for PCM (Table 4), indicating that coarse organic matter fraction can be lost through mineralization rapidly than microbial activity. In contrast, the rate of decline at 0 to 5 cm was lower for the POC/SOC ratio than for the PCM/SOC ratio, suggesting that coarse organic matter as a fraction of total soil organic matter was less sensitive to changes due to management practices compared to microbial activity as a fraction of total organic matter. The trends in reduction in the POC/SOC ratio at various depths from 2009 to 2011 were similar to trends in POC, suggesting that POC may have been reduced more than SOC due to management practices.

3.4 Management Implications

The results suggest that sheep grazing increased POC but did not increase MBC and PCM compared with tillage and herbicide applications for weed control in dryland annual continuous cropping systems. Return of a greater proportion of nonlabile than labile organic matter through feces and urine likely is the main reason for increased POC compared with MBC and PCM with sheep grazing. Another possible reason for lower MBC with sheep grazing is probably excessive grazing, because the amount of residue left in the soil after grazing was ≤ 47 kg ha⁻¹. If the amount of residue can be increased above this value by reducing grazing intensity, either by lowering the number of sheep per plot, duration of grazing, or both, soil C fractions may be enhanced with sheep grazing compared to other weed management practices. Leaving sheep for longer grazing periods during fallow may control weeds, but the practice can degrade soil health and quality by excessively grazing on crop residue. While moderate grazing may enhance crop root growth and soil quality (Franzluebbers & Stuedemann, 2003), excessive grazing with increased number of sheep per plot and/or longer duration of grazing should be curtailed. Increased amount of crop residue returned to the soil probably increased MBC and POC in CSW compared with W-P/B-F with tillage and herbicide application treatments, but longer period of sheep grazing during fallow likely increased POC in W-P/B-F with the grazing treatment. Moderate sheep grazing may increase C fractions and soil quality and health compared to tillage and herbicide application for weed control in dryland cropping systems.

4. Conclusions

Return of C inputs to the soil through feces and urine due to sheep grazing used for weed control during the fallow period reduced MBC, but increased POC compared to tillage and herbicide application, especially in CSW. Incorporation of crop residue with higher C/N ratio into the soil increased MBC with tillage on CSW compared to sheep grazing and herbicide application in CSW and W-P/B-F. Increased crop residue returned to the soil increased MBC and POC in CSW compared to W-P/B-F in all weed management practices. Removal of crop residue for hay in tillage and herbicide application treatments and due to consumption of residue by sheep in the grazing treatment reduced MBC, PCM, and POC from 2009 to 2011. Sheep grazing can increase coarse organic matter fraction relative to microbial biomass and activity compared to tillage and herbicide application for weed control in dryland cropping systems.

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