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Winter cover crop impact on soil health in Texas Rolling Plains dryland cotton

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Abstract

The Rolling Plains of Texas are historically semi-arid with sporadic, high intensity storms followed by periods of long drought. Fallow management is a common practice intended to store soil water, but leaves the bare soil exposed to erosive forces that can diminish the soil productivity. Cover crops in no-till (NT) agriculture have been proposed to increase soil health under environments with low precipitation as an alternative to fallow management. This study evaluated multiple treatments in a dryland cotton system including: (1) conventional tillage (CT); (2) NT; and NT with the following cover crops: (3) wheat; (4) Austrian winter pea; (5) crimson clover; (6) hairy vetch; and (7) mixed species cover. Soil samples were collected at 0, 3, and 6 weeks after cover crop termination and analyzed for soil organic carbon, total nitrogen, inorganic N, water-extractable organic carbon, water-extractable organic nitrogen, carbon mineralization, and phospholipid fatty acid analysis. For all parameters tested, there was no significant difference between CT and NT at any date or depth, so the addition of cover crops to NT cotton systems might be needed in order to enhance NT in regard to soil function. The multi-species mixed treatment was predicted to perform the best out of the cover crop treatments due to its combined benefits from grasses and legumes. However, the single-species Austrian winter pea treatment had 24% and 28% higher soil carbon and nitrogen than no-till without a cover crop, and can be a useful alternative to fallow management under these dryland agriculture conditions.

Abbreviations: AP, Austrian winter pea; C, carbon; CC, crimson clover; CMIN, carbon mineralization; CT, conventional tillage; HV, hairy vetch; MC, mixed species cover; N, nitrogen; NASS, National Agriculture Statistic Service; NRSC, Natural Resources Conservation Service; NT, no-till; PLFA, phospholipid fatty acid; SOC, soil organic carbon; TN, total nitrogen; USDA, United Stated Department of Agriculture; W, winter wheat; WEOC, water-extractable organic carbon; WEON, water-extractable organic nitrogen.

1 | INTRODUCTION

Today, roughly 2.2 million hectares of Texas land has been developed for cotton (*Gossypium hirsutum)* cultivation, which is significant because it makes up about 50% of the overall cotton acreage in the United States (USDA NASS, 2018). For decades, the southern Great Plains has seen cotton monoculture development due to low grain prices, increased cost of irrigation, favorable US government farm programs of 1985 and 1990, and historically a lack of insect pests (Allen et al., 2008). However, recent changes in agricultural policy have raised concerns about the profitability and financial viability of current cropping practices (Allen et al., 2008).

The southern Great Plains is often stressed with extreme drought conditions, including: hot temperatures, low precipitation, and generally unfavorable growing conditions for agricultural production (Guerro, 2011; Lane & Nichols, 1999; Lydolph, 1985). High levels of temporal and spatial climate variability with recurring periods of severe droughts have led to widespread crop failure with little residue cover (Fannin, 2012; Hansen et al., 2012). The limited precipitation that does occur usually comes in the form of intense and localized rainstorms in the late spring and summer. These intense precipitation events can cause losses of soil and nutrients in runoff (Blanco-Canqui et al., 2013). It has been difficult to achieve residue buildup and increased soil organic carbon (SOC) in fields that have been converted to NT due to that fact that crop residues rapidly degrade (Novara et al., 2016; Rasmussen et al., 1998). Current planting and tillage practices leave the soil fallow during winter, a practice that is intended to store soil water, but leaves the bare soil exposed to erosive forces that can diminish the soil productivity (Kaspar & Singer, 2011). The low biomass return of cotton, high tillage rates, and fallow periods have been shown to decrease SOC, soil organic matter, and aggregate stability, leading to soil degradation and erodibility (Blanco-Canqui et al., 2013; Peterson et al., 1998). Fallow management has been used to stabilize crop production, store soil water for subsequent crops, and reduce the chances of crop failure by forfeiting production in one season in anticipation that there will be at least partial compensation by increased crop production the next season (Nielson & Calderón, 2011; Nielson & Vigil, 2010).

Using NT management in tandem with growing cover crops as a replacement to fallow management could improve soil structure, SOC, water infiltration, water retention, and root penetration while enhancing soil microbial communities and nutrient cycling; however, these benefits are heavily influenced by soil moisture (Blanco-Canqui et al., 2013; Clark et al., 2009; Seepaul et al., 2023). No-till management with cover crops increasing the water infiltration rates of the soil can be a solution to efficiently capturing and storing more precipitation from the sporadic and unreliable storms that are common in the southern Great Plains (Blanco-Canqui et al., 2013). Cover crops can also be a defensive strategy to reduce soil crusting, soil erosion, runoff, and nutrient leaching (Seepaul et al., 2023), while also providing weed suppression by outcompeting weeds for light, water, and soil nutrients (Blaco-Canqui et al., 2015).

Core Ideas

- Offseason cover crops can replace fallow management in dryland, rainfed cotton systems in Texas.
- Cover crops added to no-till agriculture cotton systems enhance soil function.
- Single-species legume cover crop indicated higher carbon mineralization than single-species grass cover crop.
- Carbon and nitrogen values for no-till with Austrian pea were 24% and 28% higher than no-till without cover crops.
- Austrian winter pea showed greatest numerical increase of soil health improvements.

The major challenge within sustainable soil management is to conserve ecosystem service delivery while optimizing agricultural yields. Nivelle et al. (2016) demonstrated after 5 years that winter cover crops with NT management had a positive effect on the upper soil carbon (C) content, microbial enzymes, and microbial functional diversity irrespective of nitrogen (N) fertilization. Finney et al. (2017) demonstrated that there are species-specific relationships between cover crops and microbial communities, such as cereal rye and oats being associated with increases in arbuscular mycorrhizae fungi (AMF), while hairy vetch and red clover were associated with increases in non-AMF soil microbial communities. Further examination of the cover crop impacts on soil chemical parameters and biological indicators will help researchers understand the potential advantages and disadvantages of using cover crop monocultures or mixtures.

There have been recent suggestions that diverse cover crop mixtures offer more advantageous ecosystem services from enhanced soil microbial activity when compared to single-species cover crops (Calderón et al., 2016). Legume cover crops tend to decompose more rapidly than non-legume cover crops, which can reduce legume cover crop residue effectiveness at protecting the soil surface and moderating soil temperatures compared with grass cover crops (Blanco-Canqui et al., 2013). Cellulose-rich plants or plant parts degrade far more rapidly than if they were mature grasses with a higher lignin content. Hence, leafy portions of the shoot system degrade far more rapidly than the supportive stems (Edwards & Burney, 2005). The grass component scavenges residual N effectively,

while the legume adds biologically fixed N that is more readily available to the cash crop (Clark, 2008; Meisinger et al., 1991). Agronomic and soil responses from a 3-year study in Tennessee found that a multi-species mixture of legumes, grasses, and *Brassica* spp. significantly increased soybean yield, gravimetric soil water content, and soil inorganic N as compared to the less-diverse treatments and fallow control (Chu et al., 2017). A mixture of cover crop species can increase SOC more than a single-species treatment due to a greater biomass production above and below the soil (Blanco-Canqui et al., 2015; Faé et al., 2009). Comparisons between monoculture and mix species cover crops were tested in eastern Colorado and found no significant difference in water-use efficiency or dry-matter productions between both cover crops strategies (Nielsen et al., 2015). Wortman et al. (2013) determined in western Nebraska that mixtures of two or more cover crops are often more effective at weed suppression than planting a single-species due to a diversity of allelopathic interactions between the cover crops and the numerous target weed species. However, studies have shown that weed suppression is dependent on the biomass accumulation of cover crops which is largely driven by fast-growing grass monocultures or mixtures in which grasses are seeded by at least 20% of the monoculture rate (Baraibar et al., 2018; MacLaren et al., 2018). Disadvantages of cover crop mixtures may include higher seed cost, too much residue, more complicated management, and a difficulty to seed (Clark, 2008).

Based on these assumptions, we hypothesized that (1) cover crops are a better management strategy to increase soil C and N pools than tillage-dependent fallow treatments; and (2) multi-species cover crops foster more soil health benefits than single-species cover crops. The objectives of this study were to determine the effectiveness of implementing cover crops as an alternative to fallow management and their associated impacts in semi-arid Texas cropping systems.

2 | MATERIALS AND METHODS

2.1 | Experimental design

The evaluation of various cover crop options within a continuous cotton cropping system occurred at the Texas A&M AgriLife Chillicothe Research Station near Chillicothe, Texas (MRLA 78B; USDA Natural Resources Conservation Service, 2022). The test site is on a Grandfield soil series with a soil type that is described as a fine sandy loam with 0%–1% slope. Average annual precipitation (1981–2010) for the region is 710 mm. The average annual temperature is 17.2˚C as recorded by NOAA 22 km northeast of the Chillicothe Research Station (NOAA, 2018). A multi-year continuous cotton study has been ongoing at this site testing no-till (NT) and conventional till (CT) as well as various cover crop treatments with NT practices (DeLaune & Mubvumba, 2020). Soil samples were collected during the fifth year of cover crop establishment.

A randomized complete block design with four replicates was used with rainfed cotton in semi-arid environmental conditions, however the first three replicates were analyzed in this study. Plot sizes were 12.2 m × 8.1 m with 1 m spacing. Evaluated treatments included: (a) CT; (b) NT; and NT with the following cover crops; (c) hard red winter wheat (W); (d) Austrian winter field pea (AP); (e) crimson clover (CC); (f) hairy vetch (HV); and (g) mixed species cover (MC). After harvest each year, cotton stalks would be mechanically shredded. Thereafter, cool-season cover crops were planted with a NT drill with 25 cm spacing. Seeding rates were 39.2 kg ha^{-1} for AP, 22.4 kg ha^{-1} for CC and HV, and 33.6 kg ha^{-1} for W. The MC was planted at 33.6 kg ha^{-1} and comprised of rye (13.4 kg ha^{-1}), W (10.1 kg ha^{-1}), AP (6.7 kg ha^{-1}), and HV (3.4 kg ha^{-1}). The cover crops in this study were planted on November 22, 2016 and later terminated on April 20, 2017. The cotton cash crop was planted on May 30, 2017 and was not harvested due to total crop failure.

The bare soil fallow period for the CT and NT treatments for this study was approximately 6 months. The CT received the initial stalk shredding to disperse any cotton debris, then was followed by tillage with a four-row offset disc implement to a depth of approximately 10–15 cm (4–6 in.). The tillage occurred two different times during the winter. Before planting cotton, the conventional plot was reshaped with a bedder. After the cotton was planted, a field cultivator with 41 cm sweeps was used for cultivation between cotton rows. All of the treatments received an herbicide application to terminate cover crops or weeds. Glyphosate was applied at 2.3 L kg^{-1} and dicamba was applied at 0.6 L kg^{-1}. No fertilizer or irrigation was applied to any treatment at any time during this study.

2.2 | Sampling

Soil sampling times were planned around the herbicide termination of cover crops and cotton planting. The first soil sampling date was April 20, 2017 (0 weeks after the herbicide termination), May 9, 2017 (3 weeks after the herbicide termination), and May 30, 2017 (6 weeks after the herbicide termination and before cotton planting).

Soil samples (~400 g) were collected with handheld 2.54-cm diameter soil core sampler tools at two depths (0–10 cm and 10–20 cm) from three replicates of all seven treatments. Soil was homogenized by hand and collected in paper bags suitable for oven drying. A subsample (20 g) from the topsoil (0–10 cm) was taken for the phospholipid fatty acid (PLFA) analysis. The PLFA subsamples were shipped immediately to Ward Laboratories, Inc. (Kearney, NE, USA) for PLFA analysis. The remaining soil for each treatment sample was oven-dried at 60˚C for 3 days and passed through a 2-mm sieve to remove large organic debris.

2.3 | Soil physiochemical analysis

An aliquot of oven-dried, sieved soil from each treatment sample was used to determine SOC (30 mg) and total nitrogen (TN; 500 mg) concentration using an Elementar Vario Max elemental analyzer (Elementar, Langenselbold, Germany) by combustion elemental analysis (McGeehan & Naylor, 1988).

Soil inorganic N was extracted from a 2 g aliquot of oven-dried soil with 20 mL of 1 M potassium chloride (KCl).The soil + KCl solution was shaken for 1 h at 160 oscillations per minute and then filtered with Whatman No. 42 filter paper into 20 mL plastic scintillation vials (Keeney & Nelson, 1982). The filtrate was analyzed using a Skalar SANS^{++} segmented flow analyzer (Skalar, Breda, The Netherlands) for ammonium (NH_4^+) and total nitrite + nitrate (NO_2^- + NO_3^-) concentrations (Dorich & Nelson, 1983; Keeney & Nelson, 1982).

2.4 | CO_2 flush experiment

Another subset of oven-dried soil samples was passed through a 4.75 mm sieve and used to determine soil microbial respiration as an indicator of soil health. The method from Franzluebbers (2016) was used to calculate the soil microbial respiration as a measurement of carbon mineralization (CMIN) with the following exceptions. The 1 M NaOH alkali trap was diluted to 0.5 M NaOH in order to increase the sensitivity of the procedure since preliminary analysis had indicated low levels of CO_2 emission from the soils in this study. The water that was added to the dry soil in this study was applied in three layers: bottom, middle, and top.

2.5 | Water-extractable organic carbon and water-extractable organic nitrogen

Water-extractable organic C (WEOC) and water-extractable organic N (WEON) were determined from 4 g of oven-dried soil with 40 mL of deionized water and shaking for 10 min on a mechanical shaker at 160 oscillations per minute. Samples were then centrifuged for 5 min at 2095 rcf (3500 rpm), filtered through Whatman 2 V paper (Haney et al., 2012), and analyzed for WEOC and WEON using Elementar TOC Select (Langenselbold, Germany).

2.6 | Phospholipid fatty acid analysis

The PLFA analysis was conducted by Ward Labs, Inc. according to Hamel et al. (2006) with slight modifications. Total soil lipids were extracted in test tubes by shaking 2 g (dry weight equivalent) of frozen soil in 9.5 mL dichloromethane (DCM):methanol (MeOH):citrate buffer (1:2:0.8 v/v) for 1 h. Then 2.5 mL of DCM and 10 mL of a saturated KCl solution were added to each tube and shaken for 5 min. Tubes were then centrifuged at 1008 rcf (3000 rpm) for 10 min. The organic fraction was pipetted into clean vials. Lipid-class separation was conducted in silica gel columns and the vials washed twice with a small amount of DCM using a pipette. The neutral, glyco-, and phospholipids fractions were eluted by sequential leaching with approximately 2 mL of DCM, 2 mL of acetone, and 2 mL of methanol, respectively. The neutral and glycolipid fraction was discarded and the phospholipids fractions were collected in separate 4 mL vials. These fractions were dried under a flow of N_2 at 37 ± 1°C in a fume hood. The dried fractions were dissolved in a few mL of MeOH for PLFA and stored at −20°C. Samples were analyzed using an Agilent 7890A GC with a 7693 autosampler and a flame ionization detector. The abundance of individual PLFAs was expressed as ng PLFA g^{-1} dry soil (Hamel et al., 2006).

Selected terminal-branched saturated PLFAs (i15:0, a15:0, i16:0, a16:0, i17:0, and a17:0) were used as markers for Gram-positive (Gram$^+$) bacteria (Federle, 1986; Zelles, 1997). Selected monounsaturated and cyclopropyl-saturated PLFAs 16:1ω5, 16:1ω9, 17:1ω9, cy17:0, 18:1ω11, and cy19:0 was used to represent Gram-negative (Gram$^-$) bacteria and the PLFA 14:0, 15:0, and 17:0 for unspecific bacteria (Federle, 1986; Frostegård et al., 1993; Zelles, 1997). The polyenoic, unsaturated PLFA 18:2ω6c was used as an indicator of fungal biomass (Federle, 1986; Frostegård & Bååth, 1996; Huang et al., 2011). The PLFA 16:1ω11 or 20:0 was used to represent arbuscular mycorrhizal fungi (Huang et al., 2011; Olsson, 1999). The biomarkers for PLFA 20:3 at 6 and 20:4 at 6 was used as an indicator for protozoa biomass (Cavigelli et al., 1995). The rhizobia PLFA biomarkers contained 16:0, 17:0, 18:0, and 19cycloω9C fatty acids (Jarvis & Tighe, 1994). Total bacteria was calculated as sum of Gram$^+$, Gram$^-$, and unspecific bacteria. The total PLFA biomass was calculated as the sum of all the extracted PLFAs, and reported as total ng PLFA biomass g^{-1}. Individual total ng PLFA biomass g^{-1} from each treatment was used to report which cover crop can support the highest total PLFA biomass.

2.7 | Cover crop herbage characterization

Cover crop herbage samples were randomly collected from the treatment plots before the cover crop termination. Two 0.42-m^2 quadrats of cover crop herbage were then clipped at the 5 cm height from each of NT with cover crop treatments, weighed, and dried at 65°C for dry matter determination. Total C and N content was determined using combustion analysis using an Elementar Vario Max elemental analyzer

(Elementar, Langenselbold, Germany; DeLaune & Mubvumba, 2020).

2.8 | Statistical analysis

Treatment differences were evaluated using analysis of variance (ANOVA), followed by the Fisher's least significant differences (LSD) test, and linear regression analysis. Unless otherwise noted, only significant ($p < 0.05$) interactions are discussed. Analyses were conducted with the use of JMP® Pro 13.2.1 (SAS Institute, Cary, NC).

Main effects were cover crop treatments, date, and depth with randomized replicates. Relationships among selected variables were examined by pairwise correlation analysis (Haney et al., 2012). A three-way ANOVA detected main effect interactions for WEOC (depth × treatment), WEON (depth × treatment), and CMIN (depth × treatment, date × treatment). The remaining soil chemical parameters did not detect main effect interactions for most of the parameters tested, so all the replicates from each date and depth were combined ($n = 18$ replicates) to highlight the significant treatment effects that did occur (Table S1). The soil PLFA biomass values were analyzed for two-way ANOVA for treatment and date effects, but only a date effect occurred (Table S2). Relationships among treatments samples ($n = 9$ replicates) from 0–10 cm of soil were examined by pairwise correlation analysis (Haney et al., 2012).

3 | Results

3.1 | Cover crop herbage mass and characterization

Cover crop biomass from the NT + cover crop treatments were highest in HV, AP, and MC plots as reported in DeLaune and Mubvumba (2020). The biomass from CC (383 kg ha^{-1}) was significantly lower than HV (3280 kg ha^{-1}), AP (3208 kg ha^{-1}), and MC (2393 kg ha^{-1}), but it was not statistically different than W (1956 kg ha^{-1}; Figure 1). The highest percentage of C in the cover crop biomass was from the MC treatment (43.21%) and was statistically higher than CC, but not W, AP, or HV. The highest percentage of N in the cover crop biomass was from the HV treatment (3.80%) and was statistically higher than the other cover crop treatments (Table 1).

3.2 | Soil chemicals: C

The SOC values for AP (4.6 g SOC kg^{-1} soil) were significantly higher than NT (3.5 g SOC kg^{-1} soil), CT (3.4 g SOC kg^{-1} soil), and CC (3.1 g SOC kg^{-1} soil). The MC (4.3 g SOC kg^{-1} soil) treatments indicated higher SOC values compared to CT and CC. The remaining cover crop treatments were not significantly different from NT or CT (Figure 2A).

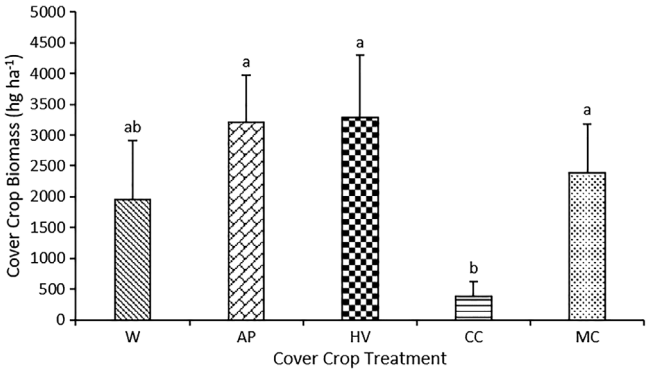

FIGURE 1 Offseason cotton winter cover crop biomass at Chillicothe Research Station as affected by treatments. Main effect means with standard deviation bars ($p < 0.05$). AP, Austrian winter pea; CC, crimson clover; HV, hairy vetch; MC, mixed species cover; W, winter wheat.

TABLE 1 Offseason cotton winter cover crop biomass C and N results at Chillicothe Research Station as affected by treatments.

Treatment	%N	%C	CN ratio
Wheat	1.02d	40.32a	40.07a
Austrian pea	2.87b	42.43a	14.93b
Hairy vetch	3.80a	41.51a	11.05b
Crimson clover	2.06c	35.72b	17.30b
Mixed cover	1.13d	43.21a	38.75a
p value	<0.0001	0.0014	<0.0001

The cover crop treatments indicated that WEOC values from AP (108 mg WEOC kg^{-1} soil) were significantly higher than NT (90 mg WEOC kg^{-1} soil), CC (87 mg WEOC kg^{-1} soil), and CT (85 mg WEOC kg^{-1} soil). The WEOC values for W (104 mg WEOC kg^{-1} soil) and MC (103 mg WEOC kg^{-1} soil) were higher than CC and CT, but not NT. (Figure 2B). The HV and CC treatments were not significantly different from NT and CT. A two-way interaction between treatment x depth occurred (Table S1). When grouped by depth ($n = 9$ replicates), significant differences were observed in the upper 0–10 cm of soil. The WEOC values for AP (130 mg WEOC kg^{-1} soil) was significantly higher than NT (99 mg WEOC kg^{-1} soil), CC (91 mg WEOC kg^{-1} soil), and CT (84 mg WEOC kg^{-1} soil). Also, the WEOC values for W (114 mg WEOC kg^{-1} soil) were significantly higher than CT (Figure 3A). In the lower 10–20 cm of soil, MC and W trended the highest but the differences were not statistically significant (Figure 3B).

The CMIN values from AP (63 mg CMIN kg^{-1} soil) were higher than the MC, W, and CC cover crop treatments. Also, AP was higher than the NT (37 mg CMIN kg^{-1} soil) and CT

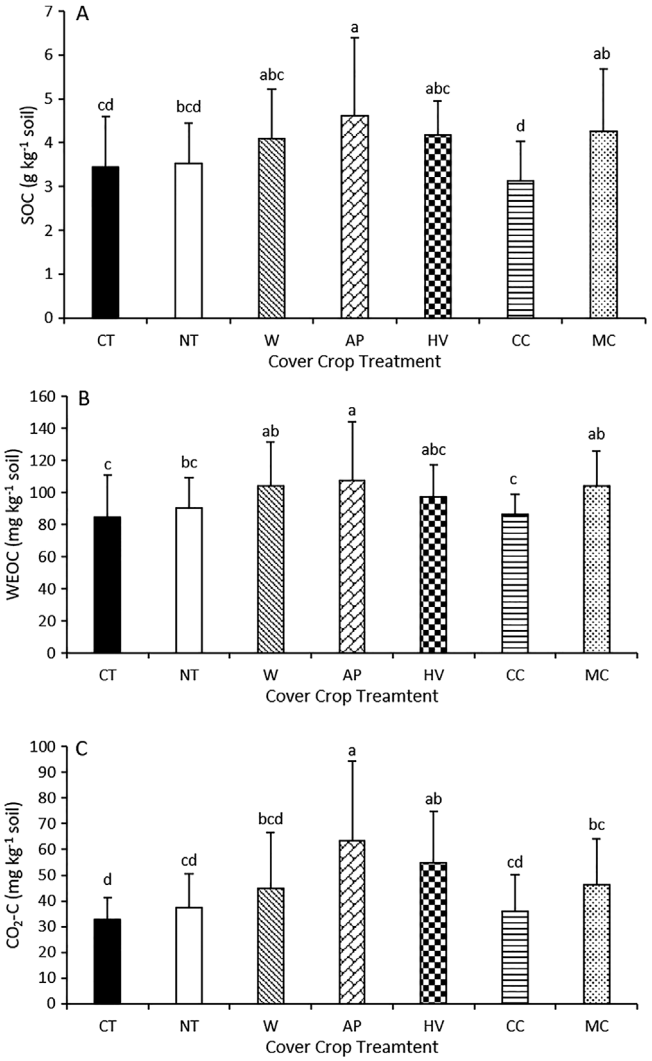

FIGURE 2 Soil carbon in 0–20 cm of soil as affected by tillage and cover crop treatments from Chillicothe Research Station measured as (A) soil organic carbon (SOC); (B) water-extractable organic carbon (WEOC); and (C) carbon mineralization (CMIN). Samples represent all dates and depths combined ($n = 18$). Main effect means with standard deviation bars ($p < 0.05$). AP, Austrian winter field pea; CC, crimson clover; CT, conventional till; HV, hairy vetch; MC, mixed species cover, NT, no-till; W, winter wheat.

(33 mg CMIN kg^{-1} soil) fallow treatments. The CMIN values from HV (55 mg CMIN kg^{-1} soil) were greater than NT, CC, and CT. Also, MC (46 mg CMIN kg^{-1} soil) indicated higher CMIN compared to CT, but was not statistically different from NT. The remaining cover crop treatments, W and CC, were not statistically different from NT or CT (Figure 2C).

Also, the CMIN values from the cover crop treatments indicated two-way interactions for treatment x date and treatment x depth (Table S1). CMIN was analyzed by separate depths by combining cover crop treatments and dates only ($n = 9$ replicates). In the upper 0–10 cm of soil, the CMIN values for AP (82 mg CMIN kg^{-1} soil) were higher than the W,

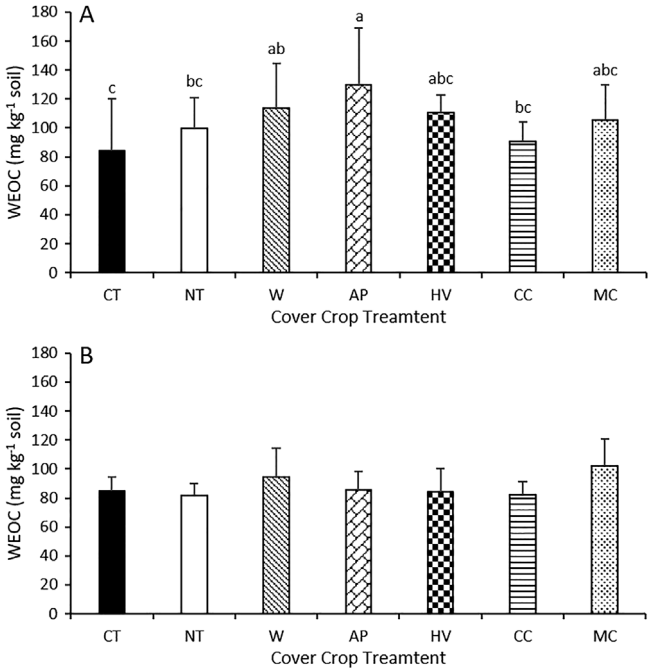

FIGURE 3 Water-extractable organic carbon separated by depth as affected by tillage and cover crop treatment from Chillicothe Research Station. Samples represent all dates combined ($n = 9$) and split by (A) 0–10 cm depth; (B) 10–20 cm depth. Statistical significance within each treatment denoted by different letters ($p < 0.05$). Error bars represent the standard deviation. AP, Austrian winter field pea; CC, crimson clover; CT, conventional till; HV, hairy vetch; MC, mixed species cover; NT, no-till; W, winter wheat.

MC, and CC cover crop treatments. Also, AP indicated significantly higher CMIN values compared to the NT (44 mg CMIN kg^{-1} soil) and CT (37 mg CMIN kg^{-1} soil) fallow treatments. The HV (68 mg CMIN kg^{-1} soil) indicated significantly higher CMIN values compared to CC, NT, and CT. The remaining cover crop treatments (W, MC, and CC) in the upper 0–10 cm soil range were not significantly different than NT and CT (Figure 4A). In the lower 10–20 cm of soil, AP (44 mg CMIN kg^{-1} soil) and HV (42 mg CMIN kg^{-1} soil) were significantly higher than all other treatments and were higher than both CT (29 mg CMIN kg^{-1} soil) and NT (30 mg CMIN kg^{-1} soil; Figure 4B). Also, in the lower 10–20 cm of soil, MC (35 mg CMIN kg^{-1} soil) was higher than single-species CC (25 mg CMIN kg^{-1} soil; Figure 4B).

The CMIN was analyzed by separate dates by combining cover crop treatments and depths ($n = 6$ replicates; Table S1). At week 0, AP (86.4 mg CMIN kg^{-1} soil) was significantly higher than NT (46.5 mg CMIN kg^{-1} soil), CC (40.5 mg CMIN kg^{-1} soil), and CT (37.5 mg CMIN kg^{-1} soil). The CMIN value for HV (71.0 mg CMIN kg^{-1} soil) was also significantly higher than CT (Figure 5A). At week 3, AP (56.2 mg CMIN kg^{-1} soil) was significantly higher than W (36.2 mg CMIN kg^{-1} soil), CC (36.0 mg CMIN kg^{-} soil), NT (31.7 mg CMIN kg^{-1} soil), and CT (30.0 mg CMIN

TABLE 2 Soil C parameters combined by treatment and depth then separated by sampling date ($n = 42$).

Date	SOC (g kg $^{-1}$ soil)	WEOC (mg kg^{-1} soil)	CO_2-C (mg kg^{-1} soil)
Week 0	4.8a	57.4a	114.2a
Week 3	3.6b	40.0b	92.6b
Week 6	3.2b	37.7b	82.3c
p value	<0.0001	<0.0001	<0.0001

Note: Statistical significance within each soil C parameter denoted by different letters ($p < 0.05$). Week 0 = April 20, 2017; Week 3 = May 9, 2017; Week 6 = May 30, 2017.

Abbreviations: SOC, soil organic carbon; WEOC, water-extractable organic carbon; CO_2-C, carbon mineralization.

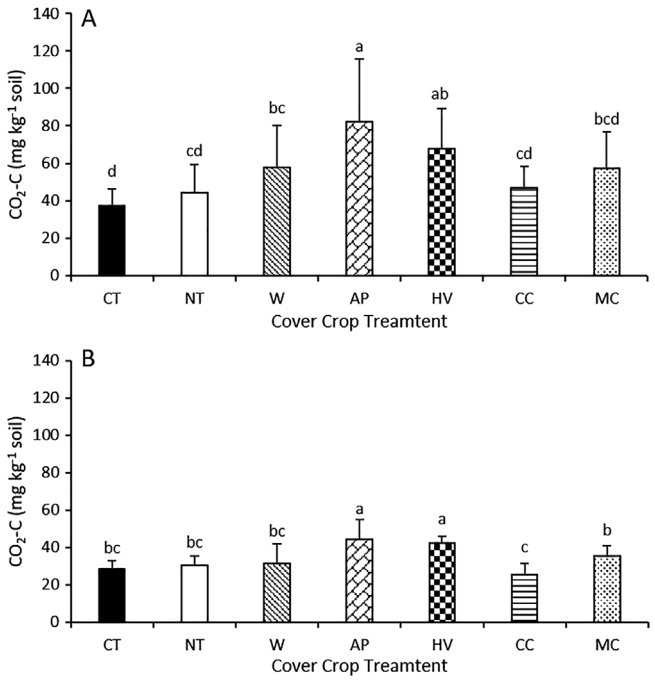

FIGURE 4 CMIN as affected by tillage and cover crop treatment statistically analyzed over time and separated by individual treatments from Chillicothe Research Station. Samples represent all dates combined ($n = 9$) and split by (A) 0–10 cm depth; (B) 10–20 cm depth. Statistical significance within each treatment denoted by different letters ($p < 0.05$). Error bars represent the standard deviation. AP, Austrian winter field pea; CC, crimson clover; CT, conventional till; HV, hairy vetch; MC, mixed species cover; NT, no-till; W, winter wheat.

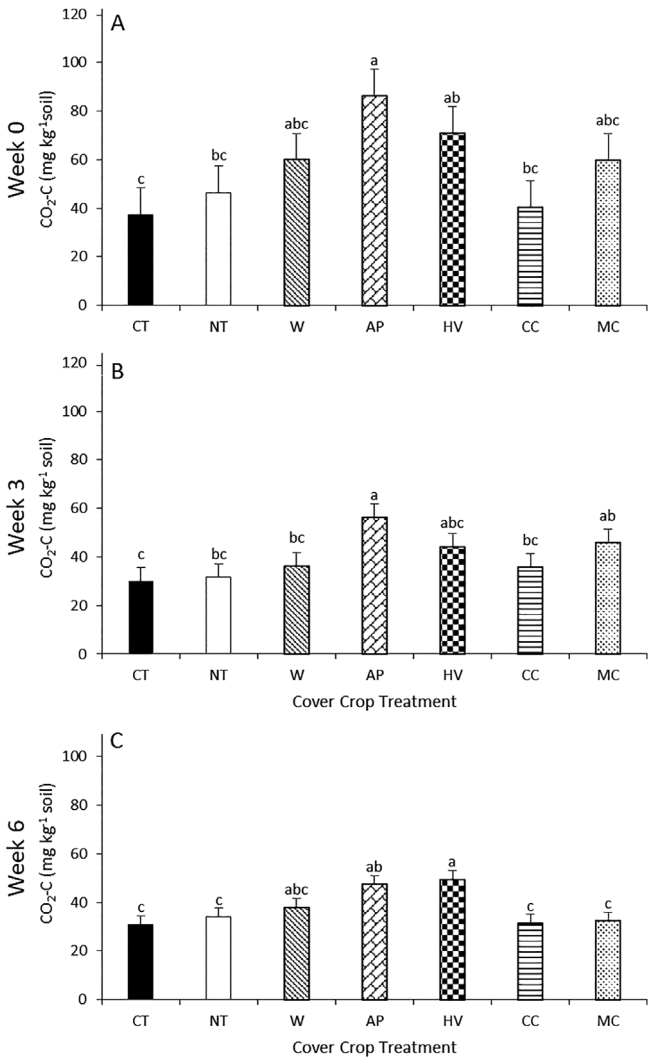

FIGURE 5 CMIN in 0–20 cm of soil as affected by tillage and cover crop treatment statistically analyzed by individual treatments and by sample date from Chillicothe Research Station. Samples represent both depths combined ($n = 6$) and separated by (A) Week 0; (B) Week 3; (C) Week 6. Statistical significance within each treatment denoted by different letters ($p < 0.05$). Error bars represent the standard error. AP, Austrian winter field pea; CC, crimson clover; CT, conventional till; HV, hairy vetch; MC, mixed species cover; NT, no-till; W, winter wheat.. Week 0 = April 20th, 2017; Week 3 = May 9th, 2017; Week 6 = May 30th, 2017.

kg^{-1} soil). Also, the CMIN value for MC (46.0 mg CMIN kg^{-1} soil) was higher than CT (Figure 5B). At week 6, the CMIN values for HV (49.5 mg CMIN kg^{-1} soil) and AP (47.5 mg CMIN kg^{-1} soil) were significantly higher than NT (34.0 mg CMIN kg^{-1} soil), MC (32.5 mg CMIN kg^{-1} soil), CC (31.5 mg CMIN kg^{-1} soil), and CT (31.0 mg CMIN kg^{-1} soil; Figure 5C).

There was a date effect for the soil C parameters measured when all treatments were combined ($n = 42$) indicated by SOC, WEOC, and CMIN were the highest during week 0 and decrease thereafter for each C parameter measured (Table 2; Table S1).

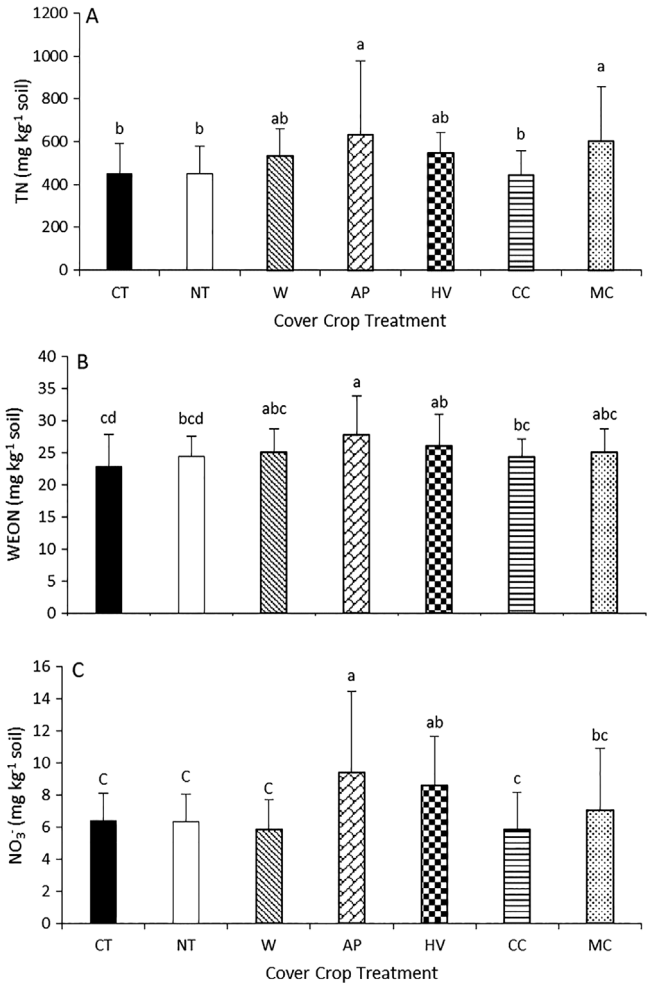

FIGURE 6 Soil nitrogen in 0–20 cm of soil as affected by tillage and cover crop treatments from Chillicothe Research Station measured as (A) total nitrogen (TN); (B) water-extractable organic nitrogen (WEON); and (C) NO_3^-. Samples represent all dates and depths combined ($n = 18$). Main effect means with standard deviation bars ($p < 0.05$). AP, Austrian winter field pea; CC, crimson clover; CT, conventional till; HV, hairy vetch; MC, mixed species cover; NT, no-till; W, winter wheat.

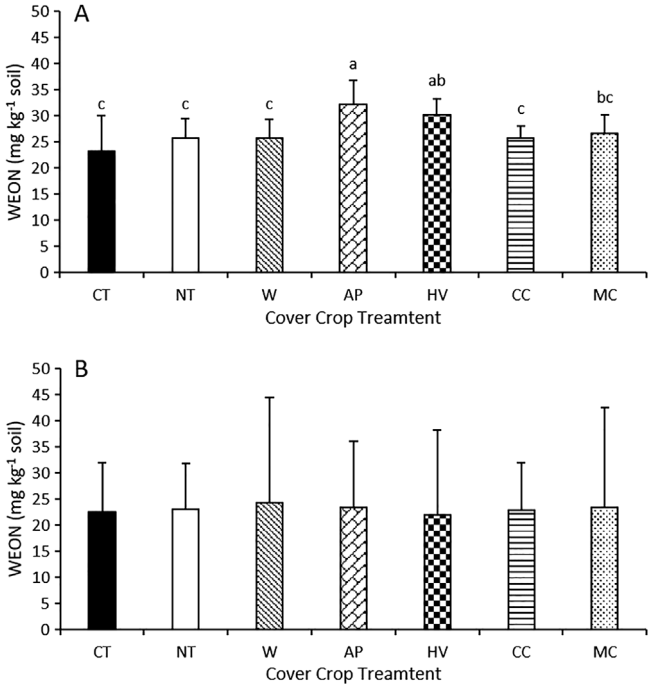

FIGURE 7 Water-extractable organic nitrogen separated by depth as affected by tillage and cover crop treatment from Chillicothe Research Station. Samples represent all dates combined ($n = 9$) and split by (A) 0–10 cm depth; (B) 10–20 cm depth. Statistical significance within each treatment denoted by different letters ($p < 0.05$). Error bars represent the standard deviation. AP, Austrian winter field pea; CC, crimson clover; CT, conventional till; HV, hairy vetch; MC, mixed species cover; NT, no-till; W, winter wheat.

3.3 | Soil chemicals: N

The cover crop treatments indicated that TN values for AP (630 mg TN kg^{-1} soil) were significantly higher than CC (444 mg TN kg^{-1} soil), NT (448 mg TN kg^{-1} soil), and CT (451 mg TN kg^{-1} soil). The MC (600 mg TN kg^{-1} soil) treatment was also higher than CC, NT, and CT (Figure 6A). The remaining cover crop treatments (HV, W, and CC) were not significantly different from NT and CT (Figure 6A).

The cover crop treatments indicated that WEON values for AP (27.8 mg WEON kg^{-1} soil) were significantly higher than NT (24.3 mg WEON kg^{-1} soil), CC (24.4 mg WEON kg^{-1} soil), and CT (22.9 mg WEON kg^{-1} soil). The WEON values from the HV (26.1 mg WEON kg^{-1} soil) treatment indicated that it was higher compared to CT (Figure 6B). A two-way interaction between treatment x depth occurred (Table S1). When separating the replicates by depth ($n = 9$ replicates), significant differences can be observed in the upper 0–10 cm of soil. The WEON values for AP (32.2 mg WEON kg^{-1} soil) were significantly higher than W, CC, and NT (25.7 mg WEON kg^{-1} soil) and CT (23.2 mg WEON kg^{-1} soil: Figure 7A). In the lower 10–20 cm of soil, all treatments indicated roughly the same amount of WEON values, and none were statistically different (Figure 7B).

The cover crop treatments indicated that soil NO_3^- levels were highest in the AP and HV treatments. The soil NO_3^- values for AP (9.4 mg NO_3^- kg^{-1} soil) were higher than NT (6.3 mg NO_3^- kg^{-1} soil) and CT (6.4 mg NO_3^- kg^{-1} soil). The soil NO_3^- values for HV (8.6 mg NO_3^- kg^{-1} soil) were higher than NT and CT (Figure 6C). The remaining cover crop treatments (MC, W, and CC) were not statistically different from NT or CT.

Total inorganic N is the sum of NH_4^+ and NO_3^-, and a majority of that total is comprised of NH_4^+ in this system. A three-way ANOVA did not detect main effect interactions for NH_4^+, and there were no treatment effects when observing NH_4^+ values. However, there was a date effect with the

TABLE 3 Soil N parameters combined by treatment and depth then separated by sampling date ($n = 42$)

Date	Total N (mg kg^{-1} soil)	WEON (mg kg^{-1} soil)	NH_4^+ (mg kg^{-1} soil)	NO_3^- (mg kg^{-1} soil)	Total inorganic N (mg kg^{-1} soil)
Week 0	622.8	26.4a	20.3c	5.2c	25.4c
Week 3	474.9b	24.0b	35.7a	7.3b	43.0a
Week 6	468.9b	24.9ab	28.0b	8.7a	36.6b
p value	<0.0004	0.0522	<0.0001	<0.0001	<0.0001

Note: Statistical significance within each soil N parameter denoted by different letters ($p < 0.05$). Week 0 = April 20, 2017; Week 3 = May 9, 2017; Week 6 = May 30, 2017.
Abbreviation: WEON, water-extractable organic nitrogen.

TABLE 4 Phospholipid fatty acid (PLFA) biomass from 0 to 10 cm with treatments combined and separated by sampling date ($n = 21$)

	Total PLFA biomas (ng g^{-1})	Total bacteria PLFA (ng g^{-1})	Total fungi PLFA (ng g^{-1})	Total Rhizobia PLFA (ng g^{-1})	Total Protozoa PLFA (ng g^{-1})
Week 0	1342.8a	518.7a	178.3a	9.6a	153.8a
Week 3	994.0a	320.6b	92.5b	5.08ab	5.3b
Week 6	413.4b	154.0c	24.1c	0.83b	0b
p value	0.0004	<0.0001	<0.0001	0.0241	<0.0001

Note: Statistical significance within each PLFA parameter denoted by different letters ($p < 0.05$). Week 0 = April 20, 2017; Week 3 = May 9, 2017; Week 6 = May 30, 2017.

collective N values from all replicates of all treatments and depths combined, and then separated by date (Table S1). During week 3 ($n = 42$ replicates), the collective NH_4^+ values and the collective total inorganic N values were statistically higher at weeks 0 and 6. Week 0 had the lowest collective NH_4^+ values (Table 3).

There was a date effect for the other soil N parameters measured when all the treatments combined ($n = 42$) indicated by the collective TN and the collective WEON being highest during week 0 and decreasing by week 3. In contrast, the collective NO_3^- values increased from week 0 to week 6 (Table 3).

3.4 | Microbial biomass estimates based on phospholipid fatty acid analysis

The mean values for soil PLFA biomass trended highest in the AP treatment for total biomass, bacteria, fungi, rhizobia, and protozoa, but the differences were not significant (Table S3). There was a date effect so all PLFA biomass values from each treatment were combined ($n = 21$ replicates) and separated by sampling date (Table 4; Table S2). The average total biomass PLFA for all replicates was highest at week 0, decreased by 35% by week 3 (not significantly different) and significantly decreased by 58% by week 6 (Table 4).

The average bacterial biomass PLFA for all replicates during week 0 had significantly decreased 38% by week 3. Then, the average bacterial biomass PLFA for all replicates during week 3 significantly decreased 58% by week 6 (Table 4). The average fungal biomass PLFA for all replicates during week 0 had significantly decreased 48% by week 3. Then, the average fungal biomass PLFA for all replicates during week 3 significantly decreased 74% by week 6 (Table 4). The average rhizobia biomass PLFA for all replicates during week 0 had decreased 47% by week 3, but it was not significant. However, the average total rhizobia PLFA for all replicates during week 0 significantly decreased 92% by week 6 (Table 4). The average protozoa biomass PLFA for all replicates during week 0 had decreased by 96% and 100% by week 3 and week 6, respectively (Table 4).

The total biomass, bacteria, and fungi were all highly correlated to SOC, WEOC, and CMIN, but not significantly correlated to TN, WEON, or any inorganic N value (Table 5). Rhizobia biomass was not significantly correlated to any soil chemical parameter tested. Protozoa biomass was highly correlated to soil chemical parameters SOC, WEOC, CMIN, inorganic N, NO_3^-, and NH_4^+ (Table 5). Protozoa PLFA was also highly correlated to microbial parameters such as the PLFA biomarkers for bacteria, fungi, and rhizobia (Table 5). All microbial PLFA biomarkers were correlated with each other (Table 5).

4 | DISCUSSION

This study examined the impact of fallow management alternatives by comparing the cover crop treatments to our fallow treatments, NT or CT. When analyzing the soil N content, the TN was highest in AP and MC treatments when compared to both fallow treatments. The smaller subset of the N pool measured as WEON was highest in AP when

TABLE 5 Phospholipid fatty acid (PLFA) biomass and soil chemical parameter correlation analysis from 0 to 10 cm of soil at Chillicothe Research Station

	Total PLFA	Bacteria PLFA	Fungi PLFA	Rhizobia PLFA	Protozoa PLFA	SOC	WEOC	CMIN	TN	WEON	Inorganic N	NH_4^+	NO_3^-
Total PLFA		<0.0001**	<0.0001**	<0.0001**	<0.0001**	0.0042**	0.0009**	0.0012**	0.2419	0.4527	0.9158	0.8790	0.3904
Bacteria PLFA			<0.0001**	<0.0001**	<0.0001**	0.0002**	<0.0001**	<0.0001**	0.0604	0.1621	0.3911	0.4960	0.3472
Fungi PLFA				<0.0001**	<0.0001**	0.0005**	0.0002**	0.0024**	0.1563	0.4484	0.4440	0.6426	0.1878
Rhizobia PLFA					<0.0001**	0.0674	0.0591	0.2556	0.5024	0.7722	0.7140	0.5216	0.4879
Protozoa PLFA						<0.0001**	<0.0001**	<0.0001**	0.0014**	0.4641	0.0032**	0.0071**	0.0409*
SOC							<0.0001**	<0.0001**	<0.0001**	<0.0001**	0.3048	0.3160	0.5917
WEOC								<0.0001**	<0.0001**	<0.0001**	0.1754	0.2171	0.3262
CMIN									<0.0001**	<0.0001**	0.0684	0.0432*	0.8264
TN										<0.0001**	0.9071	0.7079	0.4516
WEON											0.6975	0.6326	0.0028**
Inorganic N												<0.0001**	<0.0001**
NH_4^+													0.0118*
NO_3^-													

*Significance at $p < 0.05$. ** Significance at $p < 0.01$.

Abbreviations: CMIN, carbon mineralization; SOC, soil organic carbon; TN, total nitrogen; WEOC, water-extractable organic carbon; WEON, water-extractable organic nitrogen

compared to CT and NT. The soil NO_3^- levels were also highest in AP and HV treatments. Regarding the soil C content, AP and MC were the only treatments that had significantly higher SOC values compared to both fallow treatments. When analyzing the smaller, labile subset of the C pool using WEOC as a proxy, the AP, MC, and W treatments indicated greater WEOC compared to CT. The metabolically active component of soil can be measured in its simplest form as emission of CO_2^-, or CMIN, which corresponds to nutrient availability, moisture, and temperature (Haney et al., 2008; Wade et al., 2018). When analyzing the CMIN, the single-species AP and HV treatments were statistically higher than both the fallow treatments. This indicates that AP and HV had the highest soil biological activity measured as CMIN, which is a key indicator of soil health (Franzluebbers, 2016). It should also be noted that the multi-species MC treatment indicated higher CMIN than CT, but not NT. A common trend in all these soil chemical parameters tested is the high performance of the single-species legume AP cover crop treatment.

This was consistent with Doane et al. (2009) from semi-arid production in California, which reported the use of a legume cover crop was especially favorable to management of N fertility in a reduced tillage system. Blackshaw et al. (2010) from the semi-arid Canadian prairies also reported legumes, including AP, can be successfully established as an offseason ground cover. Ghimire et al. (2017) from a semi-arid research site in New Mexico reported that CMIN rates were highest in AP when compared to a fallow control. Also, Ebelhar et al. (1984) from a NT corn production in Kentucky reported that HV produced more dry matter with higher N percentage which resulted in higher N concentration in corn plants and substantially more inorganic N in the soil than with other legumes tested. Furthermore, certain legume cover crops, including HV, can provide a substantial portion of the N to a NT corn system, thereby decreasing the amount of N fertilizer needed (Ebelhar et al., 1984). This study tested CC, but the stands that were established in the semi-arid environment were not productive enough to produce adequate biomass, and this was probably due to later than ideal planting dates for legumes such as CC (DeLaune & Mubvumba, 2020). Harvest aids used in this study are not conducive to cover crop interseeding; thus, cover crops are planted immediately after cotton harvest that typically takes place between the last week of October and mid-December. These results support our first hypothesis that AP, HV, and MC are a better management strategy to increase soil C and N pools than tillage-dependent fallow treatments.

The MC crop treatment had higher TN values compared to CT, and higher SOC, CMIN, WEOC, and WEON values compared to both fallow treatments, NT and CT. The single-species legume treatments of AP did increase soil nitrogen reserves more than the MC treatment, thereby increasing the microbial capacity of the soil. This partially supports the second hypothesis, which stated that the added benefits of both grasses and legumes in the mix species blend will improve soil health more than the other treatments, but it was the single-species AP that indicated the greatest overall soil health improvements. However, the MC treatment still offers a valid alternative to fallow management. This was similar to a study in the semi-arid agroecosystems of the central Great Plains, in which the use of cover crop mixtures did not offer an additional benefit to microbial community composition and microbial activity beyond that of individual cover crops (Calderón et al., 2016). Also, Keeling et al. (1996) determined agricultural production in the southern Great Plains could obtain satisfactory ground cover if the proper species is sown and that fall rainfall is adequate for germination and plant survival. They concluded that individual treatments of wheat, rye, Austrian pea, and hairy vetch were the most dependable species, and that several legume crops, mainly small-seeded clovers, failed due to low moisture characteristic of the southern Great Plains. In Kansas, Holman et al. (2018) recorded no difference with grass–legume cover crops mixtures compared to grass cover crop monoculture stands, and Holman et al. (2021) recorded lower biomass production of cover crop mixtures with greater diversity compared to grasses alone. However, the extended duration of time between cover crop termination and the planting of the winter wheat cash crop were not the same as our study in which cover crops were immediately planted following cotton harvest. Although not quantified, the grass species of our cover crop mixture, rye, was dominant (DeLaune & Mubvumba, 2020). McDonald et al. (2019) was able to demonstrate in a semi-arid agriculture region that a no-till with a single-species wheat cover crop produced higher CMIN rates than no-till without a cover crop, and this was likely due to the cover crop providing carbon into the system by root exudates. As mentioned earlier, there have been recent suggestions that diverse cover crop mixtures offer more advantageous ecosystem services from enhanced soil microbial activity when compared to single-species cover crops, but studies like ours and others are indicating otherwise for semi-arid environments.

Although CC is a legume, it did not show the same CMIN levels as AP and HV. This could be explained by the low cover crop biomass of CC, which did not establish a prominent stand, and had the lowest biomass production. This indicates that although CC may be beneficial in some situations, it was not beneficial under the conditions tested in this study. The AP and HV plants are known for their winter hardiness (Clark, 2008; Wiering et al., 2018), but they also appear to be tolerant of the characteristic drought and heat of southern Great Plains by trending higher than the other cover crop treatments. Under dryland conditions, DeLaune and Mubvumba (2020) were able to demonstrate that AP, HV, and MC were viable cover crops in the southern Great Plains cotton systems, due to their increased water-use efficiency. The results from our study were able to support that conclusion by the increased soil chemical parameters of C and N from AP, HV, and MC. These cover crops did increase the total expenses,

especially the HV and MC treatments, but they did not significantly impact cotton yields or net returns all the while providing increased ecosystem services, such as reduced wind and water erosion, improved water infiltration and nutrient cycling, which strengthened the soil health's resiliency during periods of drought (DeLaune et al., 2020).

This study sought to use the PLFA biomass from the soil microbiome to distinguish which management practices can promote the highest PLFA biomarkers for bacteria, fungi, or rhizobia (Feng et al., 2003). No treatment differences among PLFA biomarkers were observed. However, AP trended highest in total PLFA biomarkers, as well as bacterial, fungal, and rhizobia PLFA biomarkers, which is consistent with the high performance of AP in the other parameters mentioned. This was similar to Calderón et al. (2016), which suggested that in a semi-arid environment, longer time spans may have been needed to see beneficial effects of cover crops on soil microbial community structure and soil enzyme activities. There was a date effect for all PLFA biomarker parameters tested, which indicated a general decline from week 0 to week 6. This could be due the microbial communities benefiting from the living cover crop and its active rhizosphere, which was indicated by the PLFA biomass values decreasing after the cover crop termination at week 0. Also, another contributing factor could be the semi-arid environment. Soil moisture could have been declining as the weeks progressed after the cover crop termination causing a decline in microbial activity. This might also explain the decrease in SOC, TN, WEOC, and WEON values measured from week 0 to week 6. This study was also able to observe the nitrification of the inorganic N. This process was indicated with the rise in NH_4^+ after week 0 to week 3, followed by a decline in NH_4^+ from week 3 to week 6. The decline of NH_4^+ was most likely caused by microbial oxidation, as indicated by the increasing NO_3^- values from week 0 to week 6.

5 | CONCLUSION

In this study, NT practices with cover crops improved soil quality, indicated by increased plant available nutrients, CMIN, and microbial populations. The total soil C and N nutrient pool was most improved by the single-species AP treatment indicated by increased SOC and TN when compared to fallow treatments, NT and CT. The inorganic N values were most improved by the AP and HV treatments indicated by NO_3^- values that were greater than NT and CT. The labile nutrient fraction that is readily available for soil microbes to utilize was most improved by AP indicated by increased WEOC and WEON values. Total PLFA biomass and the PLFA biomass for bacteria, fungi, and rhizobia trended highest in the AP treatment. The AP and HV treatments proved to be the most successful single-species legume cover crop treatment. The MC treatment was initially thought to combine the benefits of grasses and legumes, but the semi-arid southern Great Plains did not select for that. For all parameters tested, CT and NT never indicated a significant difference between them at any date or depth, so the addition of cover crops to NT cotton systems could potentially enhance NT in regard to soil function. A limitation of this study was that CT was not tested with cover crops to adequately compare all treatments, but CT without a cover crop acted as a control, especially since this is a common practice in the semi-arid southern Great Plains. Also, future research can test other mixtures of grasses and legumes to explore if there is a better combination of these cover crop species. Ideally, the offseason ground cover provided by cover crops promotes greater erosion control, water capture, and increased soil health. This can minimize the damaging effects of a drought, which is characteristic of this region being studied.

AUTHOR CONTRIBUTIONS

Brian A. Hux: Conceptualization; data curation; formal analysis; investigation; writing–original draft. **Paul B. DeLaune**: Conceptualization; funding acquisition; project administration; supervision; writing–review and editing. **Marie T. Schirmarcher**: Data curation; investigation. **Terry J. Gentry**: Conceptualization; formal analysis; funding acquisition; project administration; supervision; writing–review and editing. **Partson Mubvumba**: Data curation; investigation; writing–review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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