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ORIGINAL ARTICLE

Legume, cropping intensity, and N-fertilization effects on soil attributes and processes from an eight-year-old semiarid wheat system

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Abstract In the North American northern Great Plains (NGP), legumes are promising summer fallow replacement/cropping intensification options that may decrease dependence on nitrogen (N) fertilizer in small grain systems and mitigate effects of soil organic matter (SOM) losses from summer fallow. Benefits may not be realized immediately in semiarid conditions though, and longer-term effects of legumes and intensified cropping in this region are unclear, particularly in no-till systems. We compared effects of four no-till wheat (*Triticum aestivum* L.) cropping systems–summer fallow–wheat (F–W), continuous wheat (CW), legume green manure (pea, *Pisum sativum* L.)—wheat (LGM–W), and pea–wheat (P–W)—on select soil attributes in an 8-year-old rotation study, and N fertilizer effects on C and N mineralization on a duplicate soil set in a laboratory experiment. We analyzed potentially mineralizable carbon and nitrogen (PMC and PMN) and mineralization trends with a nonlinear model, microbial biomass carbon (MB-C), and wet aggregate stability (WAS). Legume-containing systems generally resulted in higher PMC, PMN, and MB-C, while intensified systems (CW and P–W) had higher WAS. Half-lives of PMC were shortest in intensified systems, and were longest in legume systems (LGM–W and P–W) for PMN. Nitrogen addition depressed C and N mineralization, particularly in CW, and generally shortened the half-life of mineralizable C. Legumes may increase long-term, no-till NGP agroecosystem resilience and sustainability by (1) increasing the available N-supply (~26–50 %) compared to wheat-only systems, thereby reducing the need for N fertilizer for subsequent crops, and (2) by potentially mitigating negative effects of SOM loss from summer fallow.

Keywords Summer fallow · Mineralizable · Microbial biomass · Aggregate stability · Soil health · Cropping system

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Introduction

Dryland agroecosystem yields in the northern Great Plains (NGP) of North America are primarily limited by low and erratic water availability. This has resulted in widespread promotion and use of summer fallow as a soil water accumulation strategy, helping to stabilize wheat (*Triticum aestivum* L.) yields (Tanaka et al.

2010; Karlen et al. 2011). However, negative summer fallow effects have been widely reported since the early twentieth century (Linfield 1902; Atkinson and Nelson 1911; Ford and Krall 1979; Carlyle 1997; Janzen 2001; Tanaka et al. 2010) with many associated with SOM decline. Recently, improved soil water storage efficiency in no-till systems has helped to eliminate summer fallow in some regions of the NGP (Tanaka and Anderson 1997; Tanaka et al. 2005).

Water-use-efficient annual legumes (i.e., pulse crops) such as pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medik.) are promising summer fallow replacements (Miller et al. 2002), and pulse crop acreage replacing summer fallow has increased tenfold in the NGP since the early 1990s (Long et al. 2014). Where summer fallow practice remains steadfast, partial-season legume green fallow crops (forages or green manure crops) have been proposed to intensify and diversify cropping (Miller et al. 2006; O'Dea et al. 2013).

Legumes are assumed to provide N benefits to subsequent wheat, but this is not consistently detected in primarily short-term studies from the NGP (Miller et al. 2002; Lupwayi and Kennedy 2007; O'Dea et al. 2013). Longer-term studies suggest that N-benefits to wheat may be realized only after multiple years of legumes in rotation (Campbell et al. 1992; Zentner et al. 2004; Walley et al. 2007; Allen et al. 2011). This delayed response may be a reality of cool, water-limited conditions in the NGP leading to low annual legume biomass contributions, slow breakdown of residues, and subsequent slow release of available N (Janzen et al. 1990; Bremer and van Kessel 1992; Beckie et al. 1997), especially in no-till systems (Schoenau and Campbell 1996; Triplett and Dick 2008). A lack of immediate benefits is potentially discouraging to producers expecting N fertilizer-type responses from legumes (O'Dea et al. 2013) while a more reasonable expectation may be a gradual buildup of the soil N pool (Ladd et al. 1981; Janzen et al. 1990).

An understanding of legume versus fertilizer N sources in agroecosystems, particularly in the NGP, remains limited, especially regarding SOM and N retention dynamics. Reduced SOM losses have been attributed to both N fertilizer additions that increased crop residues (Campbell and Zentner 1993; Paustian et al. 1997; Varvel et al. 2002) and to legume inclusion (Drinkwater et al. 1998; Gregorich et al. 2001; Fortuna et al. 2008). Recent meta-analyses also highlight, however, that N fertilizer can be inconsistent in mitigating SOM losses, and may even exacerbate SOM losses in some cases (Khan et al. 2007; Mulvaney et al. 2009; Russell et al. 2009). Questions also remain whether legumes are capable of mitigating SOM losses from fallow as well or better than well-fertilized high-residue continuous cereal systems, especially considering legumes' labile residues (Curtin et al. 2000). Priming effects on SOM decomposition and mineralization have been reported from both addition of N fertilizer and legume residues (Kuzyakov et al. 2000). Possible mechanisms of N source effects on SOM and N retention therefore remain unclear, especially in NGP agroecosystems.

To date, studies from the Canadian NGP region report that systems that are intensified with legumes (particularly) and more wheat can result in higher SOC, TN, and higher levels of several chemical, microbial, and physical soil parameters (Campbell et al. 2001; Biederbeck et al. 2005; Lupwayi and Kennedy 2007). Published studies from longer-term experiments on legume-inclusive no-till wheat systems in NGP agroecosystems are very rare though. Since legumes and intensified cropping are increasingly common and no-tillage is rapidly becoming convention in these systems, understanding soil changes resulting from these management factors is increasingly relevant (Cochran et al. 2006; Lupwayi and Kennedy 2007).

For this study, we examined potentially mineralizable soil C and N (PMC and PMN, respectively) and mineralization trends, microbial biomass-C (MB-C), and wet aggregate stability (WAS) in bulk soils from an eight-year-old dryland rotation study including no-till fallow–wheat, continuous wheat, and legume-inclusive systems. We also examined C and N mineralization in a duplicate set of soils with and without N fertilizer additions in a laboratory experiment, a relatively novel aspect of this research. Our objectives were to better understand effects of legume inclusion and cropping intensification on soils compared to traditional fallow–wheat rotations in no-till NGP agroecosystems, and to better understand N fertilizer effects on C and N mineralization from soils conditioned by these different NGP cropping systems. Understanding changes in soil parameters and soil processes associated with the use of legumes and intensified no-till wheat systems should assist with

management decisions and lead to more appropriate expectations about the results of these management changes.

Materials and methods

Site description and experimental design

Soils used in this experiment were collected from field plots in a randomized cropping system experiment with four replicates established in 2002 at Montana State University's A. H. Post Agronomy Research Farm, located 10 km west of Bozeman, MT (45°40′ N, 111° 9′ W). Soil is a silt loam (fine-silty, mixed, superactive, frigid, Typic Haplustolls) with 88 g kg^{-1} sand, 825 g kg^{-1} silt, and 86 g kg^{-1} clay in the surface 10 cm and a pH of 7.2–7.7 in the surface 15 cm. The site receives 416 mm annual precipitation and has an annual mean temperature of 6.4 °C (data from on-site Bozeman 6 W weather station, WRCC 2014). The site is less arid than most Montana wheat-growing regions, but with similar late-spring to early summer peak precipitation patterns followed by late-summer drought. Additional site characteristics, experimental design, and some management information have been described in a previous publication (Dusenbury et al. 2008) and in Miller et al. (2015). The site had a ~30-years history of intensive tillage, but had not been tilled since 1999.

We examined soils from four no-till wheat (*Triticum aestivum* L.) systems, including: fallow–wheat (F–W), legume (pea, *Pisum sativum* L.) green manure-wheat (LGM–W), continuous wheat (CW), and pea–wheat (P–W) systems; pea in the LGM–W system was either harvested as hay (2003, '05, and '07) or terminated with herbicide (2009) at full pod stage, while pea in the P–W system was harvested at maturity for grain. A summary of cropping system history and N management is presented in Table 1; SOC, TN, and residue characteristics for each system are presented in Table 2.

Soil sampling

Soil sampling occurred in April 2010 prior to spring wheat planting (used for PMN and PMC experiments), and in October 2010 after wheat harvest (used for MB-C and WAS experiments). Soils were systematically hand cored (1.7 cm diam. × 15 cm) from ten points across each plot in a zig-zag pattern (avoiding plot edges by ~1 m), excluding surface residue. The ten cores from each plot were composited and stored for 28 days at 2 °C until processing.

Potentially mineralizable carbon and nitrogen, mineralization and N addition

Measurements to determine PMN and PMC were taken over a 112-day incubation period at 30 °C, following modified methods of Drinkwater et al. (1996); repeated measurements throughout the incubation period allowed assessment of C and N mineralization trends over time. Soil samples were homogenized by sieving into two fractions (<2.5-mm, 2.5–5 mm). A final homogenized sample consisted of four parts <2.5-mm aggregates and one part 2.5–5 mm aggregates; this ratio included most of the original sample, and helped assure that soil aggregate surface areas would be standardized across samples. Roots and organic residues were not removed from samples if <5 mm.

Twelve 10-g dry-soil-equivalent subsamples from each of the original samples (four treatments × four replications) were needed so that two could be removed from each replication at each N mineralization measurement interval. Each subsample was placed into 20-mL scintillation vials, tamped to a uniform bulk density (1.0 g cm^{-3}), and water contents adjusted to 50 % water-filled pore space (WFPS). Each set of twelve vials from a given sample was split into a main and duplicate set of six each. The duplicate set of six vials from each sample was given additions of N fertilizer in the equivalent amounts of 35, 38, 23, and 36 mg kg^{-1} N (in the form of dissolved urea) for F–W, CW, LGM–W, and P–W, respectively. These amounts corresponded to 50 % of the amount of N fertilizer added to plots based on soil sampling in spring 2010 (after soil was collected for this study) and were meant to approximate an amount of N fertilizer remaining in soils after some plant uptake and/or losses. Each set of six vials from the two N treatments (N addition and control) were respectively incubated in autoclaved 950-mL Mason jars with lids fitted with a butyl rubber septum (Sigma-Aldrich Corp., St. Louis, MO; O'Dea 2011).

Table 1 Cropping system histories, soil available nitrogen to 0.6 m (kg ha^{-1}), and fertilizer nitrogen added to systems (kg ha^{-1}), 2003–2010

Cropping system[a], *nitrogen*	Year								*Average N, wheat years*[c]
	2003	2004	2005	2006	2007	2008	2009	2010	
F–W	Fallow	Win. wht	Fallow	Win. wht	Fallow	Win. wht	Fallow	Spr. wht	
Avail N (Fert N)[b]	*26 (0)*	*199 (88)*	*NA (0)*	*211 (156)*	*NA (0)*	*275 (233)*	*NA (0)*	*200 (145)*	*221 (78)*
CW	Spr. wht	Win. wht	Spr. wht	Win. wht	Spr. wht	Win. wht	Spr. wht	Spr. wht	
Avail N (Fert N)	*202 (176)*	*199 (88)*	*200 (160)*	*216 (96)*	*202 (179)*	*276 (220)*	*201 (189)*	*207 (166)*	*213 (159)*
LGM–W	Win. pea hay	Spr. wht	Win. pea hay	Spr. wht	Win. pea hay	Spr. wht	Spr. pea manure	Spr. wht	
Avail N (Fert N)	*37 (11)*	*164 (118)*	*NA (15)*	*168 (116)*	*NA (6)*	*150 (94)*	*NA (6)*	*184 (99)*	*167 (53)*
P–W	Win. pea	Win. wht	Win. pea	Win. wht	Win. pea	Win. wht	Spr. pea	Spr. wht	
Avail N (Fert N)	*37 (11)*	*178 (108)*	*NA (15)*	*191 (156)*	*NA (6)*	*255 (210)*	*NA (6)*	*191 (149)*	*204 (78)*

[a] Cropping systems are fallow–wheat (F–W), continuous wheat (CW), legume green manure–wheat (LGM–W), pea–wheat (P–W)

[b] Nitrogen rates (Fert N) determined as 5 kg N per 100 kg of targeted yield, minus available spring soil NO_3–N to 0.6 m (Avail N). Lower available N and fertilizer rates in LGM–W and P–W reflect legume N credits alloted each wheat year. *NA* Soil nitrogen data not available

[c] Average annualized soil and fertilizer nitrogen available for wheat crops in each system from 2003 to 2010. The CW system is averaged over eight wheat years while all other systems are averaged over four wheat years

Table 2 Soil organic C (SOC) and total N (TN) to 20 cm as measured in 2008, and average annual cropping system residue amounts and qualities returned to soils, 2003–2010

Cropping system[a]	Soil[b]			Residue returned[c]							
	SOC (g kg^{-1})	TN (g kg^{-1})	Soil C:N	Shoot (Mg ha^{-1})	Shoot C (kg ha^{-1})	Shoot N (kg ha^{-1})	Shoot C:N	Root[d] (Mg ha^{-1})	Root C[d] (kg ha^{-1})	Root N[d] (kg ha^{-1})	Root C:N[d]
F–W	19.1	2.26	8.4	3.51	1554	22	72	0.93	413	11	38
CW	20.5	2.38	8.6	4.77	2146	39	55	1.24	556	19	29
LGM–W	19.5	2.41	8.1	3.23	1433	42	34	1.06	468	23	21
P–W	20.9	2.40	8.7	4.40	1972	52	38	1.19	531	19	27

[a] Cropping systems are fallow–wheat (F–W), continuous wheat (CW), legume (pea) green manure–wheat (LGM–W), pea–wheat (P–W)

[b] Most recent measurements from 2008, SOC from Richard Engel, Montana State University (unpublished data, 2011); TN from McCauley (2011). Treatment differences in SOC were not determined, and no differences in TN in the 0–10 or 10–20 cm depth were reported between treatments at $P \leq 0.10$

[c] Data from Perry Miller, Montana State University (unpublished data, 2011)

[d] Root biomass calculated using shoot:root ratios used in Janzen et al. (2003) for wheat, and from Gan et al. (2009) for pea. Calculations for root C and N concentrations for wheat based on differences between root and shoot concentrations reported by Soon and Arshad (2002). Pea shoot C and N concentrations were used for root estimates based on findings by Wichern et al. (2007)

Potentially mineralizable carbon

Measurements of headspace CO_2 concentrations used to determine PMC were taken every 3.5 days from time-zero to day 28, every 7 days from day 28 to 56, and every 14 days from day 56 to 112; jars were aerated (5 min) in a fume hood following each CO_2 sampling (O'Dea 2011). Headspace CO_2 samples were taken with a

10-mL syringe, and injected directly into pre-evacuated 3.7-mL Exetainers® (Labco International Inc., High Wycombe, UK). CO_2 concentrations were determined using a Varian CP 3800 gas chromatograph (GC) equipped with a ^{63}Ni electron capture detector and a CombiPal autosampler (Agilent Tech., Santa Clara, CA). Oven and column temperatures were set at 40 and 120 °C, respectively. Headspace CO_2 readings were adjusted by subtracting output values from blank values (n = 4), dividing by the number of vials, and correcting for changing headspace volumes from evaporating water and vials removed for PMN measurements (described in following section). Cumulative C mineralized over time was calculated using the following equation modified from Robertson et al. (1999):

$$C - min = C * 12.01\left(\frac{(P \times V)/(8.3145 \times T)}{M}\right)$$

where *C-min* is mineralized C in mg kg^{-1}, *C* is the concentration (μL L^{-1}) of headspace CO_2 corrected for ambient chamber CO_2 (values from blanks), 12.01 is the molar mass of C, *P* is the atmospheric pressure (kPa) at a given elevation, *V* is the volume of the headspace chamber (L), 8.3145 is the universal gas constant in L kPa K^{-1} mol^{-1}, *T* is the ambient temperature (°K), and *M* is the sample mass (dry weight) in g. Only C-min values from days 7, 14, 28, 56, and 112 are presented in this paper.

Potentially mineralizable nitrogen

Potentially mineralizable N was measured at time-zero, and days 7, 14, 28, 56, and 112. For each measurement, one scintillation vial was removed from each incubation jar during aeration (described in previous section), capped, and immediately placed in a refrigerator at 2 °C. Soils were mixed with 50 mL of 1 *M* KCl and placed into 60 mL centrifuge tubes, shaken for 1 h at 250 rpm, centrifuged (5000×*g* at 4 °C, 10 min), and supernatants filtered through Whatman 42 (2.5 μm) ashless filter papers (Whatman plc., Maidstone, UK). Nitrate–N and NH_4–N concentrations from filtered supernatants were measured through cadmium reduction with a Lachat flow injection analyzer (Lachat Instruments, Loveland, CO; QuickChem methods 12-107-04 and 06-1B, respectively). PMN levels were defined as final NO_3–N + NH_4–N concentration minus time-zero concentration.

Microbial biomass and aggregate stability

Soil gravimetric water content (GWC) was determined after sampling (20 g, 110 °C, 24 h). Soils were sieved (1 and 2-mm, nested sieves) after reaching uniform GWC over 21 days (at 4 °C) until at least 100 g of 1–2 mm aggregates were recovered from the entire sample (O'Dea 2011).

Microbial biomass carbon

Methods for determining MB-C via substrate induced respiration (SIR) were modified from Höper (2006). Soils were pre-incubated (21 °C, 10 days), and a 10-g dry-equivalent soil subsample from the 1 to 2-mm fraction was tamped to a uniform bulk density (1 g cm^{-3}) in 20-mL scintillation jars. Substrate consisted of 1.5-g glucose dissolved in 100-mL deionized (D.I.) water, along with 5-mM NH_4 (as $(NH_4)_2SO_4$) and 1-mM PO_4 (as KH_2PO_4 and K_2HPO_4) concentrations outlined in Bollmann (2006) as additional N, phosphorus, potassium, and sulfur nutrient sources. 1 mL of this substrate plus enough D.I. water to bring WFPS to 50 % was added to each sample immediately prior to incubation. Soils were incubated at 21 °C; after a 2-h pre-incubation, time-zero measurements of headspace CO_2 concentration were taken, and again after 4 h for SIR measurements (O'Dea 2011). Sampling procedures followed those used in PMC experiments. MB-C was calculated using an equation derived from calculations presented by Höper (2006) and Anderson and Domsch (1978)

$$MB - C = 30 * V\left(\frac{C}{M * T}\right)$$

where MB-C is microbial biomass C (mg kg^{-1}), 30 is a constant (mg C-h mL-CO_2^{-1}) derived experimentally by Höper (2006), V is the incubation chamber volume (L), *C* is the measured headspace CO_2 (μL L^{-1}) concentration corrected for ambient CO_2 (as measured in four blanks), *M* is the dry soil sample mass (g), and *T* is incubation time (h).

Wet aggregate stability

Methods for measuring WAS were modified from Arshad et al. (1996) and Kemper and Rosenau (1986). Aggregate agitation in water was carried out with a

stroke length of 2.6 cm, at a speed of ~90 oscillations per min. 10-g dry equivalent subsamples from the 1 to 2-mm fraction were poured into 1-mm sieves (54-mm diam. × 30-mm deep), positioned at the surface of water in soil cans (76-mm diam. × 52-mm deep). Samples were allowed to moisten for 5 min, then agitated for 1 min to dissolve unstable aggregates. A second set of cans with 100 mL of D.I. water and 2 g of $(NaPO_3)_6$ was used to dissolve remaining stable aggregates. Both sets of cans were dried at 110 °C. Wet aggregate stability is presented as water-stable aggregates (WSA) in g kg^{-1}, calculated using the equation:

$$WSA = 10 * \left(\frac{SA}{UA + SA}\right)$$

where *SA* is stable aggregate mass (g)—0.2 g $(NaPO_3)_6$, and *UA* is unstable aggregate mass (g) (O'Dea 2011).

Statistical analysis

Carbon and N mineralization data collected over the 112-day soil incubation period were analyzed using the *nlme* package (Pinheiro et al. 2011) in R 2.13 (R Core Development Team 2011). First, to assess PMC and PMN we estimated (1) the asymptotes of cumulative C and N mineralized (not reached after 112 days) and (2) relative rates of decay as a function of first-order kinetics in long-term incubations (Stanford and Smith 1972; Stanford et al. 1974). An asymptotic ("exponential decay") nonlinear model through the origin (Pinheiro and Bates 2009) was used:

$$y_{ij} = A_i(1 - \exp\{-e^{h_i} day_{ij}\}) + \varepsilon_{ij}$$

where A is the asymptote, h is *ln* (half-life), $\varepsilon_{ij}$are normally distributed residuals with mean 0 and a power variance structure. Talpaz et al. (1981), Paustian and Bonde (1987), and Benedetti and Sebastiani (1996) assert that nonlinear, exponential decay models are appropriate for modeling mineralization trends and potentials (Smith et al. 1980). Soundness of model fit was determined with qualitative assessments of the models through standardized residual plots and likelihood ratio tests. Second, for a detailed assessment of when shifts in mineralization trends become apparent over time, we conducted analysis of variance (ANOVA) of cumulative C and N mineralization for every measured time step in the incubation period (days 7, 14, 28, 56, and 112). For both analyses, the experiment was treated as a split-plot design with cropping system as a main-plot and additional N fertilizer treatments as sub-plot, replicated four times.

Microbial biomass and WAS data was analyzed for differences between cropping system treatments with ANOVA using the *nlme* package, as a randomized complete block design with four replicates. Since cropping systems represent different combinations of residue quantities and qualities returned to soils (Table 2), we hypothesized that effects would vary on both gross and fine levels. Pre-planned contrasts therefore were designed to compare grouped combinations of cropping systems (gross level) and all individual cropping system combinations (fine level). Grouped contrasts include: (1) Wheat versus Legume rotations (F–W, CW vs. P–W, LGM–W); (2) Low versus High Intensity systems (F–W, LGM–W vs. CW, P–W); and (3) Fallow versus Alternative systems (F–W vs. CW, P–W, LGM–W). The LGM–W system could be considered "intensified" in terms of crop output and cropping frequency, but residue returns to this system differed minimally from the F–W system because the pea LGM was harvested as a forage crop from 2003 to 2007 (see Tables 1, 2). The LGM–W system is contrasted with F–W in Fallow versus Alternative because of a difference in quality of residues returned and the lack of summer fallow. The effect of fertilizer on C and N mineralized was evaluated through contrasting fertilized versus unfertilized means; if there were significant interactions between cropping system and fertilization, fertilized treatments were contrasted only with their unfertilized control in a given system. All effects were considered significant at $P \leq 0.10$.

Correlation analyses were conducted between our results and select soil and crop residue parameters listed in Table 2, using the Multivariate platform in JMP 9 (SAS Institute Inc., Cary, NC (2010)). For PMN and PMC correlations, only data from day 112 were used. Spearman's non-parametric rank correlation (ρ) was used because several of the tests did not meet distributional assumptions. Correlations with $P \leq 0.10$ were considered statistically significant.

Results

For each soil parameter measured, gross effects of grouped treatments are presented first; the Wheat

versus Legume contrast represents an effect of residue quality from legume presence, Low versus High Intensity contrast represents an effect of residue quantity from intensified cropping, and the Fallow versus Alternative contrast represents a combined effect of changing residue quantity and/or quality when summer fallow is replaced with crops. Finer-scale differences among individual cropping systems are presented second to illustrate relative strengths of individual cropping systems on soil parameters.

Potentially mineralizable carbon and nitrogen

Soil PMC and PMN are measurements of relatively finite, inherent mineralizable fractions of SOM. The nonlinear model-predicted asymptotes of PMC and PMN are best estimates of soil PMC and PMN. Cumulative mineralized carbon (C-min) and nitrogen (N-min) after 7, 14, 28, 56, 112 days illustrate mineralization trends leading to an ultimate measurement of PMC and PMN. Any effects from N added to the incubated soils are referred to as effects on the C and N mineralization process.

Potentially mineralizable carbon

Cropping system and fertilizer affected C-min from day 7 onward (Table 3). On days 7 and 14, there was a prominent Low versus High Intensity system effect, with higher C-min in the CW and P–W systems than in the F–W system on both days. By days 28 and 56, C-min in each of the Alternative systems was individually higher than in the F–W system. By day 112, both Fallow versus Alternative and Wheat versus Legume effects were prominent ($P < 0.01$); individually, C-min in the LGM–W system and the P–W system were higher than both Wheat systems, and the PMC value in each Alternative system was higher than in the F–W system. Addition of N slightly stimulated C mineralization on day 7 (4 mg kg^{-1}), had no effect on day 14, and depressed C mineralization from days 28 through 112. By day 112, N fertilizer addition had depressed C mineralization by 94 mg kg^{-1} across all soils, or by approximately 11 %.

Analysis of model-predicted asymptotes of PMC (Table 4; Fig. 1) indicated that treatment effects differed slightly from C-min results on day 112. An effect was still observed in the Fallow versus Alternative contrast (12 mg kg^{-1} difference from day 112 values), but the Wheat versus Legume system effect was greater (104 mg kg^{-1} difference from day 112 values). There was no Low versus High Intensity effect. The LGM system's asymptote PMC (1325 mg kg^{-1}) was 304 and 387 mg kg^{-1} higher than in the CW and F–W systems, respectively. The relative rate of decay of PMC was greater (shorter half-life) in High Intensity systems than in Low Intensity systems, with the greatest rate (shortest half-life) in the CW system (Fig. 1). This was also reflected in the by-day analyses where the Low versus High Intensity system effect was prominent on days 7 and 14, but the effect declined thereafter (Table 3). N addition only decreased asymptote PMC in the High Intensity systems, where N depressed C mineralization by 247 and 229 mg kg^{-1} in the CW and P–W systems, respectively.

Potentially mineralizable nitrogen

There were no main treatment effects on N-min at day 7, but there was an interaction between cropping system and fertilization (Table 3); mineralization was depressed by N addition in the CW system, and stimulated in the LGM–W system, but mean effects were small (0.9 and 2.2 mg kg^{-1}, respectively). On day 14, the Fallow versus Alternative system effect was prominent, with greater N-min in each Alternative system than in F–W. From day 28 through 112, Wheat versus Legume system effects were marginally prominent over Fallow versus Alternative effects, with the LGM–W system having consistently higher N-min than CW and F–W; notably, there was no Low versus High Intensity effect on N-min. Fertilization treatment effects from day 14 to 56 indicated that N addition depressed N mineralization across soils. By day 112, N mineralization in the F–W and LGM–W was depressed by 7.5 and 6.8 mg kg^{-1}, respectively, while N mineralization in CW was depressed most, by 16.6 mg kg^{-1}. Interestingly, N addition did not depress N mineralization in the P–W system.

The Wheat versus Legume system effect on the model-predicted PMN asymptote was more pronounced than on day 112. Specifically, PMN levels in the two Legume systems were higher than both Wheat systems by 39 mg kg^{-1} (50 %). The PMN status of the CW system differed most from day 112 results, where the model plateaued earlier in CW (Fig. 1), leading to lower PMN in CW than P–W

Table 3 Cumulative mineralized carbon (C-min) and nitrogen (N-min) as measured by day, affected by cropping systems and urea fertilizer addition

Cropping system[a]	C-min (mg kg^{-1})					N-min (mg kg^{-1})				
	Day					Day				
	7	14	28	56	112	7	14	28	56	112
F–W	68 C[b]	128 C	248 C	446 B	701 C	5.2 B	10.5 B	20.1 B	36.1 C	58.0 C
Fertilized[c]	*ns* –[b]	*ns* –	*ns* –	*ns* –	*ns* –	*4.8 b*	*ns* –	*ns* –	*ns* –	*50.5 d*
CW	98 AB	179 AB	316 AB	560 A	870 B	5.8 B	13.4 A	21.1 B	40.2 BC	60.2 BC
Fertilized	*ns* –	*ns* –	*ns* –	*ns* –	*ns* –	*4.9 c*	*ns* –	*ns* –	*ns* –	*43.6 d*
LGM–W	77 BC	150 BC	312 B	615 A	990 A	5.2 B	13.6 A	28.0 A	55.2 A	77.4 A
Fertilized	*ns* –	*ns* –	*ns* –	*ns* –	*ns* –	*7.4 a*	*ns* –	*ns* –	*ns* –	*70.6 b*
P–W	108 A	202 A	372 A	635 A	941 A	4.8 B	12.7 A	26.2 A	50.7 AB	71.2 AB
Fertilized	*ns* –	*ns* –	*ns* –	*ns* –	*ns* –	*4.7 b*	*ns* –	*ns* –	*ns* –	*67.9 ab*
Fertilization[c]										
–N	88 B	ns –	312 A	564 A	876 A	5.2 A	12.6 A	23.8 A	45.5 A	66.7 A
+N	92 A	ns –	286 B	508 B	782 B	5.5 A	11.9 B	21.6 B	39.2 B	58.1 B
Grouped contrasts[d]										
WHT–LEG=	−10	−23	−60	−122	−180	0.5	−1.2	−6.5	−14.8	−15.2
P value	*0.38*[b]	*0.15*	*0.03*	*0.01*	*<0.01*	*0.38*	*0.08*	*0.01*	*0.01*	*0.01*
LI–HI=	−31	−51	−64	−67	−60	−0.1	−1.0	0.4	0.2	2.0
P-value	*0.01*	*0.01*	*0.02*	*0.12*	*0.05*	*0.81*	*0.13*	*0.83*	*0.97*	*0.70*
F–ALT=	−26	−49	−85	−158	−233	−0.1	−2.7	−5.0	−12.6	−11.6
P-value	*0.05*	*0.02*	*0.01*	*0.01*	*<0.01*	*0.91*	*<0.01*	*0.05*	*0.03*	*0.08*
ANOVA[b]										
Cropping system										
P-value	*0.07*[b]	*0.04*	*0.03*	*0.03*	*<0.01*	*0.27*	*0.03*	*0.04*	*0.03*	*0.04*
Fertilization										
P-value	*0.05*	*ns*	*<0.01*	*<0.01*	*<0.01*	*0.18*	*0.04*	*<0.01*	*<0.01*	*<0.01*
Cropping system × fertilization										
P-value	*ns*	*ns*	*ns*	*ns*	*ns*	*<0.01*	*ns*	*ns*	*ns*	*0.08*

[a] Cropping systems are fallow–wheat (F–W), continuous wheat (CW), green (pea) manure–wheat (LGM–W), pea–wheat (P–W). Means presented for treatments are least squares means (of both unfertilized and fertilized treatments when ns and only the unfertilized treatment when significant)

[b] Upper case letters denote statistically significant ($P \leq 0.10$) differences between groups of treatments not sharing the same letter within a column. Lower case letters denote a single treatment's contrast with its unfertilized counterpart only. All differences between treatments determined by single-degree-of-freedom contrasts (ns = not significant)

[c] Means for individual fertilized treatments are only presented if a cropping system × fertilization interaction was significant in ANOVA

[d] Grouped treatments contrasted refer only to unfertilized treatments, and include wheat systems (WHT) = F–W, CW; legume systems (LEG) = LGM–W, P–W; low intensity systems (LI) = F–W, LGM–W; high intensity systems (HI) = CW, PW; fallow–wheat (F) = F–W; alternative to fallow–wheat (ALT) = CW, LGM–W, P–W. Values presented in grouped contrasts are differences between the first and second treatment group indicated

system in this analysis. This trend was also illustrated by a greater relative rate of decay (shorter half-life) of PMN in the CW systems, than the two Legume systems, which reached their asymptotes later (Table 4; Fig. 1). The addition of N affected the PMN asymptotes, indicating that the model-predicted effect on N mineralization was a 13 mg kg^{-1} depression, or approximately 13 %, across all soils.

Table 4 Nonlinear model results for asymptote and half-life (relative rate of decay) of soil potentially mineralizable carbon (PMC) and nitrogen (PMN) affected by cropping systems and urea fertilizer addition

Cropping system[a]	PMC		PMN	
	Asymptote (mg kg^{-1})	Half-life (days)	Asymptote (mg kg^{-1})	Half-life (days)
F–W	938 C[b]	62 AB	83 B	68 AB
Fertilized[c]	*757 c*	*ns –*	*ns –*	*ns –*
CW	1021 BC	47 C	74 B	52 B
Fertilized	*775 d*	*ns –*	*ns –*	*ns –*
LGM–W	1325 A	74 A	120 A	72 A
Fertilized	*1113 a*	*ns –*	*ns –*	*ns –*
P–W	1204 AB	52 BC	115 A	82 A
Fertilized	*974 c*	*ns –*	*ns –*	*ns –*
Fertilization[c]				
−N	1122 A	68 A	98 A	ns –
+N	905 B	49 B	85 B	ns –
Grouped contrasts[d]				
WHT–LEG=	−284	−8	−39	−18
P-value	*0.02*[b]	*0.20*	*<0.01*	*0.04*
LI–HI=	19	18	8	5
P-value	*0.87*	*0.01*	*0.08*	*0.56*
F–ALT=	−245	5	−20	1
P-value	*0.08*	*0.51*	*0.43*	*0.95*
ANOVA[b]				
Cropping system				
P-value	*<0.01*	*0.02*	*<0.01*	*0.05*
Fertilization				
P-value	*0.06*	*0.07*	*<0.01*	*ns*
Cropping system × fertilization				
P-value	*<0.01*	*ns*	*ns*	*ns*

Associated model results presented in Fig. 1

[a] Cropping systems are fallow–wheat (F–W), continuous wheat (CW), green (pea) manure–wheat (LGM–W), pea-wheat (P–W). Means presented for treatments are least squares means

[b] Upper case letters denote statistically significant ($P \leq 0.10$) differences between groups of treatments not sharing the same letter within a column. Lower case letters denote a single treatment's contrast with its unfertilized counterpart only. All differences between treatments determined by single-degree-of-freedom contrasts (*ns* not significant)

[c] Means for individual fertilized treatments are only presented if a cropping system × fertilization interact ion was significant in ANOVA

[d] Grouped treatments contrasted refer only to unfertilized treatments, and include wheat systems (WHT) = F–W, CW; legume systems (LEG) = LGM–W, P–W; low intensity systems (LI) = F–W, LGM–W; high intensity systems (HI) = CW, PW; fallow–wheat (F) = F–W; alternative to fallow–wheat (ALT) = CW, LGM–W, P–W. Values presented in grouped contrasts are differences between the first and second treatment group indicated

Microbial biomass carbon

Cropping system affected MB-C, though the only grouped treatment effect on MB-C was in the Wheat versus Legume systems contrast (Table 5). Individual system contrasts indicated that MB-C in the P–W system was 123 mg kg^{-1} higher than in CW and F–W systems. The MB-C level in LGM–W was individually not different from the other three systems.

Wet aggregate stability

The WAS in the High Intensity systems was 101 g kg^{-1} greater than in the Low Intensity systems, and both CW and P–W systems individually had higher WAS than both Low Intensity systems. The Fallow versus Alternative contrast also indicated a prominent treatment effect, but inspection of the data revealed that the High Intensity systems likely

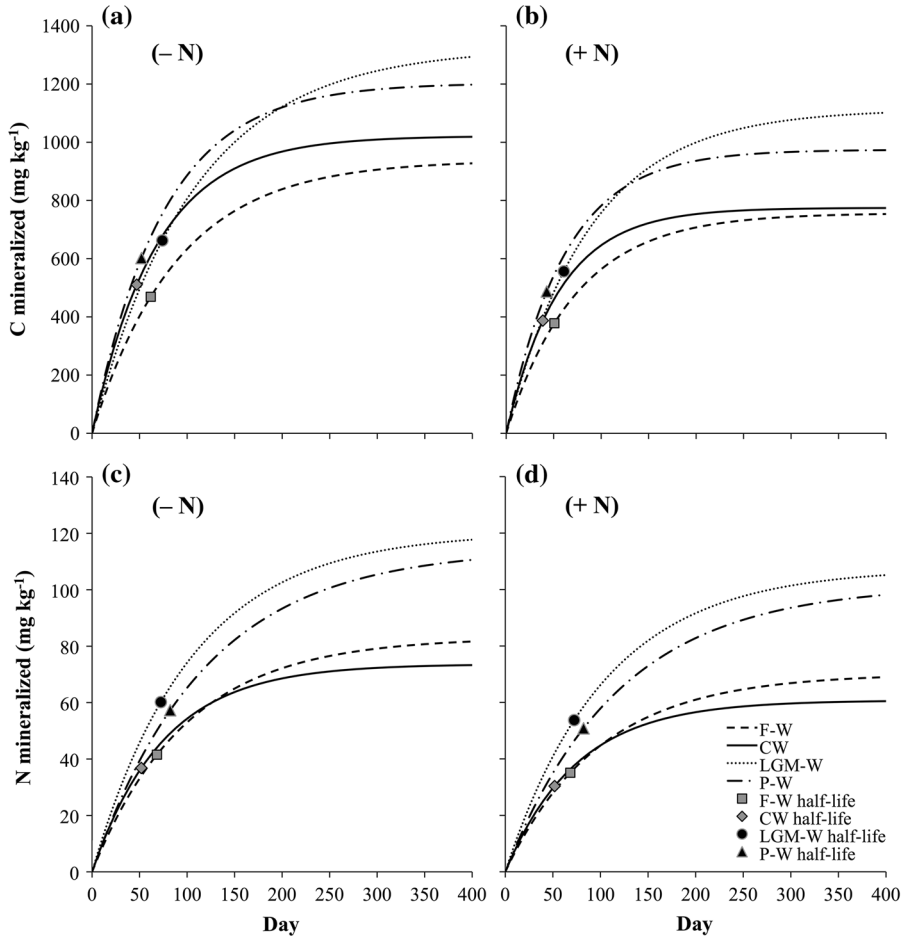

Fig. 1 Nonlinear modeled carbon mineralization without (**a**) and with (**b**) urea fertilizer and nitrogen mineralization without (**c**) and with (**d**) urea fertilizer as a function of time. The asymptote and half-life (relative rate of decay) of PMC and PMN as affected by cropping systems and urea fertilization are shown. Associated statistics presented in Table 4. Cropping systems: fallow–wheat (F–W), continuous wheat (CW), legume (pea) green manure-wheat (LGM–W), pea–wheat (P–W); Fertilization treatments: no fertilizer added (−N) and urea fertilizer added (+N). Models were developed from data collected on days 7, 14, 28, 56 and 112

strongly biased the mean of the Alternative treatments; the mean WAS value in the LGM–W system was very similar to the mean F–W value, compared to WAS in the High Intensity systems which was 105 g kg^{-1} above the mean F–W value. Additionally, the LGM–W and F–W systems exhibited no difference from each other.

Correlations among soil and crop residue parameters

Most significant correlations between background soil and residue data and our measured soil attribute parameters were relatively moderate ($\rho > 0.60$) with a few exceptions. The strongest positive correlation ($\rho = 0.74$) was between WAS and residue C returned to soils (Table 6). The other C parameters, SOC and PMC, were also moderately correlated with WAS. PMN also had a moderately strong negative correlation with residue C:N ($\rho = -0.64$). PMN and PMC were also prominently correlated ($\rho = 0.74$), as expected, because of their known interdependence on soil microbial metabolic processes.

Discussion

In this study, both crop (Wheat vs. Legume) and cropping intensity (Low vs. High) explained differences in measured soil parameters, indicating the importance of residue quality and quantity, respectively. For example, PMC, PMN, and MB-C were generally greater in the high quality residue Legume systems than in Wheat systems, and particularly the F–W system. The CW and P–W systems had much greater WAS than the low residue F–W and LGM–W

Table 5 Soil microbial biomass carbon (MB-C) and wet aggregate stability (WAS) as affected by cropping systems

Cropping system[a]	MB-C (mg kg^{-1})	WAS (g kg^{-1})
F–W	333 B[b]	101 B
CW	322 B	220 A
LGM–W	381 AB	109 B
P–W	450 A	192 A
Grouped contrasts[c]		
WHT–LEG=	−88	10
P-value	*0.03*[b]	*0.69*
LI–HI=	−29	−101
P-value	*0.41*	*<0.01*
F–ALT=	−51	−72
P-value	*0.22*	*0.01*
ANOVA[b]		
Cropping system		
P-value	*0.09*	*0.02*

[a] Cropping systems are fallow–wheat (F–W), continuous wheat (CW), legume (pea) green manure–wheat (LGM–W) pea–wheat (P–W). Means presented for treatments are least squares means

[b] Upper case letters denote statistically significant ($P \leq 0.10$) differences between groups of treatments not sharing the same letter within a column. All differences between treatments determined by single-degree-of-freedom contrasts (*ns* not significant)

[c] Grouped treatments contrasted include wheat systems (WHT) = F–W, CW; legume systems (LEG) = LGM–W, P–W; low intensity systems (LI) = F–W, LGM–W; high intensity systems (HI) = CW, PW; fallow–wheat (F) = F–W; alternative to fallow–wheat (ALT) = CW, LGM–W, P–W. Values presented in grouped contrasts are differences between first and second treatment group

Table 6 Spearman rank correlations (ρ) for soil and residue data, and measured soil attribute parameters

Parameters[a]	PMC[c]	PMN[c]	MB-C	WAS
SOC	0.52[d]	*ns*	*ns*	0.60
TN	0.59	0.43	*ns*	0.53
Soil C:N	*ns*	*ns*	*ns*	*ns*
Residue C[b]	*ns*	*ns*	*ns*	0.74
Residue N[b]	*ns*	*ns*	0.49	*ns*
Residue C:N[b]	−0.55	−0.64	−0.50	*ns*
PMC[c]		0.74	*ns*	0.45
PMN[c]			*ns*	*ns*
MB-C				*ns*

[a] Parameter abbreviations are as follows: *SOC* soil organic carbon, *TN* soil total nitrogen, *PMC* potentially mineralizable carbon, *PMN* potentially mineralizable nitrogen, *MB-C* microbial biomass carbon, *WAS* wet aggregate stability

[b] Residue data are shoot + root

[c] PMN and PMC as measured at day 112 used for correlation analyses

[d] Correlations were considered not significant (*ns*) if $P > 0.10$

systems, indicating an effect of residue quantity on WAS. These treatment effects on soil parameters were similar to those observed in tillage-managed studies from the Canadian region of the northern Great Plains. In two six-year studies in Saskatchewan, mineralizable C and N, MB-C, and WAS (to 10 cm) were higher in LGM–W rotations (including a pea green manure) than in a F–W rotation (Biederbeck et al. 1998, 2005). Mineralizable C, MB-C, and WAS in a CW system in these studies also were higher than F–W, although effects were often less than from LGM–W systems.

Our finding that MB-C did not differ between F–W and CW contrasted with previous studies. This was particularly surprising since high residue C returns from CW were expected to support more microbial biomass. In Biederbeck et al.'s (2005) tillage-based study, differences in MB-C between CW and F–W systems were related to differences in the bacterial fraction of MB-C. No-till systems likely rely more heavily on fungal, rather than bacterial decomposers (Beare et al. 1992). The SIR method we used to estimate MB-C may not detect slow-responding fungal MB-C fractions as well as the fumigation-extraction method (Wallenstein et al. 2006) used by Biederbeck et al. (2005). As a group, Legume systems supported higher MB-C in our study, but our LGM–W system alone did not differ from F–W as it did in the Biederbeck et al. (1994, 1998, 2005) studies. Our LGM–W system may not have supported higher MB-C or WAS because peas were harvested as hay for the first three, and consequently did not increase residues returned to soils compared to F–W. Our observations support this possibility because the higher residue-producing P–W system was the Legume system that individually had higher MB-C than both Wheat systems, and because our evidence suggests that higher residue returns to soils in High Intensity systems supported higher WAS. Nonetheless, WAS may also be associated with saprotrophic Basidiomycete fungi associated with more recalcitrant residues (Caesar-TonThat et al. 2011), as expected in

our CW and P–W systems which likely returned higher quantities of ligninaceous residue fractions (Lupwayi et al. 2006).

In a similar but much longer-term study, Biederbeck et al. (1994) and Campbell et al. (1993) reported that a lentil (for grain)—wheat rotation had higher mineralizable C to 15 cm, higher mineralizable N in the 7.5–15 cm depth, and higher WAS to 5 cm relative to a F–W rotation, and that the lentil-wheat rotation often equaled effects of a CW rotation on these parameters. Their CW system was the only system that supported higher MB-C (to 7.5 cm); it also had higher mineralizable C and N in the top 7.5 cm than the lentil-wheat rotation. Measurements of mineralizable C and N reported in Biederbeck et al. (1994, 1998) were from 30 and 112-day incubations, respectively, whereas results from our study indicated that mineralizable C and N measured after ~30 and 112 days, respectively, can differ from ultimate measurements (i.e., an asymptote). For example, despite indications that our CW system had higher mineralizable C and N than F–W earlier in the incubation (Table 3), the PMN and PMC asymptotes did not differ between CW and F–W (Table 4). It is also notable that the CW system studied in Biederbeck et al. (1994) had been in place for 12 years longer than their lentil-wheat rotation, which was also previously a CW system.

Biederbeck et al. (1994, 1998) found that legume rotations, along with a CW system, had higher SOC and TN than a F–W rotation, and in one study (1994) observed positive correlations similar to ours between PMC and SOC, and PMN and TN. PMN and PMC only represent a fraction of SOM, but higher amounts of PMC and PMN in Legume systems compared to Wheat systems indicate a relative accumulation of these fractions. Our results also suggest that these fractions are unlikely to decay completely in the amount of time elapsed between crop residue additions, especially in field conditions. Most systems would be yet unexhausted after >400 days in our optimized lab conditions (Fig. 1), an equivalent of ~4.9 years in field temperatures based on average annual growing degree-days at the study site (base 4.44 °C; Bozeman 6 W weather station, WRCC 2014). Nonetheless, the greater biennial time elapsed between residue additions in F–W systems highlights how mineralizable fractions could be more readily exhausted in summer fallow situations. Based on our model, we estimate that total PMN and PMC stocks could be depleted to ~25 % under field conditions in F–W during the 2 years lapse between wheat crops/residue additions.

Our study illustrated that N mineralization in legume systems can be sustained over a long period of time (Fig. 1), and appeared to be related to the C:N of residues returned to soils (Tables 2, 4, 6). Using the aforementioned estimates of in-field mineralization based on growing degree-days, total PMN stocks in the LGM and P–W systems would decline to ~60 % between annual residue additions, and are buffered by relatively greater amounts of PMN (~26–50 %) contained in these legume-conditioned soils. The CW system did not sustain N release to the same degree, with less total PMN, and total stocks potentially declining to 48 % annually due a faster relative decay rate (Table 4; Fig. 1). It also exhibited the earliest apparent N immobilization trend among the systems (Fig. 1). Compared to CW (and FW), Legume systems would theoretically increase N available to subsequent crops while simultaneously increasing potential for mineralizable fractions to accrete with lowered N fertilizer needs.

Soils given N additions in our incubation study ultimately exhibited depressed C and N mineralization from SOM. Due to microbial decomposition dynamics, N fertilizer can differentially affect mineralization where mineralization of predominately labile fractions of SOM are stimulated and that of more recalcitrant fractions are depressed (Fog 1988). Accordingly, Green et al. (1995) observed that addition of increasing rates of N fertilizer to native soil SOM depressed C mineralization, but that C mineralization rates were conversely stimulated by fresh maize residue additions. Soon and Arshad (2002) found that crop residues in fertilized plots decomposed more rapidly and mineralized more N than in unfertilized plots. We could not measure N fertilizer effects on fresh crop residues since they were not included in our study, but our results appear to agree with this dynamic regarding mineralization from SOM. When significant interactions allowed comparisons of N fertilizer effects on individual systems, the most depressive effects on mineralization were in the High Intensity systems (Tables 3, 4; Fig. 1). The High Intensity systems likely returned a relatively large fraction of ligninaceous and recalcitrant compounds to soils (Lupwayi et al. 2006), thereby exhibiting depressed mineralization from SOM when N fertilizer is added. Our results

also indicated that even though systems with legumes can have greater PMC and PMN than wheat-only systems, the mechanisms underlying why these fractions can accumulate—instead of rapidly decomposing and/or causing SOM priming—remain unclear. Fundamental differences do exist between N availability in legume systems where N fertility is partially met by residue N. Specifically, our study showed that N availability in soils from legume systems could be greater and incrementally released over a long period (Fig. 1) whereas in the systems that are more reliant on N fertilizer, (1) available N may be released more quickly from SOM initially, but may be more readily exhausted, (2) N available to crops may be available all at once, and (3) in the case of CW particularly, mineralization of SOM may be inhibited by N fertilizer—all of which have potential implications for N supply synchrony with crop uptake.

Even though legume-derived N may have some benefits over N fertilizer, the increase in N-supplying capacity of soils from legumes may be concerning. Researchers have cautioned that legume N residue needs to be managed properly in order to increase N synchrony with crop uptake demands (Crews and Peoples 2005; Gardner and Drinkwater 2009), and to improve legume-N retention (Hauggaard-Nielsen et al. 2009; Starovoytov et al. 2011). Even in semiarid Saskatchewan, a 17-year old LGM (lentil)–W–W rotation led to higher NO_3–N leaching than a F–W–W control when the N supplying capacity of the soil was underestimated (Campbell et al. 2006). Best management practices of spring soil sampling (to at least 1 m) and subsequent soil N credits should be determinants of N rates applied to crops following legumes. Accordingly, after four legume rotations in the current long-term study, the LGM–W rotation with no N fertilizer produced grain yields and protein concentrations equal to the F–W system, which received 139 kg N ha^{-1} (Miller et al. 2015). The effect of the LGM–W rotation on wheat yield was not equaled by any other legume system, including the P–W system or a pea hay-W system. This finding substantiates that increased PMN in the LGM–W system likely increased soil N-supplying power and hence wheat yield. Not only are legume-intensive systems beneficial to soil health, but through increased N supplying power, they can be more economically resilient to changes in grain protein discounts and N rate (Miller et al. 2015).

Conclusions

Intensified and legume-inclusive no-till cropping systems generally had higher levels of PMC, PMN, WAS, and MB-C than summer fallow–wheat systems. These soil parameters were most consistently higher when residue C returned to soils was high and average residue C:N ratios were lower, suggesting that residue quantity and quality both play a role. Compared to CW, legume-inclusive systems increased the N supplying capacity of soils, and generally increased the mineralizable fraction of soil C despite having returned relatively lower C:N ratio residues to soils. Compared to wheat-only systems, legume-inclusive systems also had a more sustained long-term release of mineralized-N; this aspect may have ramifications regarding plant-soil N synchrony and N losses from soils compared to fertilizer N. Legume-inclusive systems also exhibited promise to support higher MB-C, and high residue systems had two-fold higher WAS over lower residue systems. In our incubation study, we found that when we added N fertilizer to soils, mineralization from SOM was depressed, particularly in soils from CW systems. This effect could (1) theoretically increase needs for supplemental fertilizer N needs to meet crop demands and (2) affect SOM retention dynamics. Our results suggest that intensified systems positively affect soil attributes associated with SOM loss in summer fallow systems, and that intensified systems with legumes additionally benefit soil N fertility in ways that may decrease fertilizer N needs. Legumes may therefore warrant a more prominent role in intensified NGP no-till wheat systems as a soil fertility and soil-building management tool.

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