COVER CROP COMPOSITION IN LONG-TERM NO-TILL SOILS IN SEMI-ARID ENVIRONMENTS DO NOT INFLUENCE SOIL HEALTH MEASUREMENTS

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CORE IDEAS

- Effect of including a grass, broadleaf, and grass/broadleaf cover crop (CC) mixture on soil health was evaluated.
- All CC mixtures did not affect soil health measurements.
- Long-term no-till (> 10 yr), and high organic matter (mean = 41 g kg^{-1}) may explain lack of CC effect.

ABSTRACT

Evaluating the influence of grass or broadleaf cover crops on soil health measurements is common in the U.S Midwest. However, the comparison among different cover crop mixtures, including blends of both grass and broadleaf species is limited. Eleven cover crop experiments were conducted in South Dakota from 2018-2020. Cover crops were planted in the fall after small grains harvest as mixtures of dominantly grasses or broadleaves, a 50/50 grass/broadleaf mixture, and a no cover crop control. Soil and plant surface residue samples were collected in the fall before winter kill and in the spring before cover crop termination and corn planting. Soil samples were analyzed for permanganate oxidizable carbon (POXC), potentially mineralizable nitrogen (PMN), and soil respiration. Cover crops regardless of composition compared to the no cover crop control did not affect fall or spring cover crop/previous crop residue biomass in 7 of the 11 site-years, suggesting growing cover crops may accelerate decomposition of previous crop residue. Cover crops did not improve soil health measurements compared to the no cover crop control or were there differences among cover crop mixtures. Weather and soil properties (precipitation, soil organic matter, and pH) were related to differences in soil heath measurements among site-years. In the first year of planting a multi-species mixture of grasses and/or broadleaves after small grain harvest, growers should not expect to find differences in soil health measurements. Long-term trials are needed to determine whether these different cover crop mixtures over time result in changes in soil health.

Abbreviations: POXC, permanganate oxidizable carbon; PMN, potentially mineralizable nitrogen

INTRODUCTION

Planting cover crops increases crop and soil resistance to adverse weather conditions such as drought, hard rain events that cause erosion, and problematic weeds (Blanco-Canqui et al., 2015; Blanco-Canqui & Ruis, 2020; CTIC, 2017; Koudahe et al., 2022; Rorick & Kladivko, 2017). Cover crops can also increase resistance to wheel traffic compaction and improve aggregate stability (Blanco-Canqui & Ruis, 2020; Chen & Weil, 2010; A. Clark, 2012; Koudahe et al., 2022). The US has seen a 50% increase in the farmland planted with cover crops from 2012 to 2017 (Wallander et al., 2021). Specifically, during this period in South Dakota, cover crop acres increased by 89% (Bly, 2020). These cover crops are grown with the goal of improving our soils, taking up excess soil water to improve timeliness of spring crop planting, and reducing potential negative environmental effects from erosion and nutrient losses (Basche et al., 2016; Khan & McVay, 2019).

In the northern corn producing regions of the US, cover crops can be most easily added to crop rotations that include cereals due to the sufficient growing season that remains after cereal grain harvest for the cover crop to establish and increase in biomass. This longer growing season is important as cover crop biomass has been directly related to being able to identify significant changes in soil properties such as microbial biomass C, N, and C:N ratios, soil C stocks, and soil enzymes (Blanco-Canqui et al., 2015; Blanco-Canqui & Ruis, 2020; Calderón et al., 2016; Strickland et al., 2019; Tollenaar et al., 1993). Since there are many different species of cover crops to choose from, careful consideration must go into planning the best cover crop(s) to plant to achieve on-farm goals.

Cover crops can be generally categorized into two main categories: broadleaves and grasses (CTIC, 2017; Rorick & Kladivko, 2017; Snapp et al., 2005). Broadleaf species can be divided into two major categories–brassicas and legumes. Some brassica species can reduce compaction with their taproot and release compounds that suppress plant-parasitic nematodes (Gruver et al., 2010; Snapp et al., 2005). Legumes as cover crops generally have lower C:N ratio tissues compared to nonlegume cover crops, resulting in faster decomposition rates. (Gentry et al., 2013; Md Khudzari et al., 2016; Parr et al., 2011). The ability to predict the amount and timing of nutrients released from decomposing legumes is difficult as weather, soil, and management practices influence this process (Beyaert & Voroney, 2011; Kuzyakova et al., 2006; Mikha et al., 2006). However, many studies have shown that incorporating legumes into the rotation can reduce the amount of N fertilizer required to achieve optimal crop yields (Alvarez et al., 2017; A. J. Clark et al., 1997; Gentry et al., 2013; Herridge et al., 1990; Odhiambo & Bomke, 2001; Parr et al., 2011; Ranells & Wagger, 1996; Snapp et al., 2005; Yang et al., 2019).

Grass cover crop typically have the highest C:N ratio tissue, which slows down decomposition, but they do have a fibrous root system, are excellent nutrient scavengers, and leave a thick mulch after termination that helps build soil organic matter (Basche et al., 2016; Kaspar et al., 2007; Koudahe et al., 2022; Sullivan et al., 1991). Grass cover crops can also improve soil aggregate stability, soil organic matter, water infiltration rates, and decrease soil compaction with their deep penetrating fibrous root systems (Blanco-Canqui & Jasa, 2019; A. Clark, 2012; Snapp et al., 2005).

In a review of the literature (Blanco-Canqui & Ruis, 2020) reported that the cover crop species that best reduced penetration resistance and increased wet aggregate stability were in the order of legumes, grasses, and then brassicas. Of these three categories, legumes had the least impact on water infiltration. In relation to biological properties a study in Tennessee showed that planting a legume (hairy vetch; *Vicia villosa* Roth) compared to a grass (winter wheat; *Triticum* *aestivum* L.), resulted in greater enzyme activities of acidphosphatase, arylsulfatase, β-glucosidase, and L-asparaginase (Mullen et al., 1998). In comparing the effect of including a cover crop against no cover crop it was found that radishes (*Raphanus sativus*) increased permanganate oxidizable C (POXC) (Wang et al., 2017), rape (Brassica napus) increased soil respiration rates (Sanz-Cobena et al., 2014), cereal rye (*Secale cereale*) increased particulate organic matter and PMN (Moore et al., 2014; R. Norris et al., 2018), and barley (*Hordeum vulgare*) enhanced soil respiration (Sanz-Cobena et al., 2014). The different effects of these broadleaf and grass cover crops on soil properties is hypothesized to be related to root type and structure (Blanco-Canqui & Ruis, 2020; Nichols et al., 2022). Legumes and grasses have extensive fibrous root systems interacting with large volumes of soil, while brassicas generally have tap roots (tuber forming species) that interact will lower volumes of soil.

The importance of roots in improving soil properties was further emphasized in a greenhouse study using soil from a corn-soybean rotation in Nebraska. It was determined that compared to a single cover crop specie that greater cover crop species diversity increased below ground root coverage and subsequently improved soil organic matter and C, meso- and micro-aggregates, and nutrient availability (Saleem et al., 2020). Additionally, planting cover crop mixtures of grasses and legumes can result in consistently greater biomass as was found with a hairy vetch and cereal rye biculture cover crop mixture compared to only hairy vetch or cereal rye (Khan & McVay, 2019; Sainju et al., 2005). Planting a grass and broadleaf blend as a cover crop is likely an excellent option to gain the soil benefits associated with grass and broadleaf cover crops. Another option for increasing plant diversity is by including multiple grass and/or broadleaf species in the cover crop mixture. This species diversity within grasses and broadleaves would likely increase the soil area explored by roots and the consistency of cover crop biomass produced from year-to-year, increasing the likelihood of cover crops improving soil properties. Consistent biomass could be produced by the individuals species in each grass and/or broadleaf mixtures that are most adapted to that years soil and weather conditions to flourish while other species may not grow as well (Khan & McVay, 2019). However, the effect of multiple grass and/or broadleaf species on soil properties is unknown. Therefore, the objective of this research was to determine the effect of cover crop mixtures composed of multiple grass and/or broadleaf species compared to a no cover crop control on surface residue biomass and soil properties.

MATERIALS AND METHODS

This study was conducted on commercial farms in eastern and central South Dakota from the fall of 2017 to the fall of 2020 on 11 site-years with varying soil types (Table 1). The experiment was conducted as a randomized complete block design with four treatments replicated four times. The four cover crop treatments were: 1) dominantly grass mixture, 2) dominantly broadleaf mixture, 3) a 50/50 blend of grass and broadleaf species, and 4) a control (no cover crop). Each cover crop plot size was 7.5 m in length and 4.5 m in width. The dominantly grass mixture included 22.5% oats (*Avena Sativa*), 22.5% barley (*Hordeum vulgare*), 22.5% foxtail millet (*Setaria italica*), 22.5% sorghum-sudan grass (*Sorghum x drummondii*), 2.5% radish (*Raphanus sativus*), 2.5% turnip (*Brassica rapa subsp. Rapa*), 2.5% pea (*Pisum sativum*), and 2.5% lentil (*Lens culinaris*). The dominantly broadleaf mixture included 2.5% oats, 2.5% barley, 2.5% foxtail millet, 2.5% sorghum-sudan grass, 22.5% radish, 22.5% turnip, 22.5% pea, and 22.5% lentil. The 50/50 blend mixture included 12.5% of all the previously mentioned cultivars resulting in an equal quantity of grasses and broadleaf species planted.

Cover crops were planted in early to mid-August using a no-till drill after the fall harvest of winter wheat except in four site-years where cover crops followed oats (Salem 2018, Salem 2019, Beresford 2018, and Beresford 2019). The cover crops were either cold terminated during the winter months or chemically terminated in the spring 1 wk before planting. Precipitation and air temperature data were retrieved from the nearest South Dakota State University Mesonet weather station or National Weather Service station.

Table 1. Geographic location and mean values of various soil characteristics measured from fall soil samples of 11 site-years.

Site-year	**Geographic coordinates**	**Years in no-till**	**NO3-N 0-15 cm**	**NO3-N 15-60 cm**	**P**	**K**	**SOM[a]**	**pH**
		years	mg kg^{-1}				g kg^{-1}	
Beresford-2018	43°3'8.88"N 96°53'36.04"W	6	1.8	1.2	18	317	47	5.7
Garretson-2018	43°38'47.60"N 96°28'58.75"W	26	1.9	2	7	211	43	6.4
Gettysburg-2018	44°56'41.97"N 100°1'22.26"W	29	4.7	4.7	12	625	42	6.3
Salem-2018	43°44'33.75"N 97°18'0.09"W	25	7.6	6.5	19	211	45	5.8
Salem-2019	43°43'4293"N 97°18'30.36"W	26	1.7	1.7	39	254	40	6.8
Beresford-2020	43°2'24.73"N 96°53'58.29"W	7	0.8	0.4	8	205	42	6.3
Blunt-2020	44°21'12.15"N 100°0'25.99"W	20	4.2	2.8	9	551	40	6.8
Henry-2020	44°54'43.48"N 97°34'33.39"W	1	5.45	4.6	14	146	40	6.1
Mitchell-2020	43°45'1.92"N 98°7'32.94"W	28	12.8	7.2	13	314	44	6.9
Pierre-2020	44°14'24.56"N 99°59'36.09"W	30	3.5	1.9	16	490	31	6.6
Plankinton-2020	43°48'12.82"N 98°30'51.95"W	16	3	2.1	13	274	36	6.2

Note: Soil measurements are taken at the 0–15 cm depth unless noted.
[a]SOM, soil organic matter.

Plant Sampling

Surface plant tissue samples that included previous crop and cover crop residue was obtained to determine the effect of cover crops on previous crop residue biomass. These samples were collected from each cover crop treatment plot in the fall before cover crop winter kill and in the spring before chemical termination of any surviving cover crops before corn planting within two 30.5 cm^2 areas. Fall sampling dates occurred between late September and early November (depending on the first freezing event), and spring sampling took place in mid-May.

Soil Sampling and Analysis

A 12-core composite soil sample (i.d. 1.9 cm) was collected from each cover crop treatment replication in the spring one week before planting from a depth of 0 to 15 cm and 15 to 60 cm using a soil probe with an inside diameter of 1.9 cm. Soil samples were air-dried and ground to pass through a 2 mm sieve. These soil samples were analyzed for general soil fertility measurements (NO_3-N, P, K, soil organic matter (SOM), and pH) following the recommended chemical soil test procedures for the North Central Region (Nathan et al., 2015) (Table 1). Soil NO_3–N was analyzed at the 0 to 15 and 15 to 60 cm depths while all others were analyzed only at the 0 to 15 cm depth.

The 0 to 15 cm depth of the fall and spring soil samples were also analyzed for three universally recognized soil health indicators: permanganate oxidizable carbon (POXC), potentially mineralizable nitrogen (PMN), and soil respiration (Stott, 2019). These soil health measurements were chosen to focus on in this study to help us understand how carbon and nitrogen cycle through the agroecosystem under the influence of soil microbial communities. These tests have also been able to show differences faster due to changes in management practices and are relatively inexpensive to run (Aislabie & Julie R. Deslippe, 2018; Chu et al., 2019; Culman et al., 2012; Hurisso et al., 2016; Moebius-Clune et al., 2016; C. E. Norris et al., 2020).

The POXC test was done using the protocol adopted by the Cornell Soil Health Laboratory (Moebius-Clune et al., 2016) that is based on methods from (Weil et al., 2003) with minor changes described in (Culman et al., 2012). Briefly, 2.5 g of air-dried soil was placed into plastic centrifuge tubes, and 2.0 ml of 0.2 M $KMnO_4$ was added to the soil. Next, 18.0 mL of deionized water was added to the soil and put on a rotary shaker at high speed for two minutes. After shaking, the soil settled for 10 minutes. Using a pipette, 0.5 mL of the supernatant was transferred into a 50 mL plastic centrifuge tube containing 49.5 mL of deionized water. Finally, the supernatant absorbance was read directly in this centrifuge tube using a Brinkman PC 800 colorimeter spectrophotometer at 550 nm. Four standard concentrations of 0.005, 0.01, 0.015, and 0.02 M $KMnO_4$ with two controls and blanks were also used. The POXC measurement was then calculated using the intercept of the standard curves created with the standard concentration test tubes to get the total POXC concentration.

The PMN test was done using the protocol adopted for the Cornell Soil Health Laboratory (Moebius-Clune et al., 2016) based on (Drinkwater et al., 1996), while the microplate assay for colorimetric ammonium determination protocol was followed as described in (Rhine et al., 1998). Two replicates were measured out, in which one had a zero-day incubation period, and the other was incubated for seven days. The 7-day incubation replicates were completed by adding 10 mL of deionized water to the soil in a plastic centrifuge tube and incubated at 40° C for seven days. Next, 30 mL of 2.0 M KCl solution was pipetted into the plastic centrifuge tube. Next, these samples were placed on the rotary shaker for one hour and centrifuged for 10 minutes at 1500 RPMs. Finally, approximately 20 mL of the extract was poured through round filter paper into tubes. For ammonium-N determination, 50 µL of the soil extract was pipetted into 96 deep well microplates in replications of three deep wells per soil sample extract. Next, 50 µL of the citrate reagent was added and allowed to react for at least one minute. Then, 50 µL of the PPS-nitroprusside reagent was added to the wells. Finally, 25 µL of the buffered hypochlorite reagent was added to each of the wells. When it was time for the solution to start reacting, 100 µL of deionized water was added to each of the wells, covered with a thin plastic film, vortexed with a Thermo Scientific high-speed vortex, and let sit for 45 minutes undisturbed to complete color development. Two blank and 0, 2, 5, 10, 25, and 50 mg NH_4-N L^{-1} concentration standards were also prepared for comparison. After the 45-min. incubation period was complete, the micro assays were read with a Biotek Epoch spectrophotometer at 660 nm absorbance level.

In the zero-day replicate, eight g of air-dried soil was measured into a plastic centrifuge tube, and 40 mL of 2.0 M KCl solution was pipetted into the plastic centrifuge tube. Next, these samples were placed on the rotary shaker for one hour and centrifuged for 10 minutes at 1500 RPMs. Finally, approximately 20 mL of the extract was poured through round filter paper into tubes and ammonium-N was extracted follow the same procedure as the 7-d incubation measurement. The PMN measurement was then calculated by subtracting the 0-d measurement from the 7-d measurement.

The soil respiration test was done using the protocol adopted by the Cornell Soil Health Laboratory (Moebius-Clune et al., 2016) that followed methods described by (Zibilske, 1994). Two round filter papers were put into the bottom of a wide mouth mason jar with a small, perforated aluminum tray on the top of those filter papers. Twenty g of air-dried soil was measured out onto the aluminum trays. A trap assembly was installed using a pizza stand with a 10 mL beaker filled with 9 mL of 0.5 M KOH solution taped onto the pizza stand with doublesided cellulose tape. Then, 7.5 mL of deionized water was dispensed down the side of the jar to the bottom of the aluminum tray to soak the filter papers in the bottom and rewet the soil. The lid of the jar was closed and incubated for four days undisturbed. Original KOH EC was measured to obtain an initial reading before CO_2 addition could lower the EC of the solution. A blank jar, with no soil, was prepared to calculate the amount of CO_2 in the air of the jar. After four days of incubation were complete, the EC of the KOH solution was measured using a Mettler Toledo Seven Excellence Multiparameter EC meter probe. The soil respiration measurement was then calculated, comparing the used KOH EC measurement from the jar against the new KOH solution and the blank jar with no soil. The drop in EC determined the amount of CO_2 respired by the microbes in the soil sample.

Statistical Analysis

The effects of cover crop treatments on cover crop plus previous crop residue, POXC, PMN, and soil respiration were analyzed with Rstudio statistical software version 3.6.1 and interpreted using a two-way ANOVA and a linear model for all independent variables (R Core Team, 2019). A randomized complete block design was used as the experimental design with four replications in each block. Site-year, cover crop treatment, and their interaction was considered a fixed-effect, while block within each site-year was considered a random effect. Normality and constant variance assumptions were tested and shown to be met using the Shapiro-Wilk normality test and examining the residuals plots using the ggResidpanel package (Goode and Rey, 2019). Differences among plant biomass and soil health measurements caused by cover crop treatment and site-year were determined using Fishers Least Significant Difference at $P < .05$ significance level for mean separation using the agricolae package (Felipe de Mendiburu, 2017) within R statistical software. Differences among means were declared significant at $P < .05$. Site-years were analyzed separately when there was a site-year × cover crop treatment interaction. Potentially mineralizable nitrogen in the fall and spring as well as soil respiration in the spring were evaluated at only ten site-years due to insufficient amounts of soil to run the test in one site-year. Cover crop plus previous crop residue was only assessed at ten site-years in the fall and nine site-years in the spring due to missing samples. When only site-year had a significant effect on soil health measurements, the correlation between soil characteristics and weather conditions among soil health measurements was completed using Pearson's product-moment correlation in R.

RESULTS AND DISCUSSION

Weather

Cover crops were planted between early-August after small grain harvest and corn was planted in early May of the following year. The monthly average temperature departure from normal varied among site-years, but most site-years were within 2°C of normal. The only exception was the month of February, when temperatures at Pierre 2020, Blunt 2020, Mitchell 2020, and Henry 2020 dropped below average by 5°C (Figure 1a). Temperatures that terminated grass cover crops (-6°C) and broadleaf cover crops (-1°C) occurred between mid-November to early December each year. Generally, precipitation during the fall of each year was greater than normal (>50 mm above average), while in the spring, it was within 20 mm of normal (Figure 1b). However, the precipitation levels for Salem 2019 were about 50 mm above average during March through May. Overall, precipitation at each site-year was adequate to sustain cover crop growth (Barnard et al., 2015).

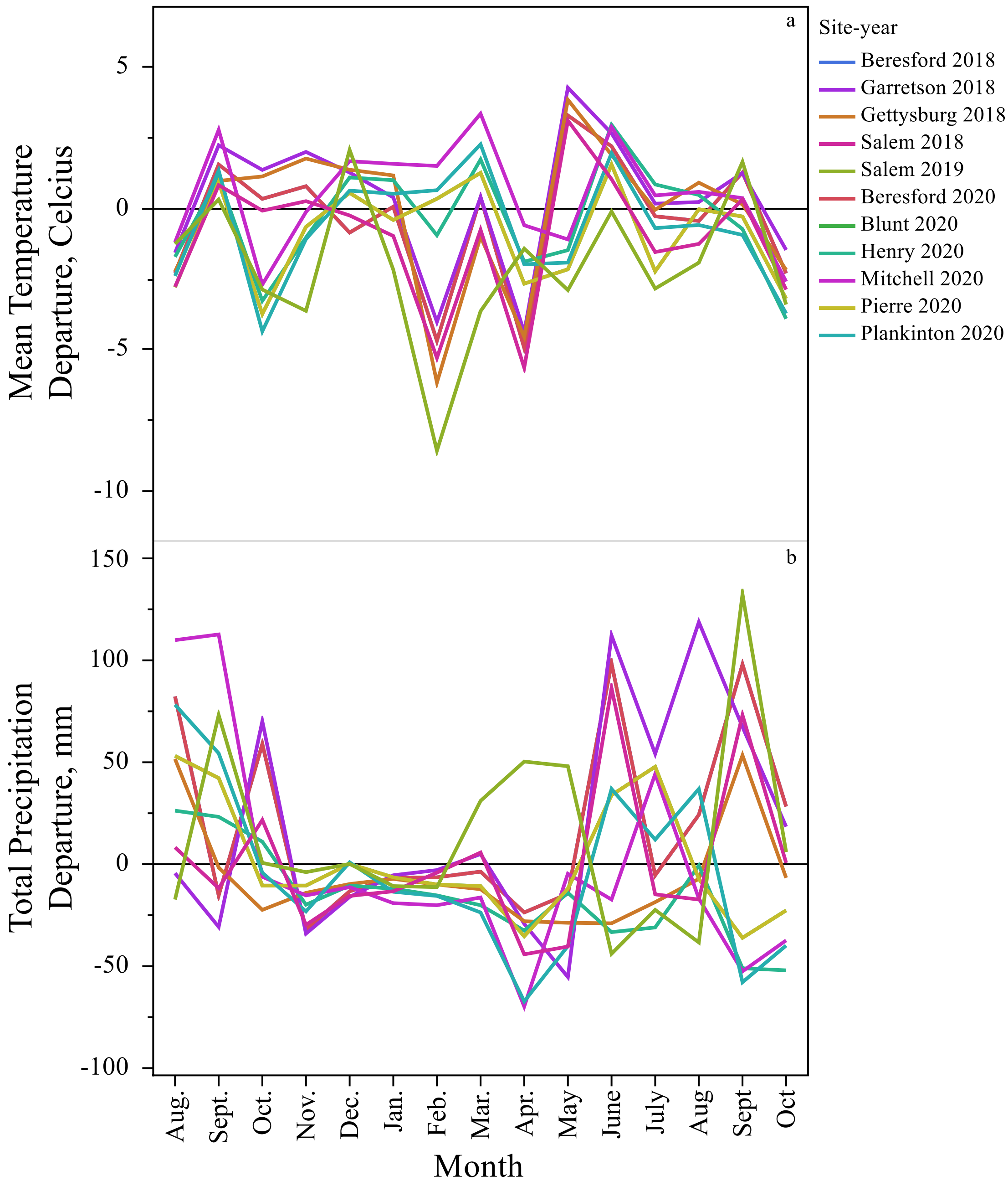

Figure 1. Monthly mean precipitation departures (a) and monthly total precipitation departures (b) from the 30-year average (1981–2010) at 11 site-years from August when cover crops were planted to October of the following year after corn grain harvest.

Biomass of Surface Residues

Both cover crop residue (living and dead) and previous crop residue were collected for surface residue biomass samples in the fall before winter kill and in the spring one week before planting corn. Therefore, both the no cover crop and cover crop treatments had biomass collected. This method was used because growing cover crops can speed up the decomposition of the previous crop residue, reducing the amount remaining in the field (Brockmueller, 2020). A high surface residue value in the control could mean little decomposition occurred, whereas a lower value implies greater decomposition of the previous cash crop residue.

The effect of including cover crops and their composition on fall and spring surface residue biomass was influenced by the site-year × cover crop interaction (Table 2). Across site-years, surface residue biomass in the fall ranged between 652 to 8349 kg ha^{-1} with a mean of 3752 kg ha^{-1} and in the spring, it ranged from 953 to 5204 kg ha^{-1} with a mean of 2662 kg ha^{-1} (Table 2). The varying weather conditions among site-years (Figure 1) was likely the cause of this wide variation in cover crop plus previous crop biomass. Other studies with similar cover crop planting dates accumulated between 210 to 1990 kg ha^{-1} in IA (Wiedenhoeft and Cambardella, 2013) and 4413 to 12096 kg ha^{-1} in central IL (Boydston and Williams, 2017). On average, these studies had similar ranges of biomass remaining on the soil surface, but the maximum values found in IL were greater than this study. The greater maximum values in IL were likely due to their warmer temperatures and a longer cover crop growing season as their cover crop would have been planted earlier and winter-killed sometime in December instead of November.

When comparing within each site-year, fall surface residue amounts were normally similar among cover crop treatments. Specifically, planting cover crops regardless of composition did not affect fall surface residue biomass in seven of the ten site-years (70%) sampled (Table 3). In the three site-years where cover crops influenced fall surface residue, two site-years had greater fall surface residue in one or more of the cover crop treatments compared to the control. Whereas, in the other site-year, fall surface residue from one or more cover crops was less than the control. Specifically, fall surface residue in Plankinton 2020 was greater with a broadleaf cover crop (8348 kg ha^{-1}) than grass (5569 kg ha^{-1}) and the control (5548 kg ha^{-1}), but the grass and control were similar. Whereas in Salem 2018, all cover crop mixtures (mean = 4020 kg ha^{-1}) had greater surface residue than the control (1667 kg ha^{-1}). In contrast to these results, in Garretson 2018, the control had the greatest fall surface residue (5281 kg ha^{-1}) and the blend had the least (3792 kg ha^{-1}) with the grass and broadleaf being similar to all treatments.

Including cover crops likely did not increase surface residue in most site-years compared to the control because including cover crops may have increased decomposition rates of the previous cash crop surface residue, resulting in similar total surface residue values. Evidence for this occurred at Garretson 2018, Beresford 2020, Mitchell 2020, and Blunt 2020, where the surface residue values of the controls were all numerically or significantly greater than where cover crops were planted. A study in southeastern SD demonstrated this possibility where they reported less previous crop residue where cover crops were growing (Brockmueller, 2020). Therefore, growing cover crops can potentially increase the previous crop residue decomposition, reducing previous crop residue and potentially increasing available nutrients for the succeeding cash crops. Overall, including cover crops regardless of the mixture in a small grain-corn rotation does not consistently affect fall surface residue. Further, when cover crops do influence fall surface residue, there is no consistent difference among cover crop mixtures. These results differ from a study in Urbana, IL, on a silty loam soil and in eastern NE on a silty clay loam soil where a grass cover crop produced greater biomass than a broadleaf cover crop (Boydston and Williams, 2017; Blanco-Canqui and Jasa, 2019). These differences may be because their studies only weighed and compared cover crop residue and did not include previous crop residue. In future studies, it would be beneficial to partition the grass and broadleaf cover crops along with previous crop residue to better understand the influence of growing cover crops on the decomposition of previous crop residues.

When comparing within each site-year, spring surface residue amounts were similar among cover crop treatments approximately 50% of the time. Specifically, planting cover crops regardless of composition did not affect spring surface residue biomass in five of the nine site-years (78%) sampled (Table 3). In the four site-years where cover crops influenced spring surface residue, the control had less or a similar quantity of spring surface residue compared to all other cover crop treatments. Compared to the control, all three cover crop mixtures had greater spring surface residue (increase of 1358–1608 kg ha^{-1}) in one site-year, the broadleaf and grass in one site-year (increase of 503–737 kg ha^{-1}), and only the blend in one site-year (increase of 550 kg ha^{-1}). In contrast, in one site-year was the control spring surface residue greater than any of the cover crop treatments, which was in Plankinton 2020 where the control had 1686 kg ha^{-1} more surface residue than the grass cover crop.

The spring surface residue biomass among the three cover crop mixtures were similar in seven of the nine site-years sampled. In the two site-years where differences occurred the broadleaf and grass cover crops had greater spring residue biomass in one site-year (increase of 339 to 573 kg ha^{-1}) and the blend cover crop had a greater biomass than the grass (increase of 1731 kg ha^{-1}) at one site-year. Overall, these results indicate that the effects of cover crops on fall and spring surface residue were generally similar regardless of the cover crop composition and when there were changes there was no consistent differences among the mixtures.

Table 2. Significance of F tests for the fixed effects of cover crop treatment, site-year, and their interactions on soil health tests including permanganate oxidizable carbon (POXC), potentially mineralizable nitrogen (PMN), soil respiration, and surface residue from samples collected in the fall and spring across 11 site-years.

	Source of variation		
Variable	**Cover crop (CC)**	**Site-year (S)**	**CC × S**
		***F*-value**	
Surface residue, fall	0.92	51.29*	2.91*
Surface residue, spring	1.36	46.77*	3.94*
POXC, fall	1.30	4.87*	0.99
POXC, spring	1.09	20.71*	0.37
PMN, fall	0.07	23.71*	0.64
PMN, spring	0.20	41.41*	0.71
Soil respiration, fall	0.04	33.04*	1.06
Soil respiration, spring	2.52	70.98*	1.42
		Numerator df	
All variables	3.00	30.00	10.00

*Significant at the 0.05 probability level.

Table 3. Effect of cover crop treatments on fall and spring surface residue biomass across 11 site-years.

	Fall				Spring			
Site-year	**Broadleaf**	**Grass**	**Blend**	**Control**	**Broadleaf**	**Grass**	**Blend**	**Control**
				kg ha^{-1}				
Beresford 2018	4254a	4478a	4430a	3948a	1991a	2021a	1794a	2290a
Garretson 2018	4360ab	4420ab	3792b	5281a	2762a	2703a	2123a	2404a
Gettysburg 2018	3115a	3160a	2989a	2590a	2816a	2912a	3217a	3199a
Salem 2018	3836a	4077a	4149a	1667b	4269a	4162a	4019a	2661b
Salem 2019	651a	1315a	681a	777a	-[b]	-	-	-
Beresford 2020	1677a	2045a	2320a	1885a	1456a	1690a	1117b	953b
Blunt 2020	2330a	2693a	2788a	3545a	-	-	-	-
Henry 2020	-	-	-	-	2212a	2712a	2213a	2151a
Mitchell 2020	6180a	3693a	4917a	5851a	2904a	3036a	2825a	2215a
Pierre 2020	5418a	5722a	4760a	-	1875ab	1862ab	2141a	1591b
Plankinton 2020	8348a	5569b	7080ab	5548b	3701ab	3473b	5204a	5159a

[a]Means followed by the same letter in a row within a sampling period are not significantly different ($P > 0.05$).
[b]Comparisons not available for this site.

Soil Health Measurements

The soil health measurements that were evaluated in these cover crop field trials were POXC, PMN, and soil respiration. In the first year of comparing cover crop mixtures, regardless of cover crop composition, cover crops did not affect any of the soil health measurements within the site-year × cover crop interaction or the main effect of cover crop (Table 2). In other short-term studies (< 4 yr), similar results were found in Illinois, Maryland, and South Dakota where including cover crops did not improve most soil physical and biological properties (Dozier et al., 2017; Rorick & Kladivko, 2017; Wang et al., 2017; Wegner et al., 2015). However, the study in Maryland determined no effect on total organic C from forage radish, but they did see an increase in POXC (Wang et al., 2017). Further, in Indiana including a cereal rye cover crop did not increase bulk density, water retention, or soil organic C and total soil N, but it did increase aggregate mean weight diameter (Rorick & Kladivko, 2017). In contrast to these results other studies in Kansas, Virginia, and Missouri did see increases in soil physical (aggregate stability) and biological soil properties (microbial biomass, activity, and structure) (Rankoth et al., 2019; Simon et al., 2022; Strickland et al., 2019).

The consistency in finding significant differences between various cover crops and no cover crops increases as length of time including cover crops increases (Blanco-Canqui & Ruis, 2020). Evidence of this occurs as studies ranging from 9 to 15 years in Iowa, California, and the Netherlands determined that inclusion of cover crops improved soil organic matter and other biological properties (N mineralization, microbial abundance and diversity, fungi:bacteria ratio) (Martínez-García et al., 2018; Moore et al., 2014; Schmidt et al., 2018). Other long-term studies in Kansas, Iowa, Illinois, and Spain (>10 yr) found including cover crops improved various soil physical properties including soil organic C, soil aggregation, mean weight diameter of dry aggregates, bulk density, or infiltration (Blanco-Canqui et al., 2011; Blanco-Canqui & Jasa, 2019; Gabriel et al., 2021; Nichols et al., 2022; Olson et al., 2014).

In addition to number of years of planting cover crops, tillage system, soil organic matter level, and soil moisture status are likely factors that influenced the lack of an effect on soil properties from including different compositions of cover crops (Blanco-Canqui & Ruis, 2020; Calderón et al., 2016). Each of the experimental fields in this study except two sites were in no-till management for greater than ten years (mean = 19 yr) with soil organic matter levels between 31 to 47 g kg^{-1} (mean = 41 g kg^{-1}) (Table 1). (Blanco-Canqui & Jasa, 2019) hypothesized that the conditions of long-term no-till and good soil organic matter levels as were found in our sites would likely result in smaller or slower effects on changing soil properties compared to sites with short-term no-till or conventional tillage practices before cover crop inclusion. Additionally, the promotion of soil biology in semiarid environments is more strongly associated with soil moisture than inclusion of cover crops (Calderón et al., 2016). Therefore, in the often water-limited environments of South Dakota, the lower quantity of precipitation may also be resulting in smaller or slower effects of including cover crops on soil biology measurements. Overall, these results indicate that in no-till systems and a semiarid environment that improving soil health measurements will take a more extended period than only the first year of implementation to have a consistent, measurable effect.

Including a cover crop did not affect soil health measurements, but site-year significantly influenced each of the soil health measurements (Table 4). Soil health measurements were related to organic matter, pH, total precipitation during the month before sampling, and temperature during the month of sampling (Table 5). Positive linear relationships among site-specific soil properties and weather variables included pH with spring PMN (R = 0.63), soil organic matter with fall POXC (R = 0.18) and spring POXC (R = 0.44), and precipitation with fall soil respiration (R = 0.25). Negative linear relationships included both pH with fall soil respiration (R = -0.21) and precipitation with fall PMN (R = -0.52). These relationships between the different soil properties and weather variables across site-years are likely what resulted in the significant effect of site-year on soil health measurements. Other studies also determined that soil organic matter was positively correlated with POXC (Hurisso et al., 2016; C. E. Norris et al., 2020) and pH was negatively related to PMN and positively related to soil respiration (Malik et al., 2018; C. E. Norris et al., 2020; Turner, 2010). In our study, precipitation was positively related to PMN, which was opposite of what other studies found in the US Midwest and Germany (J. D. Clark et al., 2020; Engelhardt et al., 2018; Zhou et al., 2009). These results indicate that there is a relationship between soil characteristics and weather patterns with soil health measurements. Therefore, these site characteristics need to be taken into consideration when comparing soil health measurements across locations.

Table 4. Effect of site-year on soil health measurements permanganate oxidizable carbon (POXC), potentially mineralizable nitrogen (PMN), and soil respiration, from fall and spring soil samples across 11 site-years.

Site Year	Fall POXC	Spring POXC	Fall PMN	Spring PMN	Fall Soil Respiration	Spring Soil Respiration
	mg kg^{-1} of soil		µg g^{-1} of soil $week^{-1}$		mg CO_2 g^{-1} of soil 4 d^{-1}	
Garretson 2018	1059a	946abc	33c	34d	1.68a	2.89a
Gettysburg 2018	869cde	900c	-[b]	-	1.47b	1.57b
Salem 2018	839e	718de	51c	40d	1.31bc	0.79de
Beresford 2018	958bc	1015ab	53c	7d	1.23c	1.13c
Salem 2019	890bcde	874c	168b	176bc	0.88de	0.81de
Blunt 2020	1054a	759d	180b	204ab	0.73ef	1.02c
Pierre 2020	858de	740d	257a	170c	0.45g	0.63e
Henry 2020	930bcde	944bc	151b	199abc	1.18c	0.93cd
Mitchell 2020	936bcd	1018a	260a	223a	0.59fg	1.08c
Plankinton 2020	892bcde	657e	176b	179bc	0.96d	1.08c
Beresford 2020	973ab	932c	231a	168c	0.45g	0.70e

[a]Means followed by the same letter in a column are not significantly different ($P > 0.05$).
[b]Comparisons not available for this site.

Table 5. Pearson correlation coefficients (R values) between fall and spring soil health measurements (permanganate oxidizable carbon (POXC), potentially mineralizable nitrogen (PMN), and soil respiration) and soil properties and weather variables; pH, organic matter (OM), soil test nitrate-N, precipitation, and temperature.

Variable[a]	OM	pH	NO_3	Precip.[b]	Temp.[c]
Fall POXC	0.18*	0.09	-0.15*	0.18*	0.11
Spring POXC	0.44*	-0.06	-0.18*	0.14	0.16*
Fall PMN	-0.27*	0.38*	0.13	-0.52*	-0.49*
Spring PMN	0.04	0.63*	0.28*	-0.03	-0.46*
Fall soil respiration	0.23*	-0.21*	-0.08	0.25*	0.40
Spring soil respiration	0.15*	-0.12	-0.11	-0.18*	0.24*

*Significant at the 0.05 probability level.
[a]Variables measured in fall or spring were correlated with soil measurements in the same season (ie. Fall PMN ~ Fall OM, Spring PMN ~ Spring OM).
[b]The precipition totals that were used were from the month of and the month prior to soil sampling.
[c]The mean temperature used was from the month of soil sampling.

CONCLUSIONS

This three-year study of cover crops on no-till soils in South Dakota, after the first year of including a multi-species grass, broadleaf, and a grass/broadleaf blend showed limited effects on changing surface residue biomass and soil health measurements compared among each other and the no cover crop control. The fact that planting cover crops regardless of composition compared to the no cover crop control did not affect fall or spring cover crop/previous crop residue biomass in 7 of the 11 site-years suggests that growing cover crops may have accelerated old cash crop decomposition. This accelerated decomposition can help build soil organic matter and improve nutrient cycling over time. In future studies, previous cash crop residues should be partitioned from fresh cover crop biomass to precisely observe how much they add to the total surface residue. The lack of an effect of including cover crops on soil heath measurements may be attributed to the experimental sites had mostly already been in no-till management for greater than 15 years and had soil organic matter levels greater than 41 g kg^{-1}. Future studies need to work on understanding the long-term effects of including cover crops in rotation after small grain crops and when transitioning from conventional tillage to no-till plus the inclusion of cover crops. Long-term studies comparing multi-species mixtures of grasses and/or broadleaves on soil health measurements are needed to determine if, and when differences begin to occur.

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