DISSERTATION

WHEN THE WELLS RUN DRY: SOIL ORGANIC CARBON DYNAMICS DURING THE TRANSITION FROM IRRIGATED TO DRYLAND CROPPING SYSTEMS

Submitted by

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ABSTRACT

WHEN THE WELLS RUN DRY: SOIL ORGANIC CARBON DYNAMICS DURING THE TRANSITION FROM IRRIGATED TO DRYLAND CROPPING SYSTEMS

In many parts of the world, irrigation must decrease due to declining water availability and increased demand from other water users. The Ogallala Aquifer, one of the biggest aquifers in the world, is one example where declining groundwater levels threaten agricultural productivity and social communities across large parts of the semiarid High Plains. In this semiarid region, irrigation is not only fundamental for crop productivity, but it also has positive effects on soil organic carbon (SOC). However, little is known about the changes in SOC dynamics during the transition from irrigated to dryland cropping systems, which has important potential implications for the long-term productivity of these agricultural systems as well as the potential for the soils of the region to be a net sink or source of CO_2. The general objective of my dissertation was to study how irrigation retirement affects SOC dynamics in semiarid agricultural systems of the Ogallala Aquifer Region. I used field experiments to quantify the early changes in crop productivity and C inputs, soil microbial communities, C outputs and SOC formation and turnover during the transition from irrigated to dryland cropping systems. Irrigation retirement had a stronger influence on C inputs than on C outputs because plants responded faster and to a greater magnitude than soil microorganisms to water limitations. Given intrinsic differences in growing season and water requirements, crops vary in their sensitivity to water stress, and wheat agroecosystems were less affected by irrigation retirement than maize agroecosystems. After three growing seasons, there was lower microbial activity and SOC formation in dryland (retired) than irrigated maize, but we did not find changes in the decomposition rate of old SOC. In winter wheat, low differences in soil moisture and crop productivity resulted in almost no changes in microbial activity and SOC dynamics after irrigation retirement. These short-term study results suggest that large losses of crop productivity and C inputs without changes in C outputs will decrease the formation of new SOC, thus affecting SOC storage on the longer term. I confirmed this outcome with on-farm observations of the longer-term effect of irrigation retirement on SOC stocks under different management options. After 7-10 years, sites that used to be irrigated and transitioned back to dryland systems had lower SOC than long-term irrigated sites and had the same SOC stocks as long-term dryland fields, confirming the relatively short legacy effect of irrigation. An exception to this was the transition from irrigated agriculture to perennial, ungrazed grasslands enrolled in the Conservation Reserve Program (CRP). Fields that transitioned into CRP were able to maintain intermediate SOC levels that did not differ from the currently irrigated controls. Taken together, the results of my dissertation indicate that there will be rapid and significant losses of SOC during the transition from irrigated to dryland cropping systems in the Ogallala Aquifer Region. These losses will occur mainly in response to changes in C inputs. Therefore, comparison of biomass and residue production could be used to rapidly identify crop and vegetation management strategies with higher potential to minimize the negative impact of irrigation retirement on SOC.

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DEDICATION

To Pechu, who used to ask me *“Qué descubriste?”*,

I’m still searching

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Chapter 1: Introduction

Land and water are two of the most important agricultural production factors (FAO, 2011) and are highly interrelated, such that actions aimed to conserve one resource may impact the other (Brauman et al., 2007; Jackson et al., 2005). Moreover, both resources are at risk, and improving their management is fundamental to face current and future global challenges (FAO, 2011; Foley et al., 2011; Pretty et al., 2010). The goal of my dissertation is to understand how irrigation retirement, a management practice to save water, impacts soil organic carbon dynamics in semiarid agricultural systems.

Irrigation is a fundamental management practice to increase the productivity of agricultural systems worldwide, especially in arid and semiarid climates where water is the main limiting factor for crop productivity (Nielsen et al., 2005; Peterson et al., 1993). However, excessive water withdrawals, declining groundwater levels, and increased competition for water resources result in a need to decrease pumping rates for agriculture (Davis et al., 2018; FAO, 2011; Richey et al., 2015; Smidt et al., 2016; Whittemore et al., 2016). The Ogallala Aquifer Region (OAR) provides one example of this situation, where rates of water withdrawal exceed the recharge rates and result in declining groundwater levels in many parts of the aquifer (Richey et al., 2015; Smidt et al., 2016). The Ogallala Aquifer is one of the most important aquifers in the world and has a great influence on crop production and social development in the High Plains of the United States (García Suárez et al., 2018; Hornbeck and Keskin, 2014). Therefore, groundwater conservation is crucial to maintaining agricultural production, rural communities, and ecosystem services in the OAR into the future. Agriculture is the main consumer of water withdrawals from the aquifer, which makes agricultural water management a key component of sustaining viable communities across the region.

To extend the life of the aquifer and to meet water compacts with neighboring states, water pumping rates must decrease sharply (Steward et al., 2013; Whittemore et al., 2016), and an increase in irrigation retirement can be expected at least in some regions of the Ogallala. The National Resource Conservation Service (NRCS-USDA) incentivizes farmers to adopt different conservation management strategies that enhance environmental sustainability of agricultural systems (Stubbs, 2015). Some of these programs, such as the Conservation Reserve Enhancement Program (CREP) or the Environmental Quality Incentives Program (EQIP) support land conversion from irrigated to dryland production. Moreover, there are cases where irrigation retirement is an economic reality for some producers as the capacity of their wells fall below a profitable level. Although there is not a precise estimation of the area already retired from irrigation in the entire region, Deines et al. (2017) estimated that approximately 190,000 hectares were retired in the Republican River Basin during the period 1999–2013. If current rates of decline continue into the future, 24% of the currently irrigated area across the OAR would need to transition to dryland crops or pastures by 2100 (Deines et al., 2020).

Declining groundwater levels and irrigation retirement are projected to have large negative impacts on crop productivity and the economies of the region's agricultural communities. Many authors have studied these impacts and how to adapt to changes in water availability (Araya et al., 2017; García Suárez et al., 2018; Golden and Johnson, 2013; Leatherman et al., 2004; Norwood, 2000; Norwood and Dumler, 2002; Schlegel et al., 2016; Stone et al., 2008; Terrell et al., 2002). There is also a good understanding of the evolution of groundwater levels, and the main hotspots of groundwater depletion have been identified (Haacker et al., 2016, 2019a; Steward et al., 2013). But, despite the long-term awareness about declining levels in the Ogallala Aquifer (Gutentag et al., 1984; Luckey and Becker, 1999), the associated environmental impacts of this situation has not received much attention until recently. There is currently growing recognition that excessive pumping rates and the related water declines may impact river ecosystems (Perkin et al., 2017) and that soil health and cropping system design have to be optimized to avoid the risk of another dust bowl (Cano et al., 2018; Deines et al., 2020; Gaskill, 2012; Stewart et al., 2010). However, there is still a lack of knowledge about the changes in soil organic carbon (SOC) dynamics during the transition from irrigated to dryland cropping systems.

SOC is the biggest non-geologic global C pool, whose dynamics affect atmospheric CO_2 enrichment and global climate change, and it also affects soil quality, biomass productivity and water quality among several other ecosystem services (Adhikari and Hartemink, 2016; Lal, 2004; Milne et al., 2015; Minasny et al., 2017; Schlesinger, 1997). Thus, understanding the effect of irrigation retirement on SOC dynamics is fundamental to predict the changes in CO_2 emissions, soil quality, and productivity that will happen under these circumstances. This will help to design sustainable agroecosystems that not only conserve water but also to consider other potential environmental impacts.

Due to an increase in biomass production, i.e., C inputs, irrigation can also improve SOC content, particularly in arid and semiarid climates (Emde et al., 2021b; Trost et al., 2013). The expected positive effect of irrigation on SOC has been confirmed in the central High Plains (Blanco-Canqui et al., 2010; Denef et al., 2008; Gillabel et al., 2007; Lueking and Schepers, 1985) where irrigated cropping systems have 15-24% more SOC stocks than their dryland counterparts (Denef et al., 2008; Gillabel et al., 2007). However, the difference in SOC is small compared to the differences in C inputs, estimated to be almost three times higher with irrigation, and so it is usually assumed that irrigation also stimulates SOC turnover (Blanco-Canqui et al., 2010; Denef et al., 2008; Gillabel et al., 2007). It has been proposed that a faster cycling of C occurs because irrigation not only increases biomass production but also increases microbial activity and soil organic matter (SOM) decomposition due to increased soil moisture (Trost et al., 2013).

Based on this previous evidence, it is expected that irrigated cropping systems in the OAR would be more productive and have more SOC, with a faster turnover rate, than dryland systems (Fig. 1.1). Therefore, I hypothesized that SOC dynamics would change following irrigation retirement and the transition to dryland systems. Irrigation retirement will have a negative effect on plant growth, decreasing the C inputs to the soil with a negative effect on SOC stocks. However, the final effect on SOC will depend on the relative differences between C inputs and outputs. For instance, because SOC has a positive impact on crop productivity (Oldfield et al., 2019) C inputs may be higher in a field that used to be irrigated (and so it has more SOC) than in a long-term dryland field. Moreover, if irrigation also affects SOC turnover (Trost et al., 2013) the decrease in soil moisture during irrigation retirement may reduce microbial activity and soil respiration, and the decrease in C inputs may be partially counterbalanced by a decrease in C outputs. Overall, the new SOC balance reached after the conversion will depend on the magnitude of these changes in SOC dynamics. If irrigation retirement directly affects different aspects of SOC dynamics, I hypothesized that there will be a legacy effect of irrigation on SOC and that it is possible to maintain at least a proportion of this SOC difference such that the retired sites will reach a new SOC balance with higher values than the long-term dryland systems.

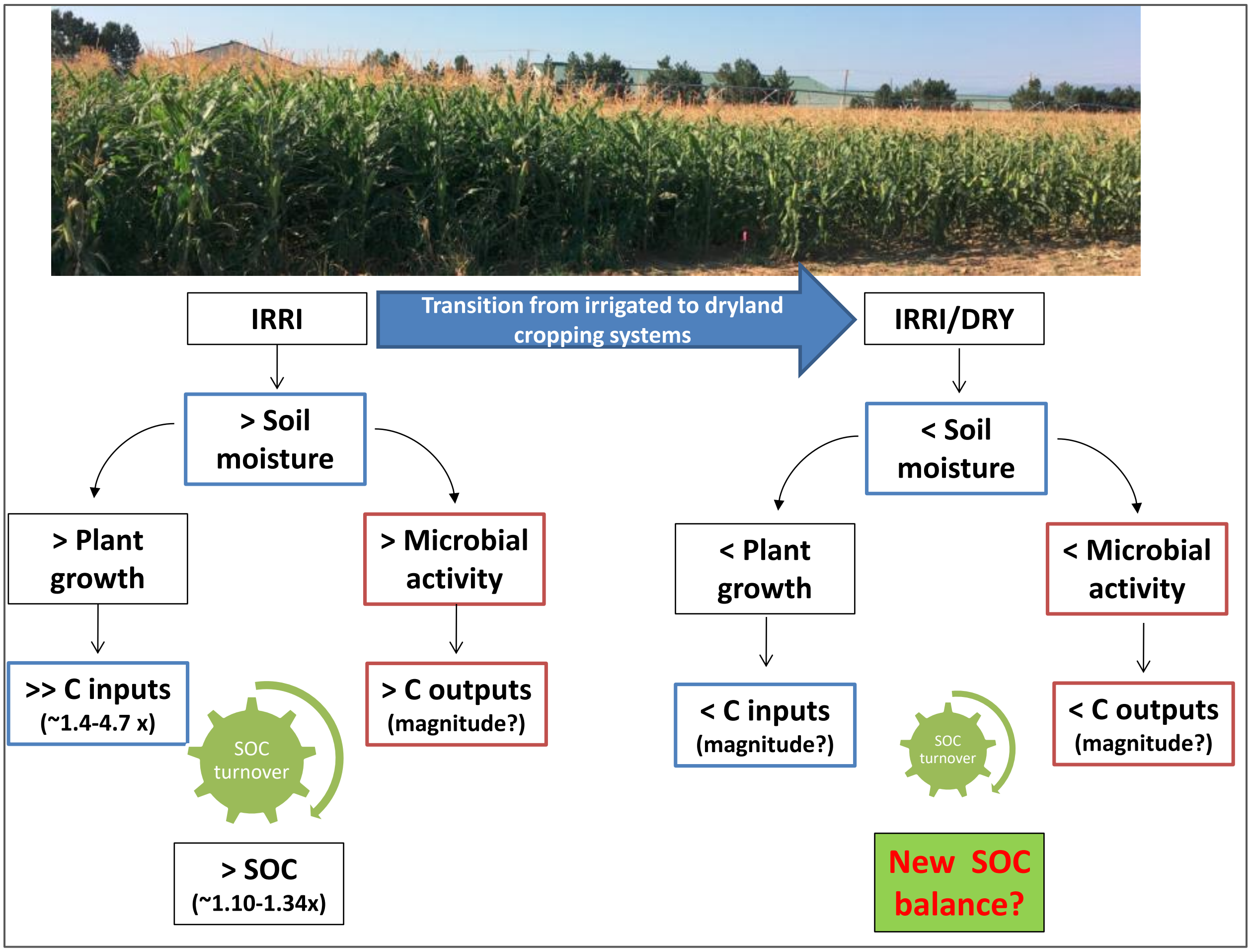

Fig. 1.1. Main effects of irrigation on SOC and hypothesized effects of the conversion from irrigated to dryland cropping systems on SOC dynamics. Diagram of basic effects of irrigation adapted from Trost et al. (2013), values summarized from studies conducted in the Ogallala Aquifer Region (Denef et al., 2008; Gillabel et al., 2007).

The general objective of my dissertation was to study how irrigation retirement affects SOC dynamics in semiarid agricultural systems of the Ogallala Aquifer Region. Specifically, I aimed to answer the following questions:

1. What is the short-term impact of irrigation retirement on the productivity and biomass partitioning of maize and wheat, the two major crops of the semiarid High Plains?
2. How do soil microbial communities respond to irrigation retirement?
3. Is the contribution of aboveground plant litter to SOM formation in no-till systems affected by irrigation?

4. What is the relative effect of irrigation retirement on C inputs and C outputs?
5. Over longer time periods following irrigation retirement, does SOC decline to pre-irrigation, dryland levels or does it stabilize at a higher, intermediate level?

In May 2017 we started a field experiment to represent the transition from irrigated to dryland cropping systems under continuous maize or wheat production. I followed this experiment for three seasons to study the early changes in SOC dynamics after irrigation retirement and took different measurements to inform the first four questions. In Chapter 2, I address the first two questions by quantifying crop production, biomass partitioning, soil microbial biomass, community composition, and activity in the irrigated and dryland (retired) treatments of both crops. To quantify the effect of irrigation on the contribution of aboveground crop residues to SOM formation (Question 3), I used an *in-situ* incubation experiment installed inside the maize treatments. There, I followed the decomposition of dual ^{13}C and ^{15}N labeled maize litter and its contribution to different SOM pools under dryland and irrigated conditions. To gain a mechanistic understanding of the changes in SOC dynamics during irrigation retirement, in Chapter 4, I integrated measurements of soil respiration dynamics, fractionation of SOM, and natural abundance ^{13}C. The last question aimed to evaluate the effect of irrigation retirement on a longer time frame and under real world conditions. For Chapter 5, we sampled working farm fields that stopped using irrigation about a decade ago and transitioned into either dryland crops or perennial grasslands and compared the SOC stocks in these fields with still irrigated and long-term dryland controls.

Chapter 2: Plant and soil microbial responses to irrigation retirement in semiarid cropping systems

Introduction

Agriculture consumes 70% of global water withdrawals (FAO, 2011). In some key agricultural areas of the world this consumption is unsustainable because it exceeds the local availability of water resources (Davis et al., 2018). Moreover, the competition for water resources is growing as municipal and industrial demands increase and water availability decreases (FAO, 2011). Thus, there is an increasing pressure to reduce agricultural water consumption. The Ogallala Aquifer Region (OAR) provides one example of this situation. The Ogallala Aquifer, one of the most important aquifers in the world, has a great influence on the production value of the High Plains of the United States (García Suárez et al., 2018; Hornbeck and Keskin, 2014). However, the rate of water withdrawals to support irrigated agriculture across many parts of the aquifer exceed recharge rates, leading to declining groundwater levels (Richey et al., 2015; Smidt et al., 2016). To extend the life of the Aquifer and to meet water requirements from other users, pumping rates for agriculture must decrease (Whittemore et al., 2016), and an increase in the transition from irrigated to dryland is expected in the region. This transition poses several challenges for the agricultural systems of the region as both crop productivity and soil health will be impacted.

Maize and winter wheat are the dominant crops in the central and northern parts of the Ogallala Aquifer Region (OAR). Maize is the predominant irrigated crop and most of the water for irrigation comes from the Ogallala Aquifer (Norwood, 2000), while wheat is the dominant crop under dryland production (Hansen et al., 2012; Rosenzweig and Schipanski, 2019). During the last decade approximately 50% of the maize area was under irrigation in Colorado, Kansas, and Nebraska, while irrigated wheat represented less than 10% of the total wheat area (USDA-NASS, 2017). In the same period, irrigation increased maize yields by 80-160%, while wheat yields with irrigation were on average 60% more than under dryland. Correspondingly, irrigated cropping systems had more crop residue production, which resulted in almost three times more C inputs than their dryland counterparts in farms of eastern Colorado and western Nebraska, the western and more water-limited portion of the northern OAR (Denef et al., 2008). This difference in C inputs resulted in 27% more soil organic carbon (SOC) in irrigated cropping systems relative to dryland systems at 0-20 cm soil depth (Denef et al., 2008). Thus, irrigated systems are more productive and have more SOC than their dryland counterparts.

Soil organic carbon plays a key role in supporting many of the agronomic functions provided by soils (Kane et al., 2021; Oldfield et al., 2019; Rawls et al., 2003; Wade et al., 2020) and is an important indicator of soil health (Cano et al., 2018; Lehmann et al., 2020). However, SOC changes slowly, and many years may be necessary to quantify its changes (Bradford et al., 2016; Smith, 2004). Soil microbial community composition and activity affect SOC formation, nutrient dynamics, and other important soil functions (Acosta-Martínez et al., 2011; Fierer et al., 2021; Kallenbach et al., 2016) and respond to environmental changes faster than total SOC (Acosta-Martínez et al., 2011; Franzluebbers et al., 1994a; Ndiaye et al., 2000). Because of this, several measurements of microbial processes have been proposed as indicators of soil health that can indicate early changes in SOC and nutrient dynamics (Acosta-Martínez et al. 2011; Cano et al. 2018; Lehmann et al. 2020; Fierer et al. 2021). Among many options, the estimation of microbial community size (microbial biomass), community composition via phospholipid fatty acid analysis (PLFA), and enzyme activity are usually suggested as biological soil health indicators informative for many of the ecosystem services provided by soils (Cano et al., 2018; Lehmann et al., 2020). In the long-term, changes in litter inputs and in microbial communities are expected to affect SOC formation and turnover (Conant et al., 2004).

While SOC tends to be greater in irrigated systems, it is unclear whether SOC-related soil functions can be maintained, including related soil microbial dynamics changes following irrigation retirement. Because soil moisture will decrease and a strong decrease in crop production is expected due to water limitation following irrigation retirement, a decline in resource availability and changes in microbial community composition and activity are expected (Cano et al., 2018; Fuchslueger et al., 2014; Ma et al., 2015; Manzoni et al., 2012). When moisture is a limiting factor, as in semiarid climates or under drought, changes in soil moisture are usually positively correlated with microbial biomass and activity (Sardans et al. 2008; Pérez-Guzmán et al. 2020), but there are also reports of microbes being unresponsive to drought (Canarini et al. 2016) or even increments in microbial biomass during summer droughts (Schaeffer et al., 2017). Differential responses of specific extracellular enzymes to moisture are also reported (Alster et al., 2013; Ren et al., 2017; Sardans et al., 2008) making it difficult to predict general changes. In addition, crop selection and substrate availability can condition microbial responses to irrigation retirement. For example, the transition from a low residue irrigated crop such as cotton to high biomass-producing sorghum may result in an increase in microbial biomass and enzyme activity, even after irrigation retirement in semiarid climates (Cotton et al., 2013). Moreover, because plants tend to produce proportionally more roots than shoots in response to drought (Zhou et al., 2018) substrate availability for microorganisms may be less affected than crop productivity. Thus, although it can be expected that irrigation retirement would have a negative impact on biomass production and soil microbial communities, it is hard to anticipate the magnitude of these effects.

Our objective was to compare the impact of the transition from irrigated to dryland cropping systems on crop production and soil microbial dynamics under the two major crops of the semiarid High Plains. To do this, we installed a transition experiment to quantify crop production, biomass partitioning, soil microbial biomass, community composition, and activity, between irrigated and non-irrigated (retired) treatments for three years in two cropping systems: continuous maize and continuous wheat. Because wheat is more adapted to the climate of this area (Farahani et al., 1998; Hansen et al., 2012), we hypothesized that dryland wheat will be a better option than dryland maize, but it is not clear if dryland wheat will be enough to maintain soil health compared to the baseline irrigated system. This information will help to anticipate longer-term changes in SOC and soil function that may take place across the landscape with larger scale irrigation retirement.

Materials and Methods

Field management and experimental design

The experiment was conducted at the Agricultural Research Development and Education Center of Colorado State University near Fort Collins (40°39'16"N, 104°59'55"W; 1555 m a.s.l.). The climate at the site is characterized by an annual precipitation of 408 mm, with an average annual temperature of 10.2°C (1981-2010 average, https://usclimatedata.com/)). The selected field was historically used for irrigated crop production managed with conventional tillage. The last tillage operation was conducted in April 2017, before installing the experiment. The soil is classified as an Aridic Haplustalf (USDA, NRCS, 2019), and the textural class was defined as a sandy clay loam (54% Sand, 14% Silt, 32% Clay). At the beginning of the experiment the soil had a bulk density of 1.26 g cm^{-3} and pH was 8.1.

In this formerly irrigated field, we initiated a factorial experiment with four treatments, consisting of all combinations of two crops (maize and wheat) and two water managements (irrigated and dryland) in a randomized complete block design with four replicates. Plots measured 48 x 14 m for maize and 24 x 14 m for wheat. As we were interested in the transition from irrigated to dryland under each continuous crop, the irrigated treatments represented controls.

The experiment was started in May 2017 at maize planting. Maize (*Zea mays*) was planted around mid-May and winter wheat (*Triticum aestivum*) between late September and early October. Wheat was harvested in July and maize was harvested in September-October. During the experiment all the treatments were managed using no-till practices, so the studied changes include both the effect of irrigation retirement and no-till adoption. Soil samples were taken at the beginning and end of each growing season, in late spring and fall. Final samples were collected in November 2019, after three maize and two wheat seasons.

Each crop was managed following local recommendations of seeding and fertilization rates. For maize, the Producers Hybrids 5218 SSTX was planted in 0.76-m rows at 84,000 and 42,000 seeds ha^{-1} for irrigated and dryland, respectively. Avery wheat was planted at 100-110 and 60-70 kg seed ha^{-1} for irrigated and dryland, in 0.19-m rows. Fertilization rates were defined based on standard soil test results from the Soil, Water and Plant Testing Laboratory at Colorado State University, and monoammonium phosphate (11-52-0) and urea (46-0-0) were broadcasted during early stages of crop development. On average, irrigated maize received 165 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1} and dryland maize received 90 kg N ha^{-1} and 40 kg P_2O_5 ha^{-1}, annually. Wheat was fertilized with 50 kg N ha^{-1} and 22 kg P_2O_5 ha^{-1} the first year for both treatments, and no fertilization was required on the second year based on soil test results. Pre- and post-emergence herbicide applications were used for weed control. For the irrigated treatments irrigation was done once per week, usually on Tuesdays, from May to September for Maize. Wheat was irrigated from May to June-July, and in 2018 it was irrigated twice after planting at the end of September. Weekly irrigation amounts were managed at the farm level for various fields at the same time, and were defined based on moisture conditions, crop phenology, and weather forecast.

Crop sampling

We quantified the effect of irrigation retirement on above- and belowground biomass production of each crop. Belowground biomass determination was done at flowering of each crop. Using a Giddings soil probe with a 6.5-cm diameter core we took four samples per plot, two in the crop row and two between the rows, and divided the samples by depth (0-10, 10-20, 20-40, 40-60, and 60-80 cm). Samples were kept in coolers before going back to the lab, where samples were refrigerated until root washing. Each sample was soaked in water and passed through three sieves of 2.0 mm, 1.0 mm, and 0.5 mm, and the roots were recovered using tweezers. Recovered roots were rinsed, oven-dried at 55-60 °C for at least

48 hours and weighed. We accounted for the sampled area and averaged the in-row and between-row samples to estimate belowground dry matter per hectare at each sampling depth. Two exceptions to this protocol were for Maize 2017, when it was impossible to sample below 60 cm, and for Maize 2019, when root recovery was affected by a failure in the root washing procedure that resulted in the loss of many samples. To overcome this problem, we estimated root biomass for Maize 2019 at crop harvest during soil sieving to 8 mm without soaking the sample in water. Thus, the estimation of root biomass on this last crop is composed mainly of coarse roots and presented as total biomass per area, as we did not record the root distribution by depth.

Total aboveground biomass production was measured at physiological maturity of each crop by sampling two representative sub-areas from the center of each plot. We sampled a total area of 9.12 and 4.56 m^2 for maize and wheat plots, respectively, weighed the entire sample and separated the grain from the stover. A representative subsample of each component was oven-dried at 55-60°C for at least 48 hours to calculate total dry matter from fresh weights. To compare the productivity between crops, we assumed than mean caloric content was 356 kcal per 100 g for maize grain and 335 kcal per 100 g of grain for wheat (FAO, 2001). We used the line-transect method (Laflen et al., 1981) to estimate soil residue cover in the maize plots at planting and harvest of the second and third year.

Soil sampling and analysis

We assessed microbial responses to irrigation retirement and crop selection by quantifying the changes in microbial community size, composition, and activity. We used chloroform fumigation to quantify the total size of the microbial community, phospholipid fatty acids to explore changes in community composition, and extracellular enzyme assays (in the last sampling) as a proxy of potential microbial activity. We collected soil samples for microbial community analysis twice a year, in spring (May-June) and fall (October-November), at 0-10 and 10-20 cm depth. We took 15-20 2-cm diameter soil cores, avoiding the borders of each plot. Cores were composited by plot and depth, placed in plastic bags, stored in coolers, and transported to the lab, where they were refrigerated for 1-2 weeks until sample processing and analysis.

To estimate soil gravimetric water content of each sample, a 10-g subsample was dried at 105°C for 48 h and reweighed. We used a simultaneous chloroform fumigation-extraction procedure to estimate microbial biomass and extractable organic carbon (C) and nitrogen (N) (Fierer, 2003). Briefly, 20-g duplicates of each sample were shaken for 4 h at 75 rpm in 100 ml of 0.5 M K_2SO_4 with or without 1 ml of chloroform, centrifuged 10 min and filtered through 0.45 µm filters. The extracts were analyzed for total organic C and N with a TOC-V-TN analyzer (Shimadzu Corp., Kyoto, Japan). We estimated microbial C and N as the difference between the chloroform-treated and the untreated subsamples and salt-extractable C and N as the amount extracted with K_2SO_4 in the untreated subsamples. Some calculations resulted in negative estimates of microbial C or N. In those cases, the extracts were reanalyzed in the TOC-V-TN analyzer and, if the estimates remained negative, the values were removed before data analysis. Based on this, we removed 6% and 5% of the microbial C and N estimates, respectively. As the main interest was to compare between treatments, we did not apply an extraction efficiency factor to estimate total microbial biomass. To estimate the proportion of inorganic N in the extracts we determined nitrate and ammonium concentrations using colorimetric analyses (Doane and Horwáth, 2003; Sims et al., 1995). Ammonium concentrations were below the detection limit in almost 90% of the samples so only nitrate results are presented.

Phospholipid fatty acids (PLFA) were used to coarsely characterize microbial community composition at 0-10 cm depth. For their extraction a subsample of fresh soil was sieved to 2 mm, cleaned of roots, lyophilized and sent to Ward Labs (Lincoln, Nebraska, US). Based on Hamel et al. (2006) lipids were extracted by shaking 2-g lyophilized soil with 9.5 ml of a 1:2:0.8 v/v/v dichloromethane (DCM)/methanol/citrate buffer solution for 1 h. Then, 2.5 ml of DCM and 10 ml of a saturated KCl solution were added, shaken for 5 min and centrifuged to remove the organic fraction. Phospholipids were isolated in solid phase extraction columns using methanol, after eluting the neutral fatty acids and glycolipids with DCM and acetone. Phospholipids were then methylated with 2 ml of $MeOH/H_2SO_4$ and 2 ml of hexane and quantified by gas chromatography on an Agilent 7890A GC (Agilent, California, US). We used the PLFA 18:2ω6 as a fungal biomarker and the following PLFAs as bacterial biomarkers: i14:0, i15:0, a15:0, 15:0, i16:0, 16:1ω7c, i17:0, a17:0, 17:0, 17:1ω8c, 18:1ω7c, 18:1ω5c, 10Me16:0, 10Me17:0, and 10Me18:0 (Frostegård et al., 1993; Frostegård and Bååth, 1996; Zelles, 1999). Bacterial PLFA biomarkers were divided into gram-positive (i14:0, i15:0, a15:0, i16:0, i17:0, a17:0), gram-negative (16:1ω7c, 17:1ω8c, 18:1ω7c, 18:1ω5c), and actinomycete (10Me16:0, 10Me17:0, 10Me18:0) functional groups.

For the last sampling, in Fall 2019, we also measured the potential activity of six soil enzymes related to cellulose degradation (β-D-cellobiohydrolase and β-glucosidase), protein degradation (L-leucine aminopeptidase and tyrosine aminopeptidase), chitin degradation (β-1,4-N-acetyl-glucosaminidase), and phosphorous mineralization (acid phosphatase). To explore whether the effect of irrigation on microbial activity was only a transient effect due to differences in water availability at sampling or whether treatment effects persisted when moisture was equalized, we compared extracellular enzyme activity in fresh and air-dry soil samples. Thus, each sample was analyzed twice, at field moisture conditions (no more than a week after sampling) and after air-drying the samples for ~2 weeks. Following the protocol of Saiya-Cork et al. (2002) soil slurries were made by homogenizing 1 g of each sample in approximately 120 mL of 50 mM, pH 8.1, tris buffer. Then, 200 µL of each slurry was pipetted into black, 96-well microplates and mixed with 50 µL of 200 µM substrate. Slurries were also mixed with buffer only or with the corresponding standards (10 mM 4 methylumbelliferone, or 7-amino-4-methyl coumarin) as negative and quenching controls, respectively. Samples were incubated at 25 °C for 4 h and the developed fluorescence read on a microplate reader (Cytation 5, BioTek, Vermont, USA) at 365 nm excitation and 450 nm emission wavelengths.

Statistical analyses

Data exploration and statistical analyses were conducted in R version 3.6.3 (R Core Team, 2020), with the packages *car* (Fox and Weisberg, 2011), *lme4* (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017), and *emmeans* (Lenth et al., 2018). For the statistical analyses of the results, we consider the factorial design of two water managements (irrigated or dryland) and two crop types (maize and wheat), arranged in a randomized complete block design with four replicates.

For the different components of biomass production, we included water management, crop type, and their interaction as fixed effects, and block as random effect, in an lmer mixed model. We also explored root distribution for each crop, but we only found an interaction between water management and depth on maize 2018 so we focused mainly on total root production. The significance of all fixed effects and their interactions were tested by Type III analysis of variance with Kenward-Roger's method. When fixed effects were significant ($p < 0.05$ unless otherwise noted in the text) means were compared using Tukey adjusted pairwise comparisons.

For the soil variables measured seasonally, we included sampling as an extra fixed effect and a random term representing each experimental unit (plot) to consider the repeated measurements design. In Fall 2017, the baseline sampling, we only sampled three plots per block, as only three treatments were planned at that time. Because of this, the baseline sampling was analyzed separately and not considered in the repeated measurement analysis of soil variables. In addition, changes in microbial community composition were explored by non-metric multidimensional scaling (NMDS) of the PLFA data with the *vegan* package (Oksanen et al., 2020). For the analysis of enzyme activity in Fall 2019, the model included the effect of sample handling (fresh vs air-dry) and the interactions with water management and crop type.

Results

Seasonal water dynamics

Annual precipitation during the experimental period was lower than the climatic average for the study site (300 mm year^{-1} for the period 2017-2019 vs climatic average of 408 mm year^{-1}). Precipitation varied both between and within years, with differences in total rain amount and in the distribution of rain events during the summer growing season (Fig. 2.1). Accumulated precipitation was highest in 2017 and lowest in 2018, but 2019 was the year with the lowest precipitation during the critical period for maize yield. Total rain during the critical period of one month around maize flowering (Otegui et al., 1995) was 88 mm, 33 mm, and 14 mm for the years 2017, 2018, and 2019, respectively. Between wheat planting and harvesting, total precipitation was 181 mm in 2017-2018 and 253 mm in 2018-2019.

Given the differences in seasonal weather and crop cycles, the number of irrigation events and total amount of water applied also varied among crops and years (Fig. 2.1). Total irrigation in maize was between 350 and 495 mm annually, which resulted in irrigated maize receiving two to five times more water than the corresponding dryland treatment. Irrigated wheat received 50-80% more water than dryland wheat (141 mm average per crop), with most of the irrigation occurring in late spring and early summer, during the reproductive stages of the crop.

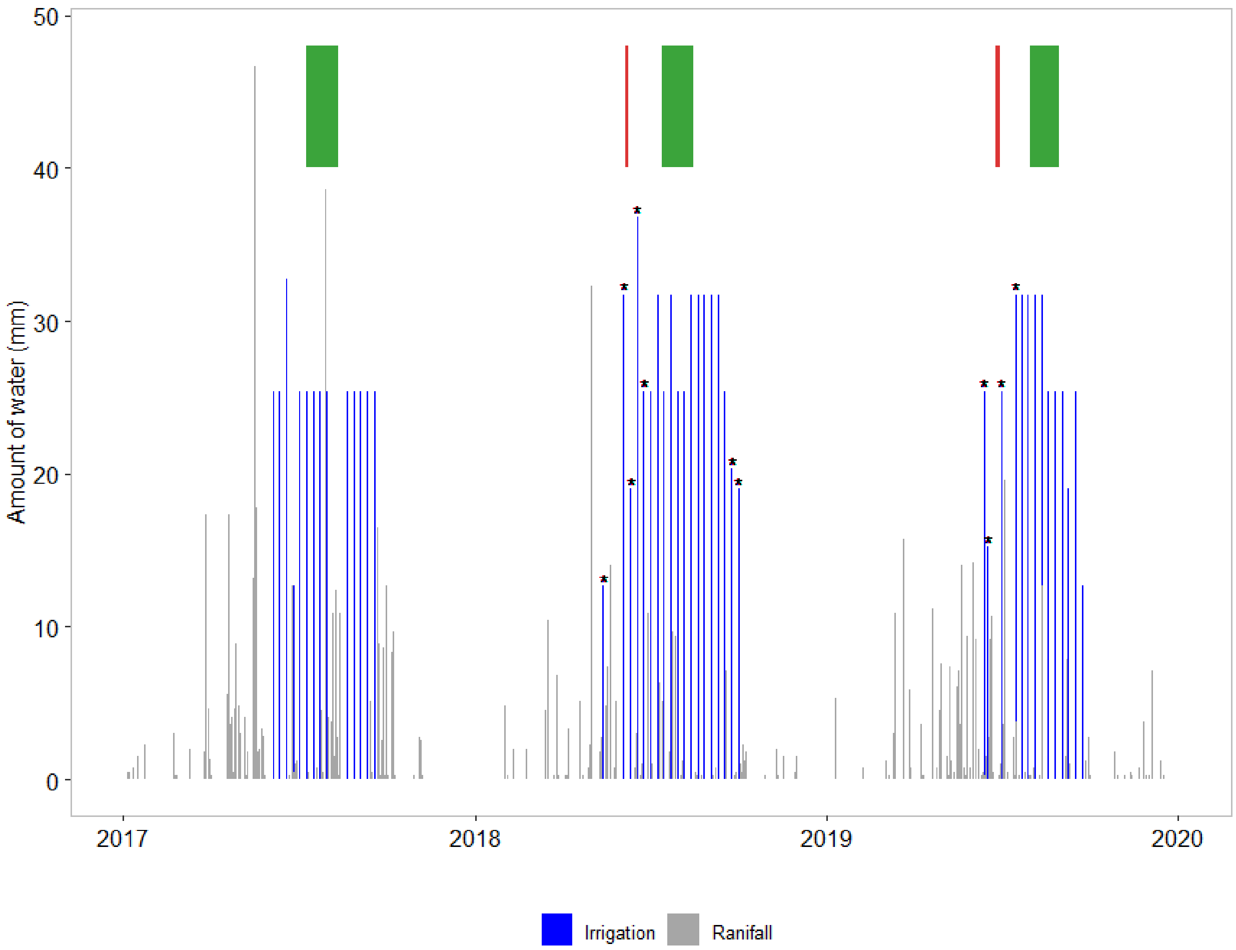

Fig 2.1. Daily precipitation and irrigation during the three years of the experiment. Asterisks denote the days when irrigated wheat was irrigated. Green areas at the top mark the one-month critical period centered in maize flowering, red bars mark wheat flowering.

The seasonal evolution of soil moisture reflected the differences in precipitation, irrigation management, and growing seasons (Fig. 2.2). Maize treatments consumed water mainly during the summer, and soil moisture recovered during the winter, while in wheat plots the recovery of soil moisture occurred after crop maturity in early summer. Irrigation increased soil moisture throughout the experiment, with a higher effect on maize than on wheat as expected due to the more intense irrigation management. Compared to irrigated maize, all the other treatments had lower soil moisture with higher seasonal variability. Gravimetric water content at flowering of each crop confirmed the observed seasonal patterns, with differences due to irrigation increasing each growing season (data not shown). In the first crop after irrigation retirement, the differences at flowering were concentrated in the 0-20 cm layer, but in the following crops irrigation effect on soil moisture was consistent in the entire profile (0-80 cm). We did not observe an important irrigation effect after the first maize crop, in fall 2017, due to late season rain events that recovered soil moisture after the peak of plant water consumption. However, during flowering at the end of July 2017 irrigated maize had 30% more gravimetric water content than the dryland treatment at 20 cm depth (0.15 vs 0.12 g water g dry soil^{-1}).

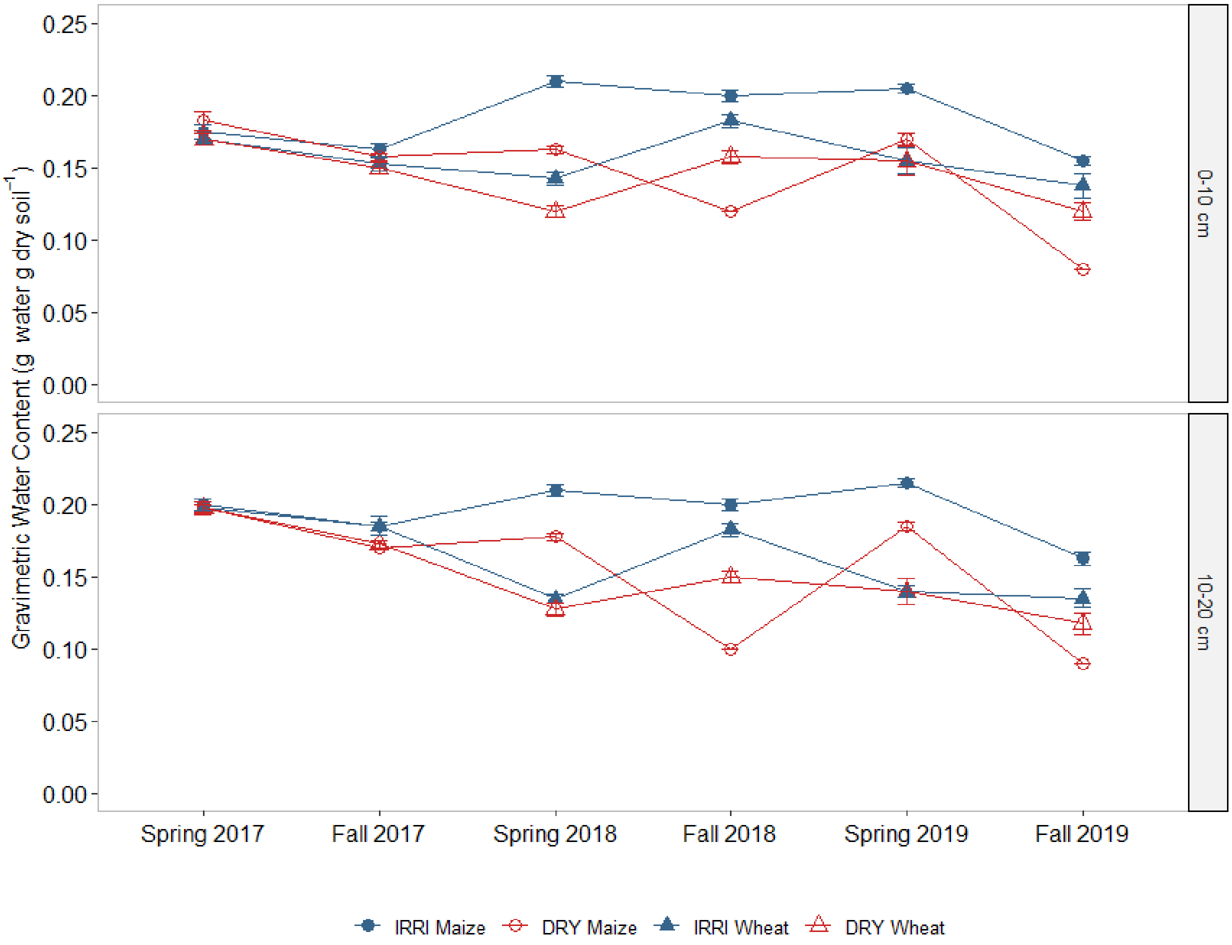

Fig. 2.2. Gravimetric water content per treatment in each sampling period at 0-10 and 10-20 cm sampling depth. Sampling point Spring 2017 corresponds to the baseline before treatment installation. Data are the mean ± 1 SE with n = 4.

Crop biomass production

Crop response to irrigation treatment varied by crop and plant component (Fig. 2.3). Irrigation retirement strongly affected maize production with 2 to 6-fold decreases in total aboveground biomass production and even stronger decreases in grain yield in dryland relative to irrigated maize. The effect of irrigation on winter wheat production was lower than on maize and statistically significant only in 2019, with a stronger effect on grain yield than on biomass production (30% vs. 20% increase). Irrigated maize had the highest biomass production, mostly explained by the high grain yield. Aboveground residue production was similar between irrigated maize and both wheat treatments, and lowest in the dryland maize. Under dryland conditions wheat was more productive than maize, with 13% higher grain yield and 90% more residue production per crop. The large differences in aboveground maize production resulted in differences in soil cover, with irrigated maize having twice the soil cover than dryland (Table 2.1).

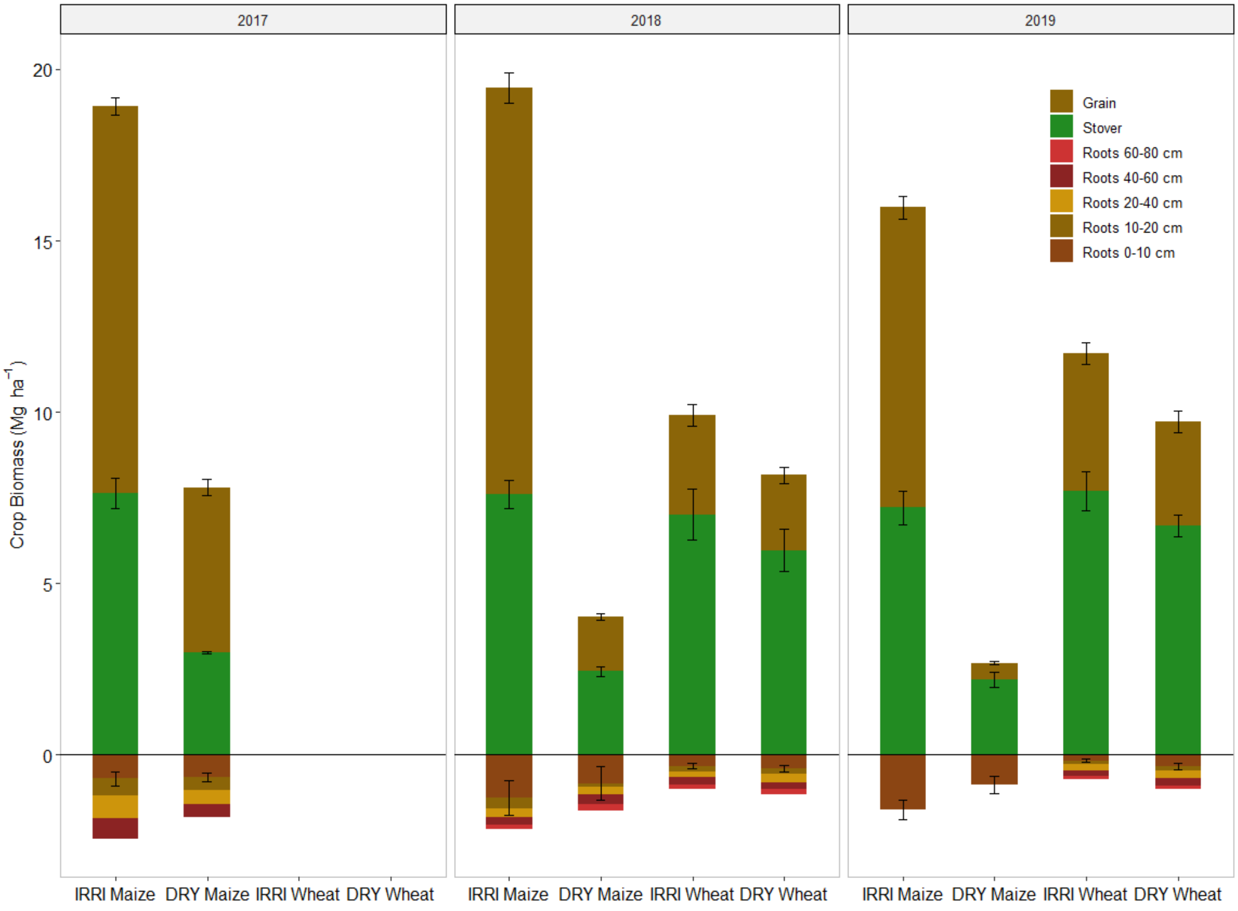

Fig. 2.3. Crop biomass production for each treatment in each growing season. Data are the mean ± 1 SE with n = 4. Root biomass production for Maize 2019 was estimated for the entire profile and no division by depth is presented.

Table 2.1. Percent of soil cover in the maize treatments at the planting of the second crop (Spring 2018), harvest of the second crop (Fall 2018) and planting and harvest of the third crop (Spring and Fall 2019). P-values correspond to the treatment effect from the type III ANOVA with Kenward-Roger's method.

		Spring 2018	Fall 2018	Spring 2019	Fall 2019
Irrigated Maize	Mean ± SE	77 ± 4.2	98 ± 0.9	92 ± 1.0	99 ± 0.3
	CV (%)	10.9	1.8	2.2	0.6
Dryland Maize	Mean ± SE	25 ± 5.3	63 ± 2.9	35 ± 2.7	57 ± 2.8
	CV (%)	41.7	9.3	15.3	9.9
	p-value	0.005	0.001	< 0.001	< 0.001

The irrigation effect on root production was lower than on aboveground biomass. There was 40-80% less belowground biomass in dryland than in irrigated maize, but in 2018 the difference was significant only at 0-10 and 10-20 cm (Fig. 2.3). Dryland wheat had almost 20% more belowground biomass than the irrigated treatment, but the difference was not statistically significant. There was also a crop effect on belowground biomass ($p < 0.05$), as wheat produced lower root biomass than maize (Fig. 2.3). Irrigation retirement increased the root to shoot ratio, and the effect was again higher in maize than in wheat (Table 2.2). The average root to shoot ratio was 2.7 times higher in dryland than in irrigated maize and 1.5 times higher in dryland than irrigated wheat.

Table 2.2. Irrigation effect on root to shoot ratio of each crop. Data are the mean ± 1 SE with n = 4; p-values correspond to the fixed effects of water management, crop type, and their interaction from the type III ANOVA with Kenward-Roger's method.

Treatment	2017	2018	2019
		Root : Shoot ratio	
Irrigated Maize	0.14 ± 0.01	0.12 ± 0.02	0.10 ± 0.02
Dryland Maize	0.23 ± 0.02	0.40 ± 0.12	0.35 ± 0.12
Irrigated Wheat	---	0.11 ± 0.02	0.06 ± 0.00
Dryland Wheat	---	0.15 ± 0.01	0.10 ± 0.02
		p-values	
Water	0.010	0.005	0.002
Crop	NA	0.035	0.001
Water:Crop	NA	0.078	0.061

Soil microbial responses to irrigation retirement

Estimates of soil microbial biomass were variable, and not always statistically significant, but microbial community size was generally lower after irrigation retirement in maize. Across the three years of the experiment with spring and fall samplings, microbial C estimated after chloroform fumigation was on average between 30 to 100% higher in irrigated than in dryland maize at 0-10 cm, but there were significant differences only between sampling times (Fig. 2.4, Table 2.3). Water management had a significant effect on microbial biomass C at 10-20 cm and decreased after irrigation retirement. The effect was again higher under maize production, with almost twice the amount of microbial C in irrigated than in dryland maize (Table 2.4). Microbial N at 0-10 cm depth was affected by the interaction of water treatment and crop, as irrigation retirement tended to decrease it in maize ($p = 0.094$) but had the opposite tendency in wheat ($p = 0.089$). Moreover, dryland wheat had higher levels of microbial N than dryland maize. Microbial N at 10-20 cm depth was not affected by irrigation or crop treatment.

Neither irrigation nor crop type had clear effects on dissolved organic C and N availability. Salt-extractable C varied only in response to sampling season at both depths (Fig. 2.4, Table 2.4). There were differences between treatments in the concentration of total salt-extractable N, but they were explained mainly by the accumulation of nitrate, which was highest in the wheat plots in Fall 2017 and in the dryland maize treatment in the last three samplings (Fig. 2.4). Moreover, there was an accumulation of inorganic N during the growing season and a loss during the winter fallow, as nitrate concentrations were higher in the fall than in the spring samplings. At 10-20 cm depth the results were consistent with the observations at surface (Table 2.4).

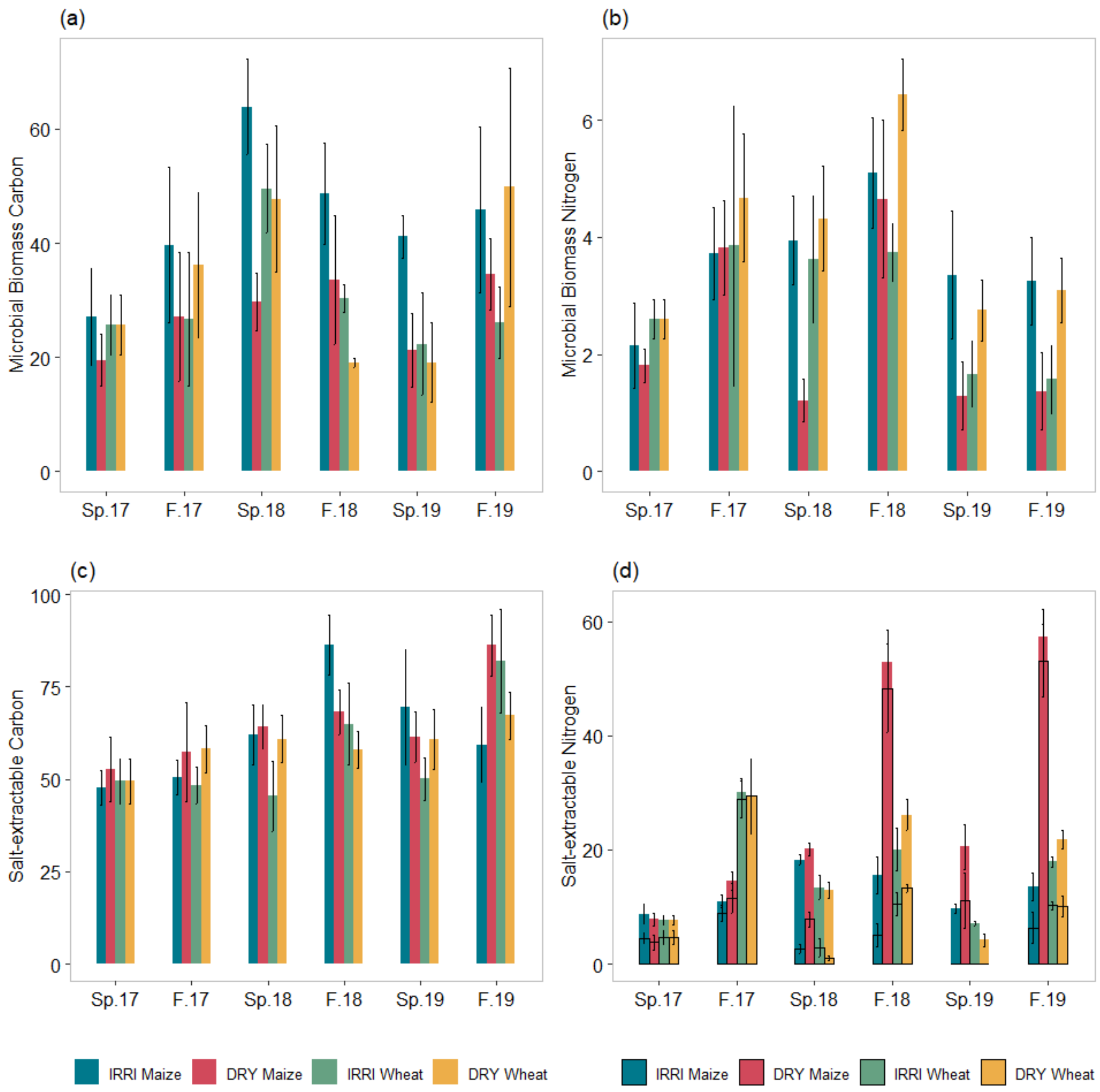

Fig. 2.4. Microbial carbon (a) and nitrogen (b) extracted after chloroform fumigation, and dissolved carbon (b) and nitrogen (b) extracted with 0.5 M potassium sulfate per treatment in each sampling period at 0-10 cm depth, in µg of nutrient per g of dry soil. Sampling point Spring 2017 corresponds to the baseline before treatment installation. Data are the mean ± 1 SE with n = 4; black rectangles over salt-extractable nitrogen represent nitrate concentration in the extracts.

Table 2.3. Treatment effects on the seasonal measures of soil microbial and salt-extractable C and N variables for 0-10cm depth. P-values of fixed effects from the Type III analysis of variance with Kenward-Roger's method.

Factor	df	Fumigation Extractable Microbial Biomass		0.5 M K_2SO_4 Extractable Nutrients		Phospholipid Fatty Acids		
		Carbon	Nitrogen	Carbon	Nitrogen	Total	Bacteria	Fungi
		p-values						
Sampling	4	**0.030**	**<0.001**	**0.002**	**<0.001**	**<0.001**	**<0.001**	**0.001**
Water management	1	0.272	0.994	0.535	**<0.001**	0.099	0.106	0.299
Crop	1	0.433	0.422	0.113	**0.001**	0.706	0.445	0.875
Sampling:Water	4	0.419	0.393	0.195	**<0.001**	0.789	0.541	0.973
Sampling:Crop	4	0.672	0.766	0.447	**<0.001**	0.657	0.832	0.475
Water:Crop	1	0.137	**0.026**	0.943	**<0.001**	0.854	0.833	0.536
Sampling:Water:Crop	4	0.734	0.821	0.076	**<0.001**	0.459	0.546	0.505

Table 2.4. Fumigation extractable microbial carbon and nitrogen, and carbon and nitrogen extracted with potassium sulfate per treatment in each sampling period at 10-20 cm depth. Data are the mean ± 1 SE with n = 4. P-values correspond to fixed effects from the Type III anova with Kenward-Roger's method.

Treatment	Sampling	Fumigation Extractable Microbial Biomass (μg g^{-1})				0.5 M K$_2$SO$_4$ Extractable Nutrients (μg g^{-1})			
		Carbon		Nitrogen		Carbon		Nitrogen	
		mean	SE	mean	SE	mean	SE	mean	SE
Irrigated Maize	Spring 17	15.66	3.47	1.34	0.08	67.32	3.68	11.36	0.93
	Fall 17	36.61	15.01	3.55	1.12	65.41	7.47	23.31	2.26
	Spring 18	50.66	2.53	6.40	2.24	94.18	14.97	17.64	1.35
	Fall 18	18.14	4.30	3.67	0.85	78.08	17.48	20.78	2.52
	Spring 19	33.80	7.06	3.91	1.38	45.40	3.88	9.33	0.42
	Fall 19	15.50	298	1.72	0.52	82.83	12.60	10.75	1.26
Dryland Maize	Spring 17	24.96	5.97	2.95	0.57	53.62	4.55	9.60	1.09
	Fall 17	34.52	10.71	2.74	0.37	66.49	5.62	27.01	2.69
	Spring 18	18.93	8.08	3.30	0.83	71.86	11.37	25.99	2.28
	Fall 18	11.46	1.96	3.95	1.68	62.88	0.39	29.71	3.02
	Spring 19	23.26	10.74	2.65	0.93	55.83	12.66	22.14	5.02
	Fall 19	5.11	2.76	1.81	0.53	81.36	7.77	22.64	3.18
Irrigated Wheat	Spring 17	40.29	11.17	3.53	1.07	53.89	5.39	11.18	0.51
	Fall 17	39.02	7.05	3.97	1.57	73.71	7.37	40.49	2.39
	Spring 18	29.45	8.48	3.81	0.71	76.62	7.36	16.72	3.47
	Fall 18	7.32	1.93	1.09	0.65	81.36	8.39	30.28	2.38
	Spring 19	57.41	17.38	3.68	0.93	58.84	10.20	4.28	0.52
	Fall 19	17.05	5.08	2.32	0.43	72.23	6.86	14.09	1.29
Dryland Wheat	Spring 17	40.29	11.17	3.53	1.07	53.89	5.39	11.18	0.51
	Fall 17	24.58	19.75	2.54	0.35	67.79	6.30	41.24	2.50
	Spring 18	15.73	6.28	4.32	0.85	73.67	9.73	17.26	2.50
	Fall 18	20.05	3.80	4.01	0.98	85.46	3.43	36.88	1.28
	Spring 19	24.27	5.94	1.83	0.47	44.98	7.75	5.00	0.72
	Fall 19	27.93	9.94	1.71	0.73	81.70	6.90	16.41	1.02

Factor	df	p-values			
Sampling	4	0.005	0.015	<0.001	<0.001
Water management	1	0.045	0.350	0.531	0.001
Crop	1	0.763	0.430	0.779	0.285
Sampling:Water	4	0.157	0.181	0.880	0.196
Sampling:Crop	4	0.243	0.799	0.800	<0.001
Water:Crop	1	0.668	0.436	0.089	0.016
Sampling:Water:Crop	4	0.290	0.370	0.324	0.312

Soil microbial biomass and total bacteria, assessed by PLFA, increased during the experimental period, with the highest values found in the last sampling (Fig. 2.5, Table 2.3). Across samplings and crops, there was a tendency for both indicators to be higher under irrigation (Table 2.3), but the tendency was not consistent over time. The response of the functional groups evaluated was similar, and the non-metric multidimensional analysis showed no differences in microbial community composition associated to either treatment or sampling time (Supplementary Fig. 2.1). The concentration of the PLFA 18:2ω6 (only marker used as a fungal biomarker) was always very low, representing less than 2% of the total microbial PLFAs identified, and it also increased over time across all the treatments.

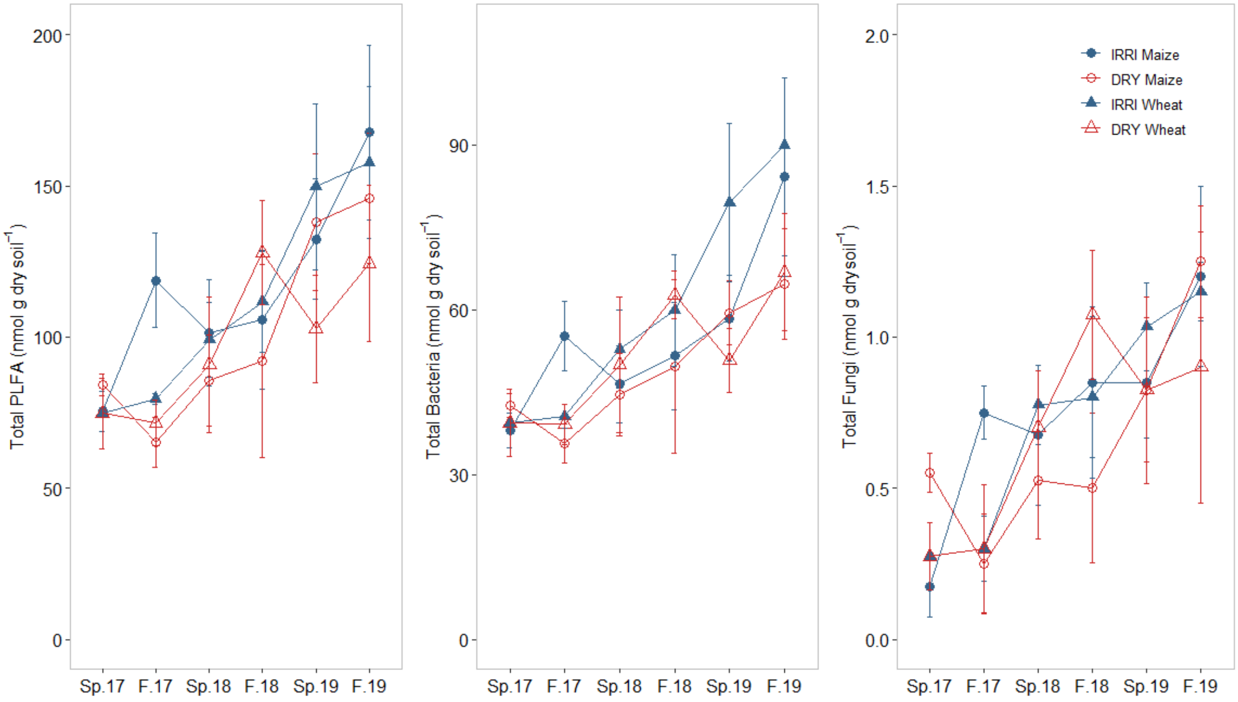

Fig. 2.5. Seasonal evolution of total, bacterial, and fungi PLFAs functional groups in each treatment. Sampling point Spring 2017 corresponds to the baseline before treatment installation. Data are the mean ± 1 SE with n = 4.

There was a significant effect of irrigation and crop type on the activity of the six extracellular enzymes evaluated, which were more often affected by irrigation retirement in maize than in wheat (Fig. 2.6, Table 2.5). Enzyme activity was correlated with gravimetric water content (r = +0.46 to +0.71), and air drying the samples decreased estimated activity in all the enzymes, but the proportional decrease was higher in the irrigated than in the dryland treatments (fresh:dry ratio was 1.64 for irrigated maize, 1.43 for dryland maize, 1.50 for irrigated wheat, and 1.31 for dryland wheat). This resulted in different interpretations of the irrigation effect depending on sample handling. When the assays were conducted using fresh samples, irrigation retirement decreased the activity of β-glucosidase, D-cellobiohydrolase, β-1,4-N-acetyl-glucosaminidase, and acid phosphatase ($p < 0.01$ in all cases), but after air-drying the effect was significant only for β-1,4-N-acetyl-glucosaminidase. For some enzymes, the effect of irrigation retirement also varied by crop type. The activity of L-leucine aminopeptidase and tyrosine aminopeptidase decreased after irrigation retirement in maize but increased when irrigation retirement was conducted in wheat. Irrigation retirement decreased the activity of acid phosphatase in maize but did not differ between wheat treatments.

While sample air-drying affected water treatment differences in enzyme activity, it did not affect the estimation of crop effect on any of the enzymes (Table 2.5). The carbon cycling enzymes β-glucosidase and D-cellobiohydrolase had higher activity in wheat than in maize treatments, consistent across irrigation management. The other enzymes, involved in nitrogen and phosphorus cycling, were higher in dryland wheat than in dryland maize, but did not differ between crop type when irrigation was applied. The activities of L-leucine aminopeptidase and tyrosine aminopeptidase were also higher in dryland wheat than in irrigated maize.

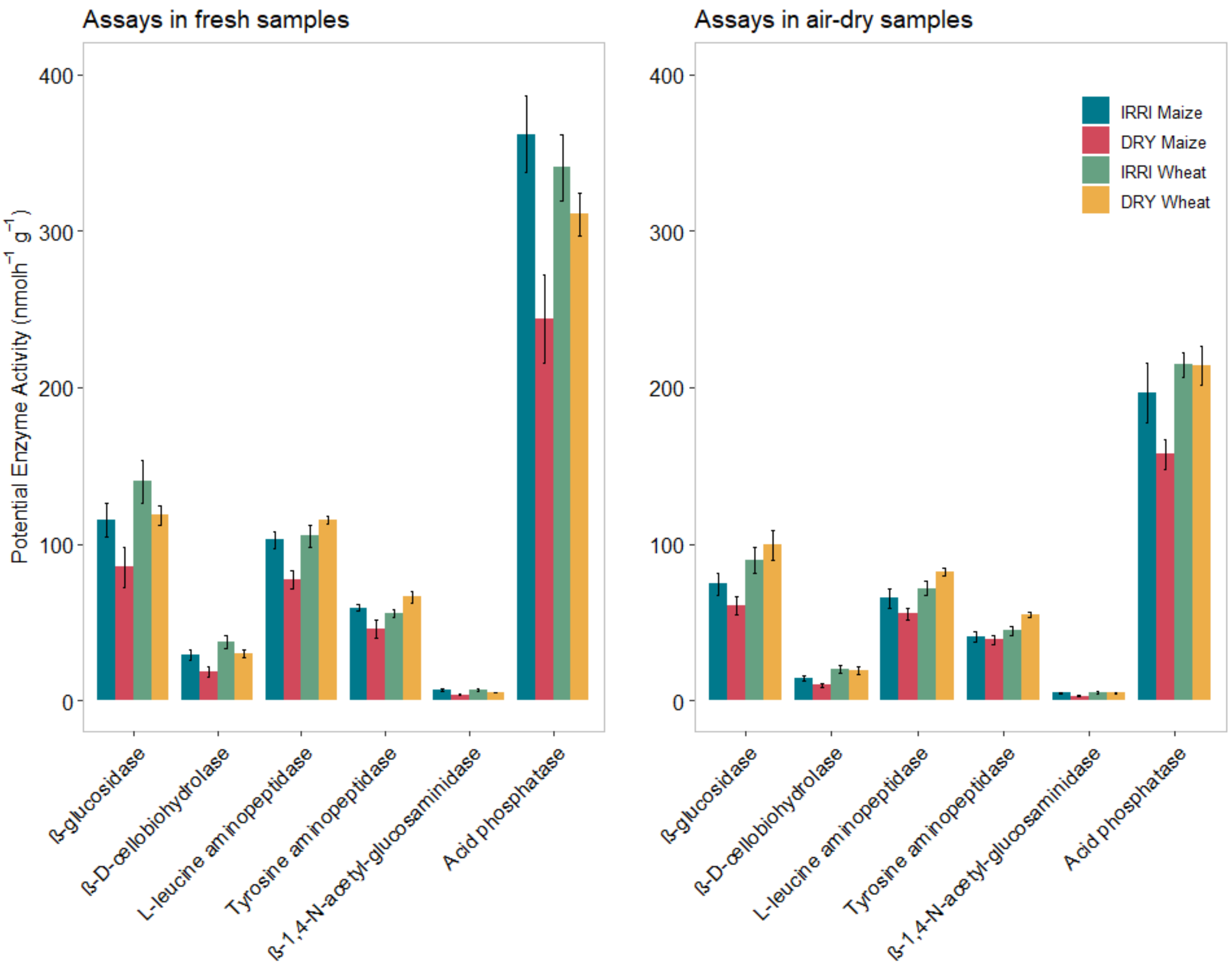

Fig. 2.6. Activity of six extracellular enzymes for each treatment in Fall 2019 as determined in fresh or air-dry soil samples. Data are the mean ± 1 SE with n = 4.

Table 2.5. Effects of treatment and sample handling on extracellular enzyme activities. P-values of fixed effects from mixed effects model, Type III analysis of variance with Kenward-Roger's method.

Factor	df	β-glucosidase	D-cellobiohydrolase	L-leucine aminopeptidase	Tyrosine aminopeptidase	β-1,4-N-acetyl-glucosaminidase	Acid Phosphatase
		p-value					
Water	1	**0.027**	**0.005**	0.266	0.570	**<0.001**	**<0.001**
Crop	1	**<0.001**	**<0.001**	**<0.001**	**0.001**	**0.005**	**0.004**
Air-drying	1	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**
Water:Crop	1	0.187	0.342	**<0.001**	**0.001**	*0.051*	**0.003**
Water:Air-drying	1	*0.055*	*0.081*	0.238	0.272	**0.044**	**0.009**
Crop:Air-drying	1	0.871	0.524	0.583	0.734	0.675	0.444
Water:Crop:Air-drying	1	0.524	0.986	0.252	0.228	0.994	0.206

Discussion

We quantified the effect of irrigation retirement on plant production and soil microbial communities under the two main crops of the Great Plains, maize and wheat. Our results suggest that the expected increase in dryland wheat under water scarcity (Cano et al., 2018; Farahani et al., 1998; Hansen et al., 2012) is a viable option not only to sustain crop production but also to minimize the negative impacts of irrigation retirement on soil health. Lower soil moisture after irrigation retirement decreased plant biomass production in both crops, with a higher effect on maize than on wheat. Despite large changes in soil moisture and plant biomass production, the effect of irrigation retirement on soil microbial community size and composition was limited. However, soil microbial activity was affected by irrigation retirement, again with a greater effect on maize than on wheat. Biomass allocation was also affected by irrigation retirement, as aboveground biomass production was more affected than roots, changing the distribution of C inputs to the soil. While transitioning to dryland wheat might help maintain surface residues, the lower root production of wheat relative to maize may affect the efficiency of SOC formation.

Crop responses to irrigation retirement

Irrigation retirement decreased total crop production and shifted biomass allocation patterns toward belowground in both crops, with more extreme effects on maize. Compared to most crops, maize has high water requirements (Araya et al., 2019, 2017; Lamm et al., 2007) and it was strongly affected by irrigation retirement, confirming the lack of suitability of this crop for dryland production where the experiment was conducted, which had lower average rainfall than the main dryland production region located further east in Colorado. Interannual variability in summer rainfall explains the high differences in the yield of dryland maize across the three years, because this crop is highly susceptible to drought stress in the reproductive stages that occur during the summer months when evaporative demand is highest (Sherrod et al., 2014). In the Great Plains there is an east-west precipitation gradient, with a mean annual precipitation of more than 500 mm on the eastern boundary of the semi-arid zone where dryland maize is more commonly grown (Hansen et al., 2012). This is almost 100 mm more precipitation than the climatic average of our experimental site and 200 mm higher than the annual precipitation during the experimental period, but still less than the total amount of water added with irrigation. Thus, our results can be considered as an extreme example of the potential impacts of irrigation retirement on crop production for the Central High Plains area of the Ogallala Aquifer. However, high variability in precipitation is a characteristic of the Great Plains (Hansen et al., 2012), and dryland maize is an important dryland rotation crop even in parts of the region with mean annual precipitation below 500 mm (Rosenzweig and Schipanski, 2019).

Winter wheat, on the other hand, is well adapted for dryland production in the Great Plains because it uses the water accumulated during cooler fall and spring months and matures before the hot and dry late summer conditions (Hansen et al., 2012). This explains the performance of this crop after irrigation retirement, with differences in grain yield due to irrigation of only 30% while maize yield was at least doubled by irrigation. Although winter wheat is a viable option to maintain crop productivity under water scarcity, the transition from irrigated maize to dryland wheat resulted in a high decrease in productivity. Annual calorie production was more than four times higher in irrigated maize than in dryland wheat (37.9 vs 8.7 x10^6 kcal ha^{-1} crop^{-1}), highlighting the net loss of productivity after irrigation retirement. However, maize is primarily used for feeding livestock whereas wheat is directly consumed by humans, making it difficult to anticipate the net system-wide effect of this transition on food production.

Biomass production and the amount of crop residues returning to the soil impact the evolution of SOC stocks (Halvorson and Schlegel, 2012; Halvorson and Stewart, 2015). After irrigation retirement the production of crop residues in maize was very low, but dryland wheat had levels of residue input per crop similar to irrigated maize. Thus, wheat appears as a more viable option than maize to maintain the levels of surface residues during the transition from irrigated to dryland cropping systems. However, both irrigation retirement and crop type affected the distribution of crop residues and the proportion of roots.

Root production plays a key role in carbon inputs because roots form proportionally more SOC than aboveground biomass residues (Fulton-Smith and Cotrufo, 2019; Mazzilli et al., 2015; Rasse et al., 2005). The higher root:shoot ratio in the dryland treatments may partially offset the negative impact of less crop production on SOC and might explain the lower than expected differences in soil microbial communities between irrigation treatments. But dryland maize produced almost 2.5 times less crop residue mass than irrigated maize. Thus, our results suggest that while shifts in biomass allocation belowground may reduce the rate of SOC depletion with irrigation retirement, the larger gross reduction in ecosystem net primary productivity with irrigation retirement will contribute to reduced SOC over time. A transition from irrigated maize to dryland wheat would help to minimize the decrease in residue inputs, but most of the wheat biomass was produced aboveground, and the average root production of wheat was even lower than the root production of the dryland maize. Thus, even though wheat seems a viable option to maintain C inputs after the transition to dryland systems, reduced allocation to roots will likely limit the proportion of new C that is converted into SOC.

In addition to the importance of crop residues as C inputs, soil cover is important for water dynamics and protection from soil erosion (García-Préchac et al., 2004; Luo et al., 2010; Stewart et al., 2019). Across the three years, irrigated maize had more soil cover than the dryland treatment (92% vs 45% soil cover). This impacted the storage of water during the fallow periods; in fall 2017 both maize treatments ended the growing season with the same soil moisture but before planting in spring 2018 gravimetric water content was higher in the irrigated treatment. Dryland maize may increase wind erosion risks relative to irrigated maize as soil cover was lower, and the distribution of residues more heterogeneous, probably the result of the residue being blown by the wind. Planting density and soil cover were lower in the dryland treatment, which resulted in the ability to generate greater wind speeds across the surface compared to the irrigated treatment, where the higher density of anchored plant stalks generated resistance for the residue to be blown. The low residue accumulation and its rapid loss during fallow increased the proportion of bare soil and the associated risk of wind erosion, an issue of high importance in the Great Plains, particularly during the spring (Farahani et al., 1998; Hansen et al., 2012). Soil cover was very similar in the wheat treatments due to similarities in planting densities and overall biomass production and no differences were observed due to irrigation. Although not measured, soil cover in dryland wheat was higher than in dryland maize, as already reported for annual crops in dryland agroecosystems (Schnarr, *in review*), resulting in another advantage of wheat over maize during the transition from irrigated to dryland.

Soil microbial responses to irrigation retirement

Besides the major changes observed in water dynamics and crop production, the effect of irrigation retirement on soil microbial communities was less evident and mainly limited to the transition under maize. Microbial biomass was lower after irrigation retirement in maize at both sampling depths, but data was variable, particularly in surface soils, and not always statistically significant. Both soil moisture and plant productivity have a positive impact on microbial biomass (Fierer et al., 2009; Pérez-Guzmán et al., 2020) but other factors that were part of our experimental set up may also affect it. Irrigated maize received on average 80% more nitrogen fertilizer than the dryland treatment, which may reduce microbial biomass (Jian et al., 2016; Liebig et al., 2002). Also, no-till management increases microbial biomass in the topsoil (Nunes et al., 2020) and the effect of reduced tillage may be more important than crop rotation (Balota et al., 2003). The use of no-till during the experiment may explain why the microbial C content in the retired treatments did not decrease compared to the baseline sampling (Fig. 2.4) and likely contributed to the increases in all PLFA biomarkers across all treatments during the experimental period. In addition to the positive effect on soil microorganisms, no-till systems favor fungal populations (Acosta-Martínez et al., 2007). The fungal biomarker 18:2ω6 also increased during the experimental period, though its abundance was very low.

The low treatment effect on PLFA indicators is somewhat surprising given the big differences in soil moisture and plant production. We think that the slow response of microbial communities to irrigation retirement may be related to the relatively lower effect of irrigation on root production. The biggest effect of irrigation retirement was on aboveground biomass production, as previously discussed, so in the long-term bigger differences in soil microbial communities between treatments can be expected as the crop residue decomposes and is transferred into the soil.

During fallow periods nitrate accumulates in the soil increasing the pool of inorganic nitrogen (Lamb et al., 1985; Smika, 1990). At the beginning of the experiment, the fallow period in the wheat plots was longer than in the maize treatments, and that was reflected by higher nitrate contents under wheat in Fall 2017. During the last two seasons, dryland maize growth was highly limited by water, with very low biomass accumulation. This resulted in low plant nitrogen uptake and an accumulation of nitrate in this treatment despite lower N fertilizer additions to dryland plots. Nitrate accumulation can have negative environmental impacts due to potential leaching losses (Dinnes et al., 2002; Gregorich et al., 2015) and nitrous oxide emissions during denitrification (Mosier et al., 2006; Robertson and Vitousek, 2009). Although the low soil moisture in dryland maize probably prevented large losses due to denitrification (Firestone and Davidson, 1989; Gregorich et al., 2015; Wrage et al., 2001), the accumulation of reactive nitrogen increases the risk of losses and environmental impacts (Schlesinger, 2009). Moreover, the decrease in nitrate concentrations between the fall and the following spring sampling (Fig. 2.4) is an indication of probable nitrogen losses during the winter and early spring.

Coincident with the tendency observed in microbial biomass carbon, irrigation retirement decreased soil enzyme activity in maize but not in wheat. Enzyme activity has been reported to be positively correlated with crop biomass production (Acosta-Martínez et al., 2011), microbial biomass carbon (Acosta-Martínez et al., 2007; Bhandari et al., 2018; Jian et al., 2016), and soil moisture (Brockett et al., 2012; Burns et al., 2013; Sardans et al., 2008). All these factors were affected by irrigation retirement and probably influenced the differences in enzyme activity found in Fall 2019. Averaged across all enzymes, irrigated maize had almost 50% more enzyme activity than the retired treatment (Fig. 2.6), but it also had more microbial biomass (Fig. 2.4). The difference in the estimated enzyme activity per unit of microbial biomass was lower (1.67 vs 1.39 nmol h^{-1} µg fumigation-extractable C^{-1} for irrigated and dryland maize, respectively) and did not vary due to water management ($p = 0.28$) or crop type ($p = 0.18$), indicating that the difference in enzyme activity in maize was likely due to larger microbial abundance rather than due to differences in specific activity.

Although enzyme activity is assumed to increase with soil moisture (Brockett et al., 2012; Burns et al., 2013; Sardans et al., 2008; Steinweg et al., 2012), the response is not consistent as individual enzymes may have positive, null, or negative correlation with soil moisture (Bell et al., 2009; Sardans et al., 2008) and variable responses to drought or irrigation treatments (Alster et al., 2013; Cotton et al., 2013; Sardans and Peñuelas, 2005; Steinweg et al., 2012). In this study, all enzyme activities were positively correlated with gravimetric water content and air-drying the soil samples decreased the estimated irrigation effect, supporting a direct effect of soil moisture on potential microbial activity. It is usually recommended to conduct enzyme assays using fresh samples (Burns et al., 2013; German et al., 2011) but the use of air-dry samples to evaluate management practices is also common (Acosta-Martínez et al., 2018; Brennan and Acosta-Martínez, 2019; Cotton et al., 2013; Dick, 2011). The decrease in enzyme activity that we observed after air-drying the samples coincides with previous findings (Bandick and Dick, 1999; Turner and Romero, 2010; Wallenius et al., 2010) although increased and unchanged activities have also been reported (Lorenz and Dick, 2011, and references therein; Rao et al., 2003). While some authors have found that sample handling did not impact conclusions (Bandick and Dick, 1999), and others detected treatment effects in air-dry samples (Acosta-Martínez et al., 2011; Cotton et al., 2013; Pérez-Guzmán et al., 2020) we found that air-drying the samples decreased the estimated irrigation effect on enzyme activity. After air-drying soil samples, enzyme activity is supposed to be stable (Lorenz and Dick 2011) and the estimated activity did not vary due to storage time for a year (Rao et al., 2003). However, samples collected at higher moisture levels would take longer to reach stability when air-drying than samples with lower initial soil moisture. The higher relative decrease in enzyme activity during air-drying samples from the irrigated treatments may be related to the potential for enzyme degradation during the drying process. When the goal is to compare management practices that influence soil moisture, our results warn against the use of air-dried samples for the estimation of extracellular enzyme activity.

The decrease in enzyme activity after irrigation retirement in maize may indicate changes in biogeochemical cycling and a potential decrease in residue decomposition (Acosta-Martínez et al., 2018; Schimel, 2018). These differences in enzyme activity occurred despite the low treatment effects on microbial biomass and no differences on microbial community composition, supporting the use of enzymes as early indicators of biological changes (Acosta-Martínez et al., 2011; Bandick and Dick, 1999; Cotton et al., 2013). However, enzyme assays reflect the potential and not necessarily the realized *in situ* activity that can also be affected by changes in soil environmental conditions (Alster et al., 2013; Schimel et al., 2017). For example, reduced water availability may increase enzyme immobilization and reduce diffusion rates, decreasing enzyme efficiency and affecting litter decomposition independently of changes in potential enzyme activity (Alster et al., 2013).

We also found a crop effect on enzyme activity that was consistent in both fresh and air-dry samples, suggesting that enzymes did not only respond to soil moisture. Higher enzyme activity in wheat than in dryland maize was expected, given the differences in biomass production and soil moisture. But the differences observed between wheat and irrigated maize cannot be explained by these factors. Litter addition stimulates enzyme activity (Dornbush, 2007; Tian and Shi, 2014), and the litter effect can be more important than the presence of growing plants (Dornbush, 2007). Given the differences in the growing season of each crop, the time between crop harvest and soil sampling varied. In Fall 2019, we sampled the soil 99 days after wheat harvest but only 22 days after the harvest of irrigated maize. The warm conditions (average temperature 14 °C) and moisture availability (32 mm accumulated precipitation) during this longer fallow time before soil sampling in wheat likely stimulated initial stages of wheat litter decomposition and enzyme activity. Non-structural plant components and nitrogen are lost at a high rate during initial stages of litter decomposition (Cotrufo et al., 2015). Although we did not observe significant differences in the pool of dissolved organic nutrients, the higher activity of L-leucine aminopeptidase and tyrosine aminopeptidase, involved in the hydrolysis of amino acid residues (Cenini et al., 2016; Koch et al., 2007), in dryland wheat compared to irrigated maize supports the idea that enzyme activity was stimulated by the wheat residue decomposition processes.

The effects of irrigation on soil microbial properties and their evolution during irrigation retirement will be affected by edapho-climatic and management variables (Calderón et al., 2016; Cano et al., 2018; Ghimire et al., 2014). The adoption of conservation practices such as no till, crop intensification and diversification, or rotation with pastures, will have positive effects on soil health (Acosta-Martínez et al., 2004; Balota et al., 2003; Cotton et al., 2013; Davis et al., 2012; Rosenzweig et al., 2018) and can help to minimize the negative impacts of irrigation retirement. Our study aimed to isolate crop type and irrigation effects on SOC dynamics. Because of this, we decided to simplify the system and keep each treatment under a continuous single crop, but we acknowledge that other management practices should be considered to decrease soil quality losses during irrigation retirement.

Conclusions

It has been estimated that 24% of currently irrigated lands in the Ogallala Aquifer will be unable to support irrigated agriculture by 2100 (Deines et al., 2020), and many farmers will have to stop irrigating and transition to dryland systems. While the transition to dryland is expected to reduce overall system productivity, little is known about the impact of irrigation retirement on soil microbial communities and SOC dynamics. After three years, we found that irrigation retirement had a stronger effect on a continuous maize agroecosystem compared to wheat cropping systems. The high decrease in C inputs and soil moisture had a negative impact on the soil microbial community but of lower magnitude than the effect on crop production. We found a lower microbial community size and activity after irrigation retirement in maize, though data was variable.

The comparison between irrigated maize and dryland wheat demonstrated that wheat is a better crop option to alleviate the negative impacts of irrigation retirement on crop production and soil microorganisms. Winter wheat productivity was less affected by irrigation retirement than continuous maize, which was expected due to the known adaptation of this crop to the climate of the OAR and lower irrigation requirements of wheat relative to irrigated maize. We found almost no differences in soil microbial dynamics in the retired treatment compared to the irrigated control after two wheat seasons with no-till management. However, there will be an inevitable decline in productivity after irrigation retirement, and the switch from maize to wheat would impact the proportion of carbon inputs belowground because root production was lower in wheat than in maize. This change in biomass allocation patterns may impact SOC formation and decrease the proportion of carbon from crop residues that remain in the system. Longer-term evaluations of SOC turnover and stocks evolution are necessary to fully assess the potential of different dryland crops and cropping systems to minimize the negative impacts of irrigation retirement on soil health.

Chapter 3: Irrigation effects on the formation of soil organic matter from aboveground plant litter inputs in agricultural systems

Introduction

Soil organic matter (SOM) plays a fundamental role in most of the ecosystem services provided by soils, from carbon (C) sequestration to nutrient cycling and plant nutrition (Adhikari and Hartemink, 2016; Milne et al., 2015; Minasny et al., 2017; Smith et al., 2015). Given the high amounts of C stored in soils (Jobbágy and Jackson, 2000; Schlesinger, 1997), management of agricultural soils to increase SOM is currently receiving much attention as a climate mitigation strategy (Minasny et al., 2017; Paustian et al., 2016). Effective management for SOM accrual requires the amount of new SOM formed from litter inputs to be higher than the amount of SOM lost through mineralization. Increasing litter inputs to the soil is one of the main management practices recommended to gain SOM in agricultural systems (Paustian et al., 2016; Rumpel et al., 2018), but its effectiveness will depend on the proportion of litter that remains as SOM after decomposition, i.e., on the efficiency of SOM formation. Thus, a clear understanding of the effect of different management practices on litter decomposition and SOM formation is fundamental for an accurate evaluation of their potential to increase SOM.

Irrigation is a management practice with the potential to increase SOM because it increases crop production and C inputs to the soil (Emde et al., 2021a; Trost et al., 2013). In semiarid climates, C inputs are 1.5 to almost 5 times higher in irrigated than in dryland cropping systems (Denef et al., 2008; Gillabel et al., 2007), but most of this difference is due to changes in aboveground crop productivity (Chapter 2). Therefore, the impact of irrigation on SOM would mainly depend on the fate of the aboveground residues and the efficiency of SOM formation from the litter layer. This is particularly relevant in no-till systems, where the residues are kept on the surface and not mixed with the soil.

The formation of SOM from aboveground litter is assumed to occur through two complementary pathways (Cotrufo et al., 2015). In the early stages of decomposition, non-structural compounds are transferred to the soil as dissolved organic matter (DOM) and utilized by microbes with high efficiency, which results in the preferential formation of mineral-associated organic matter (MAOM). Later, after a period of low SOM formation from the decomposing residue, litter fragments are physically transferred into the particulate organic matter (POM) pool (Cotrufo et al., 2015). Additionally, the physical transfer of litter fragments can be accelerated by physical disturbance (e.g., tillage) and by the activity of soil biota, inducing POM formation since the early stages of litter decomposition (Frouz, 2018; Leichty et al., 2021; Six et al., 2004). These two pools, POM and MAOM, differ not only in their formation mechanism but also in their persistence in the soil, their role in nutrient availability, and in their possibility of saturation (Cambardella and Elliott, 1992; Castellano et al., 2015; Cotrufo et al., 2019; Lavallee et al., 2020). Therefore, quantifying the effect of irrigation on litter decomposition and the pathways of SOM formation is necessary to understand the impact of this management practice on SOM and to anticipate changes in response to different water management. Irrigation can affect these pathways by changes in moisture, water fluxes, and microbial activity.

Water is a resource for microorganisms, a solvent of nutrients and metabolites, and a transport medium (Schimel, 2018; Tecon and Or, 2017), so changes in water dynamics due to irrigation may affect the dynamics of litter decomposition and SOM formation. Irrigation increases soil moisture in the litter layer, which can have a positive impact on soil microbial activity (Brockett et al., 2012; Burns et al., 2013; Sardans et al., 2008; Steinweg et al., 2012) and their access to litter substrates (Moyano et al., 2013; Schimel, 2018; Tecon and Or, 2017). This would result in higher litter decomposition under irrigation, and changes in the rate of mass loss with changes in water availability have been reported (Allison et al., 2013). However, microbial respiration has a direct response to moisture under non-limiting substrate availability (Cook and Orchard, 2008; Orchard and Cook, 1983), and irrigation can increase residue losses as CO_2

(Leichty et al., 2021), making it necessary to quantify movement of residue C into SOM and not just the losses from the litter layer.

In addition to changes in litter decomposition, irrigation can increase water percolation, which can stimulate the leaching of water solubles from the litter layer and their transport to the mineral soil. As water moves through the litter layer and the soil, it becomes enriched in DOM (McDowell and Likens, 1988), and water fluxes increase DOM movement in the soil (Park and Matzner, 2003). Thus, higher water fluxes with irrigation may result in a higher transfer of labile decomposition products into the soil and in DOM percolating deeper in the profile (Kaiser and Kalbitz, 2012). As DOM moves through the soil, it is further transformed by microorganisms and can be stabilized as MAOM (Cotrufo et al., 2015; Kaiser and Kalbitz, 2012) or can be lost though leaching (Kindler et al., 2011). Therefore, irrigation may increase the formation of MAOM due to the stimulation of DOM leaching, as has been observed with higher precipitation along a climatic gradient (Haddix et al., 2020). With greater water movement, and in large volumes in a short period of time during irrigation events, the transfer of small litter fragments into the POM pool can also be stimulated by irrigation.

The objective of this work was to quantify the relative effect of irrigation on the contribution of aboveground crop residues to SOM formation in semiarid, no-till agricultural systems. To do this, we installed an *in-situ* incubation experiment where we followed the decomposition of dual ^{13}C and ^{15}N labeled maize litter and its contribution to different SOM pools in a continuous maize cropping system with contrasting water management, dryland (DRY) and irrigated (IRRI). We hypothesized that the formation of SOM from aboveground plant litter would be slower in the dryland system due to moisture limitations, and that irrigation would increase litter-derived SOM formation in both POM and MAOM by the stimulation of DOM leaching and the physical transfer of litter fragments into the soil, respectively. We fractionated the soil by density and size to separate pools informative of the different pathways of SOM formation: DOM, light POM, heavy POM, and MAOM (Cambardella and Elliott, 1992; Christensen, 2001; Cotrufo et al., 2015; Haddix et al., 2016; Kaiser and Guggenberger, 2000; Poeplau et al., 2018; von Lützow et al., 2007). We also quantified the litter-derived SOM in different aggregate classes because aggregation is a widely recognized SOM stabilization mechanism (Fulton-Smith and Cotrufo, 2019; Six et al., 2004; Tisdall and Oades, 1982; von Lützow et al., 2007) that can be stimulated by irrigation (Apesteguía et al., 2015; Blanco-Canqui et al., 2010; Gillabel et al., 2007).

Materials and Methods

Site and experiment description

To quantify how irrigation affects SOM formation from aboveground residue decomposition, we took advantage of an ongoing experiment started in 2017 in northern Colorado, at the Agricultural Research Development and Education Center of Colorado State University, to study the effect of irrigation retirement on soil organic carbon dynamics (Chapter2). The climate at the site is semiarid, with 408 mm mean annual precipitation and a mean annual temperature of 10.2°C (1981-2010 average, https://usclimatedata.com/). The soil is classified as an Aridic Haplustalf (USDA, NRCS, 2019), and the textural class was defined as a sandy clay loam (54% Sand, 14% Silt, 32% Clay). To install our incubation experiment we selected two of the experimental treatments, transitioned from irrigated to Dryland (DRY) and still Irrigated (IRRI) maize, which had large, measured differences in water management and crop productivity in 2017 and 2018 (Chapter 2), but similar soil properties at the onset of this incubation study (Table 3.1).

The ongoing experiment is organized in a randomized complete block design with four replicates, with plots of 48 x 14 m, and has been managed without tillage since 2017. Each season, between May 10 and May 16 we planted the Producers Hybrids 5218 SSTX maize in 0.76-m rows at 42,000 and 84,000 seeds ha^{-1} for dryland and irrigated, respectively, based on standard seeding rate recommendations for the region. On average, irrigated maize received 165 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1} and dryland maize received 90 kg N ha^{-1} and 40 kg P_2O_5 ha^{-1}, annually. Pre- and post-emergence herbicide applications were used for weed control. For the irrigated treatments irrigation was done once per week, usually on Tuesdays, from May to September.

In November 2018, after maize harvest, we installed four 20-cm diameter PVC collars in each of the 8 plots. The collars were inserted approximately 5 cm into the soil between the two center rows to avoid moving them during usual field operations (planting, herbicide applications, and harvest), and we removed all the aboveground litter from them. In each plot two of the collars were randomly assigned as bare soil controls and in the other two collars we added dual ^{13}C and ^{15}N labeled maize litter, for a total of 32 collars [2 treatments (DRY vs IRRI) x 2 sampling times (6 mos and 13 mos) x 2 litter treatments (control and litter added) x 4 blocks].

We used aboveground maize residue grown in a continuous labeling chamber designed by Soong et al. (2014) with an average 4.63 atom% ^{13}C and 7.01 atom% ^{15}N enrichment. The litter was cut into pieces of ~10 cm length and mixed in a 1:1 leaf:stem ratio before adding it to each collar at rate equivalent to 486 g DM m^{-2} (13.67 g per collar). This rate of dry matter was an intermediate value of the stover produced in both treatments (3-year average of maize residue was 749 and 253 g DM m^{-2} for irrigated and dryland maize, respectively). All the collars were covered with a 6.35 mm mesh PVC net and kept free of weeds by periodically pulling out new seedlings and by herbicide applications during the usual weed management of the crop. During the fertilization of the 2019 maize crop the collars were covered to avoid fertilizer inputs.

Litter recovery and soil sampling

We followed the litter decomposition experiment for 13 months and sampled at two time points, at 6 months (in May 2019) before planting the next crop and at 13 months, in December 2019, after harvest. These sampling times were defined to represent the legacy effect of irrigation on decomposition during the winter and the direct effect of irrigation on decomposition during the 2019 growing season. At each sampling time two collars (one control and one litter-added) were sampled from each plot. We recovered all remaining litter on the soil surface within each collar by hand and stored it in a paper bag. After that, we took soil samples at 0-5, 5-10, 10-20 and 20-40 cm depth with a 6.7-cm diameter core using a Giddings probe. To have enough sample, surface samples (0-5 and 5-10 cm) were complemented with an extra sample taken with a 4.6 cm-diameter hand-core within each collar. Additionally, we used 5-cm diameter plastic cylinders to take an undisturbed sample at 0-5 cm depth within each collar for aggregate stability analysis. To avoid contamination, all the control collars were sampled first and all the collars with litter addition next, and the sampling tubes were cleaned between samplings. Samples were kept in coolers until returning to the lab where they were stored at 4°C for 1-2 weeks until further processing and analysis.

Litter and bulk soil analyses

The litter was oven-dried at 60 °C to estimate dry matter recovery and finely ground for further analyses. We analyzed four subsamples of the litter added at the beginning of the experiment and all the litter recovered in each sampling for C and N concentration, $\delta^{13}C$ and $\delta^{15}N$ in an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (EA-IRMS) in the Stable Isotope Facility at UC Davis. We corrected sample weights by ash content after combustion of 1-g subsamples in a muffle furnace at 600 °C for 5 hours.

We recorded total soil sample weight and a 10-g subsample was oven-dried at 105°C to estimate gravimetric water content. Soil samples were homogenized by passing them through an 8-mm sieve and air-dried. A subsample of ~ 200 g of the air-dried samples was further sieved to 2 mm to remove coarse rocks and plant residues before bulk soil analyses and fractionation, while the samples for aggregate analysis were only sieved to 8 mm. We used a roller mill to pulverize a 25 g subsample of the 2-mm sieved soil. Given the presence of inorganic carbon in the soil, each sample was analyzed in duplicates, one to estimate total carbon and nitrogen and the other to estimate organic carbon. Samples for total elemental concentration were weighed in tin capsules and analyzed without any extra treatment. For organic carbon analysis, samples were weighed in silver capsules, wetted, and fumigated for 8 hours with concentrated HCl (Harris et al., 2001). All samples were analyzed for elemental concentration and isotope ratios using an EA-IRMS either in the EcoCore analytical facilities at Colorado State University or in the Stable Isotope Facility at UC Davis. The litter C was not a significant contributor to the soil inorganic pool, so we focused on the litter movement into SOC pools.

Soil organic matter fractionation

We followed the incorporation of litter-derived organic matter into different SOM pools for the depths with the highest amounts of residue-derived total C, 0-5 cm at 6 months, 0-5 and 5-10 cm after 13 months of incubation. We used a density and size fractionation procedure to separate four pools of SOM: dissolved organic matter (DOM), light POM (lPOM), heavy POM (hPOM) and mineral associated organic matter (MAOM) (Mosier et al., 2021; Poeplau et al., 2018; Soong and Cotrufo, 2015). First, we added ~30 ml of DI water to 5 g of sample in a centrifuge tube and, after shaking for 15 min and centrifuging 15 min at 3400 rpm, we filtered the supernatant with a 20 µm nylon filter to recover the DOM fraction. Then, we added ~20 ml of sodium polytungstate (SPT at 1.85 g cm^{-3}) and 12 glass beads to the remaining sample and dispersed the sample by shaking it for 18 hours in a reciprocal shaker. After shaking, we rinsed and balanced the tubes with SPT, centrifuged 30 min for density separation, and aspirate the floating lPOM. We removed the residual SPT by rinsing the remaining heavy sample three times with DI and physically fractionated it into hPOM and MAOM on a 53-µm sieve.

The solid fractions were oven-dried, weighed, and ground with mortar and pestle before analyzing them in the same way as the bulk samples. For DOM, we analyzed C and N concentrations in a TOC-V-TN analyzer (Shimadzu Corp., Kyoto, Japan). After that, we acidified the samples to remove carbonates as in (Leichty et al., 2021), added a small amount of potassium sulfate (0.1-0.2 g), freeze-dried the sample, and analyzed the solid sample for its isotopic composition. Mass recovery in the SOM fractions was always within 5% of the initial sample mass. Carbon recovery was 94 ± 10% (average ± sd) and N recovery was 97 ± 13%.

Aggregate separation

We determined aggregate stability at 0-5 cm following a wet sieving protocol modified from (Elliott, 1986). Briefly, we submerged 50-g of air-dried, 8-mm sieved soil over a 2-mm sieve in deionized water for 2 minutes to allow slaking. After that period, the sample was submerged 50 times during a 2-minute interval to retain only the large macroaggregates on the sieve. For the soil that passed through the sieve, the 2-min procedure was repeated with a 250-µm sieve to isolate small macroaggregates and with a 53-µm sieve to separate free microaggregates from the silt and clay fraction. The fraction that remained over each sieve was collected, oven-dry at 60°C and weighed to estimate aggregate size distribution. The proportion of large macroaggregates was very small, less than 5%, so we combined them with the small macroaggregates for further analysis. Because sand of the same size as the corresponding aggregate fraction is not considered part of the aggregate (Elliott et al., 1991; Six et al., 2000), macro and microaggregates were corrected for sand content. To do this, we dispersed 5-g subsamples of each fraction on 5% sodium hexametaphosphate and sieved the sample through the same sieve size as the aggregate fraction to retain only the sand. Each aggregate size class was ground with mortar and pestle and samples from the 13-month sampling were analyzed in the same way as the bulk soil samples. The concentration of litter-derived C and N in each aggregate size class is presented as sand-corrected values after accounting for the mass of sand retained in each sieve.

Data analysis

We used the isotopic mixing model to estimate the contribution of litter-derived organic matter to each of the analyzed pools:

$$f_{litter} = (\delta_{sample} - \delta_{control}) / (\delta_{litter} - \delta_{control}) \qquad (3.1)$$

where $ƒ_{litter}$ is the proportion of maize litter-derived C or N in each pool, δ_{sample} is the $\delta^{13}C$ or $\delta^{15}N$ of the pool of interest (recovered litter, bulk soil, SOM fraction or aggregate size class) from the litter-amended collar, $\delta_{control}$ is the $\delta^{13}C$ or $\delta^{15}N$ of the pool of interest in the corresponding control collar, and δ_{litter} is the $\delta^{13}C$ or $\delta^{15}N$ of the original litter added at the beginning of the experiment. To facilitate the comparison among different pools, we calculated the total amount of litter-derived C and N (g m^{-2}) in each pool as the product of $ƒ_{litter}$ by the corresponding pool size. We calculated the efficiency of SOM formation from aboveground residues as the ratio of litter-derived C and N gains in bulk soil over C and N losses from the decomposing labeled litter in the litter layer (Cotrufo et al., 2015).

To test if irrigation affected the contribution of aboveground plant litter to SOM, we adjusted a linear mixed-effect model with the interaction of irrigation treatment, depth, and sampling time as fixed effects and the experimental unit (defined as block and block:treatment) as random effects. Our main interest was to quantify the effect of irrigation, so we focused on the pairwise comparisons between treatments with Kenward-Rogers degrees of freedom. We explored the residual distribution to check for homoscedasticity and normality, and log-transformed the dependent variables when necessary. We used R version 3.6.3 (R Core Team, 2020) with the packages *lme4* (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017), and *emmeans* (Lenth, 2020) for all the statistical analyses.

Results

Seasonal characterization

During the 13-month experimental period, average daily temperature was 7.8 °C, and total precipitation was 289 mm (Fig. 3.1). In the first 6 months of incubation, during winter fallow, total precipitation was 83 mm (mostly in the spring) and an average daily temperature of 1.0 °C with no management differences between treatments. During the growing season, between the two samplings, total precipitation was 206 mm, and the irrigated treatment received an extra 358 mm of water in 14 irrigation events that occurred weekly between June and September 2019. Most of the seasonal precipitation happened in the early season, with almost 80% of the summer rainfall accumulated between May and July.

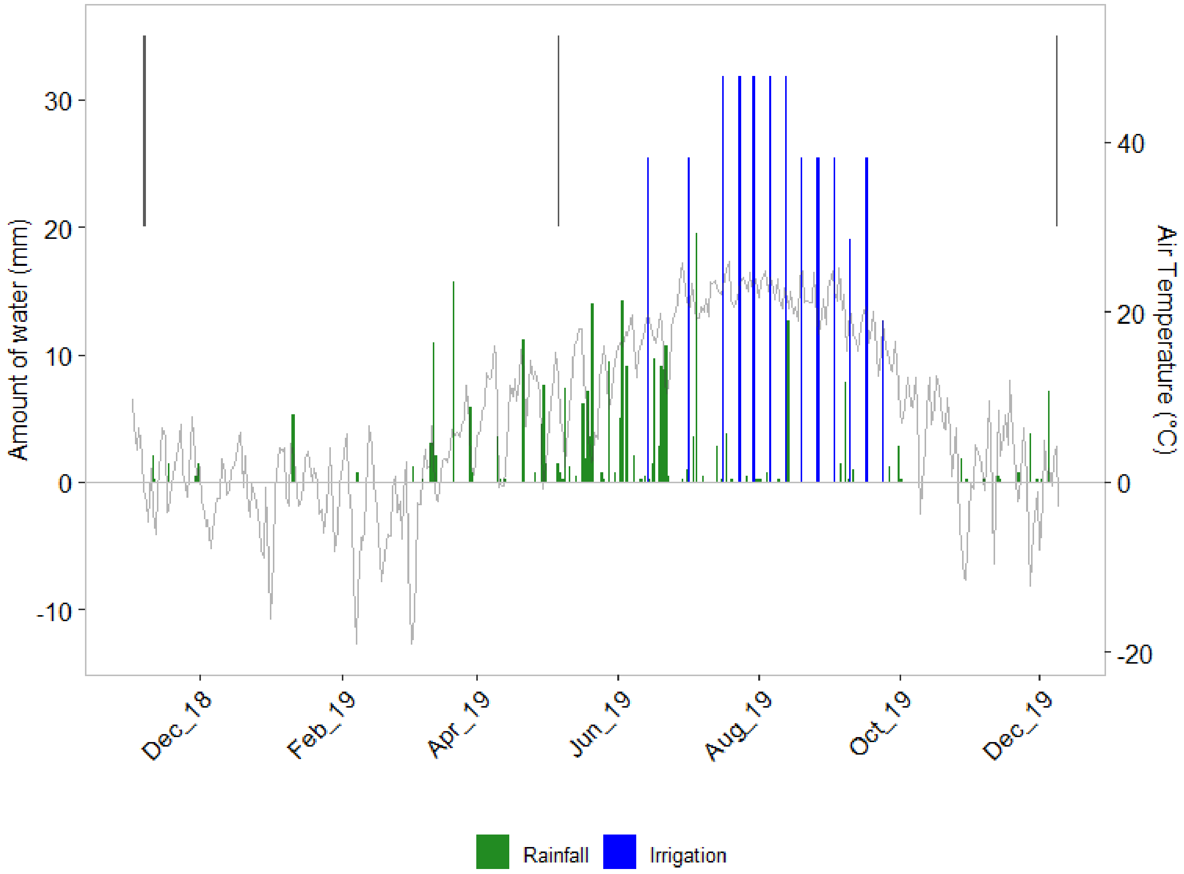

Fig. 3.1. Daily precipitation, irrigation, and air temperature (grey line) during the year of field incubation. Vertical bars at the top represent the beginning of the incubations and the two harvest periods at 6 and 13 months.

Litter decomposition and total SOM formation

Litter decomposition during the winter was low and not affected by irrigation history (p = 0.68), and after 6 months of incubation, we recovered 84% of the dry matter added in the fall (Table 3.1). After 13 months of incubation, and an entire growing season with more than a 2-fold difference in water inputs (Fig. 3.1), the litter mass remaining in the irrigated treatment was lower than the mass remaining in the dryland (Table 3.1, p = 0.056). On average, 50% of the added litter mass was lost in the dryland system and 55% under irrigation. The loss of C from the litter layer followed a very similar pattern than the loss of dry matter (Fig. 3.2). After 6 months of incubation, 82% of the added C remained in the litter layer and we did not find any difference between treatments (p = 0.92), but after 13 months the amount of C remaining in the litter layer tended to be higher in the dryland plots (90.6 ± 10.6 g C m^{-2} in DRY vs 73.1 ± 5.4 g C m^{-2} IRRI, p = 0.083). This represented a loss of 55% of the added C in the dryland treatment and a loss of 64% under irrigation.

The dynamics of N loss from the litter layer differed from those of dry matter and C during the initial stages of decomposition but were very similar after 13 months. After 6 months of incubation, the original N remaining in the litter layer was lower in the dryland system (p = 0.025), but after one year, the N remaining in the dryland system tended to be higher than the N remaining in the irrigated plots (2.15 ± 0.15 vs 1.73 ± 0.06 g N m^{-2}, p = 0.064). N losses during the winter (31% in dryland, 19% in irrigated) were higher than C losses (18%), but after 13 months of incubation, N loss was 53% in the dryland treatment and 62% in the irrigated, similar to the C loss values.

Despite the differences in litter decomposition after 13 months, irrigation did not affect the amount of litter-derived C that formed SOC at either time point. Litter-derived C was concentrated mainly in the surface soil layer, and after 6 months of incubation litter-derived C at 0-5 cm was 8.74 ± 0.31 g C m^{-2} in dryland and 7.72 ± 0.67 g C m^{-2} in the irrigated treatment (p = 0.50). After 13 months of incubation, we recovered 14.45 ± 1.37 g C m^{-2} and 15.36 ± 2.48 g C m^{-2} of litter-derived C at 0-5 cm in the dryland and irrigated treatments, respectively (p = 0.83). At 0-40 cm, litter-derived C recovery was 17.91 ± 1.18 and 17.41 ± 2.51 g C m^{-2} for dryland and irrigated plots, respectively. More than 90% of the litter-derived C recovered in the mineral soil was found above 10 cm depth, and only minor amounts were recovered at 10-20 cm (0.66 ± 0.08 g C m^{-2}) and 20-40 cm (0.44 ± 0.20 g C m^{-2}) after 13 months. When considering total litter-derived C recovery in both the litter layer and soil, there was an effect of the sampling:treatment interaction (P = 0.048), with no differences between treatments after 6 months (p = 0.99) but a tendency of higher C recovery in the dryland system at 13 months (108.50 ± 9.61 for dryland vs 90.50 ± 3.80 g C m^{-2} in irrigated, p = 0.067).

Irrigation did not affect litter-derived N in the topsoil or when accumulated at 0-40 cm (p > 0.6 in all cases), but there were some differences in depth. Contrary to our expectations, litter-derived N recovery after 13 months was higher in the dryland treatment at 10-20 cm (0.21 ± 0.03 g N m^{-2} for DRY vs 0.09 ± 0.03 g N m^{-2} for IRRI, p = 0.001) and at 20-40 cm (0.06 ± 0.01 for DRY vs 0.04 ± 0.01 g N m^{-2} for IRRI, p = 0.060). Total N recovery, in soil and litter layer, at 6 months was 3.97 ± 0.11 g N m^{-2} in DRY and 4.47 ± 0.20 g N m^{-2} in IRRI (p = 0.038), while after 13 months the recovery was higher in the dryland treatment (3.43 ± 0.15 g N m^{-2} in DRY and 2.95 ± 0.07 g N m^{-2} in IRRI, p = 0.044).

The C:N ratio of the litter-derived SOM recovered in the bulk soil at 0-5 and 5-10 cm was affected by sampling and depth (p < 0.001) but did not vary due to irrigation management (p > 0.4). C:N ratio was lower at 6 months (12.5 ± 0.5 at 0-5 cm and 7.4 ± 0.9 at 5-10 cm depth) than at 13 months (16.4 ± 0.8 and 11.4 ± 0.9) and was always higher at 0-5 cm than at 5-10 cm depth.

Efficiency of SOM formation from litter

The effect of irrigation on C recovery was related to differences in C losses from the litter layer more than to differences in SOC formation, as we did not find treatment differences in the stabilization efficiency of litter-derived C. The efficiency of SOC formation was higher during the initial litter decomposition in the off season than during the growing season (24.2% vs 11.1%, p = 0.003) but it was not affected by irrigation. After 13 months of incubation, SOC formation efficiency was 16.4 ± 1.0% for dryland and 13.6 ± 1.5% for the irrigated treatment (p = 0.15).

In the first 6 months of incubation, i.e., the winter period without irrigation events, the stabilization efficiency of litter-derived N was not affected by previous irrigation management. During the growing season, when irrigation was applied, SOM-N formation efficiency was higher in the dryland than in the irrigated treatment (47.9 ± 10.7% for dryland and 23.2 ± 7.7% for irrigated, $p = 0.03$). However, after 13 months of incubation we found weak evidence of a cumulative irrigation effect on SOM stabilization efficiency of litter-derived N (53.2 ± 4.1% for dryland and 43.1 ± 3.3% for irrigated, $p = 0.104$).

Table 3.1. Soil and litter initial characterization and characteristics of the recovered litter layer after 6 and 13 months at the dryland and irrigated maize systems. Data are average ± SE. Soil carbon data corresponds to SOC after carbonate removal. Litter dry matter (DM) corresponds to ash corrected weights.

	Baseline Soil 0-5 cm		Litter added	Litter recovered 6 months (Spring)		13 months (Fall)	
	Dryland	Irrigated		Dryland	Irrigated	Dryland	Irrigated
C (%)	1.07 ± 0.07	1.08 ± 0.08	41.31 ± 0.28	32.90 ± 0.84	34.90 ± 0.87	13.42 ± 0.65	13.24 ± 1.59
N (%)	0.13 ± 0.01	0.14 ± 0.01	0.94 ± 0.08	0.74 ± 0.02	0.86 ± 0.04	0.60 ± 0.02	0.54 ± 0.04
C:N ratio	8.1 ± 0.4	7.9 ± 0.2	45.0 ± 3.7	44.7 ± 1.8	40.6 ± 2.0	22.3 ± 0.9	24.3 ± 1.3
$\delta^{13}C$ (‰)	-20.37 ± 0.17	-19.89 ± 0.50	3347 ± 6	3256 ± 9	3235 ± 9	2640 ± 54	2673 ± 47
$\delta^{15}N$ (‰)	5.87 ± 0.17	5.72 ± 0.24	19516 ± 40	16200 ± 421	16921 ± 90	8235 ± 418	8993 ± 259
DM (g m^{-2})	----	----	435.1 ± 0.5	368.4 ± 2.1	363.7 ± 1.9	220.1 ± 7.8	196.2 ± 12.7

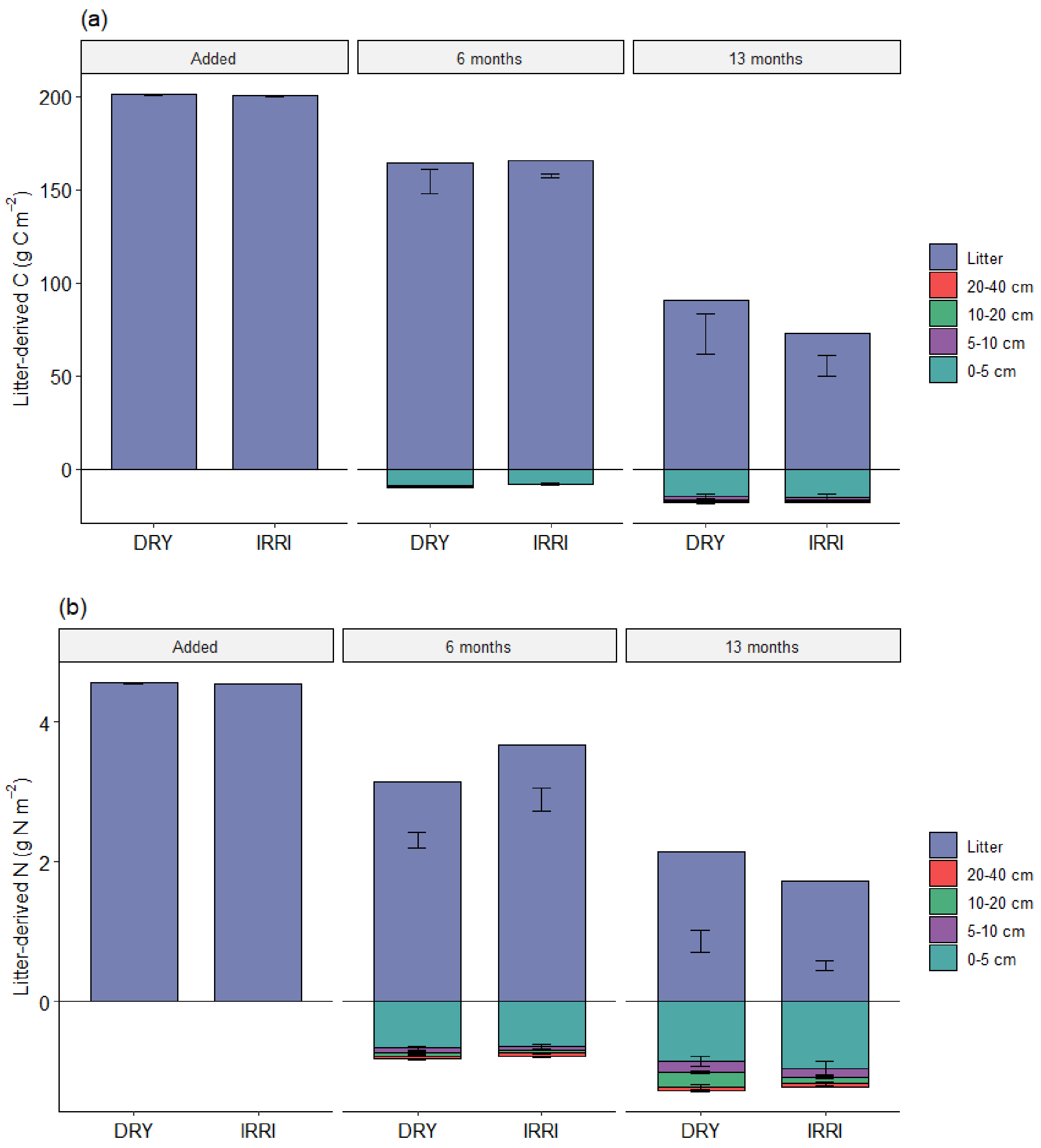

Fig. 3.2. Litter-derived carbon (a) and nitrogen (b) added and remaining in the litter and recovered in the bulk soil organic matter after 6 and 13 months of incubation in dryland and irrigated maize cropping systems. Error bars represent the standard error of the mean with n = 4.

Litter contribution to SOM fractions

To explore differences in the pathways of SOM formation, we quantified litter-derived C and N in different SOM fractions (Fig. 3.3). As expected from the bulk soil results, the amount of litter-derived C recovered in all the studied fractions increased with incubation time ($p < 0.01$) and was highest at the surface. However, irrigation only affected the DOM pool at 5-10 cm, where we found more litter-derived C in the DOM pool of the dryland treatment after 13 months of incubation ($p < 0.001$). The amount of litter-derived C found in the other SOM fractions was not affected by irrigation ($p > 0.10$). Similar to C, litter-derived N found in each SOM fraction at 0-5 cm was affected by sampling season ($p < 0.01$) but not by irrigation treatment ($p > 0.10$). The amount of N recovered in DOM was lower in the fall than in the spring, but the amount recovered in all the other fractions was higher after 13 months of incubation. In addition, similar to C, the amount of litter-derived N found at 5-10 cm was very low compared to the surface and we did not find any difference between treatments.

Irrigation did not affect the distribution of litter-derived organic matter into the different SOM fractions ($P > 0.10$), but there was a time effect as the proportional recovery in the coarse fractions increased in the second sampling ($p < 0.01$). At 6 months, 10 ± 2% of the litter-derived C was recovered in the light POM, and 2 ± 0% in the heavy POM, while after 13 months we recovered 21 ± 3% and 5 ± 0% in the lPOM and hPOM, respectively. The increase of litter-derived C in the coarse fractions was related to a decrease in the proportional recovery in both DOM (10 % at 6 months vs 7% at 13 months, $p = 0.03$) and MAOM (79 ± 1% and 67% ± 3% at 6 and 13 months, respectively, $p < 0.01$), although the majority of the litter-derived C was always recovered in the MAOM pool (Fig 3.3). Similar to C, the proportion of litter-derived N recovered in the coarse fractions (lPOM and hPOM) increased in the second sampling (13 months) while the proportion recovered in DOM decreased ($p < 0.01$). However, contrary to C, the proportion of N recovered in MAOM did not vary between samplings and even tended to increase with time (68 ± 5% and 76% ± 2% at 6 and 13 months, respectively, $p = 0.064$).

While irrigation had few effects on the quantity of litter C and N in SOM fractions, irrigation influenced the relative quality of litter residues incorporated into lPOM and MAOM, as indicated by differences in C:N ratios. After 6 months of incubation, the dryland treatment had a higher litter-derived C:N ratio in lPOM ($p = 0.020$) and MAOM ($p = 0.042$). After 13 months, we did not find any differences at 0-5 cm, but at 5-10 cm the dryland treatment had again a higher C:N ratio in the MAOM pool ($p < 0.01$) and a tendency for higher C:N ratio in the lPOM ($p = 0.07$). Between samplings, the C:N ratio of litter-derived DOM, lPOM and hPOM showed the same evolution as the total litter-derived SOM measured in bulk soil, increasing with time ($P < 0.05$) and being higher at 0-5 cm than at 5-10 cm depth after 13 months. Litter-derived MAOM showed a different trend, as the C:N ratio was higher at 6 months than at 13 months ($p = 0.052$) and did not vary with depth.

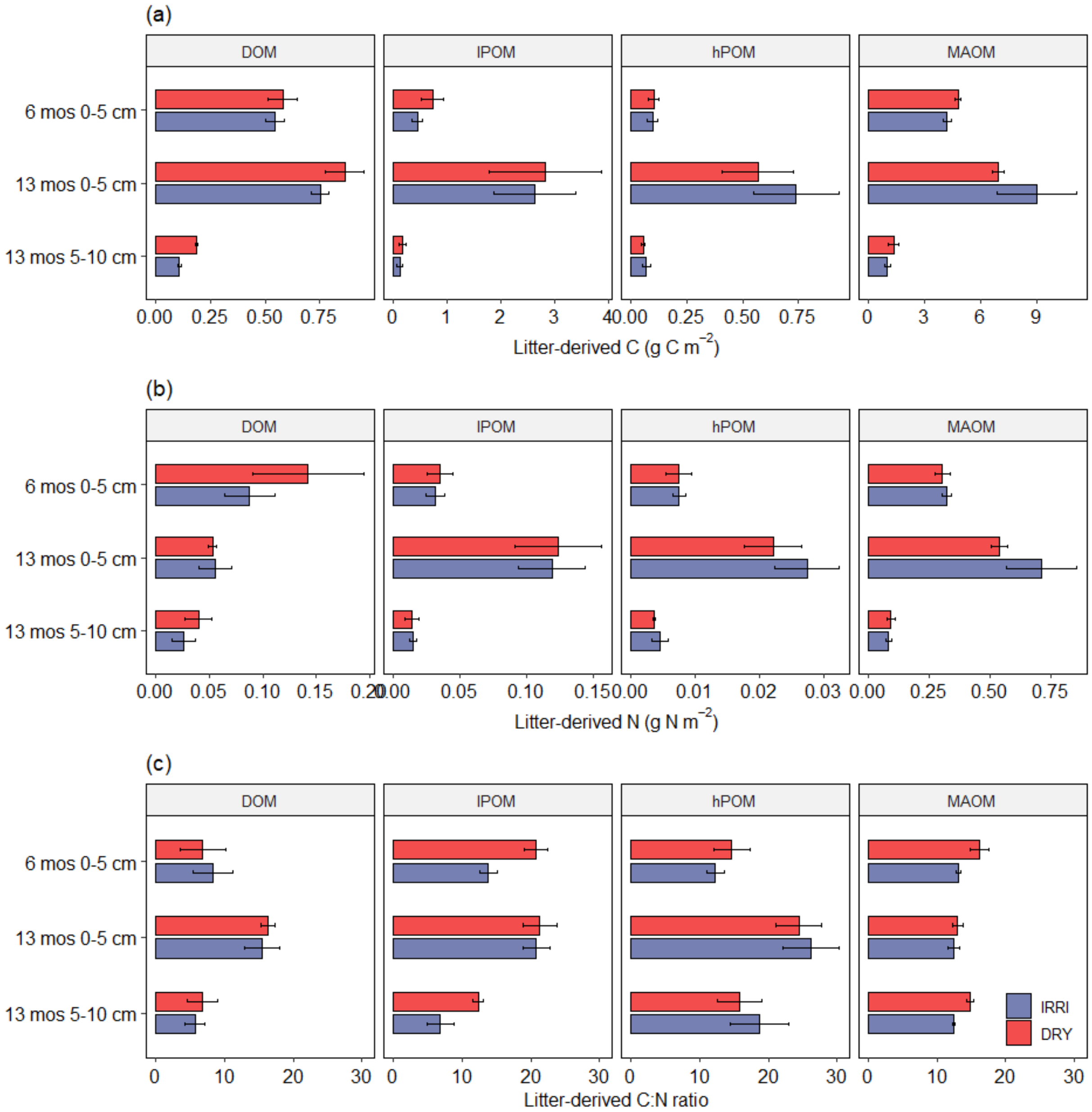

Fig. 3.3. Litter-derived carbon (a), nitrogen (b), and C:N ratio (c) in soil organic matter fractions after 6 and 13 months of incubation at 0-5 cm, and after 13 months of incubation at 5-10 cm depth in dryland and irrigated maize cropping systems. Note different scales in each fraction. Error bars represent the standard error of the mean with n = 4.

Litter contribution to aggregates

After 13 months of incubation, irrigation increased the amount of litter-derived OM found in macroaggregates at 0-5 cm but did not affect the specific concentration in any size class (Table 3.2). Total aggregate SOC stocks and the fraction of litter-derived C in the macroaggregates size class was slightly higher, but not significantly different, in the irrigated than in the dryland treatment (p = 0.21 and p = 0.14, respectively, Supplementary Table 3.1). The addition of these small differences resulted in a higher amount of litter-derived C in macroaggregates of the irrigated treatment (p = 0.040, Table 3.2). However, the sand-corrected concentration of litter-derived C in macroaggregates was not affected by irrigation (p = 0.74). Similar to C, the total amount of litter-derived N was higher in the macroaggregate size class of the irrigated treatment, but we did not find differences between treatments when the results were compared using sand-free concentrations of litter-derived N.

Given the similar irrigation effects on the aggregation of both litter-derived C and N, the C:N ratio of litter-derived SOM found in aggregates was not affected by water management (P = 0.44) but decreased with aggregate size class (P < 0.001). Averaged across treatments, the highest C:N ratios of litter-derived organic matter were found in macroaggregates (18.4 ± 1.2), with intermediate ratios in the microaggregates (15.9 ± 0.9) and the lowest values in the silt and clay (13.0 ± 0.8).

The relative distribution of litter-derived C and N in aggregates was affected by the interaction of size class and irrigation management (P = 0.031 for C and P = 0.012 for N). The irrigated treatment had a higher proportion of litter-derived C in macroaggregates (29.6 ± 8.0% vs 17.2 ± 4.0%, p = 0.080) and lower proportion in the silt and clay size (21.1 ± 4.0% vs 36.6 ± 2.4%, p = 0.034). Litter-derived N showed the same distribution, with irrigation increasing the proportional recovery in macroaggregates (25.5 ± 6.0% vs 13.8 ± 2.8%, p = 0.047) at the expense of the relative amount recovered in silt and clay (26.4 ± 4.4% vs 41.3 ± 2.6%, p = 0.015).

Table 3.2. Litter-derived C and N in different aggregate size classes after 13 months of incubation at 0-5 cm. Data is presented as sand-free concentrations and in total stocks. Data is the mean ± SE (n = 4).

	Sand-free concentration (mg g^{-1})			Stocks (g m^{-2})		
Size class	DRY	IRRI	p-value	DRY	IRRI	p-value
			litter-derived SOC			
Macroaggregates	0.68 ± 0.18	0.67 ± 0.05	0.74	1.60 ± 0.59	3.51 ± 1.23	0.04
microaggregates	0.33 ± 0.06	0.35 ± 0.05	0.75	4.00 ± 0.66	5.27 ± 0.78	0.15
Silt & Clay	0.31 ± 0.05	0.22 ± 0.03	0.32	3.11 ± 0.46	2.16 ± 0.30	0.28
			litter-derived N			
Macroaggregates	0.04 ± 0.01	0.04 ± 0.00	0.56	0.08 ± 0.03	0.19 ± 0.06	0.03
microaggregates	0.02 ± 0.00	0.02 ± 0.00	0.71	0.26 ± 0.05	0.34 ± 0.06	0.35
Silt & Clay	0.02 ± 0.00	0.02 ± 0.00	0.60	0.23 ± 0.04	0.18 ± 0.03	0.53

Discussion

We followed the decomposition of aboveground maize residue and the formation of SOM during 13 months under irrigated and dryland conditions, and found that irrigation had a larger effect on litter losses than on the formation of new SOM. As expected, there were few carry-over effects of irrigation history during the off-season, and the effect of irrigation was concentrated during the growing season. However, the overall effect of irrigation was lower than we expected, and we found very few irrigation treatment differences in the dynamics of SOM formation.

Litter decomposition was low, but SOM formation was highly efficient during the first six months of incubation. We recovered more than 80% of the added C in the litter layer. This period was characterized by low temperature and low precipitation that probably resulted in soil temperature and moisture levels below the proposed thresholds for litter decomposition (Prescott, 2010) and minimized metabolic activity (Couteaux et al., 1995). Low winter decomposition was expected, as residue accumulation is usually a factor that hinders spring crop planting in highly productive no-till systems (Halvorson et al., 2006; Halvorson and Stewart, 2015). Although the amount of organic matter lost from the litter layer was low, it formed SOM with high efficiency, probably because the losses were mostly from low C:N, more labile metabolic components. During early stages of decomposition non-structural plant compounds are preferentially lost and incorporated into mineral-stable SOM through a DOM-microbial pathway (Cotrufo et al., 2015). In addition to the high efficiency of SOM formation, we recovered almost 90% of the litter-derived C in the MAOM and DOM pools, further supporting the action of this DOM-microbial path. This early decomposition probably occurred 1-2 months before our first sampling, as temperature and precipitation events started to increase around mid-March.

There were no differences in management during the first six months of the experiment and no irrigation was applied during this fallow period. Thus, differences in litter decomposition and SOM formation would only occur if there was a legacy effect of irrigation on the soil microbial community (Allison et al., 2013; Glassman et al., 2018). Given the limited responses of the soil microbial community to irrigation found in this site (Chapter 2), the lack of treatment effect on C dynamics was not surprising. However, we did find differences in initial N losses, with a lower amount of added N remaining in the litter layer of the DRY treatment but no differences in SOM formation.

Surface decomposition of plant litter results in N mineralization and induces N_2O emissions in early stages of decomposition (Pal et al., 2013), but we were not expecting higher losses in the dryland treatment. Due to the very low plant growth in the previous season, mineral N availability in the DRY treatment was high (Chapter 2, Supplementary Table 3.1) which may have resulted in higher losses due to excess N (Gregorich et al., 2015; Mosier et al., 2006; Robertson and Vitousek, 2009). With higher N availability and low plant C inputs during the previous growing season, microbial communities may have been more C-limited during the winter, contributing to lower utilization of litter-derived N and higher N losses. Two other observations support the idea that the microorganisms from the DRY treatment used less of the litter-derived N early on due to higher soil availability combined with C limitation: (i) litter-derived C:N ratio of lPOM and MAOM was lower in the DRY treatment, but the C:N ratios of the entire fractions were very similar (Supplementary Table 3.1), supporting higher use of background mineral N in the DRY treatment, and (ii) after 13 months we recovered more litter-derived N in depths greater than 10 cm in the DRY treatment, probably due to lower immobilization in surface layers. Therefore, we hypothesize that in the DRY treatment soil microorganisms were highly C-limited, had higher availability of native N and used less litter-derived N, which resulted in higher losses during the early stages of decomposition, when the more N-rich compounds were lost from the litter. These observations are supported by the recently proposed idea that systems that are limited by C inputs have N in excess, resulting in a decoupling of C and N cycles and an open N cycle with higher losses (Cotrufo et al., 2021). However, the opposite effect was observed during the growing season, where N losses from the litter layer were higher in the IRRI than in the DRY system, suggesting that the high changes in water availability exerted a stronger effect than C limitations.

Litter decomposition was higher during the growing season, between the two samplings, coincident with warmer temperatures that are known to control litter decomposition (Gregorich et al., 2017). The early summer also coincided with a period of frequent precipitation, that may have stimulated the continuation of the initial stages of decomposition already observed at 6 months. However, rainfall was scarce after July and there were very high differences in water availability between treatments. Therefore, we expected irrigation during that period to influence SOM formation, but it only affected the losses from the litter layer.

The increased moisture availability with irrigation during a period of high temperature resulted in lower litter recovery at 13 months in the IRRI treatment. Thus, the higher litter decomposition may be related to facilitated microbial accessibility to substrates and higher microbial activity. Higher soil moisture increases solute transport and the contact between microorganisms and substrates due to the presence of water films (Moyano et al., 2013; Schimel, 2018; Tecon and Or, 2017). In addition, soil moisture stimulates potential enzyme activity (Chapter 2) and can also increase enzyme efficiency (Alster et al., 2013). With higher microbial activity more litter decomposition is expected, but its fate will be defined by the balance between the amount stabilized as SOM and the proportion lost through mineralization (Cotrufo et al., 2013).

Despite the differences in litter decay, we did not find differences in SOM formation due to irrigation. The formation of new SOC would depend on the microbial carbon use efficiency, i.e., the proportion of C substrate that microbes assimilate relative to C lost as CO_2 (Kallenbach et al., 2019). Irrigation events can increase the flux of residue-derived CO_2 (Leichty et al., 2021), probably explaining why we observed higher C losses from the litter layer but no differences in litter-derived SOC. Similarly, if microbes do not utilize all the litter-N, part of it will be mineralized and can be available as substrate for denitrifiers (Pal et al., 2013) or can be lost through leaching (Gregorich et al., 2015). Litter-derived N in the deeper samples of the IRRI treatment was low, so leaching does not appear as the probable cause of increased N losses with irrigation during the growing season. Something we did not explore was the possibility that with irrigation the higher plant growth resulted in roots scavenging the litter-derived dissolved N that moved below the collar. Because the total values of dissolved N at 5-10 cm were very similar in both treatments (1.58 ± 0.26 and 1.45 ± 0.30 $\mu g\ g^{-1}$ for DRY and IRRI, respectively) this does not appear as a probable cause, but it is something we cannot discard as we did not measure the $\delta^{15}N$ of neighboring plants. A more plausible explanation is that the irrigation events likely increased moisture variability in the litter layer as it did in the topsoil (Chapter 4), with an alternation of aerobic and anaerobic periods that may have stimulated N_2O losses during nitrification and denitrification (Firestone and Davidson, 1989; Robertson and Vitousek, 2009; Wrage et al., 2001). Indeed, higher N_2O losses with irrigation have been observed when reactive N is present (Trost et al., 2013). Our results indicate that C and N losses from aboveground litter are increased by irrigation without corresponding increases in SOM.

With higher decomposition but similar amounts of litter-derived SOM, irrigation was expected to decrease the efficiency of SOM formation from aboveground residues. This was what we observed for litter-derived N during the growing season, when the formation efficiency under dryland was double than that estimated for the IRRI treatment. But when the entire incubation period was considered, our statistical analyses did not strongly support the decreased efficiency of SOC ($p = 0.15$) or SOM-N ($p = 0.10$)

formation in the irrigated treatment. However, the proportional difference in efficiency between treatments was very similar to the relative differences in OM loss (24% more C and N losses from the litter layer and 20-23% less SOM formation efficiency in the IRRI treatment), because the differences in litter-derived OM recovered as SOM were very small (less than 5%). Moreover, if higher losses but similar SOM formation occur during the decomposition of aboveground crop residues in irrigated systems, it is expected that the differences in SOC accrual will be lower than the differences in C inputs. This is supported by much higher irrigation impacts on C inputs than on SOC that have been observed in semiarid cropping systems (Denef et al., 2008; Gillabel et al., 2007). Our results point to increased C losses from the litter layer in IRRI as one of the causes of these differences.

Our observations are somewhat contrary to our expectations, as we hypothesized that irrigation would increase DOM leaching and the transfer of litter fragments to the mineral soil. Although the proportion of litter-derived OM found in coarse SOM fractions (lPOM and hPOM) increased between samplings, we recovered most of the litter-derived OM in the MAOM pool even after 13 months of incubation. Thus, it appears that during the time-frame of our experiment, the DOM-microbial path of SOM formation dominated over the physical transfer path that usually occurs in the later stages of decomposition (Cotrufo et al., 2015). Because the DOM-microbial path dominates the decomposition of the most labile plant components, it may have been stimulated by the favorable conditions in the early summer in a similar way in both treatments. If when irrigation started most of the metabolic plant components were already lost, then irrigation would have had an effect only through the stimulation of litter fragmentation and physical transfer to the soil, which seemed unaffected according to our results. But during intermediate stages of decomposition structural plant compounds can be lost without formation of new SOM (Cotrufo et al., 2015), and this may have been the step stimulated by irrigation. The experiment ended after 13 months and we did not follow the incubation until complete litter mass loss, which limits our ability to fully assess these different phases of litter decomposition.

In experiments with similar approaches, the proportion of litter-derived OM in the lPOM was generally higher at 12 months than in our work (Cotrufo et al., 2015; Leichty et al., 2021). In a very similar experiment, (Leichty et al., 2021) recovered the majority of litter-derived C in the lPOM (56% after 12 months), while the MAOM pool represented only 36% of the litter-derived C. The absolute amount of litter-derived C that formed SOM at 0-10 cm was more than 3 times higher in (Leichty et al., 2021) than in our work, even though they only added 70% more initial litter-C. We think that these differences were caused by the litter processing approach that differed in relation to the objectives of each work. While these authors cut the litter in small pieces (< 1.5 cm) to simulate a tillage effect, we decided to use bigger pieces (~10 cm) to represent the crop residue return in our no-till system. The bigger pieces may have slowed down the decomposition process and decreased the transfer of small litter fragments into the coarse fractions. Supporting this, in our experiment, after 13 months of incubation almost 80% of the recovered litter mass corresponded to identifiable litter pieces still bigger than 2 mm.

Although we did not find a treatment effect on the distribution of litter-derived OM into different SOM fractions, we found that irrigation increased the proportion of C and N in macroaggregates. Irrigation stimulates aggregation (Apesteguía et al., 2015; Blanco-Canqui et al., 2010; Gillabel et al., 2007), and in our experiment, the higher litter-derived OM found in macroaggregates was due to increased aggregation rather than to aggregate ^{13}C enrichment. Macroaggregates have a faster turnover than microaggregates (Six et al., 2002b; von Lützow et al., 2007), and they stimulate the formation of microaggregates and MAOM inside them (Fulton-Smith and Cotrufo, 2019; Six et al., 2004; Tisdall and Oades, 1982; von Lützow et al., 2007). Because of this, we think that our observations correspond to the early steps of SOM stabilization due to soil aggregation. Our observation that litter-derived C:N ratio decreased with aggregate size class coincides with previous citations and further supports the idea of higher decomposition in smaller aggregates (von Lützow et al., 2007).

Conclusions

We followed the decomposition of aboveground crop residues and the formation of SOM under irrigated and dryland conditions in a no-till continuous maize system, and found that irrigation had a higher effect on litter losses than on the formation of new SOM. After 13 months the losses of C and N from the litter layer were 24% higher under irrigation, but there were no differences in the litter-derived C and N recovered in SOM. Moreover, irrigation did not affect the amount of litter-derived OM found in different SOM fractions, supporting low differential effects of moisture on the pathways of SOM formation. Coincident with the observations that irrigation stimulates aggregation, we found a higher amount of litter-derived OM in macroaggregates of the irrigated treatment. Given the strong influence of irrigation on aboveground biomass production in semiarid agricultural systems, much of its effect on SOM would depend on the fate of aboveground litter. Our results suggest that a smaller proportion of aboveground crop residues will form SOM in irrigated systems compared to dryland conditions and may help to partially explain why irrigation has a stronger relative effect on crop productivity and C inputs than on SOC stocks in semiarid agricultural systems.

Chapter 4: Shifts in soil organic carbon dynamics during the transition from irrigated to dryland cropping systems

Introduction

Soils are the largest reservoir of organic carbon in the biosphere (Schlesinger, 1997), and soil organic carbon (SOC) sequestration can help mitigate climate change (Lal, 2004; Minasny et al., 2017). Various agricultural management practices have been proposed that can increase SOC sequestration and help diminish greenhouse gases (Minasny et al., 2017; Paustian et al., 2016; Smith et al., 2008). Irrigation is one of these practices, as it increases biomass production which can lead to higher SOC stocks (Follett, 2001; Smith et al., 2008), particularly in arid and semiarid regions (Emde et al., 2021b; Trost et al., 2013). But, due to an increasing pressure on declining global water resources (Davis et al., 2018; FAO, 2011), irrigation will decrease in many areas, with an unknown effect on SOC. Changes in SOC over time will depend on the balance between C inputs and outputs, and a better understanding of SOC dynamics is necessary to anticipate the long-term impacts of irrigation retirement.

Because the effect of irrigation on SOC is much lower than the effect on biomass production, it is usually assumed that irrigation stimulates SOC turnover and increases the losses of SOC (Blanco-Canqui et al., 2010; Denef et al., 2008; Gillabel et al., 2007; Trost et al., 2013). It has been proposed that a faster cycling of C occurs because irrigation not only increases biomass production but also soil moisture, and thus stimulates microbial activity and SOC decomposition (Trost et al., 2013). But the direct evidence of these effects is scarce, and it is not clear which components of the C balance would be most affected by irrigation retirement. A better understanding of the mechanisms governing the response of SOC dynamics to irrigation and how they change during the transition to dryland can inform the best management practices to minimize the negative impacts of water scarcity on SOC. In semiarid ecosystems, water is the main limiting factor for crop growth, and irrigation has a strong positive effect on biomass production, increasing carbon (C) inputs (Denef et al., 2008; Trost et al., 2013). Given the direct relationship between water stress and plant growth (Lobell et al., 2013) the increased water stress after irrigation retirement will result in lower biomass production and a decrease in C inputs, but the changes in C outputs are less clear.

Soil respiration (Rs) has the greatest level of uncertainty relative to other fluxes in global terrestrial C cycle estimates (Bond-Lamberty and Thomson, 2010). Rs can be broadly divided into autotrophic (Ra, which includes root and rhizomicrobial respiration) and heterotrophic (Rh, originated from microbial decomposition of fresh litter and old soil organic matter) components (Subke et al., 2006). Rs typically increases with irrigation (Moinet et al., 2016; Sainju et al., 2008) and soil moisture (Zhang et al., 2019; Zhou et al., 2019; Zornoza et al., 2016), so a decrease in C outputs after irrigation retirement can be expected. But the response of Ra and Rh to moisture differs (Carbone et al., 2011), and irrigation may affect Rs due to changes only in Rh (Zhang et al., 2019) or only in Ra (Moinet et al., 2016). Ra does not contribute to soil C sequestration (Kuzyakov, 2006) so the effect of irrigation retirement on SOC depends on the changes in Rh. Rh is controlled by temperature, moisture, their interaction (Davidson and Janssens, 2006; Orchard and Cook, 1983; Schipper et al., 2019; Suseela et al., 2012), microbial community characteristics (Conant et al., 2004), C availability (Canarini et al., 2017; Conant et al., 2004; Cook and Orchard, 2008; Orchard and Cook, 1983), and the physical protection of soil organic matter (Moinet et al., 2016). Due to this complex set of factors influencing Rh, it is challenging to anticipate how Rh would respond to changes in environmental conditions and C inputs (Bond-Lamberty et al., 2018; Falloon et al., 2011; Moyano et al., 2013).

Detecting small changes in the large SOC pool is challenging over short periods of time (Smith, 2004). The separation of different SOM fractions and the use of ^{13}C natural abundance analysis are widely used techniques that can be integrated to increase the resolution and mechanistic understanding about changes in SOC dynamics over shorter and longer time scales (Balesdent et al., 1988; Cambardella and

Elliott, 1992; Del Galdo et al., 2003; Denef et al., 2013; Eclesia et al., 2016; Mazzilli et al., 2015; Poeplau et al., 2018; Rochette et al., 1999; Salvo et al., 2014). The physical fractionation of SOM into particulate (POM) and mineral associated organic matter (MAOM) is a practical approach to differentiate two pools with contrasting characteristics (Cambardella and Elliott, 1992; Lavallee et al., 2020). POM is formed mainly by plant materials in various stages of decomposition with low associations with the mineral matrix, so it has a faster turnover and responds rapidly to changes in management (Cambardella and Elliott, 1992; Follett et al., 2015; Lavallee et al., 2020; Salvo et al., 2014). On the other hand, the interaction of MAOM with soil mineral particles results in a more stable pool with slower turnover and more resistance to management changes (Christensen, 2001; Lavallee et al., 2020; Salvo et al., 2014). The ^{13}C natural abundance technique takes advantage of the differential discrimination between C isotopes during photosynthesis that occur in C3 and C4 plants (Farquhar et al., 1989). Because SOC retains the $\delta^{13}C$ signature of the original litter that formed it (Balesdent et al., 1993), the change between C3 and C4 vegetation allows the use of ^{13}C to trace the incorporation of new C into different pools and to quantify their turnover rates (Balesdent et al., 1988; Balesdent and Mariotti, 1996).

Our objective was to determine the effect of irrigation retirement on SOC dynamics in semiarid agroecosystems. Specifically, we aimed to quantify the changes in: (*i*) C inputs and outputs, (*ii*) different components of Rs, and (*iii*) the turnover of SOC, to identify which components are most affected following irrigation retirement. We installed a three-year transition experiment to compare biomass production, soil respiration, and SOC composition and distribution in different SOM fractions, between irrigated and non-irrigated (retired) treatments in two cropping systems: continuous maize and continuous wheat. To partition soil respiration into its different components, we installed yearly fallow subplots to estimate Rh and a long-term fallow subplot to estimate microbial respiration from native SOC. At the beginning and end of the experiment, we measured the content and isotopic composition of SOC in the bulk soil and in different SOM fractions to assess the gains of new C and the losses of old SOC in the different treatments.

While it is well established that after irrigation retirement, C inputs will decrease due to lower biomass production, less is understood regarding the response of C outputs. If moisture changes due to irrigation have a direct effect on Rh, then C outputs would decrease before inputs, and a new (intermediate) SOC balance may be expected. But if the irrigation effect is indirect, changes in C outputs would be in response to changes in C inputs, and a larger SOC loss would be expected.

Materials and Methods

Site description and experimental design

We installed our experiment at the Agricultural Research Development and Education Center of Colorado State University near Fort Collins (40°39'16"N, 104°59'55"W; 1555 m a.s.l). The climate of the site is characterized by an average annual precipitation of 408 mm and annual mean temperature of 10.2°C (1981-2010 average, https://usclimatedata.com/)). The soil is classified as an Aridic Haplustalf (USDA, NRCS, 2019), with a sandy clay loam texture (54% Sand, 14% Silt, 32% Clay). Prior to the start of the experiment, the site had alfalfa between 2004 and 2007, and after that it was managed as an annually tilled and irrigated crop field with predominance of maize and brassicas.

In April 2017, the site was tilled to homogenize the surface and incorporate previous crop residue into the soil. In May 2017 we started the experiment with two irrigation treatments: Irrigated and Dryland, and two crop types, Maize and Wheat. Given the historical field management, the dryland treatments represented the transition from an irrigated to a dryland cropping system. The irrigation treatments were arranged in a randomized complete block design with four replicates, with plots of 48 x 14 m for maize and 24 x 14 m for wheat. We followed the experiment for 2.5 years, during three maize and two wheat growing seasons.

Inside each main plot we installed 3 x 3 m subplots in a split-plot design where we removed all growing plants to represent different fallow treatments. In the first year there was only one subplot, which had residues from crops planted before the start of the experiment but no growing plants in that season (Yearly fallow). We maintained this same subplot throughout the experiment, and so during the following seasons it represented a long-term bare fallow without new carbon inputs. We installed additional subplots, in a different location each year, to have a yearly fallow subplot each season. So, after the first season each irrigation plot had three fallow treatments: Planted area (no fallow), Yearly-fallow, and Long-term fallow. We kept the subplots weed-free by hand-weeding and used a shovel to cut the borders of the subplot to a depth of ~20 cm to avoid root invasion.

Each maize season, between May 10 and May 16 we planted the Producers Hybrids 5218 SSTX in 0.76-m rows at 84,000 and 42,000 seeds ha^{-1} for irrigated and dryland, respectively, based on standard seeding rate recommendations for the region. On average, irrigated maize received 165 kg N ha^{-1} and 50 kg P_2O_5 ha^{-1} and dryland maize received 90 kg N ha^{-1} and 40 kg P_2O_5 ha^{-1}, annually. We used the Avery wheat variety, at a planting density of 100-110 and 60-70 kg seed ha^{-1} for irrigated and dryland, in 0.19-m rows. The first wheat crop was fertilized with 50 kg N ha^{-1} and 22 kg P_2O_5 ha^{-1} for both treatments, and no fertilization was required on the second year based on soil test results. Pre- and post-emergence herbicide applications were used for weed control. For the irrigated treatments irrigation was done once per week, usually on Tuesdays, from May to September in Maize and from May to June-July for wheat.

Biomass production

We quantified above- and belowground biomass production to estimate carbon inputs for each treatment. Belowground C inputs were determined by estimating root biomass production by the core method (Chapter 2). Briefly, we used a Giddings soil probe with a 6.5-cm diameter core to take four samples per plot (two in the row and two between rows) to a total depth of 80 cm and divided the core at different depth intervals (0-10, 10-20, 20-40, 40-60, and 60-80 cm, but in 2017 it was impossible to sample deeper than 60 cm). Roots were recovered with tweezers after thoroughly rinsing each sample over sieves of 2.0 mm, 1.0 mm, and 0.5 mm. For maize 2019, this method was modified slightly and at maize harvest we took six samples per plot, half in the row and half between the rows, with a 4.1-cm diameter core using the same depths intervals. We recovered the roots from these samples during soil sieving to 8 mm without soaking the sample in water. Recovered roots were oven-dried at 55-60 °C for at least 48 hours and weighed. To estimate total belowground dry matter per hectare we summed all root biomass across soil depths, accounted for the sampled area, and averaged the in row and between row samples.

Total aboveground biomass production was estimated at physiological maturity. We selected two representative subareas from the center of each plot, each one consisting of 4.6 m^2 in maize and 2.3 m^2 in wheat, and collected all the plants (total sampled area per plot was 9.12 m^2 for maize and 4.56 m^2 for wheat). We weighed the entire sample and separated grain from stover. We estimated total dry matter after oven-drying a subsample of each component at 55-60 °C for at least 48 hours. Root and aboveground stover samples were oven-dried, finely ground and analyze for C content and $\delta^{13}C$ in an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (EA-IRMS) in the Stable Isotope Facility at UC Davis.

Soil respiration

To understand the effect of irrigation and substrate availability on different components of soil respiration we measured soil CO_2 flux from the main planted area and from the fallowed subplots. Soil respiration measured in the yearly fallow subplots was used to approximate Rh (Subke et al., 2006) as these measurements included microbial respiration of native SOC and the decomposition of previous belowground crop residues but excluded living roots. Similarly, we assumed that respiration from the long-term fallow represented microbial respiration of native SOC, as no new crop residues or living roots were present in these subplots. This allowed us to assess direct irrigation effects on soil microbial respiration without the confounded effects of different carbon inputs. The exclusion of roots to estimate Rh is a simple, widespread approach that yields comparable results with other techniques (Kuzyakov, 2006; Subke et al., 2006; Suseela et al., 2012; Zhang et al., 2013), but that has some drawbacks that should be considered during the interpretation of the results. The main weaknesses of this approach are the absence of any potential priming effect due to root exudates (Kuzyakov, 2006) and the alteration of soil temperature and water dynamics (Suseela et al., 2012; Zhang et al., 2013). In our experiment, the fallow subplots affected soil moisture, but these moisture differences were small relative to the larger irrigation effects in maize. Given the lack of differences in soil temperature between subplots of the same treatment, and the lack of a strong relationship between soil respiration and moisture in the fallowed subplots we did not attempt to correct the CO_2 fluxes by moisture and temperature and conducted the comparison between the subplots using the observed fluxes.

We installed 20-cm diameter PVC collars to a depth of 5 cm into the soil, let the surrounding soil settle for at least 48 h, and used them to measure soil respiration throughout the summer growing season. One collar was inserted in each fallowed subplot and two collars were inserted within the planted area to account for spatial variability, one between crop rows and one in the crop row. Due to limited differences in the respiration between the in-row and between-row plant collars, and because together they represent the overall planted area, we averaged these values to have an integrated measurement from the planted area. Residue distribution was spatially variable, mainly in the maize dryland treatment, with spots where residue accumulated and large areas without soil cover. To avoid this affecting Rs measurements, we removed the aboveground fresh residue before installing the collars across all treatments. We measured soil CO_2 flux twice a week during the summer growing season with a LI-8100 portable infrared gas analyzer with a 20 cm diameter survey chamber (LI-8100, LI-COR, Nebraska, USA). The measurements were taken in the mornings, immediately before irrigation (Tuesdays) and two days after irrigation (Thursdays). We used the SoilFluxPro software (2015, LI-COR Biosciences, Inc.) to estimate the rate of soil respiration by fitting an exponential equation to the CO_2 concentration increase during a 2-minute measurement period.

At the time of CO_2 measurement, we also recorded soil temperature and soil moisture at 10 cm depth. We used a temperature probe connected to the LI-COR to estimate the average soil temperature at one location during the 2-minute measurement period. Volumetric water content was estimated as the average of three measurements taken around each collar with a HydroSense II hand probe (Campbell Scientific, Utah, USA).

Soil sampling and analyses

To determine the effect of irrigation, crop type, and fallow management on SOC dynamics, we sampled the planted area and the long-term fallows and compared them to baseline samples taken at the beginning of the experiment. Samples were taken in June 2017, before starting the irrigation treatments, and in November 2019 at the end of the maize growing season. In each sampling, we used a Giddings soil probe with a 4.1-cm diameter core and sampled the soil profile in the same depth intervals as we did for root biomass estimation (0-10, 10-20, 20-40, 40-60, and 60-80 cm, although only results for 0-10 and 10-20 cm are presented here). For the baseline and the final sampling of the planted areas, we integrated six samples per plot, half from the rows and half from between the rows, while we only took one core in each bare fallow subplot to avoid unnecessary disturbance. All samples were transported to the lab in coolers and kept refrigerated until further processing.

We recorded total sample weight and oven dried a 10-g subsample at 105°C for 48 hours to correct sample weight by gravimetric water content. We sieved the samples to 8 mm for homogenization and picked up any roots, if present. We let the samples air dry and sieved a subsample to 2 mm to remove rocks and plant residues. Roots in the long-term fallow subplots were negligible, confirming that we were able to exclude all root growth from the subplots. For bulk analysis, ~ 25 g the 2-mm sieved samples were pulverized in a rolling mill.

To quantify changes in SOC dynamics, we separated three SOM pools: dissolved organic matter (DOM), particulate organic matter (POM), and mineral associated organic matter (MAOM). The separation of POM from MAOM allows to efficiently quantify changes in two pools with contrasting characteristics (Cambardella and Elliott, 1992; Cotrufo et al., 2019; Eclesia et al., 2016; Lavallee et al., 2020; Mazzilli et al., 2014). In addition, because changes in water fluxes due to irrigation are expected, we included the determination of DOM in our fractionation procedure. DOM is a small fraction of total SOM but plays an important role in the vertical movement of C and its stabilization in the soil (Cotrufo et al., 2015; Kaiser and Guggenberger, 2000; Kaiser and Kalbitz, 2012; Kalbitz et al., 2000). First, to extract DOM, we shook a 10-g soil sample with ~30 ml of DI water for 15 min, centrifugate 15 min at 3400 rpm and filtered the supernatant with a 20 µm nylon filter. Then, we added 30 ml of 5 g L^{-1} Na-hexametaphosphate to the remaining sample, vortex it to loose the soil and shook the sample for 18 hours. After dispersion, we used a 53 µm sieve to separate the POM from the MAOM fraction. The DOM samples were weighed and analyzed for organic C (DOC) concentrations in a TOC-V-TN analyzer (Shimadzu Corp., Kyoto, Japan). The solid samples were oven-dried, weighed, and ground with mortar and pestle before analysis.

All bulk, POM and MAOM samples were analyzed for SOC and $\delta^{13}C$ concentrations in the Stable Isotope Facility at UC Davis. The content of inorganic carbon in the soil was high, so we removed the carbonates before analysis. To do this, we weighed the samples in silver capsules, wetted, and fumigated them for 8 hours with concentrated HCl (Harris et al., 2001). Carbon recovery in the three SOM fractions was 101 ± 4%. DOC represented a small fraction of total SOC (always less than 2%) and we did not find significant differences between treatments, so we did not analyze its isotopic composition. We used the estimated bulk density to calculate the stocks of SOC in bulk soil and in the different SOM fractions in Mg ha^{-1}. For an accurate comparison between the different samplings, all values were converted to a constant soil mass equivalent to the baseline sampling (Davidson and Ackerman, 1993; Lee et al., 2009; Solomon et al., 2002; Veldkamp, 1994).

Estimations of SOC dynamics

To quantify changes in SOC dynamics, we first estimated the proportion of new SOC incorporated during the experimental period and of the remaining old SOC utilizing the following mixing equations (Balesdent and Mariotti, 1996):

$$f_{new} = (\delta_{sample} - \delta_{control}) / (\delta_{residue} - \delta_{control}) \quad (4.1)$$

$$f_{old} = 1 - f_{new} \quad (4.2)$$

Where f_{new} is the proportion of new, crop-derived C in each pool (bulk SOC, POM or MAOM), δ_{sample} is the $\delta^{13}C$ of the pool of interest in the final sampling of the planted areas, $\delta_{control}$ is the $\delta^{13}C$ of the pool of interest in the bare fallow subplot of the corresponding treatment, $\delta_{residue}$ is the $\delta^{13}C$ of the crop residues (above- and belowground) produced by each crop during the experiment, and f_{old} is the proportion of old, native SOC. The fraction of new or old C in each pool was multiplied by the total SOC stocks of the final sampling in the planted areas to estimate the stocks of new and old SOC:

$$SOC_{new} = SOC * f_{new} \quad (4.3)$$

$$SOC_{old} = SOC * f_{old} \quad (4.4)$$

Usually, the original $\delta^{13}C$ at the beginning of the experiment is used as the control end member for the mixing model, assuming it represents the isotopic composition of the remaining native SOC at the time of sampling (Balesdent et al., 1988). Although in the long-term there is an enrichment in $\delta^{13}C$ as SOC is lost (Clay et al., 2007; Menichetti et al., 2015) this does not have a big impact in turnover estimations during decadal time frames (Christensen et al., 2011). However, when the starting point is a mixed C3/C4 soil, as in our case, if the enrichment of background $\delta^{13}C$ is not considered the contribution of fresh biomass to SOC can be overestimated for C4 and underestimated for C3 crops (Clay et al., 2007). Moreover, the $\delta^{13}C$ of native SOC may not change in the expected direction due to the preferential microbial utilization of fresh SOC (Blagodatskaya et al., 2011) or by the faster decomposition of C4-derived

SOC (Wynn and Bird, 2007). To account for the possible effect of these changes in our results, we compared the $\delta^{13}C$ of the baseline sampling to the $\delta^{13}C$ of the long-term bare fallows.

After three seasons, there were some differences in the $\delta^{13}C$ of SOC between the bare fallow subplots and the baseline sampling. The differences were not statistically significant but there was a consistent enrichment in the bulk soil and a consistent depletion of ^{13}C in the POM. The change in the POM fraction seemed to be related to the field history and the recent incorporation of maize residues that probably dominated the light POM during the baseline sampling and were rapidly decomposed during the experiment. Moreover, POM (originally lighter than MAOM and with a faster turnover) was preferentially lost during the experiment and contributed to the observed enrichment of the bulk soil. Because of these, we decided to use the $\delta^{13}C$ of the bare fallow subplots as the end member in our mixing model instead of the baseline sampling. Nevertheless, we double checked our results by making all the calculations with the baseline $\delta^{13}C$ values and, despite some differences in the estimations of new SOC between crops, the inference about the effect of irrigation on SOC dynamics did not change.

We estimated the decomposition rate (k) of old SOC assuming an exponential decay:

$$k = -\ln (SOC_{old} / SOC_{initial}) / t \tag{4.5}$$

where SOC_{old} is the remaining native SOC in the planted area or in the long-term bare fallow subplot, $SOC_{initial}$ is the original SOC stocks at the beginning of the experiment, and t is the time between samplings (2.5 years).

We also estimated the stabilization of new C inputs (h, usually defined as the humification rate) into SOC as:

$$h = (SOC_{new} / C_{input}) \tag{4.6}$$

where C_{input} is the total quantity of C inputs as plant residues estimated for each treatment.

We used these estimations of SOC dynamics to evaluate the effects of irrigation and crop type on the decomposition of old SOC (k) and on the proportion of C from crop residues that formed SOC during the entire experimental period (*h*). For easiness of interpretation, both parameters were multiplied by 100 and are presented as %.

Statistical analysis

Given the differences in growing season, soil respiration was analyzed for each crop individually. Data analysis was conducted considering the factorial design of two irrigation by three fallow treatments. The experimental structure was a split plot design with irrigation management (irrigated or dryland) as the main plot and fallow as the subplot (planted, yearly-fallow, or long-term fallow). Seasonal dynamics of soil respiration and its covariates were analyzed individually for maize 2017 and each wheat season (when not all the fallow subplots were present) and with a joint model for maize 2018 and 2019 when we had the complete comparison of the three locations. A repeated measures general linear mixed model with irrigation, fallow subplot and date, and their interactions as categorical fixed effects, and block (plus year for 2018 and 2019) as random effect was used. Subplot nested in irrigation treatment and block was defined as the experimental unit to model the correlation between measurements. Different correlation structures were compared to model the covariance structure between measurements taken from the same experimental unit, and the model with the lowest Akaike and Bayesian information criteria was selected. Respiration values were log transformed to meet the assumption of homogeneity of variances, and results were back transformed for ease of interpretation. No data transformation was needed for the analysis of soil temperature and volumetric water content. We estimated carbon outputs as the accumulated seasonal CO_2 flux by linear interpolation of the biweekly observations during each measurement period.

The interactive effects of irrigation, crop type, and fallow on SOC stocks, SOM fractions and the parameters of SOC dynamics were estimated as fixed effects in a mixed model that included block and block:treatment as random effects to account for the experimental design. Fixed effects were tested by anova with Kenward-Roger, and means were compared using Tukey adjusted pairwise comparisons with

Kenward-Roger degrees of freedom. We conducted all data analysis in R version 3.6.3 (R Core Team, 2020) with the packages d*plyr* (Wickham et al., 2020), *tidyr* (Wickham et al., 2020), *nlme* (Pinheiro et al., 2018), *lme4* (Bates et al., 2015), *lmerTest* (Kuznetsova et al., 2017), and *emmeans* (Lenth, 2018).

Results

Seasonal water and temperature dynamics

Our experimental design was effective in creating the intended differences in soil moisture between irrigated and dryland maize management systems (Fig. 4.1). Soil moisture was affected by the interaction of irrigation treatment, fallow subplot, and measurement day ($p < 0.001$). The irrigated maize received two to five times more water than the dryland (350-495 mm of irrigation per crop) across the three study years, which resulted in higher soil moisture in almost all the measurement days.

The installation of subplots with different fallow treatments also affected soil moisture, but to a lesser extent than irrigation. In the dryland maize, the yearly fallowed subplot had more moisture than the planted area during the drier 2018 and 2019 growing seasons for approximately 78% of the days ($p < 0.05$), reflecting the absence of plant transpiration and presence of plant residues. The long-term fallow areas had intermediate levels of soil moisture that were generally similar to the planted areas in irrigated maize and higher in moisture than planted areas in dryland maize. The irrigated long-term fallow subplots that had no residues from previous crops were drier than those with residues. In dryland maize, the differences in moisture between the planted and non-planted areas were greater, and yearly and long-term fallow subplots had more similar soil moisture, coincident with the low biomass production that caused low soil cover differences between them.

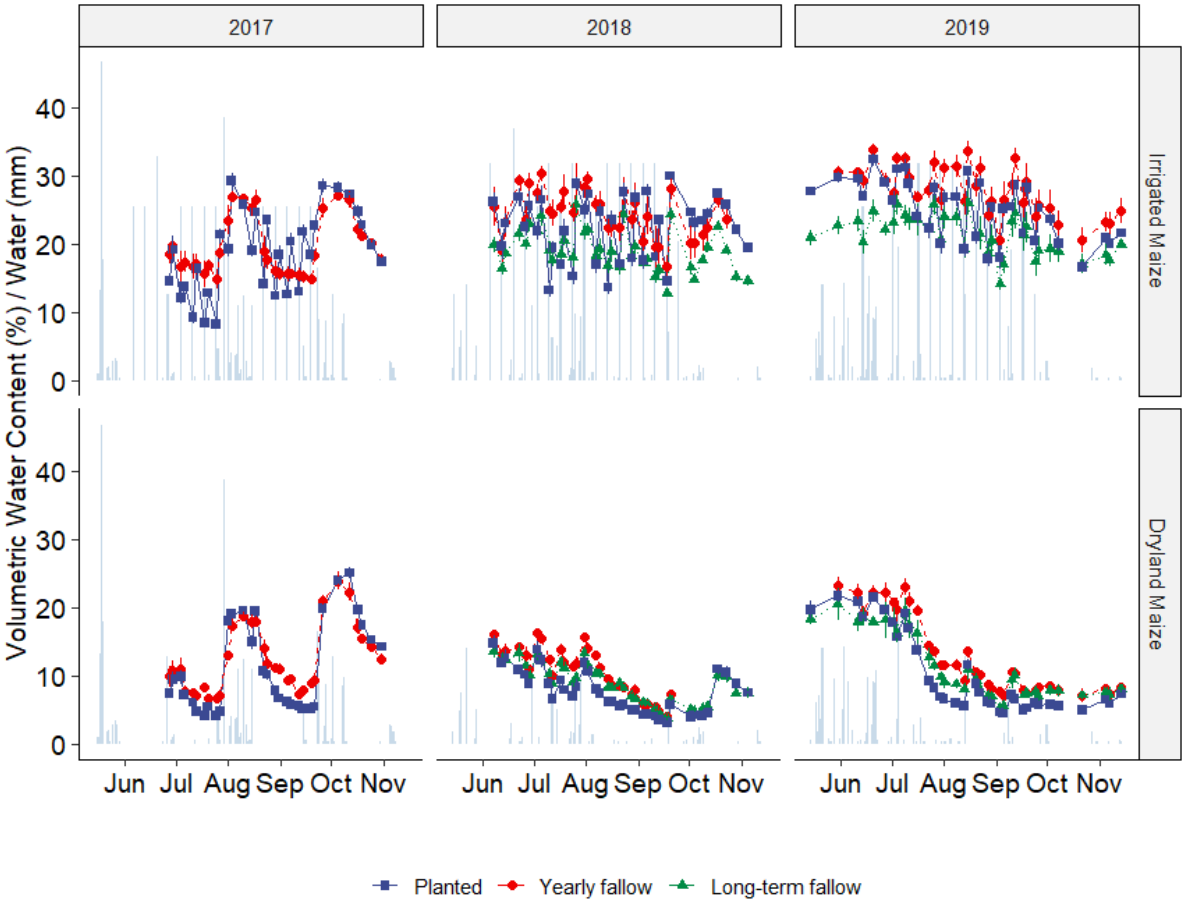

Fig 4.1. Soil moisture dynamics at 10 cm soil depth in each maize irrigation treatment and fallow management. Data are mean ± 1 SE with n=4. Bars represent water inputs from precipitation and irrigation.

Soil temperature in maize was less affected by irrigation and fallow treatments than moisture (Supplementary Fig. 4.1). During 2017, soil temperature was only affected by date ($p < 0.001$) while during 2018 and 2019 there was an interaction between irrigation and measurement date ($p < 0.001$). Only during 2019 irrigation had an important effect on soil temperature, as dryland treatments had higher soil temperature than irrigated treatments until the beginning of September (19 days, $p < 0.05$). The subplots with different fallow periods did not differ in soil temperature (effects of fallow subplot and the interactions treatment:fallow, fallow:date or treatment:fallow:date $p > 0.28$ in all cases).

The evolution of soil moisture in winter wheat treatments was different than in maize as the presence of plants had a higher impact than irrigation (Fig. 4.2). At the beginning of the measurement periods (before irrigation started but with the crop already established) soil moisture was higher in the subplots without plants than in the planted areas. After that, irrigation increased soil moisture, but the effect was not as pronounced as in maize due to the fewer irrigation events (average of 141 mm per crop). The lowest soil moisture measurements occurred in the presence of growing plants in dryland wheat and there was usually an increase in soil moisture after July as the crop reached physiological maturity and stopped consuming water. The second irrigated wheat crop was irrigated just after planting, in October 2018, to ensure plant emergence. This irrigation event likely contributed to the increased soil moisture in the subplots without plants in June 2019, which was greater than in the planted plots. Soil temperature in wheat was only affected by the measurement date ($p < 0.001$) but not by irrigation or the installation of fallows.

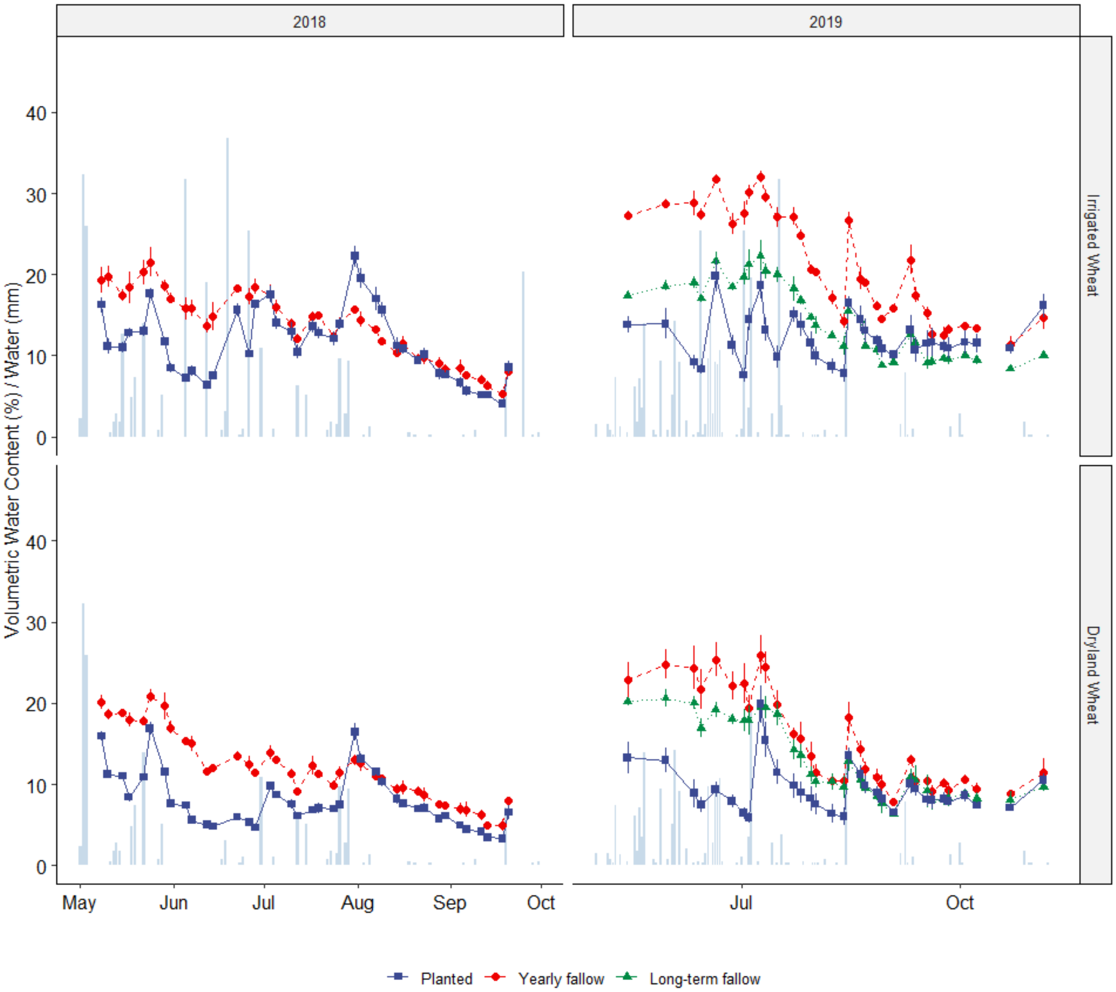

Fig 4.2. Soil moisture dynamics at 10 cm soil depth in each wheat irrigation treatment and fallow management. Data are mean ± 1 SE with n=4. Bars represent water inputs from precipitation and irrigation.

Biomass production and C inputs

Irrigation retirement strongly affected maize biomass production and C inputs (Table 4.1) with 2.4 times less inputs in the dryland than in the irrigated treatment. This difference was explained by a strong reduction in aboveground biomass production (2.96 times lower in the dryland) and a smaller but important reduction in root growth (1.47 times lower in the dryland maize). Moreover, the relative difference between treatments increased with the seasons (Chapter 2), resulting in a temporal trend in the differences in crop residue inputs between the yearly fallowed subplots. In the first year of the study, 2017, the yearly fallowed subplots had the same amount of crop residue in both treatments and differences were only due to irrigation management, but in 2018 and 2019 the differences were both in soil moisture due to irrigation management and accumulated differences in residue inputs due to the previous year differences in crop production. In contrast, the long-term fallow subplots did not receive any carbon inputs during the experiment and so they differed only in irrigation management.

The effect of irrigation on winter wheat production was lower than on maize and not always statistically significant. Irrigation increased average aboveground residue production by 16% per crop, but this difference was not significant when we estimated the accumulated C inputs. Moreover, irrigation affected the root:shoot ratio (Chapter 2) and, although not significant, belowground C inputs were 16% higher in dryland than in irrigated wheat. Because of this, and because the differences in crop production were more pronounced in 2019 (Chapter 2), irrigation had no effect on the C inputs in the wheat yearly fallow subplots.

With irrigation, crop productivity was very similar in both crops, but maize had higher root production than wheat. Irrigation retirement had a stronger effect on maize than on wheat, and dryland maize had the lowest productivity, although average root production did not differ from the other treatments. Dryland wheat had intermediate crop productivity and higher total C inputs than dryland maize during the experimental period, despite only producing two crops due to its longer, overwintering growing season compared to the three maize summer crops. As expected, the isotopic composition of crop litter reflected the different photosynthetic pathways of maize and wheat, and we did not find isotopic composition differences due to irrigation.

Table 4.1. Crop biomass, litter characteristics and total C inputs in irrigated and dryland maize and wheat crops. Grain is not included. Values represent the mean ± 1 SE with n = 4. In the same column, letters represent statistical differences between treatments ($p < 0.05$); no treatment effect on carbon concentration was found ($P = 0.29$).

Treatment	N° of crops	Crop biomass (Mg ha^{-1} crop^{-1})		Carbon concentration (%)		Total C inputs (Mg C ha^{-1})		δ^{13}C (‰)	
		Aboveground	Belowground	Aboveground	Belowground	Aboveground	Belowground	Aboveground	Belowground
IRRI Maize	3	7.49 ± 0.24 a	2.09 ± 0.18 a	42.58 ± 0.65	38.30 ± 1.54	9.57 ± 0.48 a	2.11 ± 0.26 a	−13.03 ± 0.03 a	−13.04 ± 0.19 a
DRY Maize	3	2.53 ± 0.13 c	1.42 ± 0.20 ab	41.60 ± 0.70	40.67 ± 0.87	3.18 ± 0.17 c	1.77 ± 0.16 a	−13.75 ± 0.05 a	−13.72 ± 0.29 a
IRRI Wheat	2	7.36 ± 0.46 a	0.86 ± 0.08 b	43.94 ± 0.16	40.61 ± 0.58	6.46 ± 0.52 b	0.69 ± 0.03 b	−28.21 ± 0.08 b	−26.03 ± 0.39 b
DRY Wheat	2	6.33 ± 0.35 b	1.06 ± 0.08 b	44.07 ± 0.13	39.21 ± 1.02	5.57 ± 0.36 b	0.82 ± 0.06 b	−28.11 ± 0.11 b	−25.68 ± 0.47 b

Soil respiration dynamics and C outputs

While in maize the biomass inputs were reduced by more than 2-fold in dryland relative to irrigated treatments, the effect on soil respiration (Rs) was smaller and varied between seasons. Rs in maize decreased due to irrigation retirement, but autotrophic respiration (Ra) was the component most affected, while heterotrophic respiration (Rh) from yearly fallowed subplots was only affected by irrigation in the 3rd year. Moreover, in the long-term fallow subplots, without differences in C inputs, irrigation did not affect C outputs. In wheat, with lower differences in water inputs and crop productivity, the effect of irrigation on Rs dynamics was minimal.

Irrigation retirement decreased Rs in maize, but the effect changed with time and with fallow management (Fig. 4.3). In 2017, there was no irrigation effect on total Rs ($p > 0.05$) but in the two following cropping seasons irrigation increased Rs in around 60% of the measurement days ($p < 0.05$). Rs in the irrigated maize was usually higher 7 days after irrigation than 2 days after irrigation when soil moisture was higher. This may be related to the negative correlation between soil temperature and soil moisture (Spearman correlation between temperature and moisture in irrigated maize planted area was −0.23). In the yearly fallowed subplots, there were almost no differences in Rh between irrigated and dryland treatments in the first two years, but in 2019 Rh in the irrigated maize was higher than in the dryland in 46% of the measurement days ($p < 0.05$). In the long-term fallowed subplots respiration decreased as the time without new C inputs increased, but the effect of irrigation was negligible. During the 67 measurements days of 2018 and 2019 only in six days was there a treatment effect on respiration from the long-term fallowed subplots and the effect was inconsistent; in half of these days, respiration was higher in the irrigated, and in half was higher in the dryland maize.

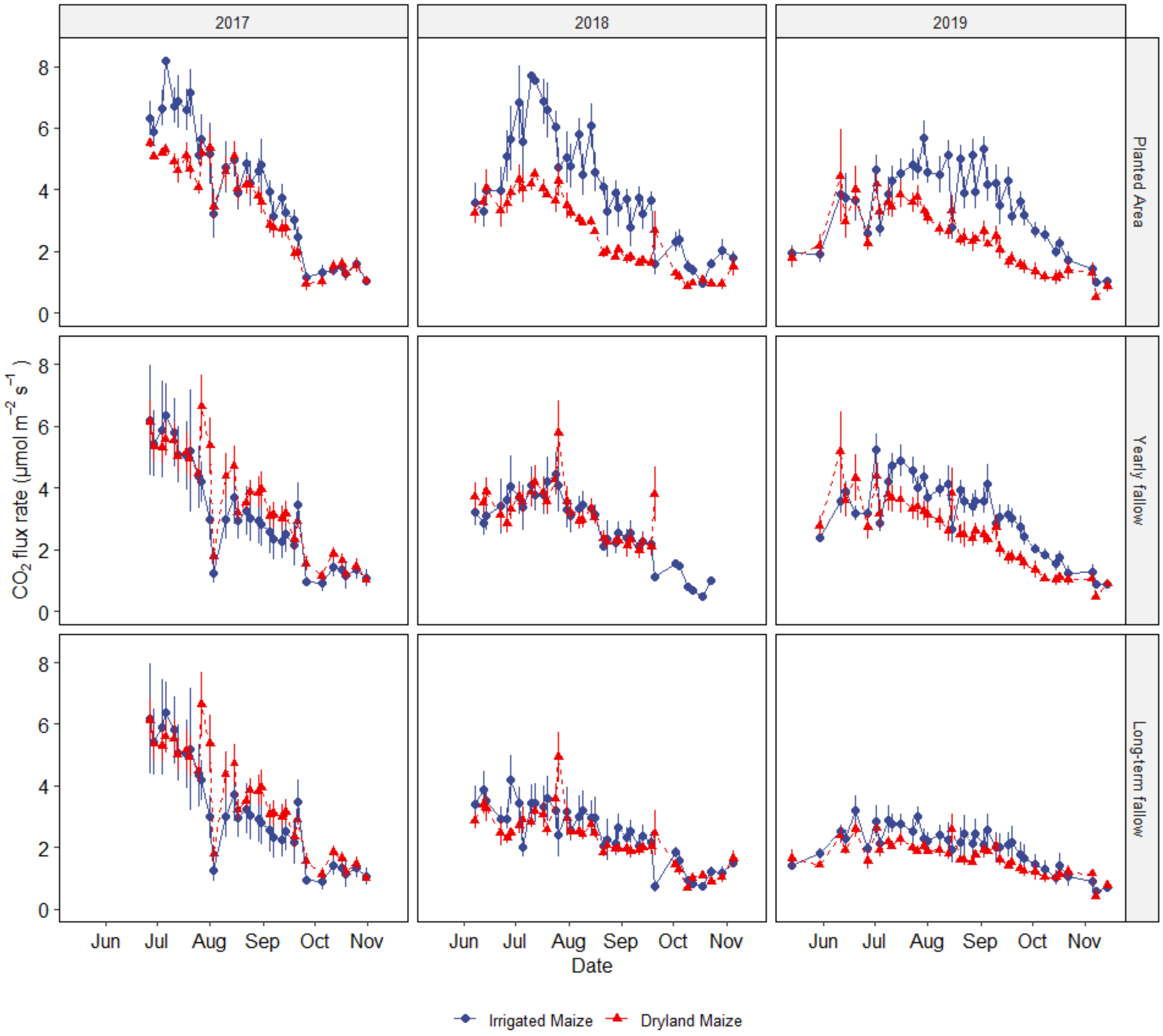

Fig. 4.3. Soil respiration dynamics in irrigated and dryland maize during three consecutive crops. For each year, upper panels represent total soil respiration in the planted areas (average of in row and between row), middle panels heterotrophic respiration (Rh, the respiration in yearly fallowed subplots) and lower panels the respiration in long-term fallowed subplots. Data for 2017, which was the first year of the long-term fallow, is shown for clarity only and was not part of the statistical analysis. Data are mean ± 1 SE with n=4.

Soil respiration did not differ greatly between irrigated and non-irrigated wheat, and the small differences were again explained by changes in Ra, not by Rh (Fig. 4.4). Only in 5% of the measurements Rs in the planted area was increased by irrigation (4 of 74 total days, $p < 0.05$), while there were no differences in Rh from the yearly fallow subplots in any year ($p > 0.10$). Similarly, respiration of native SOC from the long-term fallow subplots was not affected by irrigation. The peak seen in 2018 seemed to be related to the disturbance caused by mechanical harvest as respiration rates returned to pre-harvest values shortly after harvest.

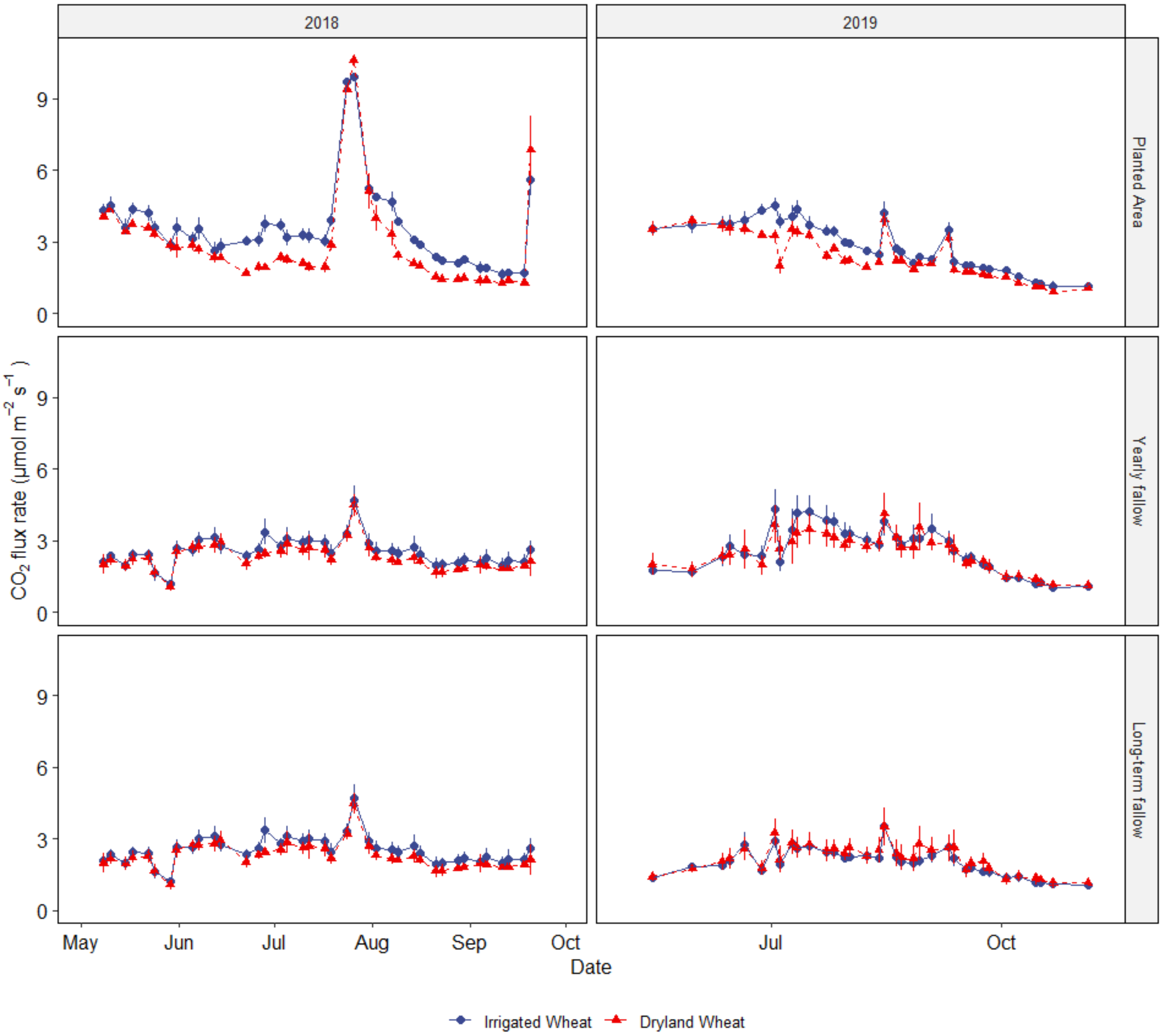

Fig. 4.4. Soil respiration dynamics in irrigated and dryland wheat during two consecutive crops. For each year, upper panels represent total soil respiration in the planted areas (average of in row and between row), middle panels heterotrophic respiration (Rh, the respiration in yearly fallowed subplots) and lower panels the respiration in long-term fallowed subplots. Data for 2018, which was the first year of the long-term fallow, is shown for clarity only and was not part of the statistical analysis. Data are mean ± 1 SE with n=4.

Irrigation retirement reduced total Rs mainly due to a decrease in Ra but with smaller changes in Rh, affecting the relative contribution of different Rs components in maize. To partition Rs, we compared CO_2 fluxes from the yearly fallowed subplots (Rh) to the fluxes of total Rs from the planted areas

(Supplementary Fig. 4.2). The slope of the relationship between Rs and Rh was higher in the dryland than in the irrigated maize (0.95 vs 0.64, $p < 0.001$), indicating that Rh dominated Rs in dryland maize while irrigation increased the relative contribution of root respiration. For wheat, the relationship between Rs and Rh was lower (slope = 0.24), with a strong effect of the atypically high fluxes observed in 2018, but it did not vary due to irrigation ($p = 0.14$).

The low effect of irrigation on Rh was related to the observation that respiration from the yearly fallowed subplots had no or very low responses to soil moisture (Supplementary Fig. 4.3). In the maize subplots, we did not find a significant association between Rh and soil moisture in any of the treatments ($p > 0.20$ for the slope of the linear response of Rh to volumetric water content). For wheat, Rh responded significantly to moisture ($p < 0.01$) but the response was low (slope = 0.02) and not affected by irrigation ($p = 0.18$).

The effects of irrigation and fallow on accumulated soil respiration varied by season (Fig. 4.5). In 2017 irrigation did not affect total C flux in any of the two evaluated areas of maize ($p = 0.12$ and 0.28 for planted and yearly fallowed, respectively). In 2018, irrigation increased C outputs relative to dryland in the planted area of both crops ($p < 0.05$) but not in any of the fallows ($p = 0.95$ and 0.51 for yearly and long-term fallowed in maize, $p = 0.45$ for yearly fallow in wheat). In 2019 irrigation impacted accumulated C outputs for maize, with reductions in total Rs ($p < 0.01$) in planted area and Rh ($p = 0.03$) in the yearly fallow, but irrigation did not impact respiration from the long-term fallow ($p = 0.13$). Accumulated C outputs from the wheat treatments in 2019 were not affected by irrigation ($p = 0.11$ for planted area, $p = 0.64$ for yearly and long-term fallows).

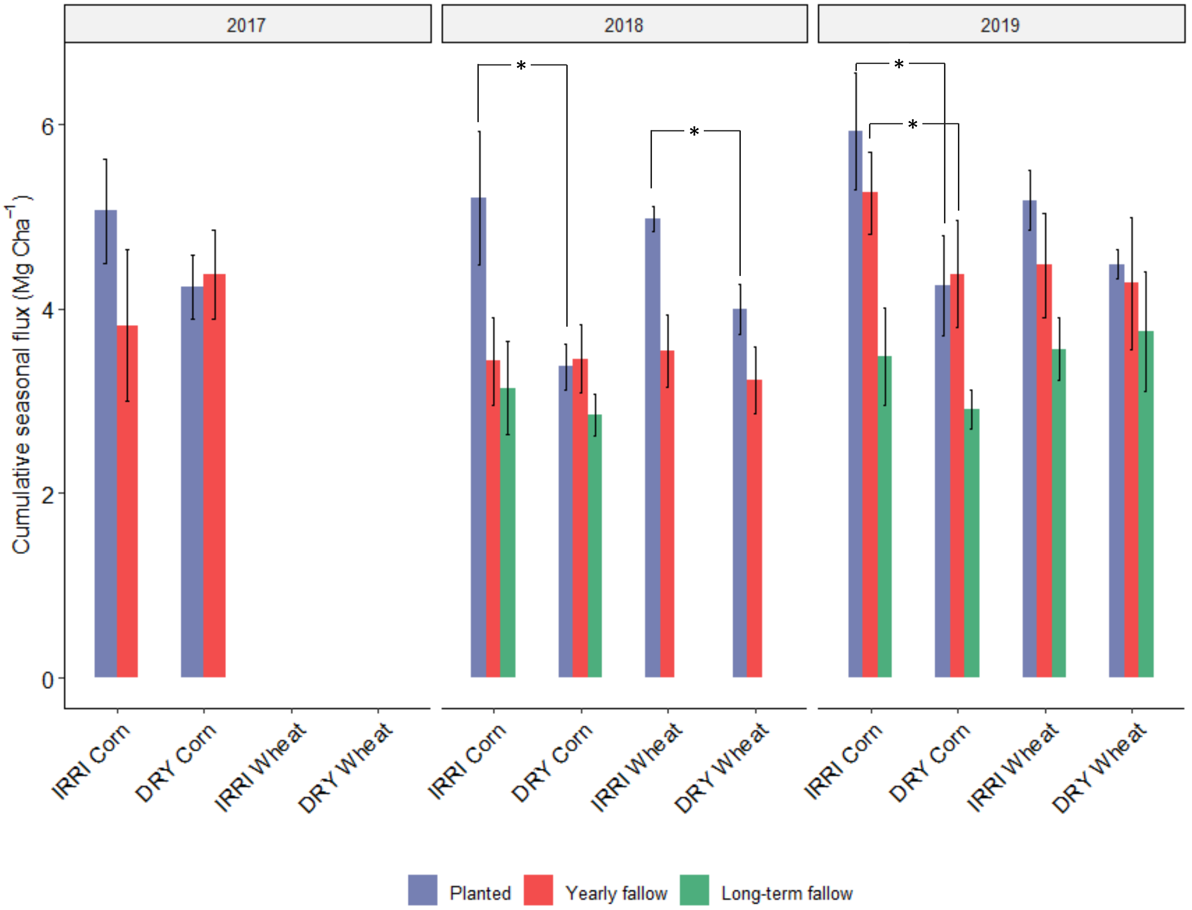

Fig. 4.5. Accumulated soil respiration in each irrigation and fallow treatment of maize and wheat. Data represents mean ± 1 SE with n=4. Asterisks denote an irrigation effect on the accumulated flux within planted or fallow treatments of the same crop ($p < 0.05$).

Changes in SOC total stocks, fractions, and isotopic composition

After three seasons, we did not find differences in total SOC stocks due to irrigation management ($p > 0.2$ for all treatment comparisons within planted areas or long-term fallows), but irrigation did affect changes in SOC over time (Fig. 4.6a). The long-term fallow subplots lost SOC compared to the baseline sampling in all treatments but had lower stocks than the planted area only in the irrigated maize treatment. At 0-10 cm, SOC stocks in the planted areas were not statistically different than the baseline sampling in any individual treatment, but in all cases the highest stocks were found at the beginning of the experiment, two months after the last tillage event. At 10-20 cm, there was a consistent loss of SOC with time both in the planted and long-term fallow areas of both crops (Fig. 4.6a, $p < 0.05$ except for IRRI Wheat $p = 0.06$).

Carbon content in the soil organic matter fractions varied with the different samplings but was not affected by irrigation or crop type ($P > 0.11$, Table 4.2). Dissolved organic carbon was highest in the baseline sampling at both depths ($p < 0.01$) but did not vary between the planted and the long-term fallow areas in any case. The long-term fallow areas had the lowest contents of POM, while the presence of growing plants was sufficient to maintain the C stocks in POM at 0-10 cm ($p > 0.7$ for all the comparisons between baseline and planted area). At 10-20 cm the planted areas had intermediate POM contents, that were usually not different from the baseline nor from the long-term fallow subplots. The amount of C in MAOM was stable during the experiment and this pool did not vary due to sampling or irrigation management in any case ($p > 0.10$).

The evolution of the isotopic composition of SOC, an indicator of the fate of new C inputs and older SOC pools, was also affected by irrigation (Fig. 4.6b). In the irrigated maize, the planted area had a higher $\delta^{13}C$ signal more similar to the maize plant isotopic signature than both the baseline and the long-term fallow subplots at 0-10 cm ($p < 0.001$), and there were also differences between the planted and fallow areas at 10-20 cm ($p = 0.03$). In contrast, in the dryland maize there were no significant differences between the three samplings at any depth ($p > 0.3$). This resulted in a cumulative effect of irrigation on soil $\delta^{13}C$ of the maize planted areas at 0-10 cm after three seasons ($p < 0.01$). In the wheat plots after two crop cycles, the planted areas were ^{13}C depleted compared to the old subplot in the dryland treatment at 0-10 cm ($p = 0.061$) but not at 10-20 cm or at any comparison in the irrigated wheat treatment ($p > 0.3$).

Both in the bulk soil and in the different SOM fractions, $\delta^{13}C$ was affected by crop type in the planted areas ($p < 0.01$) but not in the long-term fallows ($p > 0.19$), indicating that we successfully avoided new C inputs in the long-term bare fallow subplots. The $\delta^{13}C$ in the top 10 cm increased after three seasons of bare soil in all cases, but the differences were not statistically significant. However, the POM of the long-term fallow subplots became depleted ($p = 0.03$ for IRRI wheat at 0-10 cm, $p < 0.01$ in all treatments at 10-20 cm), probably due to the loss of corn-derived POM incorporated with tillage shortly before the start of the experiment.

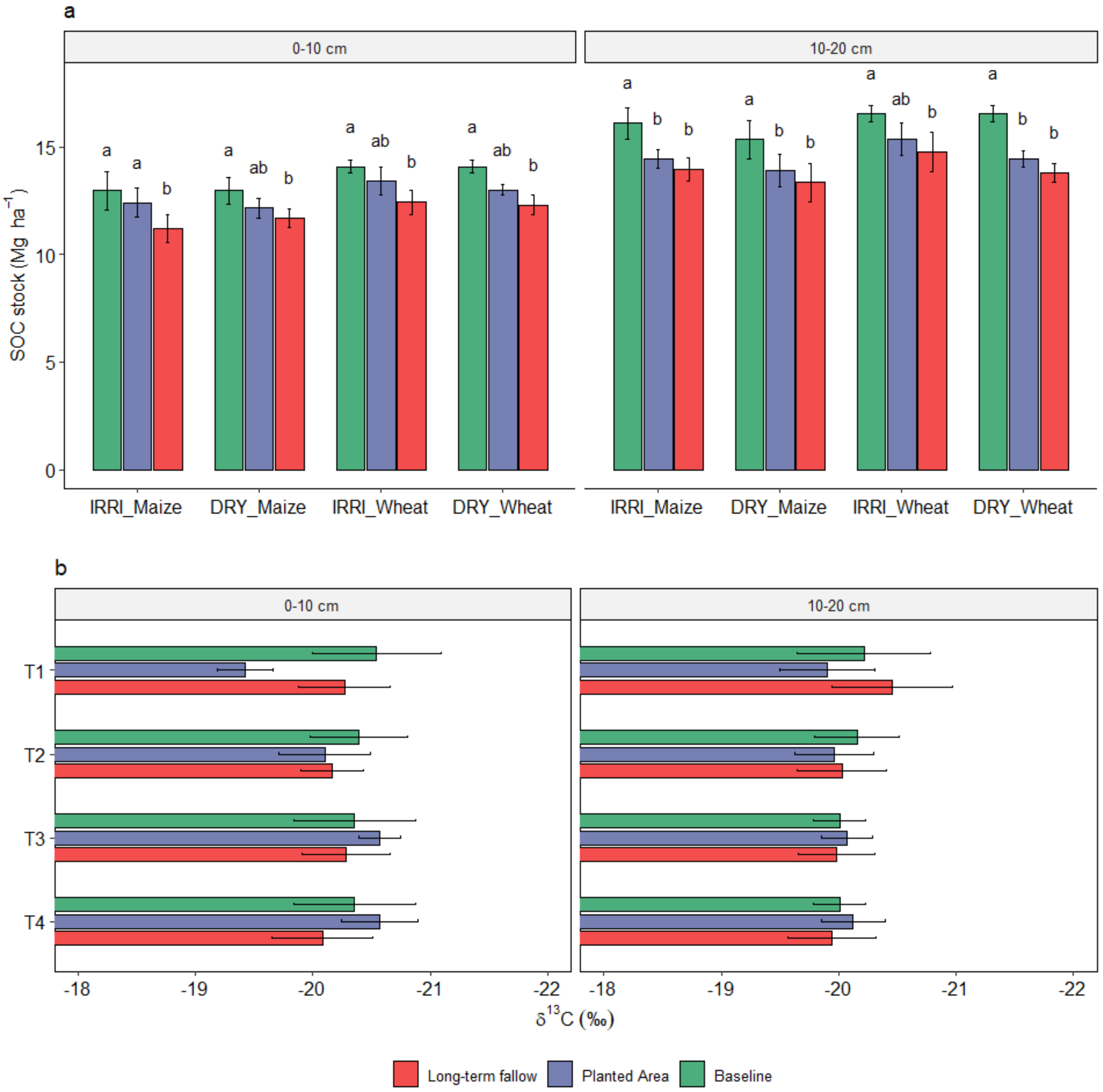

Fig. 4.6. Soil organic carbon stocks (a) and δ^{13}C values (b) at the beginning of the experiment and after three seasons in planted areas and long-term bare fallow subplots for each treatment at two sampling depths. For (b), T1 = IRRI_Maize, T2 = DRY_Maize, T3 = IRRI_Wheat, T4 = DRY_Wheat.

We also analyzed the isotopic composition of POM and MAOM and found that the POM pool was more affected by management (Table 4.2). The δ^{13}C of POM in maize plots shifted to become more maize-derived in both the irrigated and dryland planted areas at 0-10 cm ($p < 0.01$) but not at 10-20 cm ($p > 0.7$). In the wheat plots, POM values shifted toward wheat $\delta^{13}C$ signatures at both depths, becoming more depleted compared to the baseline ($p < 0.05$), but did not differ from the long-term fallows ($p > 0.7$) irrespective of irrigation management. The $\delta^{13}C$ of the larger MAOM pool was not affected by irrigation or crop type and did not vary between the different samplings in the same treatment.

Table 4.2. Carbon stocks, isotopic composition, and fraction of new carbon on different soil organic matter fractions. Data is the mean ± SE (n = 4).

Depth	Treatment	SOC stocks (Mg ha^{-1})			δ^{13}C (‰)		*f* new (%)	
		DOM	POM	MAOM	POM	MAOM	POM	MAOM
0-10 cm	Baseline	0.28 ± 0.01	1.99 ± 0.23	10.77 ± 0.42	−22.05 ± 0.60	−20.13 ± 0.28		
	IRRI Maize	0.16 ± 0.01	1.97 ± 0.20	10.82 ± 0.47	−19.86 ± 0.49	−19.75 ± 0.22	30.91 ± 5.46	6.47 ± 1.71
	DRY Maize	0.18 ± 0.03	1.89 ± 0.21	10.36 ± 0.18	−20.69 ± 0.66	−19.98 ± 0.22	22.12 ± 4.24	3.03 ± 1.27
	IRRI Wheat	0.16 ± 0.01	2.14 ± 0.14	11.47 ± 0.68	−23.15 ± 0.35	−20.42 ± 0.20	7.27 ± 7.43	2.81 ± 1.57
	DRY Wheat	0.16 ± 0.01	1.92 ± 0.11	11.13 ± 0.51	−22.75 ± 0.30	−20.21 ± 0.24	3.57 ± 7.98	2.21 ± 0.86
	Long-term fallows	0.18 ± 0.02	1.53 ± 0.15	10.50 ± 0.41	−22.72 ± 0.43	−20.17 ± 0.22		
10-20 cm	Baseline	0.37 ± 0.01	2.49 ± 0.28	12.78 ± 0.51	−21.55 ± 0.79	−19.88 ± 0.34		
	IRRI Maize	0.19 ± 0.01	2.16 ± 0.27	12.44 ± 0.35	−21.26 ± 0.80	−19.67 ± 0.35	20.45 ± 4.77	4.59 ± 2.02
	DRY Maize	0.19 ± 0.01	2.08 ± 0.27	12.02 ± 0.49	−21.73 ± 0.70	−19.71 ± 0.26	14.02 ± 3.57	0.58 ± 1.04
	IRRI Wheat	0.21 ± 0.01	2.02 ± 0.17	12.71 ± 0.64	−22.70 ± 0.57	−19.67 ± 0.19	−0.46 ± 2.17	−1.28 ± 1.38
	DRY Wheat	0.22 ± 0.00	1.99 ± 0.18	12.42 ± 0.39	−22.75 ± 0.69	−19.65 ± 0.22	−1.57 ± 9.66	−0.18 ± 1.97
	Long-term fallows	0.21 ± 0.01	1.87 ± 0.22	12.23 ± 0.54	−22.99 ± 0.64	−19.79 ± 0.33		

Changes in SOC composition

The isotope analyses allowed us to estimate gains of new, crop-derived SOC and losses of old, native SOC during the experimental period (Fig. 4.7). Irrigation increased the amount of maize-derived C that formed SOC at both depths ($p = 0.018$ and $p = 0.088$ at 0-10 and 10-20 cm, respectively), and we did not find evidence of new, maize-derived C incorporation under dryland production. In wheat, although we did not find differences due to irrigation ($p > 0.4$), the amount of new, wheat-derived SOC was significant in the dryland but not in the irrigated treatment.

More old SOC was lost from 10-20 cm than from 0-10 cm depth ($P = 0.005$), but the total amount of old SOC lost was not affected by irrigation ($P = 0.94$), crop type ($P = 0.85$) or the presence or absence of plants ($P = 0.41$, Fig. 4.7). There were no irrigation effects on the loss of old SOC from the long-term fallow plots in agreement with the respiration data. In the planted area, the estimated SOC loss was almost double in the irrigated than in the dryland maize (1 Mg ha^{-1} more), but the difference was not significant ($p = 0.18$).

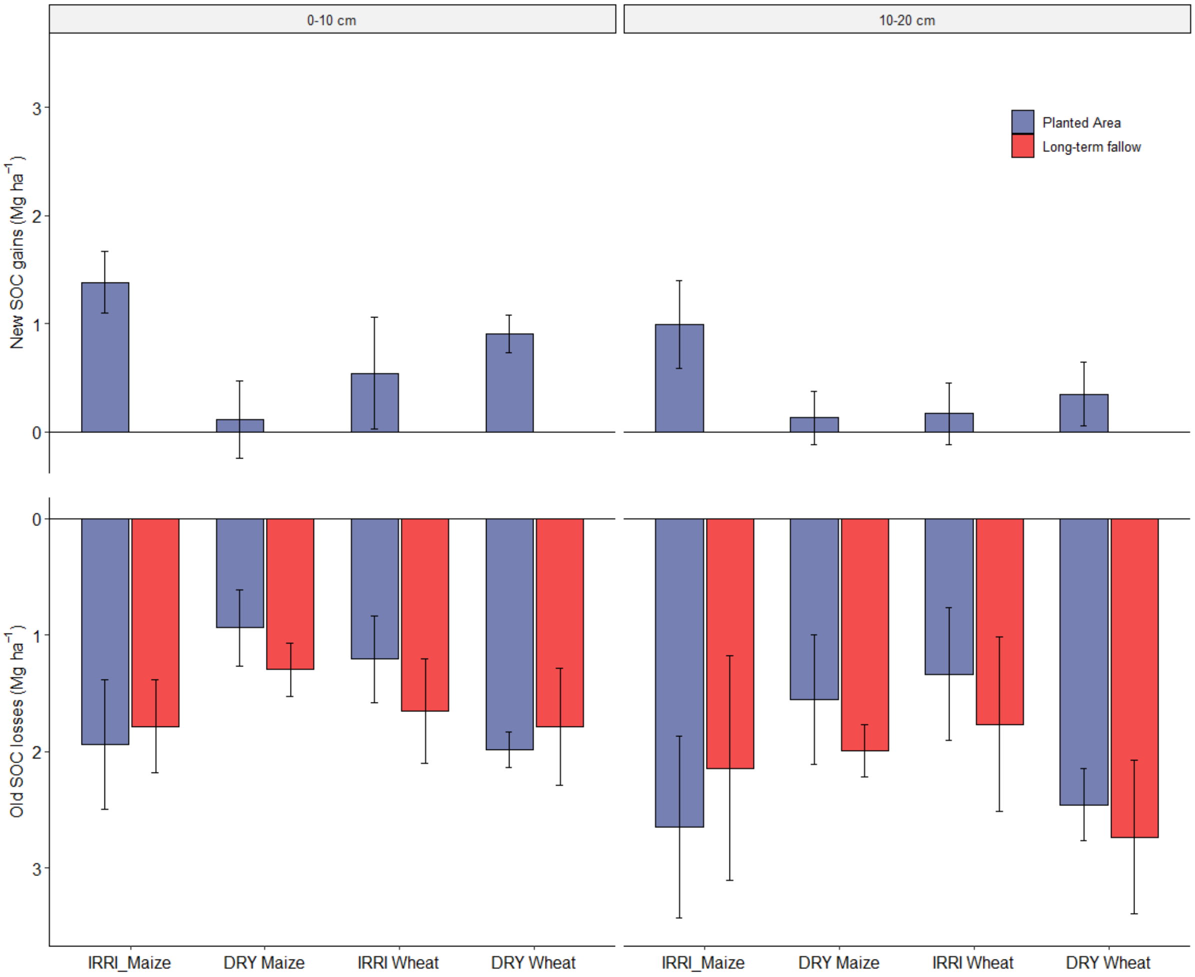

Fig. 4.7. Stabilization of new C (upper plot) and losses of native SOC (lower plot) in the planted areas and in long-term fallow subplots of each treatment at two sampling depths.

The fraction of new C was higher in POM than in MAOM ($p < 0.001$, Table 4.3) but, given the differences in pool size, the absolute amount of new C was similar in both fractions ($p = 0.9$). A higher proportion of litter-derived C was found in the surface layer ($p = 0.02$). The proportion of new C in the different SOM fractions was not affected by irrigation ($p > 0.1$) but the total amount of maize-derived C in MAOM was higher in the irrigated treatment ($p = 0.089$ and $p = 0.029$ at 0-10 and 10-20 cm depth). The estimated C losses from each fraction were not affected by the presence of plants ($P = 0.36$) and were again similar in absolute amounts between both fractions ($P = 0.67$). The irrigation effect on C losses from the different fractions was low, and we only found a tendency for higher losses of old MAOM-C in the irrigated than in the dryland maize at 10-20 cm ($p = 0.077$).

Soil organic carbon dynamics

Changes in native SOC allowed us to estimate the decomposition rate of SOC in the long-term fallow subplots and in the planted areas (Table 4.3). We did not find any difference between planted and long-term fallow areas for any specific treatment:depth estimation ($p > 0.14$ in all cases). Under maize production, the decomposition rate with irrigation tended to be higher than under dryland in the planted areas ($p = 0.080$ at 0-10 cm and $p = 0.097$ at 0-20 cm depth) but not in the long-term fallow plots ($p = 0.37$ and $p = 0.66$). We did not find any evidence of differences due to irrigation in the wheat plots ($p > 0.16$).

The decomposition rate of POM was higher than for MAOM ($p < 0.001$), but we did not find differences in the decomposition of individual fractions due to irrigation ($p > 0.16$). The decomposition rate of MAOM was not affected by the presence of plants ($p > 0.7$), but in irrigated maize the decomposition rate of POM was higher in the planted area than in the subplot ($p = 0.027$).

In the two treatments with evidence of new SOC incorporation (irrigated maize and dryland wheat, Fig. 4.7), we also estimated the stabilization rate of new C into SOC (Table 4.3), and we found very similar rates in both cases ($p > 0.9$).

Table 4.3. Decomposition rates estimated in the long-term fallow subplots and in the planted areas for bulk soil and different soil organic matter fractions, and stabilization rate of new SOC in bulk soil in each treatment, in the top 10 and 20 cm. Data are the mean ± SE with n = 4.

Treatment	Bulk soil	POM	MAOM	Bulk soil	POM	MAOM	*Stabilization of new C (%)*
	Annual decomposition rate in the long-term fallows (%)			*Annual decomposition rate in the planted areas (%)*			
				0-10 cm depth			
IRRI Maize	5.84 ± 1.11	12.81 ± 2.13	*1.96 ± 0.52*	6.47 ± 1.78	14.85 ± 2.27	*1.52 ± 0.54*	13.07 ± 2.81
DRY Maize	4.16 ± 0.66	8.87 ± 2.66	*1.56 ± 0.76*	3.02 ± 1.13	10.64 ± 1.88	*2.30 ± 0.75*	
IRRI Wheat	5.10 ± 1.50	10.87 ± 2.00	*0.46 ± 1.59*	3.68 ± 1.15	*2.11 ± 4.43*	*0.11 ± 1.71*	
DRY Wheat	5.50 ± 1.65	11.00 ± 2.74	*1.64 ± 1.37*	6.07 ± 0.41	4.71 ± 3.41	*0.99 ± 0.41*	15.37 ± 2.67
				0-20 cm depth			
IRRI Maize	5.76 ± 1.59	11.06 ± 1.97	*2.53 ± 1.03*	6.93 ± 1.82	16.55 ± 3.75	*2.68 ± 1.11*	21.65 ± 6.19
DRY Maize	4.94 ± 0.51	11.74 ± 2.69	*1.59 ± 0.60*	3.69 ± 1.37	12.05 ± 3.00	*1.66 ± 1.17*	
IRRI Wheat	4.89 ± 1.74	9.32 ± 3.03	*0.61 ± 1.50*	3.58 ± 1.32	*3.86 ± 3.94*	*0.41 ± 1.18*	
DRY Wheat	6.45 ± 1.72	11.59 ± 3.12	*2.24 ± 1.57*	6.28 ± 0.49	5.61 ± 3.47	*1.48 ± 0.55*	20.75 ± 7.50

Italics indicate estimations not different from zero (p = 0.05)

Discussion

We followed the changes in SOC dynamics for three years after irrigation retirement and the transition to dryland cropping systems. We found that irrigation retirement had a much larger effect on C inputs than on SOC turnover and C outputs. Given the differences between crops in growing season and water requirements, the effect was stronger in the maize agroecosystem. Maize biomass production was strongly affected, hence decreasing C inputs to the soil. Soil C outputs via Rh were affected by irrigation retirement only after two years of accumulated differences in C inputs, indicating that irrigation affected microbial respiration indirectly due to changes in substrate availability rather than directly due to changes in soil moisture. In addition to this temporal trend, three other lines of evidence support the indirect effect of irrigation on C outputs: (*i*) respiration of old SOC from the long-term fallow subplots (which differed in soil moisture but not in C inputs) did not differ between the irrigated and dryland treatments in any year; (*ii*) Rh in the maize systems was not affected by soil moisture; and (*iii*) isotopic analyses indicated that the differences between irrigated and dryland were due to the formation of new SOC from C inputs rather than to changes in the decomposition of old SOC.

Effects of irrigation retirement on C inputs

Irrigation retirement had a strong influence on maize-derived C inputs to the soil, as expected from the high water requirements of this crop (Araya et al., 2019, 2017; Lamm et al., 2007; Sherrod et al., 2014). Aboveground biomass production was more affected by irrigation retirement than belowground production, coincident with the usual observations that water stress affects shoot growth more than root growth (Benjamin et al., 2014; Zhou et al., 2018). In wheat, irrigation retirement decreased aboveground C inputs but proportionally increased the belowground inputs, resulting in very similar total inputs but with different distribution in the soil. The effects of irrigation retirement on above and belowground substrate availability for soil microorganisms would accumulate over time as the difference in biomass inputs increases with each growing season and the aboveground litter starts to decompose and is incorporated into the soil. In a previous experiment at the same site (Chapter 3) we followed aboveground maize litter decomposition and found that ~40% of the original C remained in the litter layer after one year, coincident with observations that total decomposition of aboveground residues may take three years or longer (Adair et al., 2008; Cotrufo et al., 2015). Therefore, more time may be needed to fully observe the differences between irrigated and dryland systems in SOC, and our observations should be taken as indicators of early changes in SOC dynamics.

Effects of irrigation retirement on soil respiration and carbon outputs

Total Rs decreased after irrigation retirement, but mainly due to a decrease in Ra. Rh did not respond to irrigation retirement during the first two years of the experiment and there were differences only after two seasons of accumulated differences in C inputs in the maize treatments. In wheat, where differences in C inputs were minimal, no irrigation effect on Rh was observed. This suggests that irrigation affected microbial respiration indirectly due to changes in substrate availability rather than directly due to changes in soil moisture. Moinet et al. (2016) also found that Rh did not respond to irrigation or moisture but it was related to the protection of SOM and the microbial accessibility to substrates. Supporting this idea, in our long-term fallow subplots, without differences in substrate availability, irrigation alone did not affect respiration.

After two years of accumulated differences in maize biomass production, Rh from the yearly fallow subplots was higher in the irrigated treatment in almost half of the days, which resulted in 20% more C outputs than in the dryland treatment during the third season. This may be the result of lower C availability in the dryland system, which has been shown to limit soil respiration (Cook and Orchard, 2008; Orchard and Cook, 1983). The delayed effect of irrigation retirement on Rh may be related to the time necessary for the initial decomposition and transfer of crop residues into the soil and suggests that SOC turnover rates may slow down in dryland systems over a longer time frame. This is coincident with field evidence of the effect of irrigation on SOC, where irrigated sites have much more crop production but only a moderate increase in SOC compared to dryland counterparts (Denef et al., 2008; Gillabel et al., 2007).

The lack of a direct irrigation effect on Rh was also evident by respiration from the maize yearly fallow subplots being insensitive to changes in soil moisture ($p > 0.20$), which agrees with other reports (Demyan et al., 2016; Metcalfe et al., 2007; Moinet et al., 2016). Usually, Rh is highest at intermediate values of soil moisture and has upper and lower thresholds related to the opposing effects of soil moisture on different processes such as substrate and oxygen transport (Moyano et al., 2013; Schimel, 2018; Suseela et al., 2012). Given the consistency and magnitude of the reduction in soil moisture after irrigation retirement and the broad range of moisture values, we were expecting moisture to limit Rh in the dryland maize. Instead, our findings suggest that substrate availability explained more of the variability in Rh than moisture, which is in agreement with other research showing that substrate availability can affect the relationship between Rh and moisture (Canarini et al., 2017; Cook and Orchard, 2008; Moinet et al., 2016).

Our results suggest that the decrease in Rs after irrigation retirement did not represent a change in net CO_2 emissions because they were dominated by changes in Ra (Kuzyakov, 2006). However, one potential limitation to our reliance on fallow subplots to estimate Rh is our inability to detect any priming effect on Rh in the planted areas, which we would expect to be greater for the irrigated maize due to the greater root biomass and activity. The lack of significant differences between the bare fallow subplots and the planted areas in the estimated decomposition rates of bulk SOC and MAOM (Table 4.3) suggests that priming was not an important process in our experiment. Moreover, there was no relationship between estimated decomposition rates and C inputs in the bulk soil or in any of the fractions ($p > 0.15$), further supporting the lack of priming in our experiment. Priming is usually observed in N-limited systems (Dijkstra et al., 2013; Diochon et al., 2016) and our fertilization rates were probably enough to prevent it, as we did not observe any sign of N deficiency and there was usually an increase in the availability of inorganic N during the growing seasons (Chapter 2). This is coincident with the direct observation in a very similar no-till, irrigated maize system managed with similar N rates, where the addition of fresh residue did not stimulate soil-derived CO_2 (Leichty et al., 2021).

Effects of irrigation retirement on SOC stocks and dynamics

Without inputs of new C during three seasons, there was a significant loss of SOC in the bare fallow subplots (Fig. 4.6). This decline was mainly explained by losses from the more labile pools, DOM and POM, although DOM was a very small fraction (< 2% of total SOC) with little influence on the estimates of total SOC. The POM pool is characterized by its rapid response to changes in management and the supply of fresh C inputs (Cambardella and Elliott, 1992; Follett et al., 2015) so its rapid decrease during a long fallow period was expected. On the other hand, the MAOM fraction is protected from decomposition by its association with the soil matrix (Lavallee et al., 2020), and the time frame of the experiment was not long enough to observe a significant decay of this pool even under bare fallow. The preferential loss of POM resulted in an increased proportion of total SOC that was MAOM; at the beginning of the experiment 82% of total SOC was in the MAOM fraction and this proportion increased to 86% after 2.5 years of bare soil.

In the planted areas, the inputs of crop residues were enough to maintain the stocks of POM and SOC in the topsoil of all the treatments. However, the highest SOC stocks were always found in the baseline sampling, coincident with the observation that the seasonal C outputs due to Rs (Fig. 4.5) were higher than the C inputs (Table 4.1). Moreover, at 10-20 cm depth there was a significant decline in the SOC stocks, suggesting that continuous cropping systems may not be always enough to maintain SOC stocks even when irrigation supports high crop productivity. However, previous works under irrigated continuous maize found stable or increasing SOC stocks with similar productivity levels, but longer experimental periods, than our work (Follett et al., 2013; Halvorson and Jantalia, 2011; Halvorson and Schlegel, 2012; Halvorson and Stewart, 2015). The significant SOC decline at 10-20 cm was probably related to the concentration of crop residues in surface after the conversion to no-till and the lower residue incorporation into the deeper layer over time. Deeper SOC losses with no till have been reported

(Angers and Eriksen-Hamel, 2008), and they may result in a general SOC loss under continuous irrigated maize in semiarid systems when the entire soil profile is considered (Stewart et al., 2017). We are currently analyzing deeper soil samples to quantify the changes in the whole profile, but we expect the primary differences to be found in the surface soils in this shorter-term experiment.

Based on the observations that C inputs were more affected than C outputs, it is expected that the transition from irrigated to dryland cropping systems would result in a general loss of SOC, particularly in the low productive dryland maize. We did not find differences in total SOC stocks due to irrigation management after three seasons, but irrigated maize was the only treatment where the planted area had significantly higher SOC stocks than the bare fallow area. The lack of significant differences was probably due to the longer times usually needed to observe changes in SOC (Acosta-Martínez et al., 2011; Bradford et al., 2016; Franzluebbers et al., 1994b; Smith, 2004), but the differences observed in irrigated maize may indicate developing changes in SOC stocks, where the dryland maize was very similar to the bare fallow subplot. In addition, compared to the irrigated treatment the dryland maize had similar losses of native SOC but very little incorporation of new C (Fig. 4.7), further supporting a rapid loss of SOC during the transitions from irrigated to dryland cropping systems.

Wheat productivity was only marginally affected by irrigation, resulting in very similar C inputs and outputs despite water management. Therefore, the lack of irrigation effect on SOC dynamics in this crop were expected. However, the mixing model results indicated a significant presence of new, wheat-derived SOC in the dryland but not in the irrigated treatment. This may be related to the observation that dryland wheat had a higher root:shoot ratio (Chapter 2) and so it partitioned proportionally more C belowground. Roots are stabilized as SOC more efficiently than aboveground residues (Fulton-Smith and Cotrufo, 2019; Jackson et al., 2017; Mazzilli et al., 2015; Rasse et al., 2005), and our results may be related to the differences in biomass partitioning, although data variability in the irrigated wheat prevents for a strong conclusion.

Particulate organic matter usually responds rapidly to changes in management, but irrigation retirement did not affect the amount of new, crop-derived C found in it. This may be related to the lower effect of irrigation retirement on root production, that in the short term can be stabilized as POM almost ten times more efficiently than maize aboveground residues (Mazzilli et al., 2015). However, POM has low stability and a fast cycling in the soil (Cambardella and Elliott, 1992; Poeplau et al., 2018) so permanent inputs of crop residues are necessary to maintain this pool. In our experiment, the mean residence time (estimated as the inverse of the decomposition rate) of POM was between 9 and 12 years, coincident with the range of values reported by other authors (Balesdent et al., 1988; Cambardella and Elliott, 1992; Mazzilli et al., 2014; Milesi Delaye et al., 2013). Therefore, it is expected that the high impact of irrigation retirement in maize productivity would result in a decline in the POM pool in a decadal time scale or less.

Although the total amount of the C stored in MAOM did not change significantly even after 2.5 years of bare fallow, irrigation retirement affected the amount of new, maize-derived C in this pool. The stable MAOM pool has residence times in the scale of centuries (Poeplau et al., 2018; Salvo et al., 2014) but it is not passive (Lugato et al., 2021) and can be efficiently formed in the short term (Cotrufo et al., 2015, 2013). Previously, we found that C released from the aboveground litter in the early stages of decomposition formed proportionally more MAOM but without differences due to irrigation (Chapter 3). Here, the use of isotopes allowed us to see changes in the new C gains associated to the differences in crop productivity, even though we did not observed changes in the total MAOM pool. Because irrigation retirement did not affect the loss of MAOM, but decreased the amount formed from new C inputs, in the long-term losses from this pool are also expected. Given the slow turnover of MAOM, these changes are expected to occur in a longer time frame than the decrease in POM. But, because the C stored in MAOM was almost six times the stored in POM (Table 4.2), the losses of C from each pool were very similar even in this short period of time (losses of old SOC were 0.92 Mg C ha^{-1} from MAOM and 0.99 Mg C ha^{-1} from

POM). Therefore, both fractions will contribute significantly to CO_2 emissions during irrigation retirement if C inputs are not enough to counterbalance the losses.

The similar decomposition rates of SOC in irrigated and dryland treatments were confirmed both in the planted areas, using isotopic analysis, and in the long-term fallow areas, by respiration and SOC measurements. With similar losses, changes in SOC after irrigation retirement would depend on the amount of C inputs and the efficiency of SOC formation. We estimated the stabilization of new C into SOC (*h*) in irrigated maize and dryland wheat and found very similar rates in both cases, evidencing low or null direct irrigation effect on the formation of SOC. This suggests that the evolution of SOC during the transition from irrigated to dryland cropping systems would be related directly to changes in crop productivity and the amount of residues returned to the soil. With the similar cycling rates found in our experiment, we expect higher differences between irrigated and dryland systems with time as differences in residue production accumulate. But we also found that irrigation stimulated the C losses from the litter layer without higher SOC formation (Chapter 3). Thus, the difference in SOC formation would be smaller than the differences in crop productivity, although still governed by the changes in C inputs.

Because C inputs appear as the primary driver, the timeframe and magnitude of changes in SOC during the transition from irrigated to dryland cropping systems would also depend on the initial composition of SOC. The proportion of POM and MAOM varies with soil type, cropping system, and management practices (Denef et al., 2013; Franzluebbers and Stuedemann, 2014; Marriott and Wander, 2006; von Lützow et al., 2007), and given the differences in the stability of each pool (Table 4.3), it would affect the rate and magnitude of change. In our experimental site, more than 80% of SOC was in the more stable MAOM pool. Although this is within the range of observed values (Follett et al., 2015; Franzluebbers and Arshad, 1997; Li et al., 2017; Marriott and Wander, 2006), POM represented a smaller proportion of total SOC at this site than in other agricultural systems (Cambardella and Elliott, 1992; Franzluebbers and Stuedemann, 2014; Halvorson and Jantalia, 2011). In soils with a higher proportion of the more active

POM pool, we would expect a decrease in crop productivity and C inputs to potentially have an even faster total SOC decay rate.

Conclusions

The combination of irrigation and fallow treatments allowed us to understand the effect of irrigation retirement on SOC dynamics in semiarid agroecosystems. Our results indicate that, after irrigation retirement, changes in C outputs occur in response to changes in C inputs and that the effect on outputs is lower than the effect on inputs. Thus, the long-term effect of irrigation retirement on SOC will likely depend on the changes in biomass production and a loss of SOC can be expected due to high decreases in inputs that are not counterbalanced by decreases in outputs. The early losses of SOC will be proportionally higher from the POM pool, which is less stable and has a faster turnover rate. Therefore, in the medium term the remaining SOC would be dominated by the more stable MAOM pool, with an expected decrease in the turnover rate of SOC. The observed differences in maize Rh during the last season appear as an early indicator of ongoing changes in SOC turnover rates after irrigation retirement. As C inputs, but not soil moisture, are driving the changes in SOC dynamics, cropping systems that minimize the negative impact of irrigation retirement on biomass production have the potential to mitigate SOC losses during the transition from irrigated to dryland cropping systems.

Chapter 5: Changes in soil organic carbon stocks after conversion from irrigated to dryland cropping systems

Introduction

In semiarid agroecosystems, water is the main limiting factor for crop productivity (Nielsen et al., 2005; Peterson et al., 1993) and irrigation decreases the yield gap (Van Ittersum et al., 2013), increasing productivity and farmers' income. As an additional positive effect, the higher biomass production under irrigation results in more carbon inputs to the soil, which can increase soil organic carbon (SOC) (Blanco-Canqui et al., 2010; Denef et al., 2008; Emde et al., 2021a; Gillabel et al., 2007; Lueking and Schepers, 1985; Trost et al., 2013). Due to its positive effect on SOC, irrigation has been proposed as a management practice with potential for SOC sequestration (Follett, 2001; Minasny et al., 2017; Paustian et al., 2016; Smith et al., 2008). However, excessive water withdrawals, declining groundwater levels, and increased competition for water resources result in a need to decrease pumping rates for agriculture (Davis et al., 2018; FAO, 2011; Richey et al., 2015; Smidt et al., 2016; Whittemore et al., 2016). This will result in an increase of irrigation retirement and the transition to dryland systems in key agricultural areas such as the Ogallala Aquifer Region (Deines et al., 2020).

The Ogallala Aquifer underlies part of eight states in the Great Plains of the United States and has a great influence on the agricultural production and social development of the region (García Suárez et al., 2018; Leatherman et al., 2004; Terrell et al., 2002). Its use for irrigation has also resulted in an estimated increase in SOC (Ogle et al., 2012), and in the western part of the region, irrigated lands have 15-19% more SOC stocks than their dryland counterparts (Denef et al., 2008). However, the water reserves of the Ogallala are declining at unsustainable rates (Haacker et al., 2016; Richey et al., 2015), and it is estimated that 24% of the currently irrigated area may need to transition to dryland systems by 2100 (Deines et al., 2020). After irrigation retirement, farmers face the decision of whether to continue crop farming under dryland conditions, transition to perennial pastures, or enroll in the Conservation Reserve Program (CRP) supported by federal incentives that requires the planting of perennial mixtures without grazing (Monger et al., 2018; Stubbs, 2015). The impact of these transitions to dryland annual crops versus perennial mixtures on SOC is not clear.

The evolution of SOC after irrigation retirement will depend on the relative changes in C inputs and outputs. Because the main effect of irrigation on SOC is due to an increase in C inputs (Denef et al., 2008; Gillabel et al., 2007; Trost et al., 2013), a loss of SOC during the transition from irrigated to dryland crops is expected. But, due to higher soil moisture, irrigation may also stimulate microbial activity, increasing SOC turnover and decomposition rates (Blanco-Canqui et al., 2010; Gillabel et al., 2007; Sainju et al., 2014; Trost et al., 2013; Verma et al., 2005), so irrigation retirement may also decrease C outputs. In addition, residue accumulation and SOC gains during irrigation may have a positive impact on water dynamics (Franzluebbers, 2002; Minasny and Mcbratney, 2017; Rawls et al., 2003; Shaver et al., 2013) and on crop productivity and stability (Bauer and Black, 1994; Díaz-Zorita et al., 1999; Loveland and Webb, 2003; Oldfield et al., 2019; Pan et al., 2009), especially under drought conditions (Kane et al., 2021). Maize and wheat, the dominant grain crops of the Great Plains, show the highest responses to SOC when its concentration is below 2% (Oldfield et al., 2019), which is the case for many soils of the Great Plains (Campbell et al., 2005; Parton et al., 1987). If the SOC gained during irrigation increases crop productivity after retirement, and if the decrease in C inputs is accompanied by a decrease in outputs, it may be possible that irrigation has a legacy effect on SOC after the transition to dryland cropping systems.

The main purpose of the CRP program is to retire highly erodible and environmentally sensitive land from agricultural production (Stubbs, 2015). The planting of perennial grassland mixtures to reach this objective may result in higher surface SOC stocks in CRP compared to dryland crops (Baer et al., 2002; Cotton and Acosta-Martínez, 2018; De et al., 2020; Follett et al., 2015; Ihori et al., 1995; Li et al., 2017; McLauchlan et al., 2006). Thus, transition from irrigated croplands to CRP may be a better strategy to maintain SOC than the transition to dryland crops. But, because irrigation systems may have C inputs similar or even higher than native prairies of the Great Plains (Denef et al., 2008; Gillabel et al., 2007), the positive effects of CRP on plant residue production credited with the positive effects on SOC relative to dryland crops, may not hold true when compared with irrigated croplands (Baer et al., 2010; Hurisso et al., 2014; Li et al., 2017; Matamala et al., 2008; Phillips et al., 2015). Thus, it is not clear if there would be a change in SOC after the transition from irrigation to CRP and the direction of this change. Compared to native vegetation, irrigation usually has a positive but variable effect on SOC (Trost et al., 2013) but, to our knowledge, no direct evaluation of the evolution of SOC after irrigation retirement and the transition to perennial grasslands has been done.

In addition to changes in C inputs and soil moisture, the fate of SOC after irrigation retirement may be influenced by the form in which SOC was accumulated during irrigation and the stability of the different pools. The size separation of soil organic matter (SOM) into particulate and mineral associated fractions allows to differentiate two pools with contrasting stability and response to management (Cambardella and Elliott, 1992; Lavallee et al., 2020). Particulate organic matter (POM) is mainly formed by plant material in various stages of decomposition and responds rapidly to changes in management (Cambardella and Elliott, 1992; Follett et al., 2015; Salvo et al., 2010), while mineral associated organic matter (MAOM) is more stable through its association with the soil matrix (Lavallee et al., 2020; Salvo et al., 2014). Thus, fractionation of SOM into these different pools can increase our understanding of the effect of irrigation and its possible legacy effect on SOC.

Our objective was to quantify changes in soil C and N stocks after irrigation retirement in semiarid agroecosystems of the High Plains. We sampled fields that stopped using irrigation about a decade ago and transitioned into either dryland crops or ungrazed CRP perennial grasslands and compared the SOC stocks in these fields with still irrigated and long-term dryland situations. Irrigation retirement will strongly decrease crop productivity, but it may also affect C outputs, and SOC gained during irrigation may have a positive impact on yields. Thus, we hypothesized that the loss of SOC gained during irrigation would not be complete and that there would be a legacy effect of irrigation on SOC, with intermediate contents in retired fields compared to both currently irrigated and long-term dryland reference situations. Based on the positive effect of perennial pastures on SOC, we also hypothesized that sites that transitioned to CRP would be able to maintain higher levels of SOC than sites that transitioned from irrigated to dryland cropping systems. To test these hypotheses, we quantified C and N stocks in the soil profile and used SOM fractions to understand the changes in the surface soil layer, where most of the changes are expected.

Materials and Methods

Study area and site location

To estimate the impact of irrigation retirement on agricultural systems of the Central High Plains, we searched for fields that had transitioned from irrigated to dryland systems. We consulted local extension agents and used irrigation maps (Deines et al., 2017) and Google Earth images to identify candidate fields. We visited the sites and interviewed the farmers to confirm that the candidate fields had been retired for at least five years after long-term irrigation with center pivots. Sandy soils were not included, and we focused on silt loams and silty clay loams soils, representative of most cultivated soils in the region (Hansen et al., 2012). We also confirmed that the fields were managed mainly without tillage and that the transition from irrigated to dryland did not involve significant changes in tillage. Based on this process, we selected six farms for sampling that included five fields retired to dryland cropping systems and three fields retired to perennial grasses under the Conservation Reserve Program (CRP). Whenever available in the same farms, we also sampled long-term, currently irrigated fields (under center pivot irrigation for approx. 30 years) and long-term dryland fields (never irrigated) for comparison purposes (Table 5.1).

The selected farm sites were in Kit Carson County, eastern Colorado, all within 30 km of Burlington (39° 18′ N, 102° 10′ W). The climate of the area is cool semi-arid, with mean annual temperature of 10.9 °C and an annual precipitation of 489 mm (1999-2020 U.S. Climate Normals, www.ncei.noaa.gov). The dominant soils are Aridic Argiustolls, with minor presence of Aridic Ustorthents and Pachic Argiustolls (NRCS, 2010). In the retired sites, the last irrigation occurred seven to ten years before sampling. The presence of wheat was more frequent in dryland than irrigated rotations (Table 5.1), coincident with previous reports from the region (Hansen et al., 2012; Rosenzweig and Schipanski, 2019). Also, dryland rotations were longer and some included summer fallows, while irrigated rotations had a higher frequency of corn and did not use summer fallows.

Table 5.1. Farm site characterization. Year of retirement corresponds to the year of the transition from center pivot irrigation to dryland. IRRI: currently irrigated fields; IRRI/DRY: fields that transitioned from irrigated to dryland crops; IRRI/CRP: fields that transitioned from irrigated crops to perennial grasslands without irrigation, DRY: long-term dryland fields. Crop key is C: corn, W: wheat, S: soybean, F: summer fallow, cc: cover crops, CRP: perennial grasses under the Conservation Reserve Program.

				Current rotations per field type			
Farm	Soil Type	Year of retirement	Year of sampling	IRRI	IRRI/DRY	IRRI/CRP	DRY
1	Richfield, Rago Weld. Silty clay loam and silt loam	2007	2017	C-C	W-C-F	CRP	W-C-F
2	Richfield, Rago Weld. Silty clay loam and silt loam	2007	2017		W-C-F		
3	Norka, Norka-Colby. Silt loam	2007	2017		W-C-F	CRP	
4	Kuma-Keith. Silt loam	2008	2017	C-S-W-cc	W-W-cc-C-C-W		W-W-cc-C-C-W
5	Norka, Kuma-Keith. Silt loam	2012	2019				
6	Norka, Weld. Silt loam	2011	2019	C-W		CRP	C-W-F-W

Sampling design

We used the selected sites to estimate, first, the effect of irrigation on soil carbon and nitrogen, and then the effect of two different management systems after irrigation retirement. For this we defined three comparisons: (i) currently irrigated fields (IRRI), (ii) formerly irrigated fields retired to dryland cropping systems (IRRI/DRY), and (iii) formerly irrigated fields retired to perennial grasses under the CRP program (IRRI/CRP). We used a paired sampling design and considered the field Corners outside the center pivot as long-term dryland controls of each field. In the case of the IRRI/CRP fields, the Corners represented the transition from dryland crops to perennial grasses, in comparison to the transition from irrigated to CRP that occurred in the Centers of the fields.

We divided each field in quadrants and defined two to four of these quadrants as sampling locations. In each location we sampled two zones, a Center zone inside the pivot as representative of the currently or formerly irrigated area and a Corner zone representative of long-term dryland management. In each zone we took four soil samples with a Giddings probe using a 4-cm diameter core to a depth of 80 cm. Each core was divided by depth (0-10, 10-20, 20-40, 40-60, and 60-80 cm), and the samples of the four cores combined per depth, bagged, and transported to the laboratory.

The selected zones in each field were inside the same soil unit and had similar topography and texture, with differences in surface clay content between Centers and Corners between ± 3%. Thus, we assumed similar soils in both zones and attributed the differences in the evaluated soil properties to management. This paired sampling approach has been useful to estimate the effect of irrigation on soil organic carbon (Denef et al., 2008; Mudge et al., 2017). But, because some fields had a history of flood irrigation and the Corners are a small proportion of the total field area, we wanted to test the validity of our assumption that the Corner zones were representative of long-term dryland management. To confirm that the Corners were representative long-term dryland controls, we also sampled fields under long-term dryland management (DRY) that had no previous records of irrigation. In these fields we used the same sampling approach already described, but we only sampled in the Center zones.

Soil analyses

Once in the lab, we recorded total sample mass and used a 10-g subsample to estimate gravimetric water content after oven-drying it for 48 hours at 105°C. We used the total dry mass and sampling core volume to estimate bulk density. Samples were air-dried, sieved to 8 mm for homogenization, and a subsample was sieved to 2-mm. Approx. 25 g of the 2-mm sieved sample was further ground on a rolling mill. We determined total carbon (TC) and nitrogen (TN) in the finely ground sample using a LECO Tru-SPEC elemental analyzer (Leco Corp., St. Joseph, MI). We also determined inorganic carbon (SIC) with a modified pressure-calcimeter method (Sherrod et al., 2002), and calculated soil organic carbon (SOC) as the difference between TC and SIC. We also sent approx. 200 g of the 2-mm sieved samples to Ward Labs (Lincoln, Nebraska, US) for texture and nutrient characterization analyses.

We used a size fractionation approach to understand the effect of irrigation on SOM dynamics (Cambardella and Elliott, 1992; Lavallee et al., 2020). We dispersed the surface soil (0-10 cm depth) by shaking a 10-g sample with 30 ml of 5 g L^{-1} Na-hexametaphosphate for 18 hours. Then, we sieved the samples on a 53 µm sieve to separate the particulate organic matter (POM, fraction bigger than 53 µm) from the mineral associated organic matter (MAOM). The collected slurries were oven-dried, weighed, and ground using mortar and pestle. All fractions were analyzed for SOC and TN in the same way as explained above for the bulk samples.

Data analysis

There were differences in bulk density between field types (Tables 5.2 and 5.3) that prevented a direct comparison of elemental stocks at fixed depth intervals (Ellert and Bettany, 1995; Lee et al., 2009;

von Haden et al., 2020; Wendt and Hauser, 2013; Wuest, 2009). Thus, we estimated carbon and nitrogen stocks on the same equivalent soil mass (ESM) for all the comparisons and considered the average of the IRRI/CRP fields, that had the lowest bulk density values, as the reference soil mass. We used a cubic spline interpolation procedure (von Haden et al., 2020; Wendt and Hauser, 2013) to quantify the total stocks at each ESM-corrected sampled depth, assuming that accumulated soil mass in the profile (0-80 cm) was 10,264 Mg ha^{-1}. Because we only analyzed SOM fractions at 0-10 cm, and the cubic spline method is not applicable to single-layer assessments (Wendt and Hauser, 2013), C and N stocks in POM and MAOM were corrected by linear interpolation. To do this, we corrected the thickness of the sampled layer in the agricultural fields based on the relationship between bulk density and depth of the IRRI/CRP fields (Solomon et al., 2002; Veldkamp, 1994). The ESM corrections allowed a more accurate comparison of element stocks between fields without the confounding effect of differences in the soil mass sampled (Ellert and Bettany, 1995; Lee et al., 2009; von Haden et al., 2020; Wendt and Hauser, 2013; Wuest, 2009), but the main results were very similar when paired comparisons were done with fixed depth calculations or with element concentrations (Table 5.3).

We used a linear mixed model to estimate the effect of irrigation and irrigation retirement on the analyzed soil variables. Depending on the variable, the interactive effects of field type, zone (Center vs Corner), and depth were considered fixed, and a random term of sampling location nested in field was included to account for the sampling design. We conducted Type III analysis of variance and adjusted pairwise comparisons with Kenward-Roger's method to assess differences between zones within each field and between fields in the same zone. The analysis of zones within fields allowed us to estimate the effect of irrigation, or irrigation retirement, compared to its own long-term dryland reference. The comparisons between fields allowed us to estimate the change after irrigation retirement compared to the currently irrigated condition, the assumed starting point of the retired fields. We used R version 3.6.3

(R Core Team, 2020) with the packages *lme4* (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017), and *emmeans* (Lenth, 2020) for all statistical analyses.

Results

Evaluation of field homogeneity and of Corner zones as long-term dryland controls

Our goal was to estimate the effect of irrigation retirement on soil organic matter (SOM) using a space-for-time substitution. For this approach to be valid, it is necessary to compare similar soils and to have a valid reference for comparison. In addition to sampling the same soil in each paired field, as already mentioned, the comparison of soil texture and cation exchange capacity across fields confirmed that we sampled similar soils across all field sites (Table 5.2) so the observed differences can be attributed to management.

We also defined two references to compare the retired sites with, the initial, long-term irrigated situation and the long-term dryland endpoint. The comparison between long-term dryland fields and the dryland Corners of irrigated or retired fields further supports the use of the field Corners as dryland references in each paired sampling (Table 5.2). We did find some differences in soil bulk density, pH, and nutrient availability within dryland fields, but the effect was explained by the Corners that transitioned to perennial grasses (CRP) being different from the dryland cropping systems. Moreover, SOC at 0-10 cm in the long-term dryland fields (12.5 ± 0.4 g C kg^{-1}) was the same as observed in the Corners of the irrigated fields (Table 5.3). Besides the differences in soil fertility that are more likely to be influenced by management, textural analyses indicated that soils were similar for fields retired to perennial grasses or dryland crops.

Table 5.2. Soil physico-chemical characterization at 0-10 cm of long-term dryland fields and dryland Corners of irrigated and retired fields. DRY: long-term dryland fields, IRRI/DRY: dryland corners used as controls of pivots retired to dryland crops, IRRI/CRP: dryland corners used as controls of pivots retired to perennial grasslands, IRRI: dryland corners used as controls of currently irrigated pivots. Values correspond to mean ± SE, p-values correspond to type III anova with Kenward-Roger's method.

Field	n	Sand %	Silt %	Clay %	CEC $cmol_c$ kg^{-1}	pH (H_2O)	bd g cm^{-3}	N-NO_3^- µg g^{-1}	P µg g^{-1}	EC mS cm^{-1}
DRY	12	36 ± 1	35 ± 1	29 ± 0	18.8 ± 0.4	7.3 ± 0.0 ab	1.27 ± 0.01 a	7.1 ± 0.7 a	53 ± 4 a	0.35 ± 0.00
IRRI/DRY	17	31 ± 0	40 ± 0	29 ± 0	19.6 ± 0.3	7.2 ± 0.0 ab	1.26 ± 0.00 a	12.7 ± 0.6 a	50 ± 1 a	0.37 ± 0.01
IRRI/CRP	11	34 ± 1	38 ± 1	28 ± 0	22.2 ± 0.5	7.6 ± 0.0 a	1.10 ± 0.01 b	1.0 ± 0.0 b	46 ± 3 a	0.29 ± 0.01
IRRI	12	33 ± 0	38 ± 0	29 ± 0	20.0 ± 0.5	7.1 ± 0.1 b	1.23 ± 0.01 a	8.4 ± 0.5 a	68 ± 3 a	0.34 ± 0.01
p-value		0.26	0.11	0.98	0.53	0.01	< 0.01	< 0.01	0.05	0.08

Carbon and nitrogen stocks in currently irrigated fields

In the currently irrigated fields, the irrigated Center zones had more SOC and TN than the dryland Corners in the surface layers (Table 5.3), with a positive effect of irrigation on SOC at 0-10 and 10-20 cm, and on TN content at 0-10 cm ($p < 0.05$, Fig. 5.1). This resulted in irrigated center pivots having more SOC and TN stocks to 80 cm depth than their dryland counterparts (9.7 Mg ha^{-1} more SOC and 0.8 Mg ha^{-1} more TN in the Center, Fig. 5.2). Related to the similar effects on SOC and TN, we did not observe differences in C:N ratios due to irrigation, with average values at 0-10 cm of 9.0 ± 0.1 for irrigated Centers and 8.8 ± 0.1 for dryland Corners. The content of inorganic carbon increased with depth but was not affected by irrigation or by any of the irrigation retirement situations (Table 5.3).

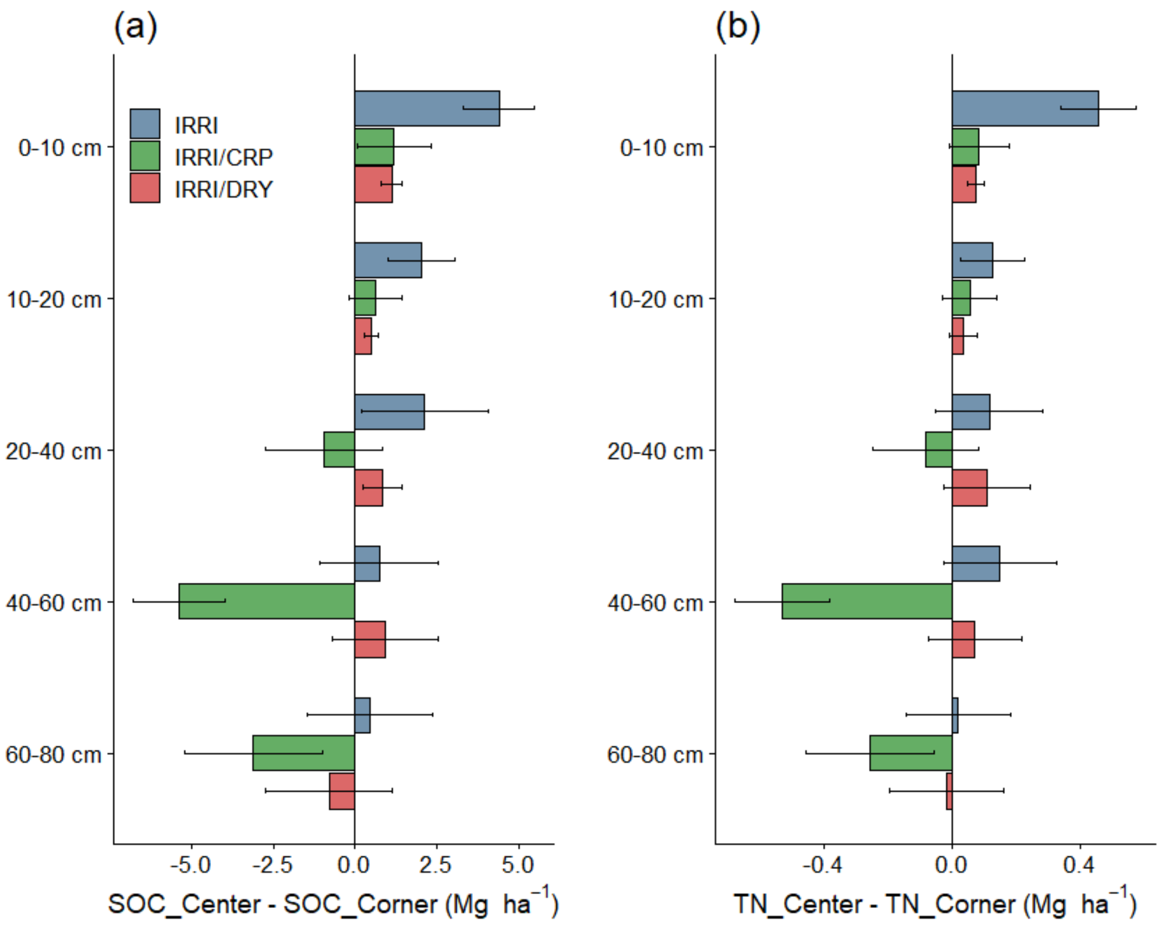

Fig. 5.1. Difference in soil organic carbon (a) and total nitrogen (b) stocks between paired Centers and Corners of currently irrigated fields (IRRI) and formerly irrigated fields that were retired to perennial grasslands (IRRI/CRP) or to dryland crops (IRRI/DRY) at different sampling depths. Corners are considered long-term dryland controls.

Carbon and nitrogen stocks after irrigation retirement

When compared to the long-term dryland reference, the legacy effect of irrigation was largely undetectable 7-10 years after irrigation retirement, as we did not find a difference in SOC and TN profile stocks between the formerly irrigated Center and long-term dryland Corners (Fig. 5.1 and Fig. 5.2). In the fields that transitioned from irrigated to dryland cropping systems (IRRI/DRY) the surface concentration of SOC was higher in the field Center than in the Corner zone (Table 5.3), but that difference was not significant after we calculated C stocks in the surface layer ($p = 0.12$, Fig. 5.1) or in the profile ($p = 0.50$, Fig. 5.2). In the retired fields that transitioned to perennial grasses (IRRI/CRP) profile stocks did not differ between previously irrigated Centers and long-term dryland Corners (Fig. 5.2, $p = 0.12$), but the field Centers had lower SOC and TN stocks at 40-60 cm depth than the Corners ($p < 0.01$, Fig. 5.1).

When compared with the currently irrigated center pivots (Fig. 5.3), the fields retired to dryland crops had lower SOC stocks at 0-10 cm (14.0 ± 0.1 vs 18.5 ± 0.3 Mg ha^{-1}, $p = 0.03$), while the sites retired to CRP showed intermediate contents (16.0 ± 0.2 Mg ha^{-1}, $p > 0.10$). However, there were no differences in SOC and TN stocks between currently irrigated or retired Center fields when comparing stocks for the entire 0-80 cm profile (Fig. 5.2). In the long-term dryland Corners, the inclusion of perennial grasses increased SOC stocks at 0-80 cm (79.5 Mg ha^{-1} in the CRP corners vs 71.1 Mg ha^{-1} average of all corners with dryland crops) but did not affect TN stocks (Fig. 5.2).

Table 5.3. Soil organic carbon, total nitrogen, and soil inorganic carbon concentrations, and bulk density at different sampling depths in paired Center and Corner zones of currently irrigated fields (IRRI) and formerly irrigated fields that were retired to perennial grasslands (IRRI/CRP) or to dryland crops (IRRI/DRY). Corners are considered long-term dryland controls. Values correspond to mean ± SE. p-values from a paired comparison of zone (Center vs Corner) in each field by depth using Kenward-Roger's degrees of freedom.

Depth	Soil Organic Carbon ($g\ kg^{-1}$)			Total Nitrogen ($g\ kg^{-1}$)			Soil Inorganic Carbon ($g\ kg^{-1}$)			Bulk density ($g\ cm^{-3}$)		
	Center	Corner	p.value	Center	Corner	p.value	Center	Corner	p.value	Center	Corner	p.value
Long-term irrigated fields (IRRI)												
0-10 cm	16.3 ± 0.2	12.5 ± 0.2	**<0.01**	1.8 ± 0	1.4 ± 0	**<0.01**	0.1 ± 0.0	0.4 ± 0.1	0.75	1.21 ± 0.01	1.23 ± 0.01	0.28
10-20 cm	9.2 ± 0.1	7.8 ± 0.2	0.07	1.2 ± 0	1.1 ± 0	0.28	0.3 ± 0.0	1.3 ± 0.3	0.41	1.38 ± 0.01	1.43 ± 0.01	**0.03**
20-40 cm	8.0 ± 0.1	7.2 ± 0.2	0.28	1.0 ± 0	1.0 ± 0	0.51	2.2 ± 0.2	3.4 ± 0.5	0.55	1.36 ± 0.01	1.33 ± 0.00	0.23
40-60 cm	6.5 ± 0.2	6.2 ± 0.2	0.70	0.8 ± 0	0.8 ± 0	0.45	7.4 ± 0.4	7.4 ± 0.5	0.85	1.33 ± 0.00	1.34 ± 0.00	0.75
60-80 cm	4.7 ± 0.2	4.7 ± 0.2	0.97	0.6 ± 0	0.6 ± 0	0.91	11.4 ± 0.2	9.2 ± 0.3	0.10	1.32 ± 0.00	1.32 ± 0.01	0.97
Fields that transitioned from irrigated to perennial grasses (IRRI/CRP)												
0-10 cm	14.7 ± 0.2	13.4 ± 0.2	0.12	1.5 ± 0	1.5 ± 0	0.19	0.2 ± 0.0	1.2 ± 0.2	0.39	1.06 ± 0.01	1.10 ± 0.01	0.10
10-20 cm	9.0 ± 0.1	8.4 ± 0.1	0.49	1.0 ± 0	1.0 ± 0	0.51	0.2 ± 0.0	1.3 ± 0.2	0.34	1.31 ± 0.01	1.33 ± 0.01	0.52
20-40 cm	7.6 ± 0.1	8.0 ± 0.1	0.66	0.9 ± 0	1.0 ± 0	0.69	2.7 ± 0.4	2.8 ± 0.5	0.99	1.31 ± 0.00	1.28 ± 0.01	0.32
40-60 cm	5.4 ± 0.2	7.3 ± 0.2	**0.02**	0.7 ± 0	0.9 ± 0	**<0.01**	8.7 ± 0.5	7.5 ± 0.6	0.31	1.32 ± 0.00	1.30 ± 0.01	0.34
60-80 cm	3.9 ± 0.1	5.0 ± 0.2	0.13	0.5 ± 0	0.7 ± 0	0.07	9.8 ± 0.4	10.2 ± 0.7	0.92	1.32 ± 0.01	1.33 ± 0.01	0.73
Fields that transitioned from irrigated to dryland cropping systems (IRRI/DRY)												
0-10 cm	12.4 ± 0.1	11.4 ± 0.1	**0.03**	1.4 ± 0	1.3 ± 0	0.14	0.1 ± 0.0	0.1 ± 0.0	0.96	1.21 ± 0.00	1.26 ± 0.00	**0.03**
10-20 cm	8.0 ± 0.1	7.6 ± 0.1	0.36	1.0 ± 0	1.0 ± 0	0.46	0.3 ± 0.1	0.3 ± 0.1	0.91	1.47 ± 0.01	1.48 ± 0.01	0.61
20-40 cm	7.2 ± 0.1	6.8 ± 0.1	0.45	0.9 ± 0	0.9 ± 0	0.33	2.5 ± 0.2	2.7 ± 0.2	0.74	1.37 ± 0.00	1.38 ± 0.00	0.78
40-60 cm	5.9 ± 0.1	5.5 ± 0.1	0.48	0.8 ± 0	0.8 ± 0	0.62	7.8 ± 0.3	8.6 ± 0.4	0.61	1.32 ± 0.00	1.32 ± 0.00	0.98
60-80 cm	3.9 ± 0.1	4.5 ± 0.2	0.21	0.6 ± 0	0.6 ± 0	0.71	10.8 ± 0.2	11.2 ± 0.3	0.86	1.33 ± 0.00	1.34 ± 0.01	0.48

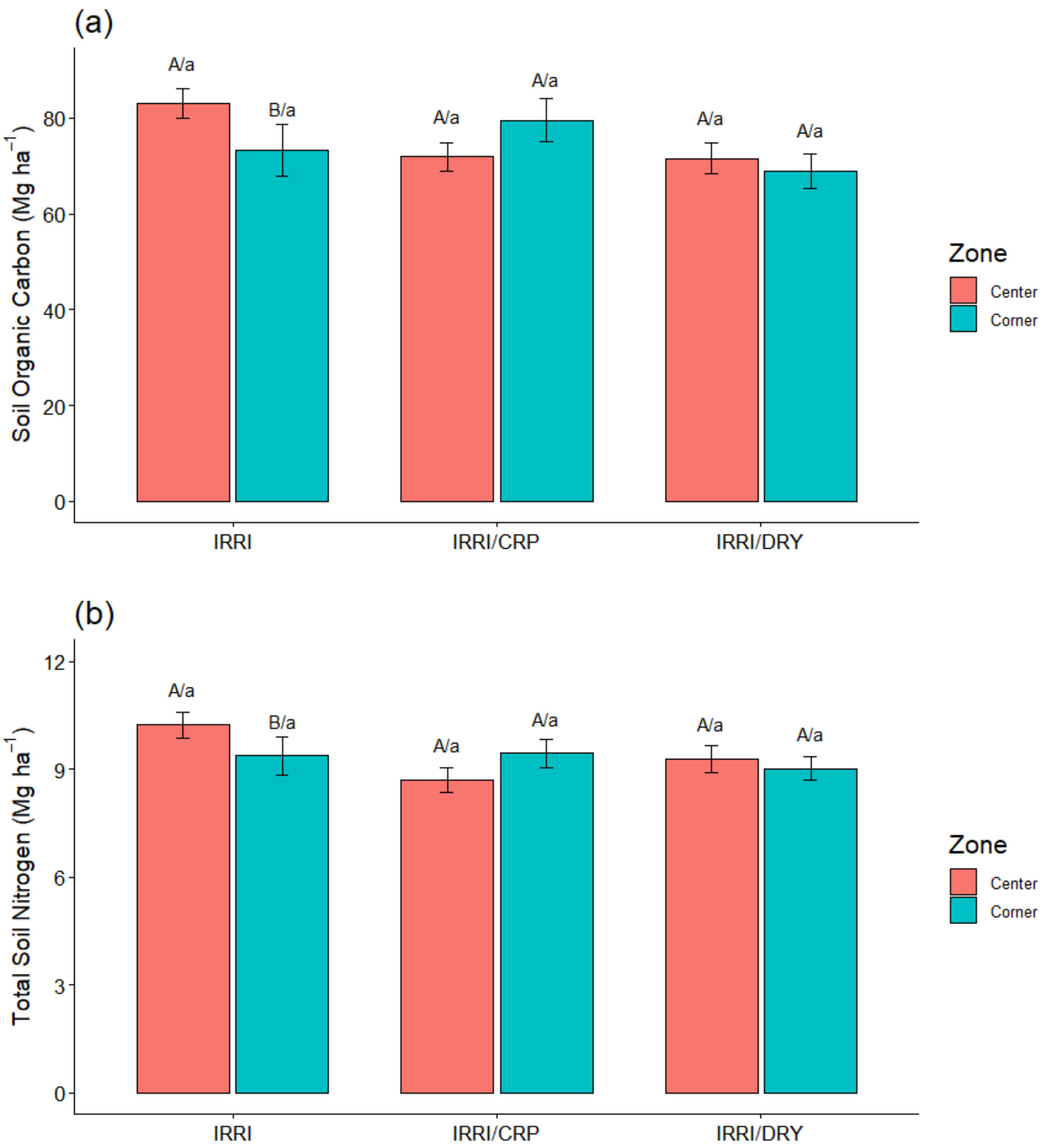

Fig. 5.2. Soil organic carbon (a) and total nitrogen (b) profile stocks at 0-80 cm depth in paired Center and Corner zones of currently irrigated fields (IRRI) and formerly irrigated fields that were retired to perennial grasslands (IRRI/CRP) or to dryland crops (IRRI/DRY). Corners are considered long-term dryland controls. Uppercase letters indicate differences between paired zones (Center vs Corner) in the same fields, lowercase letters indicate differences between fields in the same zone ($p \leq 0.05$).

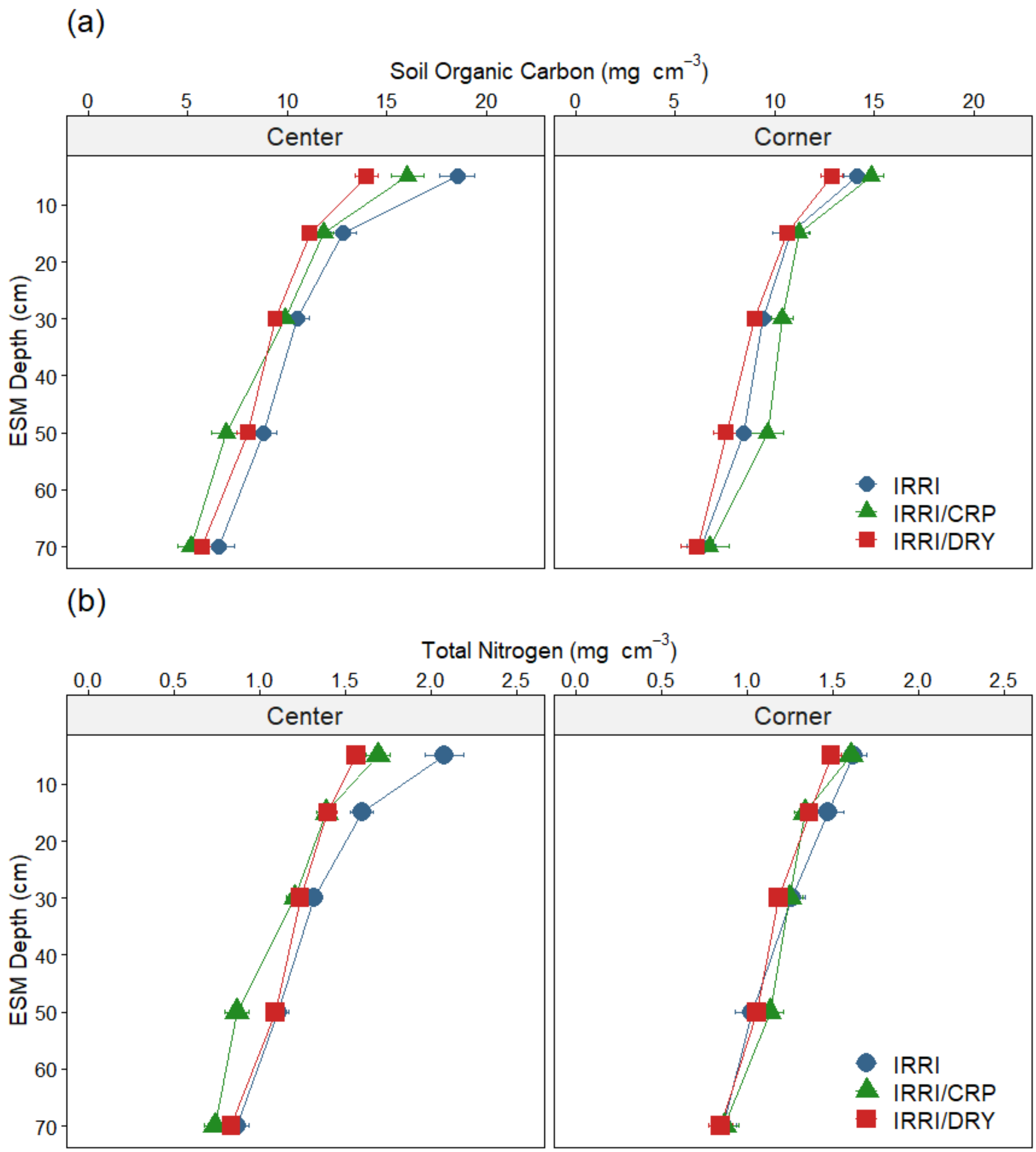

Fig. 5.3. Soil organic carbon (a) and total nitrogen (b) stock evolution with depth in Centers and Corners of currently irrigated fields (IRRI) and formerly irrigated fields that were retired to perennial grasslands (IRRI/CRP) or to dryland crops (IRRI/DRY). Corners are considered long-term dryland controls. Stocks were corrected to equivalent soil mass (ESM) using the bulk density of IRRI/CRP sites as reference.

Soil organic matter fractions

In the irrigated fields, the center pivots had more carbon and nitrogen contents both in the particulate (POM) and mineral associated (MAOM) organic matter fractions at 0-10 cm (Fig. 5.4), but the relative effect was higher in the POM than in the MAOM. There was almost 1.5 times more POM-C in the irrigated Center fields than in the dryland Corners (5.4 ± 0.1 vs 3.7 ± 0.1 Mg ha^{-1}, $p < 0.01$), while the difference in MAOM-C was only 17% (11.9 ± 0.1 vs 10.2 ± 0.1 Mg ha^{-1}, $p < 0.01$). The relative effect on N in each fraction was very similar (58% for POM, 17% for MAOM), although the C:N ratio of the POM was lower in the irrigated zones (C:N 12.9 ± 0.1 in POM of irrigated Centers vs 14.0 ± 0.2 in POM of dryland Corners, $p = 0.03$). The differential effect in each fraction resulted in a higher proportion of C found in the POM of irrigated Centers than in the POM of dryland Corners (30.2 ± 0.3% vs 26.7 ± 0.4%, $p = 0.05$).

In the fields that transitioned to dryland crops we found no differences between paired formerly irrigated Center and long-term dryland Corner zones in any of the SOM fractions evaluated. Moreover, the formerly irrigated Centers had lower C and N contents than the still irrigated center pivots in all the fractions (Fig. 5.4, IRRI Center vs IRRI/DRY Center). In the retired fields that transitioned to CRP, there was more POM-C in the Center than in the Corner zones (5.4 ± 0.1 vs 4.6 ± 0.1 Mg ha^{-1}, $p = 0.04$), but neither MAOM-C nor N in any fraction differed between paired zones ($p > 0.15$ in all the cases). Compared to the still irrigated center pivots, the Centers retired to CRP had the same amount of C and N in the POM but tended to have less in the MAOM (11.9 ± 0.1 vs 10.1 ± 0.1 Mg MAOM-C ha^{-1}, $p = 0.08$, and 1.33 ± 0.01 vs 1.14 ± 0.01 Mg MAOM-N ha^{-1}, $p = 0.07$, in IRRI vs IRRI/CRP Centers). In the long-term dryland corners, the inclusion of perennial grasses with CRP increased the POM contents but did not affect the MAOM.

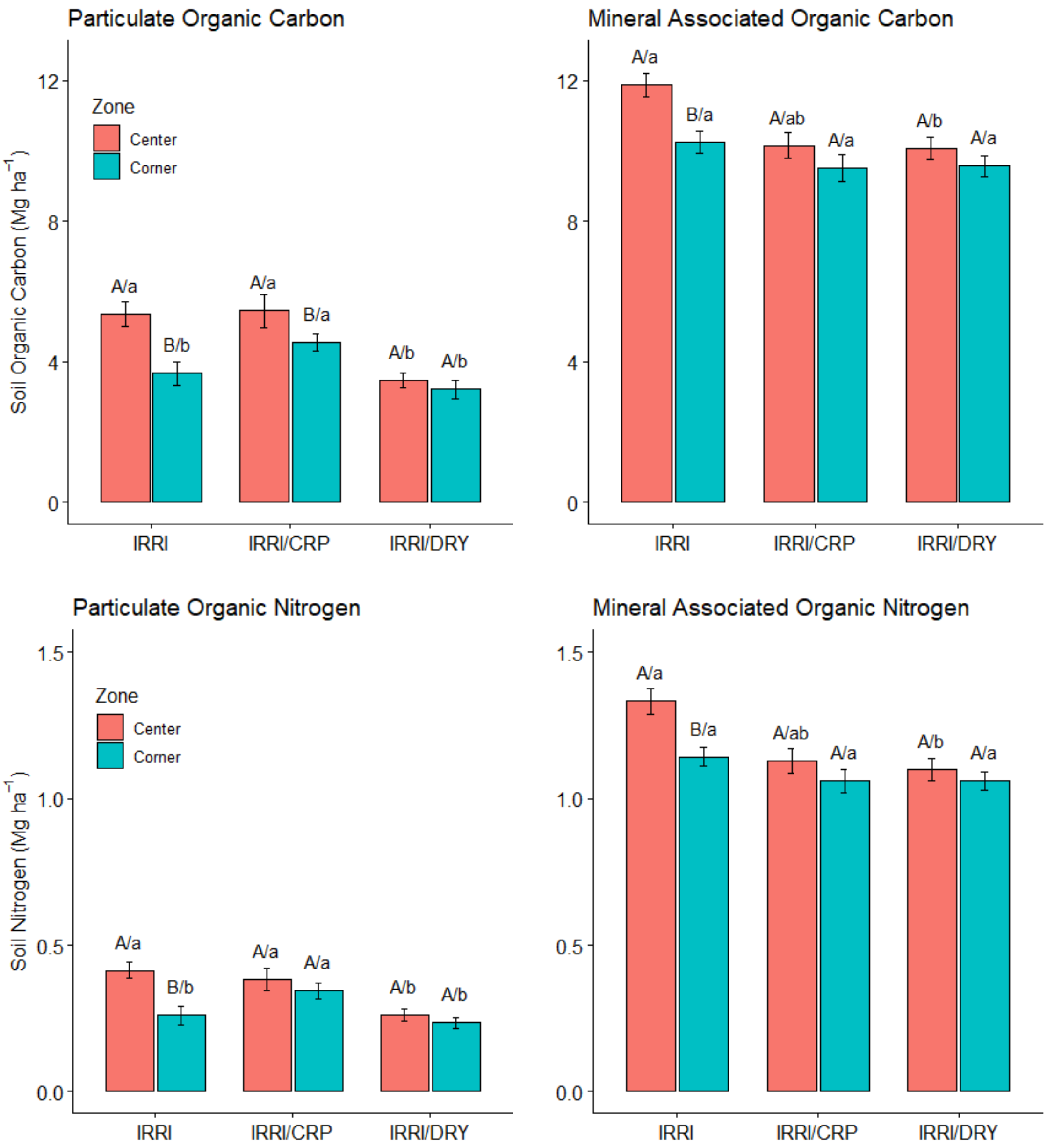

Fig. 5.4. Soil organic carbon and total nitrogen content in different soil organic matter fractions at 0-10 cm depth of paired Center and Corner zones of currently irrigated fields (IRRI) and formerly irrigated fields that were retired to perennial grasslands (IRRI/CRP) or to dryland crops (IRRI/DRY). Uppercase letters indicate differences between paired zones of the same fields, lowercase letters indicate differences between fields in the same zone ($p < 0.05$).

Discussion

Irrigation increases soil organic matter

We found a positive effect of irrigation on SOM, as currently irrigated center pivots had 14% more SOC and 9.5% more TN stocks at 0-80 cm depth than their dryland counterparts. The differences, significant at the whole profile level, were caused by an accumulation of organic matter in the surface soil layers of the irrigated systems. These findings are in close agreement with other reports from the Great Plains and other semiarid agricultural regions (Blanco-Canqui et al., 2010; Denef et al., 2008; Gillabel et al., 2007; Lueking and Schepers, 1985; Trost et al., 2013). Working on ten irrigated farms from Colorado and Nebraska, Denef et al. (2008) found that center pivots had 15-19% more SOC stocks than their dryland counterparts, and that the difference was explained by an increase in SOC at 0-20 cm. Our results and those previously cited coincide with the summary of Trost et al. (2013) that irrigation increases SOC by 11-35% in semiarid agroecosystems. However, this estimation is higher than the reported in a recent meta-analysis that assessed the irrigation effect based on experiments that followed the evolution of SOC through time (Emde et al., 2021a). In that work, the authors found that the highest effect or irrigation was in semiarid climates, under sprinkler irrigation, and that the effect was more important in the surface soil layers. Although these findings coincide with ours, they estimated a relative increase of only 8% in the full profile and of 15% at 0-10 cm depth (Emde et al., 2021a). These differences in magnitude may be related to the different experimental approaches (Christie et al., 2019; Franca et al., 2016; Yanai et al., 2000), but all agree on the positive effect of irrigation in semiarid climates and on the concentration of the effect in surface soil layers. The higher SOC with irrigation is caused by an increase in plant productivity that results in higher C inputs (Denef et al., 2008; Gillabel et al., 2007; Trost et al., 2013), which also explains the stratification of the effect.

Soil inorganic carbon (SIC) increased with depth, was more variable than SOC (Table 5.3), and we did not find differences between irrigated center pivots and dryland corners, coincident with previous reports (Denef et al., 2008). As expected based on the lack of differences in the currently irrigated sites, and the previously reported no effect of CRP on SIC (Phillips et al., 2015), irrigation retirement did not affect SIC in any of the evaluated conditions. Moreover, the evaluation of total carbon stocks (data not shown) indicated a significant difference due to irrigation in the surface, explained by the effect on SOC, but no differences in total carbon stocks at 0-80 cm, again in agreement with the findings of Denef et al. (2008).

Small legacy effect of irrigation on soil organic matter

Irrigation has a great effect on crop productivity, which results in higher C inputs and an accumulation of SOC in irrigated systems (USDA-NASS 2017; Lal 2004; Denef et al. 2008; Fig. 5.2). After irrigation retirement, water will be the main limitation for crop development in semiarid regions, and we expected that SOC accumulated during irrigation would be lost, resulting in a decline in SOC in retired fields compared to the still irrigated fields. This was confirmed by our study, as the fields retired to dryland crops had significantly less SOC at 0-10 cm than the currently irrigated fields (Fig. 5.3). Although we did not find statistical differences when comparing total stocks to 80 cm, the differences between irrigated and retired fields were very similar to the differences between the currently irrigated Center and the paired dryland Corner zones discussed in the previous section (Fig. 5.2), further supporting the decline in SOC and TN stocks after the transition from irrigated to dryland cropping systems.

Irrigation can impact both C inputs and outputs. Because irrigation also affects SOC turnover and C outputs (Trost et al., 2013), we expected that water limitation would decrease C outputs after irrigation retirement. We also expected that the higher SOC concentration in irrigated fields may have a positive impact on crop yields (Oldfield et al., 2019) and that crop yield and litter inputs in a retired field would be higher than in the long-term dryland counterpart. Thus, we hypothesized that SOC would be intermediate in a retired field. However, we did not find evidence to support this hypothesis because there were very few differences between the fields retired to dryland crops and their long-term dryland controls. We did find a small difference in SOC concentration at 0-10 cm, but that difference was not enough to cause an effect on SOC stocks. Moreover, SOC and TN stocks were the same in the retired fields than in the long-term dryland corners of the irrigated fields, both in the profile and in the surface layer. Our results suggest that all the SOC and TN gained during irrigation is lost approximately a decade after the transition to dryland cropping systems. Thus, any change in SOC turnover or a positive effect of SOC on crop yield was not enough to counterbalance the high impact of water stress on crop productivity and C inputs.

After irrigation retirement, the transition to perennial grasses appears as an option to mitigate, at least partially, the negative impact on SOM. Since the transition from dryland crops to CRP usually increases SOC (Ihori et al. 1995; Baer et al. 2002; McLauchlan et al. 2006; Follett et al. 2015; Li et al. 2017), we hypothesized that the transition from irrigated agriculture to CRP would allow the maintenance of SOC stocks compared to the transition to dryland crops. This hypothesis was partially supported by our data, because while the retirement to dryland crops resulted in a decrease in SOC stocks at 0-10 cm, the sites retired to CRP did not differ from the currently irrigated sites. However, the sites retired to CRP had intermediate SOC stocks that did not differ from the sites retired to dryland crops either. Thus, although CRP may be a better option than dryland crops, it may not be enough to retain all the SOC gained with irrigation. But CRP did have a positive effect on SOC stocks in the long-term dryland Corners, that represent almost a quarter of the total area in each field. Therefore, the transition from irrigated agriculture to perennial grasses maintained SOC stocks in the formerly irrigated areas and increased them in the Corner zones, resulting in an overall advantage compared to the transition to dryland crops. However, this advantage would come at a cost for farmers, that will have to resign income while the land enrolled in CRP is generally not available for production or grazing (Deines et al., 2020; Stubbs, 2015). For a wide adoption of perennial grasslands after irrigation retirement larger incentives will likely be required to offset economic losses with the transition.

Several factors may explain the intermediate SOC stocks in the sites retired to CRP. Compared to dryland cropping systems, even though many authors found a positive effect of CRP, this effect may be site or texture dependent and cannot be generalized to all the situations (Baer et al., 2010; Bowman and Anderson, 2002; De et al., 2020). Because not all the area under irrigated agriculture is suitable for dryland cropping (Deines et al., 2020) it may have happened that soil quality in the sites retired to CRP was lower than in the sites retired to dryland crops. We did not find differences in soil type or textural analyses between the retired sites that would suggest differences in initial SOC stocks, but the sampling design does not allow us to totally discard the possibility that there were previous differences between fields. Also, we found a low number of sites retired to CRP, which may have affected the statistical power of the comparison. Moreover, accumulation of SOC under CRP may take time (De et al., 2020), and estimations of the time needed to recover SOC stocks to levels similar to natural systems vary from decades (Baer et al., 2010, 2002; McLauchlan et al., 2006) to centuries (Matamala et al., 2008). We sampled sites that have been under CRP for 10 years or less, but SOC may accumulate linearly for at least 40 years after the introduction of perennial grasses (McLauchlan et al., 2006). We did find differences in other soil properties already reported to be affected by CRP, such as lower bulk density and nitrate concentrations (Baer et al., 2010; De et al., 2020), which may indicate that the restoration process is still under development. A longer time frame may be necessary to accurately estimate the effect of irrigation retirement to CRP and to quantify SOC stocks under steady state conditions.

Interestingly, in the sites retired to CRP we found that the long-term dryland Corners, that transitioned into CRP, had higher SOC and TN stocks at depth than the formerly irrigated center pivots (Fig. 5.1). Although there were no significant differences in depth between similar zones of the different fields (Fig. 5.3), the formerly irrigated zones under CRP tended to have less SOC at depth (40-60cm) than the other center fields, while the opposite occurred in the long-term dryland corners. Thus, the differences between zones in the sites retired to CRP cannot be explained only by an accumulation in the

Corners or by a loss in the Center zones, and do not seem to be related to differences in historical land use. Usually, the positive effect of CRP has been reported based on surface soil samples (Baer et al., 2010; Bowman and Anderson, 2002; De et al., 2020; Follett et al., 2015; Ihori et al., 1995; Li et al., 2017; McLauchlan et al., 2006) and those that sampled deeper than 20-30 cm found no effects at depth (Hurisso et al., 2014; Matamala et al., 2008; Phillips et al., 2015). Recently, Yang et al. (*in review*) conducted a continental-scale analysis of CRP effects on SOC and found that, while SOC increased at 0-5 cm, that gain was offset by a decrease at 30-100 cm, but the mechanisms behind these changes are not clear.

Compared to annual crops, perennial grasses under CRP have more root production at depth (Matamala et al., 2008; Phillips et al., 2015) and of lower quality as indicated by its higher C:N ratio (Baer et al., 2010, 2002; Matamala et al., 2008). In addition, during transitions to CRP, C stocks recover faster than N stocks (Matamala et al., 2008) and a decline in N availability may occur (Baer et al., 2010; McLauchlan et al., 2006). We found that the CRP sites had very low nitrate concentrations but enough phosphorus availability (Table 5.2) which further supports the possibility of N limitations. In N-limited systems an increase in soil C inputs may stimulate microbial activity and result in a positive priming effect (Dijkstra et al., 2013; Diochon et al., 2016), increasing the microbial mineralization of deep, old SOC (Fontaine et al., 2007). Together with a deeper root system, CRP sites have more microbial biomass per unit of SOC than croplands even at depth (Li et al., 2017; Matamala et al., 2008). If the grassland was more productive in the formerly irrigated center pivot than in the long-term dryland zone of the sites retired to CRP, more root growth may have resulted in a priming effect and in a loss of SOC. However, we do not have enough evidence to test this possibility, and future studies that quantify root development and the evolution of C inputs and SOC stocks in depth are necessary.

Use of fractionation to understand changes in SOM dynamics due to irrigation

The analysis of SOM fractions provides more insights into the source of SOC gains and losses. The currently irrigated fields had more C and N in both POM and MAOM than their long-term dryland controls, coincident with the concept that irrigation impacts both plant productivity and microbial activity (Cotton et al., 2013; Denef et al., 2008; Trost et al., 2013). However, the effect was bigger in the POM pool, which is mainly formed by plant-derived compounds (Lavallee et al., 2020), further supporting changes in C inputs as the main driver of SOC responses to irrigation (Denef et al., 2008). The crop rotations in the irrigated fields were dominated by corn and wheat (Table 5.1). These crops produce low quality residues with a high C:N ratio (Mazzilli et al., 2015; St. Luce et al., 2014) that would contribute proportionally more to POM than to MAOM formation (Cotrufo et al., 2015, 2013). We did find that C:N ratio of the POM pool was lower under irrigation, probably related to differences in fertilization. Optimum N fertilization rates are higher under irrigation (Rudnick et al., 2016) which may increase POM-N (Divito et al., 2011) and decrease the C:N ratio of SOM (Jagadamma et al., 2008).

The high increase in litter inputs due to irrigation had the highest effect on the POM pool, which represented ~30% of total SOM in the irrigated fields. POM is reported to be sensitive to management changes (Cambardella and Elliott, 1992; Follett et al., 2015), with low mean residence time and few protection mechanisms against decomposition (Lavallee et al., 2020). Thus, C and N accumulated in the POM fraction can be rapidly lost after changes in management and a decrease in litter inputs as expected during irrigation retirement. Indeed, we found a decrease in POM after irrigation retirement and the transition to dryland cropping systems. We also found a decrease in MAOM after irrigation retirement, confirming that this pool is also vulnerable, especially in croplands where it represents most of the SOM (Lugato et al., 2021). Nevertheless, the impact of irrigation retirement on POM was much greater than the impact on MAOM, resulting in a higher proportion of SOM in the MAOM pool of dryland systems. The contribution of POM to total SOC of dryland fields (26%) was very similar to previous reports from the region (Cambardella and Elliott, 1992) and did not vary due to past irrigation management, indicating that all POM accumulated during irrigation was rapidly lost.

The transition from irrigated croplands to CRP maintained SOM stocks in the soil surface due to the preferential accumulation of POM. Under CRP, POM contents were similar to the irrigated fields and higher than under dryland crops. Perennial grasses from the CRP program have been shown to increase the pool of POM (Follett et al., 2015; Hurisso et al., 2014; Li et al., 2017) and our results suggest that they can maintain it at the same levels as irrigated systems. Moreover, the inclusion of perennial grasses in the long-term dryland corners resulted in an increase in POM, coincident with the findings of previous authors. However, after the transition to CRP, POM contents were still higher in the formerly irrigated center pivots than in the dryland corners. Because POM protection against decomposition is low (Lavallee et al., 2020) this difference may be the result of a legacy effect of irrigation on grassland productivity but, as mentioned in the previous section, more evidence is needed to test this possibility.

The results of our fractionation analysis indicate that, although CRP can maintain SOM levels after irrigation retirement while dryland crops cannot, the majority of this effect is due to accumulation of POM. Given the low stability of POM already discussed, questions arise about its persistence in the long-term. This is particularly relevant as it is expected that 30 to 40% of fields under CRP may eventually transition to dryland croplands, with only a negligible fraction (~ 2%) going back to irrigation (Barnes et al., 2020; Sullins et al., 2021). Previous reports indicate that transitions from CRP to dryland crops can rapidly lose the gained SOM (Abraha et al., 2018; Bowman and Anderson, 2002; Cotton and Acosta-Martínez, 2018; Phillips et al., 2015), probably related to our findings that most of the SOC gains in CRP are in the POM pool. Soil aggregation is a mechanism of POM stabilization (Six et al., 2004) and irrigation can stimulate the accumulation of POM inside microaggregates (Gillabel et al., 2007). However, occlusion in aggregates provides short-term protection to decomposition (Lavallee et al., 2020) and, although we did not measure it, the similar POM content in fields retired to dryland crops compared to long-term dryland Corners suggests that aggregation was not an effective protection mechanism after irrigation retirement.

Summary and Conclusions

We used a space-for-time substitution to estimate the legacy effect of irrigation on soil carbon and nitrogen stocks in agricultural systems of the Central High Plains. Our results confirmed the previously reported positive effect of irrigation on SOC and TN stocks (Denef et al., 2008; Emde et al., 2021a; Trost et al., 2013). However, the legacy effect of irrigation was low, and after 7-10 years of irrigation retirement we did not find differences between zones that used to be irrigated (Center) and long-term dryland zones (Corner) of the same fields. This low legacy effect after the transition to dryland cropping systems is related to the preferential accumulation, during irrigation, of C and N in the less stable particulate organic matter (POM) pool at 0-10 cm soil depth.

The transition to perennial grasses may provide an option to mitigate, at least partially, the negative impact of irrigation retirement on SOC. Sites retired to CRP had intermediate SOC stocks, that did not differ from the still irrigated nor from the sites retired to dryland crops. In addition, the long-term dryland Corners that went into CRP had higher stocks than the dryland cropping systems, further supporting the positive impact of perennial systems. However, the advantage of CRP over transitions to dryland crops is explained by the accumulation of C and N in the POM fraction, which poses some questions about the stability of SOM in these systems after changes in management.

Chapter 6: Summary and Conclusions

Many areas of the Ogallala Aquifer are being depleted at unsustainable rates (Richey et al., 2015; Smidt et al., 2016). To extend the life of the Aquifer and to meet water compacts with neighboring states, water pumping rates must decrease sharply (Steward et al., 2013; Whittemore et al., 2016), and an increase in irrigation retirement is expected. The general objective of my dissertation was to estimate how irrigation retirement might affect SOC dynamics in semiarid agricultural systems of the OAR. I used field experimentation to understand crop and soil microbial responses during the transition from irrigated to dryland cropping systems and to quantify the early changes in SOC dynamics. I used observations from retired farm sites to quantify the longer-term changes in SOC dynamics under different land use types. Taken together, the results of my dissertation suggest that there will be rapid, important losses of SOC during the transition from irrigated to dryland cropping systems.

During the first years after irrigation retirement, crop productivity and C inputs were more affected than soil microorganisms (Chapter 2). Productivity responses to irrigation were crop dependent, but irrigation retirement consistently had a higher impact on above- than on belowground biomass production, resulting in higher root:shoot ratios in dryland than in irrigated treatments. As expected, the cornerstone dryland crop for the region, winter wheat, was less affected by irrigation than maize. The effect of irrigation retirement on soil microbial dynamics was low, and our results suggest any changes in microbial activity were indirectly affected by changes in C inputs rather than directly due to differences in soil moisture (Chapters 2 and 4). The determination of natural abundance ^{13}C in different SOM fractions (Chapter 4) further confirmed C inputs as the main drivers of changes in SOC dynamics during the transition from irrigated to dryland cropping systems. The decomposition rate of old SOC was not affected by irrigation directly, but the irrigation retirement effects on productivity affected the amount of new, crop-derived SOC in maize.

Contrary to my initial hypothesis that C outputs would also decline with irrigation retirement, high losses of crop productivity coincided with minimal changes in C outputs following the transition to dryland management. This suggested that irrigation retirement will result in the loss of SOC gained during irrigation. This expectation was confirmed in Chapter 5, where I used a space for time substitution to quantify the legacy effect of irrigation on SOC. Fields that used to be irrigated and transitioned to dryland cropping systems 7-10 years ago had very similar SOC stocks to long-term dryland fields, and this was related to the preferential accumulation during irrigation, and subsequent loss after its retirement, of C in the POM pool. Sites that entered CRP and transitioned from irrigated agriculture to perennial grasslands showed an advantage over the sites retired to dryland crops, with intermediate SOC stocks that did not differ from the still irrigated nor from the sites retired to dryland crops.

Both in the designed experiment and in the commercial fields, soils were characterized by moderately fine texture (28-32% clay, 14-40% silt) and a high proportion of SOC in the MAOM pool (70-80%). Although the studied soils are representative of a big area, the distribution of soils with coarse texture (Cano et al., 2018; Farahani et al., 1998; Hansen et al., 2012; Li et al., 2017), and a widespread distribution of the proportion of C found in the POM pool (Follett et al., 2015; Franzluebbers and Arshad, 1997; Halvorson and Jantalia, 2011; Li et al., 2017) are also common in the OAR. Because texture is a soil property with high incidence in SOC content and in the protection of SOC against decomposition (Castellano et al., 2015; Cotrufo et al., 2019; Jobbágy and Jackson, 2000; Lal et al., 2015; Parton et al., 1987; Six et al., 2002a; von Lützow et al., 2006; Wiesmeier et al., 2019), the potential for SOM protection would be lower in sandier soils. In these cases, a faster decay of SOC after irrigation retirement can be expected if management changes are not enough to maintain C inputs during the transition to dryland. Similarly, if irrigation retirement occurs in soils where POM represents a larger proportion of total SOC, the loss of SOC after irrigation retirement may occur at a faster rate.

All my results indicate that C inputs are more affected by irrigation than C outputs and that the direct effect of moisture on SOC turnover rates is low. This is somewhat contrary to the assumption that a faster cycling of C occurs because irrigation increases soil moisture, which stimulates microbial activity and SOM decomposition (Trost et al., 2013). Moreover, because the observed effect of irrigation on C inputs is an order of magnitude higher than the effect on SOC stocks, SOC turnover should be higher under irrigation (Blanco-Canqui et al., 2010; Denef et al., 2008; Gillabel et al., 2007). I think that this incongruence may be explained by the differences found in the previous chapters that accumulate over longer time frames.

First, the differences in C inputs may be lower than previously assumed and concentrated in the surface. Estimations of C inputs are based on available data, usually grain yield alone (Denef et al., 2008; Gillabel et al., 2007) or from aboveground observations at the most (Blanco-Canqui et al., 2010), and the application of fixed harvest index and root:shoot ratios. Because irrigation retirement affected plant production in the order grain yield > aboveground residue > belowground biomass (Chapter 2), these assumptions likely overestimate the real differences in C inputs between irrigated and dryland systems. In addition, C losses from the litter layer tended to be higher under irrigation, but irrigation did not affect the amount of residue-derived C found in the soil (Chapter 3). Thus, the differences in grain yield and aboveground biomass production cannot be directly extrapolated as differences in inputs available for SOC formation. Because the complete decomposition of aboveground litter may take longer than the time frame considered in this dissertation (Adair et al., 2008; Cotrufo et al., 2015), observations that consider a longer range of time will be useful to completely assess the fate of aboveground residues.

In addition to a possible overestimation of C inputs, some of the differences I found in soil microbial activity and in SOM fractions seem to foreshadow developing differences between irrigated and dryland systems. During the last season of measurements, both extracellular enzyme activity and heterotrophic respiration were lower in the dryland maize. Moreover, old SOC was preferentially lost from the particulate organic matter (POM) fraction relative to the more stable mineral associated pool, resulting in a proportional retention of SOC in fractions with lower decomposition rates. Therefore, in the long-term SOC turnover rate of retired sites may become slower than irrigated systems as the POM pool size shrinks, but the differences will be caused by differences in substrate availability rather than by a direct moisture effect. Given the importance of aboveground residues and the sensitivity of the enzyme analyses, the exploration of microbial activity in the litter layer may be helpful to further explore these developing differences.

Soil organic carbon plays a fundamental role in the mitigation of CO_2 emissions and the fight against climate change, both as a cause and as part of the solution (Lal, 2004; Minasny et al., 2017; Paustian et al., 2016). The observed losses of SOC during irrigation retirement indicate that fields transitioning from irrigated to dryland cropping systems to save water will likely become an increased source of CO_2. Therefore, a full assessment of water conservation strategies in the OAR should also consider the expected changes in SOC to avoid, paraphrasing Jackson et al. (2005), trading C for water. Because the loss of SOC will be related to the decrease in crop production and C inputs, regional knowledge and ongoing research can be used to identify opportunities to mitigate the negative effect of irrigation retirement on SOC.

The design of cropping systems with high levels of residue production and retention will be critical for the conservation of SOC during the transition out of irrigation. Due to experimentation purposes and time limitations, I only considered continuous monocultures of two crops, maize or wheat, but I acknowledge that this is not representative of the best management practices for agricultural conservation. Cropping system intensification, diversification, and perennialization are effective management practices to improve soil health, increase SOC and other ecosystem services (Hobbs et al., 2008; Kelly et al., 2020; Rosenzweig et al., 2018; Sanford et al., 2021; Tamburini et al., 2020), and they should be evaluated to quantify their impact during irrigated to dryland transitions. Perennial grasslands have been proposed as an effective way to combine agricultural production and SOC sequestration

(Conant et al., 2017), and they appear as another possibility worth exploring during the transition out of irrigation. My observations at the farm level coincide with previous works about the positive impacts of prairie restorations, and transition into the CRP program is one promising practice to avoid high losses of SOC after irrigation retirement. Therefore, those areas with lower potential to support crop yield under dryland production should be prioritized in the design of conservation programs that incentivize the adoption of perennial systems.

The OAR is not homogeneous and there is variability in climate, soil types, and groundwater reserves across the region (Cano et al., 2018; Farahani et al., 1998; Haacker et al., 2016; Hansen et al., 2012; USDA, NRCS, 2019). This results in variability in cropping systems, productivity, and the capacity to support dryland farming (Cano et al., 2018; Deines et al., 2020), which may all impact the evolution of SOC after irrigation retirement. The identification of priority areas for water conservation should contemplate this vast array of conditions across the aquifer. There is recent and ongoing work studying several of these issues in the OAR that aids in the identification of sensitive areas. For example, estimations of the distribution of groundwater availability and its projected evolution (Haacker et al., 2016, 2019b) allowed the identification of areas with higher probability of water depletion (Deines et al., 2020; Haacker et al., 2016), and the potential of these areas to support dryland crops (Deines et al., 2020). There are also adjusted models to predict crop productivity under different water management scenarios (Araya et al., 2017; Masasi et al., 2019; Sharda et al., 2021; Sima et al., 2019; Xiang et al., 2020). Other researchers are considering the social and economic impacts of different agricultural water management scenarios (Araya et al., 2019; Lauer and Sanderson, 2020; Manning et al., 2020; Mitchell-McCallister et al., 2021; Shepler et al., 2019). However, to my knowledge there is no consideration of potential changes in SOC in any of these simulations.

Due to the large potential spatial extent of irrigation retirements, including estimations of SOC evolution into different agricultural water management scenarios would be very useful to inform policies.

However, integrating modeling approaches to simulate the diverse hydrologic, ecological, economic, and agronomic dynamics of these systems presents several challenges and can be complicated (Haacker et al., 2019c). Recently, Xiang et al. (2020) integrated an agronomic model with a groundwater flow model and were able to jointly simulate groundwater availability and crop yields under irrigated and non-irrigated scenarios. Given the observation that C inputs are the main drivers of changes in SOC during irrigation retirement, the differences in crop productivity and residue production between irrigated and dryland systems could be used as a proxy to estimate the transition options with higher potential to minimize the negative impact of irrigation retirement and the areas more sensitive to SOC losses, and where to focus further research efforts.

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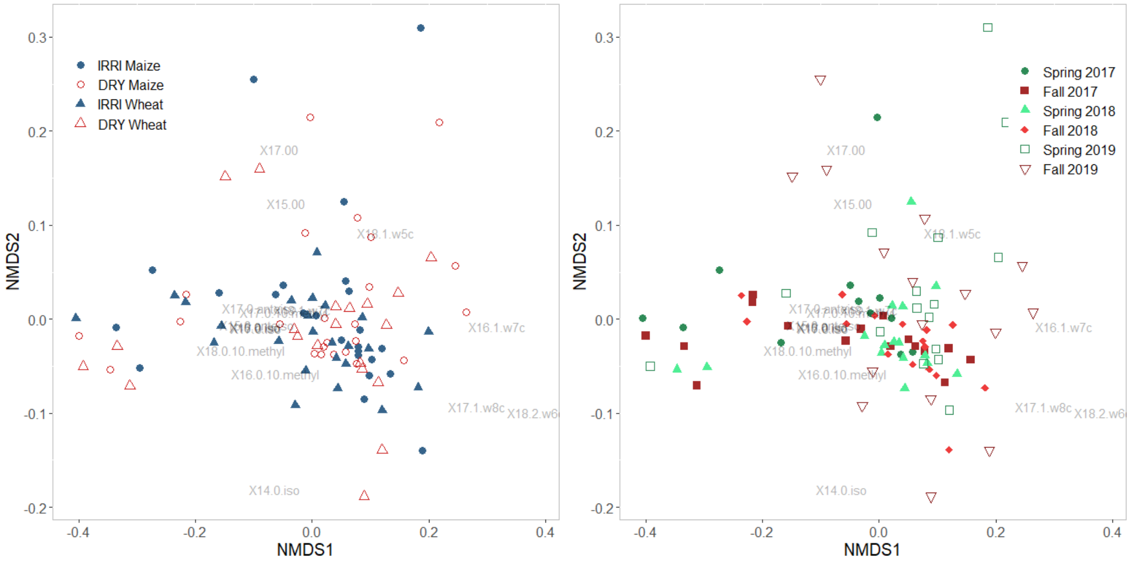
Appendix 1: Supplementary Information for Chapter 2



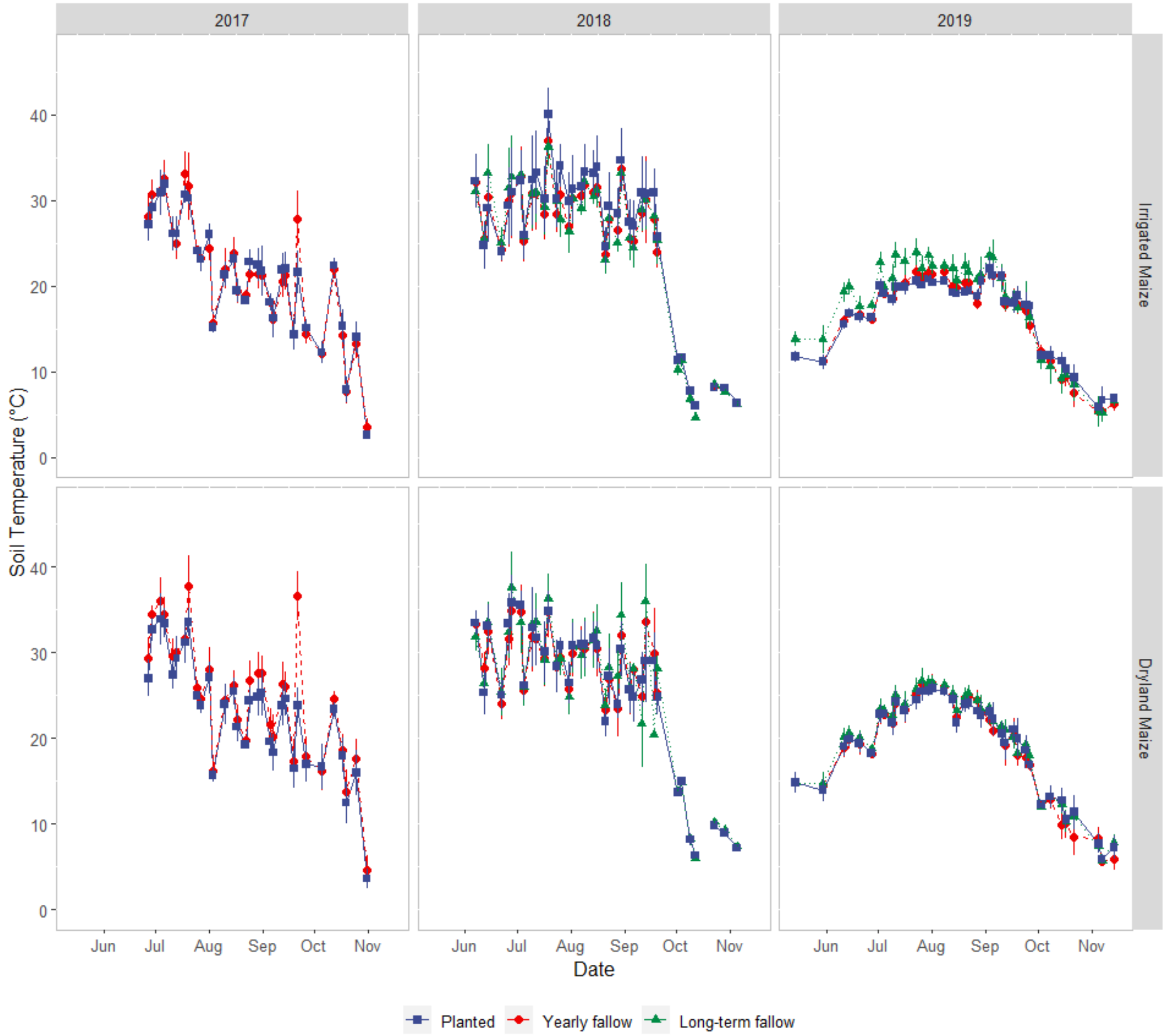
Supplementary Fig. 2.1. Non-metric multidimensional analysis exploring changes in soil microbial community structure indicated by the relative concentration of selected PLFA, biomarkers of different functional groups, for different treatments (left panel) and sampling periods (right panel).

Appendix 2: Supplementary Information for Chapter 3

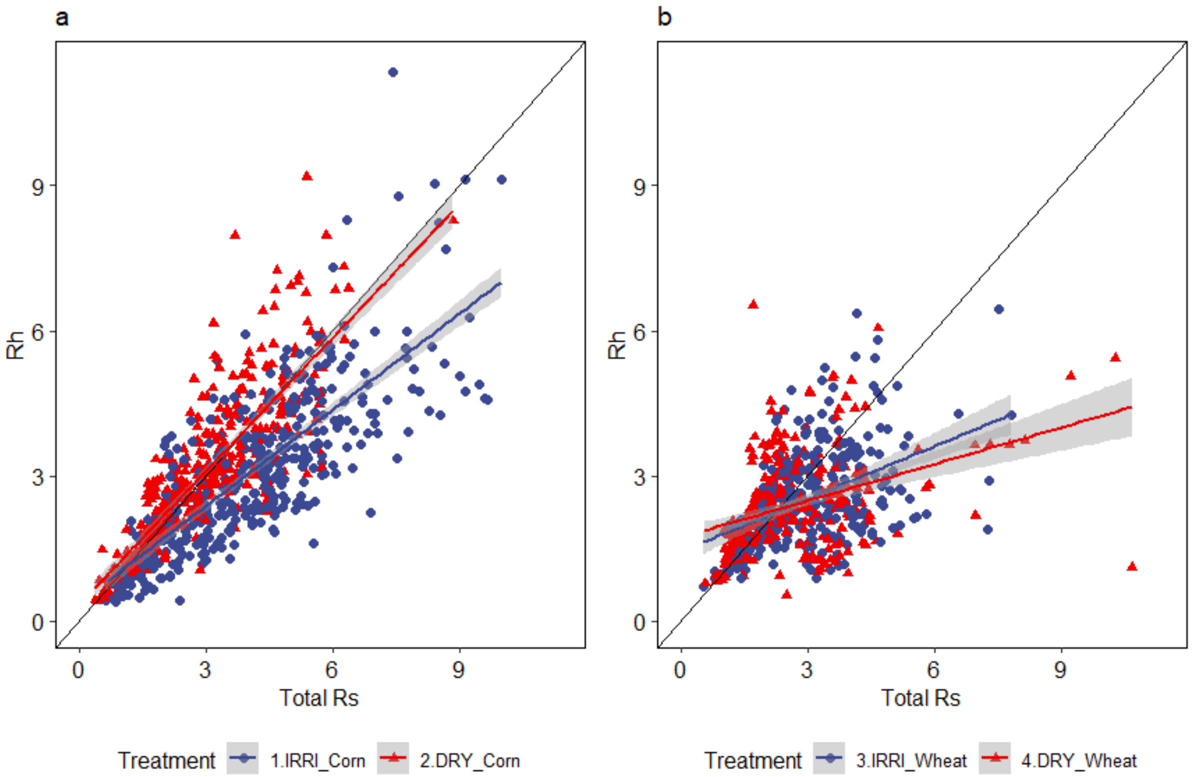
Supplementary Table 3.1. Total carbon and nitrogen stocks, and C:N ratio in the bulk soil and different soil organic matter fractions after 6 and 13 months of incubation at 0-5 cm, and after 13 months of incubation at 5-10 cm depth in dryland and irrigated maize cropping systems. Data is the mean ± SE with n = 4.

	SOC (g C m^{-2})		TN (g N m^{-2})		C:N	
Sample	DRY	IRRI	DRY	IRRI	DRY	IRRI
			6 months – 0-5 cm			
Bulk	675.88 ± 47.78	683.26 ± 55.94	87.27 ± 6.95	86.37 ± 5.92	7.79 ± 0.37	7.90 ± 0.25
DOM	11.53 ± 1.05	11.61 ± 0.69	4.51 ± 2.56	1.59 ± 0.27	5.47 ± 2.20	7.83 ± 1.16
LF	114.07 ± 11.48	121.78 ± 28.93	8.87 ± 1.21	8.95 ± 2.12	13.04 ± 0.46	13.69 ± 0.59
POM	25.91 ± 2.44	24.86 ± 2.55	5.39 ± 0.68	4.21 ± 0.62	5.11 ± 0.95	6.24 ± 0.98
MAOM	437.18 ± 18.53	432.49 ± 41.85	57.74 ± 2.36	55.47 ± 5.25	7.57 ± 0.03	7.79 ± 0.16
			13 months – 0-5 cm			
Bulk	572.55 ± 62.27	663.54 ± 73.56	66.69 ± 6.76	77.26 ± 7.63	8.57 ± 0.07	8.56 ± 0.14
DOM	12.70 ± 0.94	14.32 ± 1.39	1.09 ± 0.13	1.35 ± 0.24	11.87 ± 0.55	11.07 ± 1.02
LF	112.83 ± 23.25	104.14 ± 22.83	8.58 ± 1.69	8.29 ± 1.89	13.10 ± 0.26	12.67 ± 0.28
POM	26.44 ± 2.13	28.29 ± 3.90	2.75 ± 0.17	3.26 ± 0.43	9.62 ± 0.43	8.67 ± 0.31
MAOM	383.60 ± 41.22	467.66 ± 69.31	52.39 ± 4.88	64.14 ± 8.87	7.30 ± 0.14	7.26 ± 0.15
Macro	78.70 ± 18.00	125.64 ± 42.97	7.37 ± 1.64	13.51 ± 4.66	10.62 ± 0.10	9.33 ± 0.03
micro	307.12 ± 43.19	367.89 ± 36.65	36.99 ± 4.95	42.41 ± 4.47	8.29 ± 0.08	8.70 ± 0.12
S&C	176.00 ± 19.29	159.46 ± 2.68	21.87 ± 2.38	19.61 ± 0.44	8.05 ± 0.09	8.14 ± 0.05
			13 months – 5-10 cm			
Bulk	666.33 ± 93.15	671.23 ± 55.30	80.71 ± 10.01	79.79 ± 6.30	8.21 ± 0.20	8.43 ± 0.35
DOM	14.35 ± 1.76	14.29 ± 0.79	1.58 ± 0.26	1.45 ± 0.30	9.36 ± 0.83	10.65 ± 1.30
LF	103.85 ± 31.35	96.71 ± 26.02	7.97 ± 2.50	7.69 ± 2.23	13.23 ± 0.30	12.87 ± 0.39
POM	30.49 ± 4.43	29.63 ± 4.52	5.63 ± 1.08	5.52 ± 0.83	5.58 ± 0.32	5.61 ± 0.86
MAOM	528.95 ± 73.45	571.66 ± 24.38	69.14 ± 9.45	74.51 ± 3.01	7.65 ± 0.06	7.67 ± 0.11

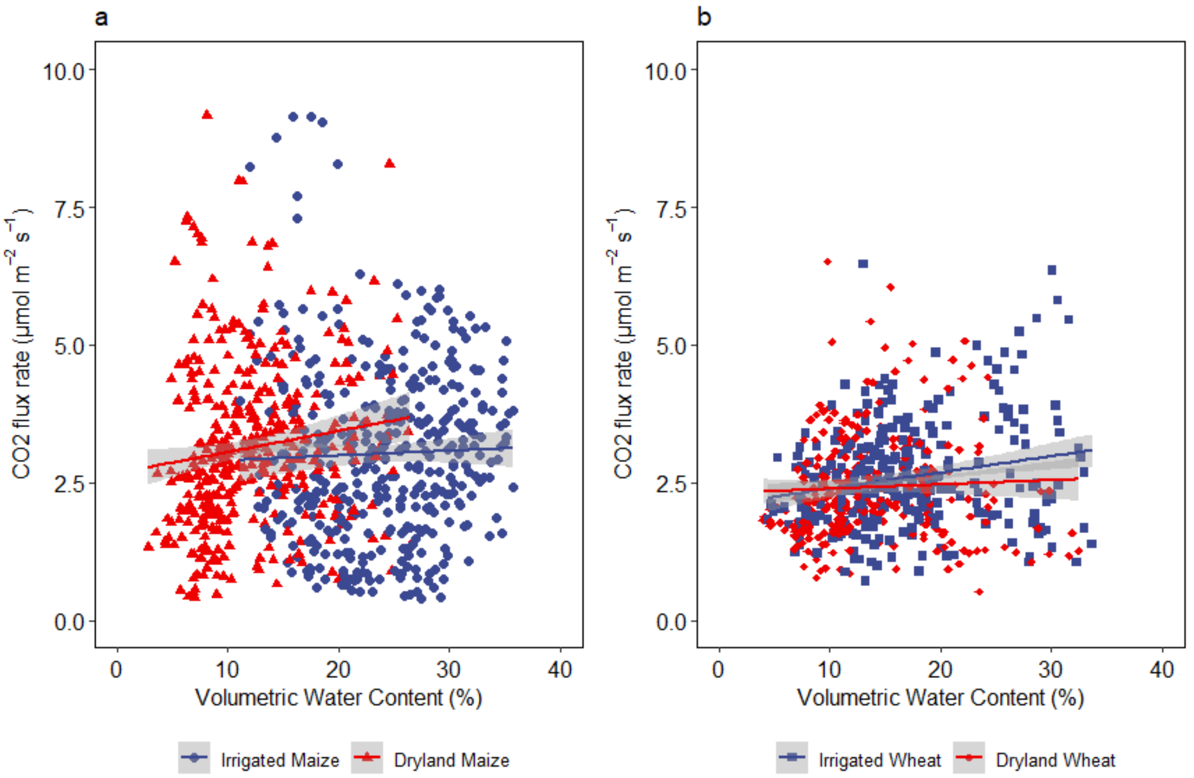
Appendix 3: Supplementary Information for Chapter 4



Supplementary Fig. 4.1. Soil temperature dynamics at 10 cm soil depth in each maize treatment and fallow management. Data are mean ± 1 SE with n=4.



Supplementary Fig. 4.2. Partitioning of soil respiration in irrigated and dryland maize (a) and wheat (b). Total Rs estimated as soil respiration in the planted area, Rh estimated as soil respiration in yearly fallow subplots. All fluxes in µmol CO_2 m^{-2} s^{-1}.



Supplementary Fig. 4.3. Soil heterotrophic respiration response to soil moisture in irrigated and maize (a) and wheat (b). Rh estimated as soil respiration in yearly fallow subplots.