# IMPACT OF CROPPING PRACTICES AND TILLAGE ON GREENHOUSE GAS EMISSIONS AND SOIL PROPERTIES DYNAMICS IN SOUTH CENTRAL TEXAS

#### A Dissertation

by

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#### **ABSTRACT**

Over the last two centuries, changes in land use and management from agricultural activities have impacted environmental quality and soil productivity.

Although soils store substantial amounts of carbon (C) (second largest sink after oceans), intensive tillage practices have accelerated the decomposition of soil organic matter and the release of carbon dioxide (CO<sub>2</sub>) into the atmosphere. The greenhouse gas (GHG) mitigation potential of soils, is often overlooked, and limited data is available for accurately quantifying emissions. The objective of this dissertation was to investigate the impact of cropping practices and tillage on GHG emissions in southcentral Texas. We performed three research studies that quantified GHG emissions, soil C balance, and soil physical and chemical properties from long-term conventional and transitioning organic systems.

In the first study, the dynamics of soil CO<sub>2</sub> emissions ( $R_s$ ) and soil environmental conditions as influenced by long-term tillage were investigated at an experimental site established in 1982.  $R_s$  was measured in the monoculture soybean (*Glycine max* (L.) Merr.) and winter wheat (*Triticum aestivum* L.)/soybean rotation cropping systems under conventional and no-tillage practices in 2016. Rotational cropping showed similar soil temperatures between tillage treatments, but higher soil moisture with no-till reduced  $R_s$ . Monoculture soybean showed the opposite trend to rotational cropping, with higher soil temperatures under conventional till that possibly suppressed microbial activity and  $R_s$ . In the second study, we quantified GHG emissions and soil properties from an

unmanaged cropland that transitioned into organic corn (*Zea mays* L.) production on a Vertisol in 2016. Four production systems were evaluated including fall/winter cover crops and reduced tillage practices during 2017 and 2018. During 2017, *R*<sub>s</sub> was higher than in 2018 in all production systems, but the average treatment effect was similar between years. No-tillage resulted in higher GHG emissions as soil cracking occurred. The third study investigated cover crop decomposition (legume vs cereal/legume mixture) through an incubation experiment and a litter bag experiment in 2018. We identified possible stages in microbial growth and activity that regulated nutrient mineralization-immobilization. This research provided important insights into GHG emissions and C and N balance from agricultural systems in Texas.

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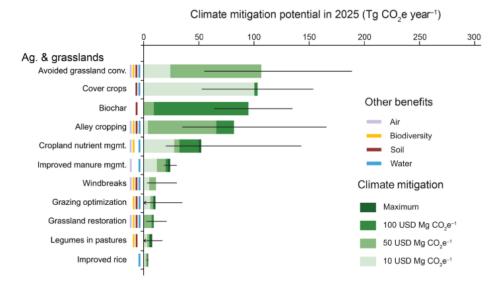
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#### 1. INTRODUCTION

Over the last two centuries, changes in land use and management from agricultural activities have impacted environmental quality and soil productivity (Schlesinger and Andrews, 2000; McGuire et al., 2001; Swift, 2001). Although soils store substantial amounts of carbon (C) (second largest source after oceans), intensive tillage practices have accelerated the decomposition of organic matter and release of carbon dioxide (CO<sub>2</sub>) into the atmosphere (Sabine, 2014; Amundson et al., 2015). On a global scale, 11% of the greenhouse gasses (GHG) are emitted from land use, such as through deforestation, land clearing and tillage for agriculture, and degradation of soils (IPCC, 2014). It is estimated that an emission of 1 Pg of soil C could enrich atmospheric CO<sub>2</sub> by 0.47 ppm (Kimble et al., 2002). If emissions are not curbed, current CO<sub>2</sub> concentration of 408.8 ppm is expected to increase by the end of the century to 600-700 ppm (Anwar et al., 2018). Besides CO<sub>2</sub>, nitrous oxide (N<sub>2</sub>O) and methane (CH<sub>4</sub>) are also GHG emitted from agricultural activities with higher radiative forcing than CO<sub>2</sub> (Parton et al., 2015). The greenhouse warming potential (GWP) of N<sub>2</sub>O is approximately 300 times more than that of CO<sub>2</sub> and CH<sub>4</sub> is 25 times more potent as a GHG than CO<sub>2</sub> (IPCC, 2007).

The adoption of sustainable management practices can mitigate GHG emissions from agriculture. Large-scale practices that can potentially mitigate climate change by increasing C sequestration and reducing GHG emissions are called 'natural climate solutions' (NCS) (Figure 1.1). Land use activities that increase sinks are believed to

impact emissions directly and can at least partially ameliorate the 6.1 Pg CO<sub>2</sub>eq yr<sup>-1</sup> emissions produced from agricultural activities (Griscom et al., 2017). Potential NCS opportunities to mitigate climate change include increasing C sequestration in above-and below-ground biomass, the addition of organic matter inputs through practices such as cover cropping, and reduction of organic matter decomposition rates (Doran and Smith, 1991; Ruis and Blanco-Canqui, 2017). Incorporating cover crops in a cropping program can also help in nutrient management through increasing nutrient availability, improving soil properties, and contributes to long-term sustainability (Johnson et al., 2007; Hill et al., 2017). On average, cover crops could enhance soil organic C by 0.32±0.08 Mg ha<sup>-1</sup> annually, showing great potential as a sequestration strategy (Poeplau and Don, 2015). Implementing conservation practices such as cover cropping is expected to increase C inputs into the soil by 103 Tg CO<sub>2</sub> eq yr<sup>-1</sup> (Fargione et al., 2018).



**Figure 1.1** Climate mitigation potential of NCS (natural climate solutions) in the United States. Black lines indicate the 95% CI or reported range. Ecosystem service benefits linked with each NCS are indicated by colored bars (Fargione et al., 2018).

Quantifying the C and nutrient losses from agricultural activities continues to be a challenge given the complexity of soil dynamics, driving factors that regulate fluxes and pool sizes, and the time-scale over which soil processes occur. An increase in soil organic C is known to improve soil resilience, soil structural stability, microbial processes, water retention, and crop productivity (Houghton, 2007). Soil C storage in croplands is notably affected by intensive tillage practices and crop intensification that modify soil physical, chemical, and biological properties (Johnson et al., 2007). Conventional tillage practices are expected to increase wind and water soil erosion, land degradation, and organic matter oxidation. On the other hand, conservation tillage (for example, no-tillage and strip tillage) has been shown to reduce soil and water losses, enhance soil productivity, reduce runoff and erosion, and sequester soil organic C (Mitchell et al., 2013). Overall, conservation tillage practices can reduce net GHG

emissions and improve soil and environmental quality. There is an urgency to develop and determine the impact of practices that can positively impact the environment as well as identify environmental variables that reduce soil GHG emissions (Han et al., 2017; Ruis and Blanco-Canqui, 2017). The GHG mitigation potential of soils is often overlooked, and high-frequency soil flux measurements are needed to accurately quantify emissions from soils.

In this research, three field experiments were performed to quantify GHG emissions, soil C balance, and soil physical properties from long-term conventional and transitioning organic cropping systems in south central Texas. In the first study, the dynamics of soil CO<sub>2</sub> emissions as influenced by long-term tillage practices were investigated by measuring fluxes from an experimental site that has utilized monoculture and rotational cropping systems under conventional and no-tillage practices since 1982. In the second study, GHG emissions from an unmanaged cropland transitioning into organic grain crop production were quantified. The third study investigated C and nitrogen mineralization from cover crops in organically managed soils.

This research provided important insights into GHG emissions and C sequestration potential from agricultural systems in south central Texas. The outcomes from this project include a more comprehensive understanding of the diurnal, seasonal, and inter-annual C fluxes from agroecosystems and their relationship with climate, agricultural management, and plant and soil processes. The results will also improve estimations of C pools and fluxes from dynamic agricultural systems under future climate change scenarios.

# 2. IMPACT OF LONG-TERM TILLAGE ON SOIL CO<sub>2</sub> EMISSIONS AND SOIL CARBON SEQUESTRATION IN MONOCULTURE AND ROTATIONAL CROPPING SYSTEMS

#### 2.1. Introduction

Soil CO<sub>2</sub> flux, or soil respiration  $(R_s)$ , is a major component of the terrestrial carbon (C) cycle (Lloyd and Taylor, 1994; Schlesinger and Andrews, 2000). Agricultural soils globally account for nearly 25% of CO<sub>2</sub> released to the atmosphere from anthropogenic sources (Hutchinson and Campbell, 2007; Gao et al., 2017). In natural ecosystems, dead biomass undergoes slow decay as a result of microbial activity that converts part of the biomass carbon to CO<sub>2</sub> which is returned to the atmosphere as  $R_s$ . The remainder of the biomass C undergoes a complex sequence of transformations in soils and eventually becomes associated with soil particles and aggregates that limit further decomposition (Swift, 2001; Lal, 2002; Six et al., 2002). This "stable" or "occluded" C can potentially persist in the soil profile for thousands of years, effectively sequestering it to the lithosphere in a manner that can at least partially counteract the effects of fossil fuel burning (Torn et al., 1997; Amundson et al., 2015; Castellano et al., 2015). Cultivation, however, produces dramatic changes in soil physical and biological characteristics and sets the stage for rapid decay of organic C. Post and Kwon (2000) reported that up to 50% of organic C in the top 20 cm of the soil is usually lost within three to five decades of cultivation of agricultural soils.

In addition to the influence of soil tillage on decomposition and C accumulation, cropping system intensification has also been found to affect soil organic C (Dou et al., 2008; Rosenzweig et al., 2018). Crop rotation may increase both aboveground and belowground residue inputs. Numerous studies have focused on understanding the effect of crop rotation in soil fertility, water availability, plant growth, and crop yield (Franzluebbers et al., 1995c; Adiku et al., 2008; Tan et al., 2015). However, the major C contribution in rotational systems often results from root exudation and decomposition (Keiluweit et al., 2015; He et al., 2016). Root decomposition impacts water storage, microbiome diversity, and nutrient cycling (Van Der Krift et al., 2001; Tiemann et al., 2015). The interrelationship of environmental driving factors (i.e., soil temperature and moisture) and surface soil respiration provides an indication of below ground C decomposition rates under different cropping intensities.

A majority of previous studies that investigated soil CO<sub>2</sub> flux from agroecosystems have been based on manual static chamber measurements (Hendrix et al., 1988; Paul et al., 1999; Rochette and Hutchinson, 2005; Storlien et al., 2014). However, the manual static chamber method is laborious. Thus measurements are usually made at weekly, bi-weekly or monthly intervals (Rochette et al., 1991; Pendall et al., 2001; Elder and Lal, 2008; Ussiri and Lal, 2009). Due to inadequacies in temporal resolution, static measurements could potentially lead to significant over- or underestimation of cumulative soil CO<sub>2</sub> fluxes over the entire growing season (Hendrix et al., 1988). Besides, our understanding of diurnal cycles of soil respiration and its short-term responses to management-related perturbations such as tillage and fertilization is still

limited (Görres et al., 2015). Recent advancements in automated chamber technologies have increased the number of studies that use high-frequency soil respiration measurements. However, the majority of such studies have been conducted in undisturbed ecosystems (Carbone et al., 2008; Chang et al., 2008).

In this study, we present results from two field experiments performed to investigate high-frequency CO<sub>2</sub> flux measurements from a long-term cropping systems site in south central Texas. This site was established in 1982 and includes different tillage and cropping sequences. Specific objectives of this study were (1) to determine the effect of tillage management practices and cropping systems on the magnitude of soil respiration measured at half-hourly intervals, (2) to investigate changes in soil environmental conditions as affected by tillage and cropping sequences, and (3) to determine the sensitivity of soil respiration to varying soil temperature and moisture conditions in the field.

#### 2.2. Materials and Methods

#### 2.2.1. Long-term Study

The study was conducted in a long-term cropping systems experiment established in 1982 at the Texas A&M University Research Farm (30°32'N, 96°26'W, 68.6 m a.m.s.l.) near College Station located in south central Texas. The climate is humid subtropical with mean annual precipitation of 992 mm (NOAA. 2016), annual average minimum air temperature of 15°C, and an average maximum of 27°C. Daily air temperature and precipitation for the measurement period were obtained from a weather station located approximately 350 m from the experimental site (Figure 2.1). The soil at

the site is a Weswood silty clay loam (fine-silty, mixed, superactive, thermic Udifluventic Haplustepts) with 39 % silt, 23 % sand, 38 % clay in the top 30 m of the profile.

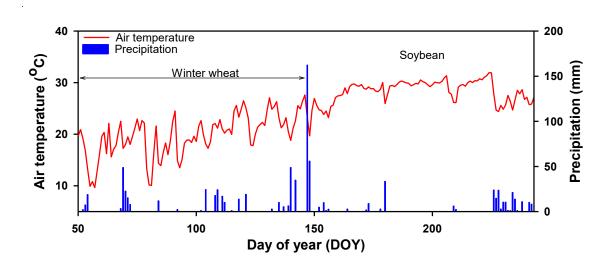


Figure 2.1 Daily air temperature and precipitation during the measurement period.

The experiment was established as a split design with cropping systems (winter wheat/soybean rotation and soybean monoculture) as main plot and tillage as split plot (conventional and no-tillage) in a randomized complete block with four replications. The size of each plot was 12 x 4 m. We conducted continuous soil CO<sub>2</sub> flux measurements from conventional and no-tillage winter wheat (in rotation with soybean) and soybean monoculture plots. Winter wheat received a total nitrogen application of 68 kg N ha<sup>-1</sup>, broadcast as ammonia nitrate (NH<sub>4</sub>NO<sub>3</sub>), in two equal split applications shortly after emergence and 60 days after planting. Soybean did not receive N fertilizer. Conventional tillage consisted of disking to a depth of 25 cm three to four times after harvest. No-tillage had no soil disturbance other than that resulting from planting. Winter wheat was

planted in 0.18 m wide rows on 14 November 2015 (DOY 318) and harvested on 25 May 2016 (DOY 146). Soybean was planted in 1 m wide rows on 25 April 2016 (DOY 116) and harvested on 10 October 2016 (DOY 284).

#### 2.2.2. Soil CO<sub>2</sub> Flux Measurements

Soil respiration was measured using an automated soil CO<sub>2</sub> flux system (Model LI-8100A, LI-COR Biosciences, Lincoln, NE, USA) with four long-term chambers (Model 8100-104C, LI-COR Biosciences, Lincoln, NE, USA; headspace volume of 4.8 L). All four chambers were connected to a multiplexer (LI-8150, LI-COR Biosciences, Lincoln, NE, USA) and an infrared gas analyzer (IRGA) that measured CO<sub>2</sub> and water vapor (H<sub>2</sub>O) concentrations simultaneously at 1 Hz frequency. Chambers were placed over PVC collars (20 cm diameter, height 11.43 cm, and area 317.8 cm<sup>2</sup>) inserted to a depth of approximately 5 cm. These collars were installed permanently throughout the winter wheat and soybean growing seasons. In winter wheat plots, soil chambers were installed between the rows with two chambers each in conventional tillage and no-tillage plots. Because of wider row spacing in soybean plots, one chamber was installed close to the crop row (10 cm from the plant stem) and one in between the crop rows (50 cm from the plant stem). Similar to winter wheat plots, two chambers were installed in conventional tillage and two in no-tillage soybean plots. Measurements in the winter wheat plots started on 23 February (DOY 54) and ended on 25 May (DOY 146). Measurements in the soybean plots began on 12 May (DOY 133) and ended on 6 October (DOY 280). No measurements were taken between 26 May (DOY 147) and 16 July (DOY 198) due to instrument malfunction. Measurements from the soybean plots

were resumed on 17 July (DOY 199). Soil chambers were programmed to measure soil CO<sub>2</sub> flux sequentially from all four chambers at half-hourly intervals, with the actual measurement period lasting 3-minutes. At the beginning of the measurement period, the automated closure mechanism of the soil chamber gently lowered the chamber bowl cover onto the soil collar. After the measurement was completed, the chamber moved away from the soil collar. Raw data were saved on a Secure Digital (SD) memory card onboard the IRGA. Soil CO<sub>2</sub> flux during the measurement period was estimated by plotting the increase in CO<sub>2</sub> concentration against time using SoilFluxPro software (version 4.0.5, LI-COR Biosciences, Lincoln, NE, USA).

#### 2.2.3. Environmental Measurements

Water content reflectometer sensors (Model CS655, Campbell Scientific, Logan, UT, USA) were used to monitor soil volumetric water content (VWC) and temperature continuously at 5, 10, 20 and 30 cm depths. Data from all sensors were measured at 30-second intervals using a CR1000 datalogger (Campbell Scientific, Logan, UT, USA) and saved as 30-minute average values. Sensors were installed near the chamber in each tillage treatment. Precipitation and air temperature data were obtained from a weather station located at 375 m from the experimental site.

#### 2.2.4. Soil Carbon and Nitrogen

Soil samples were collected from both soybean and winter wheat plots in November 2015. Eight soil cores (2.5 cm diameter) were collected along a transect line from the crop row to the middle-row. Soil cores were sectioned and composited into four depth intervals (0–5, 5–10, 10–20, and 20–30 cm). Soil samples were air dried and

ground to pass an 80 mesh (0.18 mm) sieve to determine total soil nitrogen (TN), total C (TC), and soil organic C (SOC) via thermal combustion analysis (Schulte and Hopkins, 1996). Soil bulk density was determined at 10, 20 and 30 cm depths at the beginning and end of the growing season by the soil core (4.5 cm diameter) method (Blake and Hartge, 1986). Three soil cores were collected per plot and oven dried at 105°C. The potential of conservation tillage in sequestering soil C was estimated by comparing SOC measured at winter wheat planting with historical measurements performed at the experimental site (Franzluebbers et al., 1994; Dou et al., 2007; González-Chávez et al., 2010).

#### 2.2.5. Data Processing and Statistical Analysis

Half-hourly mean soil CO<sub>2</sub> flux was calculated as the average of the two soil chambers located within a treatment plot. The temperature dependence of soil CO<sub>2</sub> flux was evaluated by estimating the  $Q_{10}$  value (the factor by which soil CO<sub>2</sub> flux increases with an increase in temperature of 10°C). Soil temperature was separated into 2°C bins, with soil fluxes averaged over each bin to capture the diurnal pattern of soil respiration and temperature (Falge et al., 2001). A nonlinear function of the form  $R_s = \beta_0 e^{\beta_1 T_s}$  was fitted to the measured  $R_s$  and  $T_s$ .  $Q_{10}$  was then calculated using equation 2.1 and the parameters  $\beta_0$  and  $\beta_1$ , which corresponded to the intercept of the exponential curve and rate of increase in  $R_s$ , respectively.

$$Q_{10} = e^{10\beta_1}$$
 [Eq. 2.1]

Pair-wise t-tests were used to compare soil temperature and moisture at each depth between cropping systems. A two-way analysis of variance (ANOVA) was performed to assess the effect of long-term tillage and cropping systems with depth on

SOC, TC, and TN. All data processing and statistics were performed using SAS statistical software (SAS Institute, 2017).

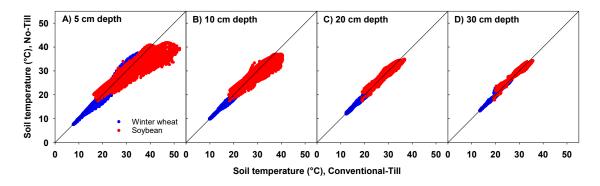
To investigate the effect of temporal frequency of soil CO<sub>2</sub> flux measurements on C emissions between cropping systems and tillage practices, we calculated cumulative C emissions by interpolating fluxes at three-time intervals. The half-hour frequency used the 30-minutes soil CO<sub>2</sub> flux records to calculate cumulative emissions. The daily frequency estimated the daily average soil CO<sub>2</sub> flux from measurements collected from 0900 h to 1100h. The weekly frequency calculated cumulative emissions from 7-day average fluxes measured from 0900 h to 1100h. We used a penalized regression spline to fit a curve that connected all data points using PROC EXPAND. Cumulative growing season emissions were estimated by integrating the underlying area and expressed as C losses.

#### 2.3. Results and Discussion

#### 2.3.1. Soil Temperature in Conventional and No-Tillage Plots

Scatter plots were constructed by plotting half-hourly records of soil temperature from conventional tillage plots against no-tillage plots at four depths for both crops (Figures 2.2A-D). In general, conventional tillage plots had higher soil temperatures compared to no-tillage plots (more points below the 1:1 line in scatter plots). For winter wheat, the mean soil temperatures in conventional and no-tillage plots were 20.1 and 19.9°C at 5 cm, 19.8 and 19.7°C at 10 cm, 19.7 and 19.4°C at 20 cm, and 19.5 and 19.2°C at 30 cm depths, respectively. The diurnal fluctuations in soil temperature in

conventional and no-tillage winter wheat plots were similar throughout the growing season at all depths with no significant differences (data not shown).



**Figure 2.2** Soil temperature at A) 5 cm, B) 10 cm, C) 20 cm, and D) 30 cm depth measured at 30-minute intervals during the growing season of the rotation winterwheat/soybean and monoculture soybean under conventional- and no-tillage.

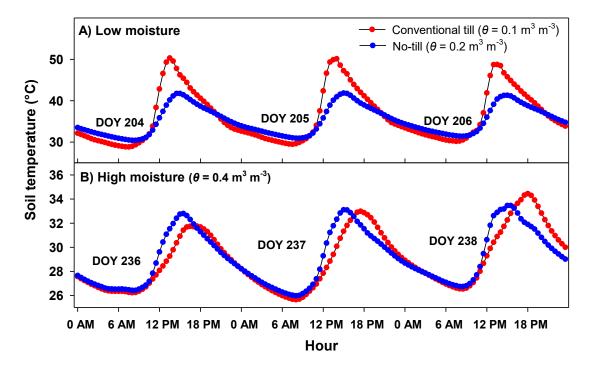
For soybean, differences in soil temperature due to tillage were more pronounced in the topsoil (5 cm depth). The mean soil temperatures in conventional and no-tillage soybean plots were 32.3 and 30.9°C at 5 cm, 31.3 and 30.4°C at 10 cm, 30.3 and 29.8°C at 20 cm, and 29.8 and 29.7°C at 30 cm depths, respectively. Unlike winter wheat, soil temperatures in soybean plots were significantly different between tillage treatments (*P* < 0.0001). The magnitude of differences in soil temperature between conventional and no-tillage plots was more pronounced under low soil moisture conditions (Figure 2.3A). For example, on DOY 204-206 under drier conditions (VWC < 0.2 m³ m⁻³), the maximum soil temperature at 5 cm depth with conventional tillage was at least 13°C higher than with no-tillage (Figure 2.3A). Additionally, there was a 1 h lag in time when the peak temperatures were observed in conventional and no-tillage plots. As soil moisture content increased to saturation, the diurnal amplitude of temperature became

similar for both conventional and no-tillage (Figure 2.3B). For example, on DOY 236-238, differences between maximum air temperatures for conventional tillage and no-tillage decreased to approximately 1°C after VWC increased to 0.4 m<sup>3</sup> m<sup>-3</sup> following precipitation (Figure 2.3B). On those days, the lag in peak soil temperatures increased to 3 h between no-tillage and conventional treatments.

Our results, which showed large differences in soil temperature between conventional and no-tillage in wider row cropping systems, are in agreement with findings from previously conducted research in similar systems (Johnson and Lowery, 1985; Amos et al., 2007). Residue accumulation on the surface of no-till plots also plays a major role in decreasing soil temperature compared to conventionally managed plots due to the low thermal conductivity of crop residues (Fabrizzi et al., 2005). Greater residue amounts on the surface in no-tillage soybean plots at our study site reduced heat loss that resulted in higher temperatures at nighttime compared to conventional plots. During daytime, residues in no-tillage plots increased surface reflectivity and decreased heat penetration which resulted in lower soil temperatures compared to conventional plots (Chen and McKyes, 1993). The narrow row spacing (0.18 m) and high residue input with winter wheat that helped reflect solar radiation and insulate the soil surface are possible reasons for no differences in soil temperature as affected by tillage for this crop.

The time lag in daily soil temperature peaks in conventional and no-tillage soybeans (Figure 2.3) can be explained by the changes in heat capacity and thermal conductivity induced by tillage. Heat flux through soil is affected by water content, soil

structure and bulk density, all of which are strongly impacted by tillage (Potter et al., 1985; Arshad and Azooz, 1996; Shen et al., 2018). On DOY 204-206, VWC in no-tillage plots was higher (0.2) than in conventional plots (0.1). Higher VWC increased the soil heat capacity and thermal conductivity, resulting in more energy required to warm those soils. The difference in the diurnal amplitude of soil temperature between tillage treatments under dry conditions suggested that drier soils cool and warm faster than wet soils. Soil disturbance reduces aggregate among aggregates, increasing thermal conductivity and producing large temperature fluctuations in conventional tillage (Abu-Hamdeh, 2000). Thus, tillage exerts a stronger effect in damping soil temperature during warm and dry conditions. Under moist conditions (DOY 237-238), the lag in maximum temperatures is explained because of differences in heat capacity, organic matter, bulk density and aggregation between tillage treatments that alter soil thermal diffusivity and the ability of the soil to store heat when temperature varies (admittance). Overall, notillage plots generally have more SOC than conventional plots, higher water content, and lower temperatures, suggesting that no-tillage will transmit heat faster into the soil (Johnson and Lowery, 1985; Licht and Al-Kaisi, 2005).



**Figure 2.3** Soil temperature measured at 5 cm depth for three consecutive days during A) low moisture and B) high moisture of the monoculture soybean under conventional-and no-tillage.

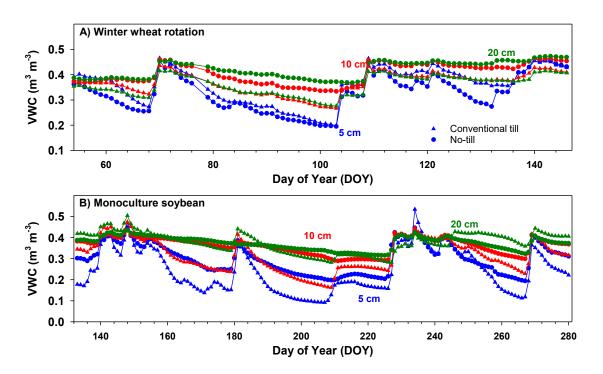
#### 2.3.2. Soil Moisture in Conventional and No-Tillage Plots

Daily average records of soil VWC under conventional and no-tillage winter wheat and soybean are presented in Figure 2.4. Soil moisture was different between tillage treatments at all depths (P < 0.05). The no-tillage winter wheat plots had higher soil moisture at 10 and 20 cm depths throughout the season compared to conventional tillage plots. However, the conventional tillage winter wheat plots had higher soil VWC at 5 cm depth compared to no-tillage plots on most days during the growing season (Figure 2.4A). Differences in VWC between conventional and no-tillage winter wheat plots were more pronounced during drying periods after precipitation. High VWC in the

topsoil of conventional plots could be attributed to increased water infiltration in those plots due to loosening of soil followed by tillage operations. Previous studies have reported that in the short-term, tillage may increase rapid soil water infiltration and hydraulic conductivity by increasing soil porosity (Azooz and Arshad, 1996; Ruiz and Blanco-Canqui, 2017). However, in the long-term, infiltration rates may decrease due to structural deterioration, and low residue and surface SOC accumulation (Guzha, 2004, Franzluebbers, 2002). The narrow row spacing and active wheat growth in the midseason that increased groundcover and reduced water loss could explain why small differences in moisture were observed at 5 cm depth in the mid and late seasons (Amos et al., 2007).

Soil water content at the 5 cm depth under soybean was greater with no-tillage than with conventional tillage by an average of 27%, with differences being consistent throughout the season (Figure 2.4B). The effect of tillage on soil moisture was most notable during drying periods after precipitation and in the topsoil layers, i.e., DOY 160-180, 184-208, and 252-268. In these three periods, VWC at 5 cm depth in no-tillage was on average 48% higher than in conventional tillage. The differences in VWC between tillage treatments decreased with depth and showed the least differences at 20 cm depth, where no-tillage was on average 10% higher. Similar results were found by Franzluebbers et al. (1995a), where a large difference in soil moisture occurred at 5 cm. This effect was likely related to more rapid residue decomposition with conventional tillage where residues are incorporated whereas in no-tillage, residues remained on the soil surface. These results emphasize the role of conservation tillage in enhancing soil

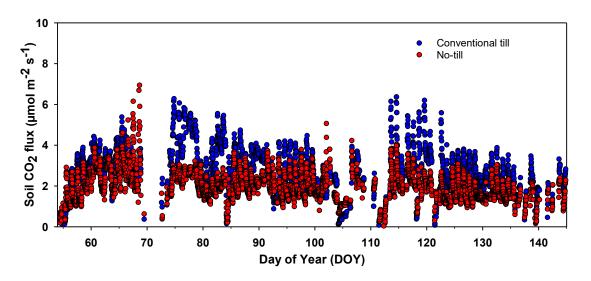
water storage that can help sustain root activity during drought periods (Merrill et al., 1996). Conventional tillage stimulates a more rapid drying rate in soils because of higher soil temperatures measured with this treatment. Soil temperature was negatively correlated with moisture at all depths and tillage treatments. A stronger correlation was found under no-tillage, likely due to the effect of crop residue on the soil surface acting as a barrier, reducing latent heat losses and maintaining soil water content (Wagger and Denton, 1992; Shen et al., 2018). YSC1990120656



**Figure 2.4** Daily volumetric soil water content (VWC) measured at 5, 10 and 20 cm depth in A) winter wheat rotation and B) monoculture soybean under conventional- and no-tillage plots.

#### 2.3.3. Half-Hourly Soil Respiration Measurements

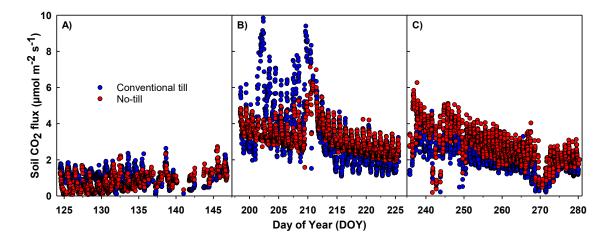
During the majority of the growing season, the conventionally managed winter wheat exhibited higher soil CO<sub>2</sub> emissions compared to no-tillage (Figure 2.5). On average, half-hourly soil CO<sub>2</sub> flux from conventionally managed winter wheat was 16% higher  $(2.46 \pm 1 \, \mu \text{mol m}^{-2} \, \text{s}^{-1})$  compared to no-tillage  $(2.06 \pm 0.97 \, \mu \text{mol m}^{-2} \, \text{s}^{-1})$ . Higher soil CO<sub>2</sub> flux from conventional tillage could be attributed to higher soil temperature and moisture at 5 cm depth compared to no-tillage (Figure 2.4A). Additionally, conventional tillage wheat had better crop growth resulting in higher yield (data not shown) compared to no-tillage, increasing the contribution from root respiration to above-ground soil CO<sub>2</sub> flux. Plant productivity is known to regulate diurnal and seasonal variation of soil respiration. In studies conducted in forest ecosystems (Tang et al., 2005; Vargas et al., 2011), half of the contribution to soil CO<sub>2</sub> flux came from plant roots as carbohydrates were translocated downwards to roots for respiration.



**Figure 2.5** Soil CO<sub>2</sub> flux measured at half-hour intervals from winter wheat plots under conventional and no-tillage.

Emissions from soybean plots were lowest at the early vegetative stage (DOY 124–146; Figure 2.6A), reached maximum at reproductive stage (DOY 198–225; Figure 2.6B) and started declining during the maturity period (DOY 230–280; Figure 2.6C). During the early growth period (Figure 2.6A), emissions from conventional tillage soybean were higher than with no-tillage, which could be due to the combined effect of soil disturbance and significantly higher topsoil temperatures in conventional tillage plots (P < 0.001) (Figure 2.4B). During the early bloom stage (DOY 198-211; Figure 2.6B), daytime soil CO<sub>2</sub> emissions from conventional-tillage plots were substantially higher than from no-tillage. Although topsoil was drier with conventional tillage (VWC 0.1), soil moisture at 20 cm depth was close to field capacity (VWC 0.4). Similar to winter wheat, soybean had better crop growth with conventional tillage resulting in higher yield compared to no-tillage. During the early bloom stage, the soybean root system expands rapidly (Torrion et al., 2012), potentially increasing the contribution of root respiration to total soil CO<sub>2</sub> flux during this time. After a rainfall event on DOY 209 (9.4 mm), daytime soil CO<sub>2</sub> emissions from no-tillage became higher than conventionaltill on most days until the end of the growing season (Figures 2.6B and 2.6C). Two possible explanations for this include (1) availability of more labile C in the topsoil of no-tillage plots after rainfall events due to greater amounts of decomposing residues (Kessavalou et al., 1998; Adiku et al., 2008; Liang et al., 2015), and (2) higher soil moisture in no-tillage plots compared to conventional plots favoring higher microbial activity with no-tillage. In addition, peak day-time soil temperature exceeded the optimum for microbial activity (approximately 40°C) by several degrees in

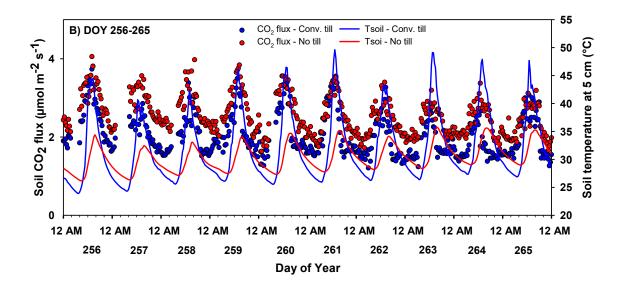
conventional-tillage plots, thereby potentially reducing respiration in those plots (Conant et al., 2011; Blagodatskaya et al., 2016; Liu et al., 2018).



**Figure 2.6** Soil CO<sub>2</sub> flux measured at half-hour intervals from soybean plots under conventional and no-till during the A) vegetative, B) early-bloom, and C) maturity growth stages.

Our data showed that night-time soil CO<sub>2</sub> emissions were always higher in notillage plots after the onset of the reproductive stage in soybean. An example data set is presented in Figure 2.7 for DOY 256–265. This trend in soil respiration might be explained by the soil temperature and moisture dynamics in the no-tillage plots. The higher night time soil respiration with no-tillage could be associated with the higher night time temperatures in those plots. During the day, soil temperature in no-tillage was lower due to less radiant heat transfer as a result of residue accumulation. Crop residues also act as a barrier to soil evaporation, thus increasing soil moisture content. However, from 1000 h to 0800 h, soil temperatures in no-tillage were higher because the surface residues hindered the escape of longwave radiation resulting in warmer soils. The amplitude of temperature variation was higher in conventional compared to no-tillage

plots. Soil disturbance associated with tillage modifies the pattern of heat transport into the soil profile (Hillel, 1998), mainly by initially reducing thermal conductivity and increasing the number of soil macropores resulting in rapid changes in soil temperature.



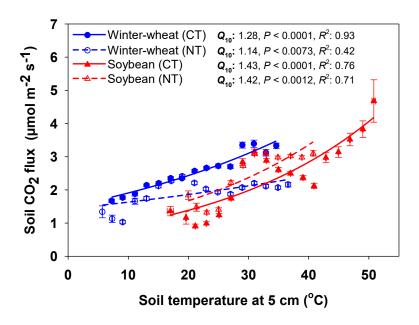
**Figure 2.7** Diurnal soil CO<sub>2</sub> flux and soil temperature measured at 5 cm depth in conventional and no-till plots soybean monoculture during DOY 256-265.

#### 2.3.4. Soil Temperature, Moisture, and Soil CO<sub>2</sub> fluxes

For winter wheat, the  $Q_{10}$  values estimated were 1.28 for conventional vs. 1.14 for no-till plots (Figure 2.8). Overall, we observed that conventional-tillage exhibited a more rapid increase in soil  $CO_2$  emissions with an increase in temperature compared to no-tillage. Although we did not observe differences in soil temperature in winter wheat as affected by tillage (P = 0.191), sensitivity to temperature was lower in no-tillage plots. The higher VWC found in no-till soil (Figure 2.4) may explain the lower temperature-dependency of  $CO_2$  emissions for no-tillage as indicated by the  $Q_{10}$  value. No-tillage

systems that accumulate more crop residue on the soil surface may have reduced temperature sensitivity that results in lower  $CO_2$  fluxes, and may indicate  $Q_{10}$  is partly regulated by plant residue in no-tillage. Meyer et al. (2018) found that soil respiration was significantly affected by soil moisture, which also showed a positive relationship with soil total N and SOC.

Although no difference in soil temperature between tillage treatments was observed with winter wheat at 5 cm depth, deeper soil layers showed a positive relationship with CO<sub>2</sub> fluxes. This result indicated that soil CO<sub>2</sub> emissions are not only regulated by topsoil layer conditions, but also by contributions from deeper soil layers under certain scenarios. For example, high CO<sub>2</sub> fluxes in winter wheat under conventional till were observed when VWC ranged from 0.2 to 0.4, whereas in no-till plots, higher fluxes were occasionally observed when VWC was between 0.25 at 5 and 30 cm, and 0.4 at 10 and 20 cm (Figures 2.9A-D). This trend was observed in both cropping systems, and may partially be explained because deeper layers contain more sand because of the alluvial stratification associated with the depositions of this soil and the resultant textural changes throughout the soil profile. Our results suggested that measuring temperature and moisture in deeper soil layers can help account for spatial variability along the soil profile and its contribution to total CO<sub>2</sub> flux. Xiao et al. (2015) found similar results in bare soil and concluded that soil drying increased air-filled pore space, which allows CO<sub>2</sub> diffusion from a deeper soil layer to the soil surface.

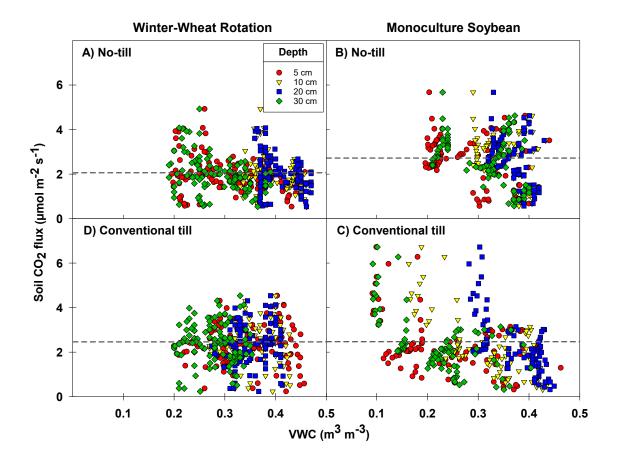


**Figure 2.8** Soil CO<sub>2</sub> flux versus soil temperature at 5 cm depth for winter wheat (blue) and soybean (red). Solid symbols are conventional tillage (CT) and open symbols are no-tillage (NT). Soil temperature was separated into 2°C bins and averaged over each bin.

For soybean, Q<sub>10</sub> values estimated were similar between conventional-tillage and no-till (Figure 2.8). Unlike winter wheat, we did not observe an increase in soil CO<sub>2</sub> flux with soil temperature as the growing season progressed. During the early season, conventional-till plots showed a greater soil CO<sub>2</sub> flux response to soil temperature (Figure 2.6A). Temperature-dependency of soil CO<sub>2</sub> emissions in mid-growing season showed more dispersion with conventional than no-till (Figure 2.6B). This result could be due to the high temperatures observed during the summer that exceeded optimum thresholds for microbial activity and respiration (Figure 2.6B). Late season emissions were higher in no-till plots and occurred when soil temperature ranged from 20 to 35°C, whereas in conventional-till plots soil temperature ranged from 20 to 50°C. The lower

temperature range in soil temperature could have enhanced microbial activity and increased C losses that resulted in higher CO<sub>2</sub> emissions in no-till. This explanation was also supported by the narrower soil moisture range in no-till plots where VWC ranged from 0.2 to 0.45 compared to conventional plots that ranged from 0.09 to 0.45 VWC throughout the soil profile (Figures 2.9B-D).

Conventional plots showed warmer conditions at 5 cm (maximum temperature ~ 50°C) that decreased respiration as it exceeded the optimum threshold for microbial activity (Figure 2.2). Meyer et al. (2018) defined soil temperatures between 5-25°C and soil moisture between 30-75% water holding capacity as optimum thresholds for microbial activity. Higher CO<sub>2</sub> fluxes observed in conventional till occasionally occurred when VWC was around 0.1 at 5 and 30 cm, whereas at 10 and 20 cm depth VWC was around 0.25 (Figure 2.9B-D). The drier conditions that occurred during this period may have increased gas diffusivity, considering that high soil water content acts as a barrier for gas movement, and may possibly explain the high fluxes observed under lower moisture content. The greater fluxes also occurred during the most active plant growth period in which more root respiration and water uptake likely occurred. The observed changes in soil temperature and moisture induced by tillage practices might explain why monoculture systems are more likely to result in higher CO<sub>2</sub> emissions compared to rotational crops (Figure 2.7). Crop rotation tends to increase belowground microbiome diversity and soil fertility contributors, such as soil C and N and microbial biomass (Tiemann et al., 2015).



**Figure 2.9** Relationship of daily average soil CO<sub>2</sub> flux with volumetric water content (VWC) in no-till plots in (A) monoculture soybean and (B) winter wheat/soybean rotation, and conventional till plots in (C) monoculture soybean and (D) winter-wheat/soybean rotation. The dashed lines denote the mean soil CO<sub>2</sub> emission during the winter wheat and soybean measurement periods.

### 2.3.5. Temporal Resolution of Soil CO<sub>2</sub> Measurements

Cumulative soil CO<sub>2</sub>-C emissions estimated at three measurement frequencies in each cropping system are presented in Table 2.1. For winter-wheat, conventional tillage showed higher C emissions than no-tillage independent of the sampling frequency at which soil flux was measured. At half-hour and daily measurements, conventional till emitted 17% more C than no-tillage, while when measurements were done weekly

conventional till emitted 13% more C. Average soil CO<sub>2</sub> emission flux at all measurement intervals evaluated were similar in conventional till plots, while emissions in no-tillage were potentially overestimated by 8% when measured weekly from 0900 h to 1100 h.

Unlike winter wheat, the effect of tillage on C emissions in the soybean monoculture varied depending on the sampling frequency at which the fluxes were measured. Cumulative emissions calculated using half-hour soil CO<sub>2</sub> fluxes were 10% higher from no-tillage than conventional till plots. However, at the larger daily or weekly time intervals, no differences in C emissions were found between tillage practices. Also, daily and weekly measurements overestimated conventional tillage C fluxes by 16% and 18%, respectively, compared to half-hour fluxes. No-tillage practices were less affected by flux measurement frequency, with total emission overestimated by 3% using daily and by 6% using weekly flux measurements compared to half-hour fluxes.

Automated systems that provide high temporal frequency soil CO<sub>2</sub> flux measurements have previously been shown to improve the capability to relate fluxes to rapid changes in soil moisture and temperature compared to less intensive manual systems (Savage and Davidson, 2003). Similar results were found in this study, in which flux differences among sampling intervals were mostly observed during the warmer months of the soybean growing season, but not with winter wheat. Cumulative C emissions from interpolation of half-hour soybean data indicated that conventional tillage systems emitted less C than no-tillage, while fluxes were similar when interpolated at daily or weekly emissions (Table 2.1). Under variable environmental

conditions, high-frequency measurements are recommended and likely better captured treatment effects on soil CO<sub>2</sub> fluxes.

**Table 2.1** Cumulative soil CO<sub>2</sub>-C emissions (kg ha<sup>-1</sup>) estimated using half-hour, and average daily and weekly fluxes from 0900 h to 1100 h.

Measurement	Wheat rotati	ion	Soybean monoculture		
frequency	Conventional tillage	No-tillage	Conventional tillage	No-tillage	
Half-hour	2876	2384	3947	4334	
Daily	2922	2418	4568	4455	
Weekly	2956	2566	4641	4580	

## 2.3.6. Long-Term Effect of Tillage on Soil Carbon Sequestration

Measurements of SOC and soil total nitrogen of the same reported study were compiled in 1991 (Franzluebbers et al., 1994), 2002 (Dou et al., 2007), and 2016 (Zapata et al., 2018) to determine the long-term effect of crop intensification and tillage on carbon sequestration (Table 2.2). Both crop intensification and tillage had a significant effect on long-term C sequestration. Overall, from 1991 to 2016 SOC was lower in the soybean monoculture compared to the winter wheat rotation. Likewise, no-tillage always showed higher SOC compared to conventional tillage. SOC varied among years, with a slight decrease observed from 2002 to 2016 that is likely due to differences in analytical methodologies to determine C. We also observed that soil total N steadily increased in both tillage practices, with no-tillage showing a higher N content.

After 34-years, the increase in SOC content in no-till winter wheat/soybean rotation plots compared to conventionally managed plots was 46% at 5 cm, 17% at 10 cm, 9% at 20 cm, and 40% at 30 cm. In the monoculture soybean plots, the increase in SOC content in no-tillage plots was 53% at 5 cm, 27% at 10 cm, 8% at 20 cm, and 45% at 30 cm depth compared to conventional-tillage plots. Although SOC was higher in

winter wheat, the increase in SOC in the topsoil was similar in both cropping systems. In 2016, when comparing only the effect of cropping intensity on SOC, crop rotation increased SOC an average of 62% at 5 cm, 57% at 10 cm, 33% at 20 cm and 25% at 30 cm depth compared to monoculture. The large increase in SOC in rotational systems is expected as crop rotation often increases both above and below ground residue inputs. In addition, when comparing only the effect of tillage on SOC, no-tillage increased SOC an average of 33% at 5 cm, 17% at 10 cm, 8% at 20 cm and 30% at 30 cm depth compared to conventional tillage. Thus, crop intensification had the greater effect on SOC accumulation compared with tillage.

Although there was no significant interaction between crop rotation and tillage, we observed that monoculture under no-tillage yielded similar results to that of crop rotation under conventional till. Reduced tillage and crop intensification are known strategies that increase SOC in the long-term (Dou et al., 2008), but in this study these two strategies did not contribute similarly to C accumulation. The effect of high crop residue input plus soil disturbance was comparable to having low residue input in undisturbed soils (soybean). The different qualities of residue from cereals (high C:N) and legumes (low C:N) could explain the lower SOC observed with soybean in 2016, in which C accumulation was promoted by the slower decomposition of crop litter from winter wheat (Fontaine et al., 2004).

Total soil N increased in the long-term at all depths under winter wheat and soybean, with a greater increase under conventional plots (Table 2.2). After 14 years (from 2002 to 2016), soil N at 5 cm depth increased 72% in conventional till and 32% in

no-till in the rotational winter wheat treatment. For the same depth, soil N in monoculture soybean under conventional till increased 135% compared to 40% in no-till. At deeper soil layers (10 and 20 cm), a similar effect of tillage in enhancing N accumulation was observed. The N depletion of in the topsoil layer in winter wheat was expected as non-leguminous rotation crops enhance nutrient uptake compared to soybean monoculture. These results could also indicate greater potential nitrogen losses to leaching in conventional till.

Crop intensification showed a significant effect in the C:N ratio, with lower values observed in conventional plots (P < 0.05) (Table 2.2). Although year included as a covariate had a significant effect on N, but not SOC, we found that in the long-term, inadequate N may have limited organic matter decomposition after SOC accumulated over the years. We also found that C:N ratio for the top 30 cm was lower in conventional than no-till, with an average of 4.6 and 6 in winter wheat, and 3.4 and 4.2 in soybean, respectively. Greater differences in C:N ratio with tillage in winter wheat could be due to lower nitrogen availability. Our results also indicated that the C:N ratio decreased with depth in agreement with previous studies (Franzluebbers et al., 1995b). No-tillage showed a higher rain pulse response compared to conventional practices. The consequences for undisturbed soil regarding  $CO_2$  losses can offset the gain in carbon sequestration observed over 33-years of no-tillage. Higher  $CO_2$  pulses have been reported to be associated with increased decomposition of labile organic matter and nitrogen mineralization (Birch, 1958).

**Table 2.2** Historical records of soil organic carbon (SOC, g kg<sup>-1</sup>) and total nitrogen (TN, g kg<sup>-1</sup>) in 34-years of winter wheat and soybean under conventional tillage and no-tillage measured at 5, 10, 20, and 30cm depth.

			Conv	entional	till		No	o-tillage			
Year	Variable*	able* Soil depth (cm)									
		5	10	20	30	5	10	20	30		
		Winter wheat/Soybean rotation									
1991 <sup>1</sup>	SOC	8.84	8.38	6.51		17.26	8.55	8.00			
$2002^{2}$	SOC	11.20	8.71	6.18		16.84	8.89	8.24			
_00_	TN	1.11	0.86	0.54		1.68	0.88	0.75			
2016	SOC	10.15	9.58	6.96	4.93	14.80	11.17	7.55	6.91		
2010	TN	1.91	1.91	1.51	1.28	2.21	1.88	1.45	1.33		
			C			14					
2002?	202	0.60		oybean 1	nonocu		<b>7</b> .00	<b>5</b> .40			
$2002^{2}$	SOC	8.69	7.24	6.03		12.57	7.92	7.43			
	TN	0.76	0.67	0.59		1.36	0.82	0.72			
2016	SOC	6.08	5.83	5.26	3.87	9.32	7.42	5.68	5.59		
-	TN	1.79	1.68	1.53	1.42	1.90	1.81	1.63	1.54		

\*Units: SOC = g kg<sup>-1</sup>, STC = g kg<sup>-1</sup>, STN = g kg<sup>-1</sup>. <sup>1</sup> Franzluebbers et al., 1994, <sup>2</sup>Dou et al., 2007.

# 3. COVER CROPPING AND SOIL MANAGEMENT EFFECTS ON GREENHOUSE GAS EMISSIONS AND SOIL PROPERTIES IN TRANSITIONING ORGANIC SYSTEMS

#### 3.1. Introduction

Organic farming relies on manure, organic amendments, and plant residues to supply nutrients for plant growth. Organic matter inputs undergo a microbial decomposition process, in which enzymes catalyze biochemical reactions while mineral nutrients are slowly released (Acosta-Martinez et al., 2007). Nutrients produced during soil organic matter mineralization are either absorbed by plant roots, leached into groundwater, or lost into the atmosphere (Mäder et al., 2002; Seufert et al., 2012). The intermediate gaseous losses that occur during mineralization include several greenhouse gases (GHG), such as carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O). The amounts of GHG emitted and their production rate is regulated by several factors including soil temperature, soil moisture, residue exposure, C/N ratio, and soil disturbance (Miller et al., 2008; Johnson et al., 2017; Kravchenko et al., 2018). By avoiding mineral fertilizers, organic systems have significant potential to reduce the emission of GHG (Wittwer et al., 2017). However, results are inconsistent regarding the environmental impact of organic farming practices and their GHG emission potential (Kirchmann and Thorvaldsson, 2000; Decock, 2014; Han et al., 2017).

The adoption of sustainable management practices can mitigate GHG emissions from organic agriculture. Large-scale practices that can potentially mitigate climate

change by increasing carbon (C) sequestration and reducing GHG emissions are called 'natural climate solutions' (NCS) (Fargione et al., 2018). Land use activities that increase C sinks are believed to impact emissions directly and can at least partially ameliorate the 6.1 Pg CO<sub>2</sub>eq yr<sup>-1</sup> emissions produced from agricultural activities (Griscom et al., 2017). Potential NCS opportunities to mitigate climate change include increasing soil organic carbon (SOC) sequestration via plant biomass, addition of increased organic matter inputs through practices such as cover cropping, and reduction of organic matter decomposition rates (Doran and Smith, 1991; Johnson et al., 2007; Ruis and Blanco-Canqui, 2017). Incorporating cover crops into the cropping plan can also help in nutrient management through increasing nutrient availability, improving soil properties, and contributing to long-term sustainability (Negassa et al., 2015; Hill et al., 2017). On average, cover crops could potentially enhance soil organic C by 0.32±0.08 Mg ha<sup>-1</sup> annually, showing great potential as a sequestration strategy (Poeplau and Don, 2015). Implementing conservation practices that include cover crops is expected to increase C inputs into the soil by 103 TgCO<sub>2</sub>e yr<sup>-1</sup> (Fargione et al., 2018).

Incorporation of soil conservation practices (i.e., no-tillage, reduced-tillage, striptillage) in organic production can help reduce soil and water losses, and enhance soil productivity and sustainability (Kern and Johnson, 1993; Houghton, 2007; Franzluebbers, 2002). Conservation tillage is known to decrease net GHG emissions and improve soil and environmental quality by increasing C sequestration. However, the impact of transitioning unmanaged cropland land into newly established organic production systems on soil properties and GHG emissions remains uncertain. Although

tillage is a major component for weed suppression in organic systems (Clark et al., 2017), it can also impact SOC accumulation negatively by increasing soil disturbance and organic matter oxidation (Kern and Johnson, 1993; Krauss et al., 2017; Lal and Kimble, 1997). Integrating conservation tillage practices with crop intensification can reduce the impact of tillage and increase soil C input (Wittwer et al., 2017).

Nevertheless, several studies have indicated that organic systems have a larger impact on the environment than conventional systems because a greater area of land is required to produce similar crop yields, resulting in higher GHG emissions (Johnson et al., 2007; Krauss et al., 2017; Searchinger et al., 2018).

Quantifying the C and nutrient losses from agricultural activities continue to be a challenge due to the complexity of soil dynamics, driving factors that regulate nutrient and gaseous fluxes, and time-scale over which soil processes occur. Overall, conservation practices are over-generalized, and recommendations do not consider site-specific factors (i.e., weather variability, soil type, etc.) (Decock, 2014). As transitioning unmanaged cropland to organic systems is common among organic producers, studies are needed to develop regionally specific production practices. In the Texas Blacklands, high-clay Vertisols exhibit specific soil properties (i.e., shrink-swell, preferential flow) under dry-wet conditions that can directly impact management recommendations (Corbeels et al., 1999). Clay soils associated with smectitic mineralogy are considered to increase C sequestration because of the large surface area and adsorption capacity (Weisbrod et al., 2009). However, there is evidence that smectite is also sensitive to high temperatures that could result in the loss of sequestered C (Michels et al., 2015). Further

research that investigates the uncertainties and risk of transitioning into organic production practices in soils dominated by smectitic clays can help to provide recommendations with a positive impact on the environment.

The overarching goal of this study was to investigate GHG emissions and changes in soil physical and chemical properties in a transitioning organic cropping system that evaluated cover crops and reduced tillage practices in a Vertisol in south central Texas. We hypothesize that organic cropping systems that incorporate manures and plant residues through tillage will have higher SOM decomposition and GHG emissions compared to reduce tillage organic cropping systems. A second hypothesis is that cover crops will increase soil ground cover and water retention leading to a decrease in soil temperature and GHG emissions. To test these hypotheses, we established a transitioning organic corn (*Zea mays* L.) cropping systems experiment in south central Texas. Specific objectives of this study were to (i) investigate soil GHG emissions from a field transitioning from an unmanaged cropland to an organic production system, (ii) determine the effect of C and N inputs from cover crops on GHG emissions at the field scale, and (iii) determine the effect of winter cover crop mixtures on soil properties and water availability in transitioning organic corn cropping systems.

#### 3.2. Materials and Methods

#### 3.2.1. Research Site and Management

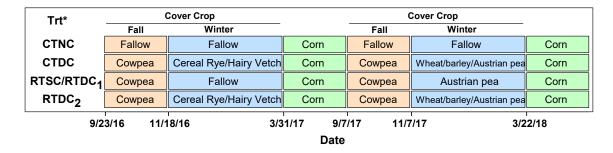
The experiment was conducted at the Texas A&M University-Brazos River Farm located approximately 16 km south-east of College Station, TX (30°32'N, 94°26'W, 68 m a.m.s.l.). The climate is characterized as humid subtropical, with mean annual

precipitation of 992 mm, minimum average air temperature of 15°C, and maximum average air temperature of 27°C (NOAA. 2016). Mean daily precipitation and air temperature for the study period was obtained from a weather station located approximately 512 m from the experimental site. The soil is characterized as Ships clay (very-fine, mixed, active, thermic Chromic Hapludert) with 44.5% clay, 17.6% sand, and 37.9% silt. The experiment was managed according to the National Organic Program (NOP) guidelines as the land transitioned to certified organic production. The NOP standards for organic production include a buffer zone (approx. 15m) and the use of approved synthetic and non-synthetic substances. The site remained as unmanaged cropland for 7-years prior to clearing and tilling to establish the study in September 2016. The experiment was arranged as a split-plot randomized complete block design with three replications. The main factor was the main crop in the 3-yr rotation cornsorghum-soybean and the split plots the cropping systems (Figure 3.1). The size of each plot was 300 by 3 m.

Four organic production systems that evaluated double cover cropping and tillage practices during the growing season of corn (*Zea mays* L.) were implemented in 2016-2017 and 2017-2018 seasons. Double cover cropping included both summer (planted immediately after harvesting corn) and winter cover crops. After the initial land preparation, cowpea (*Vigna unguiculata* L., var. Iron Clay) was planted on 23 October at a seeding rate of 56 kg ha<sup>-1</sup>. After 55 days (17 November 2016), the cowpea was shredded and incorporated before planting the winter cover crops. The winter cover crop treatment planted was a premix of cereal rye (*Secale cereale* L.) and hairy vetch (*Vicia* 

villosa Roth.) at a seeding rate of 146 kg ha<sup>-1</sup>. Winter cover crops were mechanically terminated with a roller-crimper on 29 March 2017 before planting the main crop. Corn was planted in 0.76 m wide rows on 31 March 2017 (75,600 seeds ha<sup>-1</sup>) and harvested on 31 July 2017. On 7 September 2017, the fall cowpea cover crop was planted at a seeding rate of 39 kg ha<sup>-1</sup>. The fall cover crop grew for 61 days and was shredded and incorporated on 7 November 2017 before planting the winter cover crops. Although hairy vetch/rye is a popular winter cover crop among farmers in the south eastern U.S., roller crimping was less effective in terminating the winter cover crop in our study as the biomass production was low due to late planting. Hence for the 2017-2018 season, the winter cover crop mixture was changed to wheat (Triticum aestivum L.)/barley (Hordeum vulgare L.)/Austrian winter pea (Pisum sativum L.) (72.1 kg ha<sup>-1</sup> of wheat, 25.1 kg ha<sup>-1</sup> of barley, and 38 kg ha<sup>-1</sup> of Austrian pea). Additionally, the reduced tillagesummer cover crop only treatment (RTSC) was changed to a reduced till double cover crop treatment with cowpea as the fall cover crop and Austrian pea as the winter cover cop (seeding rate of 56 kg ha<sup>-1</sup>). Winter cover crops were terminated on 21 March 2017. Corn was initially planted on 22 March 2018, but due to poor germination, the main crops were replanted on 16 April 2018 (79,040 seeds ha<sup>-1</sup>) and harvested on 16 August 2018. In summary, the conventional tillage production systems included a no cover crop (CTNC) and a double cover crop treatment (CTDC). The reduced tillage treatments included a single fall cover crop treatment (RTSC) and a double cover crop treatment in 2016-2017 (RTDC<sub>1</sub>), and two double cover crop mixtures (RTDC<sub>1</sub> and RTDC<sub>2</sub>) in 2017-2018 (Figure 3.1). Both fall and winter cover crop residues were incorporated only in

conventional tillage plots, whereas in reduced tillage, crop residues remained on the surface. In reduced tillage plots, tillage was done prior to planting of cover crops. In 2016-2017 growing season, poultry litter was applied on 3 March 2017 at a rate of 1,937 kg ha<sup>-1</sup>, and a fish emulsion was applied at planting of corn as a starter fertilizer at a rate of 112.2 L ha<sup>-1</sup> (56.1 L N ha<sup>-1</sup>) (Appendix A). In 2017-2018 growing season, turkey compost was applied on 14 March 2018 at a rate of 4,694 kg ha<sup>-1</sup> (Appendix A). The starter fertilizer applied in 2018 was Chilean nitrate at a rate of 28 kg N ha<sup>-1</sup> as per the NOP guidelines.



**Figure 3.1** Cover crop rotations in a transitioning organic cropping system that evaluated fall/winter cover crops and tillage practices in organic corn. \*CTNC: tillage with no cover crop, CTDC: tillage with a double cover crop, RTSC: reduced tillage with fall cover crop in 2016-2017, RTDC<sub>1</sub>: reduced tillage with a double cover crop in 2016-2017, and RTDC<sub>2</sub>: reduced tillage with a double cover crop.

#### 3.2.2. Biomass Measurements

Aboveground biomass was determined for the fall/winter cover crops, corn, and weed biomass. Cover crop biomass was collected before mechanical termination from a random 1 x 1 m area in each plot (n = 36). Biomass samples were oven dried at 65°C to estimate dry biomass production. Corn biomass was collected at harvest by sampling

five plants radomly from each plot. Weed biomass was collected at harvest in 2018 from a random 1 x 1 m area in each plot. Residues were analyzed for total C and N. Total C was determined by dry combustion analysis and total N using the Kjeldahl technique (Miller et al., 1997). Analyses were performed at the Texas A&M AgriLife Extension Service Soil, Water and Forage Testing Laboratory in College Station, TX.

#### 3.2.3. Greenhouse Gas Flux Measurements

Immediately after planting corn, soil chambers were installed for GHG flux measurements. Soil CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O fluxes were measured using an automated soil CO<sub>2</sub> flux system (Model LI-8100A, LI-COR Biosciences, Lincoln, NE, USA) connected to a Picarro GHG analyzer (Model G2508, Picarro Inc., Santa Clara, CA, USA) which uses laser spectroscopy for measuring gas concentrations. As the LI-8100A had the capability to measure only CO<sub>2</sub> and water vapor (H<sub>2</sub>O) concentrations, integrating this system with a G2508 analyzer allowed measurements of CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O concentrations (Zapata et al., 2018). The LI-8100A analyzer control unit and the G2508 were placed inside an air-conditioned trailer in the field. The LI-8100A analyzer control unit was connected to a multiplexer (Model LI-8150) placed outside the trailer. The multiplexer controlled eight long-term soil chambers (Model LI8100-104C) to automatically measure soil surface gas concentration when chambers were in the closed position. Soil chambers were connected to the multiplexer using 30 m long cables. Each chamber was placed over a PVC collar (20 cm diameter, height 11.4 cm, and area 317 cm<sup>2</sup>) inserted to a depth of approximately 5 cm. Two chambers were installed in each of the four treatments. One chamber was placed close to the crop row and the second

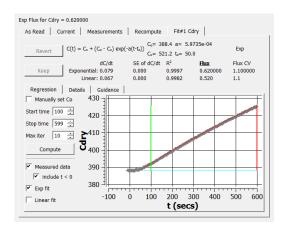
chamber was placed in between the crop rows to capture the spatial variability in gaseous emissions. Soil chambers were programmed to measure soil fluxes sequentially from all eight chambers. In 2017, the measurement time for each chamber lasted for 3-minutes, which allowed up to 48 measurements from each treatment plot during a 24 hr period. In 2018, the measurement time was increased to 10-minutes due to the low diffusion rate of CH<sub>4</sub> and N<sub>2</sub>O observed in the previous year. This reduced the number of measurements to 12 per treatment plot during a 24 hr period.

Flux measurements were made during the early-, middle-, and late-season growth of corn in both years. Each measurement campaign lasted for 7-10 days. In 2017 soil fluxes were measured on DOY 132–140 (early), DOY 164–171 (middle), and DOY 193–201 (late). In 2018, measurements were made on DOY 106–110 (after the cover crop was rolled/crimped), DOY 129–136 (early), DOY 150–157 (middle), and DOY 174–184 (late). The change in gas concentration inside the chamber was used to calculate GHG fluxes by plotting the increase in gas concentration against time using the SoilFluxPro software (version 4.0.5, LI-COR Biosciences, Lincoln, NE, USA). Data quality control was conducted before and after GHG flux calculations. After fluxes were calculated, measurements with a coefficient of determination ( $R^2$ ) < 0.9 or a coefficient of variation > 2% were removed (Figure 3.2). Flux measurements that did not meet these criteria were mostly due to leakage, damaged tubing, or instrument malfunction.

# a) Poor fitting

#### Exp Flux for Cdry = -0.030000 As Read | Current | Measurements | Recompute | Fit#1 Cdry | $C(t) = C_x + (C_o - C_x) \exp(-a(t-t_o))$ $C_o = -a = - C_x - t_o = - C_x$ dC/dt SE of dC/dt R<sup>2</sup> Exponential: -0.00212535 - - Linear: -0.00212535 0.000605466 0.0241455 Regression Details Guidance 402 Start time 100 ÷ 400 Stop time 599 🛨 398 Max iter 10 🛨 ≥396 394 392 ✓ Measured data 390 ✓ include t < 0 Exp fit Ó 100 200 300 400 500 ✓ Linear fit

# b) Good fitting



**Figure 3.2** Comparison of a poor exponential regression fitting (a) and a good exponential fitting (b) used to calculate soil fluxes in SoilFluxPro.

# 3.2.4. Soil Chemical Properties

To investigate the effect of transitioning the land to organic gFigurerain production on soil chemical properties, soil samples were collected before establishing the cropping systems in 2016 and periodically throughout the growing season.

Immediately after land preparation for plot establishment, a Giddings probe (Giddings Machine Co., Fort Collins, CO) was used to collect 12 soil cores of 5 cm diameter and 60 cm length from the field for developing baseline soil characteristics. After establishing the cropping systems, six soil cores of 5 cm diameter and 60 cm length were collected per plot. Soil cores were sectioned and composited into three depth intervals (0–15, 15–30, and 30–60 cm) for each treatment. Soil samples were air dried and ground to pass an 80 mesh (0.18 mm) sieve and were analyzed for total soil nitrogen (TN), total C (TC), and soil organic C (SOC) via thermal combustion method (Schulte and Hopkins, 1996). During the corn growing season, six soil cores (2.5 cm diameter) per plot were

collected monthly from 0–15 cm depth to determine plant available N in the form of ammonium (NH<sub>4</sub>-N) and nitrate (NO<sub>3</sub>-N). Soil analyses were performed at the Texas A&M AgriLife Extension Service Soil, Water and Forage Testing Laboratory in College Station, TX.

### 3.2.5. Soil Physical Properties

Soil water content reflectometer sensors were installed in each expeirmental plot at 5, 15, and 25 cm depths to monitor volumetric water content and temperature (Model CS655, Campbell Scientific, Logan, UT, USA). Data from all sensors were measured at 30-second intervals using a CR1000 datalogger (Campbell Scientific, Logan, UT, USA) and averaged to output half-hour values. Bulk density was measured in-situ during the off-season from six soil cores collected per plot from 0–15, 15–30, and 30–60 cm depth intervals using a sectioned cylinder (40.4 cm<sup>3</sup>). Soil bulk density was determined as mass per unit volume of oven-dried mass of undisturbed soil core (Blake and Hartge, 1986). Bulk density measurements were made before plot estasblishment in 2016 and after harvesting of corn in 2017.

### 3.2.6. Data Analysis

Data processing and analysis were performed using SAS software (SAS Institute, 2017). The effect of cover cropping and soil management on crop biomass and soil inorganic N were tested using a two-way ANOVA in PROC GLM. Fischer's LSD test was used for means separation. Statistical significance was determined at  $\alpha = 0.05$  probability unless otherwise stated. Statistical analyses were performed separately in 2017 and 2018 for each sampling date.

We used a penalized regression spline to fit a curve that connects all data points using PROC EXPAND. Soil flux was log-transformed as data showed positive skewness. Cumulative growing season GHG emissions were estimated by linearly interpolating between measurements and integrating the area under the curve. We coupled standardized fluxes with soil temperature, and moisture for all depths and statistically significant correlations were identified using Pearson correlations. Average daily  $CO_2$  flux was compared among treatments using a mixed effect model with days nested within treatments during the early, middle, and late seasons (Petrakis et al., 2018). The temperature dependence of soil  $CO_2$  flux was evaluated by estimating the  $Q_{10}$  value (the factor by with soil  $CO_2$  flux increases with an increase in temperature by  $10^{\circ}C$ ). A nonlinear function of the form  $R_s = \beta_0 e^{\beta_1 T_s}$  was fitted to the measured  $R_s$  and  $T_s$ .  $Q_{10}$  was then calculated using equation 3.1 using the parameters  $\beta_0$  and  $\beta_1$ , which correspond to the intercept of the exponential curve and rate of increase in  $R_s$ , respectively. We compared  $Q_{10}$  estimates among cover crop treatments

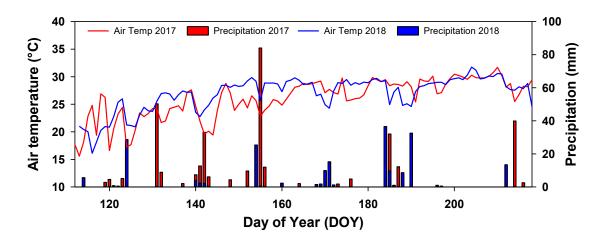
$$Q_{10} = e^{10\beta_1}$$
 [Eq. 3.1]

#### 3.3. Results and Discussion

#### 3.3.1. Environmental Conditions

The amount and distribution of precipitation received during the corn growing season at the site varied greatly in 2017 and 2018. The total precipitation received at the study site was 343 mm in 2017 and 203 mm in 2018. Of the 343 mm of cumulative precipitation in the 2017 growing season, 50% of it fell between planting (DOY 90) and DOY 155. Despite the greater precipitation received in 2017, drought conditions were

experienced towards the late season. In 2018, the majority of precipitation events occurred during the late season and drier conditions prevailed early in the season. Thus, 50% of the precipitation fell between planting on DOY 106 and DOY 184. Drier conditions were accompanied with higher air temperature during most of the early growing season in 2018. The mean air temperature during the study period was 26.1°C in 2017 and 28.4°C in 2018 (Figure 3.3). Overall, the 2018 growing season was warmer than in 2017.



**Figure 3.3** Daily mean air temperature and precipitation during the growing season of corn in 2017 and 2018.

# 3.3.2. Aboveground Biomass Production

Cover crop residue inputs after mechanical termination can impact soil GHG emissions and crop productivity (Table 3.1). Average cowpea biomass production was 436 kg ha<sup>-1</sup> in 2016 and 738 kg ha<sup>-1</sup> in 2017. The aboveground cowpea biomass in our

study for both years was considerably lower than the average biomass reported by Clark (2007) that ranged from 3,360 to 4,500 kg ha<sup>-1</sup>. In both years, cowpea was grown for less than 61 days as the planting of cowpea was delayed due to wet conditions until late summer or early fall. This is a typical situation in south eastern Texas, in which farmers are hesitant to adopt fall cover crops due to the short planting window in the fall before the rainfall season that restricts the early harvest and cowpea establishment.

As the study was initiated in fall 2016, biomass production of winter cover crops was similar across the tillage treatments in the 2016-2017 growing season. The average rye/hairy vetch mixture aboveground biomass was 2,377 kg ha<sup>-1</sup>. The biomass production in this study was similar to the biomass reported for winter cover crops that were grown for shorter periods (approx. 3-4 months) (Hayden et al., 2014; Poffenbarger et al., 2015; Duval et al., 2016; Sainju et al., 2018). The recommended growth stage for effective mechanical crimping of rye/vetch mixture is past anthesis (Mirsky et al., 2009). As roller crimping in our study was done prior to the anthesis stage, it was less effective in terminating the winter cover crop. The biomass production from the rye/hairy vetch mixture was 30% below the recommended weed biomass suppression threshold of 8,000 kg ha<sup>-1</sup> reported by Mirsky et al. (2013).

Average winter cover crop biomass in the 2017-2018 season from the wheat/barley/Austrian pea mixture was 2,767 kg ha<sup>-1</sup> in conventional tillage and 2,734 kg ha<sup>-1</sup> in reduced tillage plots. The Austrian pea treatment established in 2017-2018 season produced an average aboveground dry biomass of 2,490 kg ha<sup>-1</sup>. Although there were no significant differences in aboveground biomass of cover crop mixtures between

years (P > 0.05), overall, biomass production was slightly higher in 2018 because cover crops were established earlier than the previous year. Similar to 2016-2017, the biomass production in the 2017-2018 season from the winter cover crop was 30% below the recommended weed biomass suppression threshold.

Differences in aboveground corn biomass at harvest were observed in both 2017 and 2018, with tillage having the largest impact on crop biomass production. Both conventional tillage treatments (no-cover crop and double cover crop) had the highest aboveground biomass production (19,731 kg ha<sup>-1</sup> in CTNC and 16,101 kg ha<sup>-1</sup> in CTDC). Among the reduced tillage treatments, biomass production was the highest in the double cover crop treatment (7,770 kg ha<sup>-1</sup> in RTDC) compared to the single cover crop treatment (4,489 kg ha<sup>-1</sup> in RTSC). Reduced tillage without a winter cover crop had significantly higher weed pressure compared to the other treatments and the growth and yield of corn was significantly impacted in those treatment plots. In 2018, above ground biomass production of corn was significantly lower in all treatments, with an average of 3,810 kg ha<sup>-1</sup> in CTNC, 2,311 kg ha<sup>-1</sup> in CTDC, 1,354 kg ha<sup>-1</sup> in RTDC<sub>1</sub>, and 914 kg ha<sup>-1</sup> in RTDC<sub>2</sub>. Overall, the low plant productivity observed in 2018 was due to poor crop establishment, late replanting, and dry conditions during the early season.

The average weed biomass production and its estimated C and N concentration were calculated at harvest (Table 3.1). Overall, weed density was more pronounced in reduced tillage than conventional tillage plots due to the effect of tillage on reducing weed pressure and increasing crop competitiveness. On average in 2018, weed biomass in plots that implemented tillage was 1,852 kg ha<sup>-1</sup> in CTNC and 1,837 kg ha<sup>-1</sup> in CTDC.

Reduced tillage showed a much higher weed biomass production, with an average of 2,623 kg ha<sup>-1</sup> in RTDC<sub>1</sub> and 2,330 kg ha<sup>-1</sup> in RTDC<sub>2</sub>. The dominant weed specie was Johnsongrass (*Sorghum halepense* (L.) Pers.). Caldwell et al. (2014) reported higher weed biomass (4-years average) in organic corn under reduced tillage (1,176 kg ha<sup>-1</sup>) than conventional tillage that cultivated the soil for weed suppression (172 kg ha<sup>-1</sup>).

As the results of our study demonstrated, several factors could impact successful integration of cover crops in organic production systems. Although the planting window for corn in south east Texas begins in early March, planting delays (primarily due to inclement weather) could affect the establishment of fall cover crops after harvest.

Additionally, the inclusion of a fall cover crop as part of a double cover crop system (fall and winter cover crops after harvest of corn) could potentially delay the planting and establishment of winter cover crops. As cover crops offer a range of benefits in organic production systems, timely establishment is one of the most critical factors affecting its success for soil fertility and weed management purposes (Clark, 2007; Mirsky et al., 2013).

**Table 3.1** Aboveground biomass, and carbon (C) and nitrogen (N) inputs during 2017 and 2018 from fall and winter cover crops, manure application, corn biomass, and weed biomass. Values in parenthesis are standard deviation.

Year	Source	Aboveground biomass		C	N		
		kg ha <sup>-1</sup>	<b>%</b>	kg ha <sup>-1</sup>	<b>%</b>	kg ha <sup>-1</sup>	
2017	Fall cover crop						
	Cowpea	436 (60)	44.0	192 (26)	2.3	10 (1.4)	
	Winter cover crops						
	Rye/Vetch	2377 (970)	44.0	1046 (427)	2.5	59.4 (24.3)	
	Poultry litter	1936	44.0	852	2.5	48.8	
2018	Fall cover crop						
	Cowpea	738 (323)	44.0	325 (142)	2.3	17 (7.4)	
	Winter cover crops						
	Austrian pea	2491 (713)	43.6	1086 (311)	4.0	99.6 (28.5)	
	Austrian						
	pea/Wheat/Barley	2751 (555)	44.6	1227 (248)	3.0	82.5 (16.7)	
	Turkey compost	4694	41.3	1939	1.1	52.1	
	Weed biomass	2503	44.0	1101 (532)	1.0*	25 (12.1)	

Source: \* Schwinning et al. (2017).

# 3.3.3. Soil Chemical Properties in Transitioning Organic Systems

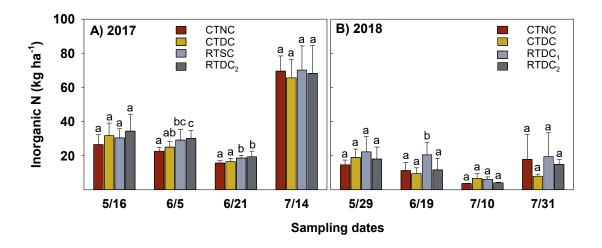
Initial soil sampling before transitioning into organic farming showed that soil chemical properties were similar across the field (Table 3.2). After the transition year to organic production, we observed an increase in NO<sub>3</sub>-N and a decrease in soil electrical conductivity, extractable available P, and extractable K, Ca, Mg, S, and Na, with no significant differences among cropping systems (P > 0.05). On average, soil NO<sub>3</sub>-N increased from 0.5 to 6.95  $\mu$ g g<sup>-1</sup> soil after the first year of transitioning into organic production. This increase in soil inorganic N could be due to the decomposition of cover crop and weed biomass residues, and manure, which decompose slowly and release mineral nutrients into the soil. Besides the contribution from aboveground biomass,

rhizodeposition and root decomposition enhance microbial activity, mineralization and therefore, N availability (Van Der Krift et al., 2001). Despite the increase in soil N availability, overall soil nutrient status and electrical conductivity slightly declined because of crop intensification that increased plant uptake of nutrients from the soil.

The 2017 in-season measurements of inorganic N in the top soil later (0-15 cm depth) showed that soil inorganic N declined until the third sampling in late-June but drastically increased on mid-July before corn harvest (Figure 3.4A). The increase in inorganic N observed in the last sampling was mostly driven by an increase in soil NH<sub>4</sub>-N (data not shown). Similar trends in inorganic N were found in 2018, but values were generally lower than in 2017 (Figure 3.4B). These results can be explained because the drought conditions in late-summer (Figure 3.1) resulted in (1) a reduced N uptake by the crop due to water stress and (2) an increase in SOM decomposition that increased N mineralization and inorganic N release (Anand et al., 2011). Besides, it could possibly indicate that N mineralization was not synchronous with corn N demand.

The baseline SOC content was 1.4±0.23% at 0-15 cm, 0.71±0.11% at 15-30 cm, and 0.67±0.09% at 30-60 cm depths (n = 12). Although there were no significant differences in SOC among treatments, SOC decreased after the land was converted to cropland. Average SOC contents were 1.24±0.19% at 0-15 cm, 0.71±0.09% at 15-30 cm, and 0.68±0.07% at 30-60 cm. The slight decrease in SOC content in the topsoil layer (0-15 cm) could be due to soil cultivation and enhanced SOC losses via oxidation or mineralization (Lal, 2002). Although no significant differences in SOC were found among treatments, conventional tillage without any cover crop showed the lowest SOC

(Table 3.2). These SOC measurements were comparable to the measurements from Wright and Hons (2005) in a study that was conducted in the same area that evaluated tillage practices and crop rotation for 20-yrs without cover crops.



**Figure 3.4** Soil inorganic N (NO<sub>3</sub>-N + NH<sub>4</sub>-N) measured during the corn growing seasons of A) 2017 and B) 2018. Error bars are standard errors (n = 3). CTNC: tillage with no cover crop, CTDC: tillage with a double cover crop, RTSC: reduced tillage with fall cover crop in 2016-2017, RTDC<sub>1</sub>: reduced tillage with a double cover crop in 2016-2017, and RTDC<sub>2</sub>: reduced tillage with a double cover crop.

**Table 3.2** Soil chemical properties (0-15 cm depth) before establishing an organic system in 2016 and 1-year after the transition in 2017. Numbers in parenthesis are standard errors. All soil samples were collected in September of each year.

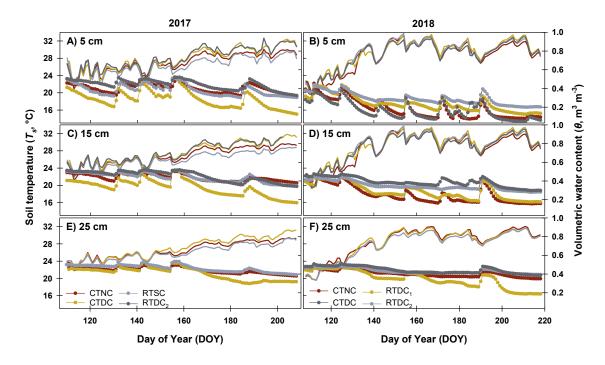
Year	Treatment*	pН	Cond	SOC	Total N	NO <sub>3</sub> -N	P	K	Ca	Mg	S	Na
1 Cai	Treatment	pm	μmhos/cm	%		ppm						
2016		7.6	329.4	1.4		0.5	32.9	393.8	10377.6	365.5	19.5	16.4
		(0.1)	(44.2)	(0.23)		(0.4)	(13.5)	(31.8)	(1337.7)	(48.1)	(2.8)	(4.1)
2017	CTNC	7.9	180	1.15	1365.26	6.9	18.9	256.8	8294.8	297.5	14.5	12
		(0.1)	(30.1)	(0.16)	(186.42)	(0.8)	(4.9)	(26.9)	(829.3)	(34.4)	(1.1)	(3.8)
	CTDC	7.8	161	1.23	1475.25	6.8	18.5	267.4	8240.2	296.2	14.1	10.7
		(0.1)	(24.6)	(0.2)	(203.11)	(0.3)	(5.6)	(36.8)	(772.9)	(35)	(1.2)	(2.9)
	RTSC	7.8	165.3	1.33	1680.26	7	22.8	285.5	8083.1	292.6	14.1	11.3
		(0.1)	(24.2)	(0.2)	(199.86)	(0.5)	(8.7)	(37.5)	(719.9)	(32.1)	(1.1)	(3.7)
	RTDC	7.9	176.6	1.27	1509.57	7.1	20.2	269.3	8081.8	291.8	14	12
		(0.1)	(21)	(0.2)	(219.58)	(0.8)	(6)	(33.4)	(807)	(34.5)	(1.6)	(3.3)

<sup>\*</sup> CTNC: tillage with no cover crop, CTDC: tillage with a double cover crop, RTSC: reduced tillage with fall cover crop in 2016-2017, RTDC<sub>1</sub>: reduced tillage with a double cover crop in 2016-2017, and RTDC<sub>2</sub>: reduced tillage with a double cover crop.

# 3.3.4. Effects of Cover Cropping and Tillage on Soil Temperature and Moisture

The effect of cropping system practices on soil temperature and moisture varied between years (Figure 3.5). During the early season in 2017, soil temperature conditions were similar among treatments (Figures 3.5A, 3.5C, and 3.5E). Differences in soil temperature among cover crops and tillage practices were more noticeable after DOY 155 when drought conditions occurred (Figure 3.3). Overall, reduced tillage showed higher soil temperatures than conventionally tilled plots at all depths. Similarly, soil moisture was the lowest in the no-cover crop with reduced tillage plots (0.1 m<sup>3</sup> m<sup>-3</sup>), while similar conditions were observed among the other cover crop treatments.

In 2018, soil temperature was similar among cover crop mixtures at all depths (Figures 3.5B, 3.5D, and 3.5F). Conventional tillage with no-cover crop had the lowest soil temperature during the early season. Towards the middle and late seasons, soil temperature was similar due to more precipitation events occurring. Soil moisture showed different trends that varied with depth. Thus, at 5 cm depth, conventional tilled plots (CTNC and CTDC) showed the lowest moisture content throughout the growing season, while at 15 cm depth the tilled with no cover crop and reduced tillage with Austrian pea had the lowest values. The low soil moisture in conventional tillage was due to more plant growth as tillage reduced weed pressure.



**Figure 3.5** Soil temperature (line) and volumetric water content (circle) measured in corn plots at 5, 15, and 25 cm depth using TDR sensors in 2017 (left) and 2018 (right). CTNC: tillage with no cover crop, CTDC: tillage with a double cover crop, RTSC: reduced tillage with fall cover crop in 2016-2017, RTDC<sub>1</sub>: reduced tillage with a double cover crop in 2016-2017, and RTDC<sub>2</sub>: reduced tillage with a double cover crop.

# 3.3.5. GHG Fluxes during the Crop Growing Season

### 3.3.5.1. Soil CO<sub>2</sub> Flux

Emissions during 2017 were higher in all treatments compared to 2018, but the average treatment effect was similar between years (Figure 3.6). Hence, in 2017, average CO<sub>2</sub> flux rate from CTNC plots was  $7.15 \pm 3.8 \, \mu mol \, m^{-2} \, s^{-1}$ , followed by the CTDC with  $8.49 \pm 5.38 \, \mu mol \, m^{-2} \, s^{-1}$ , RTSC with  $9.28 \pm 4.94 \, \mu mol \, m^{-2} \, s^{-1}$ , and RTDC with  $9.36 \pm 5.68 \, \mu mol \, m^{-2} \, s^{-1}$  (Table 3.3). Conversely, the average soil CO<sub>2</sub> emissions in 2018 from plots under CTNC was of  $2.01 \pm 1.24 \, \mu mol \, m^{-2} \, s^{-1}$ , followed by the CTDC with  $3.39 \pm 1.24 \, \mu mol \, m^{-2} \, s^{-1}$ , followed by the CTDC with  $3.39 \pm 1.24 \, \mu mol \, m^{-2} \, s^{-1}$ , followed by the CTDC with  $3.39 \pm 1.24 \, \mu mol \, m^{-2} \, s^{-1}$ , followed by the CTDC with  $3.39 \pm 1.24 \, \mu mol \, m^{-2} \, s^{-1}$ , followed by the CTDC with  $3.39 \pm 1.24 \, \mu mol \, m^{-2} \, s^{-1}$ , followed by the CTDC with  $3.39 \pm 1.24 \, \mu mol \, m^{-2} \, s^{-1}$ , followed by the CTDC with  $3.39 \pm 1.24 \, \mu mol \, m^{-2} \, s^{-1}$ , followed by the CTDC with  $3.39 \pm 1.24 \, \mu mol \, m^{-2} \, s^{-1}$ , followed by the CTDC with  $3.39 \pm 1.24 \, \mu mol \, m^{-2} \, s^{-1}$ , followed by the CTDC with  $3.39 \pm 1.24 \, \mu mol \, m^{-2} \, s^{-1}$ 

 $1.34~\mu mol~m^{\text{--}2}~s^{\text{--}1},~RTDC_1~with~3.26\pm2.17~\mu mol~m^{\text{--}2}~s^{\text{--}1},$  and  $RTDC_2~with~3.08\pm1.25~\mu mol~m^{\text{--}2}~s^{\text{--}1}.$ 

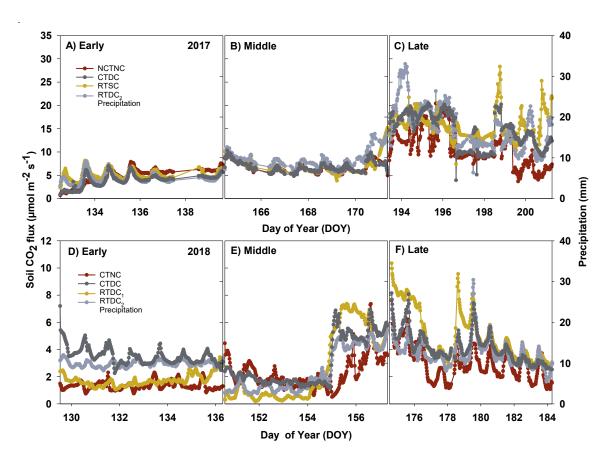
**Table 3.3** Average soil CO<sub>2</sub> flux ( $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) during the early-, middle-, and late-season of corn in 2017 and 2018 in plots that planted winter cover crop mixtures. CTNC: tillage with no cover crop, CTDC: tillage with a double cover crop, RTSC: reduced tillage with fall cover crop in 2016-2017, RTDC<sub>1</sub>: reduced tillage with a double cover crop in 2016-2017, and RTDC<sub>2</sub>: reduced tillage with a double cover crop. Values in parenthesis are the standard deviation. Different letters in the same row are significantly different (LSD, P = 0.05)

Season	CTNC	CTDC	RTSC/RTDC <sub>1</sub>	RTDC <sub>2</sub>	
2017					
Early	4.72 (1.81) <i>b</i>	3.9 (1.35) <i>a</i>	5.1 (1.11) <i>c</i>	4.36 (1.12) <i>b</i>	
Middle	6.73 (1.17) <i>a</i>	6.78 (1.12) <i>a</i>	7.02 (1.83)a	8.36 (1.85) <i>b</i>	
Late	9.35 (4.85) <i>a</i>	13.34 (5.38) <i>b</i>	$14.23 \ (4.05)c$	14 (6.06) <i>d</i>	
Average	7.15 (3.8) <i>a</i>	8.49 (5.38) <i>b</i>	9.28 (4.94) <i>c</i>	9.36 (5.68) <i>c</i>	
2018					
After-planting	0.66 (0.13)a	$2.26 \ (0.68)b$	2.93 (0.6)c	2.99 (1.38) <i>d</i>	
Early	1.34 (0.34)a	$3.48 \ (0.57)d$	$1.72 \ (0.45)b$	2.95 (0.26)c	
Middle	2.24 (1.15)a	3 (1.8) <i>c</i>	2.64 (2.6)b	2.4 (1.6)b	
Late	2.94 (1.18) <i>a</i>	4.11 (1.1) <i>c</i>	4.97 (1.87) <i>d</i>	$3.71 \ (1.04)b$	
Average	2.01 (1.24)a	3.39 (1.34) <i>b</i>	3.26 (2.17)c	$3.08 \ (1.25)d$	

The growing season is the most dynamic period and fluxes are expected to vary as corn develops and cover crop decompose. To determine cropping system impact on CO<sub>2</sub> emissions, we compared early-, middle-, and late-season separately. During the early season of 2017, the RTSC showed the highest CO<sub>2</sub> emissions followed by the CTNC and RTDC treatments with similar emissions (Figure 3.6A, Table 3.3). The middle season fluxes were identical in most cropping systems, but RTDC exhibited higher emissions (Figure 3.6B, Table 3.3). Towards the late season, soil CO<sub>2</sub> emissions

drastically increased in all treatments and significant differences were found among treatments as follows CTNC < CTDC < RTSC < RTDC (Figure 3.6C, Table 3.3).

In 2018, we were able to immediately measure GHG fluxes after cover crops were rolled-crimped and corn was planted. Overall, cover crop mixtures increased CO<sub>2</sub> emissions compared to the treatment with no cover crop (CTNC) and differences among treatments varied depending on the season (Table 3.3). The early season of corn showed that the average magnitude of the emissions were as follows CTNC < RTDC<sub>1</sub>< RTDC<sub>2</sub> < CTDC (Figure 3.6D, Table 3.3). However, in the middle season CTDC showed higher emissions than reduced tillage plots (Figure 3.6E, Table 3.3). Unlike the 2017 growing season, in 2018 emissions slightly declined during the late season and were in order the magnitude as follows CTNC < RTDC<sub>2</sub> < CTDC < RTDC<sub>1</sub> (Figure 3.6F, Table 3.3).



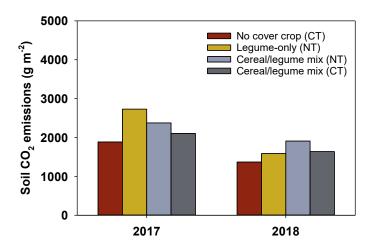
**Figure 3.6** Soil CO<sub>2</sub> flux and precipitation measured during the early-, middle-, and late-seasons of corn in 2017 and 2018 in plots that planted winter cover crop mixtures. CTNC: tillage with no cover crop, CTDC: tillage with a double cover crop, RTSC: reduced tillage with fall cover crop in 2016-2017, RTDC<sub>1</sub>: reduced tillage with a double cover crop in 2016-2017, and RTDC<sub>2</sub>: reduced tillage with a double cover crop.

Over the two-year study period (2017-2018), cumulative soil CO<sub>2</sub> fluxes differed among cropping systems (Figure 3.7). In 2017, which was characterized as a warm year, emissions were higher than in 2018 which was a cooler year (Figure 3.1). Emissions in 2017 were 100% higher in corn for all treatments compared to 2018. Cover crops and reduced tillage increased emissions in both years. For example, when comparing emissions in corn against the CTNC treatment in 2017, it was estimated that the fall

cover crop only (RTSC) increased emissions by 44.7%, RTDC<sub>2</sub> by 47.3%, and CTDC by 11.6%. During 2018, the RTDC<sub>1</sub> increased emissions by 41.8% compare to CTNC, while RTDC<sub>2</sub> and CTDC increased losses by 47.3% and 61.6%, respectively. The cumulative soil CO<sub>2</sub> fluxes measured in corn from our study were lower from those reported by Wilson and Al-Kaisi (2008) in continuous corn and corn-soybean rotation. Unmanaged cropland converted into cropland has shown reduced soil CO<sub>2</sub> emissions under less intensive tillage (Jabro et al., 2008). In our study, emissions were lower from CTNC followed by the RTDC<sub>1</sub>, the RTDC<sub>2</sub> and CTDC. No cover crop showed higher VWC up to 30-days after cover crops were terminated, but then decreased and no differences were observed among cover crop mixtures (Figure 3.5). The Austrian pea with reduced tillage treatment showed the lowest soil water content for all the depths. Also, reduced tillage treatments showed the highest soil emissions that could be due to more soil cracking occurring during drying when soil is undisturbed (Figures 3.6-3.7). Towards the late season of corn, emissions were notably higher in plots that included cover crops mixtures, with no difference among treatments (Figure 3.6F).

Winter cover cropping increased emissions compared to the control with bare soil that did not include plant residues and was managed under conventional till (Figures 3.6-3.7). Using winter cover crops resulted in more complex diurnal patterns of soil CO<sub>2</sub> fluxes (Figure 3.6) compared to bare soil (Xiao et al., 2015) because cover crops directly affect root distribution and therefore, contribution to soil respiration. The soil CO<sub>2</sub> fluxes measured during the decomposition of crop residues and development of corn showed high variability due to the intercropping that increase the contribution from autotrophic

and heterotrophic respiration (Bavin et al., 2009; Vargas et al., 2010). Although cover crops were rolled/crimped, root activity has shown to continue for months after termination (Sanders et al., 2018). Also, rhizodeposition can increase soil C inputs more easily than shoot decomposition because roots exudates in the form of organic acids (i.e., lactate, acetate, oxalate, malate, and citrate) are adsorbed to clay mineral surfaces (Austin et al., 2017). In both years, it was observed that cycles of drying and wetting stimulated SOM mineralization, leading to the rapid release of inorganic N and CO<sub>2</sub>. (Figures 3.4-3.6).

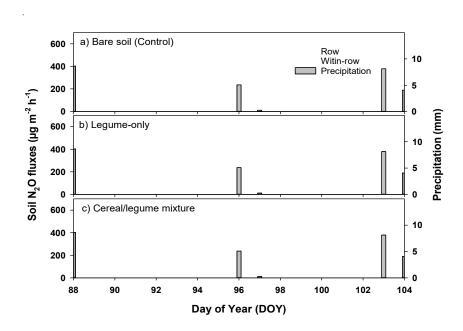


**Figure 3.7** Cumulative soil CO<sub>2</sub> emissions measured during the 2017 and 2018 growing season of corn. CTNC: tillage with no cover crop, CTDC: tillage with a double cover crop, RTSC: reduced tillage with fall cover crop in 2016-2017, RTDC<sub>1</sub>: reduced tillage with a double cover crop in 2016-2017, and RTDC<sub>2</sub>: reduced tillage with a double cover crop.

#### 3.3.5.2. Soil N<sub>2</sub>O Flux

During the first year of transitioning into organic, we did not observe soil  $N_2O$  emissions possibly due to the low soil N status (Table 3.2) and the lack of denitrification

conditions (Weier et al., 1993). In 2018, a peak in N<sub>2</sub>O emissions was observed during the early season after cover crops were mechanically terminated through roller-crimping and a rain event on DOY 88 (Figure 3.8). Several studies have reported that N<sub>2</sub>O emissions are highly variable and occur sporadically as conditions are favorable (Drury et al., 2008; Han et al., 2017; Miller et al., 2008). On average, N<sub>2</sub>O emissions were higher in the legume-only (60.13 μg m<sup>-2</sup> h<sup>-1</sup>) than the cereal/legume mixture (50.76 μg m<sup>-2</sup> h<sup>-1</sup>) treatment, while the CTNC showed the lowest emissions (11.93 μg m<sup>-2</sup> h<sup>-1</sup>). N<sub>2</sub>O emissions measured were higher in the row than within-rows in all treatments. Emissions from RTDC<sub>1</sub> and RTDC<sub>2</sub> were 84 and 46% higher, respectively.



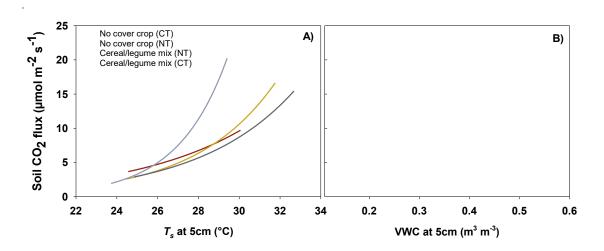
**Figure 3.8** Soil nitrous oxide (N<sub>2</sub>O) emission measured in the row and within-rows after cover crops were rolled-crimped in 2018.

# 3.3.6. Drought Conditions and CO<sub>2</sub> Emissions

Soil temperature and soil CO<sub>2</sub> fluxes showed a positive relationship in all cropping systems (Figure 3.9A). A larger increase in CO<sub>2</sub> emissions with soil temperature was observed in the reduced tillage with a double cover crop treatment. On the other hand, soil moisture and CO<sub>2</sub> fluxes showed a negative association that was stronger in reduce tillage plots (Figure 3.9B). Overall, soil respiration is expected to increase with soil moisture (Curiel Yuste et al., 2007; Yan et al., 2018). However, in this study, drought conditions caused soil shrinkage, exposed cracks and increased the exposed soil surface area. Previous studies have found that soil cracks can produce pathways for preferential flow even after cracks are not visible on the soil surface.

The higher flush in CO<sub>2</sub> observed in undisturbed soils can be an indicator of more carbon mineralization, soil microbial biomass, and net nitrogen mineralization (Franzluebbers et al., 2000). Also, it can indicate more C exchange from deep SOC into the atmosphere, which varies depending on the soil type and climatic conditions (Balesdent et al., 2018). Furthermore, several biophysical factors can influence the gas movement observed in this study. An early study suggested that crack depth and width, and wind speed are factors affecting water loss from shrinking soils (Ritchie and Adams, 1974). Wind speed was the primary factor controlling evaporation as it increased the vapor deficit by replacing cool and moist air with warm and dry air near the crack surface. Adams et al. (1969) found that evaporation within the crack was affected by temperature and vapor pressure of the air moving through the top of the shrinkage crack.

Nachshon et al. (2012) reported that convection could increase the rate of movement of air inside the cracks two orders of magnitude faster than gas diffusion rates.



**Figure 3.9** Relationship between soil CO<sub>2</sub> flux and A) soil temperature ( $T_s$ ) and B) volumetric water content (VWC). CTNC: tillage with no cover crop, CTDC: tillage with a double cover crop, RTSC: reduced tillage with fall cover crop in 2016-2017, RTDC<sub>1</sub>: reduced tillage with a double cover crop in 2016-2017, and RTDC<sub>2</sub>: reduced tillage with a double cover crop.

# 4. IMPACT OF CEREAL AND LEGUME COVER CROP RESIDUES ON CARBON AND NITROGEN MINERALIZATION IN ORGANIC SYSTEMS

#### 4.1. Introduction

Maintaining soil fertility to meet crop nutrient demands is a major challenge in organic production systems (Mäder et al., 2002; Seufert et al., 2012). Non-synthetic fertilizer sources, such as livestock manure, poultry manure, and compost, are generally used to fulfill the demand for nutrients in organic cropping systems. Organic fertilizer sources are known to release nutrients slowly and are applied based on soil phosphorus levels, which often do not necessarily meet crop nitrogen (N) requirements (Berry et al. 2002; Mäder et al. 2002). Incorporating cover crop residues in organic systems can help in soil fertility management by increasing nutrient availability, improving soil properties, and enhancing long-term sustainability (Johnson et al., 2007; Hill et al., 2017).

The amount of nutrients released from cover crop residues depends on several factors including the species, growth stage, and the quantity of biomass and its chemical composition (Gardner and Sarrantonio, 2012; Lavallee et al., 2018; Mitchell et al., 2013). Residues become part of the soil organic matter (SOM) complex through the processes of decomposition and mineralization. During the mineralization process, SOM is transformed into inorganic forms via microbial activity that breaks down complex compounds into simpler compounds and molecules (Galloway, 2014). The initial mineralization processes include aminization and ammonification, and ammonium (NH<sub>4</sub><sup>+</sup>) is released into the soil. Plants utilize NH<sub>4</sub><sup>+</sup> as a N source; however, it also

quickly undergoes further microbial transformations to become nitrate (NO<sub>3</sub>-). These processes are collectively called nitrification (Anand et al., 2011; Robertson and Groffman, 2007). Nitrate-N is another major source of N for plants, but it is easily lost from the soil system through denitrification and leaching. All mineralization processes are affected by microclimatic factors, such as soil moisture and temperature, which influence soil microbial activity and the time needed for decomposition of cover crop biomass residues. Therefore, knowing the rate that nutrients are released into the soil from cover crop residue decomposition and the factors affecting decomposition and mineralization processes, can increase our understanding of nutrient management constraints and opportunities in organic systems (Manzoni et al., 2012; Sinsabaugh et al., 2013).

The amount of nutrients released from crop residues depends on the net mineralization rate (i.e., the difference between nutrient mineralization and immobilization). The C:N ratio of residues is a major factor in determining the net mineralization rate (Cabrera et al., 2005; Van Der Krift et al., 2001). The C:N ratio that is ideal for soil microbial activity is 24:1, with approximately 66% of the C used for producing energy and the remaining C for maintenance (Melillo et al., 1989). Cover crop residues with a high C:N ratio such as cereal residues can result in rapid immobilization of N in soils and limit the availability of N for crops during early stages of decomposition (Johnson et al. 2007; Adiku et al. 2008). Conversely, legume residues generally have low C:N ratios and provide high-quality litter, resulting in rapid N mineralization and release of plant available N (Hadas et al., 2004). Recently, Lavallee

et al. (2018) found that low C:N ratio residues enhanced the C and N contribution to stable soil C pools, wherein residue quality did not affect the rate of decomposition but did affect microbial efficiency.

Both carbon dioxide (CO<sub>2</sub>) and nitrous oxide (N<sub>2</sub>O) are intermediate gaseous products of SOM decomposition and mineralization (Kravchenko et al., 2018; Miller et al., 2008). Most mineralization studies involve incubations that measure CO<sub>2</sub> production to estimate mineralization rate and nutrient release in soils (Franzluebbers et al., 2000; J. M.-F. Johnson et al., 2007; Stenger et al., 1995). Nitrous oxide is also produced as an intermediary product due to microbial denitrification in soils. However, only few studies have investigated both C and N gaseous losses simultaneously that are produced during the mineralization processes in soils (Dörsch et al., 2004; Huang et al., 2004; Qiu et al., 2016). Xu et al. (2008) and Negassa et al. (2015) investigated the association between CO<sub>2</sub> and N<sub>2</sub>O fluxes from soils and found a positive linear correlation at different spatial scales. Both these studies used empirical relationships to estimate N<sub>2</sub>O fluxes using soil CO<sub>2</sub> fluxes.

In this study, we conducted a soil incubation experiment under laboratory conditions and a litter bag experiment in the field to investigate C and N losses under different cover crop residue inputs. In the incubation experiment, microbial activity was indirectly measured from CO<sub>2</sub> and N<sub>2</sub>O production and was linked to C and N mineralization rates. We also measured methane (CH<sub>4</sub>) flux dynamics in addition to CO<sub>2</sub> and N<sub>2</sub>O fluxes as CH<sub>4</sub> is consumed by methanotrophic microbes under aerobic conditions for their metabolism or CH<sub>4</sub> is released in soils under anaerobic conditions

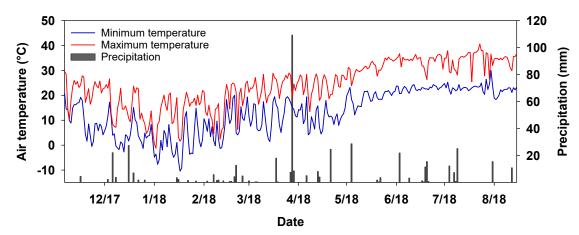
due to methanogenesis (Topp and Pattey 1997; Oertel et al. 2016). Our hypothesis is that cover crop addition can increase gaseous losses of C and N as a result of high N inputs from legume residues. We also investigated the relationship between CO<sub>2</sub> and N<sub>2</sub>O produced during residue decomposition and identified possible stages in microbial growth and activity that regulate mineralization-immobilization.

#### 4.2. Materials and Methods

## **4.2.1. Study Site Description**

An organic cropping systems field study was established in October 2016 at the Texas A&M University Research Farm near College Station, Texas (30°33'N, 96°25'W, 68 m a.m.s.l.) to investigate tillage and cover cropping practices suitable for corn production in the south central Texas region. The climate of the region is characterized as humid subtropical with annual precipitation of 992 mm, and an average minimum air temperature of 15°C and maximum of 27°C (USDA plant hardiness zone 8) (Clark, 2007). Average daily minimum and maximum air temperature and precipitation for the winter cover crop and corn growing seasons were obtained from a weather station close to the experimental site (Figure 4.1). The soil at the experiment site is classified as Ships clay (very-fine, mixed, active, thermic chromic hapluderts). Soil texture from 0-15 cm depth is 44.5% clay, 17.6% sand and 37.9% silt. Four corn (*Zea mays* L.) cropping systems that included fall and winter cover crops and tillage practices were established as a randomized complete block design with three replications (Figure 4.2). Production systems included (1) conventional tillage with no cover crop, (2) reduced tillage with a

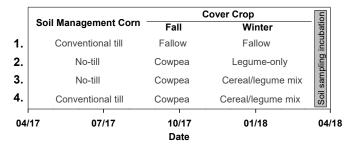
legume cover crop, (3) reduced tillage with cereal/legume cover crop mixture, and (4) conventional tillage with cereal/legume cover crop mixture.



**Figure 4.1** Average daily minimum and maximum air temperature and precipitation during the winter cover crop and corn growing seasons (November 2017 – August 2018) in an organic cropping systems experiment established near College Station, TX.

The experiment was managed according to the National Organic Program (NOP) guidelines as the land transitioned to certified organic production. The site was uncultivated and remained as grassland for 8-years before clearing and tilling to establish the study in September 2016. Cowpea (*Vigna unguiculata* L.) was planted as a fall cover crop on 23 October 2016 at a seeding rate of 56 kg ha<sup>-1</sup>. Cowpea grew for 55 days and was shredded and incorporated before planting the winter cover crops on 17 November 2016. The winter cover crop treatment planted in 2016 was cereal rye (*Secale cereale* L.)/hairy vetch (*Vicia villosa* Roth.) mixture (146 kg ha<sup>-1</sup>). Winter cover crops were mechanically terminated with a roller-crimper on 29 March 2017 before planting the main crop. Corn was planted on 31 March 2017 (75,600 seeds ha<sup>-1</sup>) and harvested on

31 July 2017. Tillage treatments were initiated during the corn growing season and consisted of chisel plowing three-times to a depth of approximately 20 cm. On 7 September 2017, the soil was tilled before planting the fall cowpea cover crop at a rate of 39 kg ha<sup>-1</sup>. The fall cover crop grew for 61 days and was shredded and incorporated on 7 November 2017 before planting the winter cover crops. The cereal/legume mixture planted was 64.3 kg ha<sup>-1</sup> of wheat (*Triticum aestivum* L.), 25.1 kg ha<sup>-1</sup> of barley (*Hordeum vulgare* L.), and 38 kg ha<sup>-1</sup> of Austrian winter pea (*Pisum sativum* L.). The legume only treatment was Austrian pea at a rate of 56 kg ha<sup>-1</sup>. Winter cover crops were terminated on 21 March 2018.



**Figure 4.2** Cover crop and tillage treatments in an organic corn cropping systems experiment established near College Station, TX. Soil samples for the incubation study were collected in spring 2018.

## 4.2.2. Incubation Experiment

## 4.2.2.1. Soil Sample Collection and Preparation

Six soil cores were randomly collected from 0-15 cm depth from each treatment before the winter cover crops were terminated in March 2018 (Figure 4.2). The soil samples were composited, homogenized, and pre-incubated at 4°C for 15 days to stabilize soil respiration (Meyer et al., 2018). After pre-incubation, the soil was passed

through a 4-mm sieve to remove roots, residues, and stones. Soil samples were then airdried in the laboratory and ground to pass through a 2-mm sieve. A subset of the ground samples was analyzed for soil chemical properties (Table 4.1), and the remaining samples were used in the incubation experiment.

## 4.2.2.2. Cover Crop Biomass Collection and Preparation

Aboveground biomass production was determined for all cover crops five times during the growing season before mechanical termination. Biomass samples were collected from a 1 x 1 m area in each plot (n = 18). Samples were oven dried at 65°C to estimate dry biomass production. The cover crop biomass samples collected prior to mechanical termination on 21 April 2018 was used in the incubation experiment. To use in the incubation experiment, the oven-dry biomass was ground and passed through a 2mm sieve. The chemical composition of cover crop residue was characterized for each treatment prior to the beginning of the incubation. Residues were analyzed for total C and N, lignin, cellulose, and hemicellulose. Total C was determined by dry combustion analysis and total N using the combustion method (Padmore, 1990b; Miller et al., 1997). The acid detergent lignin (ADL) procedure was used for determining the lignin content (Padmore, 1990a). Cellulose and hemicellulose were indirectly estimated by measuring neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Mertens, 1992). Thus, cellulose was calculated by subtracting lignin from ADF, and hemicellulose content calculated by subtracting ADF from NDF (Bjorndal, 1980). Analyses were performed at the Ward in Kearney, NE.

**Table 4.1** Soil chemical properties at the beginning of the incubation from plots that evaluated cover crops mixtures and tillage.

Treatment	pН	NO <sub>3</sub> -N	P	K	Ca	Mg	S	Na	
Treatment	hm				μg g <sup>-1</sup>				
No cover crop (CT)	7.9	17.4	16.8	325.5	8674.5	329.3	17.7	14.5	
Legume-only (NT)	8.0	9.6	20.3	330.9	8054.8	306.1	16.0	15.3	
Cereal/legume mixture (NT)	8.1	8.7	14.6	281.5	8820.7	328.1	15.0	17.3	
Cereal/legume mixture (CT)	8.1	9.0	8.8	252.5	9750.6	345.2	15.5	18.6	

CT: Conventional tillage; NT: reduced tillage.

#### 4.2.3. Laboratory Incubation and Gas Sampling

The incubation experiment was initiated on 10 April 2018 and lasted for 146 days. Fifty grams each of air-dried and 2-mm sieved soil samples were placed in 266 mL plastic cups, and soil moisture content was adjusted to 60% water-filled pore space (WFPS) assuming a soil bulk density of 0.9 g cm<sup>-3</sup> and particle density of 2.65 g cm<sup>-3</sup>. Plastic cups were then placed inside mason jars (946.35 mL) with lids equipped with a butyl rubber septa for gas sample collection. Rubber septa were replaced periodically throughout the study. Cover crop biomass was placed on top the soil inside the plastic cups without mixing with the soil to simulate field practices, where cover crops were terminated mechanically by roller-crimping and the above-ground residues were not incorporated into the soil. The amount of residue applied to each incubation cup was 283 g m<sup>-2</sup> which was equivalent to the average above-ground biomass obtained in the field on an area basis. Incubation jars were stored in the dark at approximately  $25 \pm 1.4$  °C. The WFPS was maintained constant during the incubation period by weighing the incubation cups every time gas samples were collected and adding de-ionized water as necessary. The laboratory incubation experiment was laid out as a completely randomized design using soils collected from the four production systems (conventional tillage with no cover crop, reduced tillage with a legume cover crop, reduced tillage with cereal/legume cover crop mixture, and conventional tillage with cereal/legume cover crop mixture). Each treatment had 12 replicates, and each mason jar was considered an experimental unit.

Carbon and N mineralization rates were calculated from the evolution of gasses into the jar headspace and changes in soil C and N contents over time. To quantify the production of gases from decomposition, gas samples were collected 18 times during the incubation period and analyzed by gas chromatography for CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O concentrations. During the incubation period, the jars were sealed to prevent the loss of gases into the atmosphere. At each sampling, 20 mL air samples were drawn using airtight syringes and samples were immediately injected into pre-evacuated 12 mL glass vials (Labco Exetainers, UK) before analyzing for CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O concentrations using a gas chromatograph. After the gas sample was collected, the jars were opened, and the headspace air was mixed using a fan before closing the jars again for continued incubation. Immediately after closing the jar, a second gas sample was collected to determine the initial gas concentrations inside the jar headspace. The initial gas concentration was subtracted from the gas concentration measured at the next sampling to determine the gas evolution during the incubation period.

Gas samples were analyzed using a Varian 450 Gas Chromatograph (Bruker Daltronics Inc. Billerica, MA) fitted with a <sup>63</sup>Ni electron capture detector (ECD), a thermal conductivity detector (TCD), and a flame ionization detector (FID) in series, with a Combi-PAL autosampler (CTC Analytics AG, Zwingen, CH). A 2-mL subsample was auto-injected and split into two sample loops, delivering 500 μL to both the ECD, and the TCD and FID in series. For the N<sub>2</sub>O measurements, the system was configured with a 0.5 m HayeSep N backflush column (Hayes Separations, Inc., Bandera, TX) followed by a 2 m HayeSep D analytical column. The carrier gas was P10

(90% Ar, 10% CH<sub>4</sub>). Methane and CO<sub>2</sub> analyses were carried out using a 0.5 m HayeSep N backflush column and a 2 m Poropak QS (Waters Corp., Milford, MA) analytical column with Ultra High Purity helium carrier gas. Hydrocarbon-free air and Ultra High Purity hydrogen gas were supplied to the FID for combustion. Calibration curves were developed for each detector using a commercial blend of N<sub>2</sub>O (0.26, 1.01, 5.1, 25.0, 75.1, 150, 300 ppm), CH<sub>4</sub> (1.50, 5.01, 10.0, 100, 499, 2000, 10000 ppm) and CO<sub>2</sub> (301, 501, 998, 5000, 10000, 20000, 50100 ppm) in air (Scott Specialty Gases, Plumsteadville, PA). Precision analysis expressed as the coefficient of variation for 30 replicate injections of 0.26 ppm N<sub>2</sub>O 1.5 ppm CH<sub>4</sub> and 301 ppm CO<sub>2</sub> was 1.78, 0.81, and 2.23%, respectively. A minimum of 4 laboratory blanks and four replicates of the lowest three calibration standards were analyzed with each sample analysis run.

# 4.2.4. Carbon and Nitrogen Mineralization

Changes in the headspace gas concentration over time was used to calculate C and N mineralization rates of the added crop residues. The cumulative evolution of CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O with time was fitted to a double exponential model that describes cover crop mineralization and estimates active and slow C and N pools (Eq. 4.1)

$$C_m = C_a(1 - e^{-k_a t}) + C_s(1 - e^{-k_s t})$$
 [Eq. 4.1]

where  $C_m$  is the cumulative C or N mineralized during the incubation period (t),  $C_a$  is the active C or N pool,  $C_s$  is the slow C or N pool,  $C_s$  is the active pool mineralization rate, and  $C_s$  is the slow pool mineralization rate. The model was fitted to all data points in each sampling date and cover crop treatment. After the model was

fitted, the estimated cumulative gas production in each cover crop treatment was used to determine the net mineralization of C and N.

Soil organic carbon (SOC), total nitrogen (TN), nitrate-N (NO<sub>3</sub>-N), ammonium-N (NH<sub>4</sub>-N) and pH were measured from five destructive samplings performed at 0, 13, 32, 63, and 146 days after incubation (DAI). Soil inorganic N or plant available N was calculated as the sum of NO<sub>3</sub>-N and NH<sub>4</sub>-N. Remaining organic N in soils was estimated as the difference between TN and inorganic N. Mineralization rates were estimated by fitting measured inorganic N concentrations with a single-exponential model that best fitted the data than a linear regression (J. M.-F. Johnson et al., 2007). In this relationship  $N_t$  is defined as the N mineralized at time t (days),  $N_0$  as the potentially mineralizable N, and k is the N mineralization rate constant (Eq. 4.2).

$$N_t = N_0(1 - \exp(-kt))$$
 [Eq. 4.2]

The nitrification/denitrification processes that result in N<sub>2</sub>O production during cover crop decomposition was also examined by determining the association between N<sub>2</sub>O emissions and inorganic N using Eq. 4.2. In this case,  $N_0$  corresponded to the potential N<sub>2</sub>O losses during cover crop decomposition (replaced by  $N_{N_2O}$ ) and k was the rate of N<sub>2</sub>O emissions per unit of inorganic N released into the soil (replaced by  $k_{N_2O}$ ). To investigate the relationship between C and N emissions, the cumulative evolution of N<sub>2</sub>O emissions as a function of CO<sub>2</sub> was fitted to a sigmoidal function (Eq. 4.3)

$$N_2 O = a/(1 + \exp^{\left(-\frac{\text{CO}_2 - b}{c}\right)})$$
 [Eq. 4.3]

where  $N_2O$  and  $CO_2$  are the nitrous oxide and carbon dioxide cumulative emissions ( $\mu g \ g^{-1}$  soil) recorded during the incubation, a is the limit on  $N_2O$  emissions, b

is the  $CO_2$  emissions inflection point, and c is the slope of the curve. The a, b, and c parameters estimated were compared and provided an approximation of changes in C and N use efficiency.

## 4.2.5. Litter Bag Experiment

## 4.2.5.1. Sample Preparation

Litter bags (0.2 by 0.2 m dimensions) were fabricated using nylon mesh (1-mm size) and filled with aboveground cover crop residues collected from the field (Sec. 2.1) on 10 March 2018. Cover crop biomass was oven-dried at 60°C and clipped into 0.2 m pieces before placing inside the bag. The amount of residue placed in the litter bag was based on the average field cover crop biomass estimates. Because there were no significant differences in biomass production among treatments at the last sampling date (Figure 4.3), the same amounts of dry biomass were placed in all cover crop treatment plots. Litter bags were sealed with safety pins to allow the removal of weeds growing inside over time and pinned directly to a bare soil surface. Bags were placed between the crop rows after planting of corn on 22 March 2018. Twelve bags were placed in each cover crop treatment plot and replicated three times. The litter bags remained in place throughout the corn season, except when they were temporarily removed from conventional tillage plots during cultivation.

# 4.2.5.2. Litter Bag Sample Collection and Analysis

Three litter bags per treatment plot (n = 9) were collected four times during the corn growing season at 48, 88, 120, and 142 days after placement. Litter bags removed from the field were placed in paper bags before transporting to the laboratory. Litter

content was oven-dried at 60°C, weighed, and ground. Residues collected were analyzed for dry weight, and C and N concentrations. The C and N concentration were measured by dry combustion analysis (Leco CN628 analyzer, St. Joseph, MI). The change in dry biomass, and C and N concentrations were expressed as a proportion of the initial values and were fitted to an exponential decay model of the form

$$P(t) = aexp^{-bt}$$
 [Eq. 4.4]

where P(t) is the proportion of mass or N or C remaining at a given time from litter bags collected from the field, a is the proportion of initial mass or N or C content, b is the decay constant of the initial mass or N or C content, and t is the time in units of growing degree days (GDD). Daily air temperature from a weather station on-site was used to calculate GDD using a base temperature of  $10^{\circ}$ C. The decay exponential model was fitted to each cover crop treatment (reduced tillage with a legume cover crop, reduced tillage with cereal/legume cover crop mixture, and conventional tillage with cereal/legume cover crop mixture). The model fitted was used to estimate the half-proportion decomposition between treatments and expressed in terms of GDD.

#### 4.2.6. Data Analysis

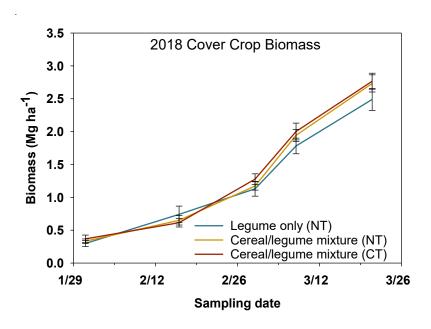
Data processing and statistical analyses were performed using SAS statistical software (SAS Institute, 2017). Repeated measures analysis of variance (ANOVA) was done to test the effect of cover crop residues on soil chemical properties in PROC MIXED. Since the measurements were correlated across time during the incubation, time was treated as a within-subject factor and cover crop residue as between-subject factors. Curve fitting to estimate C pool sizes and mineralization rates was done using

Marquardt algorithm in PROC NLIN. The statistical difference among treatments means was determined using least significant difference (LSD) multiple comparisons in PROC GLM. All statistical tests were considered significant at P < 0.05.

#### 4.3. Results

# 4.3.1. Cover Crop Biomass Production and Quality

Aboveground biomass production was similar between treatments, with no statistical differences between the legume-only and cereal/legume mixture at all sampling dates (Figure 4.3). Biomass production of the Austrian winter pea treatment was slightly lower than the cereal/legume mixture in the last two samplings before mechanical termination. Before mechanical termination, the aboveground biomass of the cereal/legume mixture in conventionally tilled plots ranged from 1.96 to 4.05 Mg ha<sup>-1</sup> and averaged at 2.76 Mg ha<sup>-1</sup>, whereas the aboveground biomass yield of the cereal/legume mixture in reduced tillage plots ranged from 1.84 to 3.66 Mg ha<sup>-1</sup> and averaged at 2.73 Mg ha<sup>-1</sup>. For Austrian winter pea, the average biomass before mechanical termination ranged from 1.22 to 3.73 Mg ha<sup>-1</sup> and averaged at 2.49 Mg ha<sup>-1</sup>.



**Figure 4.3** Aboveground biomass production (Mg ha<sup>-1</sup>) during the winter period in 2018. Error bars are standard errors (n = 18).

The chemical composition of Austrian winter pea and Austrian winter pea/wheat/barley mixture is presented in Table 4.2. Biomass C concentration was similar in both Austrian winter pea and Austrian winter pea/wheat/barley mixture, but differences were observed in total N, lignin, cellulose, and hemicellulose contents. The Austrian winter pea biomass contained 38% more N compared to the Austrian winter pea/wheat/barley mixture (4.9% vs. 3.5%). Lignin content was 22% higher in the cereal/legume mixture compared to the legume-only treatment (3.2% vs. 2.8%). Although the cereal/legume mixture contained only 17% more cellulose than the Austrian winter pea treatment, significant differences were observed in hemicellulose content, in which the mixture treatment showed 78% more hemicellulose than the Austrian winter pea residue.

**Table 4.2** Chemical composition on a dry-weight basis for the legume-only and legume/cereal mixture cover crop aboveground biomass.

Treatment	С	Crude Protein	N	C:N	Lignin	NDF	ADF	Cellulose	Hemicellulose	Ca	P	K	Mg	S	Zn	Fe	Mn	Cu	Mo
								%									ppm		
Legume only	43.5	24.3	4.9	8.9	2.8	28.6	25.6	22.6	3.0	1.2	0.3	3.8	0.3	0.2	49.6	838.0	48.5	9.5	2.6
Cereal/legume mixture	43.9	17.6	3.5	12.5	3.5	43.6	30.1	27.3	13.5	0.9	0.3	3.4	0.2	0.2	41.4	177.0	34.3	7.9	3.6

<sup>\*</sup> Neutral detergent fiber (NDF), Acid detergent fiber (ADF).

#### 4.3.2. Carbon Dioxide, Nitrous Oxide, and Methane Fluxes

The rate of CO<sub>2</sub>-C production for the cover crop treatments during the 146-day incubation period is presented in Figure 4.4A, and the cumulative CO<sub>2</sub>-C production is presented in Figure 4.4B. Cover crop additions stimulated soil C mineralization compared to the no-cover crop treatment. Cumulative CO<sub>2</sub>-C emission was 619.65 μg g<sup>-1</sup> soil in the no-cover crop treatment. Among the other treatments, CO<sub>2</sub> production was the highest in the legume-only treatment with total CO<sub>2</sub>-C emission of 3376.47 μg g<sup>-1</sup> soil. The legacy effect of previous tillage had less impact on C mineralization in the cereal/legume mixture as the cumulative CO<sub>2</sub>-C emissions from reduced and conventional tillage soil treatments were similar in magnitude (3175.67 μg g<sup>-1</sup> soil and 3137.88 μg g<sup>-1</sup> soil, respectively).

Cover crop residue addition significantly increased  $N_2O$  fluxes compared to the no-cover crop treatment (Figure 4.4C-D). After 20 DAI,  $N_2O$ -N fluxes declined significantly, and the rate of emission was similar in all treatments for the remaining period of incubation (Figure 4.4C). Cumulative  $N_2O$  emission was highest in the legume-only treatment (0.982  $\mu g$  g<sup>-1</sup> soil) and lowest in the no-cover crop treatment (0.041  $\mu g$  g<sup>-1</sup> soil). Cumulative  $N_2O$ -N emissions from the reduced tillage cereal/legume treatment had higher emissions (0.409  $\mu g$  g<sup>-1</sup> soil) than the conventional tillage cereal/legume treatment (0.329  $\mu g$  g<sup>-1</sup> soil).

Unlike CO<sub>2</sub> and N<sub>2</sub>O, mostly CH<sub>4</sub> consumption was observed in all treatments during the incubation period (Figure 4.4E-F). Slight CH<sub>4</sub> emissions were observed in the legume-only treatment during the first 15 DAI. Overall, the legume-only treatment

showed lower CH<sub>4</sub> consumption followed by the cereal/legume mixtures, and the nocover crop treatment. The total CH<sub>4</sub>-C consumption was of 0.023  $\mu g$  g<sup>-1</sup> soil in the nocover crop treatment, 0.024  $\mu g$  g<sup>-1</sup> soil in the reduced and conventional tillage treatments with the cereal/legume mixture, and 0.014  $\mu g$  g<sup>-1</sup> soil in the legume-only treatment.

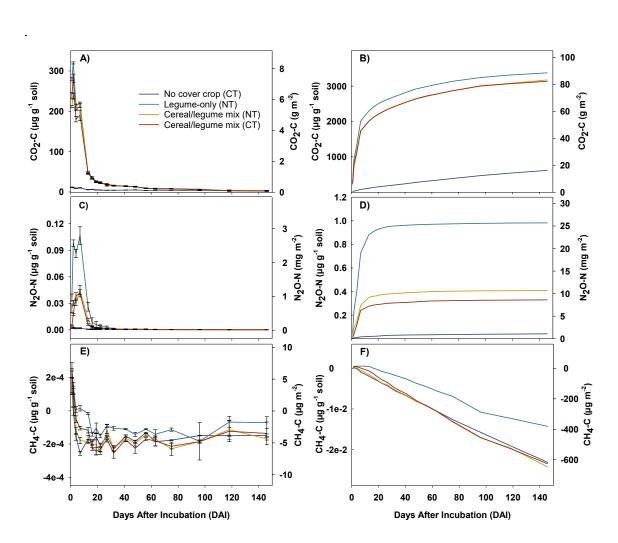


Figure 4.4 Rate (left) and cumulative (right) emissions of (A-B) carbon dioxide (CO<sub>2</sub>), (C-D) nitrous oxide (N<sub>2</sub>O), and (E-F) methane (CH<sub>4</sub>) during the 146 days incubation period. Incubation was conducted using soils collected from an organic corn field at 25±1.4°C in the laboratory. Cover crop treatments include no cover crop, Austrian winter pea (legume-only) and wheat/barley/Austrian winter pea mixture (cereal/legume mixture). Error bars are standard errors.

#### 4.3.3. Carbon Pool Sizes under Different Cover Crops

Active and slow C pools and their mineralization rates were estimated by fitting the double-decay exponential model to the cumulative CO<sub>2</sub> emission data from the incubation study (Table 4.3; Figure 4.4B). The regression was significant for all the treatments (P < 0.0001). Cover crop residue inputs increased active C pool sizes ( $C_a$ ) but did not affect active mineralization rates ( $k_a$ ). The legume-only treatment showed the highest  $C_a$  of 2207.6  $\mu$ g g<sup>-1</sup> soil. No significant differences were found in  $C_a$  of the cereal/legume mixtures managed under conventional and reduced tillage (1869.2 and 1983.9  $\mu$ g g<sup>-1</sup> soil, respectively). Although the slow C pool sizes ( $C_s$ ) estimated were not significantly different among treatments, the Austrian winter pea/wheat/barley mixture in both tillage practices showed the highest  $C_s$ . The slow C mineralization rates ( $k_s$ ) increased with residue inputs compared to the no-cover crop treatment, with the cereal/legume mixture with reduced tillage having the lowest  $k_s$ .

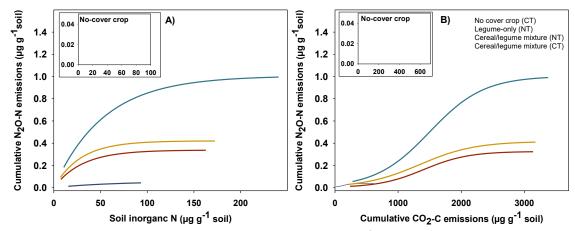
**Table 4.3** Estimated active ( $C_a$ ) and passive ( $C_s$ ) C-pools ( $\mu g \, g^{-1} \, soil$ ), and active ( $k_a$ ) and slow ( $k_s$ ) mineralization rates (day<sup>-1</sup>) on an area basis. Standard errors of the coefficients estimated are presented in parenthesis.

Treatments	Ca	$k_a$	$C_s$	ks
No cover crop (CT)	45.64	0.184	1232.6	0.004
No cover crop (C1)	(3.75)	(0.0297)	(63.18)	(0.0003)
Legume-only (NT)	2207.6	0.18	1317.1	0.016
Leguine-only (N1)	(124.7)	(0.0153)	(90.61)	(0.0045)
Cereal/legume mixture (NT)	1983.9	0.157	1462.6	0.012
Cereal/legume mixture (NT)	(143.1)	(0.0169)	(159.3)	(0.0048)
Canad / la cruma miretuma (CT)	1869.2	0.18	1419.3	0.016
Cereal/legume mixture (CT)	(103.9)	(0.0149)	(73.97)	(0.0034)

CT: Conventional tillage, NT: Reduced tillage in the previous growing season before collecting soil sample for incubation.

#### 4.3.4. Turnover of Carbon and Nitrogen in Soils

Figure 5A presents the model fitted (Eq. 4.2) to the soil inorganic N measured at five destructive sampling dates performed at 0, 13, 32, 63, and 146 DAI. As incubation progressed, an increase in soil inorganic N (NO<sub>3</sub>-N + NH<sub>4</sub>-N) was observed in all the treatments (Figure 4.5A). Overall, NO<sub>3</sub>-N contributed the most to the inorganic N pool compared to NH<sub>4</sub>-N (data not shown). The initial inorganic N in soils before incubation was as follows; no-cover crop treatment (15.5  $\mu$ g g<sup>-1</sup>) > legume-only (10.7  $\mu$ g g<sup>-1</sup>) > legume/cereal under conventional tillage (7.5  $\mu$ g g<sup>-1</sup>) > legume/cereal under reduced tillage (7.2 µg g<sup>-1</sup>). Table 4.4 presents the parameters of the exponential model, potentially mineralized N content  $(N_0)$  and the rate of N mineralization (k). Estimated  $N_0$ and k differed among cover crop treatments, with the legume-only treatment showing the highest values. After 63 DAI, 45% of the  $N_0$  was mineralized in the no-cover crop treatment, 87% in the legume-only treatment, and 54 and 64% in the cereal/legume mixture in reduced tillage and conventional treatments, respectively. At the end of the incubation,  $N_0$  corresponded to 83% in the no-cover crop treatment, 100% in the legumeonly, and 82 and 88% in the cereal/legume mixture in reduced and conventional tillage, respectively.



**Figure 4.5 A)** Soil inorganic N (NO<sub>3</sub><sup>-</sup>+ NH<sub>4</sub><sup>+</sup>) (μg kg<sup>-1</sup> soil) measured (symbol) and exponential model fitted (line) that estimated potentially mineralizable N and mineralization rates. **B)** Soil organic C (mg g<sup>-1</sup> soil) measured at 0, 13, 32, 63, and 146 days of incubation. Cover crop treatments include no cover crop, Austrian winter pea (legume-only) with reduce tillage, and wheat/barley/Austrian pea mixture (cereal/legume mixture) with reduced (NT) and conventional tillage (CT). Error bars are standard errors.

**Table 4.4** Potentially mineralizable nitrogen ( $N_0$ ,  $\mu$ g N g soil<sup>-1</sup>) and mineralization rates (k, day<sup>-1</sup>) estimated from soil samples collected after 0, 13, 32, 63, and 146 days of incubation. Standard errors of the coefficients estimated are presented in parenthesis.

Treatments	$N_{\theta}$	<u>k</u>	$R^2$
No cover crop (CT)	111.23 (39.69) a†	0.0114 (0.0076) a	0.86
Legume-only (NT)	238.84 (10.81) c	0.0374 (0.0051) b	0.99
Cereal/legume mixture (NT)	209.94 (28.65) bc	0.0118 (0.003) a	0.98
Cereal/legume mixture (CT)	184.21 (17.04) ab	0.015 (0.0029) a	0.99

CT: Conventional tillage, NT: Reduce tillage. † Means followed by the same letter are not significantly different.

The effect of cover crop residue type on soil inorganic N was significant starting 13 DAI (P < 0.05) (Table 4.5) according to repeated measures ANOVA. The legume-only treatment showed the highest inorganic N, followed by the legume/cereal cover crops in soil under conventional and reduced tillage. After 146 DAI, the legume-only

and legume/cereal mixture additions to the soil increased soil inorganic N content by 300% and 82%, respectively, when compared to the no-cover crop treatment.

**Table 4.5** Repeated measures analysis of variance for the effect of cover crop residue type on soil chemical properties during the incubation period.

Variable	D	AI	Cover c	rop type	DAI*Cover crop type		
Variable	F	<i>P</i> -value	F	<i>P</i> -value	F	<i>P</i> -value	
Inorganic N	391.77	<.0001*	230.04	<.0001*	22.8	<.0001	
$NO_3$ -N	392.89	<.0001*	230.24	<.0001*	23.36	<.0001	
NH <sub>4</sub> -N	6.03	0.001*	3.76	0.020	1.1	0.395	
Organic N	22.08	<.0001*	47.16	<.0001	4.13	0.001	
Total N	28.92	<.0001	80.62	<.0001	5.08	0.0001	
Inorganic C	8.03	0.004*	0.41	0.745	0.69	0.663	
Organic C	1.21	0.325	61.71	<.0001	0.47	0.919	
Total C	4.04	0.038*	9.22	0.001	1.17	0.369	
pН	22.41	<.0001*	16.25	<.0001	1.73	0.137	

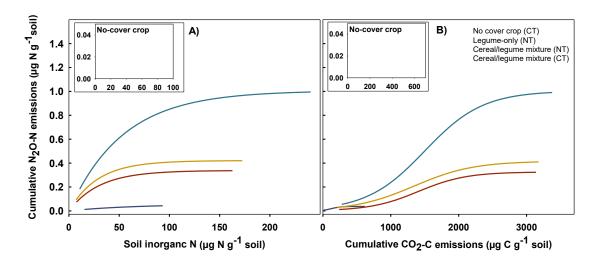
<sup>\*</sup> Significant at P < 0.05.

Initial SOC was different among treatments, with the legume-only treatment showing the highest value. While SOC did not change significantly during the incubation period, initial differences observed among treatments continued through the cover crop decomposition. SOC showed a slight increase in all the treatments up to 15 DAI, but after this period SOC decreased and remained constant (Figure 4.5). Average SOC was 15.15 mg g<sup>-1</sup> in the legume-only with reduced tillage treatment, and 13.12 mg g<sup>-1</sup> in the cereal/legume mixture with conventional tillage and 13.11 mg g<sup>-1</sup> with reduced tillage. No-cover crop treatment showed similar SOC to that of the cereal/legume mixture treatment with an average SOC of 13.27 mg g<sup>-1</sup>. Soil inorganic C was not affected by cover crop treatments (Table 4.5). Although there were no significant

differences in soil pH among soil samplings, a slight decline was observed with biomass residue inputs (data not shown).

# 4.3.5. Soil Inorganic Nitrogen and N2O Fluxes

Figure 4.6A presents the relationship between cumulative N<sub>2</sub>O-N fluxes and soil inorganic N (NO<sub>3</sub>-N + NH<sub>4</sub>-N) measured at 0, 13, 32, 63, and 146 DAI. A single-exponential model was fitted to the data [Eq. 4.2] to determine potential N<sub>2</sub>O losses  $(N_{N_2O})$  and the rate of N<sub>2</sub>O emissions per unit of inorganic N released into the soil  $(k_{N_2O})$ . Overall, soil inorganic N showed a positive non-linear relationship with N<sub>2</sub>O emissions (P < 0.01). The no-cover crop treatment showed potential  $N_{N_2O}$  losses of 0.056 µg N<sub>2</sub>O-N g<sup>-1</sup> soil  $(R^2 = 0.84)$ . Among the cover crop mixtures, the legume-only showed the highest  $N_{N_2O}$  of 1.01 µg N<sub>2</sub>O-N g<sup>-1</sup> soil  $(R^2 = 0.96)$ , followed by the cereal/legume mixture in reduced tillage and conventionally managed soil with 0.42 and 0.34 µg N<sub>2</sub>O-N g<sup>-1</sup> soil  $(R^2 = 0.94)$  and 0.92), respectively.



**Figure 4.6 A)** Relationship between cumulative N<sub>2</sub>O fluxes and soil inorganic N (NO<sub>3</sub>-N+NH<sub>4</sub>-N) measured at 0, 13, 32, 63, and 146 days after incubation and **B**) relationship between cumulative carbon dioxide (CO<sub>2</sub>) and nitrous oxide (N<sub>2</sub>O) emissions (μg g<sup>-1</sup> soil) during the incubation study. Lines are the exponential (A) and sigmoidal (B) model fitted and symbols measured data. Cover crop treatments include no cover crop, Austrian winter pea (legume-only) with reduce tillage, and wheat/barley/Austrian winter pea mixture (cereal/legume mixture) with reduced (NT) and conventional tillage (CT).

#### 4.3.6. Cumulative N2O and CO2 Fluxes

Figure 4.6B presents the relationship between cumulative  $CO_2$  and  $N_2O$  emissions measured during the incubation. A positive association was found between cumulative  $CO_2$  and  $N_2O$  emissions in all treatments. Initially, a linear increase in both GHGs was observed, followed by a slow or null increase in  $N_2O$  emissions, but a continuous increase in  $CO_2$ . The a, b, and c parameters estimated that describe the sigmoidal trend observed are presented in Table 4.6. There were significant differences among the parameters estimated that were affected by the cover crop residues. However, the inflection point in  $CO_2$ , c, was similar between soils managed under reduced tillage in legume-only and cereal/legume mixtures (428.55 and 436.96  $\mu g$  g<sup>-1</sup> soil, respectively).

The no-cover crop treatment showed the lowest maximum  $N_2O$  emissions and rates estimated. The maximum increase in  $N_2O$  emissions, a, was the highest in the legume-only treatment, with a value of 1.003  $\mu g \, N_2O$ -N  $g^{-1}$  soil. The cereal/legume mixtures showed maximum  $N_2O$  emissions of 0.326 and 0.416  $\mu g \, N_2O$ -N  $g^{-1}$  soil under conventional and reduced tillage soils, respectively.

**Table 4.6** Parameters estimated (a, b, and c) and coefficient of determination  $(R^2)$  of the sigmoidal function that relates  $CO_2$  and  $N_2O$  cumulative emissions during the incubation under several cover crop treatments. Standard errors of the coefficients estimated are presented in parenthesis.

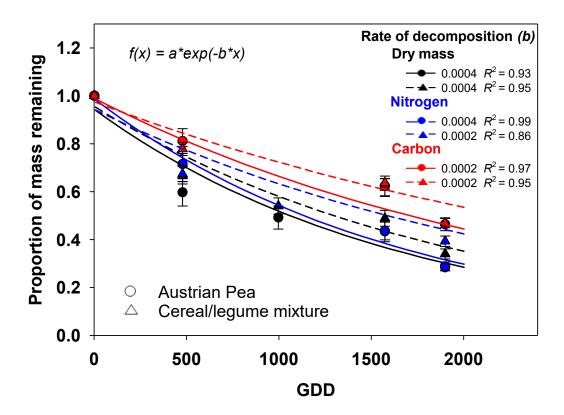
Treatments	a	b	c	$R^2$
No cover crop (CT†)	0.037	123.88	69.66	0.973*
	(0.001)	(7.06)	(7.14)	
Legume-only (NT)	1.003	1500.28	428.55	0.997*
	(0.009)	(22.6)	(18.01)	
Cereal/legume mixture (NT)	0.416	1344.23	436.96	0.998*
	(0.003)	(16.59)	(12.69)	
Cereal/legume mixture (CT)	0.326	1406.89	353.05	0.997*
	(0.003)	(18.52)	(13.85)	

<sup>†</sup>CT: Conventional tillage, NT: Reduced tillage in the previous growing season before collecting soil sample for incubation. \*Significant to *P*-value < 0.001.

#### 4.3.7. Litter Bag Decomposition

The exponential decay model provided a good fit to the proportion of mass loss, and C and N content with GDD ( $R^2 > 0.85$ ). Dry mass losses increased with time, with no significant differences in the rate of decomposition (b) between cover crop mixtures (Figure 4.7). Similar decomposition patterns were observed in C and N concentrations in the litter bag study. Although no significant differences were observed in the slope, N

concentration had a higher rate of loss in the legume-only treatment compared to the cereal/legume mixture. In addition, the C decay constants were the lowest and similar for all cover crops. Based on the exponential decay model fitted (Eq. 4.4), 50% of the dry mass loss occurred at 1060 GDD (74 days) in the legume-only treatment and at 1296 GGD (88 days) in the cereal/legume mixture. Considering N content, 50% of the loss occurred at 1135 GDD (79 days) in the legume-only and 1587 GGD (104 days) in the cereal/legume mixture. Half of the C concentration was lost at 1708 GDD (110 days) and 2226 GDD (> 125 days).



**Figure 4.7** Proportion of total dry mass, and N and C contents in litter bags containing legume-only (solid line) and cereal/legume mixture (dash line) against growing degreedays (GDD). Each point represents the mean of nine replicates at a given time. Total dry mass and N and C concentrations are presented on a dry-weight. Error bars are standard errors.

#### 4.4. Discussion

## Effect of cover crop biomass and composition on decomposition loss of residues

Cover cropping is a crucial management practice to improve soil fertility and control weeds in organic cropping systems. The average aboveground dry matter production of the winter cover crops in our study (approx. 2.5 Mg ha<sup>-1</sup>) was 31% of the proposed threshold biomass for effective weed suppression (8 Mg ha<sup>-1</sup>) in organic cropping systems (Mirsky et al., 2013). Studies have suggested that biomass contribution from cereals is generally higher compared to legumes (Clark et al., 2017; Duval et al., 2016). In our study, no differences were found between cover crop mixtures (Figure 4.2). The overall low biomass production of Austrian winter pea and the wheat/barley/Austrian winter pea mixture were likely due to late fall planting (7 November 2017) or early termination (21 March 2018). Seeding rates and combinations of cover crop mixtures are also important in determining the final biomass yield (Poffenbarger et al., 2015a). Bauer et al. (1993) reported similar Austrian winter pea aboveground biomass production in South Carolina (USDA plant hardiness zone 8) and Kuo et al. (1997) indicated similar results in Washington. Biomass production of the cereal/legume mixture was lower than a study evaluating cover crop mixture proportions using cereal rye/hairy vetch, in which aboveground biomass varied from 5 to 6 Mg ha<sup>-1</sup> (Hayden et al., 2014).

Although biomass production was similar, the chemical composition of cover crop residues varied significantly. The Austrian winter pea cover crop provided the most N, with a lower C:N ratio than the cereal/legume mixture. Cellulose and hemicellulose

varied significantly between treatments, with hemicellulose showing the highest content in the cereal/legume mixture (Table 2). Hemicellulose content of the legume-only treatment was lower and typical of leaf tissue. Johnson et al. (2007) found that N concentration and lignin content are highly correlated to active decomposition rates, while hemicellulose, N concentration, and the C:N ratio are associated with slow residue decomposition rates. Therefore, the high hemicellulose content and C:N ratio in the cereal/legume mixture were expected to result in much slower decomposition and mineralization rates than legume only additions.

To better understand cover crop biomass decomposition, we combined a laboratory incubation with a field experiment that used litter bags installed after mechanical termination of the cover crop. The mass loss and changes in C and N content under field conditions were evaluated from litter bags (Figure 4.7) and compared the results with decomposition under controlled conditions (Figure 4.4). Studies from northern regions of the United States indicate that 50% of the mass loss occurs in < 1-yr (Johnson et al., 2007; Poffenbarger et al. 2015). However, in the southern region where this study was performed 50% of the mass loss occurs in < 100 days. The rapid residue mass loss observed in Texas could be due to the effect of temperature and precipitation in litter decomposition (Figure 4.1). Unlike differences in C decomposition observed in the incubation study, no differences were observed in the proportion of mass and C losses between the legume-only and cereal/legume mixture. The legume-only showed a lower portion of N remaining that is in agreement with the initial low C:N ratio

measured in the residue and high N mineralization rate (k) estimated in incubated soils (Table 4.4).

#### Carbon and Nitrogen Mineralization from Cover Crops

Quantifying the amount of N released from biomass decomposition and mineralization can provide valuable insights into the potential contribution of plantavailable N from winter cover crops to the subsequent main crop. Overall, cover crops increased C and N mineralization rates due to the large and diverse residue inputs added to the soil that increased C supply for microbial activity. During the incubation, soil NH<sub>4</sub>-N was low and showed to be a transitioning N pool during mineralization (data not shown) (Stenger et al., 1995). As evident from the results of the incubation study, the N rich residue of Austrian winter pea increased  $N_0$  and k (Table 4.4) and resulted in the production of more plant available N, mainly NO<sub>3</sub>-N (Figure 4.4). These results are also in agreement with the large active C pools estimated in the Austrian winter pea treatment (592 kg C ha<sup>-1</sup>) that indicated more N could potentially be mineralized. The active C pool, which includes microbial biomass and dissolved C, has rapid turnover rates and is sensitive to land and soil management practices (Paustian et al., 1992). This is in agreement with the high hemicellulose content found in the cover crop mixture (Table 4.2) that reduced the rate of N mineralization (Table 4.4). The potentially mineralizable N with no-cover crop was similar to that estimated in disturbed soils with high-clay and N content by Cabrera and Kissel (1988).

Although tillage was expected to have an impact on active C pools in the short-term, no differences in the C pool sizes were estimated between conventional and

reduced tillage soils under the same cover crop type (517 kg C ha<sup>-1</sup> in reduced tillage and 505 kg C ha<sup>-1</sup> in conventional tillage). The tillage practices were established in our study was only for 2-years, and further changes in the active pools could be expected in the long-term. The active C pool estimated in the no-cover crop treatment (13 kg C ha<sup>-1</sup>), was lower than the values reported by Kaiser et al. 2014 of 50 kg C ha<sup>-1</sup> that were determined using a one-pool model. The overall mean residence time (MRT) estimated as the reciprocal of the mineralization rates indicated that the active C pool showed the lowest MRT of 6-days in most treatments, while the slow C pool ranged from 63 to 250 days. Paul et al. (1999) reported higher MRT in no-cover crop soil under different management for the active and slow C pool that ranged from 10 to 22 days and 3.1 to 4.4 years, respectively.

Several studies have suggested that increasing mineralization or immobilization turnover can potentially reduce N losses through leaching and denitrification (Franzluebbers et al. 1995b; Stenger et al. 1995). Harper et al. (2005) found a decrease in  $N_2O$  emissions with cover crops residue possibly due to an increase in ground cover that reduces soil gas exchange and air transport near the surface. However, we found that  $N_2O$ -N emissions increased during cover crops decomposition (Figure 4.4). The legume-only treatment showed high  $N_0$ , k, and  $N_2O$ -N losses. The greater  $N_2O$  emissions were due to a low C:N ratio and higher N mineralization from legumes that increased  $CO_2$ -C and soil  $NO_3$ -N (Figure 4.3-4.4, Table 4.3) (Chen et al., 2007). Residue quality has shown to impact microbial activity (Peichl et al., 2013). Crop residue that decomposes easily enhance the release of labile C pool compared to low-quality litter.

The slow C pool sizes estimated were higher in the cereal/legume mixture compared to the legume-only residue (Table 4.3). This result can be explained because N immobilization is positively correlated to high C:N ratio inputs associated to high molecular weight molecules (i.e., lignin, cellulose) (Table 4.2) (Gardner and Sarrantonio, 2012). A larger slow C pool can be related to an increase in C sequestration (Manzoni et al., 2012). The no-cover crop treatment showed that even without biomass additions, C and N mineralization occurring in natural soils could impact losses in the long-term. Similar results are observed in fallow land that produce a steady decline in organic matter (Walela et al., 2014).

## Carbon and nitrogen availability affect nitrous oxide dynamics

Nitrogen mineralization is closely associated to soil C cycle, and CO<sub>2</sub> production during the conversion to soil inorganic N. The release of CO<sub>2</sub> during the incubation that is an indicator of the rate of organic C decomposition from cover crop residues showed a significant correlation with soil parameters. For example, a positive correlation was found between CO<sub>2</sub> production and inorganic N (R = 0.81, P < 0.01) and N<sub>2</sub>O-N emissions (R = 0.8, P < 0.01). We investigated the changes in soil inorganic N mineralized and N<sub>2</sub>O emissions among cover crop residues and estimated potential  $N_{N2O}$  losses and the rate of N<sub>2</sub>O production,  $k_{N2O}$  (Figure 4.6A). Although there were no significant differences in  $k_{N2O}$  during decomposition,  $N_{N2O}$  differed among cover crop residues as follows no-cover crop < cereal/legume mixture < legume-only.

It has been suggested that the correlation between soil CO<sub>2</sub> and N<sub>2</sub>O emissions are soil and site-specific (Xu et al., 2008) and follows a linear relationship (Huang et al.

2004; Miller et al. 2008). However, in this study, the association between CO<sub>2</sub> and N<sub>2</sub>O followed a logistic function similar to the residue decomposition curve that is characterized by three stages (Figure 4.6B). Initially, the easily decomposable C fraction decay (sugars, amino acids), followed by the intermediate (cellulose, hemicellulose), and the slowly decomposable fractions (lignin) (Qiu et al., 2016). A similar trend in terms of C and N gaseous losses was observed during residue decomposition. Initially, an increase in labile C decomposition resulted in a flush of CO<sub>2</sub>. During this phase, microbial activity growth and N substrate reach a critical concentration before mineralization can occur (Paul, 2007). Secondly, a linear increase in both, CO<sub>2</sub> and N<sub>2</sub>O was observed that was associated with mineralization occurring and microbes assimilating N from organic sources. Lastly, a decline in N<sub>2</sub>O emissions, but a continuous increase in CO<sub>2</sub> occurred as a result of N immobilization by the microbes. The relationship followed the same trend independent of C additions from cover crop residues. Previous studies have suggested that C availability controls N assimilation (Stevenson and van Kessel, 1996; Johnson et al. 2007). Two reasons can explain the flattering in N<sub>2</sub>O emissions; first, the large C emissions from microbial respiration observed can be associated with N limitation, mainly because most of the N was consumed by microbes (Manzoni et al., 2012). Second, C inputs initially enhanced microbial activity and oxygen consumption that stimulated denitrification, but once C is consumed there is a decline in denitrifiers activity and therefore, in N<sub>2</sub>O emissions (Miller et al., 2008).

## Cover crop inputs affect CH<sub>4</sub> uptake

The net flux of CH<sub>4</sub> from soil depends on various factors including pH, substrate, soil texture, the presence of electron acceptors, C mineralization, moisture, and temperature (Goulding et al., 1996; Powlson et al., 1997). Although during the incubation, mostly CH<sub>4</sub>-C consumption was observed in all treatments (Figure 4E-F), under high N inputs from the legume-only residues, CH<sub>4</sub>-C oxidation decreased. The inorganic N released during mineralization could have inhibited methane monooxygenase, an enzyme required to initiate CH<sub>4</sub> oxidation (Smith et al., 2018; Topp and Pattey, 1997). These results are in agreement with the negative correlation observed between CH<sub>4</sub>-C consumption and inorganic N (R = -0.61, P < 0.01). Similar results were found by Sitaula et al. (1995), where N fertilization (NH<sub>4</sub>NO<sub>3</sub>) reduced CH<sub>4</sub> consumption in 15 to 38%.

An increase in microbial activity during mineralization could have enhanced C consumption as the energy source. These results also highlight the linkage between the C and N cycle and factors driving processes occurrence (i.e., consumption, production) and the implication for cover crop expansion. Quantifying the amount of methane produced by a soil sample indicates the activity of anaerobic methanogenic bacteria (Moiser et al., 1991). Although soils generally act as a sink for atmospheric CH<sub>4</sub> under not oxygen-limited conditions, cover crops can potentially decrease the sink for atmospheric CH<sub>4</sub>, but further research is needed to evaluate cover crops inputs and its effect of GHG emission in a field scale.

## 5. CONCLUSIONS

In chapter 2, we investigated the effect of tillage management practices and cropping systems on the magnitude of soil respiration measured at high-frequency intervals. Soil CO<sub>2</sub> emissions from monoculture and rotational systems were compared, and changes in soil environmental conditions were quantified to better understand the long-term effect of tillage. Although rotational cropping of winter-wheat showed similar soil temperature profiles in conventional and no-till, higher soil moisture in no-till plots reduced soil CO<sub>2</sub> emissions compared to conventional plots. Residues accumulated on the soil surface under no-till likely decreased the temperature-dependency of CO<sub>2</sub> emissions that resulted in lower emissions and indicates  $Q_{10}$  is regulated by plant residue in no-tillage. On the other hand, monoculture soybean showed the opposite trend to rotational cropping, with extremely high soil temperatures under conventional till that suppressed microbial activity and soil CO<sub>2</sub> emissions. The optimum soil temperature range in no-till increased C losses in soybean that resulted in higher CO<sub>2</sub> emissions. Conversely, SOC measurements did not agree with our findings in soil CO<sub>2</sub> fluxes, wherein no-till always showed the highest SOC regardless of the crop sequence. Intensive cropping can increase. This information could be used to improve our understanding of the relationship among of C fluxes and pool sizes measurements that serve as indicators of C sequestration.

In chapter 3, we quantified emissions from transitioning organic systems in a Vertisol under different cover cropping and soil management practices. Soil CO<sub>2</sub> flux was the most dynamic contributor to GHG when land-use changed into agricultural

production. The low soil N status likely resulted in null N<sub>2</sub>O flux during the first year and sporadic N<sub>2</sub>O flux during the second year of transition. The CO<sub>2</sub> flux reported in this study were generally higher than previously reported. Soil cracking was a significant factor for increased CO<sub>2</sub> emissions towards the late season of 2017 due to soil shrinkage during drought conditions. Overall, reduce tillage showed the highest flux when the soil was extremely dry and was associated with more soil cracking under undisturbed conditions. Cover cropping and soil tillage influence cumulative CO<sub>2</sub> emissions similarly in both years, despite the production of different cover crops in 2018. Winter cover crop mixtures that were mechanically terminated before corn planting enhanced CO<sub>2</sub> emissions during the growing season, probably due to an increase in residue decomposition and root respiration from both crops. Although integrating cover cropping increased CO<sub>2</sub> emissions, this could be offset by the CO<sub>2</sub> uptake from cover crops. More uncertainties in determining GHG emissions and C and N dynamics are expected from intercropping systems. Additional research is also needed to understand the mechanism of gas transport in Vertisols, which is crucial to accurately estimate fluxes as well as develop soil management practices that can lead to C sequestration.

In Chapter 4, we estimated cover crop residue decomposition rates and the fate of intermediate products can improve our understanding of the environmental impact of agricultural practices. In this study, cover crop mixtures determined C and N mineralization rates and gaseous losses as CO<sub>2</sub> and N<sub>2</sub>O. The legume-only treatments increased N availability in the short-term that was supported by a greater active C pool size and active mineralization rate. Conversely, the cereal/legume mixture showed a

greater passive C pool and passive mineralization rates. Carbon mineralization was the same between conventional and no-till soils with the cereal/legume mixture. However, N<sub>2</sub>O emissions were different between tillage and were higher in soil collected from reduced tillage plots. Our results also suggested that N<sub>2</sub>O emissions were limited by the active C pool sizes that vary among C and N inputs from crop residues. These results show the coupling between C and N cycles.

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APPENDIX A

CHEMICAL COMPOSITION MANURE AND COMPOST

Element	Units	Poultry manure	Turkey compost
Dry matter		89.00	49.10
Carbon			41.30
Nitrogen	%	2.52	1.11
Phosphorus		1.23	0.18
Potassium		3.87	0.35
Calcium		2.95	2.02
Magnesium		0.75	0.18
Sodium		1.21	0.07
Zinc	ppm	359.81	65.19
Iron		185.80	4945.70
Copper		395.82	12.71
Manganese		329.92	189.00
Sulfur		6388.31	1149.59
Boron		78.15	4.36