



Article Impact of Cover Crops and Poultry Litter on Selected Soil Properties and Yield in Dryland Soybean Production

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Abstract: Soil biological properties are important for the stabilization and preservation of a good soil structure. Management practices can affect the diversity and population of microorganisms, which could beneficially change soil properties and promote a more sustainable dryland crop production. This study was established near Pontotoc, MS, USA (34°07' N, 88°59' W) on an Atwood silt loam (fine-silty, mixed, semiactive, thermic Typic Paleudalf) to evaluate the impacts of cover crops, planting dates and fertilizer sources (poultry litter, inorganic fertilizer and no fertilizer) on selected biologically related soil properties in a no-tillage, dryland soybean production. Soil analyses included total carbon and nitrogen, permanganate oxidizable carbon (POXC), easily extractable glomalin-related soil protein (EE-GRSP), water stable aggregate (WSA) and soil pH. Cover crop production and soybean yield were also determined. The results indicated that the fertilizer source had an impact on total nitrogen, EE-GRSP and soybean yield. Total N was 6% higher with poultry litter at the early planting date compared to no fertilizer (control) (p < 0.0018) and at the late planting date, when total N and EE-GRSP were increased by 11% and 13%, respectively, with poultry litter compared to no fertilizer. Additionally, soil pH was reduced by 0.25 units in the poultry litteramended treatment. Soybean yield was increased by 68% and 51% in early-planted soybean and 42% and 40% in late-planted soybean with poultry litter and inorganic fertilizer, respectively, compared to no fertilizer. This study revealed that biological soil properties and soybean yield were influenced by poultry litter application. The results showed no significant effects of cover crops over the short time period of the study.

Keywords: soil microorganisms; cover crop; poultry litter; glomalin; dryland

1. Introduction

The adaptation of management practices is a priority for sustainable crop production in a dryland system with poor soil quality. Low soybean yield in dryland systems can be directly related to a lack of moisture in critical plant development stages during the growing season [1]. More than half of Mississippi state soybean hectares are produced under a dryland system and productivity is typically low if timely rainfall events do not occur [2]. Soil moisture is an important determinant of crop yield in a dryland cropping system [3]. Soil structure influences moisture status in the root zone and soil microorganisms are important for the stabilization and preservation of a good soil structure. In some North Mississippi fields, wells are drilled as deep as 200 m [4] At this depth, irrigation is not economically feasible. Farmers need an irrigation alternative that can increase yield with low cost. Thus, research is needed to determine management practices for a more sustainable dryland soybean production system.



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The use of cover crops and poultry litter as a management practice has been adopted in many no-tillage systems. No-tillage maintains soil moisture status by influencing evaporation, drainage and runoff after rainfall events [5,6]. The inclusion of cover crops in a no-tillage production system enhances soil physical properties and microbial enzymatic activities [7,8]. Cover crops have multifunctional properties that enhance soil C, soil porosity, water-stable aggregates (WSA) and soil organic matter [9-11]. Residue from the above-ground cover crop production increases soil organic matter, while below-ground root exudates diversify the energy sources available to soil microbes that improve soil properties [12], which in turn benefit microbial communities [13–15]. Improved activities include enhanced nutrient cycling, better soil quality and ultimately increased cash crop yield [16]. The inclusion of legumes as a cover crop can also fix atmospheric N, which can reduce the rate of applied N fertilizer required by the cash crop [16]. In the US, the use of winter cover crops in the farm management system has increased by about two million ha (5 million ac) between 2012 and 2017 [17]. The optimum application of inorganic fertilizer and organic fertilizer such as poultry litter remarkably improves the soil health, which is defined as the vital functional capacity of soil for the sustainable development of plants, animals and humans [18]. Cover crop termination dates and methods depend on the aim of their integration into a cropping system. Two weeks of delay in the termination of cereal rye and hairy vetch increased biomass accumulation by 39% and 61%, respectively and growing cover crops until three weeks before planting the cash crop increased mycorrhizal fungi population [19,20]. Lawson et al. [21] observed a 60% increase in cover crop biomass with a month delay in termination on a Briscot loam (coarse-loamy, mixed, superactive, nonacid, mesic Fluvaquentic endoaquept) in Washington.

Field studies have demonstrated profitable results of cover crop integration on the enhancement of soil properties, ultimately increasing the cash crop yield. A 15 year study in Kansas noted an improvement in aggregate stability and organic C on a Geary silt loam (fine-silty, mixed, superactive, mesic Udic Argiustoll) over time [9]. However, there may not be a short-term effect (2–5 years) of cover crops on soil total organic C because it responds slowly to changes in management practices. Therefore, labile C may be used as a short-term indicator of management practices [22,23]. Research also indicates that fertilizer treatments based on soil test results increase soybean yield in dryland soybean systems [24]. The results of Ren et al. [25] showed that inorganic fertilizer increases soil labile C but there was no change in total C and total N content on a silt loam soil in a six-year study. Geisseler and Scow [26] found that inorganic fertilizer increases organic C by 12.8% and microbial biomass C by 15.1% in a review of studies with at least a five-year observation period. The application of poultry litter increases total C, N, soil pH and N mineralization in comparison to inorganic fertilizer [27]. Poultry litter improves soil properties, plant nutrient availability and plant development, ultimately leading to an increase in crop yield [28–31]. It also increases beneficial fungal and bacterial populations such as arbuscular mycorrhizal fungi (AMF) and nitrifying bacteria and suppresses pathogens such as *M. incognita* [32]. Mierzwa-Hersztek et al. [33] reported that nitrifying bacteria activities increased 50% with the addition of poultry litter in a two-year study on loamy sand soil, which might be the result of higher C substrate. Poultry litter contains organic N, which serves as a slowrelease fertilizer and it can help fulfill the soybean N requirement during peak stages of development [34,35].

Soil health, the capacity of soil to function as a living system and support the development of plant and animals, can be improved with changes in management practices. These changes are measured as water-stable aggregates (WSA), permanganate oxidizable C (POXC) and microbial respiration [36,37]. A 15 year study in the Californian San Joaquin Valley showed an improvement of soil properties with the use of cover crops in a no-tillage cash cropping system [38]. This study noted an improvement in the water infiltration rate, WSA, total C and total N in the upper 15 cm of soil compared to a no-cover-crop standard tillage system. Aldridge et al. [33] and Amado et al. [33] demonstrated a higher C content in surface soil with cover crops in no-tillage treatments compared to fallow treatment. Permanganate oxidizable C is composed of soil microbial biomass, fresh organic material, particulate organic matter and root exudates that have a relatively short mineralization time (2–5 years). The determination of permanganate oxidizable C is an economical and fast method to quantify biologically active labile C in soil, which can be used to indicate a change produced by a management practice [37,39]. The rapid response of POXC to changes in soil health due to management practices may help farmers to develop a new strategy to increase soil health.

Easily extractable- glomalin-related soil protein (GRSP) is a labile fraction of GRSP and is believed to be a recently produced glomalin; it mainly contains glycoprotein, which is produced by arbuscular mycorrhizal fungi (AMF) [40,41]. The soil protein EE-GRSP is also a part of the C-based soil component that performs as an adhesive material which may enhance aggregation and stimulate plant growth [42–46]. Thermostable EE-GRSP also helps in enhancing drought tolerance in plants [47]. Studies reveal that management practices influence AMF and GRSP production in soil by influencing the root exudates and microbial population [44,48,49]. Cover crops have been shown to improve the AMF population in cash crops, which indirectly affects GRSP production and WSA formation [50]. Zhang et al. [51] reported improved fungal energy sources and habitats and enhanced hyphae development for glomalin production on a Cambisol with poultry litter.

Therefore, cover crop and fertilizer use as part of a farming system may improve soil properties. Cover crops may improve aggregate stability with the exudation of polysaccharides, root mucilage and the root system itself [52]. The addition of poultry litter increases the C content of the soil, which may improve macroaggregates. A study by Zhang et al. [51] noted that glomalin, microbial biomass C and organic C in soil were highly influenced by poultry litter in a Cambisol soil in China.

Many studies have shown that these management practices change soil properties including soil organic matter, soil aggregate stability, soil organic C, total C, total nitrogen (N), soil pH and glomalin [22,53–55]. In contrast, some studies have shown no effect of cover crops on soil properties. For example, a two-year study on a five-year crop rotation field showed no differences regarding aggregate stability and organic C due to cover crop and cattle manure application in a Albic Stagic Luvisol in the Estonian area of northern Europe [56]. Long-term studies in various physiographic regions of North Carolina showed no impact of cover crops on soil aggregation. The same study showed differences regarding biological properties but these were inconsistent [57]. The discrepancy in research results warrants further investigation. Limited research has been conducted with a dryland soybean production system that has elucidated the need for the combined impact of no-tillage, cover crop and fertilizer sources on biologically related soil properties. In addition, the impact of these applications may vary among different site factors; thus, the impacts cannot be generalized to other regions with different conditions.

The purpose of this project was to evaluate the combined effects of cover crop and fertilizer source in no-tillage conditions on dryland soybean production with the overarching goal of determining ways to help producers improve soil health and increase soybean yield. The soil health indicators of total C, total N, permanganate oxidizable carbon (POXC), easily extractable glomalin-related soil protein (EE-GRSP), WSA and soil pH were determined to measure the short-term effects of management practices on biologically related soil characteristics. We hypothesized that the combination of cover crop and fertilizer source will beneficially change soil biological properties in a no-till, dryland soybean system. Moreover, the cover crop production and soybean yield would increase with cover crop and fertilizer treatment over time. The objective of this study was to determine the effects of cover crops, inorganic fertilizer and poultry litter on biologically related soil properties in a no-tillage, dryland soybean system.

2. Materials and Methods

2.1. Study Site

A cover crop study was conducted at the Pontotoc Ridge-Flatwoods Branch Experiment Station in Pontotoc County, MS, USA (34°07′ N, 88°59′ W). The study was initiated in October 2017 and continued until September 2020. The soil series at the study site was an Atwood silt loam (fine-silty, mixed, semi-active, thermic Typic Paleudalf) with 13.9% clay, 17.6% sand and 68.5% silt (measured by pipette-method) on a 3% slope (Soil Survey, Natural Resources Conservation Service (NRCS)). The study was conducted under no-tillage, rainfed conditions. Prior to this experiment, corn and soybean were planted in 2016 and 2017, respectively. An initial soil test determined a pH of 6.67 and 1.57% organic matter. The monthly mean maximum and minimum air temperature, total monthly rainfall for the study period and 30 year mean (1981–2010) at the experiment station were taken from reports by the National Oceanic and Atmospheric Administration [58] (Tables 1 and 2).

Table 1. Monthly mean maximum, minimum temperatures ($^{\circ}$ C) and rainfall for the first, second and third year of study and 30 year mean monthly normal temperature at Pontotoc, MS.

Maximum Temperature (°C)				Minimum Temperature (°C)				Monthly Total Rainfall (mm)				
Month	Year 1	Year 2	Year 3	Mean	Year 1	Year 2	Year 3	Mean	Year 1	Year 2	Year 3	Mean
October	24.6	22.9	35.6	22.9	9.5	10.3	3.9	10.1	46	41	165	109
November	21.4	15.1	23.3	16.9	7.2	4.0	-10.0	5.2	37	150	99	120
December	14.6	11.2	21.1	11.2	1.1	-0.6	-4.4	0.6	158	96	226	164
January	9.0	14.5	20.6	9.9	-5.4	-1.1	-5.6	-0.9	166	189	189	118
February	16.3	15.5	21.7	12.4	4.7	2.0	-2.8	1.1	334	348	281	134
March	19.2	17.1	30.0	17.3	3.6	-1.5	-2.8	5.2	76	86	208	127
April	20.9	23.5	27.2	21.9	5.1	9.3	3.3	9.6	209	240	146	137
May	29.8	29.2	30.6	26.3	16.7	15.7	11.1	14.9	120	151	125	146
June	31.4	30.2	32.2	29.9	19.6	17.8	13.9	19.1	176	191	205	123
July	32.6	32.1	34.4	31.8	21.3	20.2	19.4	20.8	32	153	186	110
August	32.2	32.4	35.6	31.8	20.4	21.1	17.2	20.2	100	87	121	102
September	30.5	34.3	32.2	28.7	19.5	18.6	9.4	16.3	161	3	50	93

Year 1 = October 2017–September 2018; Year 2 = October 2018–September 2019; Year 3 = October 2019–September 2020; Mean = 30 years (1981–2010).

	Cover Crop		Fertilize	er Source	So	Soil		
Planting	Biomass Collection	Killing	Poultry Litter	Inorganic Fertilizer	Planting	Cultivar	Harvest	Soil Sampling
Early Plan	nting Date							
30 October 2017	12 April 2018	19 April 2018	10 April 2018	10 April 2018	01 May 2018	Asgrow [®] 46X6	04 October 2018	07 May 2018
29 October 2018	02 April 2019	17 April 2019	02 April 2019	02 April 2019	30 April 2019	Asgrow [®] 45X8	30 September 2019	08 May 2019
05 November 2020	07 April 2020	04 April 2019	16 April 2020	16 April 2020	05 May 2020	Asgrow [®] 45X8	06 October 2020	05 May 2020
	La	te Planting Da	ite					
30 October 2017	10 May 2018	14 May 2018	10 April 2018	10 April 2018	24 May 2018	Asgrow [®] 46X6	19 October 2018	04 June 2018
29 October 2018	08 May 2019	08 May 2019	02 April 2019	02 April 2019	23 May 2019	Asgrow [®] 45X8	30 September 2019	28 May 2019
05 November 2020	11 May 2020	11 May 2020	16 April 2020	16 April 2020	01 June 2020	Asgrow [®] 45X8	06 October 2020	03 June 2020

Table 2. Cover crop and soybean operation, fertilizer application and soil sampling dates from early and late planting dates in Pontotoc, MS.

2.2. Experimental Design

The experimental design was a randomized complete block design with a split-plot treatment structure of two factors and three replications. The main plot factor was cover crops and fertilizer source was the sub-plot factor. There were two soybean planting dates (i.e., early and late planting date/ early and late-terminated cover crop) which were studied separately. Each planting date consisted of 15 whole plots of 167.2 m² (1800 ft²) each and 45 sub-plots (74.4 m² each (800 ft²) for each soybean planting date. The five cover crop treatments included cereal rye (*Secale cereale*), vetch (*Vicia villosa*), wheat (*Triticum aestivum*), mustard (*Brassica rapa*) and cereal rye (CC-mix) and native vegetation (allowing naturally seeded weeds to grow- control). The three fertilizer treatments were poultry litter, inorganic fertilizer (phosphorus, potassium and sulfur) and no-fertilizer control.

2.3. Field Methods

Cover crops were drill-seeded at the rate of 91.91 kg ha⁻¹ of cereal rye, 22.42 kg ha⁻¹ of vetch, 94.15 kg ha⁻¹ of wheat, 71.73 kg ha⁻¹ of cereal rye and 16.81 kg ha⁻¹ of the mustard mixture in both years. Cover crops were killed with N,N'-dimethyl-4,4'-bipyridinium dichloride (paraquat), N-(phosphonomethyl) glycine (glyphosate) and 3,6-dichloro-2methoxybenzoic acid (dicamba) in 2018, 2019 and 2020, respectively Herbicides were applied twice at an interval of about 10 days before planting herbicide-resistant soybean. Poultry litter application was based on an equivalent amount of P_2O_5 as inorganic fertilizer. The poultry litter analysis was conducted by the nutrient analysis laboratory United States Department of Agriculture- Agricultural Research Service (USDA-ARS) Mississippi, USA. Based on the soil test, potassium (K), phosphorus (P) and sulfur (S) were recommended for the production of 2690 kg ha^{-1} of soybean in this soil. The recommended inorganic fertilizer rates were P_2O_5 at 134.5 kg ha⁻¹, K_2O at 33.63 kg ha⁻¹ and S at 22.42 kg ha⁻¹ based on Lancaster macronutrient extraction method soil test recommendation from Southern Soil Labs (Yazoo City, MS, USA). Phosphorus was applied as triple superphosphate at 292.5 kg ha⁻¹. Potassium was applied at 56.04 kg ha⁻¹ as muriate of potash. Sulfur was applied as 90% elemental S at 24.7 kg ha⁻¹. Poultry litter was surface-broadcasted at 4695.3 kg ha⁻¹ in 2018, 4483.4 kg ha⁻¹ in 2019 and a similar amount in 2020. Each plot was treated with the same treatment each year. Soybean was planted with a no-till planter at

316,160 seeds per ha⁻¹. Each sub-plot had eight rows of soybean. All the field operations are listed in Tables 3 and 4.

Table 3. Chemical composition of poultry litter applied to the soybean plots at Pontotoc Ridge– Flatwoods Branch Experiment Station, Pontotoc, MS, USA. TN: total nitrogen; TC: total carbon.

Year	Moisture %	TN (g kg ⁻¹)	TC (g kg $^{-1}$)	К	Р	Cu	Zn	Fe	Mn
					-g			 mg	
				kg-	1		kg ⁻¹ —		
2018	24.1	31	250	25.7	13.0	0.400	0.424	1.617	0.656
2019	25.9	32	229	28.8	17.5	0.113	0.416	0.859	0.455

Table 4. Main effects of cover crop on cover crop production in 2018, 2019 and 2020 and average soybean yield in early and late-terminated cover crops.

	Cove	r Crop Production (kg	ha ⁻¹)
Cover Crop	2018	2019	2020
Early-termin	ated cover crop		
Cereal rye	1645 ¹ (200) ² a	3639 (326) a	1986 (288) a
CC-mix	1768 (288) a	3035 (442) a	2037 (316) a
Native veg.	418 (138) b	3140 (690) a	1228 (174) b
Vetch	685 (16) b	1953 (260) a	1313 (142) b
Wheat	1770 (108) a	3532 (379) a	2026 (207) a
<i>p</i> value	0.0014	0.2108	0.0244
Late-termina	ited cover crop		
Cereal rye	4425 (292) a	4228 (478) a	3467 (468) ab
CC-mix	4284 (368) a	4408 (330) a	4008 (457) a
Native veg.	2254 (52) b	2632 (350) a	2544 (346) bc
Vetch	1546 (212) b	3545 (383) a	2473 (330) c
Wheat	4386 (363) a	2989 (664) a	3244 (294) abc
<i>p</i> value	< 0.0001	0.3641	0.0031

¹ Means followed by different letters in columns are significantly different at the 0.05 level using Fisher's protected least significant difference (LSD) test and variables with no letters in columns are not significantly different; Native Veg = Native vegetation, CC-mix = cereal rye and mustard; ² Standard error in parenthesis.

2.4. Soil Sampling and Preparation

Soil sampling was conducted at soybean planting/after cover crop termination. Soil sub-samples from the top 0–10 cm were collected from the middle of each planted row in a plot and homogenized. Approximately 500 g of soil was placed in a Ziploc bag and immediately stored in a cooler. For long-term storage, samples were frozen in a -20 °C freezer upon arrival in the laboratory. For total C, total N, POXC, EE-GRSP, WSA and pH, a soil sample was air-dried overnight, ground, passed through a 2.0 mm sieve and stored at room temperature [59].

2.5. Soil Analyses

For total soil C and total nitrogen (N), one gram of air-dried soil sample was sent to the MSU soil testing lab and measurement was done with a dry combustion analyzer. For this method, samples were weighed in a crucible and introduced to a resistance furnace. Samples were oxidized above 950 °C under purified oxygen [60] and converted to CO_2 and N₂, which was detected by an N₂ analyzer and CO_2 detector. Permanganate oxidizable C (POXC) or active carbon (C) were measured using the procedure given by Culman et al. [37]. Briefly, air-dried soil samples (2.5 g) were stored in 50 mL polypropylene tubes, to which 18 mL of deionized water and 2.0 mL of 0.2 M KMnO₄ stock solution were added. Tubes were shaken at 180 oscillations per minute for exactly 2 min and then allowed to settle for 10 min. After 10 min, 0.5 mL of supernatant was transferred to a second 50 mL of the tube holding 49.5 mL of deionized water and mixed. The absorbance was read with a spectrophotometer at 550 nm. POXC was calculated using the formula given by Weil et al. [39]:

$$POXC\left(mg\,kg^{-1}\,soil\right) = 0.02\,mol\,L^{-1} - (a + b \times abs) \times \left(9000\,mg\,C\,mol^{-1}\right) \times (0.02\,L\,solution\,Wt^{-1})$$

where 0.02 *mol/L* = initial solution concentration of KMnO₄; *a* = intercept of a standard curve; *b* = slope of the standard curve; *Abs* = unknown absorbance; 9000 = milligrams of carbon oxidized by 1 mole of MnO₄; changing from Mn⁷⁺ \rightarrow Mn⁴⁺; 0.02 *L* = volume of reacted stock solution; *Wt* = weight of air-dried soil sample in kg.

Easily extractable glomalin-related soil protein (EE-GRSP) was extracted from soil solution as described by Reyna and Wall [61]. One gram of air-dried soil sample was mixed with 8.0 mL of 20 mM of sodium citrate (pH 7.0) in a 50 mL centrifuge tube. Tubes were autoclaved at 121 °C for 30 min. Immediately after autoclaving, the tubes were centrifuged at $5000 \times g$ for 15 min. The supernatant containing EE-GRSP was separated from the centrifuge tube. This supernatant was assayed using the Pierce Bicinchoninic Acid (BCA) Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and detected in a spectrophotometer at 562 nm absorbance using bovine serum albumin (BSA) as standard. For EE-GRSP determination, 100 μ L of supernatant was added to 2.0 mL of standard working reagent in a glass tube. Tubes were incubated at 37 °C for 30 min and the absorbance of samples and standards was read at room temperature. The easily extractable-GRSP concentration was calculated as mg/g of dry soil. Water-stable aggregate (WSA) was measured by using the wet-sieving method [62]. Four grams of air-dried soil sample (1.0–2.0 mm) was placed in the sieve of a wet sieving apparatus and wet-sieved in a can of deionized water for 3.0 min. This can was replaced by another can with a dispersing solution (2.0 g of NaOH/ L) and sieved until only sand particles were left in the sieve. The soil collected in the can was oven-dried overnight at 110 °C. The weight of soil in the can was measured. Water-stable aggregate was calculated using the formula below:

$$WSA = \frac{Wds}{(Wds + Wdw)} \times 100\%$$

where WSA = water stable aggregate; Wds = weight of aggregate dispersed in dispersing solution; Wdw = weight of aggregate dispersed in deionized water.

Soil pH was measured on soil:water (1:1) slurry following the procedure by Thomas [63]. Ten grams of air-dried soil sample was weighed into a 50 mL beaker. Ten milliliters of deionized water was added and mixed well. The soil suspension was allowed to stand for 10 min and soil pH was determined with silver chloride and a combination electrode.

For cover crop production, cover crop vegetation was collected from a 1 m^2 area using a quadrat in each plot before the termination of cover crop. Samples were dried at 65 °C for 1–2 weeks in a drier to constant weight and the final weight was recorded. Five plants were measured from each of two middle rows in all plots. Soybean was harvested from the four middle rows of each plot with a plot combine.

2.6. Statistical Analysis

Data were analyzed using a general linear model ANOVA to determine the effects of treatments on variables. In 2018, fertilizers were not applied until after cover crops were terminated. Therefore, cover crop production was analyzed with a one-factor experiment with a randomized complete block design. In 2019 and 2020, fertilizer source was considered as a split-plot factor due to the possibility of residual fertilizer effects on cover crop production and analyzed with a randomized complete block design with split-plot treatment structure. Total C and total N, WSA, EE-GRSP, POXC, pH and soybean yield were analyzed as a randomized complete block design with a split-plot treatment structure using cover crop as the whole plot and fertilizer as a split plot. The soybean planting dates were analyzed separately. Data were analyzed using the general linear model PROC GLIMMIX of SAS 9.4

(SAS Institute Inc., SAS Campus Drive, Cary, NC 27513, USA) with mean values separated at the 0.05 level of probability using Fisher's protected least significance difference (LSD).

3. Results

This study focused on the effects of cover crops and fertilizer sources on selected biologically related soil properties, cover crop production and soybean yield. The data for no year/treatment (cover crop and fertilizer source) interaction were combined except for the effect of cover crops on cover crop production and the effect of fertilizer source on EE-GRSP (Table 5). There was no cover crop and fertilizer source interaction effect on biologically related soil properties except in terms of the soil pH at the late planting date. Therefore, the data were presented as the main effects of the cover crop and fertilizer source. Soybean received a total rainfall of 308 mm in 2018, 431 mm in 2019 and 512 mm in 2020. The rainfall in September 2018 was 161 mm, which greatly reduced the soybean seed quality and yield. Therefore, soybean yield data from 2018 were excluded.

Table 5. Probability values (*p*-values) and numerator of degrees of freedom (df) associated with the sources variance on soil properties and soybean yield components determined as effects of cover crop and fertilizer source in a no-tillage dryland system at Pontotoc, MS.

Effect	Df	C (g kg ⁻¹)	N (g kg ⁻¹)	WSA %	EE-GRSP (mg g ⁻¹)	POXC (mg kg ⁻¹)	pН	Yield (kg ha ⁻¹)
Early Plant	ting Date							
Y ¹	2	< 0.0390	0.0413	< 0.0001	0.0097	0.0075	< 0.001	< 0.001
CC	4	< 0.2452	0.1466	0.3696	0.2566	0.5448	0.0123	0.4356
Y* ² CC	8	0.9997	1.0000	0.7305	0.9993	0.9941	0.9579	0.8835
F	2	0.0265	0.0002	0.0978	0.2134	0.0066	0.0044	< 0.0001
Y*F	4	0.6733	0.7078	0.7682	0.8216	0.4450	0.1176	< 0.0001
CC*F	8	0.4397	0.3402	0.1950	0.3836	0.0462	0.0797	0.2760
Y*CC*F	16	0.1640	0.2622	0.7265	0.3456	0.3407	0.9422	0.9361
Late Plant	ing Date							
Y	2	< 0.001	< 0.001	0.0002	< 0.001	0.0089	0.0006	0.0686
CC	4	0.8794	0.7914	0.5534	0.7464	0.9088	0.2786	0.3837
Y*CC	8	0.9003	0.9331	0.3842	0.9904	0.3723	0.9593	0.9631
F	2	0.0189	0.0004	0.3615	< 0.001	0.0276	0.0006	< 0.001
Y*F	4	0.4436	0.4131	0.0085	0.0366	0.2616	0.2463	0.0404
CC*F	8	0.4647	0.3534	0.1986	0.0419	0.4682	< 0.001	0.9979
Y*CC*F	16	0.8793	0.7221	0.7623	0.8709	0.9692	0.0971	0.3407

¹ Abbreviations: C = total carbon; N = total nitrogen; WSA = water stable aggregate; EE-GRSP = easily extractable-glomalin-related soil protein; POXC = permanganate oxidizable carbon; LAI = leaf area index; Y = year; CC = cover crop; F = fertilizer source and ² Interaction between factors.

3.1. Cover Crop Production

Cover crop production in the early-terminated cover crop was higher in 2019 compared to 2018 and 2020. This could be due to the abundant rainfall in the spring and nutrient carry-over in soil and plant residue from 2018. The yields of wheat, CC-mix and cereal rye biomass were greater than native vegetation and vetch in both early and late-terminated cover crops (Table 4). Biomass yield with vetch and native vegetation was not different (Table 4). Cover crop production was higher with late termination because it was allowed to grow for approximately 4 weeks longer compared to the early-terminated cover crop. In addition, fertilizer was applied to both early and late planting dates at the early planting termination. This would also have benefited the growth of cover crops in the late-terminated cover crop during this four-week time period. In 2019, cover crop production was not different from native vegetation in both early (ranged from 1953 to 3639 kg ha⁻¹) and late-terminated cover crops (ranged from 2989 to 4408 kg ha⁻¹) (Table 4). However, vetch biomass was lower compared to other cover crops at early termination. Overall, cover crop production were typically higher than native vegetation except at early termination in 2019 (Table 4).

In 2018, cover crop production was collected only from no-fertilizer plots. Fertilizer source showed no effect on cover crop production in 2019 but had a significant effect in 2020 (Figure 1). In early-terminated cover crop, cover crop production accumulation was high with poultry litter (2195 kg ha⁻¹) and inorganic fertilizer (1966 kg ha⁻¹) compared to no fertilizer (993 kg ha⁻¹) (p < 0.0001) (Figure 1a) in 2020. In late-terminated cover crop, the highest cover crop production was with poultry litter (4056 kg ha⁻¹) followed by inorganic fertilizer (3233 kg ha⁻¹) compared to no fertilizer in 2020 (2152 kg ha⁻¹) (p < 0.0001) (Figure 1b). The biomass accumulation was higher in late-terminated cover crop compared to early-terminated cover crop.





3.2. Total Carbon and Total Nitrogen

Including cover crops in farming showed no effect on total C and total N in both early and late planting dates (Table 6). Total C ranged from 11.8 (g kg⁻¹) to 18.3 (g kg⁻¹) in early planting date and 17.2 (g kg⁻¹) to 18.0 (g kg⁻¹) in late planting date. Total N ranged from 1.6 (g kg⁻¹) to 1.9 (g kg⁻¹) and 1.8 (g kg⁻¹) to 1.9 (g kg⁻¹) in early and late planting dates, respectively. With increases in cover crop production, higher organic matter addition, an increase in total C and total N was expected especially in the late planting date. Total C was not influenced by poultry litter and inorganic fertilizer. Total N content was 6% higher with poultry litter compared to no fertilizer (p < 0.0018). Inorganic fertilizer had a similar effect on total N compared to no fertilizer, indicating less effect of inorganic fertilizer on total N than poultry litter. In the late planting date, poultry litter influenced the total N by 11% compared to no fertilizer (p < 0.0197) (Table 7). The effect of inorganic fertilizer was similar to both poultry litter and no fertilizer.

Table 6. Main effects of cover crop on mean soil total carbon (C), total nitrogen (N), permanganate oxidizable carbon
(POXC), easily extractable glomalin-related soil protein (EE-GRSP), water-stable aggregate (WSA), soil pH and soybean
yield with <i>p</i> -value at early and late planting dates.

Cover Crop	Total C ¹ (g kg ⁻¹)	Total N (g kg ⁻¹)	POXC (mg kg ⁻¹)	EE-GRSP (mg g ⁻¹)	WSA %	pH	Yield (kg ha ⁻¹)		
Early-Planting Date									
Cereal Rye	17.1 (0.6)	1.7 (0.0)	595 (26)	89 (3)	54 (32)	5.84 (0.07)	3641 (293)		
CC-mix ²	18.3 (0.6)	1.9 (0.1)	598 (24)	97 (3)	54 (2)	5.75 (0.07)	3787 (251)		
Native Veg. ³	16.0 (0.5)	1.6 (0.1)	541 (18)	89 (2)	56 (2)	5.94 (0.07)	3790 (257)		
Vetch	11.8 (0.7)	1.9 (0.1)	560 (22)	89 (3)	54 (2)	5.63 (0.07)	3773 (269)		
Wheat	16.0 (0.5)	1.6 (0.0)	537 (21)	87 (3)	51 (2)	5.94 (0.06)	3623 (291)		
<i>p</i> -value	0.6306	0.5288	0.8171	0.6101	0.5093	0.1708	0.9538		
			Late-Plan	ting Date					
Cereal Rye	17.7 (0.7)	1.9 (0.1)	580 (22)	83 (3)	61 (2)	5.71 (0.06)	3228 (136)		
CC-mix	17.4 (0.7)	1.9 (0.1)	562 (20)	80 (3)	59 (2)	5.76 (0.09)	3160 (130)		
Native Veg.	17.2 (0.6)	1.8 (0.0)	548 (22)	80 (3)	57 (1)	5.89 (0.10)	2993 (137)		
Vetch	17.7 (0.7)	1.9 (0.1)	564 (23)	82 (4)	59 (2)	5.64 (0.09)	3222 (145)		
Wheat	18.0 (0.7)	1.9 (0.1)	578 (24)	77 (2)	60 (1)	5.82 (0.08)	2995 (133)		
<i>p</i> -value	0.9705	0.9389	0.8477	0.9037	0.5268	0.7360	0.6624		

¹ Variables in column with no letters are not significant at the 0.05 level using Fisher's protected LSD; ² CC-mix = cereal rye and mustard;

³ Native Veg = Native vegetation; Standard error in parenthesis.

Table 7. Main effects of fertilizer source on soil total carbon (C), total nitrogen (N), permanganate oxidizable carbon (POXC), easily extractable glomalin-related soil protein (EE-GRSP), water stable aggregate (WSA) and soil pH.

Fertilizer Source	Total C ¹ (g kg ⁻¹)	Total N (g kg ⁻¹)	POXC (mg kg ⁻¹)	EE-GRSP (mg g ⁻¹)	WSA %	рН
E						
Inorganic	16.8 (0.5) ² a	1.7 (0.0) b	558 (23) a	89 (2) a	52 (2) a	5.76 (0.06) a
No Fertilizer	16.7 (0.5) a	1.7 (0.0) b	550 (15) a	89 (2) a	54 (2) a	5.90 (0.05) a
Poultry Litter	17.8 (0.5) a	1.8 (0.0) a	592 (18) a	93 (2) a	55 (2) a	5.80 (0.05) a
<i>p</i> -value	0.0727	0.0018	0.0843	0.2672	0.2297	0.1296
1	Late-Planting Dat	e				
Inorganic	17.6 (0.5) a	1.9 (0.0) b	557 (15) a	80 (2) b	60 (1) a	5.71 (0.07) b
No Fertilizer	17.0 (0.5) a	1.8 (0.0) ab	550 (18) a	75 (2) b	59 (1) a	5.91 (0.07) a
Poultry Litter	18.3 (0.5) a	2.0 (0.0) a	580 (16) a	85 (2) a	58 (1) a	5.66 (0.06) b
<i>p</i> -value	0.1962	0.0197	0.3629	0.0010	0.5973	0.0030

¹ Variables with different letters in column are significantly different and variables with same letter are not significant at the 0.05 level using Fisher's protected LSD; ² Standard error in parenthesis.

3.3. Permanganate Oxidizable Carbon

Both cover crop and fertilizer showed no significant effect on POXC in early and late planting dates (Tables 6 and 7). The POXC in early planting date ranged from 537 to 598 mg kg⁻¹ and in late planting date it ranged from 546 to 580 mg kg⁻¹. It was expected that POXC would be higher in cover crops plots compared to the native vegetation plots.

3.4. Easily Extractable Glomalin-Related Soil Protein

Easily extractable glomalin-related soil protein (EE-GRSP) was not influenced by cover crops in both planting dates. Fertilizer source showed a significant effect on EE-GRSP in late planting date. In the late planting date, with poultry litter addition, EE-GRSP increased

by 13% compared to no fertilizer (p < 0.001) (Table 7). There was no difference between the no-fertilizer addition and inorganic fertilizer treatments in either planting date group (Table 7). Cover crops showed no effect on EE-GRSP in three years of study while poultry litter showed an effect on late planting date. This may be because of the availability of energy from poultry litter for a longer period than an early planting date.

3.5. Water Stable Aggregate

There was no difference in water stable aggregates (WSA) among cover crops and fertilizer sources in both early and late planting dates (Tables 6 and 7). The average WSA was 54 and 59 mg kg⁻¹ in early and late planting dates. Water stable aggregates are formed by organic matter and biological activities. The biological activities and organic carbon may not be enough to show significant effects of cover crop and inorganic fertilizer in three years of study in dryland soybean farm.

3.6. Soil pH

Cover crops had no effect on soil pH in early planting (Table 6). There was cover crop and fertilizer interaction in the late planting date. Higher soil pH was with native vegetation x no fertilizer (6.3) compared to cover crop and fertilizer interaction (p < 0.005). Lower soil pH was with vetch × poultry litter (5.3). Soil pH with vetch was low compared to native vegetation in both early and late planting dates. Fertilizer source did affect soil pH in the late planting date (Table 7). In the late planted soybean area, soil pH was 0.25 units lower with poultry litter compared to no fertilizer treatment (Table 7). There was no difference between no fertilizer and poultry litter treatment (Table 7). Inorganic fertilizer and no fertilizer effect were similar. In the poultry litter applied plots, cover crop growth was relatively higher than no fertilizer.

3.7. Soybean Yield

Cover crops did not affect soybean yield in both early and late-planted soybeans in the three years of the study (Table 6). The use of fertilizer had a positive effect on both early and late planting dates. At the early planting date, the yield increased by 68% and 51% with poultry litter and inorganic fertilizer compared to no fertilizer, respectively (Figure 2a). Soybean yield with poultry litter and inorganic fertilizer increased by 42% and 40% compared to no fertilizer in late-planted soybean, respectively (Figure 2b).



Figure 2. Fertilizer source effect on soybean yield (**A**) early planting date and (**B**) late planting date. The soybean yield is the average of two years (2019 and 2020). In 2018, heavy rainfall greatly reduced the soybean yield and thus these data were not included. Different letters indicate significant differences at $p \le 0.05$.

4. Discussion

4.1. Cover Crop Production

The variation in rainfall and temperature might be a possible reason for the variation in biomass yield between years [64]. Furthermore, the selected mustard variety had low cold-tolerance in the fall of 2017 and it did not survive through the winter months to serve as a cover crop in 2018. The increased cover crop production in 2019 could be due to the high rainfall during the cover crop growth period. More biomass in cereal rye and wheat compared to native vegetation was also reported by Lawson et al. [21], where the biomass with rye and rye–vetch mix was on average 62% higher compared to vetch alone on Briscot loam in a six-year study near Washington State University. In addition, the biomass yield was higher with late versus early-terminated cover crop, which was also similar to the results of Lawson et al. [21]. Other research indicates that an accumulation of cover crop production in the soil surface will increase organic matter [23]. In a dryland system, the addition of organic matter could increase the water-holding capacity because it can retain about 20 times more water than its weight [65]. Our results showed that, overall, cereal rye, CC-mix and wheat added more cover crop biomass to the soil compared to native vegetation.

4.2. Total Carbon and Nitrogen

Total C was expected to be high at the late planting date compared to the early planting date due to the accumulation of more cover crop biomass. Total N when poultry litter was applied was 11% higher than the no-fertilizer and inorganic fertilizer treatments (Table 7). Similar results with no considerable effects of cover crop on total C and total N were reported by Acuna and Villamil [66] in two locations in Illinois. Olson et al. [67] reported no effect of cover crops on C content in an eight-year study. Watts et al. [68] also reported higher N accumulation with poultry litter in northeast Alabama. However, some cover crop studies showed an increase in total C and total N in short-term studies. Tao et al. [68] observed increased soil organic matter and total N with the addition of hairy vetch on a loam soil. Muchanga et al. [69] reported a 3% increase in C content over a two-year period with hairy vetch as a cover crop in a high-tunnel tomato system. Harun [70] showed a 26% increase in soil organic C with winter wheat compared to control in a laboratory study on a Cumberland silt loam (fine, mixed, semiactive, thermic Rhodic Paleudalfs) in Tennessee. Garcia-Gonzalez et al. [71] showed increased C and N with barley and vetch cover crop in a 10 year study on irrigated Topic Calcisol soil. This contrast with our results might be due to the slow effects of cover crop on total C and total N in a dryland system. Cover crops supply a C energy source to soil microbes and after cover crop termination, soil microbes decompose the crop residue. During the cover crop decomposition process, soil microbes respire large amounts of CO2 and only a small fraction of C is added to the soil, which results in a slow build-up of total C [72]. Another reason could be the rapid mineralization of C due to the soil type and the hot and humid climate of Mississippi [53,73]. This shortterm field study was subjected to various other factors such as irregular rainfall patterns and temperature variations, which could also be a reason that the results from our study disagree with others. In addition, the study used a no-tillage system, meaning that the cover crop production was not incorporated into the soil and the sample was collected after removing the surface biomass and this results in a reduced effect of cover crop on soil C. Adeli et al. [74] reported a 19% increase in total C with poultry litter compared to no-added-fertilizer treatments on this soil at the same branch station over three years. Parker et al. [75] also reported increased total C in soil over a four-year period with poultry litter and cover crops in Alabama, suggesting a longer time frame may be necessary to see a difference in treatments. We were applying about 3.2 g of N kg^{-1} of poultry litter and the increased N content with poultry litter in our study might be due to the build-up of N over time since an average of 40–70% of the nitrogen in poultry litter is released within six weeks of application and soil samples were collected after four weeks of poultry litter application [75].

4.3. Permanganate Oxidizable Carbon

Cover crop and fertilizer source had no effect on permanganate oxidizable C (POXC) content at both planting dates. These results agree with Jagadamma et al. [76] who reported no difference in POXC in the top 15 cm but they did see a difference in the top 0–7.5 cm of soil with crop rotation and cover crop management practices at three locations in Tennessee. White et al. [77] reported a 26% increase in POXC with cover crop and manure in California after eight years of study and Jokela et al. [78] observed increases in POXC with cover crop but no effect with manure addition on a Bertrand silt loam after 13 years study in Wisconsin. Steele et al. [79] also reported a significant effect on POXC with cover crop in a no-tillage system on Coastal Plains silt loam but not on Piedmont line loam after 12 years. Soil texture directly influences C content in the soil and POXC is a fraction of C [80,81]. In clay soils, POXC binds to clay particles and makes it less available to soil microbes but in sandy and silty soils, POXC is easily available to soil microbes for conversion into more stable C forms [82]. The reason for the lack of an effect could be due to the increased microbial abundance by treatments that could have rapidly oxidized and transformed POXC to a stable form of soil organic C [82].

4.4. Easily Extractable Glomalin-Like Soil Protein

Cover crops had no influence on easily extractable-glomalin-related soil protein (EE-GRSP) at both early and late planting dates. However, Garcia-Gonzalez et al. [50] reported increased EE-GRSP with a vetch–barley mix compared to fallow after two years. Balota et al. [83] also observed a 50% increase in EE-GRSP with a cover crop and no-tillage system after 23 years. Precipitation directly affects the AMF hyphal length, a warm moist environment can greatly influence AMF population and decline hyphae viability [84,85]. Since EE-GRSP is produced in hyphae, a decline in hyphae viability reduces EE-GRSP production. High moisture reduces AMF population; the reason for this could be the decreased availability of energy sources for AMF and other anaerobic fungi may have suppressed AMF population [86].

Fertilizer sources showed a significant effect on EE-GRSP in late-planted soybean areas. EE-GRSP is produced by AMF and it works as a binding agent of water-stable aggregate, which makes it an indirect method of predicting soil aggregation and fungal population. Studies have shown a correlation between EE-GRSP and AMF [87–89]. About one-third of soil microbial biomass is occupied by AMF and its activity influences EE-GRSP production [90]. Zhang et al. [51] reported that poultry litter improved fungal energy sources and habitat and enhanced hyphae development for EE-GRSP production.

4.5. Water Stable Aggregate

There was no difference in water-stable aggregates (WSA) among cover crops and fertilizer sources at both early and late planting dates (Tables 6 and 7). Sanchez de Cima et al. [56] reported decreased WSA due to high rainfall. Irregular rainfall with varied intensity dispersed soil particles and reduced soil aggregation [91]. There was irregular and highly intensive rainfall in 2019, which could have been a reason for the reduction in WSA (Table 1). Another reason for reduced WSA could be the high rate of organic matter decomposition by soil microbes in the second year. Hetrick et al. [91] reported a lower level of soil aggregation with a decrease in organic matter. Song et al. [92] noted an accumulation of C due to crop residue left on the soil surface with no-tillage, which increased WSA by 35.18% compared to conventional tillage after three years on estuarine alluvial soil. In another study, WSA increased by 55% with a cereal rye compared to no cover crop in southeast Indiana [93]. The addition of organic matter added to soil in our study may not have been high enough to promote WSA content in a two-year study [94].

WSA content was influenced by fertilizer source. Our study disagreed with statements by USDA-NRCS [95] and Liu et al. [46] who reported that WSA improves with high C, EE-GRSP and more biological activity with poultry litter, as these are binding agents of soil

particles. Soil biological activities are responsible for the formation of soil aggregates [96]. The increased WSA with poultry litter may be due to the high POXC, which provides a readily available source of energy for soil microbes to catalyze their activities [97]. Another reason might be the higher production of EE-GRSP with poultry litter that binds the soil particles [45]. Furthermore, soil microbial mycelia and root mucilage boost aggregation [98]. Adeli et al. [74] reported a 7% increase in WSA with poultry litter compared to no fertilizer on Atwood loam after four years in a no-tillage system. The shorter period of our study might be a reason for the lack of effects of cover crop and fertilizer source on WSA.

4.6. Soil pH

The soil pH did not show a significant difference among the treatment plots over the three years study. Vetch-poultry litter plots showed a modest low pH compared to no fertilizer–native vegetation treatment, which was not consistent among planting dates and years. Vetch produces ammonia by using N from the air rather than the soil, resulting in a lower soil pH, which might be a reason for the lower pH with vetch [99]. The results of this study was in agreement with Liebig et al. [64], who reported no effect of summer cover crops on soil pH after three years of late-seeded cover crops.

4.7. Soybean Yield

Cover crops did not affect soybean yield in both early and late-planted soybeans in our three years study period. Similar results were found by Acuna and Villamil [66] and our results also agreed with Freitas et al. [100] who showed that the use of cover crops did not affect the no-till soybean yield on Rhodic Hapludox soil in a dryland system. Fertilizer use showed a significant effect on soybean yield for both early and late-planted soybean. Similar results were reported by Ayolagha and Peter [101] on Ultisols of Ogoni land in the Niger Delta. Our results suggest that the impact of cover crops and fertilizer on biologically related soil properties was strongly influenced by rainfall and temperature during the growing period.

5. Conclusions

This study represents, to our knowledge, one of the few studies on the cover crop and planting date impacts on biologically related soil properties in dryland soybean production in the southeastern USA. The main goal of this study was to evaluate the agronomic impacts of winter cover crops, soybean planting date and fertilizer management on soil properties. Soybean yields were not affected by cover crop and fertility treatments, perhaps because the agronomic benefits of these practices on dryland soybean production may take several years to appear. While the short-term effects of cover crop and organic fertilizer combinations appear promising with respect to soil fertility and yield, additional long-term experiments in no-tillage dryland soybean production are clearly needed.

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