Geoderma 406 (2022) 115497

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Response of soil organic matter to cover cropping in water-limited environments

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A R T I C L E I N F O

Handling Editor: Ingrid Kögel-Knabner

Keywords:
Cover crops
Soil health
Soil organic carbon
Soil aggregates
Minimum data set

A B S T R A C T

Cover crops are promoted to improve soil health and soil carbon (C) sequestration in agroecosystems, yet responses of various soil organic carbon (SOC) and nitrogen (N) components to cover cropping have not been quantified for water-limited environments. This study evaluated the response of SOC and N components to different cover crops and mixtures in limited irrigation winter wheat (*Triticum aestivum* L.)-sorghum (*Sorghum bicolor* L. Moench)-fallow rotation. Cover cropping treatments included pea (*Pisum sativum* L.), oats (*Avena sativa* L.), and canola (*Brassica napus* L.); mixtures of pea + oats (POmix), pea + canola (PCmix), pea + oats + canola (POCmix), and a six-species mixture (SSmix) of pea + oats + canola + hairy vetch (*Vicia villosa* Roth) + forage radish (*Raphanus sativus* L.) + barley (*Hordeum vulgare* L.); and a fallow. Cover crop treatments were arranged in a randomized complete block design within each phase of the crop rotation. Soil samples were collected in the summer of 2019 and 2020 from the 0–15 cm depth of study plots established in fall 2015 and analyzed for various soil organic matter (SOM) components. Soil inorganic N was 7–36% lower with cover crops than fallow. The PCmix had 48–73% greater 24-h-carbon dioxide-carbon (CO_2-C) than fallow, canola, and SSmix at termination time. Thirty-six days after termination, particulate organic carbon (OC) content was 61–69% higher with pea, SSmix, and POCmix than fallow. The SOC content was 9.3–22% greater with oats than pea, canola, POmix, and SSmix. Similarly, total N content with oats was 10% and 22% higher than with SSmix and canola, respectively. The increase in SOC and total N were primarily observed in intermediate-size aggregates (250 µm–2 mm and 53–250 µm). However, the minimum data set of soil health included SOC, soil pH, labile organic nitrogen (LON), mineral-associated organic nitrogen (MAON), and microbial biomass carbon (MBC). While this study showed a diverse response of SOC and N components to various cover crop treatments, oats and their mixture as cover crops had greater SOC and total N than other cover crops. Cover cropping could improve soil health in crop-fallow rotations in water-limited environments.

1. Introduction

Increasing SOC storage, a proxy for SOM and soil health, through improved management practices is a crucial component of increasing the resilience of agriculture and mitigating climate change (Lal, 2004). Plant tissue is the primary source of SOM, whereas the secondary sources are microflora, fauna (primarily invertebrates), and organic amendments (manure, compost, biochar) (Turan, 2020; Sönmez et al., 2016). Therefore, SOM accumulation in agroecosystems depends on the balance between biomass carbon (C) inputs and C losses through mineralization, leaching, and erosion (Liu et al., 2006). Management practices that increase cropping intensity and diversity, maintain residue cover, and reduce soil disturbance can increase SOC storage and improve soil health (Cano et al., 2018).

Cover crops are increasingly considered in recent years to increase SOM storage and enhance soil health because of their potential to increase biomass C and N inputs, rhizodeposits, and soil microbial activity (Sainju et al., 2007; Thapa et al., 2021). Grass cover crops with dense,

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https://doi.org/10.1016/j.geoderma.2021.115497
Received 13 May 2021; Received in revised form 12 September 2021; Accepted 20 September 2021
Available online 1 October 2021

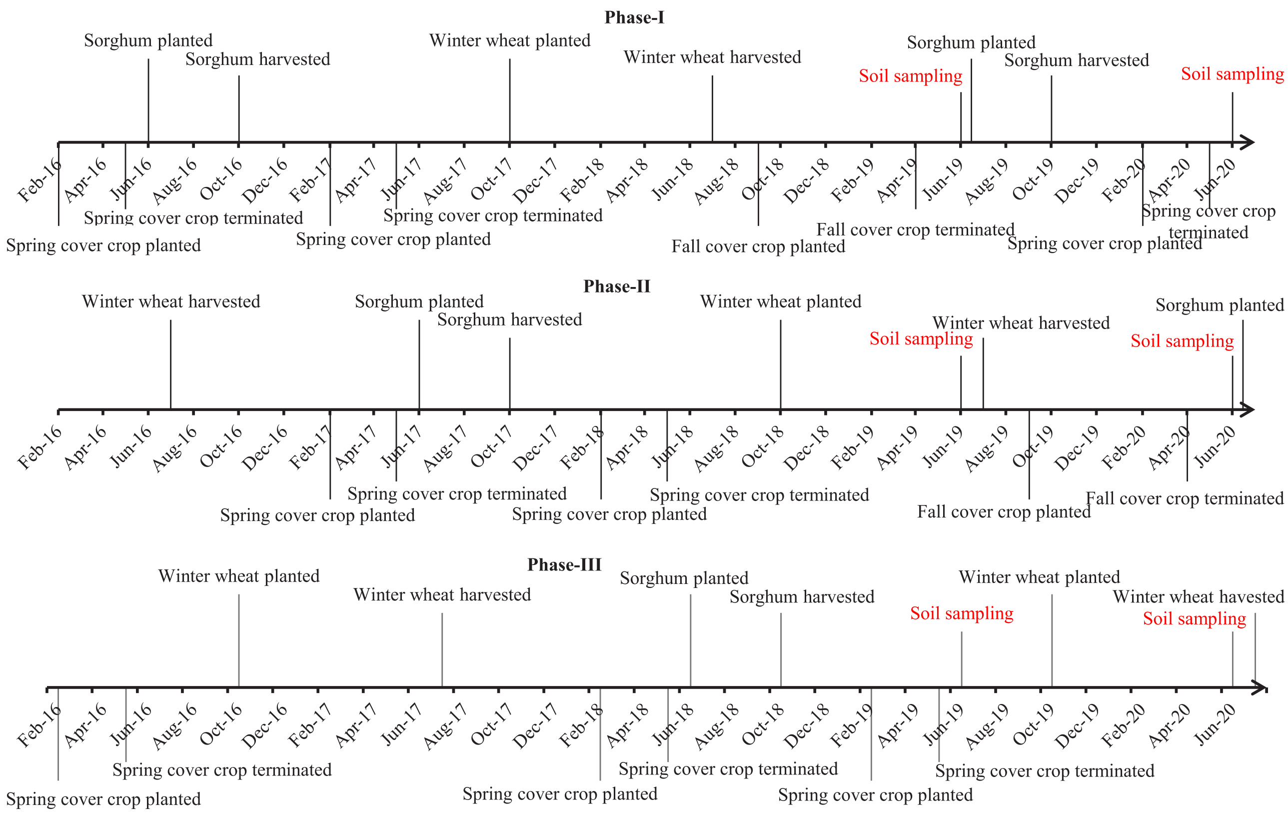

Fig. 1. Diagram of winter wheat-fallow/cover crop-sorghum-fallow/cover crop rotation and timeline of soil sampling. Each phase of the rotation corresponds to a randomized complete block design (RCBD) with three replications.

Table 1
Means of cover crop (CC) biomass, carbon to nitrogen (C:N) ratio, ground cover (%), and lignin (%) by treatment.

Treatment †	Spring CC biomass	Fall CC biomass (2019)	C:N ratio (spring CC biomass)	Ground cover (spring CC)	Lignin (spring CC)
	kg ha^{-1}			%	
Fallow	–	–	–	–	–
Pea	1440c ‡	2993 bc	10.8 d	40.3 ab	5.13 a
Oats	2538 a	900c	26.1 a	31.8c	3.39b
Canola	1915 bc	5380 ab	13.5c	35.1 bc	3.62b
POmix	2343 ab	1873c	21.2b	35.9 bc	3.24b
PCmix	1652c	6567 a	11.8 cd	44.7 a	4.85 a
POCmix	2177 ab	2177c	21.5b	32.9 bc	2.94b
SSmix	2415 a	2190c	23.4b	35.3 bc	3.07b

† POmix: pea + oats; PCmix: pea + canola; POCmix: pea + oats + canola; SSmix: six species mixture of pea + oats + canola + hairy vetch + forage radish + barley.
‡ Mean values followed by different lower case letters in a column indicate a significant difference among treatments ($p = 0.05$, Fisher's protected least significant difference (LSD) test).

fibrous root systems produce a greater amount of biomass with a high C:N ratio, increasing soil C contents (Ghimire et al., 2017; Ghimire et al., 2019). Legume cover crops fix atmospheric N in their root nodules and supply N to subsequent crops (Dabney et al., 2001). They produce high-quality residue (low C:N ratio) and favor early mineralization and rapid recycling of soil nutrients (Ghimire et al., 2017). Multispecies cover crops help in SOC accumulation, nutrient cycling and provide multiple other ecosystem services benefits, including weed suppression, nitrate leaching reduction, and erosion control (Finney et al., 2016; Chu et al., 2017).

Cover crops also increase SOC sequestration and soil health through increased soil aggregation and protection of organic residues in soil aggregates (Sainju et al., 2003; Blanco-Canqui et al., 2011). Soil aggregation indirectly protects SOM from mineralization and oxidation by reducing the access of substrates to microorganisms (Six et al., 2002) and reducing soil loss through wind and water erosion (Jien and Wang, 2013). In addition, cover crops help in aggregate formation by providing a food source to soil microorganisms. Diverse microorganisms produce various organic polymers that bind soil particles into aggregates (Zhang et al., 2012). Increased enmeshing action of cover crop roots could also bind soil particles together, thereby improving aggregation. Studies reported that SOC and N sequestration due to cover cropping occurs particularly in intermediate and small-size (<2 mm) aggregates (Sainju et al., 2003; Mendes et al., 1999). Improved knowledge of how cover crops and mixtures affect soil aggregation and SOC and N distribution in aggregates of different size classes will help design best management practices that improve soil health and C sequestration.

While sequestering SOC and N is critical for improving soil health and resilience of water-limited environments, the response of SOC and N to management changes has been slow in the southern High Plains (SHP) region of the USA (Bronson et al., 2004). Low precipitation and high weather variability have accelerated the loss of SOM and nutrients and posed additional challenges to soil health and sustainability of agriculture in the region (Acosta-Martinez et al., 2014; Pérez-Guzmán et al., 2020). Since water levels in the Ogallala Aquifer, the region's primary source of irrigation water, have been declining, cropping systems are rapidly transitioning to limited irrigation or dryland production (Cano et al., 2018). Transitioning to limited irrigation or dryland management will decrease plant C inputs, resulting in soil health degradation. A recent study by Ghimire and Khanal (2020) reported a 14% and 13% decline in SOC and total N, respectively, from 0 to 30 cm depth during the transition from limited irrigation to dryland cropping while cover cropping under limited irrigation maintained SOC. Studies show that integrating cover crops in a crop rotation in such a transition can

Table 2
Selected soil health indicators in response to various cover crop treatments in 2019 and 2020.

Treatment †	pH	Inorganic N ¶	LON	24 h-CO_2-C ¶	72 h-CO_2-C ¶	MBC	SOC ¶	Total N	Particulate OC ¶	Particulate ON ¶	MAOC ¶	MAON ¶	WAS (%)
	Phase-I δ												
		kg ha^{-1}											
Fallow	7.3 ‡	15.4	19.9	29.8c	75.6	607	18,138 ab	1521 ab	6583	235	10,076	1051	29.4b
Pea	7.3	12.9	19.0	38.3 abc	97.2	560	17,366b	1494 ab	5470	205	11,798	1248	34.7 ab
Oats	7.3	9.50	18.8	48.2 ab	128	641	19,173 a	1640 a	6027	200	12,868	1337	39.9 a
Canola	7.4	12.9	18.3	34.5 bc	87.0	484	15,783c	1345c	4305	140	11,410	1182	28.2b
POmix	7.3	13.4	20.2	38.6 abc	85.3	575	17,535b	1496 ab	5443	226	12,017	1228	36.8 ab
PCmix	7.2	18.2	25.4	51.5 a	123	655	18,625 ab	1544 ab	5942	150	12,552	1328	34.4 ab
POCmix	7.2	11.4	20.1	48.3 ab	106	660	18,176 ab	1551 ab	5690	189	12,448	1343	35.4 ab
SSmix	7.1	17.8	21.2	34.7 bc	85.8	647	17,482b	1487 bc	5316	155	12,092	1260	43.8 a
	Phase-II												
Fallow	7.3	27.7 a	29.3	16.2c	40.2	686	16,707	1630	3659b	221	12,969	1387	21.0
Pea	7.6	25.8 ab	27.2	19.1 bc	49.9	803	18,963	1813	6167 a	432	13,763	1338	28.8
Oats	7.4	20.2 bc	24.4	23.2 abc	61.9	649	18,599	1760	4834 ab	279	12,770	1298	36.3
Canola	7.5	21.6 abc	25.3	21.8 abc	45.5	729	17,898	1786	5365 ab	409	12,317	1298	30.7
POmix	7.5	17.8c	21.2	16.9c	48.7	650	16,769	1626	4248 ab	288	12,422	1299	28.0
PCmix	7.2	23.8 ab	25.2	28.9 ab	57.1	839	17,746	1696	5093 ab	291	12,414	1326	28.7
POCmix	7.4	19.6 bc	22.7	30.7 a	61.4	733	18,302	1710	5893 a	399	12,311	1244	29.1
SSmix	7.3	19.6 bc	23.2	29.9 a	63.2	750	18,733	1791	6143 a	413	12,540	1313	29.7
	Phase-III												
Fallow	7.5	11.3	14.9	51.5	107	947	19,756	1775	6784	240	12,853	1420	30.7 cd
Pea	7.7	12.8	17.3	44.8	92.9	999	19,715	1752	6656	228	12,996	1427	32.7 bcd
Oats	7.5	13.8	19.0	46.1	95.2	946	18,382	1600	5186	95	13,056	1434	37.8 abc
Canola	7.7	12.1	18.8	42.3	88.5	847	18,164	1501	6203	128	11,995	1199	33.4 bcd
POmix	7.3	13.5	19.3	57.5	111	920	20,035	1755	6621	266	13,101	1376	38.8 ab
PCmix	7.5	10.9	13.8	49.5	109	935	19,606	1672	6353	93	13,098	1428	35.2 bcd
POCmix	7.5	13.2	16.4	45.8	95.3	903	18,638	1572	6189	163	12,615	1339	30.3 d
SSmix	7.6	12.5	18.2	49.9	111	1091	21,558	1608	7191	132	13,880	1314	42.5 a

† POmix: pea + oats; PCmix: pea + canola; POCmix: pea + oats + canola; SSmix: six species mixture of pea + oats + canola + hairy vetch + forage radish + barley; N: nitrogen; LON: labile organic nitrogen; 24 h-CO_2-C: 24 h-carbon dioxide-carbon; 72 h-CO_2-C: 72 h-carbon dioxide-carbon; MBC: microbial biomass carbon; SOC: soil organic carbon; Total N: total nitrogen; particulate OC: particulate organic carbon; Particulate ON: particulate organic nitrogen; MAOC: mineral-associated organic carbon; MAON: mineral-associated organic nitrogen; and WAS: wet aggregate stability

¶ Back-transformed means of inorganic N, 24 h-CO_2-C, 72 h-CO_2-C, SOC, Particulate OC, Particualte ON, MAOC, and MAON.

‡ Mean values followed by different lower case letters in a column indicate a significant difference among treatments ($p = 0.05$, Fisher's protected least significant difference (LSD) test).

δ Phase-I: at cover crop termination time; Phase-II: 36-days after cover crop termination; Phase-III: a year after cover crop termination (or during active wheat growth stage).

moderate soil temperature, reduce evaporation, and enhance microbial activities (Nilahyane et al., 2020; Thapa et al., 2021; Ghimire et al., 2019), which could increase SOC storage and ultimately improve soil health.

Quantifiable changes in the SOC and N take several years in water-limited environments. Measurements of labile SOM components and microbial activity could respond quickly to improved management practices such as no-tillage and cover cropping (Sainju et al., 2012a; Thapa et al., 2021). Active fractions of SOC and N, including inorganic N, MBC, particulate OC and ON, and potential C and N mineralization, varied seasonally due to changes in the amount of plant residues returned to the soil (Sainju et al., 2012a,b). Therefore, these fractions could serve as an early indicator of changes in SOM storage. Evaluating multiple SOM pools helps identify the relative response of these pools and ways to improve soil health and resilience in low-productivity semiarid environments. In light of the rapid expansion of drylands and desertification worldwide (Reynolds et al., 2007; D'Odorico et al., 2013), understanding the impacts of cover crops on soil health could provide valuable insight into soil health management in arid and semiarid regions.

The primary objective of this study was to evaluate the effect of diverse cover crops (single as well as in mixture) on SOM components and other soil health indicators under a limited irrigation winter wheat-fallow/cover crop-sorghum-fallow/cover crop rotation. The secondary objective was to determine the relationships among different soil health parameters at systems scale (without accounting phases) to identify the minimum data set for soil health assessment in water limited-environment.

2. Materials and methods

2.1. Study site

The study was conducted in 2019 and 2020 at the New Mexico State University Agricultural Science Center (ASC) near Clovis, NM (34°35′ N, 103°12′ W; elevation 1368 m). The experiment was established under no-tillage management in fall 2015 in a field that was previously under conventional management of irrigated corn and sorghum production for

Table 3
Aggregate proportion (%), aggregate-associated soil organic carbon (SOC) and total N concentrations in response to various cover crop treatments in 2020.

Treatment †	Aggregate proportion (%)				Aggregate-associated SOC conc. (g C kg^{-1})				Aggregate -associated total N conc. (g N kg^{-1})			
	2–8 mm	250 μm-2 mm	53–250 μm	<53 μm	2–8 mm	250 μm-2 mm	53–250 μm	<53 μm	2–8 mm	250 μm-2 mm	53–250 μm	<53 μm
					Phase-I δ							
Fallow	65.8 ‡	22.8	9.08	2.31	9.36 ab	21.1	16.9	10.9	0.80 abc	1.89	1.42	1.00
Pea	63.6	22.6	11.0	2.73	9.68 ab	24.8	19.5	11.0	0.84 ab	2.19	1.61	1.01
Oats	63.9	23.4	9.98	2.69	9.87 a	23.8	16.8	11.6	0.82 ab	2.14	1.30	1.05
Canola	65.8	22.2	9.55	2.46	8.61c	21.2	15.3	10.4	0.73c	1.84	1.18	0.92
POmix	61.8	23.7	11.5	2.93	9.63 ab	23.6	17.6	10.3	0.86 a	2.13	1.44	0.93
PCmix	64.7	22.6	9.92	2.85	9.90 a	24.2	17.5	11.8	0.85 a	2.23	1.40	1.07
POCmix	63.5	24.5	9.51	2.46	9.55 ab	34.6	15.1	11.1	0.79 abc	3.19	1.19	0.97
SSmix	64.9	21.8	10.4	2.93	9.06 bc	22.4	14.4	10.4	0.77 bc	2.05	1.20	0.95
					Phase-II							
Fallow	49.4 ab	38.6	10.2	1.87	8.41	21.3	18.3	11.2	0.74	1.84	1.67	1.04
Pea	42.8 bc	38.9	15.6	2.69	9.61	35.7	28.2	12.6	0.79	2.76	2.45	1.12
Oats	45.6 abc	39.9	12.1	2.31	10.4	30.1	20.8	12.3	0.94	2.63	1.62	1.06
Canola	38.9c	44.0	14.8	2.30	10.0	28.2	23.1	12.4	0.89	2.39	2.03	1.08
POmix	43.1 bc	43.4	11.7	1.86	8.48	22.6	19.9	11.2	0.76	2.06	1.66	0.97
PCmix	54.1 a	33.4	9.96	2.52	9.41	24.6	18.0	12.4	0.80	2.14	1.50	1.12
POCmix	40.7 bc	43.2	13.7	2.32	9.95	40.8	20.0	12.4	0.84	3.38	1.74	1.10
SSmix	44.7 bc	40.2	12.9	2.25	10.7	28.2	18.5	12.6	0.92	2.37	1.59	1.12
					Phase-III							
Fallow	51.7	29.8	14.8	3.69	10.1b	26.2	19.9	11.0	0.96	2.27	1.80	1.08
Pea	58.8	25.4	12.9	2.90	10.3b	31.6	25.7	11.7	0.97	2.70	2.15	1.13
Oats	50.5	28.6	17.3	3.70	8.98b	26.6	19.8	10.6	0.89	2.41	1.82	1.05
Canola	59.4	28.2	9.7	2.61	9.30b	26.5	21.0	11.2	0.89	2.33	1.89	1.08
POmix	59.3	26.5	11.6	2.64	9.40b	29.1	20.8	11.4	0.94	2.55	1.95	1.10
PCmix	59.2	25.7	12.3	2.69	10.3b	24.5	19.7	11.5	0.95	2.08	1.66	1.09
POCmix	63.5	23.6	10.3	2.57	10.3b	40.8	17.8	11.5	0.97	3.50	1.64	1.09
SSmix	61.4	25.7	10.4	2.45	13.1 a	31.3	18.9	12.6	1.14	2.60	1.66	1.17

† POmix: pea + oats; PCmix: pea + canola; POCmix: pea + oats + canola; SSmix: six species mixture of pea + oats + canola + hairy vetch + forage radish + barley.
‡ Mean values followed by different lower case letters in a column indicate a significant difference among treatments (p = 0.05, Fisher's protected least significant difference (LSD) test).
δ Phase-I: at cover crop termination time; Phase-II: 36-days after cover crop termination; Phase-III: a year after cover crop termination (or during active wheat growth stage).

several years. The study site has a semiarid climate with a mean annual temperature of 14.1 °C and a mean annual precipitation of 437 mm, approximately 70% of which occurs from May through September. The study area experiences high seasonal and inter-annual variability in precipitation with short-term drought periods, which often occur within a crop growing season. Soils are characterized as Olton clay loam (*Fine, mixed, super active, thermic Aridic Paleustolls*) under the USDA soil classification system, with 43.7% sand, 21.5% silt, and 34.8% clay contents. Soil bulk density measured at the time of plot establishment was 1.2 g cm^{-3}, gravimetric soil moisture content was 17.5%, soil pH was 8, and electrical conductivity was 0.4 dSm^{-1}. The SOM content, inorganic N, and available P were 14.5 g kg^{-1}, 5.6 kg N ha^{-1}, and 29.7 kg P ha^{-1}, respectively, at 0–15 cm depth.

2.2. Experimental design and treatments

The experiment had three phases of crop rotation (Fig. 1), eight treatments, and three replications. The rotation phases were winter wheat, sorghum, and fallow. All phases of the crop rotation were present each year, and cover crops were planted in each fallow period before winter wheat and sorghum. Within each rotation phase, cover crop treatments were arranged in a randomized complete block design. Treatments included fallow (no cover crop); three sole cover crops: pea, oats, and canola; and four cover crop mixtures: pea + oats (POmix), pea + canola (PCmix), pea + oats + canola (POCmix), and a six-species mixture (SSmix) of pea + oats + canola + hairy vetch + forage radish + barley. The plot size for each treatment was 18.3 m × 12.2 m.

Each year, experimental plots were treated with N-phosphonomethyl glycine 53.8% (glyphosate) at the rate of 0.38 L ha^{-1} and 2,4-D ester (6 lb gal^{-1}, LV-6) at the rate of 0.87 L ha^{-1} with ammonium sulfate and nonionic surfactant at rates of 20 g L^{-1} and 5 mL L^{-1}, respectively, two weeks before cover crop planting. Cover crops for this study were planted in late February following sorghum harvest in October of the previous year (spring cover crops) and in September (fall cover crops) following the previous year's wheat harvest in June. During 2016–2018, however, cover crops after both wheat and sorghum were planted in late February and terminated in mid-May. All cover crops were planted using a 20-ft wide plot drill (Great Plains 3P600, Salina, KS). The seeding rates for sole cover crops were 22.4, 44.8, and 4.5 kg ha^{-1} for pea, oats, and canola, respectively. The seeding rates for hairy vetch, forage radish, and barley were 11.2, 4.48, and 44.8 kg ha^{-1}, respectively. Cover crop species used in two-, three-, and six-species mixtures used 50, 33, and 16.6%, respectively, of the sole seeding rates.

Irrigation water of 39.6 mm and 28 mm was applied to cover crops in 2019 and 2020, respectively, for seed germination, after which no additional irrigation was applied. Spring cover crops were maintained in plots for three months and fall cover crops were maintained for seven months before being chemically terminated. The flowering stage of oats was used as a reference to terminate all cover crops. After termination, the cover crop residues were left on the soil surface.

Samples of aboveground fresh cover crops were hand clipped from four random 0.25 m^2 quadrat areas at the time of termination to estimate biomass production and C input from cover crops. The dry biomass yield was determined after oven drying the samples at 65 °C for 72 h.

Table 4
Aggregate-associated soil organic carbon (SOC) and total N stocks in response to various cover crop treatments in 2020.

Treatment †	Aggregate-associated SOC stock (kg C ha^{-1})				Aggregate-associated total N stock (kg N ha^{-1})			
	2–8 mm	250 μm-2 mm	53–250 μm	<53 μm	2–8 mm	250 μm-2 mm	53–250 μm	<53 μm
	Phase-I δ							
Fallow	12,354 ‡	6985	3063	502	1055	622	256	46.2
Pea	11,684	6737	4140	572	1017	597	333	53.3
Oats	12,211	7220	3269	597	1033	652	261	53.8
Canola	10,440	5700	2669	473	887	500	204	42.0
POmix	11,415	7040	3864	583	1018	638	316	52.7
PCmix	12,319	6819	3342	641	1059	637	265	58.4
POCmix	11,577	8705	2753	523	961	803	221	45.8
SSmix	11,231	6250	2804	584	958	572	227	53.2
	Phase-II							
Fallow	8613	11,978	3960 bc	426b	763	1040	367 bc	39.5 bc
Pea	8190	16,562	9065 a	676 a	678	1306	786 a	59.7 a
Oats	9496	16,276	4593 bc	540 ab	860	1421	364 bc	46.6 abc
Canola	7975	17,012	6877 ab	571 ab	706	1428	606 ab	50.1 abc
POmix	7381	13,029	4431 bc	421b	670	1185	378 bc	36.0c
PCmix	10,393	11,690	3751c	637 a	875	1021	310c	57.3 a
POCmix	8104	17,919	5487 bc	579 ab	684	1466	476 bc	51.5 ab
SSmix	9460	15,403	4815 bc	568 ab	817	1290	409 bc	50.7 ab
	Phase-III							
Fallow	10,162 bc	11,152	5747	792	970	975	522	77.1
Pea	11,603 bc	9587	6152	649	1088	810	521	62.9
Oats	8959c	10,011	6756	785	885	906	620	77.9
Canola	10,655 bc	9814	3938	563	1020	865	354	55.0
POmix	10,866 bc	9934	4861	590	1087	868	456	56.8
PCmix	11,981 bc	8506	4762	602	1105	722	396	57.1
POCmix	12,627b	9534	3691	576	1185	824	336	54.3
SSmix	16,097 a	10,447	3691	602	1411	872	325	56.3

† POmix: pea + oats; PCmix: pea + canola; POCmix: pea + oats + canola; SSmix: six species mixture of pea + oats + canola + hairy vetch + forage radish + barley.
‡ Mean values followed by different lower case letters in a column indicate a significant difference among treatments (p = 0.05, Fisher's protected least significant difference (LSD) test).
δ Phase-I: at cover crop termination time; Phase-II: 36-days after cover crop termination; Phase-III: a year after cover crop termination (or during active wheat growth stage).

The dried plant materials were ground to pass through a 0.5-mm screen to estimate total organic carbon, total N, and lignin contents. The ground cover (%) in the field was estimated at the time of cover crop termination by using a mobile app CANOPEO, which estimates green area based on color ratios of red to green (R/G) and blue to green (B/G) and an excess green index (2G-R-B). The fall cover crops in 2020 were killed by snow and freezing temperatures, and thus biomass yield was not determined.

2.3. Winter wheat and sorghum management

Winter wheat varieties TAM 113 (2015–2018) and TAM 114 (2019–2020) were planted in the second week of October using a plot drill (Great Plains 3P600, Salina, KS) at a seeding rate of 62 kg ha^{-1} with the drill spacing maintained at 0.25 m. All the experimental plots received 70 kg N ha^{-1} and about 12 kg sulfur (S) ha^{-1} each year via fertigation during the spring season. Sorghum cultivar NK5418 was planted in the first week of June using a no-till drill (John Deere, Moline, IL) at a seeding rate of 123,553 seeds ha^{-1} with the row spacing maintained at 0.76 m. All sorghum plots received 97 kg N ha^{-1} and 15 kg S ha^{-1} from a mixture of urea, ammonium nitrate, and ammonium thiosulfate in liquid form at the time of planting each year. About 50% of the crop water requirement was applied to both crops only at critical growth stages, such as jointing, booting, heading, and grain filling, because of limited water availability for irrigated crop production. Winter wheat received 124 mm and 239 mm, while sorghum received 138 mm and 242 mm of irrigation water in 2019 and 2020, respectively.

2.4. Soil sampling and laboratory analysis

Soil samples were collected from 0 to 15 cm depth of all phases of crop rotation during summer (first week of June 2019 and 2020) (Fig. 1). The sampling time represented three different phases of fields after cover crop termination: at termination time (phase-I), 36 days after termination (phase-II), and a year after termination or the active wheat growth stage (phase-III). The fallow plots were considered as a control to compare changes in SOM components and other soil health indicators due to cover cropping. Three soil cores were collected diagonally from each plot using a core sampler (2 cm diam.), composited and thoroughly homogenized, and all visible plant materials (roots, stems, and leaves) and crop residues were removed by hand. Soil samples were transported to the laboratory, and approximately 20-g subsamples were used for soil moisture estimation. About 200-g subsamples were stored in a refrigerator at 4 °C for inorganic N, labile organic nitrogen (LON), 24-h-carbon dioxide-carbon (24-h-CO_2-C), 72-h-CO_2-C, and MBC estimation. The rest of the samples were air-dried for pH, SOC, total N, particulate OC and ON, mineral-associated organic C (MAOC) and N (MAON), wet aggregate stability (WAS), aggregate-associated SOC, and total N estimation. Approximately 10-g air-dried subsamples were ground to estimate SOC and total N.

In the laboratory, gravimetric soil moisture was estimated by oven drying 20-g field-moist soil samples at 105 °C for 24 h. Soil pH was determined on a 1:5 soil to water suspension using an electrode standardized against known buffer solutions. Soil inorganic N concentration was determined as a sum of nitrate (NO_3^-) and ammonium (NH_4^+) ions measured in an automated flow injection N analyzer (Timberline Instruments, LLC, Boulder, CO). For this, 5 g of soil was extracted with 25 mL of 1 M potassium chloride (KCL). The LON concentration was measured by boiling 5-g soil samples for 4 h with 25 mL 1 M KCL in a Pyrex glass tube in a 100 °C water bath. After boiling, the extract was filtered and NO_3^- and NH_4^+ were analyzed as inorganic N (Gianello and Bremner, 1986). The 24-h-CO_2-C and 72-h-CO_2-C concentrations were

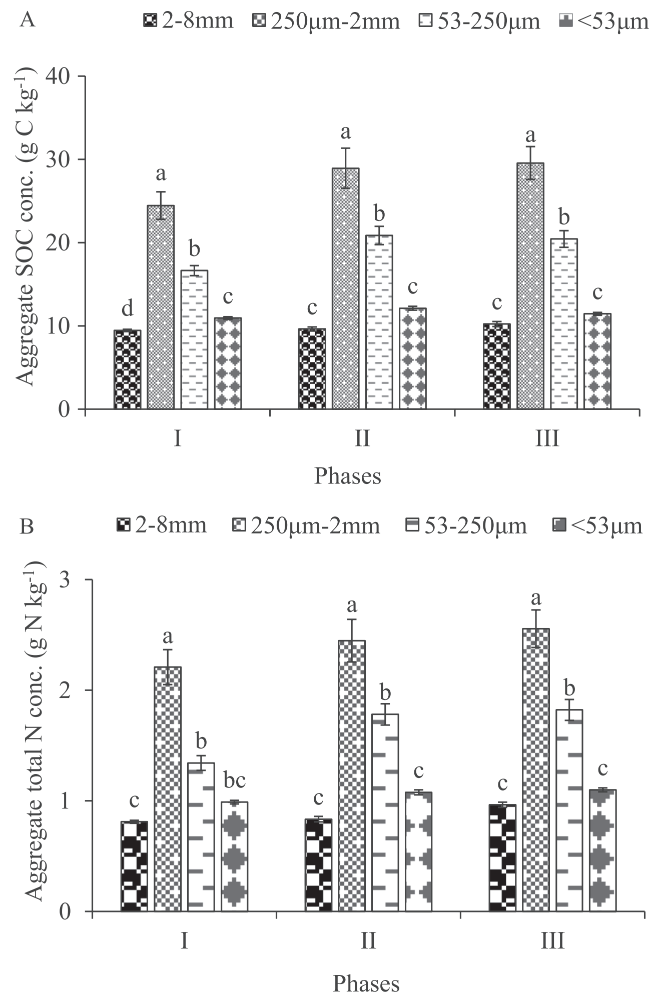

Fig. 2. Soil organic carbon (SOC) [A] and total N [B] concentrations among aggregate size classes within each phase in 2020. Different lowercase letters accompanied with standard error bars indicate a significant difference among aggregate size classes within each phase ($p = 0.05$, Fisher's protected least significant difference (LSD) test). Phase-I: at cover crop termination time; Phase-II: 36-days after cover crop termination; Phase-III: a year after cover crop termination (or during active wheat growth stage).

estimated by aerobic incubation of approximately 20-g soil samples at field capacity moisture (23% *v/v*) in 1-L mason jars (Zibilske, 1994) and measuring CO_2 released from incubation jars using an infrared gas analyzer (LI-COR Inc., Lincoln, NE). For soil MBC measurement, 10 g of soil samples were fumigated for 48 h with chloroform ($CHCl_3$) and then incubated for 72 h using the protocol for 72-h-CO_2-C estimation (Jenkinson and Powlson, 1976).

The SOC and total N were analyzed using a dry combustion method in a LECO C:N analyzer (LECO Corporation, St. Joseph, MI). The particulate OC and ON, MAOC, and MAON were determined following a procedure outlined by Cambardella and Elliott (1992). Briefly, 10-g air-dried subsamples were dispersed in 30 mL of 5 g L^{-1} sodium hexametaphosphate by shaking for 15 h on a reciprocal shaker. The dispersed soil samples were passed through a 53-µm sieve. The soil slurry that passed through the sieve containing mineral-associated and water-soluble C and N was dried at 50 °C overnight and analyzed for MAOC and MAON concentrations. The difference between SOC and total N values obtained from a non-dispersed bulk soil sample and those for MAOC and MAON was considered as Particulate OC and ON, respectively. The WAS was estimated using a Cornell Sprinkler Infiltrometer in which rainfall was simulated from 1 m height to the soil samples (2–4 mm) (Almajmaie et al., 2017). About 80 g of air-dried samples (<8 mm) were oscillated in a nest of sieves mounted on a Sieve Shaker (Gilson Company Inc., Lewis Center, OH) for 5 min to separate the soil into the following aggregate size classes: large macroaggregates (2–8 mm), small macroaggregates (250 µm–2 mm), microaggregates (53–250 µm), and a mineral fraction (<53 µm). The sand-corrected aggregate-associated SOC and total N for 250 µm–2 mm and 53–250 µm size classes were then analyzed as SOC and total N. Soil bulk density was determined by collecting four undisturbed soil cores (2.3 cm diam. × 15 cm depth) using a hand probe within each plot, oven drying soil samples at 105 °C for 24 h, and dividing the weight of the oven-dried sample by the volume of the core. The contents of all soil C and N fractions (kg ha^{-1}) were determined by multiplying their concentrations (ppm or mg kg^{-1}) by soil bulk density (g cm^{-3}) and the thickness of the soil layer.

2.5. *Statistical analysis*

Data for spring cover crop biomass, C:N ratio, ground cover and lignin (%), soil pH, SOC and N fractions, and WAS were analyzed using a MIXED model procedure in SAS (v 9.4, SAS Institute Inc., Cary, NC). These data were analyzed by phase to get a more robust estimate of the cover crop effects on selected SOM dfractions and soil health at different times after cover crop termination. Data from 2019 and 2020 were pooled to analyze by crop rotation phases. This analysis considered treatment as a fixed factor whereas replication and phase (replication) were random terms in the statistical model. The aggregate proportion and aggregate-associated SOC, and total N were determined from 2020 sampling only. In this analysis, treatment was considered a fixed factor, and replication was a random term to compare treatment differences within each aggregate size class. The comparisons of aggregate-associated SOC and total N concentrations among four aggregate size classes within each phase were also conducted using replication and replication × aggregate size as random terms in the model. A post hoc Fisher's protected least significant difference (LSD) was used to separate means when treatment and aggregate size effect were significant at $p = 0.05$ unless otherwise stated. The normality of residuals was checked for all the data using the Shapiro-Wilk test. The data that did not meet the normality criteria (inorganic N, 24-h-CO_2-C, 72-h-CO_2-C, SOC, particulate OC and ON, MAOC, and MAON) were log-transformed for statistical analysis, back-transformed means were reported in the results. Relationships among different soil health indicators were assessed using a Pearson correlation procedure (PROC CORR) in SAS at the system scale (without accounting phases). Simple linear regression analysis was performed on treatment averages obtained from each phase to predict MBC and WAS as a function of SOC and particulate OC. The principal component analysis was employed on soil properties, except aggregate-associated SOC and total N, using a correlation matrix structure in SAS to understand relationships among various soil C and N fractions and other soil health indicators. A minimum data set of soil health was selected from principal component analysis outputs following the criterion described in Rezaei et al. (2006). Briefly, only the principal components (PCs) with eigenvalues > 1 were considered for identifying the minimum data set. Within each PC, indicators receiving weighted loading values within 10% of the highest weighted loading were selected for the minimum data set. When more than one variable was retained within a PC, the linear correlation coefficient (r) was examined to determine if any variable could be considered to be redundant, and variables were eliminated if r was > 0.7.

3. Results

3.1. *Cover crop biomass and quality*

The spring and fall cover crop biomass productions were significantly different among cover crop treatments (Table 1). Oats, on average, produced the greater biomass as a spring cover crop. The oats biomass was 33, 54, and 76% higher than that of canola, PCmix, and

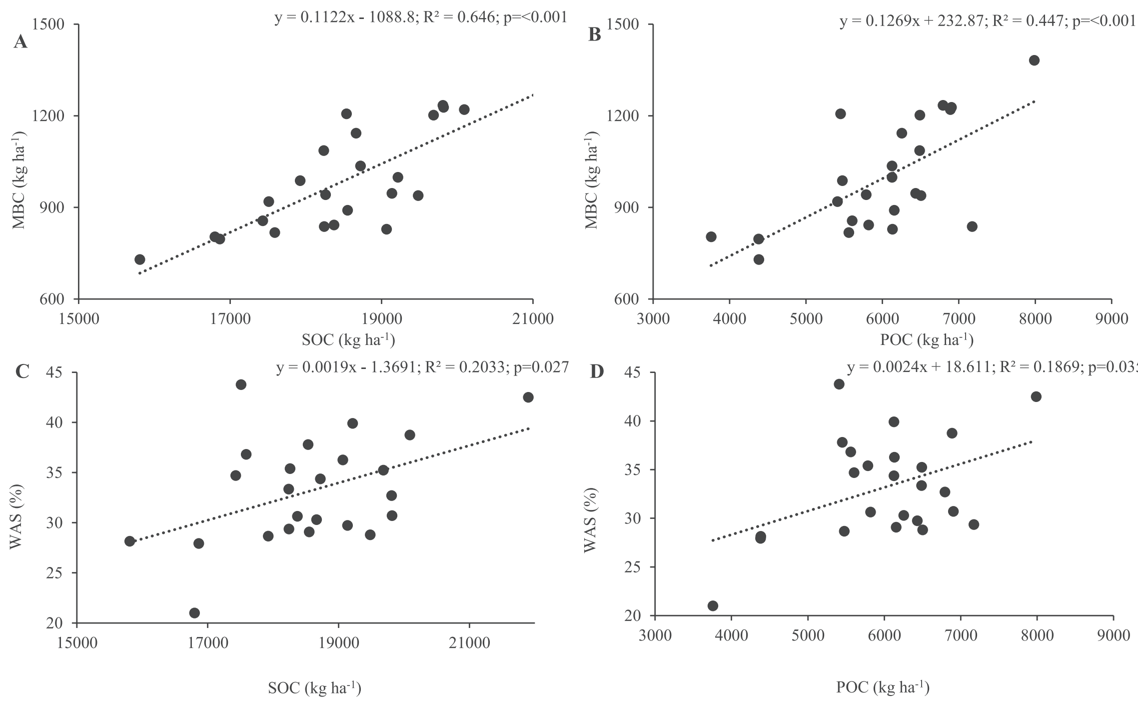

Fig. 3. Simple linear regression between soil organic carbon (SOC) and microbial biomass carbon (MBC) [A], particulate organic carbon (OC) and MBC [B], SOC and wet aggregate stability (WAS) [C], and particulate OC and WAS [D]. Each data point represents the treatment average obtained from each phase (8 × 3 = 24).

Table 5
Pearson correlation coefficients (r) among selected soil health indicators at 0–15 cm soil depth (n = 144).

Variable †	pH	Inorganic N	LON	24 h-CO2-C	72 h-CO2-C	MBC	SOC	Total N	particulate OC	Particulate ON	MAOC	MAON
Inorganic N	−0.42*											
LON	−0.36*	0.87*										
24 h-CO2-C	−0.52*	0.20*	0.10									
72 h-CO2-C	−0.49*	0.13	0.12	0.91*								
MBC	0.19*	−0.11	−0.13	0.09	0.19*							
SOC	−0.22*	0.01	0.09	0.40*	0.40*	0.16						
Total N	−0.01	−0.06	0.09	−0.09	−0.01	−0.01	0.74*					
Particulate OC	−0.16	−0.03	0.05	0.31*	0.34*	0.19*	0.84*	0.62*				
Particualte ON	0.04	−0.02	0.09	−0.23*	−0.14	−0.08	0.41*	0.73*	0.49*			
MAOC	−0.11	0.05	0.11	0.27*	0.26*	−0.01	0.63*	0.50*	0.18*	0.12		
MAON	−0.10	−0.10	−0.05	0.14	0.15	0.07	0.58*	0.69*	0.22*	0.06	0.68*	
WAS	0.12	−0.26*	−0.17*	−0.03	−0.02	0.30*	0.06	−0.04	0.09	−0.13	−0.07	−0.01

*Significant at p = 0.05
† Inorganic N: inorganic nitrogen; LON: labile organic nitrogen; 24 h-CO_2-C: 24 h-carbon dioxide-carbon; 72 h-CO_2-C: 72 h-carbon dioxide-carbon; MBC: microbial biomass carbon; SOC: soil organic carbon; Total N: total nitrogen; particualte OC: particulate organic carbon; particualte ON: particulate organic nitrogen; MAOC: mineral-associated organic carbon; MAON: mineral-associated organic nitrogen; and WAS: wet aggregate stability

pea, respectively. The cover crop mixtures with oats, i.e., SSmix, POmix, and POCmix, also produced more biomass than treatments without oats (Table 1). Similarly, SSmix biomass was 26, 46, and 68% higher than that of canola, PCmix, and pea, respectively. In fall cover crops, oats were mostly winter-killed, so biomass production was higher under PCmix, followed by canola and pea, and lower under oats than other treatments. Treatments such as oats, POmix, POCmix, and SSmix were not different from each other (Table 1).

Cover crop biomass samples varied in quality as indicated by C:N ratio and lignin content (%) (Table 1). Oats and its mixtures had higher C:N ratios than pea, canola, and PCmix (Table 1). The C:N ratio followed the trend as: oats (26.1) > SSmix (23.4) > POCmix (21.5) > POmix (21.2) > canola (13.5) > PCmix (11.8) > pea (10.8). The lignin (%) was 34–74% higher under pea and PCmix compared to other treatments (Table 1). The PCmix had the higher ground cover (%), which was similar to pea but significantly greater than other treatments.

3.2. Soil C and N components

Soil inorganic N content differed significantly among treatments in crop rotation phase-II. It was 37–56% greater under fallow than under oats, POmix, POCmix, and SSmix (Table 2). Also, the soil inorganic N content under pea was 45% higher than under POmix. The 24-h-CO_2-C content varied significantly among treatments in phase-I and phase-II. The PCmix had 48–73% higher 24-h-CO_2-C content than fallow, canola, and SSmix in phase-I, while in phase-II, POCmix and SSmix had 57–90% higher 24-h-CO_2-C content than fallow, pea, and POmix. The SOC and total N contents varied significantly among treatments in phase-I. The SOC and total N contents followed similar trends, with the higher SOC under oats than under other treatments. Soils under oats had 9.3–22% greater SOC content than pea, canola, POmix, and SSmix. Similarly, total N content in soils under oats was 10% and 22% higher than under SSmix and canola, respectively.

The particulate OC content, the labile fractions of SOC, differed

Table 6
Principal component (PC) loading matrix (factor loading) for selected soil health indicators.

Variables †	PC1	PC2	PC3	PC4	PC5
pH	−0.194	**0.418**	−0.035	0.023	0.190
Inorganic N	0.059	−0.424	0.400	0.088	0.332
LON	0.097	−0.347	**0.454**	0.127	0.358
24 h-CO_2-C	0.255	−0.383	−0.353	0.064	−0.163
72 h-CO_2-C	0.269	−0.346	−0.354	0.127	−0.126
MBC	0.079	0.099	−0.305	0.315	**0.607**
SOC	**0.475**	0.100	−0.027	0.080	−0.025
Total N	0.382	0.296	0.241	−0.073	−0.031
Particulate OC	0.373	0.124	0.008	0.443	−0.208
Particulate ON	0.229	0.284	0.375	0.320	−0.165
MAOC	0.358	0.013	−0.059	−0.458	0.238
MAON	0.338	0.139	−0.044	**−0.506**	0.195
WAS	−0.008	0.190	−0.291	0.282	0.386
Eigen values	4.014	2.639	2.009	1.377	1.049
Proportion	0.309	0.203	0.155	0.106	0.081
Cumulative	0.309	0.512	0.666	0.772	0.853

† Inorganic N: inorganic nitrogen; LON: labile organic nitrogen; 24 h-CO_2-C: 24 h-carbon dioxide-carbon; 72 h-CO_2-C: 72 h-carbon dioxide-carbon; MBC: microbial biomass carbon; SOC: soil organic carbon; Total N: total nitrogen; Particulate OC: particulate organic carbon; Particulate ON: particulate organic nitrogen; MAOC: mineral-associated organic carbon; MAON: mineral-associated organic nitrogen; and WAS: wet aggregate stability.

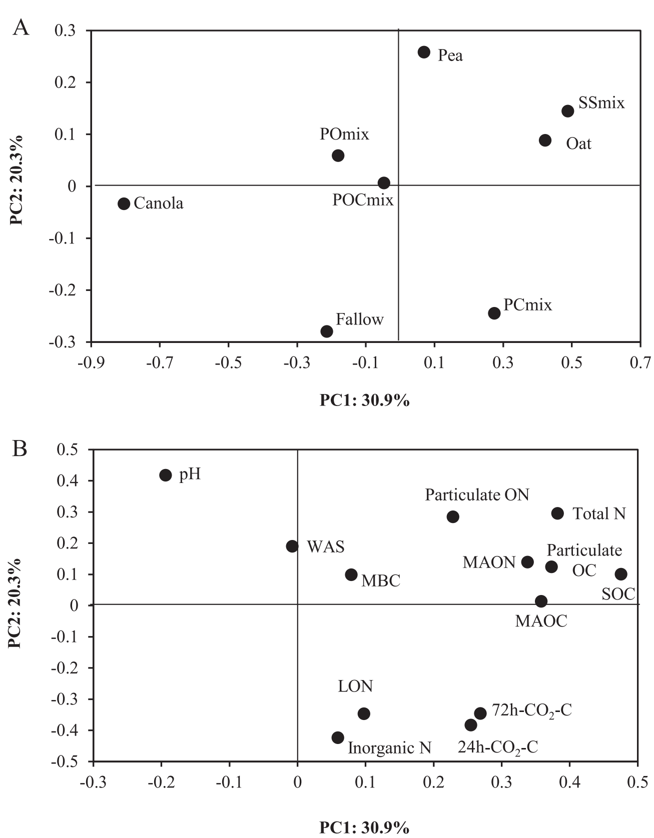

Fig 4. Principal component analysis of selected soil health indicators and their loading score. Each data point is a plot where PC1 and PC2 in pairs applied to the eigenvectors of the group (treatment) mean [A] and individual soil health indicators [B]. POmix: pea + oats; PCmix: pea + canola; POCmix: pea + oats + canola; SSmix: six-species mixture of pea + oats + canola + hairy vetch + forage radish + barley. Inorganic N: inorganic nitrogen; LON: labile organic nitrogen; 24 h-CO_2-C: 24 h-carbon dioxide-carbon; 72 h-CO_2-C: 72 h-carbon dioxide-carbon; MBC: microbial biomass carbon; SOC: soil organic carbon; Total N: total nitrogen; POC: particulate organic carbon; PON: particulate organic nitrogen; MAOC: mineral-associated organic carbon; MAON: mineral-associated organic nitrogen; and WAS: wet aggregate stability.

significantly among treatments 36 days after cover crop termination (phase-II). The particulate OC content under pea was similar to SSmix and POCmix, but significantly greater than under fallow. It was 61–69% greater under pea, SSmix, POCmix than under fallow (Table 2). Treatments such as oats, canola, POmix, and PCmix were not different from one another. The particulate ON, the easily decomposable fractions of N, MAOC, and MAON, did not vary among treatments in all phases.

3.3. *Aggregate size distribution, soil carbon and nitrogen in aggregates, and wet aggregate stability*

Among various aggregate size fractions, cover cropping affected the 2–8 mm size fraction in phase-II. The PCmix had the highest proportion of these aggregates, which was similar to fallow and oats but significantly greater than other treatments (Table 3). Treatments such as pea, canola, POmix, POCmix, and SSmix were not different from one another. Cover cropping affected both aggregate-associated SOC and total N concentrations in the 2–8 mm size in phase-I, but only aggregate-associated SOC concentration for the same size in phase-III. The PCmix and oats exhibited a higher aggregate-associated SOC concentration than other treatments (Table 3). Similarly, POmix and PCmix had a higher aggregate-associated total N concentration than other treatments in phase-I. In phase-III, SSmix had the higher aggregate-associated SOC concentration compared to other treatments for the 2–8 mm size.

The aggregate-associated SOC and total N stocks for the 53–250 μm and < 53 μm sizes significantly varied among treatments in phase-II (Table 4). The aggregate-associated SOC and total N stocks for the 53–250 μm and < 53 μm sizes followed a similar trend, with the higher levels under pea than other treatments. Specifically, the aggregate-associated SOC and total N stocks for the 53–250 μm size were 65–142% and 65–154% higher under pea than under fallow, oats, POmix, PCmix, POCmix, and SSmix, respectively (Table 4). The aggregate-associated SOC and total N stocks for the < 53 μm size were 50–61% and 45–66% greater under pea and PCmix than under fallow and POmix, respectively (Table 4). In phase-III, the treatment effect was observed only in aggregate-associated SOC stocks for the 2–8 mm size; it was significantly greater under SSmix than other treatments (Table 4).

The aggregate-associated SOC and total N concentrations also varied among aggregate size classes within each phase. The highest amount of SOC was accumulated in the 250 μm–2 mm size, followed by 53–250 μm, which was significantly greater than in the 2–8 mm and < 53 μm size classes in all phases (Fig. 2). The aggregate-associated SOC and total N concentrations between 2 - 8 mm and < 53 μm sizes were not significantly different, except for total SOC concentration in phase-I (Fig. 2).

The WAS varied significantly among treatments in phase-I and phase-III. The SSmix had the highest WAS (43.8%), which was similar to oats (39.9%) but significantly greater than fallow (29.4%) and canola (28.2%) in phase-I. Treatments such as pea, POmix, PCmix, and POCmix remained intermediate of fallow, canola, oats, and SSmix. In phase-III, the WAS under SSmix was significantly higher than under fallow, pea, canola, PCmix, and POCmix (Table 2).

3.4. *Linear regression, correlation, and principal component analyses*

Regression analysis performed on treatment averages obtained from each phase showed increased MBC and WAS with SOC and particulate OC contents (Fig. 3). The Pearson correlation analysis showed that most of the SOC and N components were positively correlated with total SOC and N (Table 5). Several labile C and N fractions were significantly correlated to each other, except for a negative correlation between 24-h-CO_2-C and Particulate ON (Table 5).

Considering principal components receiving eigenvalues > 1, about 86% of the variation among selected soil health indicators was explained by the first five principal components for minimum data set selection

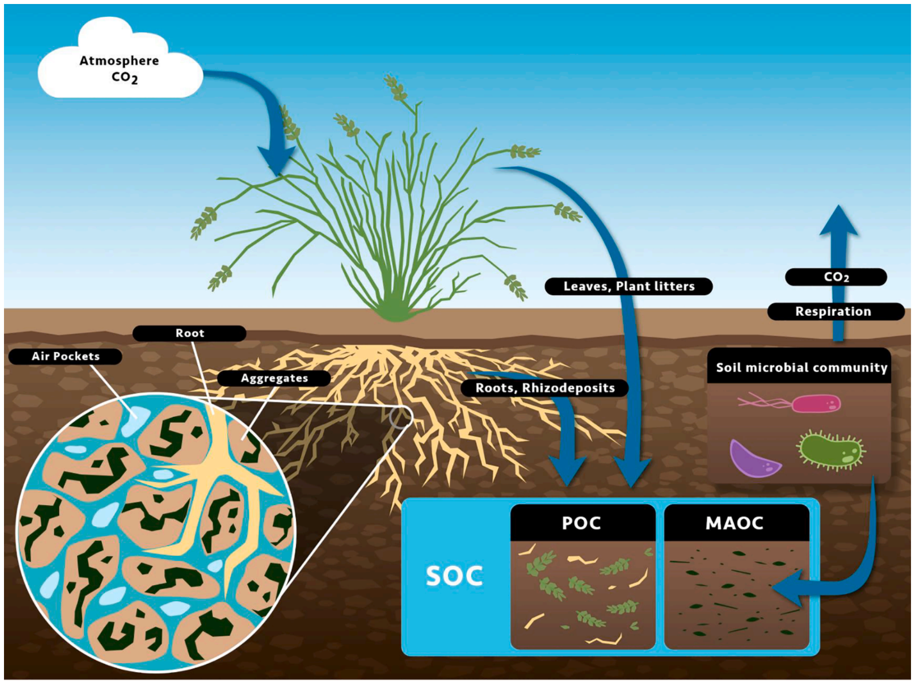

Fig 5. Conceptual diagram illustrating soil organic carbon components and dynamics under cover crop-integrated cropping system. SOC: soil organic carbon, POC: particulate organic carbon, and MAOC: mineral-associated organic carbon.

(Table 6). The first, third, fourth, and fifth principal components had only one highly weighted variable within 10% of the highest factor loading; they were SOC, LON, MAON, and MBC, respectively (Table 6). For the second principal component, inorganic N and pH were within 10% of the highest factor loading. The inorganic N was eliminated because it was highly correlated with LON ($r = 0.87$; Table 5). Therefore, the indicators selected for the minimum data set comprised SOC, pH, LON, MAON, and MBC. The first two principal components were graphed to see the relative response of various soil health indicators.

The first two principal components [PC1 and PC2] explained about 51% of the variation among selected soil health indicators (Fig. 4). The PC1 represented 30.9% of the variation and was mainly associated with cover crop biomass addition, while PC2 represented 20.3% of the variation and was mainly related to species diversity for nutrient pool factors. When plotting PC1 and PC2 in pairs applied to the eigenvectors of the group (treatment) means, there was a clear distinction between fallow and cover crop treatments (Fig. 4a). Among cover crops, canola showed a clear separation from other cover crops. The response of SSmix and oats as cover crops varied along PC1 while the response of pea and PCmix varied along PC2 (Fig. 4a). The magnitude of differences between POmix and POCmix was relatively small. All the C and N components had a positive loading, while soil pH had a negative loading along PC1 (Fig. 4b). Along PC2, total N, particulate OC and ON, MAON, soil pH, WAS, MBC, and SOC had a positive loading, whereas inorganic N, LON, 24-h-CO_2-C, and 72-h-CO_2-C had a negative loading (Fig. 4b). The loading on WAS along PC1 and MAOC along PC2 was close to zero.

4. Discussion

4.1. Cover crops played a crucial role in SOC and N accumulation

Organic residue addition through cover cropping often increases SOC and N storage (Jian et al., 2020; Mazzoncini et al., 2011). This study evaluated SOC and N components in the bulk soil, soil aggregate size distribution and stability, and SOC and total N distribution within aggregate size classes to assess soil health with the introduction of partial fallow replacement cover crops in a limited irrigation winter wheat – sorghum – fallow. It appears that oats, with higher biomass production than other cover crops, increased SOC and N accumulation, while combinations of oats with other cover crops improved the overall soil health. Specifically, higher SOC and total N contents in soils under oats than other cover crops at phase-I was likely related to the biomass C addition. The aboveground spring cover crop biomass was greater with oats than with pea, canola, and PCmix (Table 1). Grass cover crops often have dense root systems and produce more root biomass, contributing to greater root-derived C in the soil (Amsili and Kaye, 2020). The C:N ratios of spring pea, oats, and canola biomass samples at the time of termination were 10.8, 26.1, and 13.5, respectively (Table 1). Higher C:N ratio of oats compared to pea and canola may have slowed down the mineralization rate of the residue, thereby increasing SOC and total N contents. Previous studies also showed that SOC and total N are usually greater with non-legumes than legume or brassica cover crops (Sainju et al., 2003; Ghimire et al., 2019).

The particulate OC, a relatively undecomposed fraction of SOC, was higher under pea, SSmix, and POCmix than other treatments at phase-II. Lower C:N ratio of pea and canola compared to oats may have favored decomposition, increasing particulate OC content within a month of cover crop termination. Treatments containing pea and canola in the mixture, i.e., SSmix and POCmix had greater particulate OC contents. The lack of cover crop C inputs under fallow resulted in the lower particulate organic C contents. Fallow plots also had more N at the time of cover crop termination, which accelerates the rate of residue decomposition lowering particulate OC concentration. In line with this, Bradford et al. (2008) observed lower particulate OC levels in mesocosms with N addition. The MAOC and MAON contents did not differ among treatments in all phases (Table 2). The MAOC and MAON indicate more processed fractions of SOM. It may take more than ten years to produce detectable changes in slow-cycling SOM components such as MAOC and MAON (Conant et al., 2011).

There is a direct relationship between C inputs from residues and C accumulation, soil aggregation, and SOC stabilization in aggregates (Kong et al., 2005). It appears greater amounts of SOC and particulate organic C in the soil influence the availability of C to soil microbes, which in turn increases the proportions of water-stable aggregates

(Fig. 5). The observation of greater WAS under oats supported our hypothesis of the positive responses of biomass addition from grass cover crops on SOC accumulation and soil aggregation. The significant positive linear relationship of SOC and particulate organic C with MBC and WAS further confirmed the crucial role SOC could play in the formation of stable aggregates. Grass cover crops produced greater root biomass and root length density than legume cover crops (Amsili and Kaye, 2020); the increased enmeshing action of oat roots under no-tillage may have also improved soil aggregation (e.g. Fig. 5). The mixture of legumes, grasses, and brassica cover crops with SSmix diversified microbial substrate availability supported diverse microbial communities, which in turn produced a variety of organic polymers that bind soil particles to form stable aggregates (Zhang et al., 2012). A previous study at the same site also reported higher fungal biomass with oats and SSmix as cover crops (Thapa et al., 2021). Higher fungal abundance also supports higher soil aggregation (Lehmann et al., 2020).

The greater soil inorganic N content observed under fallow than under cover crop treatments at cover crop termination was due to the absence of plants for N uptake in fallow plots. However, after 36 days of cover crop termination (phase-II), pea had higher soil inorganic N content among cover crops suggesting rapid recycling of N taken up by legume cover crops. Legume residues, such as pea with a low C:N ratio, decomposed more rapidly and increased soil inorganic N compared to non-legume residues (Soon and Arshad, 2002). In addition, soils under pea could have increased inorganic N content through atmospheric N fixation. Studies suggest up to 20 kg ha^{-1}N addition with legume cover crops in the semiarid environment (Blackshaw et al., 2010). We did not observe high nodulation in legumes used in this study, but they certainly contributed to N addition. Cover crop treatments with a high C:N ratio, however, support more SOC accumulation (Shahbaz et al., 2017; Ghimire et al., 2017). The greater 24-h-CO_2-C content with PCmix than other treatments at phase-I was associated with increased C inputs from cover crop residue because the fall biomass was greater with this cover crop than other treatments. On average, PCmix produced greater biomass than other cover crops in fall cover cropping (Table 1). The POCmix and SSmix had greater 24-h-CO_2-C content than other treatments at phase-II, possibly delaying decomposition due to the presence of oat residues with a higher C:N ratio.

4.2. Highest soil organic matter storage in intermediate-sized aggregates

The observation of greater SOC and total N concentrations in 250 μm–2 mm and 53–250 μm than in 2–8 mm and < 53 μm aggregates suggests that C and N sequestration through cover cropping may occur mostly in intermediate-sized aggregates than in large aggregates or the organo-mineral complex of soils. Microaggregates typically form around organic debris (Six et al., 1998; Cambardella and Elliott, 1993), protecting organic matter from decomposition. Previous studies have also reported higher SOC and N accumulation in intermediate-sized aggregates (Zhang et al., 2012; Sainju et al., 2003; Mendes et al., 1999). The lower aggregate-associated SOC and total N concentrations under canola in 2–8 mm size aggregates at phase-I was possibly due to lower biomass inputs with this cover crop (Table 1). The rhizosphere of living canola roots releases a fumigant-like compound (*2-phenylethyl isothiocyantae*), which could also affect soil microbial communities related to SOM formation and aggregation (Rumberger and Marschner, 2003). While glucosinolates produced by brassica roots improve nutrient availability and disease suppression (Mazzola and Mullinix, 2005), their role on SOM and soil aggregation has not been documented yet. The highest aggregate-associated SOC and total N stocks under pea in 53–250 μm size and under pea and PCmix in < 53 μm size at phase-II also suggest a role of particulate organic matter on soil aggregation. Higher-quality residues such as pea, either individually or in mixtures, increase residue decomposition (Soon and Arshad, 2002), ultimately increasing aggregate associated as well as mineral-associated SOM formation (Six et al., 1998; Cotrufo et al., 2013).

4.3. Permanence of cover crop derived carbon in a hot, dry environment

Despite several benefits of cover crops at phase-I and phase-II, there were no significant differences among treatments on SOM components at Phase-III, suggesting that labile SOC and N accumulated due to cover cropping was short-lived in this hot, dry climate. High soil moisture and temperature in the summer may have created the ideal condition for the rapid mineralization of SOM. A previous study estimated the need for at least 5 Mg ha^{-1} of cover crop residue to maintain SOC stocks in the semiarid SHP region (Ghimire et al., 2017). However, annual cover crop biomass input, on average, was only 2.1 Mg ha^{-1} (Table 1), and the cash crop residue input was not significantly different between cover crops and fallow plots. In line with this, Blanco-Canqui et al. (2013) also reported no effects on soil properties nine months after cover crop termination in the semiarid climate of southwest Kansas. These results suggest cover crops should be grown in each fallow phase in crop-fallow rotations for several years to increase SOC accumulation and improve soil health.

4.4. Minimum data set of soil health indicators for water-limited environments

A minimum data is desirable to keep the cost of soil health assessment manageable for farmers and landowners. Out of 13 soil health indicators assessed in this study, SOC, pH, LON, MAON, and MBC represented the minimum data set of soil health. The minimum data set identified in this study relates to major agroecosystem functions. The SOC is critical for the stabilization of soil structure, retention and release of plant nutrients, and soil water storage (Villarino et al., 2019). The SOC also serves as a source of energy for microbial proliferation. Diverse microbial communities enhance the resilience of cropping systems by help in the formation and stabilization of diverse organic compounds, contributing to SOC sequestration. Newly added C through cover cropping can be stabilized in the soil mainly via three mechanisms: (i) physically via isolating inside soil micro- and macroaggregates, (ii) chemically via strong bonding with silt and clay particles (organo-mineral clusters), and (iii) bio-chemically via re-synthesizing into recalcitrant SOM compounds (Fig. 5). The mineral-associated organic matter is relatively stable, protected from decomposition through association with soil mineral surface, and thus persist for much longer periods (Kögel-Knabner et al., 2008). Compared to particulate organic matter, mineral-associated organic matter has more microbial-derived compounds (Six et al., 2002; Sanderman et al., 2014; Kögel-Knabner et al., 2008), suggesting more microbially processed product, which stabilizes SOC in the profile for many years. In addition, microflora such as fungi via hyphal network and production of a broad range of organic polymers help bind soil aggregates, improving structural stability while reducing soil erosion potential. Most of the mineralized N in soils is also considered to be derived from the LON, a microbially derived organic N, due to its rapid turnover rates (Ros et al., 2011). Soil pH indicates acidity or alkalinity of soil that affects solubility and availability of minerals or nutrients and the activity of soil microorganisms. Therefore, the indicators included in the minimum data set represented major soil functions related to soil water storage, nutrient availability and crop productivity, and SOM sequestration, a foundation for resilient and productive agroecosystems. However, the minimum data set developed for one region may not be applicable for other regions because soil health responses vary between soil type, climate, crop, and soil management practices (Lehman et al., 2015). Comprehensive assessment of soil health at a regional and global scale will help in identifying universal and region-specific indicators for soil health assessment and management. The minimum data set we identified in this study could be applicable to water-limited environments such as semiarid SHP and similar other agroecosystems across the world.

5. Conclusions

This study revealed the benefits of cover cropping to increase SOM components and overall soil health under limited irrigation winter wheat-sorghum-fallow rotation. The principal component analysis identified SOC, pH, LON, MAON, and MBC as a minimum data set of indicators to assess soil health in semiarid croplands. Oats and their mixture with other species had higher biomass as a spring cover crop compared to pea, canola, and PCmix. Pea as a cover crop, either individually or in mixtures, had higher inorganic N and particulate OC, and aggregate-associated SOC and total N stocks in 53–250 μm and < 53 μm sizes, highlighting the role of high-quality residues in improving N availability, particulate and aggregate associated SOC formation. The greater particulate organic C under SSmix and POCmix indicates that a mixture of legumes, grasses, and brassicas as cover crops diversifies substrate availability and supports higher microbial activity and nutrient turnover. Overall, SSmix and oats had higher WAS than fallow. The SOC and total N sequestration occurred particularly in intermediate-sized aggregates (250 μm–2 mm and 53–250 μm) in this soil. Although not all treatment comparisons were significant in all phases, this study highlights the potential of integrating cover crops in crop rotations for improving soil health and resilience of cropping systems in semiarid regions. Mixture of species with higher biomass and C:N ratio, such as oats with legume and brassica, could diversify substrate availability and quality and improve overall soil health and resilience.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by project No. 2016-68007-25066 of the USDA National Institute for Food and Agriculture's Agriculture and Food Research Initiative, with additional funding from the USDA Natural Resources Conservation Service, New Mexico. The study plots were established with funding from the New Mexico State University Agricultural Experiment Station. We appreciate Frank Sholedice III for help in editing this manuscript and Evan Evans and NMSU Innovative Media Research & Extension for graphic image.

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