



*Research article*

## **Carbon dioxide mitigation potential of conservation agriculture in a semi-arid agricultural region**

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**Abstract:** The Texas High Plains (THP) region is one of the largest upland cotton (*Gossypium hirsutum* L.) producing regions in the world. Cotton is a versatile crop with uses for both food and fiber products. Conservation management practices such as no-tillage and cover crops have been used to reduce wind erosion on the THP but are also associated with mitigating and reducing greenhouse gas (GHG) emissions from soil. Although row-crop agriculture has been linked to GHG emissions across the world, cotton production in the THP ecoregion has not been thoroughly evaluated for its contribution to GHG production. This research quantified the soil flux of carbon dioxide (CO<sub>2</sub>-C) from continuous cotton production systems on the THP after implementing three tillage practices: (1) no-till with a winter wheat cover crop (NTW); (2) no-till winter fallow (NT); and (3) conventional tillage winter fallow (CT). In addition, the timing of nitrogen fertilizer application was evaluated within each tillage system. Five N treatments were implemented: (1) an unfertilized control; (2) 100% pre-plant (PP); (3) 100% side-dressed (SD); (4) 40% PP 60% SD; and (5) 100% PP with a nitrogen stabilizer product (STB). Tillage practice affected CO<sub>2</sub>-C flux rates in spring 2016 and 2017 with the NTW system having greater CO<sub>2</sub>-C flux than the NT and CT systems. In summer 2017, the NTW system had a greater flux of CO<sub>2</sub>-C than the NT or CT systems. In fall/winter 2016, the NTW and CT systems had a greater CO<sub>2</sub>-C flux than the NT system. Cumulative emissions of CO<sub>2</sub>-C were affected by N treatment in 2016, with later season applications of N fertilizer increasing emissions compared to the STB treatment and the control. In 2017, cumulative emissions of CO<sub>2</sub>-C were greater in the NTW system than in the NT and CT system. However, a greater amount of CO<sub>2</sub>-C was assimilated by the wheat cover crop from the atmosphere

than was lost from the soil which reduced net C losses from the system. With continued use of no-tillage and a cover crop, lower net soil CO<sub>2</sub>-C losses should result in a greater rate of soil organic C gain, positively impacting the sustainability of cotton production in the THP.

**Keywords:** carbon dioxide; carbon assimilation; no-tillage; cover crops; nitrogen timing; carbon balance; soil greenhouse gas emissions

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## 1. Introduction

Cotton (*Gossypium hirsutum* L.) is the major crop produced on the Texas High Plains (THP) accounting for approximately 34% of Texas, and 15% of USA production [1,2]. Due to the prominence of cotton production in the study area, it was prudent to assess the soil and environmental effects of the management practices used for its production. Cotton is versatile in the use of its products with applications in both food and fiber. The long history of cotton production on the THP includes the Dust Bowl era of the 1930's which led to encouragement by government agencies to make production more sustainable. Specifically, the use of cover crops and reduced tillage was encouraged to reduce wind erosion. Wind erosion has been significantly reduced since the Dust Bowl era and subsequent uptake of conservation tillage and cover crops [3]. Not only on the THP, but across Texas, conservation tillage and cover crops have seen an increase in use, with 38% of cropped area using conservation tillage and 6% of cropped area using cover crops, particularly wheat (*Triticum aestivum*), in 2012 [4].

Along with the benefit of reducing wind erosion, conservation management practices, such as reduced or no-till and cover crops, have been reported to reduce net greenhouse gas (GHG) emissions [5,6], likely related to sequestration of C through plant photosynthesis in agricultural soils [7]. In most monoculture systems, less C is sequestered from carbon dioxide (CO<sub>2</sub>) assimilation by the cash crop than can be sequestered when a cover crop is used [8]. In addition, the sequestered C within the plant can be protected by reducing tillage and leaving the plant residue on the soil surface [8]. Studies such as Roberts and Chan [9] have reported a reduction in CO<sub>2</sub> flux due to the use of less intensive tillage in a laboratory simulation compared to intensive tillage simulation. The majority of studies regarding the reduction in GHG emissions through the use of conservation management practices have been undertaken in regions climatically different than the THP. Thus, the information only aids in guiding research on the THP towards practices that will potentially succeed in the semi-arid climate of the THP. Of the few studies on the THP, a study related to C conservation in soils found that under dryland conditions, no-till used in a wheat-sorghum rotation conserved greater C than stubble mulch tillage due to less soil disturbance and a shorter fallow period [8]. Research conducted in the southern part of the THP determined that soil organic carbon (OC) was not different between conventional tillage and no-till with a rye cover 19 years after implementation [10]. This 19-year study indicates the potential for a long delay between treatment implementation and soil OC increases in no-till systems.

There are six key roles of soil in the provision of ecosystem services, one of which is to modify the atmosphere [11]. This is accomplished via the production of GHGs including CO<sub>2</sub>, nitrous oxide (N<sub>2</sub>O), and methane (CH<sub>4</sub>) as well as oxygen and other non-GHGs [11]. It is estimated that agricultural soil emissions account for less than 10% of GHG emission in the USA [12], but by

examining the ways in which GHG production can potentially be mitigated the overall production of GHGs in the USA can be reduced. Greenhouse gas production in the soil is complex and heterogenous [13], and is influenced by microbial processes [14], soil moisture content [15,16] and temperature [17,18] among others.

Carbon cycling in agricultural systems, specifically CO<sub>2</sub> transformations, is more active than other GHGs due to its consumption during photosynthesis and use in plant production [8]. Despite the fact that plant residue will be decomposed, and a portion of residue C will be converted to CO<sub>2</sub>, the net emission of CO<sub>2</sub> from agriculture is minor compared to the amount of CO<sub>2</sub> cycled within the system [19]. The relationship between C cycling and CO<sub>2</sub> emissions goes deeper than the decomposition of plant residue and its subsequent role in CO<sub>2</sub> emissions. Plant primary production has been correlated with soil respiration across all major biomes on Earth [20]. In cotton production, soil respiration increases over the growing season following a pattern similar to primary productivity of the cotton plant [21]. One factor related to plant primary productivity is the release of exudates from plant roots. The release of carbonaceous root exudates has been reported to stimulate respiration rates [22] and increase microbial density around plant roots in the field [23]. When considering agricultural CO<sub>2</sub> emissions, Koizumi et al. [14] determined that the major source of CO<sub>2</sub> from soil was microbial and root respiration with 45% of total soil respiration reported to be due to rhizosphere respiration in corn (*Zea mays* L.) [24]. Thus, root exudation potentially plays a major role in soil respiration by adding an additional carbon source and increasing microbial density.

In addition to temperature and carbon input impacts on soil respiration, nitrogen (N) fertilizer application has been associated with soil respiration. Numerous studies related to this relationship have determined that the effects vary among vegetation types, soils, and sites as well as the source of N addition [19,20]. Due to the variable results regarding the relationship between N fertilization and soil respiration, and the desire to adhere to the best application methods of fertilizer as outlined by Smith et al. [13], understanding how fertilizer application timing affects soil respiration in conservation tillage systems is important for increasing the sustainability of cotton production.

The objective of this study was to evaluate the short-term impacts of tillage practices (conventional tillage, no-till with a winter wheat cover crop, and no-tillage without a cover crop) coupled with different N fertilizer application timings on CO<sub>2</sub> emissions from the soil-atmosphere interface in THP cotton production. This study also evaluated N fertilizer application timing effects on C assimilation by a wheat cover crop and the relationship between cover crop sequestered C and CO<sub>2</sub> emissions.

## 2. Materials and methods

### 2.1. Cropping system and experimental design

This study was conducted at the Texas A&M AgriLife Research and Extension Center in Lubbock, Lubbock County Texas (33.687 °, -101.827 °). The 30-year annual (1981–2010) averages of rainfall and temperature for this area are 486 mm and 15.9 °C, respectively [25]. The soil was an Acuff loam (fine-loamy, mixed, superactive, thermic Aridic Paleustolls) [26]. The field plots had been planted in cotton for 11 of the last 17 years. Corn (*Zea mays* L.) planted in 2003 and 2014, and sorghum (*Sorghum bicolor*) was planted in 2002, 2008, 2009, 2014, and 2015. The study was designed as a split plot with tillage as the main plot and N fertilizer application arranged as the split plot in randomized complete blocks with three replicates. Each of the 45 plots were four rows

wide (row width 1 m) by 15 m long. Main plot tillage treatments included: (1) no-till with a winter wheat cover crop (NTW); (2) no-till winter fallow (NT); and (3) conventional tillage (CT). The N fertilizer application timings were: (1) no-added N (control); (2) 100% of N applied in a pre-plant application (PP); (3) 100% of N side-dressed applied (SD); (4) 40% of N applied PP and 60% SD applied (SPLIT); and (5) 100% of N applied PP with a N stabilizer product (STB). Side-dress N fertilizer was applied at the cotton growth stage of pinhead square. The stabilizer product used was Limus<sup>®</sup> Nitrogen Management (BASF Corporation, United States) a dual action urease inhibitor. Prior to implementing tillage treatments in fall 2015, the site was under conventional tillage for at least 60 years.

Wheat (TAM 304) was planted on 25 January 2016, and 22 November 2016 at a seeding rate of 67 kg ha<sup>-1</sup> (19 cm row spacing). The wheat planted on 25 January 2016 was a re-plant after a failed stand from an earlier planting in November 2015. Wheat cover was chemically terminated on 13 April 2016 and 20 April 2017 using glyphosate [N-phosphonomethyl glycine] at 2.2 kg ha<sup>-1</sup> active ingredient (a.i.) in 2016 and at 3.5 kg ha<sup>-1</sup> a.i. in 2017. Nitrogen fertilizer was applied at a total rate of 168 kg N ha<sup>-1</sup> as urea ammonium nitrate (UAN, 32-0-0) via knife injection using a coulter fertilizer applicator about 10 to 15 cm from the cotton row. Pre-plant (PP) N fertilizer treatments on 10 May 2016 and 11 May 2017, and in-season or SD applications occurred on 13 July 2016 and 20 July 2017. Cotton (DP 1321 B2RF) was planted on 26 May 2016 and 6 June 2017 at a rate of 123,553 seeds ha<sup>-1</sup>.

In all tillage systems for both 2016 and 2017, the field was prepared by shredding stalks with a four-row John Deere shredder (Moline, Illinois, USA), and the conventional tillage was then disked with a four-row John Deere offset disk to a depth of 5–8 cm. On 22 February 2016 and 22 March 2017, trifluralin [ $\alpha,\alpha,\alpha$ -trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine] was applied at 0.8 kg ha<sup>-1</sup> a.i. to all plots and incorporated using a four-row spring tooth harrow to a depth of 5–8 cm in the CT system. Planting beds were then re-formed using a bed lister for the CT system. Tillage was conducted in the CT system one time in each growing season using a sweep cultivator to a depth of 10 cm. All tillage systems were scratched with a rotary hoe, to 1 cm depth, about a week after planting in 2017 in order to prevent soil crusting and reduced seed emergence. Glyphosate was applied once for weed control in 2016, other than for wheat termination, at a rate of 2.7 kg ha<sup>-1</sup> a.i. on 3 May 2016. In 2017, glyphosate was applied once, at a rate of 3.4 kg ha<sup>-1</sup> a.i. with 0.8 kg of spray adjuvant (ammonium sulfate) on 17 June 2017. In addition, 2,4-D (2,4-dichlorophenoxyacetic acid) was applied on 6 April 2016 at a rate of 2.1 kg ha<sup>-1</sup> a.i. in the CT system. Plots were irrigated using furrow irrigation on 1 July 2016, 27 July 2016, 13 August 2016, 6 June 2017, and 30 July 2017 with approximately 150 mm of water applied per event.

## 2.2. Soil characterization

Soil samples were collected before pre-plant applications of N-fertilizer on 8 April 2016 using a 5.1 cm diameter Giddings probe (Giddings Machine Company, Windsor, CO) to a depth of 15 cm. Two cores from each plot were composited to provide a representative soil sample. Soil samples were dried at 60 °C for 7 days, and mechanically ground using a flail grinder to pass through a 2 mm mesh screen and stored at room temperature until nutrient analysis.

Samples were analyzed for extractable soil nutrients including P, K, calcium (Ca), magnesium (Mg), and sulfur (S) using a procedure adapted from Mehlich [27] and measured using inductively coupled

plasma spectroscopy (ICP). Residual soil inorganic nitrate-N ( $\text{NO}_3^-$ -N) was extracted using 1 M KCl (1:5 soil to extraction ratio) and analyzed by cadmium reduction to nitrite prior to analysis using flow injection spectrometry (FIALab 2600, FIALab Instruments Inc., Bellevue, WA). A 1:2 soil to deionized water slurry was used to determine pH with actual determination made using a pH probe [28]. A subsample of each composite soil sample was finely ground with a ring-and-puck grinder to pass a 150  $\mu\text{m}$  mesh screen and analyzed for OC and total N (TN) by combustion [29–31]. Inorganic and OC was separated using differential heating with the primary furnace set at 650  $^\circ\text{C}$  with a 2  $\text{L min}^{-1}$   $\text{O}_2$  flow rate for OC. The same instrument was used for TN analysis at 900  $^\circ\text{C}$  [32,33].

### 2.3. Gas flux

The flux of  $\text{CO}_2$  at the soil-atmosphere interface was measured using a Gaset DX-4040 portable FTIR (Fourier Transform Infrared) multi-gas analyzer (Gaset Technologies, Helsinki, Finland) integrated with a 20 cm diameter Li-Cor survey gas chamber (Li-8100-103, Li-Cor Biosciences, Lincoln, NE USA). The gas library for this study included: water vapor,  $\text{CO}_2$ ,  $\text{CH}_4$ ,  $\text{N}_2\text{O}$ , ammonia ( $\text{NH}_3$ ), and carbon monoxide (CO). PVC collars (19.5 cm diameter, 11 cm height) were installed semi-permanently within the field between the center two rows of each plot, about 15 cm from the center of the third crop row, and within 3 m of the center of the plot. The PVC collars were hammered into the soil so that the bottom of the collar was at least 3 cm below the soil surface. Collars were removed and re-installed as needed for field operations including tillage in the CT system and fertilizer application in all plots. Prior to beginning measurements, the DX-4040 gas analyzer was prepared for measurements according to manufacturer protocol, including sample cell purging and background spectrum collection. The Li-Cor chamber was deployed on the PVC collar for approximately 480 seconds, with a sampling frequency of 20 seconds, totaling about 24 data points per measurement series (plot). Gas concentrations, using the Gaset DX4040 with the Li-Cor survey chamber as described above, were measured monthly, as well as 1, 3, and 7 days post fertilizer application as weather permitted. Monthly measurements were collected as long as the ambient temperature remained within 6  $^\circ\text{C}$  of daily starting temperature over a period of two to three days. Gas measurements following fertilizer application were conducted in a single day when possible, as these samples were collected to determine  $\text{CO}_2$  flux rates 1, 3, and 7 days after N fertilizer application.

The gas concentrations collected over the deployment time for each plot were plotted versus time and fit with a linear regression. A significant flux was determined if the linear regression analysis reported a coefficient of determination ( $r^2$ ) of 0.7 or greater. However, the  $r^2$  values for regressions of  $\text{CO}_2$  concentration rarely fell below 0.98. The minimum detectable concentration difference for  $\text{CO}_2$  flux using the Gaset DX4040 was reported to be 5 ppm (J. Cornish, Gaset Technologies, personal communication, 8 Nov 2016). The slope of the linear regression of the gas concentrations versus time, when determined to be significant, was then converted to gas flux ( $\text{mg CO}_2\text{-C m}^{-2} \text{h}^{-1}$ ) using the ideal gas law equation:

$$PV = nRT \quad (1)$$

where  $P$  was the average atmospheric pressure for the sampling period (about 0.89 atm),  $V$  was the measured flux of gas corrected for the volume of the collar and chamber,  $n$  was the moles of gas for which the equation is solving in order to determine gas flux over a period of time and area,  $R$  was the

ideal gas law constant ( $0.08206 \text{ L atm mol}^{-1} \text{ K}^{-1}$ ), and  $T$  was the absolute temperature (K). Headspace within the collar was measured before each sampling event to insure proper gas flux conversion. Air temperature data was collected from the National Center of Environmental Information weather station at the Lubbock International Airport, Lubbock, TX ( $33.6656^\circ$ ,  $-101.8231^\circ$ ), 1 km from the field site, in 1 hour blocks. With a roughly 12–15 minute sampling period per plot, the temperature was converted to K, and the closest data point to the sample time was used.

The growing season was broken into three parts, which also reflect some climatic differences, across which daily flux rates were averaged: spring (April to May), summer (June to September), and fall/winter (October to March). Seasons were determined by the cotton growing season, where spring included measurements from cover crop termination to planting, summer included the cotton growing season until just prior to harvest, and fall/winter included measurements following harvest to just prior to cover crop termination 2016 and the end of data collection for the study in 2017. Cumulative  $\text{CO}_2\text{-C}$  emissions were calculated from cover crop termination (23 April 2016, 28 April 2017) through cotton harvest (14 November 2016 and 15 November 2017) in each year. In addition to during the cotton growing season (May through November), gas fluxes were measured during the winter following the first year (December 2016 to March 2017) to assess the winter flux of  $\text{CO}_2\text{-C}$  and the contribution of winter wheat growth to  $\text{CO}_2\text{-C}$  emissions. Cumulative emissions were calculated by averaging the  $\text{CO}_2\text{-C}$  flux of the two most recent sampling events, and then multiplying the average flux by the amount of time between measurements.

#### 2.4. Cover crop herbage mass

The winter wheat cover crop was hand harvested before termination from two areas ( $1 \text{ m}^2$ ) in each NTW plot on 11 April 2016 and 21 April 2017. Samples were dried at  $60^\circ \text{C}$  for seven days to determine herbage mass on a dry matter basis before being mechanically ground to pass a 2 mm sieve. Carbon and TN concentration determined via combustion analysis using an Elementar Vario Max CN (Elementar, Ronkonkoma, NY USA) at the Texas A&M AgriLife Research and Extension Center in Vernon, TX [29–31]. Assimilation of N and C by the cover crop was determined by multiplying the concentration of N and C within the herbage mass by the dry weight of the herbage mass.

#### 2.5. Carbon balance

In the case of this study,  $\text{CO}_2$  assimilation was estimated as the amount of C produced by the wheat cover crop because it was the only difference between tillage treatments. In addition, the amount of C lost from the system was estimated by measuring the  $\text{CO}_2$  flux from the soil:atmosphere interface. In the NTW system, assimilated wheat-C and  $\text{CO}_2\text{-C}$  were compared to evaluate the potential of C storage. Emissions of  $\text{CO}_2\text{-C}$  due to the wheat cover crop were estimated by subtracting the  $\text{CO}_2\text{-C}$  emissions from the NT system from the  $\text{CO}_2\text{-C}$  emissions from the NTW system. The estimate of  $\text{CO}_2\text{-C}$  could be considered the emissions that were a direct result of the presence of the wheat cover crop.

## 2.6. Statistical approach

Data was analyzed using Proc GLIMMIX at a significance level of  $p < 0.1$  using SAS version 9.3 (SAS Institute Inc., Cary, NC). The GLIMMIX procedure is a generalized linear mixed model which combines the characteristics of generalized linear models and mixed models. Random effects can be incorporated into the GLIMMIX procedure and it can be used to fit statistical models to data with nonconstant variability, in addition to where the response is not normally distributed [34]. Statistical analysis of daily fluxes determined that the average daily flux in 2016 was greater than the average flux in 2017 ( $p < 0.0001$ ), because of this effect, and climatic differences between years, all data collected for this study was analyzed within year. Statistical analysis within year determined that season had an effect on the flux of CO<sub>2</sub>-C, and thus analysis was conducted within season for both years of the study. Main-plot (NTW, NT, CT) and split-plot factors (control, PP, SD, SPLIT, STB) were treated as fixed effects and replication was treated as a random effect. The interaction between main plot and split plot was also investigated using factorial analysis included in the GLIMMIX program. Fisher's protected LSD ( $p < 0.1$ ) was used to separate means of significant effects. The relationship between CO<sub>2</sub>-C and daily maximum temperature was determined using Proc CORR. The CORR procedure computes Pearson's correlation, polyserial correlation coefficients, three nonparametric measures of association, and the associated probabilities [35]. Pearson's correlation is a parametric measure of association for two variables [35].

## 3. Results and discussion

### 3.1. Soil characterization

Before the start of this study in 2016, soil parameters were determined within each tillage system because no N treatments had been implemented at the time of sampling. Soil pH averaged 7.4 (0–15 cm depth) across tillage systems with the CT system having a generally greater pH (7.5) than the NTW and NT systems (Table 1). Averaged across tillage systems, OC at 0–15 cm was 5.2 g kg<sup>-1</sup>, and TN was 0.709 g kg<sup>-1</sup>. Nitrate-N (NO<sub>3</sub><sup>-</sup>-N) was determined to be affected by tillage system with the NTW system having less NO<sub>3</sub><sup>-</sup>-N than the CT and NT systems (Table 1). This reduction in NO<sub>3</sub><sup>-</sup>-N levels was likely due to the use of residual N by the wheat cover crop prior to sample collection in 2016 [36]. Differences between tillage systems were not determined for P, K, Ca, Mg, S, and Na. According to the Texas A&M AgriLife Extension Soil, Water, and Forage Testing Laboratory, K concentrations were very high; Ca and Mg concentrations were high; S concentrations were rated as moderate to high; P concentrations were moderate; and Na concentrations were very low.

### 3.2. Carbon dioxide flux rates

Statistical analysis of daily fluxes determined that the average daily flux in 2016 was greater than the average flux in 2017 ( $p < 0.0001$ ), thus years were analyzed separately. The two years of this study had different climatic conditions which can affect soil CO<sub>2</sub> respiration [17,18] (Figure 1). In both years of the study, the average CO<sub>2</sub>-C fluxes measured during the summer season (June to September) were greater than the fall/winter (October to March 2016, October to December 2017)

and spring seasons (April and May), with the CO<sub>2</sub>-C fluxes measured during the spring also being greater than the fall/winter fluxes in 2016 ( $p < 0.0001$ ). Increasing CO<sub>2</sub> fluxes have been reported to be correlated with increasing soil temperature [17,18]. Soil temperature data was not available for this study, so air temperature is used as a proxy. On the THP, temperatures generally increase from spring to summer, and then decrease through the fall (Figure 1). The correlation of temperature and CO<sub>2</sub>-C flux was determined using Pearson's correlation to be positive and significant in both years. The correlation coefficient in 2016 was 0.349 ( $p < 0.0001$ ) and 0.381 ( $p < 0.0001$ ) in 2017. While the study conducted by Lamberty and Thomson [37] excluded agricultural sites, they concluded that because of the positive relationship between soil temperature and respiration rates global soil respiration will continue to increase due to climate change (i.e. temperature increases).

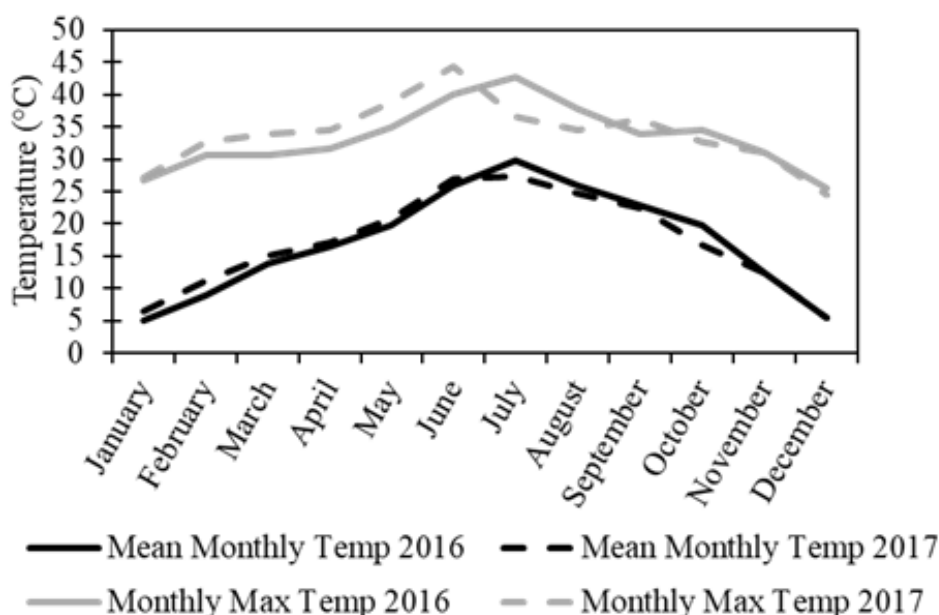
**Table 1.** Soil characterization of samples collected at a depth of 0–15 cm following cover crop termination in April 2016.

Tillage system <sup>a</sup>	pH	OC <sup>b</sup>	TN <sup>c</sup>	NO <sub>3</sub> <sup>-</sup> -N	P	K	Ca	Mg	S	Na
		g kg <sup>-1</sup>								
NTW	7.4	5.3	0.692	0.4b	42	423	1859	823	13	29
NT	7.4	5.4	0.745	6.9a	49	463	1993	809	14	36
CT	7.5	5.1	0.690	6.8a	46	419	1931	852	11	32
<i>p</i> -value	0.901	0.264	0.305	0.028	0.604	0.188	0.519	0.337	0.528	0.217

<sup>a</sup> NTW, no-till with winter wheat cover; NT, no-till winter fallow; CT, conventional tillage winter fallow.

<sup>b</sup> OC, organic carbon.

<sup>c</sup> TN, total nitrogen.

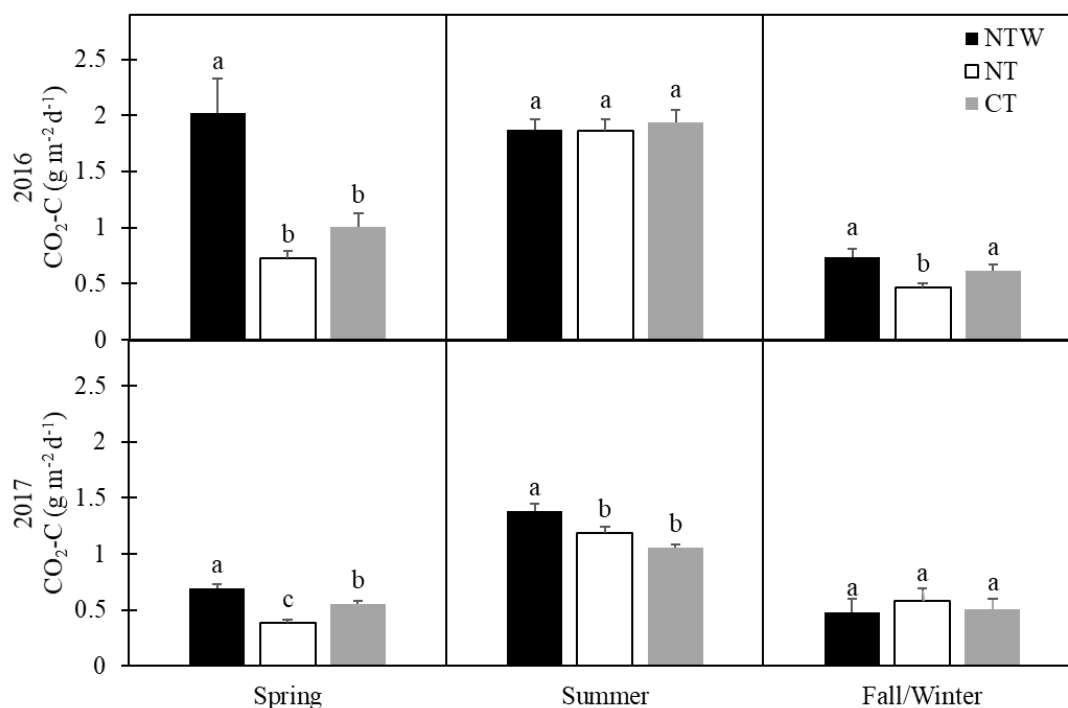


**Figure 1.** Max and mean monthly temperature in 2016 and 2017. Data was collected at the Lubbock International Airport (33.6656 °, -101.8231 °) located 2 km south of the research site.



In addition to microbial respiration, the increase in CO<sub>2</sub>-C flux from spring to summer follows a similar pattern to that of primary production by cotton plants [21]. Primary production has been correlated with soil respiration across all major biomes [20]. Root exudation of carbonaceous substances has been shown to increase CO<sub>2</sub> production in numerous systems. For example, respiration rates in an Italian forest soil were stimulated for up to three days after laboratory addition of carbonaceous low-weight root exudate compounds [22]. However, as reported by Van Hees et al. [38], the type of root exudate affects the destination of the C in the root exudate, where some exudates, such as organic acids, are evolved to CO<sub>2</sub> [38]. Root respiration and root exudates have the potential to change the rate of CO<sub>2</sub>-C emission as the cotton plant develops, with root exudates being associated with greater microbial density within the field [23]. Less, or potentially altered, root exudation and root respiration due to the poor plant stand (data not shown), is likely the reason for the reduced CO<sub>2</sub>-C emissions in 2017. Previous studies determined that rhizosphere respiration accounts for up to 45% of soil respiration in a maize cropping system [24]. The nature of the measurements collected in this study did not allow for differentiation of soil respiration in a semi-arid cotton cropping system. Further investigation of the cotton plant's contribution to soil respiration will be necessary to understand the separation of CO<sub>2</sub>-C sources (plant and microbial) in semi-arid agricultural soils.

When analyzed within season, tillage system affected CO<sub>2</sub>-C emissions in spring and fall/winter 2016, and spring and summer 2017 (Figure 2a,c,d,e), but N treatment had no effect. The NTW system produced the greatest flux of CO<sub>2</sub>-C in spring 2016, spring 2017, and summer 2017. In fall/winter 2016, the NTW and CT systems produced a greater flux than the NT system (Figure 2c). In addition, the CT system produced a greater flux than the NT system in spring 2017 (Figure 2d). Greater CO<sub>2</sub>-C production during the spring and summer for the NTW system was expected due to the decomposition of the wheat cover crop, which would result in a C addition to the soil [39,40]. In addition, carbonaceous root exudates have been shown to increase CO<sub>2</sub> emissions [23]. The greater flux of CO<sub>2</sub>-C in the NTW system compared to the NT system in fall/winter 2016 is likely due to the actively growing cover crop in the NTW system, which was providing carbon to the system via root exudates [22]. The greater flux of CO<sub>2</sub>-C produced in the CT system compared to the NT system in fall/winter 2016 and spring 2017 was likely due to the tillage action performed on the CT system. This has been observed in sorghum systems where the incorporation of crop residue briefly increased CO<sub>2</sub> evolution from CT systems compared to NT system [41]. The peak fluxes of CO<sub>2</sub>-C determined during this study are comparable to CO<sub>2</sub>-C fluxes determined previously on a forested slope in North Carolina [39], and on the Northern China Plain [42]. both of which peaked between 2 and 3 g CO<sub>2</sub>-C m<sup>-2</sup> day<sup>-1</sup>. In a corn production system in Canada, peak CO<sub>2</sub>-C production was about 6 g CO<sub>2</sub>-C m<sup>-2</sup> day<sup>-1</sup> [24], or about 3 times greater than peak CO<sub>2</sub>-C production in 2016 and 4 times greater than peak CO<sub>2</sub>-C production in 2017. Emissions of CO<sub>2</sub>-C in 2016 and 2017 were roughly 2.5 times and 3.3 times less, respectively, than CO<sub>2</sub>-C emissions from a crop rotation study on the semi-arid Canadian prairie in 2012, where emissions averaged 40–50 kg ha<sup>-1</sup> d<sup>-1</sup> [43]. Carbon dioxide-carbon emissions produced in this study were similar to emissions determined in an Australian cotton-wheat rotation in 2009 and 2010 [44]. Scheer et al. [44] examined irrigation effects on CO<sub>2</sub>-C flux rates and determined production from the low irrigation was about 1.6 g CO<sub>2</sub>-C m<sup>-2</sup> day<sup>-2</sup> and 2.3 g CO<sub>2</sub>-C m<sup>-2</sup> day<sup>-1</sup> from the high irrigation. Compared to the flux rates reported in this study, the lowest average daily flux rates determined (Spring 2017, NT) were about one fourth the lowest rates determined in the Australian study and the highest rates were about equal.



**Figure 2.** Carbon dioxide flux rates ( $\text{g m}^{-2} \text{d}^{-1}$ ) averaged by tillage system within seasons of spring (a and d), summer (b and e), and fall/winter (c and f) in 2016 (a, b, c) and 2017 (d, e, f). Spring was from April-May, Summer was from June-September, and Fall/Winter was from October-March. Tillage treatments included no-till with a wheat cover crop (NTW), no-till winter fallow (NT), and conventional tillage (CT). Wheat cover was terminated prior to the first sampling event in 2016, and after one sampling event in 2017. CO<sub>2</sub>-C means within season with the same letter are not different at  $p < 0.1$ .

### 3.3. Cumulative emissions

Final cumulative CO<sub>2</sub>-C emissions were greater in 2016 compared to 2017 ( $p < 0.0001$ ). This is attributed to the potential reduction in root respiration due to reduced plant stand in 2017 (data not reported). Tillage effects on cumulative CO<sub>2</sub>-C emissions were not determined in 2016, but there was a N treatment effect (Table 2, Figure 3). The interaction of tillage and N treatment was not significant. In 2016, the SD treatment produced greater cumulative CO<sub>2</sub>-C from April to December than the STB N treatment and both the SD and SPLIT treatments produced greater cumulative emissions of CO<sub>2</sub>-C than the control (Figure 3b). Nitrogen fertilizer applications late in the growing season (SD and SPLIT treatments) coupled with warmer soil temperatures in July and August increased soil CO<sub>2</sub>-C respiration likely because of greater microbial activity [17,37]. The STB treatment did not produce significantly greater CO<sub>2</sub>-C emissions than the control in 2016. This lack of difference may be due to the action of the urease inhibitor product, which would have reduced the production of CO<sub>2</sub> due to the breakdown of the urea in the soil from which all of the C present can be considered to be emitted as CO<sub>2</sub> [19]. The lack of tillage effect in 2016 may be due to a delay in the various effects of implementing conservation practices [10]. A delay in soil chemical response is not uncommon when implementing tillage systems, although many studies are conducted years after

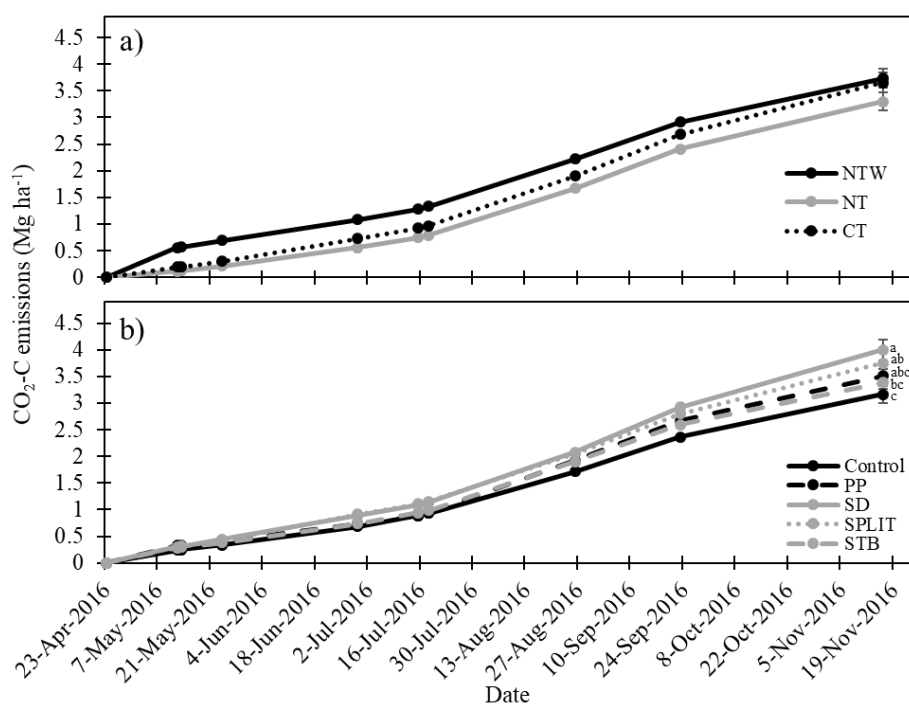
initial implementation, and thus any initial delays are not usually reported [45,46]. A delayed response can be expected on the THP, where after 19 years, no differences were determined in soil OC concentration [10].

In 2017, tillage system affected cumulative CO<sub>2</sub>-C emissions, but N treatment did not (Table 2). The NTW system produced greater CO<sub>2</sub>-C than the NT and CT systems (Figure 4a). Like the daily flux increase in the NTW system in 2017 (Figure 2), greater cumulative CO<sub>2</sub>-C emissions in the NTW system is likely due to the wheat residue acting as a C and energy source, stimulating microbial activity [39,40]. In addition, the NT system produced greater emissions than the CT system.

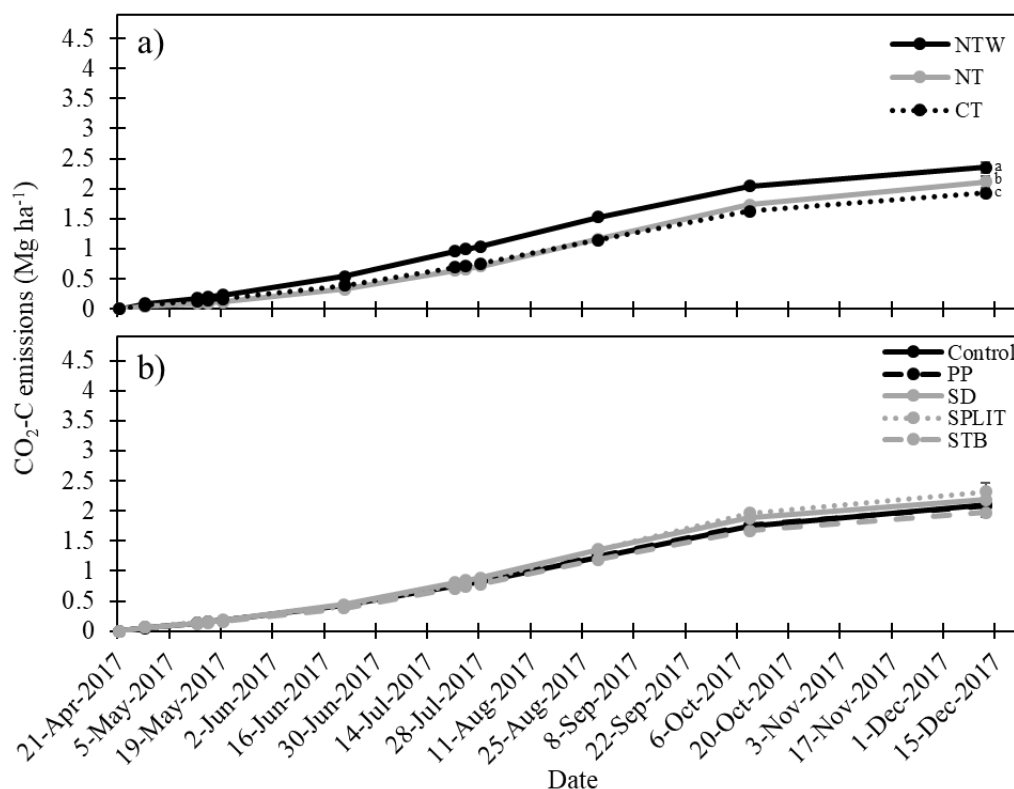
**Table 2.** ANOVA results for tillage system, nitrogen (N) treatment, and interaction effects on cumulative CO<sub>2</sub>-C emissions in 2016 and 2017.

Year	ANOVA ( <i>p</i> -value)		
	Tillage system	N treatment	Tillage × N
2016	0.142	0.068a	0.785
2017	0.003	0.190	0.723

<sup>a</sup> Bolded *p*-values are considered significant (*p* < 0.1).



**Figure 3.** Cumulative CO<sub>2</sub>-C emissions produced in 2016 averaged by main factor a) tillage system and b) nitrogen (N) fertilizer treatment. Control, no added nitrogen (N) fertilizer; PP, 100% pre-plant application of N fertilizer; SD, 100% side-dressed application of N fertilizer; SPLIT, 40% PP, 60% SD application of N fertilizer; STB, 100% PP application of N fertilizer with N stabilizer product. NTW, no-tillage with a winter wheat cover crop; NT, no-tillage winter fallow; CT, conventional tillage winter fallow. There were no differences in CO<sub>2</sub> emissions due to tillage in 2016. Cumulative CO<sub>2</sub>-C emissions within main factor with the same letter are not different at *p* < 0.1.



**Figure 4.** Cumulative CO<sub>2</sub>-C emissions produced in 2017 averaged by a) tillage system and b) nitrogen (N) fertilizer treatment. Control, no added nitrogen (N) fertilizer; PP, 100% pre-plant application of N fertilizer; SD, 100% side-dressed application of N fertilizer; SPLIT, 40% PP, 60% SD application of N fertilizer; STB, 100% PP application of N fertilizer with N stabilizer product. NTW, no-tillage with a winter wheat cover crop; NT, no-tillage winter fallow; CT, conventional tillage winter fallow. There were no differences in CO<sub>2</sub> emissions due to N treatment in 2017. Cumulative CO<sub>2</sub>-C emissions within main factor with the same letter are not different at  $p < 0.1$ .

### 3.4. Wheat herbage mass

Cover crop herbage mass was significantly different between the two years ( $p < 0.0001$ ), with greater herbage mass produced in 2016 than 2017. In 2016, there were no differences among N treatments (Table 3) due to no N treatments being applied prior to planting the cover crop in 2015/2016. However, in 2017 N treatment had a significant effect on herbage mass, with the SD and SPLIT treatments producing more than the control and the STB treatment (Table 3). Herbage mass was greater with the PP treatment than the control but not the STB treatment. The lack of differences among N treatments in 2016 was expected as no N treatments had been applied prior to seeding wheat in January 2016, while the differences in 2017 are likely due to greater residual N with the later season N treatments (SD and SPLIT) applied in 2016 than the control and the PP and STB treatments. Presumably, less of this later applied N was used by the cotton plant, and thus more was available for cover crop use.

The concentration of C in the wheat herbage mass was different between years ( $p < 0.0001$ ),

and among N treatments in 2016 and 2017 (Table 3). Similar to herbage mass treatment effects in 2017, wheat C in 2017 was greater with the SD and SPLIT treatments than the STB treatment and the control. Differences in C assimilation by wheat were determined in 2017 ( $p = 0.020$ ), with the SPLIT and SD treatments resulting in greater C assimilation than the STB treatment and the control (Table 3). The PP treatment had greater C assimilation than the control. The separation of N treatment means for C assimilation are identical to the separation of means for the herbage mass produced and C concentration.

**Table 3.** Wheat cover crop herbage mass, carbon (C) concentration, assimilated C, CO<sub>2</sub>-C emissions due to the wheat over crop, and the estimated C balance assuming 100% sequestration of wheat assimilated C in 2016 and 2017.

Year	Treatment <sup>a</sup>	Herbage mass kg ha <sup>-1</sup>	Total C g kg <sup>-1</sup>	Assimilated C	Wheat CO <sub>2</sub> -C kg ha <sup>-1</sup>	Estimated C balance <sup>c</sup>
2016	CONTROL	2440	412 a <sup>b</sup>	1005	135	870
	PP	2640	406 c	1072	414	658
	SD	2927	409 b	1197	128	1069
	SPLIT	2850	409 b	1166	921	245
	STB	2247	409 ab	919	580	339
	<i>p</i> -value	0.279	0.023	0.316	0.717	
	CONTROL	822 c	393 c	323 c	289	34
2017	PP	1547 ab	400 ab	619 ab	60	559
	SD	1655 a	405 a	670 a	329	341
	SPLIT	1795 a	403 a	723 a	373	350
	STB	1092 bc	396 bc	432 bc	-56	488
	<i>p</i> -value	0.027	0.054	0.020	0.612	

<sup>a</sup> Control, 0 added N fertilizer; PP, 100% preplant; SD, 100% side-dressed; SPLIT, 40% preplant, 60% side-dressed; STB, 100% preplant with N stabilizer.

<sup>b</sup> Treatment means within year with the same letter are not significantly different at  $p < 0.1$ .

<sup>c</sup> Estimated C Balance equals wheat CO<sub>2</sub>-C production subtracted from assimilated C at 100% C sequestration efficiency.

### 3.5. Carbon balance

The carbon balance of a system is the net fluctuation of C in and out of the system. Through photosynthesis, atmospheric CO<sub>2</sub> was assimilated by the wheat cover crop of which a portion can be converted and stored in the soil as more stable forms of C [18]. For the STB treatment in 2017, there were greater CO<sub>2</sub>-C emissions in the NT system than in the NTW system, resulting in a negative value for CO<sub>2</sub>-C emissions as a result of the wheat cover crop (Table 3).

Nitrogen treatment had no effect on the estimated wheat CO<sub>2</sub>-C emissions in 2016 or 2017 (Table 3) The average estimated CO<sub>2</sub>-C emissions resulting from the wheat cover crop was 435 kg ha<sup>-1</sup> in 2016 and 199 kg ha<sup>-1</sup> in 2017. Compared to the average amount of assimilated wheat-C in 2016 (1072 kg ha<sup>-1</sup>) and 2017 (558 kg ha<sup>-1</sup>), less C was lost as CO<sub>2</sub> due to the presence of the wheat cover crop compared to the amount of C assimilated by the wheat (2016:  $p = 0.003$ ;

2017:  $p = 0.0052$ ) (Table 3). Over time this should result in an increase of soil OC. Although there was greater average CO<sub>2</sub>-C lost with the NTW system than the NT and CT systems in 2017 (Figure 4), the overall effect of C lost to the atmosphere may be mitigated through C assimilation by the wheat cover crop and the conversion of that C into a stable form of soil C. However, the conversion of C in the soil to more stable forms will not be 100% efficient [47], so the amount of C stored in the soil will be much less than the amount assimilated by the wheat cover crop. Curtin et al. [48] conducted a similar analysis on semi-arid Canadian prairies using wheat to potentially restore C stocks in soil that had been subjected to long-term cropping. Their results did not indicate an increase in soil C stock due to the planting of crested wheatgrass as a cash-crop, but this may have been in part because of using conventional tillage. In semi-arid regions, an increase in soil OC can be slow [49], which was demonstrated by the lack of changes of soil OC observed two years after implementing conservation tillage with a wheat cover crop in the THP. This is especially true when using a grain cover crop on the high plains, where after 19 years of a rye winter cover, there was no difference in soil OC between a no-tillage rye cover treatment and conventional tillage [10].

#### **4. Conclusions**

Emissions of CO<sub>2</sub>-C were affected by tillage system, N-fertilizer management, and climatic conditions over the two-year study. We did not observe short-term impacts of using conservation tillage to reduce gross CO<sub>2</sub>-C emissions. In fact, the NTW treatment tended to increase CO<sub>2</sub>-C emissions from the soil surface compared to the NT and CT systems over the two-year study. Although the NTW system produced greater gross CO<sub>2</sub>-C emissions than the NT system in 2017, the amount of C assimilated in the wheat cover crop was greater than the estimated CO<sub>2</sub>-C emissions resulting from the wheat cover crop (calculated as the difference in emissions between the NTW and NT systems). While not all of the assimilated CO<sub>2</sub>-C in the wheat cover will be converted to a stable C pool, overtime this should result in greater soil OC. This research demonstrated the potential C sequestration benefits of using conservation tillage in conjunction with a winter wheat cover crop in a semi-arid region. We recommend that continued research should be conducted to better understand the changes in CO<sub>2</sub>-C emissions after subsequent years of conservation tillage and cover crop use, and to better understand the dynamics of C sequestration when using a cover crop in a semi-arid agricultural region.

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#### **Conflict of interest**

All Authors declare no conflicts of interest in this paper.

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