

THESIS

RESIDUE-DERIVED CARBON TRANSFORMATIONS WITH ALTERED RESIDUE
MANAGEMENT IN AN IRRIGATED CORN SYSTEM IN COLORADO

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ABSTRACT

RESIDUE-DERIVED CARBON TRANSFORMATIONS WITH ALTERED RESIDUE MANAGEMENT IN AN IRRIGATED CORN SYSTEM IN COLORADO

Soil plays an essential role in storing carbon (C) and nutrients regulating climate and sustaining food production. As such, it is important to understand how human activities affect C cycling in soil. Agricultural lands, particularly croplands, comprise some of the most managed areas of the world. We have the potential to positively impact C sequestration in croplands by understanding how residue management affects the C mineralization as well as the formation and stabilization of soil organic C (SOC). No-till (NT), which leaves residue on the surface, has been proposed as the superior residue management for building SOC due to a decreased rate of residue and existing SOC decomposition. Conventional tillage (CT), which breaks aggregates while incorporating residue into the soil, is thought to result in less SOC storage through increased residue and SOC decomposition, and soil erosion. However, NT does not always result in higher SOC storage than CT when measuring total profile stocks and the gains might not be stable in a changing climate. I studied a semi-arid, irrigated, corn system where gains in surface SOC but losses at depth had been observed because of conversion to NT. I studied the effect of altered residue management by mimicking NT and CT residue placement and soil disturbance and followed the residue-derived C into soil respiration (CO₂) and physically defined SOC pools.

I applied isotopically labeled (¹³C) residue either on the soil surface (NT) or incorporated into the soil (CT), which allowed me to track losses and transformations of residue C under different residue placement. A third treatment with surface-applied residue following soil

disturbance tested the effect of disturbing aggregates during tillage. I collected soil respiration measurements and determined its isotopic composition for twelve months following residue addition to understand how residue location and soil disturbance affected the rate of total CO₂ efflux, and of their component fractions (i.e., derived from residue vs. derived from soil). As reported in Chapter 2, I found that incorporated residue had higher residue-derived respiration in the first six months (“off season”), but surface-applied residue had higher residue-derived respiration during the later six months (“growing season”). Growing season respiration rates were higher and driven largely by warmer temperature and more available water (i.e. irrigation events) than in the off season. Thus, the growing season trend dictated the full year trend with surface residue more vulnerable to loss as CO₂ efflux compared to incorporated residue that was likely protected from decomposition by the soil matrix presumably from either aggregation or mineral associations.

Following, I tracked the fate of the remaining residue C by measuring SOC pools with varying levels of stability and report these results in Chapter 3. I harvested incorporated and surface-applied residue treatments six months after residue addition. I found that incorporated residue had more residue-derived C in bulk soil compared to surface-applied residue treatments. The larger pool of residue SOC within the incorporated residue treatment was due to increased residue decomposition compared to slower surface residue decomposition. Based on the two pathways of SOC formation proposed in Cotrufo et al., (2015a), residue C was tracked into both mineral-associated and particulate organic matter (MAOM and POM respectively). We hypothesized more MAOM formation due to the importance of the DOC-microbial pathway at the initial stages of decomposition with a smaller pool of POM due to the physical transfer path becoming important later in decomposition. After six months of residue decomposition, residue

C was greater in POM than MAOM fractions for all treatments (either marginally or significantly), indicating the importance of POM formation pathways in both surface-applied and incorporated residue treatments. After accounting for differences in decomposition, I found that incorporated and surface-applied SOC formation efficiencies of residue decomposition (i.e., the fraction of residue C decomposed that resulted in SOC versus being lost through mineralization) were similar when SOC pools were summed. However, the SOC formation efficiency of surface-applied residue with soil disturbance was 40% lower than either surface-applied or incorporated residue, indicating that soil disturbance inhibited SOC formation when residue was surface applied. It is surprising that surface-applied residue POM formation was high in the short term because surface residue had to enter the soil matrix over time unlike incorporated residue. The high amount of POM likely formed through biotic transformation by soil fauna in contrast to POM protection likely through aggregation in the incorporated residue treatment. Thus, it appears that POM formation was more important than MAOM formation for both surface-applied and incorporated residue management. My results show that intact NT systems, particularly with earthworms and fungal networks may efficiently transfer POM C belowground during the initial six months of decomposition. At 12 months, I predict that protection of incorporated residue will be more pronounced compared to surface residue, since surface residue appears to be vulnerable to decomposition after irrigation events. Longer-term results (~3 years) will elucidate whether CT residue management continues to store more residue SOC than NT, potentially explaining why irrigated, NT systems might not consistently store more SOC. Climate change will likely increase the use of irrigation to meet crop water needs, thus we need to continue exploring the complexities of SOC formation and stabilization within irrigated NT and CT in order to sequester C for the health of our soil and planet.

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CHAPTER 1: INTRODUCTION

Soil plays a large role in regulating ecosystem processes such as carbon (C) cycling. For maximum productivity, ecosystems need to balance C and nutrients flowing in and out of the soil (Janzen, 2006). Human activities, such as agricultural practices, have fundamentally altered C and nutrient fluxes and stores (Schlesinger and Andrews, 2000). One such way is by affecting the formation and stabilization of C stored in soil organic matter (SOC). While decomposing SOC supports plant productivity, SOC decomposition has been progressing at a high rate which affects soil fertility and releases greenhouse gases (Lal, 2013). To protect soil health and avoid exacerbating climate change, it is important to understand how to manage agricultural land for both short-term productivity and long-term sustainability. Approximately 33% of Earth's land surface is used for agriculture of which a third is used to grow crops often in an intensively managed manner (i.e. fertilization, tillage) (DeFries et al., 2005). Over the past 200 years, cropland soils have lost SOC at an increasing rate following management that alters biomass quantity and quality and creates environments favorable to decomposition (i.e. harvest, tillage) (Albrecht, 2002; Sanderman et al., 2017). Thus, there is a need to better understand how to tailor cropland management to build SOC rather than deplete it further.

Residue, a general term for non-grain crop biomass, has the potential to increase SOC levels in croplands if returned to the soil (Paustian et al., 2016). While residue characteristics affect the potential for residue to form SOC (i.e. residue quantity and quality), recent work has focused on how SOC formation pathways are impacted by residue chemistry, the soil mineral matrix and the location of residue addition to the soil (Cotrufo et al., 2015a, 2013; Six et al., 2002; Sokol et al., 2018). In the context of agroecosystems, no-till (NT) and conventional tillage

(CT) alter the location of residue within the soil. NT typically stores more SOC in the top 30 cm through decreased residue decomposition by leaving residue on the soil surface and avoiding soil disturbance (Six et al., 2004a). CT involves mechanically disrupting surface soil to produce a suitable seedbed for next season's crop by disrupting weed cycles and increasing spring soil temperatures. By incorporating surface residue, CT may increase residue and existing SOC decomposition by changing the microenvironment surrounding residue and SOC (Mitchell et al., 2016). Studies comparing tillage effects on SOC stocks generally measure surface SOC stocks and overall find more SOC within NT systems (Palm et al., 2014). When deeper measurements are made, down to a meter, results are more mixed with CT demonstrating higher SOC in layers directly below the layer of residue incorporation (Angers and Eriksen-Hamel, 2008). CT residue is generally mineralized more rapidly and throughout the plow layer which might create a more persistent pool of SOC deeper in the soil profile (Smith et al., 2007). Increasing temperatures and changing moisture regimes add uncertainty to the fate of SOC gains in NT and CT systems (Heimann and Reichstein, 2008). Studying how residue C is transformed into soil respiration efflux and SOC pools will provide a better understanding of the mechanisms of SOC formation and stabilization depending on residue location, ultimately enabling the agricultural system to tailor management for C sequestration.

Water availability is a major driver of both plant productivity and residue decomposition. Thus, recent studies have shown that irrigated systems do not see an increase in SOC despite greater plant productivity (Denef et al., 2008; Follett et al., 2013; Gillabel et al., 2007). When comparing the C sequestration potential of CT irrigated/dryland systems, Gillabel et al., (2007) found higher C storage in the topsoil (0-20 cm) under irrigation than dryland, resulting from an increase of microaggregate-protected C, but not macroaggregate-C. In contrast, NT

irrigated/dryland systems showed no significant difference in SOC stocks when the entire profile (0-75 cm) was examined, indicating different pathways of SOC formation under irrigated, NT and CT (Denef et al., 2008). Follett et al., (2013) found a loss of older SOC in CT compared to NT after eight years, indicating that SOC formation under CT might be counteracted by loss of older SOC following soil disturbance. Additionally, Stewart and Halvorson, (2017) found a decrease in subsurface SOC 12 years after conversion from irrigated NT to CT, which demonstrated that SOC in irrigated systems cycles quickly and NT SOC appears to be decomposing faster than its replaced at depth. Together these studies suggest that irrigated, NT and CT systems have different pathways of SOC formation and stabilization that effect the potential of these systems to sequester C for the long-term.

The majority of aboveground residue C in an agricultural system is mineralized to CO₂ with between 0-30% remaining in the soil system after residue has decomposed (Castellano et al., 2015). Soil respiration efflux is therefore indicative of temporal respiration of residue, soil and root-derived CO₂ rates across seasons (Conant et al., 2004). Although there is evidence to suggest that NT and CT residue decompose at different rates (Mitchell et al., 2016), we are not as confident in the environmental conditions driving residue versus existing SOC decomposition in semi-arid, irrigated systems (Follett et al., 2013). We do not know how residue location and soil disturbance will affect C source of soil respiration efflux in an irrigated system, which motivated my first research question:

- (1) How does residue location and soil disturbance effect the magnitude and proportion of soil respiration efflux from either residue or SOC sources?

While roughly 90% of residue C becomes CO₂, the small portion of remaining residue C has the potential to become incorporated into SOC pools which cycle on a timescale from years to centuries (Urbanski et al., 2018; von Lützow et al., 2007). Two pathways of SOM formation are proposed in Cotrufo et al., (2015): a dissolved organic carbon (DOC)-microbial path and a physical transfer path. The DOC-microbial path relates to microbial utilization of non-structural compounds released early in decomposition, contributing to MAOM formation. The physical transfer path relates to fragmentation, physical transfer into the mineral soil (for above ground residue) and possible protection of residue-derived particulate organic matter (POM) within aggregates. Thus, it is expected to be higher during the later stages of decomposition, when the residue is more brittle (Cotrufo et al., 2015). Both processes are important to consider when understanding how residue C contributes to the formation of SOC distinctively in MAOM and POM, since these pathways could be altered depending on when and how available residue is for mineralization. On one hand, we hypothesize that more slowly decomposing NT residue will allow soil fauna to utilize and transform residue into POM (Bossuyt et al., 2005; Frouz, 2018). On the other hand, we hypothesize that more quickly decomposing CT residue will allow greater adsorption of DOC/microbial products on soil particles resulting in MAOM formation.

Irrigation not only affects residue decomposition rate but has the potential to alter the magnitude and depth of SOC within the soil profile by influencing the movement and production of DOC. Irrigated systems have the potential to deliver DOC deeper in the soil profile through preferential flow pathways created by inter-aggregate pores and earthworm channels (Rumpel and Kögel-Knabner, 2011a). The location of residue (i.e. surface vs. incorporated) will likely affect how quickly and efficiently DOC percolates into deeper soil depths. During the off season, residue decomposes more slowly due to low temperatures and moisture but is likely to

decompose more during the growing season with higher temperatures and irrigation water. High water availability will increase DOC percolation into deeper soil profiles with CT residue-derived DOC likely reaching deeper depths due to incorporation. Additionally, the more time NT residue-derived DOC spends within the top centimeters of soil, the higher the likelihood that the DOC will be processed by microbes, thus affecting its potential fate as a precursor of MAOM. We do not know how residue location and soil disturbance will affect the temporal importance of pathways for residue-derived SOC formation and its long-term stabilization, which motivated my second research question:

- (2) How does residue location and soil disturbance effect the magnitude and proportion of residue derived SOC formation and stabilization of DOC, POM, and MAOM?

The objective of my thesis is to improve our understanding of how altered residue management of aboveground residue contributes to SOC formation and stabilization in an irrigated system by tracing residue C in both loss as CO₂ and transformation to SOC. In the following two chapters, I address this objective through measurements of isotopically labelled soil respiration and SOC fractions from a long-term no-tilled field experiment in a semi-arid, irrigated, corn system in Colorado.

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CHAPTER 2: NO-TILL RESIDUE VULNERABLE TO FAST MINERALIZATION DURING GROWING SEASON CLIMATIC FLUCTUATIONS IN AN IRRIGATED CORN SYSTEM

2.1 Introduction

Conventional agricultural management has depleted cropland soil of half of its original soil organic carbon (SOC) stocks by stimulating heterotrophic decomposition of C substrates and increasing soil C loss through erosion (Paustian et al., 2000; Sanderman et al., 2017). SOC loss decreases soil fertility and increases emissions of the greenhouse gas carbon dioxide (CO₂), affecting our ability to produce food, fiber, and fuel while contributing to climate change (Paustian et al., 2016). One method of restoring SOC in croplands is through crop residue management (Lal, 2008). The general idea is that increasing residue inputs and decreasing decomposition will result in an increase in SOC. No-till (NT) is a conservation agriculture practice proposed to increase SOC by slowing down residue decomposition through physical protection inside aggregates, decreasing soil temperature, and reducing erosion through residue cover (Follett, 2001; Ogle et al., 2005; Paustian et al., 1997; Shen et al., 2018; West and Post, 2002). In contrast, tillage breaks aggregate structures and incorporates residue within the topsoil. Incorporating residue increases residue and soil C mineralization, and stimulates surface erosion by exposing the mineral soil (Balesdent et al., 2000; Six et al., 2002). However, while NT residue initially decomposes slower than tilled residue (Mitchell et al., 2016), there is uncertainty surrounding the longer-term fate of the remaining residue C and its incorporation as SOC. Increases in SOC under NT are often site-specific and might be vulnerable to loss in the face of warming temperatures and altered moisture regimes (Gregorich et al., 2017a; Helgason et al.,

2014; Ogle et al., 2012; Stewart et al., 2017a; Wu et al., 2011). Thus, there is a continued need to evaluate the fate of SOC under different management practices.

Residue decomposition rates in NT and till are largely influenced by specific microclimates experienced by surface (“NT”) and incorporated (“till”) residue (Guérif et al., 2001). Production of CO₂ (i.e. soil respiration) is used to measure residue decomposition with temperature, moisture, and C substrate availability influencing the rate of decomposition (Helgason et al., 2014). In semi-arid systems, low soil moisture typically dampens the magnitude of temperature-driven increases in soil respiration (Conant et al., 2004). Irrigation has been introduced in many semi-arid croplands, effectively removing a moisture limitation during high temperature periods (“growing season”) with potentially contrasting effects on residue decomposition depending on residue/soil contact. In the context of residue microclimates, surface residue respiration stalls during low, post-harvest (“off season”) temperatures, but may experience heightened respiration following growing season, irrigation events due to its exposure to extreme surface climatic fluctuations (Helgason et al., 2014). Conversely, incorporating residue directly into the soil may increase off season, residue respiration due to higher temperatures within the soil compared to the surface, but may decrease respiration during the growing season through soil matrix protection from climatic fluctuations.

Residue C input is important for increasing SOC stocks, but the stability of the existing SOC stocks is important to maintain current SOC stores. SOC is composed of residues at different stages of decomposition which vary in their accessibility to further decomposition (Paul, 2016). Changes to the soil microenvironment can put these stocks at risk of increased decomposition (Six et al., 2002). Residue C is introduced to agroecosystems post-harvest which may influence SOC pools through enhanced or diminished mineralization of SOC, known as the

priming effect (PE) (Kuzyakov et al., 2000). A positive PE is an increase in heterotrophic respiration following the introduction of fresh substrates (Dimassi et al., 2014). Under tillage, fresh residue becomes incorporated into the soil matrix and closely associated with soil particles. This association can serve to either mineralize SOC or form new, mineral-associated SOC depending on the availability of mineral sites for SOC stabilization, the time until soils re-aggregate, and the satisfaction of microbial nitrogen needs (Bronick and Lal, 2005; M. J. Castellano et al., 2015; Six et al., 2000).

Studies quantifying SOC pools in paired NT/till, irrigated systems typically only report SOC stock changes but do not follow residue losses in respiration throughout the growing season (Gillabel et al., 2007; Schmer et al., 2014), exemplifying the need for more holistic approaches to capture temporal residue respiration in irrigated systems. The objectives of this study were (1) to determine the relative importance of residue location and soil disturbance on respiration by partitioning residue and soil CO₂ efflux and (2) to identify abiotic drivers of these potential differences. We used isotopically labeled aboveground residue to partition residue and soil C into CO₂ efflux over one year in an irrigated corn field. Tracing residue decomposition using stable isotopes is a tool used in numerous studies to track residue C decomposition pathways apart from that of soil C (Di Lonardo et al., 2017; Hahn et al., 2004; Mitchell et al., 2016; Soong and Cotrufo, 2015; Stewart et al., 2017b). We hypothesized that (a) total C respiration would be greater for treatments with incorporated residue than surface-applied or no residue due to (b) greater soil C respiration following soil disturbance and incorporation of residue C substrates, and (c) residue C respiration would be greater for surface-applied residue (“NT”) than incorporated residue (“till”) due to greater residue C respiration following (d) climatic fluctuations during the growing season in this irrigated corn system.

2.2 Materials and methods

2.2.1 *Experimental site and design*

To assess the impact of residue location and soil disturbance on residue and SOC decomposition, the experiment was conducted at a NT, continuous corn field at the Agricultural Research and Development Center near Fort Collins, CO (40°39'6" N, 104°59'57" W; 1535 m above sea level). The soil series is a Fort Collins clay loam (fine-loamy, mixed, mesic Aridic Haplustalf). The climate at this site is semi-arid with an average annual precipitation of 408 mm and a mean annual temperature of 10.1°C. The site has been under NT since 2008 (Halvorson and Stewart, 2015). Every year in late April/early May, corn (*Zea mays* L.) was hand-planted into full residue from the previous year's harvest. Fertilizer, in the form of SuperU (slow release N product, Koch Agronomic Service, Wichita, KS), was annually surface-band applied to corn at a rate of 135 kg N ha⁻¹ in late May. During the growing season, corn received an average of 35 mm of irrigation water from a linear-move sprinkler system once a week (early June – mid September).

On November 1st, 2017, within 24 hours of harvesting corn, PVC collars (15-cm height, 10-cm diameter) were pounded 10 cm into the ground to contain labeled residue additions. The corn stover from the most recent harvest was removed by hand from inside the collars. Residue additions were applied 48 hours later, on November 3rd, 2017. We added residue at a rate of 6709 kg ha⁻¹, determined from previous estimates of corn stover C addition rates for the experimental plot (Halvorson and Stewart, 2015). PVC caps were constructed by cutting a 10-cm diameter hole from PVC caps and fixing a fiberglass screen (2 mm²) to prevent loss of residue or inclusion of corn stover from the plot.

The experiment was a random block design with three treatments and two controls randomly assigned within four replicate rows spaced 0.7 meters apart in a 38 m² area. The treatments explored the effect of the location of residue relative to soil particles to mimic the effect of tillage on residue decomposition. The treatments included surface-applied (SA) or incorporated residue (MIX). A third treatment with surface-applied residue after soil disturbance (SAD) accounted for the unavoidable effect of broken soil aggregates following residue/soil mixing. The disturbed soil treatments involved removing the top 10 cm of soil from the collar, placing the soil in a plastic bag, and homogenizing the soil before returning it to the PVC collar. In the MIX treatment, the soil was mixed in a bag with labeled residues. Two controls without residue addition explored respiration rates of undisturbed (CU) and disturbed (CD) soil.

2.2.2 Isotopically labeled residue

To trace residue C in soil respiration, 4.3707 atom% ¹³C-labelled aboveground grass (*Andropogon gerardii*) residue was used. The residue was grown in a continuous labeling chamber at Colorado State University as described in Soong et al., 2014. Following physiological maturity, aboveground biomass, thereafter, referred to as “residue”, was harvested, air-dried, and cut into <1.5 cm sections. Aliquots of residue were weighed and placed in separate bags for field application. A residue subsample was oven-dried (55 °C), ground, and analyzed for C (45% ± 0.5) and N (0.98% ± 0.1) amount and isotopic signature using an isotope ratio mass spectrometer (Europa 20-20, Sercon Ltd, Crewe, England). We chose to use this ¹³C labeled grass residue because it has been used extensively in labeled residue experiments around the world; thus, making this experiment part of a broader network of experiments (e.g., Cotrufo et al., 2015; Soong and Cotrufo, 2015) for later synthesis. *Andropogon gerardii* is suitable for this

study because it has a similar tissue chemistry and C: N ratio as corn (bluestem leaves =46:1, corn stover =57:1) grown with high nitrogen additions (Halvorson and Stewart, 2015).

2.2.3 Soil respiration and isotopic analyses

CO₂ fluxes were captured periodically from November 2017- October 2018 using a static chamber approach (Mosier et al., 2006). Chamber lids were constructed from air tight PVC lids using guidelines from the USDA-ARS GRACEnet protocol (Parkin and Venterea, 2010). Gas samples were collected in the morning, between 8:30 and 12:00 h, to consistently estimate an average daily flux without the effect of diurnal variation (Nichols et al., 2016). Chamber lids were deployed in-field for 45 minutes with measurement intervals of 15 minutes. A longer deployment time (> 30 minutes) was necessary to ensure accurate capture of robustly labeled ¹³CO₂ over a large range of seasonally dependent fluxes.

At the beginning of each gas efflux sampling event, soil temperature and moisture were measured using Decagon Devices EC-TM soil moisture and temperature probes (Decagon Devices Inc., Pullman, WA). The probes were positioned at a soil depth of 10 cm, and data was logged at the beginning of each gas sampling event. A temperature probe (Taylor Precision Products, Inc., Las Cruces, NM) was used to capture hourly air temperature fluctuations at the soil surface during each gas sampling. Air temperature readings were used when adjusting gas flux calculations for temperature-dependent effects.

The gas sampling process involved placing a PVC lid over a collar and using a 60-ml polypropylene syringe to extract a 50 ml sample from the headspace. The resulting gas was immediately injected into two pre-evacuated exetainer vials (12 ml) with rubber butyl septum (Exetainer vial from Labco Limited, High Wycombe, Buckinghamshire, UK). One vial was analyzed for CO₂ amount using a fully automated gas chromatograph (Varian 3800, Varian Inc.,

Palo Alto, CA) equipped with a thermal conductivity detector. The other vial was analyzed for $\delta^{13}\text{C}$ amount using an isotope ratio mass spectrometer (Europa 20-20, Sercon Ltd, Crewe, England). Sampling frequency was once a week in the weeks following residue application (November 5th-21st), once a month during the off season (December-April), and twice a week during the growing season (April 26th-October 26th). The twice weekly gas sampling took place one day before and two days after irrigation events to capture soil respiration fluctuations due to changes in water availability.

2.2.4 Data Analyses

Daily CO_2 fluxes were determined by linearly interpolating known gas fluxes as described by Nichols et al., (2016). Outlier flux rates were discarded if a replicate's flux fell below three standard deviations from the average of the other three treatment replicates. The outlier flux was replaced by the average of the other three treatment replicates in order to linearly interpolate a replicate's flux between sampling days. Keeling plots were used to determine the $\delta^{13}\text{C}$ of the CO_2 efflux, which was then partitioned using a two end-member mixing model into residue and soil CO_2 flux (Pataki et al., 2003). The residue C contribution to the total efflux was assessed as:

$$f_{\text{residue}} = \frac{(\delta_S - \delta_B)}{(\delta_{\text{residue}} - \delta_B)}$$

where f_{residue} represents the fraction of residue C in the resulting CO_2 . δ_S , δ_B , and δ_{residue} represent the $\delta^{13}\text{C}$ of the CO_2 sample, the averaged controls, and the initial residue respectively.

Cumulative gas flux was the sum of the daily fluxes from November 5th, 2017- October 26th, 2018. Residue and soil cumulative CO_2 fluxes were obtained by multiplying each replicate's daily flux with the residue and soil contribution to the flux, respectively. Seasonality was a significant factor in cumulative CO_2 fluxes; thus, each treatment was divided into off season,

growing season, and full year fluxes. Off season is operationally defined as the period from the first gas sampling (November 5th, 2017) until corn was planted (April 26th, 2018). Growing season is defined as the period from when corn was planted until roughly a year after residue application (October 26th, 2018). The presence and direction of a priming effect was evaluated by comparing the average, cumulative soil flux of the controls (CD and CU) and the labeled residue treatments. Cumulative control soil CO₂ fluxes were averaged across CD and CU because there was not a statistically significant difference between the fluxes of the undisturbed (CU) and disturbed (CD) controls.

Variables related to temperature, moisture, and residue C substrate were tested in a statistical model described in the *Statistics* section of the methods. Residue C remaining was calculated and included in model fitting because the amount of C available influences temporal C respiration with more remaining residue C relating positively to decomposition rate (Eberwein et al., 2015). This variable was obtained by subtracting cumulative residue C loss (in mg C) over time (2370 mg initial C). The remaining residue C was converted into a percent for easier visual assessment of decomposition dynamics.

Treatment-specific temperature and moisture variables were created to assess climatic differences experienced by surface versus incorporated residue. Residue temperature and moisture accounted for climate experienced by surface-applied residue (air temperature/water applied) and incorporated residue (soil temperature/soil moisture). Water applied was defined as the sum of precipitation and irrigation water that fell within three days of a gas sampling event to avoid assigning irrigation water to multiple gas sampling events. Water applied was normalized by dividing water applied by a constant that produced the same full year average for both soil moisture and water applied.

2.2.5 Statistics

A linear mixed effects regression including the interaction between treatment and season was used to determine differences among treatments and controls for cumulative total, soil, and residue CO₂ fluxes for seasonal and full year CO₂ fluxes. A two-way analysis of variance (ANOVA) was run to determine significance of the interaction between treatment and season, after which pairwise comparisons were made for treatment differences separated by season. Paired disturbed/undisturbed treatments (CD/CU and SAD/SA) were not significantly different, so contrasts were run to increase the statistical likelihood of identifying significant differences between controls, incorporated residue, and surface-applied residue treatments for cumulative total, soil, and residue CO₂ fluxes. Treatment and contrast differences were considered significant when $p < 0.05$. ANOVA statistical analyses were performed in R version 3.5.0 (R Core Team, 2018). To determine climatic variables responsible for magnitude of total, soil, and residue CO₂ fluxes, a multiple regression stepwise model was fit to the data using SAS statistical software (“SAS Institute,” 2004). A stepwise regression was chosen to inform statements about the variables that explained the most variation in CO₂ production by season. Total, soil, and residue CO₂ fluxes were log-transformed to achieve normally distributed data. The following variables were tested: soil temperature, air temperature, residue temperature, soil moisture, residue moisture, and percent residue C remaining. Partial R-squared values were reported instead of other statistical measures of fit such as the Akaike information criterion because our variables exhibited linear relationships after log-transformation. The top three statistically significant variables were reported for the total, soil, and residue models in order of highest partial R-squared value.

2.3 Results

2.3.1 *Effect of Seasonality on CO₂ fluxes*

Total, soil, and residue daily CO₂ fluxes were significantly higher during the growing season than during the off season (Fig. 2.1a-c; Table 2.1). Consequently, cumulative fluxes are separated into off season, growing season, and full year for the following analyses. Overall the residue accounted for 20% of the total CO₂ efflux, with a higher average contribution in the off season (23%) than the growing season (17%), resulting in a significant interaction between season and source (Table 2.1).

2.3.2 Total CO₂ fluxes

Total CO₂ efflux of treatment pairs with and without soil disturbance (CU/CD, and SA/SAD) were not significantly different from each other (Fig. 2.2a) as well as for soil and residue CO₂ efflux (data not shown). Thus, we pooled the data across disturbed and undisturbed treatments to compare controls and residue location (MIX, SURFACE) for all fluxes. Controls had significantly lower cumulative CO₂ efflux than surface-applied residue treatments during both growing season (2019 kg C ha⁻¹ < 2595 kg C ha⁻¹) and full year (2631 kg C ha⁻¹ < 3169 kg C ha⁻¹) (Fig. 2.2b). The MIX treatment had significantly lower cumulative CO₂ than surface-applied residue treatments during the growing season (2120 kg C ha⁻¹ < 2595 kg C ha⁻¹), but only slightly lower during the full year (2963 kg C ha⁻¹ < 3169 kg C ha⁻¹) (Fig. 2.2b). Most of the cumulative total CO₂ was produced during the growing season with 77% of control, 71% of incorporated and 82% of surface-applied total CO₂ occurring between April 26th and October 26th, 2018. From off season to growing season, CO₂ for CONTROL tripled, MIX doubled, and SURFACE quadrupled.

2.3.3 Priming of Soil CO₂ Flux

An increase in CO₂ after a residue addition indicates a positive priming effect. There were not any significant differences in soil CO₂ efflux when treatments were partitioned by presence

and location of residue, indicating no priming of soil C mineralization from the residue addition (Fig. 2.3).

Most of the cumulative soil CO₂ was produced during the growing season with 77% of control, 74% of incorporated and 82% of surface-applied soil CO₂ occurring between April 26th and October 26th, 2018. From off season to growing season, CO₂ for CONTROL and MIX tripled, and SURFACE quadrupled.

2.3.4 Effect of residue placement on residue mineralization

During the off season, the MIX treatment respired two times more residue C (217 kg C ha⁻¹) compared to the surface-applied residue treatments (103 kg C ha⁻¹) (Fig. 2.4). The opposite was observed during the growing season, in which the surface-applied residue treatments (556 kg C ha⁻¹) respired double the MIX residue C (331 kg C ha⁻¹). Over 12 months, 19% less residue C was respired from MIX (548 kg C ha⁻¹) compared to surface-applied residue (669 kg C ha⁻¹). Much of the cumulative residue CO₂ was produced during the growing season with 56% of MIX and 83% of surface-applied residue CO₂ occurring between April 26th and October 26th, 2018. From off season to growing season, residue CO₂ increased slightly in MIX and quadrupled in the SURFACE treatment. After a year, between 15.2-20.1% of MIX residue and 18.5-26.0% of SURFACE residue C was respired as CO₂.

2.3.5 Temperature and moisture drivers of residue, soil, and total CO₂ fluxes

Air and soil temperature, water, and residue C availability varied over the course of the year (Fig. 2.5-6). During the off season, the average air temperature was 1.7 °C while the average soil temperature was 3.8 °C. Growing season temperatures were higher with an average air temperature of 17.4 °C and average soil temperature of 14.9 °C. Average soil moisture during the

off season and growing season did not vary based off soil moisture measurements taken during gas sampling days ($0.31 \text{ m}^3\text{m}^{-3}$ and $0.34 \text{ m}^3\text{m}^{-3}$ respectively).

As mentioned in the *Data Analyses* section, the remaining residue C, residue temperature, and residue moisture variables were created in order to account for treatment-level differences in residue C availability, temperature, and moisture experienced due to residue location. The models selected captured 57, 62, and 36% of the variability in the full year total, soil, and residue CO_2 flux (Table 2.2).

2.4 Discussion

2.4.1 *The highest total CO_2 during growing season was produced by surface-applied residue treatments*

Over the course of 12 months, surface-applied residue treatments produced the most total CO_2 (Fig. 2.3b). This is contradictory to our hypothesis that incorporating residue would lead to the largest production of total CO_2 . We hypothesized stimulated total CO_2 from increases in soil CO_2 due to decomposition following residue addition and soil disturbance. After seeing an increase in total CO_2 from the surface-applied residue treatment, total CO_2 was separated into soil and residue fluxes to understand the dynamics responsible for this result. Differences between cumulative total CO_2 of control and residue treatments were not apparent until the growing season, when fluxes were higher than during the off season. The year results were influenced by a large increase in growing season residue CO_2 efflux from surface-applied treatments, since the soil CO_2 did not show any significant treatment differences. Although residue CO_2 did not account for a large portion of total CO_2 produced in the system (20%), its growing season decomposition affected the overall trend of CO_2 production from our irrigated system. De Graaff et al., (2010)

found similar results in which an intermediate amount of fresh C substrates increased total CO₂ without stimulating soil CO₂ production.

2.4.2 Soil-derived CO₂ production was not stimulated by soil disturbance or residue addition

Over the course of 12 months, neither soil disturbance nor residue addition stimulated soil-derived CO₂ production (Fig. 2.3b) which contradicts our initial hypothesis that both would lead to larger production of soil CO₂. We expected stimulated soil CO₂ from the disruption of aggregates and release of previously-protected soil C. One possible explanation is broken aggregates in our system quickly re-formed with the help of calcium carbonate, re-protecting exposed SOC (Wuddivira and Camps-Roach, 2007). Calcium acts as a bridge between negative exchange sites on mineral surfaces and negatively charged organic compounds. Our results are contradictory to a residue decomposition experiment in Australia which found stimulated soil CO₂ after mixing in residue (Mitchell et al., 2016), but it is possible that their results are due to lower re-aggregation in their soil without carbonates present.

We expected residue addition to produce a positive priming effect by stimulating SOC decomposition. Studies have found positive priming following residue addition and incorporation (Mitchell et al., 2016; Shahbaz et al., 2017), but others have found no effect of residue addition (Qiao et al., 2014) on soil-derived CO₂. An explanation for our absence of priming coincides with the relatively high level of inorganic N in our fertilized soil (135 kg N ha⁻¹ yr⁻¹). Both soil N and residue N content are important in predicting residue decomposition dynamics. Knorr et al., (2005) conducted a meta-analysis in which fertilization rates above 125 kg N ha⁻¹ yr⁻¹ inhibited SOM decomposition. Additionally, Stewart et al., (2015) found lower decomposition of high-quality residue in a high soil N environment indicating an interaction between residue and soil N on SOM decomposition dynamics. Therefore, the high-N residue and soil N in this system might be

satisfying microbial N requirements without the need to decompose SOM pools in search of N (Kuzyakov et al., 2000) resulting in no priming effect.

2.4.3 Residue-derived CO₂ production was highest for surface-applied residue over 12 months

Over the course of 12 months, more residue CO₂ was produced from surface-applied residue than incorporated residue (Fig. 2.4). This was consistent with our initial hypothesis, yet contradictory to other residue decomposition studies which found larger residue CO₂ efflux from incorporated residue (Coppens et al., 2007; Fu et al., 2000; Mitchell et al., 2016). Our unique result may derive from the seasonality of temperatures and moistures in our semi-arid, irrigated system absent in other labeled-residue studies either completed within one season (Coppens et al., 2007; Fu et al., 2000) or in a subtropical climate (Mitchell et al., 2016). The off season was dominated by low temperatures and moisture, while the growing season experienced high temperatures and regular pulses of moisture from irrigation events (Fig. 2.5). Differences in seasonal climate likely affected residue respiration, since substrate decomposition is dependent on soil temperature and moisture conditions (Henriksen and Breland, 2002; Liu et al., 2006; Sokol et al., 2018).

During the off season, surface-applied residue decomposition was lower due to freezing air temperatures (Fig. 2.5). In contrast, incorporated residue decomposition was stimulated by the combination of higher soil temperatures (compared to air), enhanced soil aeration, and increased contact between soil and residue which provided ideal decomposition conditions and an available C substrate (Al-Kaisi and Yin, 2010; Henriksen and Breland, 2002). This stimulation persisted only a few weeks into the off season (Fig. 2.1c), but strongly influenced the cumulative residue CO₂ efflux (Fig. 2.4). During the growing season, surface-applied residue decomposition was stimulated by high average daily air temperatures and showed large spikes in residue CO₂ efflux

after irrigation events (Fig. 2.1c). Weekly irrigation events likely removed any water limitation which would limit CO₂ production in our semi-arid system as supported by previous studies which have identified regular pulses of water as a driver of CO₂ production (Huxman et al., 2004; Xu et al., 2004). In contrast, incorporated residue decomposition was lower due to lower soil temperatures (compared to air) and possible protection of remaining residue from irrigation events within the soil matrix. Helgason et al., (2014) found that rainfall regime produced fewer variable effects on residue-C decomposition for incorporated residue compared to surface residue.

On average, our residue lost similar amounts of residue C as CO₂ (21% of total residue C) after 12 months than a laboratory-run decomposition experiment with our same residue species (18%) over 1 year (Soong et al., 2015). This is far below what Mitchell et al., (2016) found in Australia (44%) after 12 months, but their experimental site received higher annual temperatures (20°C) and precipitation (1110 mm). Besides climatic differences, their experiment was conducted with a higher quality residue (3% N) which stimulated faster decomposition (Schomberg et al., 2010). Our lower residue C loss as CO₂ shows the impact of distinct locales on residue decomposition results, especially when short-term studies with the same residue species are compared in climatically different settings.

2.4.4 Soil and residue CO₂ are largely driven by soil temperature and residue moisture respectively

Using stepwise regression modeling, the majority of total and soil CO₂ variability was explained by soil temperature while variability in residue CO₂ fluxes was best explained by residue moisture (Table 2.2). Soil temperature is regularly included as a main factor when modeling soil CO₂, but it was surprising that none of the moisture variables were large contributing factors of

soil CO₂ efflux (Table 2.2). We expected the most variability to be explained by the interaction of temperature and moisture, as reported in both global-scale and semi-arid specific models of residue decomposition (Adair et al., 2008; Del Grosso et al., 2005). In our system, regular pulses of water from irrigation events likely elevated moisture-limited conditions, lessening the contribution of moisture variables in explaining trends in soil CO₂.

The top driver of residue CO₂ changed between the off season and growing season with the off season influencing the full year, top residue CO₂ driver. Residue moisture explained more residue CO₂ variability during the off season (31%) than during the growing season (11%). During the off season, residue moisture was an average of 0.12 m³m⁻³, which was well below the growing season average (0.42 m³m⁻³). While soil temperature was not a significant driver of off-season residue CO₂, it explained 24% of growing season residue CO₂ (Table 2.2). Soil temperature varied widely between the seasons, with the off-season average temperature well below the growing season (4.1 vs. 17.1 °C). These results suggest that temperature becomes a main driver of residue CO₂ production only when moisture is not limiting. Our results differ from Gregorich et al., (2017) who found an overwhelming influence of temperature (either air or soil) on labeled residue decay in diverse climatic conditions at Canadian sites. In their model, moisture did not increase their model fit, but 9/10 of their sites had lower mean annual air temperatures and higher mean annual precipitation compared to our site. Thus, it is reasonable that moisture is often a limiting factor and drives residue CO₂ production in our semi-arid, irrigated system compared to colder/wetter Canadian sites.

Edaphic variables explained only 36% of the residue CO₂ variability as compared to 55-60% of the variability in total and soil CO₂. It is possible that including microbial carbon use efficiency (CUE) would increase the model fit as CUE determines the proportion of residue C

respired by microbes as opposed to incorporated into microbial biomass (Liu et al., 2018). Nonetheless, these simple regression models still explained a third of residue CO₂ variability with only three variables

2.5 Conclusions

Our 12-month, in-field residue decomposition experiment of surface-applied and incorporated residue revealed seasonally dependent total, soil, and residue CO₂ production with the off season producing lower CO₂ than the growing season across all treatments. During the growing season, total CO₂ production was highest for surface-applied residue compared to incorporated residue and controls without residue. Although the surface-applied treatments did not produce significantly higher total CO₂ than incorporated residue over the full year, there was significantly higher total CO₂ for the surface-applied treatments during the growing season. Soil CO₂ efflux was not different between surface, incorporated, or no-residue treatments during either season or over the full year. Soil disturbance did not increase soil CO₂ efflux likely due to our soil reaggregating quickly in the presence of high inorganic C content. Addition of residue did not prime SOC because inorganic N additions satisfied microbial N needs. Residue CO₂ efflux was significantly higher for incorporated residue during the off season, but higher for surface residue during the growing season and full year. The top variable explaining residue CO₂ efflux during the off season and growing season were residue moisture and soil temperature respectively. These results highlight the vulnerability of residue to decomposition when a moisture-limited system becomes wet. These results indicate that surface residue is more vulnerable to decomposition during growing season conditions than incorporated residue which can reduce the amount of surface residue C remaining for SOC formation in this semi-arid, irrigated corn system.

Table 2.1. Results of average total, soil, residue, and residue contribution to daily CO₂ fluxes separated by season. Numbers in parentheses represent one standard error (n=260 and n=760 for off season and growing season respectively).

Flux C Source	CO ₂ Flux (kg C ha ⁻¹ day ⁻¹)		p-value
	Off Season	Growing Season	
Total	2.6 (0.1)	7.8 (0.1)	<0.001
Soil	2.1 (0.1)	6.8 (0.1)	<0.001
Residue	0.5 (0.1)	1.0 (0.1)	<0.001
Contribution of Residue to Total	23.3% (1.5)	17.2% (0.6)	<0.001

Table 2.2. Stepwise regression results for models fit to total, soil, and residue CO₂.

Total Flux - Year			
Variable	Partial R-Squared	Model R-Squared	P-value
soil temperature	0.538	0.538	<0.001
residue moisture	0.025	0.563	<0.001
soil moisture	0.007	0.570	<0.001
Soil Flux - Year			
Variable	Partial R-Squared	Model R-Squared	P-value
soil temperature	0.609	0.609	<0.001
residue temperature	0.009	0.618	<0.001
residue moisture	0.003	0.621	0.037
Residue Flux - Year			
Variable	Partial R-Squared	Model R-Squared	P-value
residue moisture	0.221	0.221	<0.001
soil temperature	0.085	0.306	<0.001
residue C	0.057	0.363	<0.001
Residue Flux - Off Season			
Variable	Partial R-Squared	Model R-Squared	P-value
residue moisture	0.311	0.311	<0.001
soil moisture	0.099	0.41	<0.001
water applied	0.076	0.486	<0.001
Residue Flux - Growing Season			
Variable	Partial R-Squared	Model R-Squared	P-value
soil temperature	0.237	0.237	<0.001
residue moisture	0.111	0.348	<0.001
residue C	0.078	0.425	<0.001

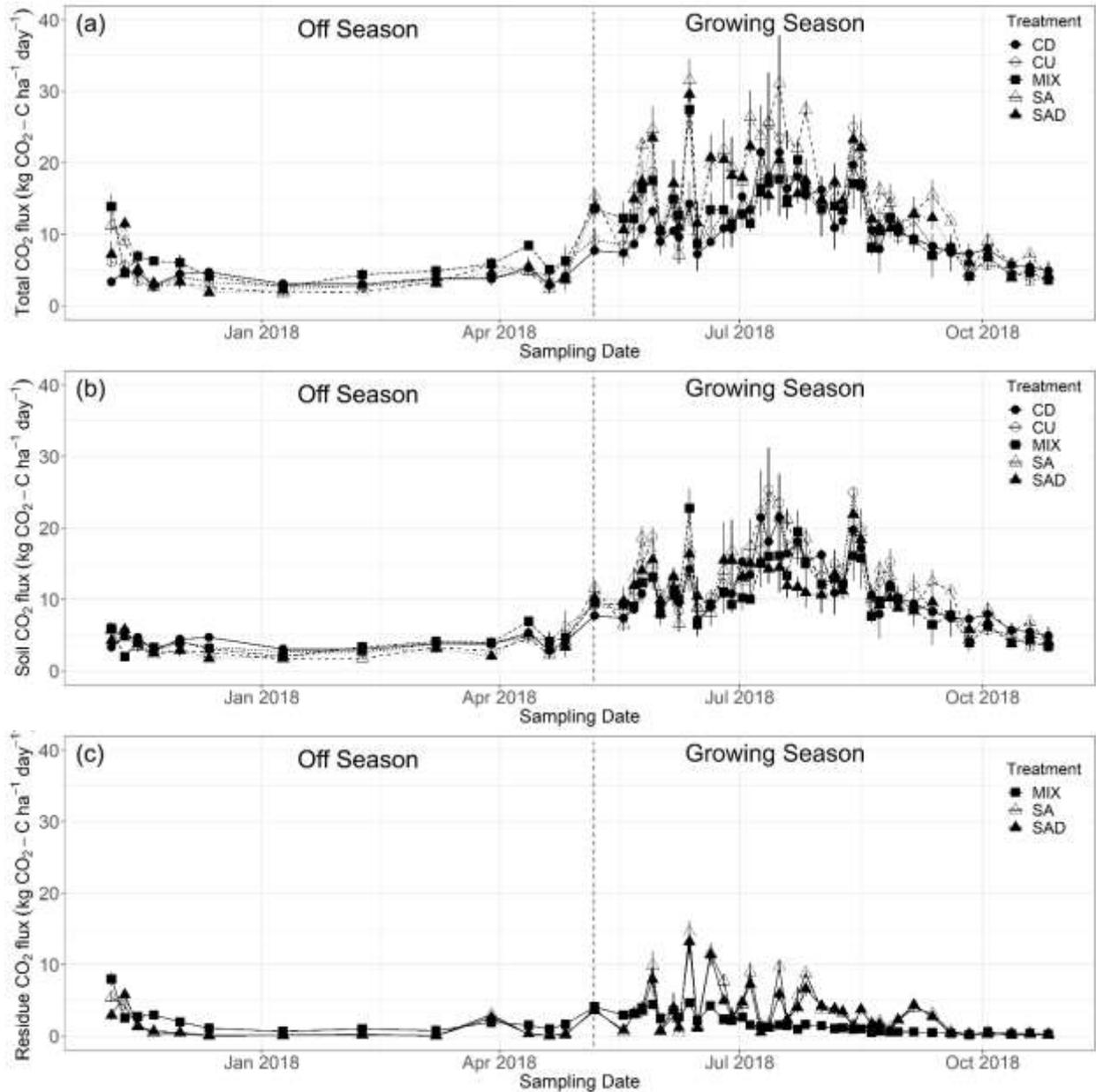


Figure 2.1. Temporal dynamics of CO₂ fluxes (November 2017–October 2018) for no residue controls (CD, CU) and residue-added treatments (MIX, SA, SAD). CD: no residue with soil disturbance, CU: no residue, MIX: incorporated residue, SA: surface-applied residue, SAD: surface-applied residue with soil disturbance. (a) Total CO₂ flux in all treatments. (b) Soil CO₂ flux in all treatments. (c) Residue CO₂ flux in all residue-added treatments. Error bars represent one standard error (n=4).

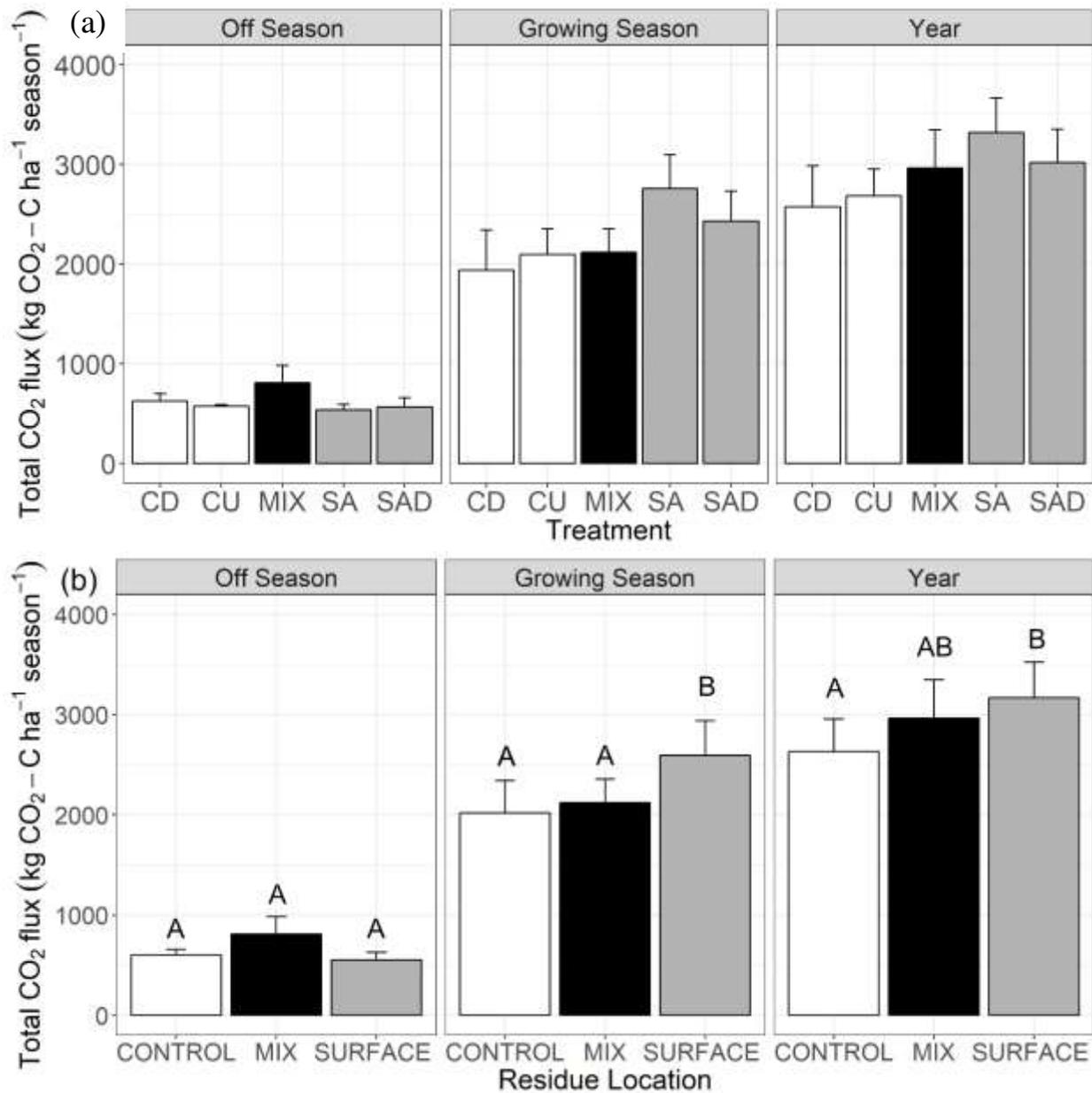


Figure 2.2. Cumulative total CO₂ fluxes (November 2017-October 2018) separated by season for (a) no-residue controls (CD, CU) and residue treatments (MIX, SA, SAD), and (b) contrast by residue presence and location (CONTROLS without residue vs. MIX or SURFACE-applied residue). Error bars represent one standard error (n=4). Letters denote significance (p<0.05).

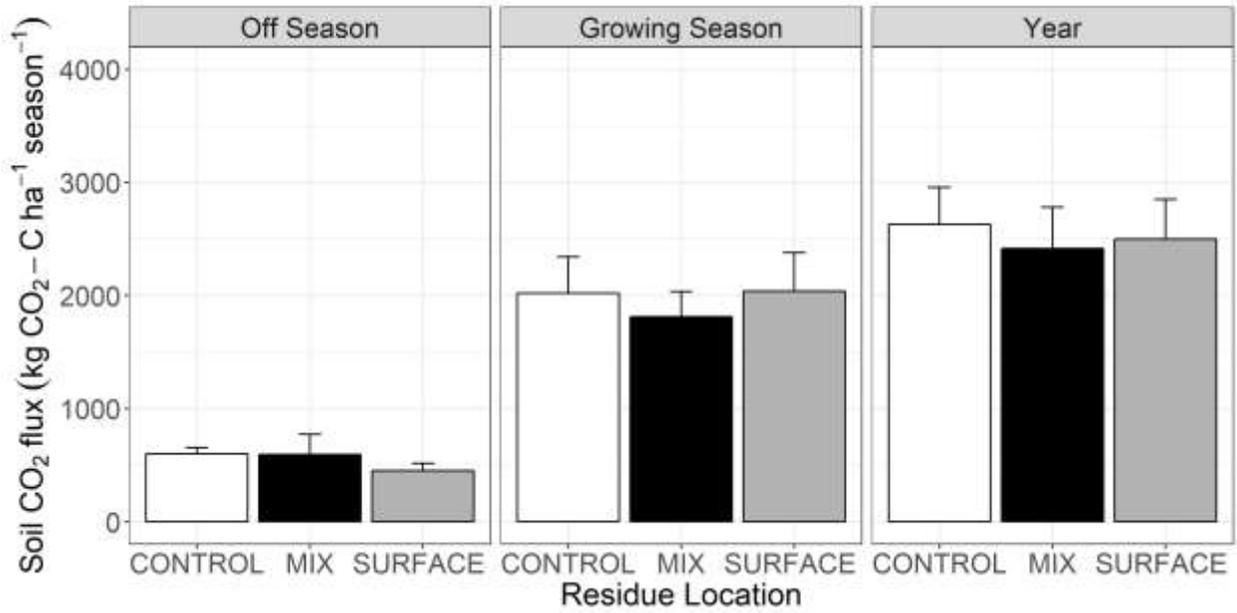


Figure 2.3. Cumulative soil CO₂ fluxes (November 2017-October 2018) separated by season and contrasted by residue presence and location (CONTROLS without residue vs. MIX or SURFACE-applied). Error bars represent one standard error (n=4).

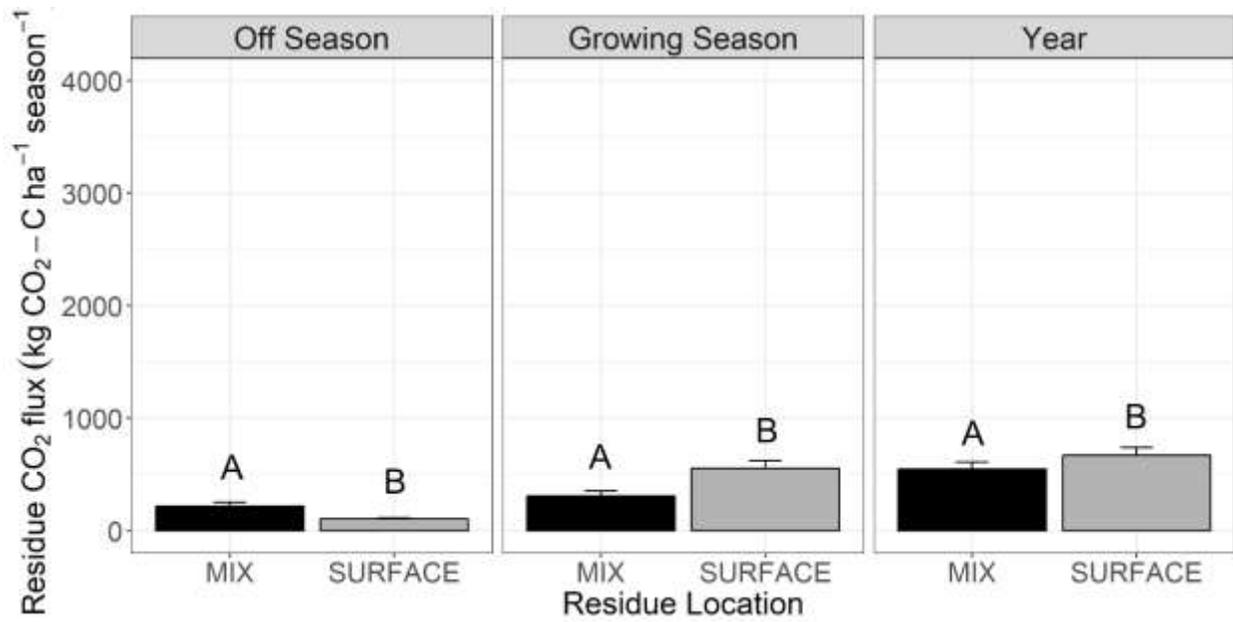


Figure 2.4. Cumulative residue CO₂ fluxes (November 2017-October 2018) separated by season and residue location (MIX or SURFACE-applied residue). Error bars represent one standard error (n=4). Letters denote significance (p<0.05).

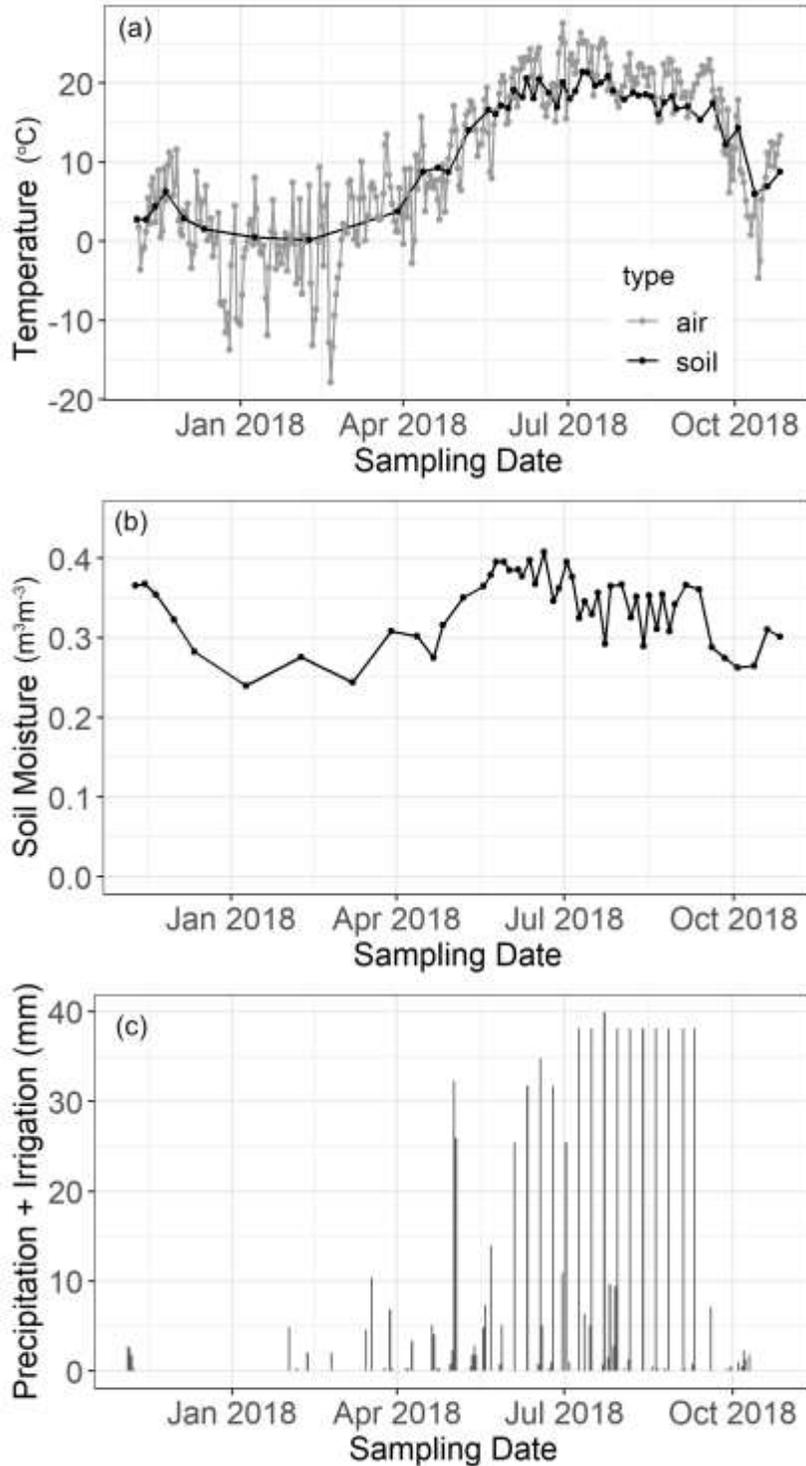


Figure 2.5. Air and soil temperature (10 cm depth) (a), soil moisture (10 cm depth) (b), and water delivery at the study site for the duration of the experiment (November 2017 – October 2018).

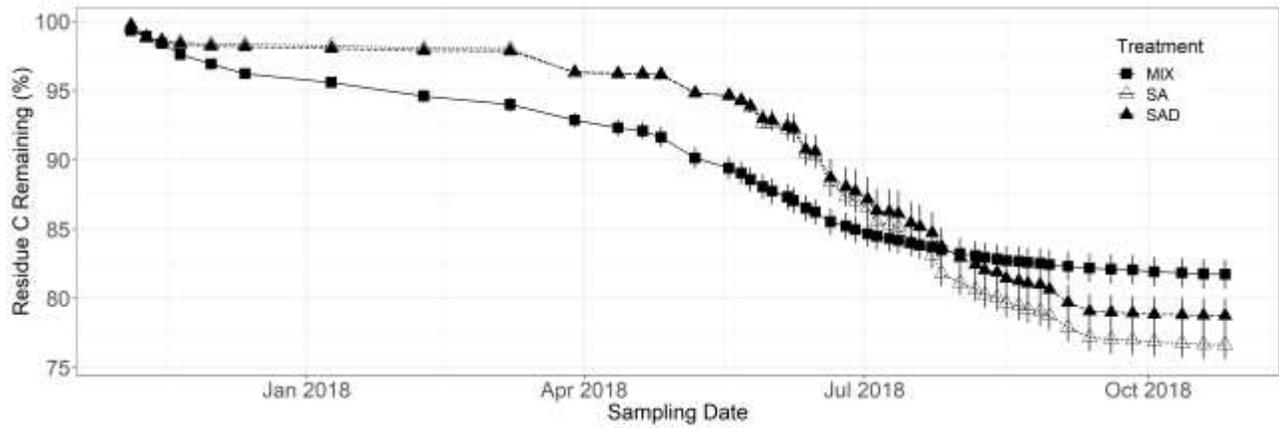


Figure 2.6. Calculated percent of residue carbon remaining by treatment (November 2017 – October 2018). MIX: incorporated residue, SA: surface-applied residue, SAD: surface-applied residue with soil disturbance. Error bars represent one standard error (n=4).

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CHAPTER 3: SIMILAR SHORT-TERM FORMATION EFFICIENCY OF NO-TILL AND TILL RESIDUE-DERIVED SOIL ORGANIC CARBON

3.1 Introduction

The formation and stabilization of soil organic carbon (SOC) is essential to our efforts to build soil health while limiting future emissions of greenhouse gases (Follett, 2001). SOC is formed from decomposing animal and plant products and contributes to soil health by improving soil structure, water retention, and nutrient pools (Lal, 2004). In agroecosystems, SOC stocks can be increased with more efficient SOC formation and lowered SOC decomposition (Paustian et al., 2016). Conservation tillage practices, such as no-till (NT), reduce soil disturbance which generally increases surface SOC stocks by limiting SOC decomposition. Conventional tillage (CT) decreases SOC stocks through increased mineralization of previously-stored SOC by creating microclimates favorable to decomposition and through soil erosion (Al-Kaisi and Yin, 2010; Conant et al., 2011). Many studies have arrived at this conclusion by measuring total SOC amounts in surface soil from NT and CT fields (Luo et al., 2010). By focusing on SOC stocks in the surface soil, where NT SOC concentrates, deeper SOC stocks are ignored. When studies have measured whole-profile SOC down to a meter, similar amounts of SOC have been found in NT and CT systems (Angers and Eriksen-Hamel, 2008; Baker et al., 2007; Powlson et al., 2014) suggesting a translocation of SOC below the plough layer. Additionally, profile-wide SOC stocks are sometimes not protected after conversion from CT to NT in irrigated systems (Stewart et al., 2017a). Therefore, there is a need to determine how stable NT and CT SOC stocks are in irrigated systems.

Understanding the stability of SOC in NT and CT systems may help elucidate why NT does not always store more SOC than CT. Total SOC amount lumps SOC pools instead of considering their different mean residence times based on how physically or chemically accessible they are for decomposition (Janzen, 2015). Techniques have been developed to physically and chemically separate SOC into pools with distinct residence times (Jagadamma and Lal, 2010; Poeplau et al., 2018). We separated soil into POM and MAOM pools with distinct density and size characteristics: dissolved organic C (DOC), light particulate organic matter (lPOM), heavy POM (hPOM), silt-sized, and clay-sized particles (von Lützow et al., 2007). Mineral-associated organic matter (MAOM) includes both silt- and clay-sized fractions, yet silt and clay possess different capacities to store SOC. As a general rule, clay minerals have more reactive sites and surface area than silt which correlates to a greater capacity to bind SOC (Kögel-Knabner et al., 2008). Therefore, separating the silt- and clay-sized particles clarifies the importance of texture in determining SOC potential.

Two distinct paths of SOC formation have been proposed: a DOC-microbial path and a physical transfer path (Cotrufo et al., 2015a). The DOC-microbial path is important early in decomposition when DOC production is high (Soong et al., 2015) and result in the formation of MAOM while the physical transfer path is more important later in decomposition, when the litter is expected to be more brittle and susceptible to fragmentation and forms POM. DOC is produced from the leaching of water-soluble residue components or microbial assimilation and turnover. The DOC-microbial path forms MAOM when biotically or abiotically produced DOC adheres to soil particle surfaces. POM formation occurs through physical fragmentation of partly decomposed residue by soil fauna and other agents, and can be protected through aggregation (Six et al., 2002; Six and Paustian, 2014). POM can be further separated into a light and heavy

fraction (IPOM and hPOM respectively) with the heavier fraction associated with sand-sized particles (Helfrich et al., 2006; Sollins et al., 2006). While all SOC pools are continually decomposing, MAOM has a longer average residence time on the order of centuries and even up to millennia compared to a residence time of years to decades for IPOM and hPOM respectively (Gaudinski et al., 2000; Lehmann and Kleber, 2015; Lützow et al., 2006). Thus, recent studies have focused on understanding how MAOM is formed for its long-term C sequestration potential (Córdova et al., 2018; Lavallee et al., 2018).

There is evidence for soils reaching a threshold for MAOM formation from studies showing no increase in MAOM after increasing C input levels (Six et al., 2002; Stewart et al., 2008). Agroecosystems with a history of intensive management typically have not reached MAOM saturation but saturation has been shown when soil C and/or plant inputs reach above the capacity of minerals to store C (Castellano et al., 2015; Stewart et al., 2009). Although MAOM typically has a lower decomposition rate than POM and is therefore thought to contribute more to long-term C storage (Christensen, 2001), POM constitutes a pool of SOC that theoretically has no saturation limit (Stewart et al., 2008). Thus, both POM and MAOM formation are important for building SOC.

When considering NT and CT SOC pathways, it is important to consider how residue proximity to soil particles affects the prominence of MAOM and POM formation. Based off the two paths of SOC formation proposed by Cotrufo et al., (2015), we have two contrasting hypotheses about how NT and CT residue management influence SOC formation. (1) NT forms and stabilizes more SOC through slower surface decomposition which leads to more efficient residue transformation into POM through the physical transfer path. (2) CT forms and stabilizes more SOC through immediate contact with reactive soil surfaces which leads to more efficient

transformation into MAOM through the DOC-microbial path. Irrigated agroecosystems add a layer of complexity, increasing the movement of DOC within the soil profile, potentially affecting the importance of the DOC-microbial path, and the magnitude of MAOM formation (Denef et al., 2008). The current challenge is to assess the importance of POM and MAOM formation rates between CT and NT residue management in irrigated systems to determine the stability of residue-derived C over time.

Studies quantifying SOC pools in paired NT/CT, irrigated systems typically report bulk C amounts and rarely follow residue into specific SOC pools (Denef et al., 2008; Gillabel et al., 2007; Stewart et al., 2017a). We used isotopically labeled aboveground residue to trace residue C decomposition into bulk soil and SOC fractions to assess differential SOC stabilization with altered residue management. The use of labeled residue to trace residue into SOC is an established tool used in studies around the globe (Di Lonardo et al., 2017; Hahn et al., 2004; Mitchell et al., 2016; Soong and Cotrufo, 2015; Stewart et al., 2017b). We hypothesized that surface-applied residue management (“NT”) would have less residue-derived C in the bulk soil than incorporated residue (“CT”) after six months, and these differences would be apparent in SOC pools. Within SOC pools, we hypothesized that there would not be treatment differences in POM pools until later in residue decomposition, but surface-applied residue treatments would have less residue-derived C in silt- and clay-sized fractions (MAOM) than incorporated residue due to lower contact with reactive soil surfaces. Overall, we are hypothesizing that the DOC-microbial path will be more important than the physical transfer path in short-term SOC formation particularly when residue is incorporated (“CT”).

3.2 Materials and methods

3.2.1 Experimental site and design

The study site was a NT, continuous corn plot at the Agricultural Research and Development Center near Fort Collins, CO (40°39'6" N, 104°59'57" W; 1535 m above sea level). Soils are classified as a Fort Collins clay loam (fine-loamy, mixed, mesic Aridic Haplustalf). Soil characteristics for 0-10 cm are as follows: pH of 8.0 (Alluvione et al., 2009), a bulk density of 1.4 g cm⁻³, and a SOC amount of 14.6 g kg⁻¹ (data not shown). The experimental plot has been under NT since 2008 (Halvorson and Stewart, 2015), and field operations are described in detail in Chapter 2.

PCV collars (15-cm height, 10-cm diameter) were pounded in within a day of corn harvest (Nov. 1st, 2017). Corn stover from the most recent harvest was identified by its lighter color and removed from inside the collars by hand. Collars have a two-fold purpose to limit the movement of labeled and exogenous residue out of or into the experimental area. Unfortunately, collars also limit soil movement, which limits a considerable SOC loss in the form of soil erosion following CT (Six et al., 2000). Aboveground *Andropogon gerardii* residue was grown in a continuous labeling chamber at Colorado State University using established methods outlined in Soong et al., (2014). The initial residue had a $\delta^{13}\text{C}$ of 3088‰ determined through isotopic analysis of oven-dried residue using an isotope ratio mass spectrometer (Europa 20-20, Sercon Ltd, Crewe, England). Aliquots of labeled residue were applied within three days of corn harvest (Nov. 3rd, 2017). Residue was added at a rate of 6709 kg ha⁻¹, determined from previous estimates of corn stover C addition rates when fertilizer was added at a conventional rate of 135 kg N ha⁻¹ (Halvorson and Stewart, 2015).

The study was a randomized block of three treatments in four replicate rows for each of four blocks. The treatments included surface-applied residue (SA) or residue mixed with soil

(MIX). The MIX treatment consisted of removing the top 10 cm of soil from inside the collar, disturbing it within a plastic bag containing residues, and returning it to the same soil volume. A third treatment with surface-applied residue after soil disturbance (SAD) accounted for the unavoidable effect of broken soil aggregates following residue/soil mixing. Plots were established for destructive sampling over three years, and we report initial results from 0 months (baseline) and 6 months.

3.2.2 Residue and soil sampling

Baseline soil cores were collected two weeks after experimental set-up (November 16th, 2017). Briefly, eight 6.25 cm-diameter soil cores were taken from directly north and south of the four replicate rows of collars. Each core was separated into the following depths: 0-5, 5-10, 10-20, 20-40, 40-60, 60-80, 80-100 cm. Each soil sample was processed and analyzed for bulk SOC stocks with the top three depths fractionated to quantify isotopic end members for SOC pools when determining the contribution of C from residue versus native SOC. Six months after residue addition, four collars from each of three residue-addition treatments were carefully excavated from the surrounding soil. A 6.25 cm-diameter soil core was taken from the soil directly underneath each extracted collar to account for translocation of label below the collar. Each deep soil core was separated into the depths outlined above, starting with the 10-20 cm depth. The intact collar and surface residues were placed in pre-labelled plastic bags and transported in coolers for processing in the USDA-ARS laboratory in Fort Collins, CO. In the SA collars, undecomposed residue was picked by hand for roughly 30 minutes per collar, dried in a 55°C oven, sieved to remove soil (500 µm sieve size), and weighed. The soil inside the collars was removed and divided into 0-5 and 5-10 cm depths. Separation of the 10 cm of collar soil into 0-5 and 5-10 cm was important to capture the isotopic signal of the slowly

decomposing, surface-applied residue treatments (Mitchell et al., 2018). Soil cores for each depth were weighed intact for bulk density determination. Soil moisture was calculated by weighing hand-homogenized soil before and after oven drying for 48 hours at 110°C. All soil depths (0-100 cm) were sieved to 8 mm with any residue > 8 mm set aside to determine undecomposed residue weight.

3.2.3 Bulk soil analysis

For each soil sample, a subsample of 100 g was sieved to 2 mm to remove identifiable plant material and rocks. Sieved soils were air dried for one week and a 10g subsample was ground on a roller table until aggregates were pulverized. Organic C content and its isotopic composition were measured by weighing soil into silver capsules after fumigating with 1 M hydrochloric acid for 8 hours to remove carbonates (Stewart et al., (2018), using an isotope ratio mass spectrometer (Europa 20-20, Sercon Ltd, Crewe, England)..

3.2.4 Dissolved organic carbon (DOC) extraction

A detailed description of the DOC extraction can be found in Davidson et al., (1987). Briefly, aliquots of field moist soil (8.00 ± 0.004 g) were shaken with 40 mL deionized water in a 50 mL polypropylene centrifuge tube on a wrist action shaker for 30 minutes. Samples were then centrifuged for 15 minutes at 7,000 rpm to settle soil particles out of suspension. Samples were filtered using a device consisting of funnels with Whatman GF/F filters on top of syringes connected by a 0.45 μm filter. Inorganic C was removed from DOC by acidification of samples. Drops of 1 N phosphoric acid were added until the pH of the solution was between 3 and 2.5. The acidified extracts were left for 1 hour to allow for venting of inorganic C as carbon dioxide (Davidson et al., 1987). Extracts were returned to neutral pH ($7 - 7.5 \pm 0.05$) by adding

drops of 1 M sodium hydroxide. Samples were freeze-dried (Labconco, Kansas City, MO), and subsequent salts were measured into tin capsules and analyzed for elemental and isotopic analyses using an isotope ratio mass spectrometer (Europa 20-20, Sercon Ltd, Crewe, England).

3.2.5 Soil organic carbon (SOC) fractionation

Soil was fractionated by size and density into pools with different pathways of formation (Poeplau et al., 2018). In summary, eight grams of soil were added to 30 mL of sodium polytungstate (1.85 g/cm^3) in a 50 mL polypropylene centrifuge tube. Twelve glass beads were added, and the sample was shaken on a reciprocal shaker for 18 hours for aggregate dispersal. The dispersed suspension was centrifuged for separation of the light POM (IPOM) which is made of partly decomposed plant structural compounds from heavier fractions. IPOM was aspirated through vacuum filtration and rinsed thoroughly to remove residual SPT. The remaining heavy fraction was rinsed three times with deionized water to remove remaining SPT from sample. After the third rinsing, the heavy fraction was wet sieved over a $53 \mu\text{m}$ sieve until the rinse water ran clear. The fraction on top of the sieve represents sand associated SOC (hPOM). The $<53 \mu\text{m}$ fraction was poured into clean centrifuge tubes and centrifuged until silt settled to the bottom and clay remained in suspension. The clay was aspirated into a vacuum filtration system and transferred to pre-weighed aluminum pans. The silt/clay separation was repeated at least twice more, after which the silt fraction was rinsed into a pan. All fractions were dried for at least two days in oven at 55°C , transferred to labelled vials, ground, weighed, and analyzed for elemental and isotopic analyses using an isotope ratio mass spectrometer (Europa 20-20, Sercon Ltd, Crewe, England). The total fractions mass recovery was within $\pm 7\%$ of the initial mass. Recovery of C in SOC fractions was $94 \pm 15\%$ of bulk soil C. Only depths with residue-derived C in the bulk soil (i.e., 0-5 and 5-10 cm) were fractionated.

3.2.6 Data analyses

The residue-derived C contribution to the bulk soil, DOC, and SOC fractions was determined by comparing the isotopic signature of the baseline and six-month soil samples (post-residue addition) using the isotopic mixing model:

$$f_{residue} = \frac{(\delta_S - \delta_B)}{(\delta_{residue} - \delta_B)}$$

where $f_{residue}$ represents the fraction of residue-derived C in the resulting SOC pool. δ_S , δ_B , and $\delta_{residue}$ represent the $\delta^{13}C$ of the sample, the baseline soil, and the initial residue respectively. For bulk soil, DOC, and SOC fractions, the δ_B was the average (n=4) of the baseline soil samples. The $f_{residue}$ was multiplied by C amount (g C kg⁻¹ soil), bulk density (g cm⁻³), and depth of soil layer (m) to determine the amount of residue-derived C in SOC pools on a surface base (m²). Efficiency of SOC formation was calculated by dividing the amount of residue-derived C in SOC pools by the amount in all SOC pools and CO₂. Efficiency calculations are described in more detail in Lavallee et al., (2018).

3.2.7 Statistics

All statistical analyses were carried out using R software (R Core Team, 2018). The data was tested for normality and log-transformed when necessary. Bulk density and SOC pool sizes were tested for treatment and harvest (baseline, 6 month) differences using the emmeans package and no differences were found. The effect of treatment (MIX, SA, SAD) and depth (0-5, 5-10, etc.) on residue-derived C in SOC pools was tested using linear mixed effects models with treatment replicate as a random factor using the lmerTest package. We averaged over the 0-5 and 5-10 cm depth to consider treatment effects over the depth in which residue was incorporated (0-10 cm).

The effect of treatment on SOC formation efficiency from 0-10 cm was tested using linear mixed effects models with replicate as a random factor. Pairwise comparisons were run using the emmeans package to determine specific treatment differences.

3.3 Results

3.3.1 Residue-derived C in CO₂ and undecomposed residue

Residue-derived C in CO₂ was measured for 12 months, analyzed, and results are described and interpreted in Chapter 2. After 12 months, between 18-23% of residue-derived C was lost as CO₂ with most of the loss occurring during the growing season (Table 3.1). CO₂ made up to 40% for the MIX and 14-17% for the SA/SAD of the residue-C transformed during the off season (Table 3.1).

3.3.2 Residue-derived C in bulk soil and DOC

Residue C amounted to 3% of bulk soil C and 8% of DOC within the 0-10 cm depth. DOC contributed an average of 1.4% of the bulk soil residue C. Residue-derived C amount in both bulk soil and DOC was significantly greater in the MIX treatment than the SA and SAD treatments for the 0-10 cm depth (Fig. 3.1-2). Residue-derived C in bulk soil and DOC for the SAD treatment were significantly lower than the SA treatment in the 0-5 cm depth. Traces ($0.8 \pm 0.4 \text{ g C m}^{-2}$) of residue-derived C were recovered in the 10-20 cm depth only in the MIX treatment with no residue-derived C below the 0-5 cm depth for SA or SAD (Fig. 3.1-2).

3.3.3 Residue-derived C in lPOM and hPOM fractions

Averaged over the treatments, the majority (53%) of residue-derived C was recovered in the lPOM fraction in the 0-10 cm depth. The lPOM $\delta^{13}\text{C}$ values were highly variable, ranging from

76-466‰ (Table 3) which produced IPOM residue-derived C amounts between 7.8 and 14.6 g C m⁻² (Table 3.4). The SA treatment had significantly more residue-derived IPOM C than SAD within the 0-5 cm depth (Fig. 3.3a). Averaged over the 0-10 cm depth, the MIX treatment had significantly higher IPOM residue-derived C compared to SAD and SA treatments. The hPOM fraction contained minimal (2%) residue-derived C (Fig. 3.3b). Although residue-derived C amounts were low, the SA treatment was significantly higher than the SAD treatment within the 0-5 cm depth. Averaged over the 0-10 cm depth, the MIX treatment had a higher amount of residue-derived C in hPOM than the SAD and SA treatments (Fig. 3.3a).

3.3.4 Residue-derived C in silt- and clay-sized fractions

Within the 0-10 cm depth, an average of 18% and 25% of residue-derived C was found in the silt- and clay-sized fractions respectively. In the 0-10 cm depth, residue-derived C amounts for silt and clay were similar for SA and SAD, but clay had significantly higher SOC than silt in the MIX treatment ($p < 0.001$). Within the 0-5 cm depth, the SA and SAD treatments contained similar amounts of residue-derived C in the silt and clay-sized fractions (Fig. 3.3c). The MIX treatment had the highest residue-derived C in the silt- and clay-sized fractions within the 0-10 cm depth (Fig. 3.3 c-d). To compare the importance of SOC formation pathways, we combined pools related to POM (IPOM+hPOM) and MAOM (silt+clay). When separated by treatment, we found the highest MAOM and POM formation in the MIX treatment compared to SA and SAD treatments (Fig. 3.4). Within treatments, MIX and SA showed significantly and slightly ($p = 0.05$) higher POM than MAOM formation respectively (Fig. 3.4).

3.3.5 Residue-derived SOC efficiency

Six months after residue addition, the MIX treatment had 26% of the initial residue-derived C transformed into CO₂ (28%) and SOC pools (72%) (Table 3.4). The SA treatment had 12% of the initial residue-derived C transformed into CO₂ (29%) and SOC pools (71%). The SAD treatments had 9% of the initial residue-derived C transformed into CO₂ (41%) and SOC fractions (59%). SOC efficiency calculations showed significantly lower stabilization rate for the SAD treatment for summed SOC pools and slightly lower ($p=0.07$) for the IPOM pool (Fig. 3.5). The MIX and SA treatments were not significantly different from each other in terms of separate or summed SOC formation efficiency (Fig. 3.5). When SOC pool formation efficiencies were summed, the MIX treatment had similar and 20% higher efficiency compared to SA and SAD treatments respectively (Table 3.4).

3.4 Discussion

3.4.1 Residue incorporation increased residue-derived SOC

There was minimal (9-26%) residue decomposition within the treatments after six months due to low temperatures and moisture during the off season. Even with decomposition-inhibiting conditions, we were still able to distinguish results in residue-derived SOC depending on residue location and soil disturbance. Six months after residue addition, the incorporated residue treatment had more residue decomposition (i.e. ¹³CO₂ loss) compared to surface-applied residue treatments which confirmed our hypothesis presented in Chapter 2. This result is similar to the results of Mitchell et al., (2016), who found incorporation increased residue decomposition 21% compared to surface-applied residue. On the other hand, the surface-applied residue treatments had an average of 60% lower residue C in bulk soil compared to the incorporated residue treatment, showing that incorporation increased SOC formation. This result confirms other studies that question find CT increases SOC (Angers and Eriksen-Hamel, 2008; Baker et al.,

2007). In a study comparing CT and NT SOC after 16 years, higher bulk soil C was similarly found under CT management when considering the whole depth of residue incorporation (0-30 cm) (Gregorich et al., 2010). Both Gregorich et al., (2010) and our study site have high clay soils which may be important for aggregate protection of residue C following incorporation. Additionally, a meta-analysis comparing NT and a deep tillage method (full-inversion tillage) found higher amounts of SOC under tillage near or at the bottom of the layer of residue incorporation (Angers and Eriksen-Hamel, 2008), suggesting that deep tillage distributes SOC more widely than NT within the soil profile. Mechanisms by which CT could increase bulk C compared to NT are: (1) close association of residue with mineral surfaces enhances MAOM formation (Angers and Eriksen-Hamel, 2008), and (2) incorporation embeds residue within the soil matrix, creating aggregate protection as the residue decomposes to form POM (Gregorich et al., 2010; Gunina and Kuzyakov, 2014; Plaza et al., 2013).

3.4.2 Residue incorporation did not increase SOC decomposition

Although the MIX treatment showed greater residue-derived SOC after six months, loss of existing SOC following disruption of soil aggregates and addition of residue could negate any SOC accrual due to greater losses of native SOC than residue SOC formation (Kuzyakov et al., 2000). Surprisingly, we did not observe an increase in soil-derived CO₂ (proxy for native SOC decomposition) from the MIX treatment after six months (Chapter 2). This is in contrast to results observed in Mitchell et al., (2016), who found an increase in soil-derived CO₂ in their incorporated residue treatment compared to their surface-applied residue treatments. The absence of increased SOC decomposition in our system is likely due to low temperatures and soil moisture conditions that created unfavorable decomposition conditions during the six-month incubation. Additionally, lowered decomposition could be partially attributed to the high level of

calcium carbonates and clay, and high soil N in our fertilized system reforming broken aggregates and satisfying microbial N needs respectively (Stewart et al., 2015; Wuddivira and Camps-Roach, 2007). This is in contrast to possible lower re-aggregation in the sandy soil of the non-fertilized experimental plots used in Mitchell et al., (2016).

3.4.3 Soil disturbance decreased bulk and DOC residue-derived C amount

Six months after residue addition, the treatment with surface-applied residue and soil disturbance (SAD) had lower residue-derived C than surface-applied residue (SA) in bulk soil within the 0-5 cm depth. This result indicates that soil disturbance inhibited SOC formation. Within specific SOC pools, the SAD treatment had significantly lower residue-derived C than the SA treatment in DOC, IPOM, and hPOM within the 0-5 cm depth (Fig 2-3). This lower residue-derived C stabilization of surface-applied residue with disturbance could be linked to changes in preferential flow pathways and soil fauna within the soil profile following soil disturbance. Preferential flow pathways are important for transport of surface DOC into deeper soil profiles (Rumpel and Kögel-Knabner, 2011b), thus we theorize that soil disturbance disrupted flow pathways that had developed in our NT system since its establishment in 2008. In addition, the DOC-microbial path is likely important during the early stages of residue decomposition (Cotrufo et al., 2015b); thus, changes in flow pathways might have prevented DOC from moving down into surface soil. The MIX treatment was likely not affected by the soil disturbance because the DOC produced from incorporated residue did not need to rely on flow pathways to percolate into the soil profile, but instead formed SOC by directly adsorbing to mineral surfaces (Sokol et al., 2018). Additionally, soil faunal activity is important in physically fragmenting residue, thus forming POM. Surface-applying residue after disturbing the soil likely led to compacted soil conditions which decreased short-term soil fauna abundance, decreasing

the size of POM formed by faunal residue transformation and distribution (Crittenden et al., 2014).

3.4.4 Short-term SOC formation was in the IPOM fraction

In all treatments, the majority of residue-derived C was recovered in the POM fraction accounting for an average of 54% of residue-derived C in the 0-10 cm depth. The IPOM fraction made up the 87-95% of residue-derived C within the POM pool. The IPOM and hPOM fractions represent SOC pools that are thought to form through the physical transfer path and contain largely unprocessed residue (Baldock et al., 1992; Cotrufo et al., 2015a). IPOM has undergone limited decomposition while hPOM contains some relatively more decomposed residue (Gregorich et al., 1996). hPOM often plays a minor role in SOC because it represents sand-sized particle associations which exhibit weak interactions with SOC (Lützow et al., 2006). Contrary to our hypothesis, we found roughly half of residue-derived C in the IPOM pool regardless of residue location (Fig. 3a). Physical fragmentation, which forms POM, was likely accelerated after homogenizing residue by cutting it into small segments (<1.5 cm). Alternatively, POM formation might be high in this NT system due to increased aggregation by reactive soil particles and high soil faunal activity. Six et al., (2002) highlighted the importance of residue protection within aggregates, determining that aggregate formation and turnover are crucial mechanisms for the protection of POM. The soil at our site is high in clay and inorganic C which have the potential to form aggregates around POM (Gregorich et al., 2010; Oades and Tisdall, 1982). In addition, soil fauna affect SOC formation by assimilating and redistributing residue within the soil (Frouz, 2018; Six et al., 2004b). After a 21-day incubation, Bossuyt et al., (2005) found that earthworms are responsible for protecting residue C within microaggregates created through ingestion and excretion of residue within casts. In terms of SOC mechanisms, Angst et al.,

(2017) found that earthworms are physically separating residue and decomposer communities rather than altering the chemistry of the residue. While it seems unlikely that soil fauna would be very active during the cold temperatures that persisted for much of the six-month study, 67% of the average daily air temperatures were above freezing (Chapter 2, Fig. 2.5).

3.4.5 Residue incorporation increased mineral-associated residue-derived C

The silt- and clay-sized fractions represent SOC pools that form through chemical processes (Six et al., 2002), soil aggregation, and the DOC-microbial path (Cotrufo et al., 2015a). Our results agreed with our hypothesis, showing significantly higher MAOM formation when residue was incorporated from increased residue decomposition within the soil. During the six months following residue addition, the average soil temperature was warmer (3.8 °C) than the air temperature (1.7 °C), kinetically favoring incorporated residue decomposition (Gregorich et al., 2017b). Greater residue decomposition in the MIX treatment likely provided a larger pool of substrates for adsorption on mineral surfaces in immediate contact with the residue (Mitchell et al., 2018).

We expected more residue-derived C in clay than silt because of greater reactive surface area of clays. However, only incorporated residue showed a significant increase in clay-sized residue-derived C compared to silt. The residue is still in the beginning stages of decomposition; therefore, we may see differential C stability between silt and clay within surface-applied residue treatments as time progresses (von Lützow et al., 2007). Another possibility for why silt and clay are not stabilizing residue C differently might be that the high level of calcium carbonate at our field site is forming strong bonds between minerals and DOC regardless of whether the particle is silt or clay-sized (Singh et al., 2016). The soils at our study site have been depleted of SOC

from past conventional agricultural management, thus they likely have not reached a level of C saturation, suggesting that there is still plenty of room for more C stabilization as the residue continues to decompose.

3.4.6 Efficiency of residue-derived C was the same for incorporated and surface-applied residue

Incorporated residue lost more residue C as CO₂ after six months; thus, it is in a later stage of decomposition than surface-applied residue. Surprisingly, MIX and SA treatments had the same efficiency of residue-derived C in SOC while the SAD treatment had a significantly lower C efficiency. This result highlights the differential impact of residue location and soil disturbance on SOC formation. In our NT system, it appears that both aggregation and soil fauna (i.e. earthworms) are producing POM with similar efficiencies. Soil disturbance appears to limit residue fragmentation which may be due to a short-term decrease in soil fauna following compacted soil conditions (Crittenden et al., 2014).

3.4.7 Long-term predictions for the fate of residue-derived C

The overarching objective of this study was to assess SOC formation and stabilization over the course of three years. SOC formation path and relative pool sizes change over time, thus our residue-derived SOC results are subject to change (Cotrufo et al., 2015b; Mitchell et al., 2018). We predict an increase in the importance of the physical transfer path in surface soil over time. As the water-soluble pool of residue C is leached and transformed, the remaining, structural residue C will be fragmented and distributed by soil fauna, adding to the POM within the topsoil. We predict an increase in the DOC-microbial path deeper within the profile with growing season irrigation events. Recently, the Microbial Efficiency-Matrix Stabilization framework has been developed into a model (MEMS v1.0) which includes measurable, distinct SOC pools

(Robertson et al., 2018). Within the model, leached DOC is included as a distinct SOC pool important in deep SOC formation. Weekly irrigation events during the growing season (early June – mid September) will likely increase the pool of leached DOC below the layer of residue incorporation (>10 cm). While subsoil SOC formation is not as well understood as surface SOC, formation of MAOM from DOC is proposed as an important pathway (Rumpel and Kögel-Knabner, 2011a). Therefore, we expect a translocation of DOC and its resultant MAOM deeper within the soil profile in soil samples taken 12 months after residue addition.

3.5 Conclusion

This study demonstrated that incorporating residue (“CT”) increased the total amount of residue-derived C in bulk soil compared to surface-applied residue (“NT”) due to greater formation of POM and MAOM from aggregation and mineral associations in both silt- and clay-sized SOC fractions. Although the MAOM formation pathway was hypothesized to occur first and POM second, we found a larger pool of POM than MAOM in both incorporated and surface-applied/undisturbed residue treatments. These results highlight the importance of physical fragmentation of residue within the early stages of decomposition. We normalized residue-derived C by the amount of residue C respired and found no difference between CT and NT treatments. The NT/disturbed treatment showed significantly lower residue-derived C amount in the bulk soil and POM, along with lower efficiency of residue-derived SOC. This result demonstrates the negative effect of soil disturbance apart from residue location (i.e. incorporation) on SOC formation. Long-term results will elucidate whether NT and CT residue efficiency results will change over the growing season and over years. Residue was cut for homogenization before field application; thus, artefacts might have been created within the POM pools. The importance of the physical transfer path early in residue decomposition in forming

POM was surprising and stresses the need for further research on the importance of existing soil structure on aggregate formation and soil faunal activity in short-term residue decomposition dynamics. It is important to remember that this site is a NT system in which soil fauna was not affected outside of the collars; thus, NT soil fauna proved to be a key driver of POM formation in our system and would likely be negatively impacted in a conventionally tilled system. Over the long-term (>12 months), we predict increased POM formation in the surface soil from physical fragmentation of remaining residue and increased MAOM formation in subsoils from leached DOC following irrigation events. Future results will elucidate whether the growing season irrigation events will contribute to C sequestration or promote residue decomposition in semi-arid agroecosystems.

Table 3.1.

Residue-derived C-CO₂ (g m⁻²) and remaining residue (%) after 6 and 12 months (initial residue C – residue C-CO₂) for MIX: incorporated residue, SA: surface-applied residue, SAD: surface-applied residue with soil disturbance. Numbers in parentheses represent one standard deviation (n=4).

Treatment	Residue-derived C		Remaining residue mass	
	6 m	12 m	6 m	12 m
	Residue-derived C-CO ₂ g m ⁻²		% of initial	
MIX	21.73 (3.39)	54.81 (6.33)	92.8 (1.12)	81.84 (2.1)
SA	10.04 (0.45)	70.1 (6.13)	96.67 (0.15)	76.78 (2.03)
SAD	10.58 (1.02)	63.77 (7.2)	96.49 (0.34)	78.88 (2.39)

Table 3.2.

Delta ^{13}C values (‰) for baseline, MIX: incorporated residue, SA: surface-applied residue, SAD: surface-applied residue with soil disturbance. Fractions are light (l) and heavy (h) POM, and silt- and clay-sized soil organic matter (0, 6, and 12 months after residue addition). Numbers in parentheses represent one standard deviation (n=4).

Fraction	Residue-added treatments						
	Baseline	MIX		SA		SAD	
	0 m	6 m	12 m	6 m	12 m	6 m	12 m
	$\delta^{13}\text{C}$ (‰)						
light POM†							
0-5 cm	-17 (1)	238 (75)	-	300 (174)	-	197 (158)	-
5-10 cm	-17 (1)	318 (107)	-	-18 (0)	-	-18 (0)	-
0-10 cm	-17 (1)	278 (73)	-	141 (87)	-	90 (79)	-
heavy POM†							
0-5 cm	-22 (3)	41 (12)	-	61 (40)	-	20 (32)	-
5-10 cm	-20 (2)	95 (63)	-	-22 (0)	-	-22 (0)	-
0-10 cm	-21 (2)	68 (37)	-	19 (20)	-	-1 (16)	-
silt-sized							
0-5 cm	-19 (1)	42 (3)	-	34 (16)	-	26 (11)	-
5-10 cm	-18 (2)	56 (25)	-	-19 (0)	-	-19 (0)	-
0-10 cm	-19 (1)	49 (11)	-	8 (8)	-	3 (6)	-
clay-sized							
0-5 cm	-19 (1)	48 (4)	-	40 (21)	-	29 (13)	-
5-10 cm	-19 (0)	57 (13)	-	-19 (0)	-	-19 (0)	-
0-10 cm	-19 (1)	52 (6)	-	10 (11)	-	5 (6)	-

†POM, particulate organic matter

Table 3.3.

Average residue-derived C mass (g m^{-2} soil) for baseline, MIX: incorporated residue, SA: surface-applied residue, SAD: surface-applied residue with soil disturbance. Fractions are light and heavy POM, and silt- and clay-sized soil organic matter (0, 6, and 12 months after residue addition). Numbers in parentheses represent one standard deviation ($n=4$).

Fraction	Residue-added treatments						
	Baseline	MIX		SA		SAD	
	0 m	6 m	12 m	6 m	12 m	6 m	12 m
	Residue-derived C g m^{-2} soil						
bulk soil							
0-5 cm	-	25.13 (4.07)	-	27.54 (11.16)	-	16.61 (3.21)	-
5-10 cm	-	30.03 (7.62)	-	0.26 (0.23)	-	0.27 (0.5)	-
0-10 cm	-	55.19 (7.42)	-	27.84 (11.1)	-	16.88 (3.39)	-
IPOM†							
0-5 cm	-	12.76 (6.61)	-	14.58 (7.01)	-	7.79 (5.58)	-
5-10 cm	-	17.64 (8.16)	-	0 (0)	-	0 (0)	-
0-10 cm	-	30.41 (8.34)	-	14.61 (7.02)	-	7.8 (5.61)	-
hPOM†							
0-5 cm	-	0.7 (0.14)	-	1.23 (0.57)	-	0.52 (0.25)	-
5-10 cm	-	1.1 (0.44)	-	0 (0)	-	0 (0)	-
0-10 cm	-	1.81 (0.57)	-	1.23 (0.57)	-	0.52 (0.25)	-
silt-sized							
0-5 cm	-	4.45 (0.51)	-	3.77 (1.1)	-	3.18 (0.69)	-
5-10 cm	-	5.15 (1.06)	-	0 (0)	-	0 (0)	-
0-10 cm	-	9.6 (0.67)	-	3.78 (1.1)	-	3.18 (0.69)	-
clay-sized							
0-5 cm	-	6.09 (0.54)	-	5.25 (2.09)	-	4.27 (1.05)	-
5-10 cm	-	6.89 (1.16)	-	0 (0)	-	0 (0)	-
0-10 cm	-	12.98 (0.64)	-	5.26 (2.07)	-	4.27 (1.04)	-

† POM, particulate organic matter

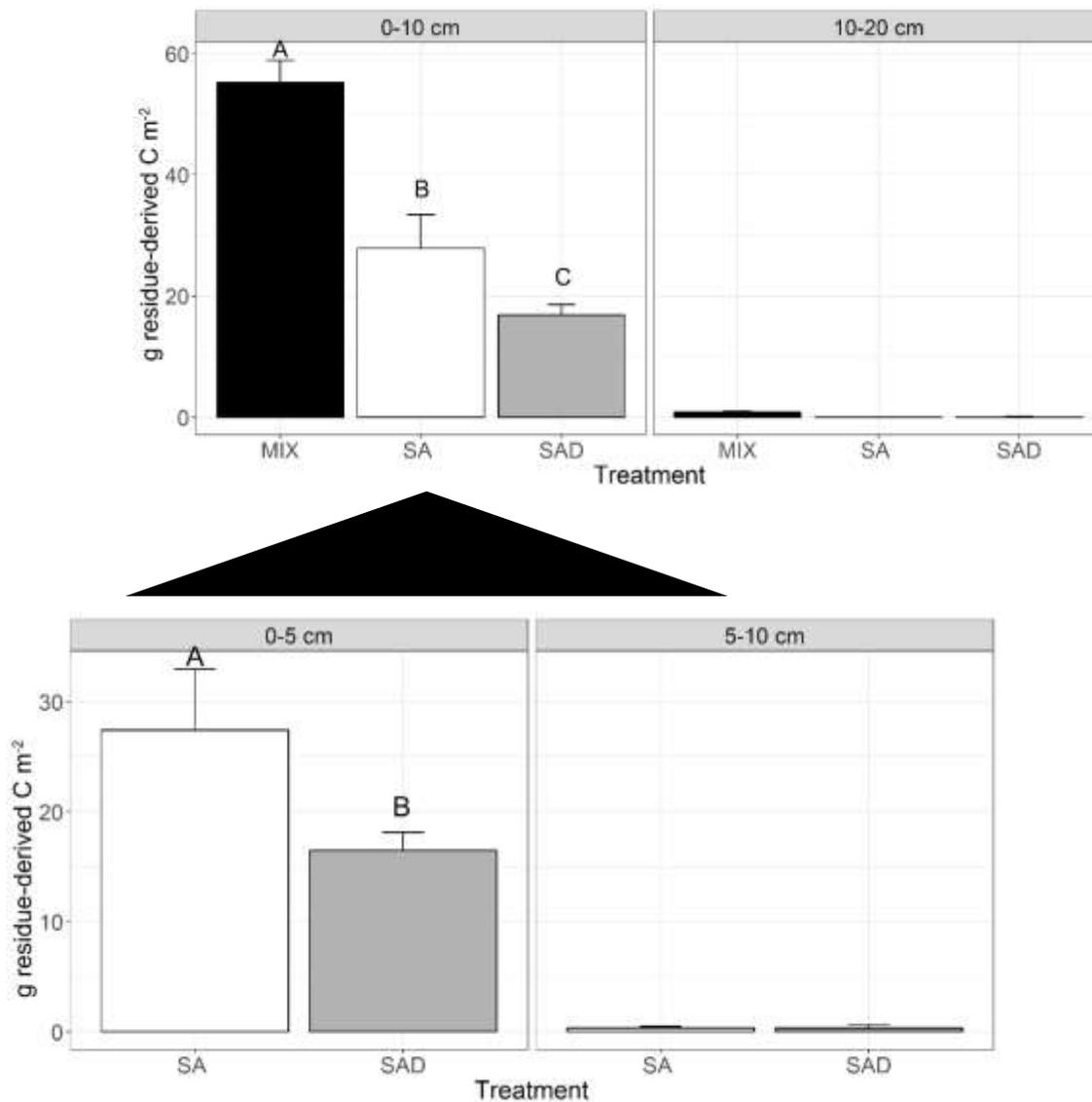


Figure 3.1. Residue-derived C (g residue-derived C m⁻² soil) in bulk soil for MIX: incorporated residue, SA: surface-applied residue, SAD: surface-applied residue with soil disturbance. 0-10 cm depth was calculated by averaging across 0-5 and 5-10 cm depths. Error bars represent standard errors (n=4). Letters denote significance (p<0.05).

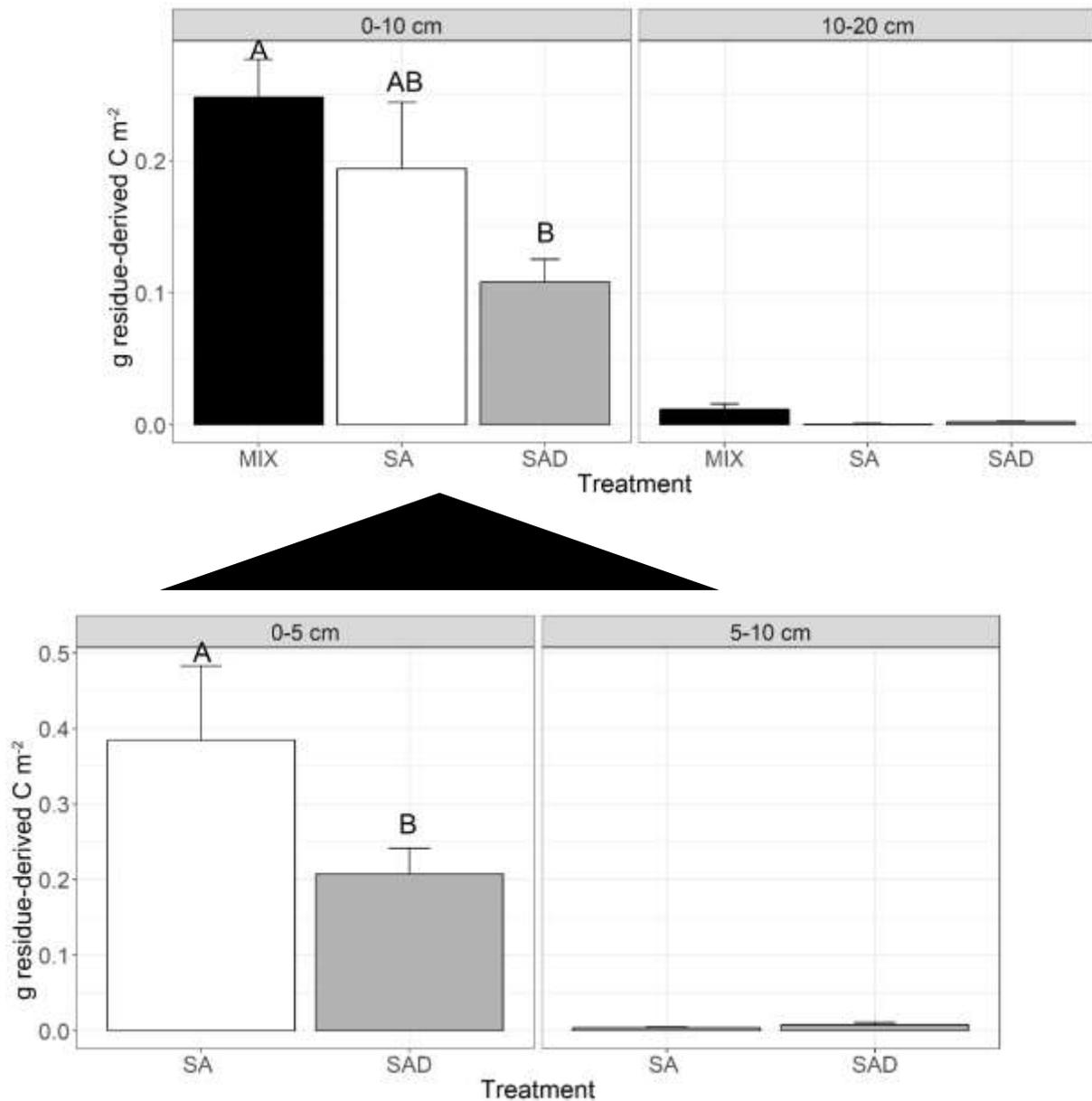
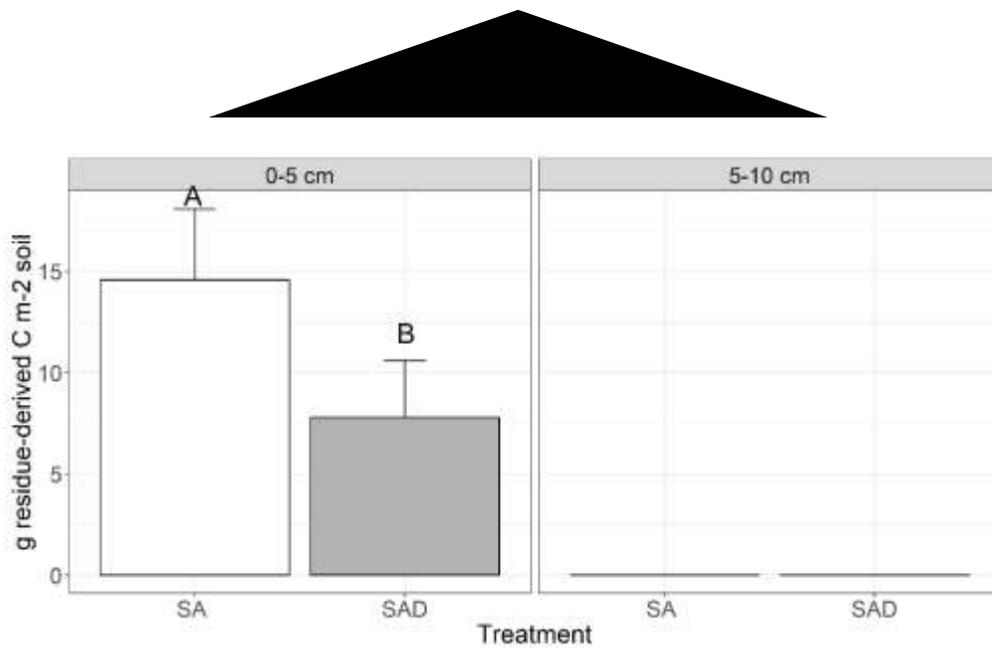
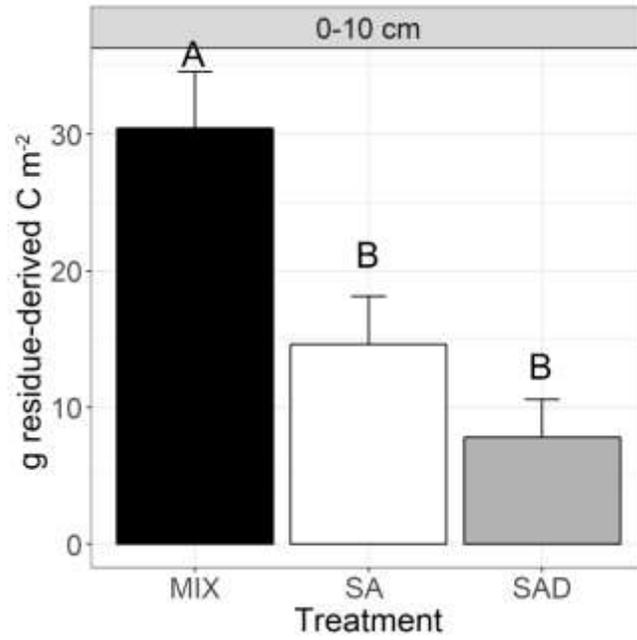
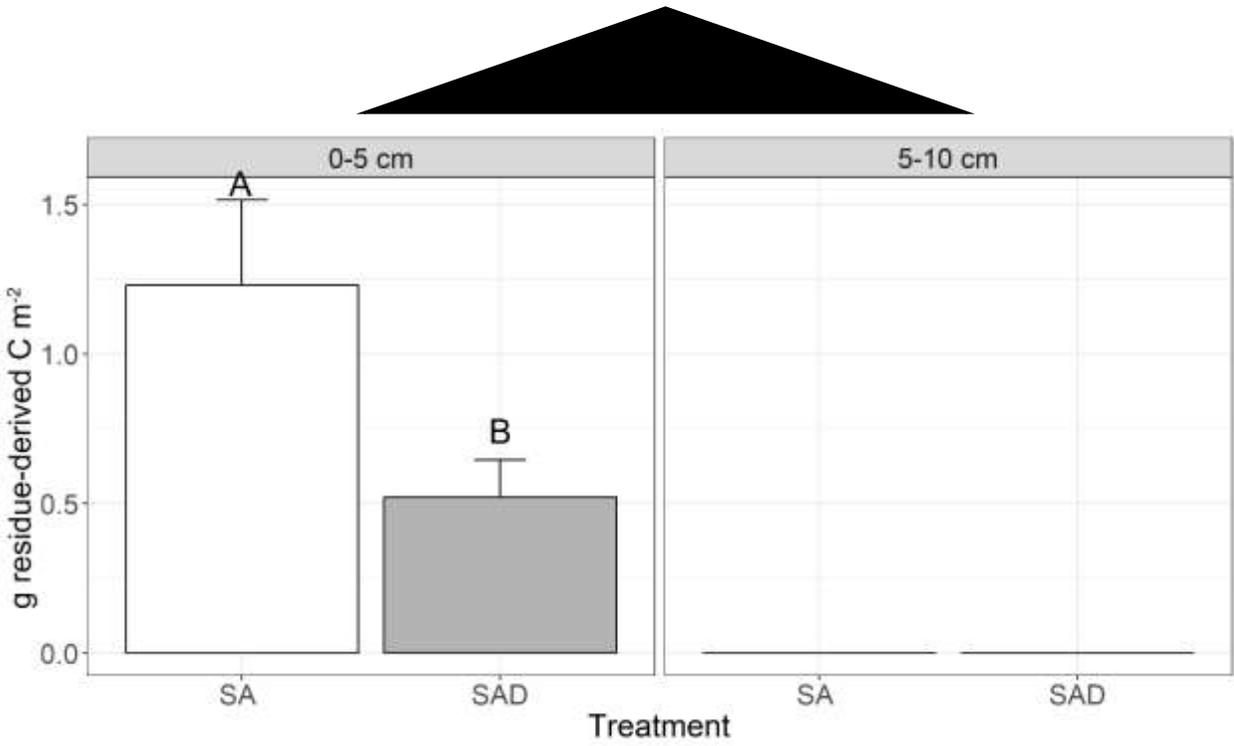
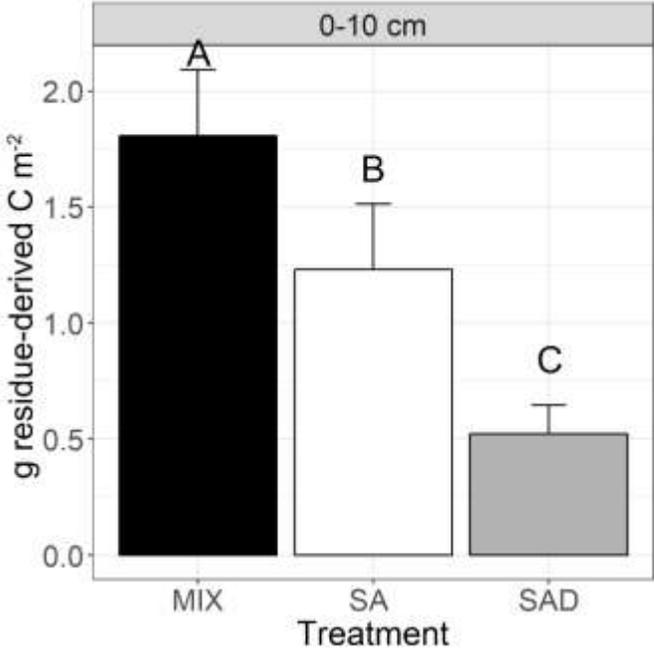


Figure 3.2. Residue-derived C (g residue-derived C m⁻² soil) in dissolved organic carbon for MIX: incorporated residue, SA: surface-applied residue, SAD: surface-applied residue with soil disturbance. 0-10 cm depth was calculated by averaging across 0-5 and 5-10 cm depths. Error bars represent standard errors (n=4). Letters denote significance (p<0.05).

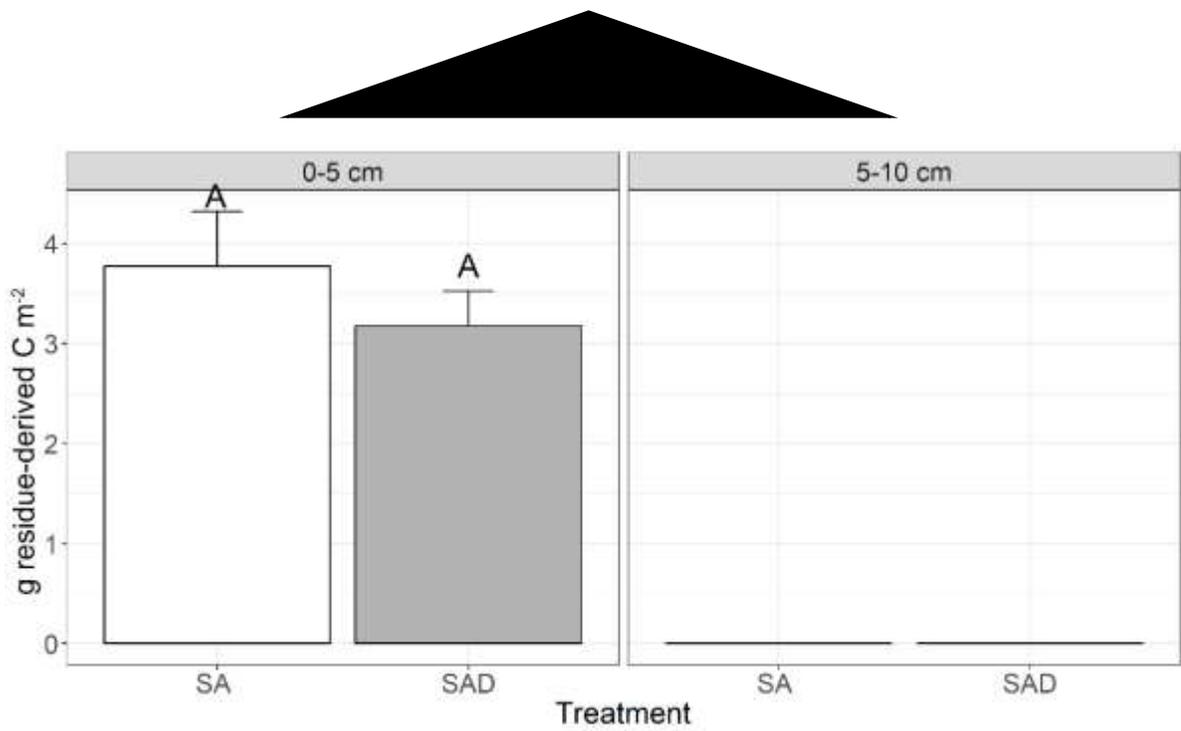
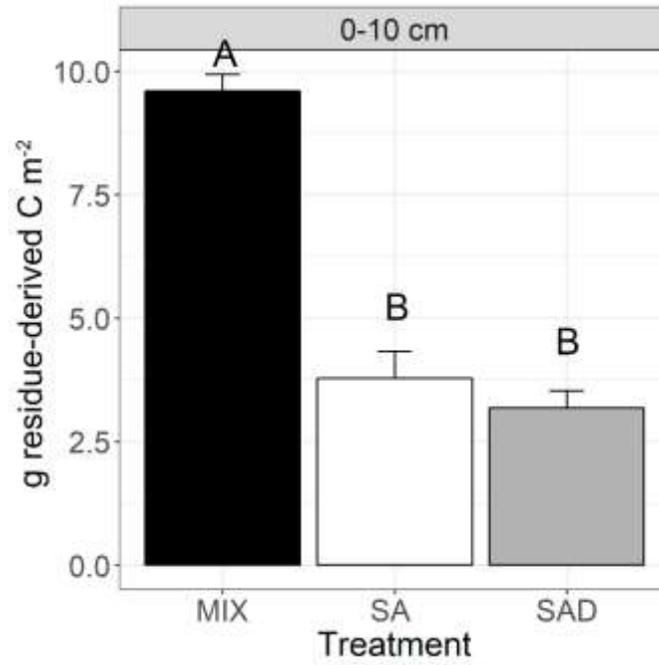
(a) IPOM



(b) hPOM



(c) silt-sized



(d) clay-sized

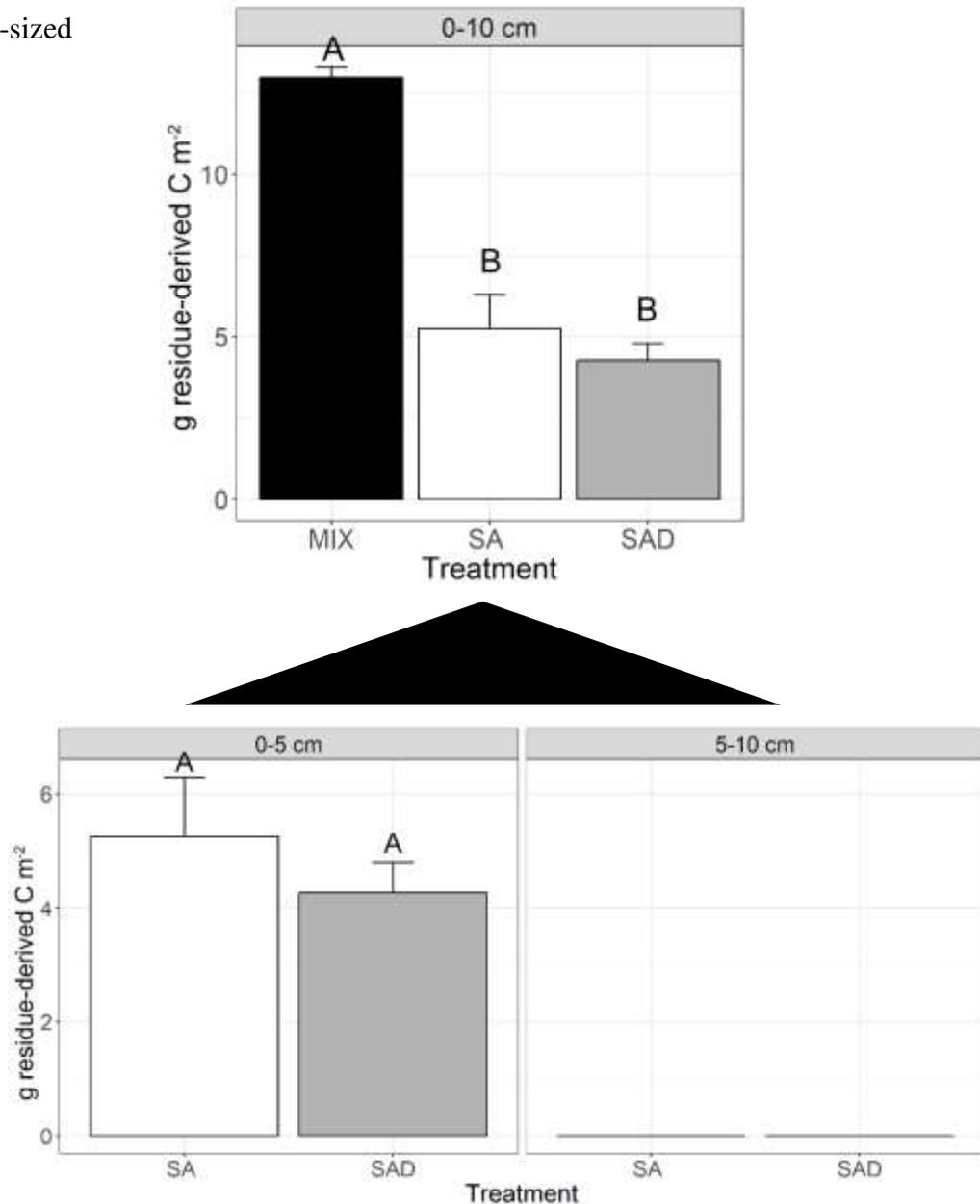


Figure 3.3. Residue-derived C (g residue-derived C m⁻² soil) in soil organic matter fractions for MIX: incorporated residue, SA: surface-applied residue, SAD: surface-applied residue with soil disturbance. Panels denote residue-derived C in separate SOC fractions: (a) light particulate organic matter (lPOM), (b) heavy particulate organic matter (hPOM), (c) silt-sized particles, (d) clay-sized particles. 0-10 cm depth was calculated by averaging across 0-5 and 5-10 cm depths. Error bars represent standard errors (n=4). Letters denote significance (p<0.05).

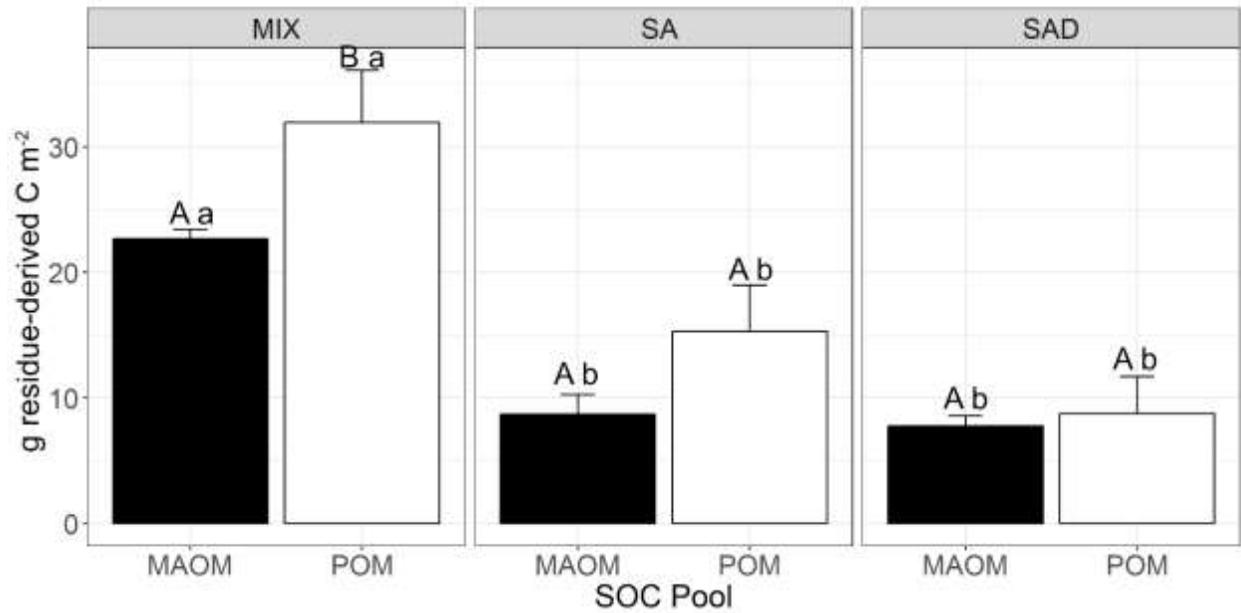


Figure 3.4. Residue-derived C (g residue-derived C m⁻² soil) in summed soil organic matter fractions for MIX: incorporated residue, SA: surface-applied residue, SAD: surface-applied residue with soil disturbance. SOC fractions include: minerally-associated organic matter (MAOM) and particulate organic matter (POM). Error bars represent standard errors (n=4). Uppercase letters denote significance (p<0.05) within treatments. Lowercase letters denote significance (p<0.05) across treatments.

Table 3.4. Residue-derived C (g residue-derived C m⁻² soil) in CO₂ and SOC pools after six months for MIX: incorporated residue, SA: surface-applied residue, SAD: surface-applied residue with soil disturbance. SOC fractions: (a) light particulate organic matter (lPOM), (b) heavy particulate organic matter (hPOM), (c) silt-sized particles, (d) clay-sized particles. Error bars represent standard errors (n=4).

Treatment	Residue-derived C						
	CO ₂	DOC	lPOM	hPOM	silt-sized	clay-sized	Undecomposed residue
	Residue-derived C- CO ₂ g m ⁻²	Residue-derived C g m ⁻²					
MIX	21.73 (3.39)	0.25 (0.06)	30.41 (8.34)	1.81 (0.57)	9.6 (0.67)	12.98 (0.64)	224.22 (11.43)
SA	10.04 (0.45)	0.19 (0.1)	14.61 (7.02)	1.23 (0.57)	3.78 (1.1)	5.26 (2.07)	265.89 (10.36)
SAD	10.58 (1.02)	0.11 (0.04)	7.8 (5.61)	0.52 (0.25)	3.18 (0.69)	4.27 (1.04)	274.53 (7.67)

Table 3.5. Normalized residue-derived C (g residue-derived C (g residue-derived C in SOC + CO₂)⁻¹) in SOC pools after six months for MIX: incorporated residue, SA: surface-applied residue, SAD: surface-applied residue with soil disturbance. SOC fractions: (a) light particulate organic matter (lPOM), (b) heavy particulate organic matter (hPOM), (c) silt-sized particles, (d) clay-sized particles. Error bars represent standard errors (n=4). Calculations based on (Stewart et al., 2009).

Treatment	Residue-derived C					
	lPOM	hPOM	silt-sized	clay-sized	DOC	SOC
	(g residue-derived C (g residue-derived C in SOC + CO ₂) ⁻¹)					
MIX	0.39 (0.06)	0.02 (0.01)	0.13 (0.01)	0.17 (0.02)	0 (0)	0.72 (0.03)
SA	0.4 (0.08)	0.03 (0.01)	0.11 (0.01)	0.15 (0.03)	0.01 (0)	0.7 (0.08)
SAD	0.27 (0.11)	0.02 (0.01)	0.12 (0.02)	0.16 (0.03)	0 (0)	0.58 (0.09)

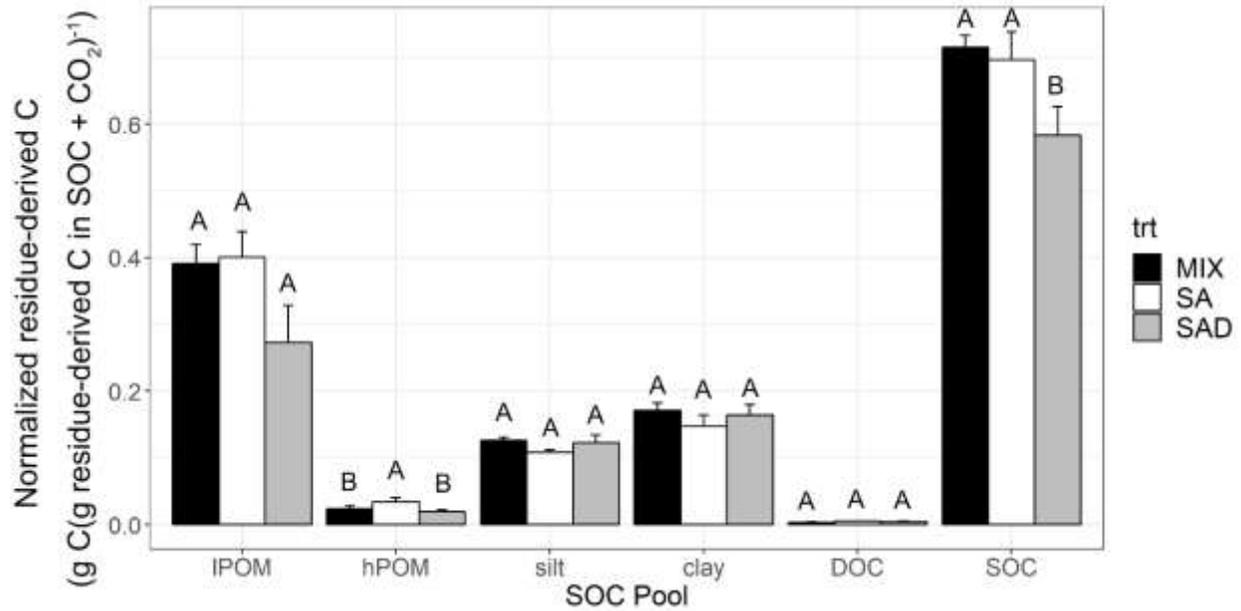


Figure 3.5. Normalized residue-derived C ($\text{g C (g residue-derived C in SOC + CO}_2\text{)}^{-1}$) in soil organic matter fractions for MIX: incorporated residue, SA: surface-applied residue, SAD: surface-applied residue with soil disturbance. SOC fractions include dissolved organic carbon (DOC), light and heavy particulate organic matter (IPOM and hPOM), silt- and clay-sized OM. Error bars represent standard errors ($n=4$). Letters denote significance ($p<0.05$) between treatments within an SOC pool.

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CHAPTER 4: CONCLUSION

The primary objective of my thesis was to better understand how residue management (i.e. NT and CT) affects CO₂ loss and SOC formation in an irrigated corn system. My primary research questions were:

- (1) How does residue location and soil disturbance effect the magnitude and proportion of soil respiration efflux from either residue or soil C sources?
- (2) How does residue location and soil disturbance effect the magnitude and proportion of residue-derived SOC formation and stabilization of DOC, POM, and MAOM?

In Chapter 2, I addressed the first question and found that residue location and season had a significant effect on the amount of residue-derived CO₂ over the course of 12 months. Incorporated residue had higher residue-derived CO₂ during the off season, but surface residue had higher residue-derived CO₂ during the growing season and full year. Surface residue experienced much greater decomposition particularly after irrigation events suggesting that available water in this semi-arid system stimulates surface residue decomposition during high temperature seasons (“growing season”). A greater loss of residue C in NT limits the potential of the remaining residue C to form SOC in this semi-arid, irrigated corn system.

In Chapter 3, I addressed my second question about residue C protection and found that residue location had a significant effect on the amount of residue-derived C in both bulk and SOC pools six months after residue addition. After six months, the incorporated residue treatment had an average of 60% more residue-derived C in the bulk soil compared to the averaged surface-applied treatments. When I normalized SOC formation by residue-derived respiration, incorporated and surface-applied/undisturbed residue treatments were not different

from each other. Surprisingly, the surface-applied/disturbed residue treatment exhibited significantly lower residue-derived SOC efficiency than either the incorporated or undisturbed, surface-applied residue. This result was due to significantly lower IPOM and hPOM formation in the surface soil when the soil was disturbed. Soil disturbance may have inhibited preferential flow pathways and reduced soil fauna activity. Soil disturbance of the incorporated treatment was less important due to increased residue decomposition via soil microclimate and POM formation via aggregation. The similarity in SOC stabilization between incorporated residue and surface-applied/undisturbed suggest that soil structure and fauna were very important for POM formation in this NT system.

There was an average of 29 and 43% more residue C in POM than MAOM for incorporated and surface-applied/undisturbed treatments. The higher importance of POM formation pathways is likely due to aggregation and soil fauna processing which are unique to our high-clay, long-term NT system. A small amount (7-25%) of residue was decomposed after 6 months because of cold, dry season conditions which may help explain why surface-applied and incorporated residue had similar SOC efficiencies. Based off our Chapter 2 results, residue might continue to decompose on the surface after incorporated residue has become protected within the soil matrix. If incorporated residue has a higher SOC efficiency after the growing season, this might explain why NT does not consistently store more profile wide SOC than CT in temperate systems.

My thesis research has focused on advancing our understanding of residue decomposition in the context of varying residue and tillage management in agroecosystems and shows that these decisions can change the short-term C stabilization mechanisms. I chose to study an irrigated corn system after previous studies highlighted uncertainties surrounding the fate of residue C and long-term SOC storage in an irrigated NT system. While my results focus on the first 12 months

after residue addition, this project will continue for another two years to understand the longer-term results of residue decomposition under NT and CT residue management. Our results suggest that while NT management slows residue decomposition during the off season, CT management protects residue from surface climatic fluctuations during the growing season. After 6 months (off season) the long-term NT field this experiment was established in provided resident biota for short-term POM formation for surface-applied residue. Surface-applied residue POM might have been formed by microaggregate encapsulation following earthworm digestion, while incorporated residue POM may have been protected by aggregation. Even though our clay loam soil has a high potential for MAOM formation, this study demonstrated the importance of biotic pathways of POM formation for short-term residue SOC dynamics.