THESIS

THE EFFECTS OF IRRIGATION RETIREMENT ON SOIL CARBON DYNAMICS OF A CONTINUOUS MAIZE AGROECOSYSTEM

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ABSTRACT

THE EFFECTS OF IRRIGATION RETIREMENT ON SOIL CARBON DYNAMICS OF A CONTINUOUS MAIZE AGROECOSYSTEM

Over half of the world's fresh water is used in crop production and, in some key agricultural regions, use far exceeds local water availability and recharge rates. With the increasing strain on freshwater resources caused by climate change and a growing population, agriculture is under pressure to reduce its water consumption and large areas of currently irrigated farmland across the Western U.S. will likely transition into dryland agriculture over the coming decades. The effects this will have on global soil carbon (C) dynamics, however, remain unclear. In 2016, a study was established in Northern Colorado to understand how stopping irrigation affects soil C turnover in a no-till, continuous maize agroecosystem. Earlier results showed limited responses of the soil microbial community to irrigation retirement, but differences in soil heterotrophic respiration (R_h) rates were detected after two years of accumulated differences in plant residue inputs, thus suggesting a possible co-limitation of water and available C to the microbial community. We continued this experiment through 2022 to further explore the relationship between soil moisture and C inputs in shaping the soil microbial community under the new watering regimes and the consequential effects on soil respiration (R_s) as an indicator of soil organic C (SOC) turnover rates. Two seasons of data collection in 2021 and 2022 showed decreases in available soil water, bacteria, fungi, protozoa and actinomycetes fatty acid methyl ester (FAME) biomarkers, activities of four extracellular enzymes and soil autotrophic respiration in response to both reductions in irrigation and plant inputs, with strong

interactive effects between the two factors. However, plots under dryland conditions had higher concentrations of dissolved organic carbon (DOC) and muted differences in soil R_h when compared to their irrigated counterparts; differences in R_h between fallow treatments with (YF) and without residue inputs (LTF), on the other hand, were more pronounced. Soil R_s in fallow plots was consistently, positively correlated with field soil temperature, while correlations with moisture were weak or even negative, thus suggesting soil moisture was not a strong direct driver of R_h. We investigated the direct and indirect influences of variables collected monthly across two seasons on soil R_h to test our hypothesized model using structural equation modeling. In contrast to the cumulative treatment level impacts of plant inputs and irrigation, monthly soil moisture measurements had a stronger, direct effect on R_h than substrate availability as estimated by water-extractable DOC. The final model only explained 24% of the variability in soil R_h. Changes in global C dynamics can be expected with transition of land areas from irrigated to dryland agriculture. However, focusing on soil health, resource conservation practices and the resiliency of the soil microbiome can be the key to minimize the potential negative impacts of this transition.

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BACKGROUND AND RATIONALE

It is estimated that 70 percent of the world's fresh water is utilized to support crop production (FAO, 2011). Global crop production depends on water received as both precipitation and irrigation from surface water bodies and aquifers (Davis et al., 2017). In regions like the Great Plains, withdrawal rates to support agriculture far exceed local freshwater availability and aquifer recharge rates (McGuire (USGS SRI), 2011). Climate change studies predict shifts in precipitation timing and amount (Sherwood and Fu, 2014; Southworth et al., 2000). This, along with increasing global temperatures, could potentially further impact recharge rates and water availability. Moreover, due to rapid population growth, municipal and industrial water demands continue to increase, further putting pressure on limited freshwater sources. In some regions, this decline in resources is causing and/or will cause some farmers to transition from fully irrigated to deficit or complete dryland farming (Wheeler et al., 2008; Amosson et al., 2009; Rudnick et al., 2019).

The effects that these transitions will have on soil health and carbon (C) dynamics remain unclear. The retirement of irrigation will reduce crop productivity, thus reducing crop residue inputs to the soil with likely cascading impacts on multiple soil functions (Conant et al., 2004). Soil functions are regulated, in general, by soil biological communities and, thanks to their rapid response to environmental changes, they have proven to be effective predictors of soil health changes (Acosta-Martínez et al., 2011; Cano et al., 2018, Kallenbach et al., 2016; Lehmann et al., 2020; Fierer et al., 2021).

Although negative impacts on soil microbial communities can be expected in response to irrigation retirement (Siebelec et al., 2020; Hueso et al., 2011), the magnitude of these effects can vary. In climates with limited water availability, soil microbial biomass has been reported to decrease along with soil moisture (Carbone et al., 2011, Davidson et al., 1998; Wu et al., 2011; Manzoni et al., 2012; Sardans et al., 2008; Pérez-Guzmán et al., 2020), have no response (Canarini et al., 2016) or even increase when exposed to drought (Schaeffer et al., 2017). Further, drought drives shifts in the composition of the soil microbial community. Long-term drought has been reported to reduce bacterial and fungi abundance (Maestre et al., 2015), with bacteria being generally more sensitive to drought than fungi (Evans and Wallenstein, 2012).

The high temporal variability of available water in soils affects soil microbial activity (Porporato et al., 2004). Microorganisms may opt for dormancy (Jones and Lennon, 2010) or shift extra-cellular enzyme (EE) production to optimize C uptake when water is limited and conditions become unfavorable (Vetter et al., 1998; Allison, 2012; Moorhead et al., 2012). Soil EE activity tends to decrease under drought conditions, but the effects can be inconsistent (Sardans et al., 2008; Alster et al., 2013; Ren et al., 2017). Drought experiments have revealed decreases in activities of β-glucosidase (BGLUC) (Pohlon et al., 2013), leucine aminopeptidase (LAP) (Schreckinger et al., 2021), β-cellobiosidase (BCELL) and N-acetyl-β-glucosaminidase (NAG) (Weitntraub et al., 2007) enzymes. On the other hand, an experiment which subjected a Central-European grassland to 100 years of extreme drought reported limited EE activity responses to reduced soil moisture (Kreyling et al., 2008), suggesting high adaptability of the soil microbial communities to drought (Sinsbaugh, 1994; Williamson and Wardle, 2007).

While soils may experience limited activity during drought periods, rewetting events (i.e., rainfall, irrigation) create large surges in activity (Austin et al., 2004; Borken and Matzner,

2009). Hence, even small increases in soil moisture after a long dry period may trigger large pulses in CO_2 efflux (Birch effect) (Birch, 1958; Reichstein et al., 2002; Carbone et al., 2011, Kim et al., 2012, Göransson et al., 2013). For this reason, soil respiration (R_s) has the greatest level of uncertainty relative to other fluxes in global terrestrial C cycle estimates (Bond-Lamberty and Thompson, 2010). Soil respiration can be divided into autotrophic (R_a) and heterotrophic (R_h) components (Subke et al., 2006). Plant-derived R_a includes root and rhizomicrobial respiration, while R_h originates from microbial decomposition of litter and soil organic matter and has been reported to represent between 40 and 80% of R_s in maize cropping systems (Zhang et al., 2013; Li et al., 2019). Soil respiration generally increases with soil moisture (Cook and Orchard, 2008; Zornoza et al., 2016; Zhang et al., 2019; Zhou et al., 2019), therefore, a decrease in R_s can be expected after transitioning into a non-irrigated system. Since R_a does not contribute to soil C sequestration (Kuzyakov, 2006), the effect of the transition into dryland agriculture on SOC depends heavily on the changes in R_h. Soil temperature and moisture (Schipper et al., 2019), microbial community composition and activity (Trost et al., 2013; Ma et al., 2015; Calderon et al., 2016) and C availability (Orchard and Cook, 1983; Cook and Orchard, 2008; Canarini et al., 2017) all influence R_h, but it is still challenging to anticipate how R_h will respond to shifts in environmental conditions and C inputs (Falloon et al., 2011; Moyano et al., 2013; Bond-Lamberty et al., 2018).

A transition experiment was established in 2016 to determine the effect of irrigation retirement on SOC dynamics—more specifically C outputs— in a continuous maize agroecosystem in the High Plains of the United States (Nuñez et al., 2022). This experiment focused on comparing biomass production and soil respiration between irrigated and non-irrigated (dryland) continuous maize. Fallow subplots were installed to partition soil respiration

into its different components: a yearly fallow (YF) subplot where no new plants were allowed to grow but plant residue was retained from the previous year has served to represent R_h derived from recent and older residue and SOC sources, and a long-term fallow (LTF) subplot to estimate R_h from native SOC. After three years, a significant decrease in soil moisture and biomass production was observed in the plots retired from irrigation relative to the irrigated treatments. Despite lower biomass—hence reduced C returns into the soil— the irrigation retirement effect on soil microbial community size and composition was limited. Lower extra-cellular enzyme activity (EEA) was observed in the dryland maize system suggesting that irrigation retirement caused a reduction in soil microbial activity. Soil C outputs via R_h (YF plots), however, were affected by the change in irrigation regime only after two years of accumulated differences in litter inputs. Respiration of native SOC from LTF plots was not affected by irrigation. These observed effects, or the lack thereof, seem to point out that the irrigation treatment affected microbial respiration indirectly, due to changes in substrate (labile C) availability, rather than direct effects of soil moisture alone.

Drought affects the mechanisms of dissolution, diffusion and transport that allows microbial access to substrates (Schimel, 2018). In microbial dynamics, water acts as a resource, a solvent and a way of transport to produce complex biogeochemical processes (Tecon and Or, 2017; Moyano et al., 2013). With very few exceptions, the substrates used by microbes as energy sources are water soluble, often referred to as dissolved organic carbon (DOC) (Cleveland and Yavitt, 1997; Schimel, 2018). Because most substrates are water soluble, connected water-filled pores are vital to allow transport of resources to microbes (Skopp et al., 1990; Moldrup et al., 2001; Bailey et al., 2017; Tecon and Or, 2017). Schimel & Schaeffer (2012) argued that microbial physical access to soil C is the primary control on whether that carbon is decomposed

(and respired to CO_2) rather than other factors such as microbial community composition or the chemical quality of the substrates.

A model proposed by Manzoni et al. (2014) and based on the structure proposed by Schimel and Weintraub (2003) aims to assess microbial responses to changes in moisture by coupling diffusion limitations due to low moisture in the system to osmoregulation, dormancy and extra-cellular enzyme synthesis in soil microbes. In this model, soil organic C receives a litterfall input that is decomposed by enzymatic reactions to soluble compounds that are then allocated by the same community for growth, enzyme production and synthesis of osmolytes, which together explain variability in heterotrophic soil respiration. Using the conceptual framework proposed in this model and based on results reported by Nuñez et al. (2021, 2022), we designed an experiment to explore the relationship between soil moisture and available C substrates in the shaping of the microbial community and their activity, measured as R_h in the field and EEA in the lab.

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CHAPTER 1

LINKING SOIL MICROBIAL CO_2 RELEASE TO SUBSTRATE AND MOISTURE IN A DRYLAND AGROECOSYSTEM

1 Introduction

Global fresh water sources are under increasing stress. Agriculture consumes almost three quarters of global water withdrawals (FAO 2011) and in some key agricultural regions this consumption far exceeds local availability of water resources (McGuire (USGS SRI), 2011; Davis et al., 2018). As municipal and industrial demands increase (FAO 2011) and water availability decreases, the pressure to reduce agricultural water consumption grows and the number of farms transitioning to dryland agriculture in regions like the High Plains is expected to increase (Whittemore et al., 2016; Nuñez et al., 2022). Irrigated systems are far more productive than their dryland counterparts, therefore, the transition to dryland agriculture poses many challenges to not only crop yield, but also soil C dynamics and soil health.

Soil C plays a key role in many soil functions (Rawls et al., 2003; Wade et al., 2020; Kane et al., 2021) and is a good indicator of soil health (Cano et al., 2018; Lehmann et al., 2020). Unfortunately, SOC changes slowly and may take many years for changes in this pool to be quantifiable (Bradford et al., 2016). The soil microbial community, on the other hand, responds more quickly to changes in the environment (Acosta-Martinez et al., 2011) and microbial community abundance and composition has proven to be an effective benchmark of soil health, given its direct relationship to SOC formation, nutrient dynamics and other soil functions (Acosta-Martínez et al., 2011, Kallenbach et al., 2016; Cano et al., 2018; Fierer et al., 2021). In

addition, microbially produced soil EEAs are considered useful indicators of nutrient turnover and microbial activity, particularly under environmental stress (e.g., drought) (Kardol et al., 2010, Burns et al., 2013).

Global soils contain more C within the first meter than the atmosphere (Scharlemann et al., 2014) and a small change in soil C could have important effects on the atmospheric CO_2 concentrations (Köchy et al., 2015). Microbial respiration (R_h) can account for up to 90% of the total soil CO_2 efflux depending on the ecosystem (Hanson et al., 2000; Curiel Yuste et al., 2004; Tang and Baldocchi, 2005; Churchland et al., 2013; Savage et al., 2013), and represents the C losses from the decomposition of litter and SOC by microorganisms (Subke et al., 2006). Studies suggest that seasonal and environmental factors drive soil R_h (Reichstein et al., 2003; Sperlich et al., 2016; de Dato et al., 2017), however, mechanisms at biome, regional and global scales remain uncertain. The study of the soil microbial community can provide insight into how the transition into dryland agriculture will affect soil processes, especially C turnover, and, in turn, expand current knowledge on the direct drivers of soil R_h. Decreases in soil moisture and plant productivity are a given when stopping irrigation, thus decreases in water and substrates (labile C) available to the soil microbiome are also expected and possible cascading effects in soil R_h will be observed.

The decline in soil moisture expected with the retirement of irrigation is likely to impact soil microbial abundance and community composition. Water acts as a resource, a solvent and a way of transporting substrates to soil microbes (Moyano et al., 2013; Tecon and Or, 2017). Thus, water availability directly and indirectly regulates soil communities and their function. Most of the changes in community structure are driven by death— organisms die from physiological stress, leaving other organisms dominating the community when activity and growth are limited

(Schimel, 2018). However, microbial communities have exhibited resilience by quickly returning to their original state after a disturbance, and, in certain cases, microbial composition may change but functionality remains unaffected (Allison and Martiny, 2008). It has been observed that microbial biomass often remains stable through month-long dry periods (Aponte et al., 2010; Landesman and Dighton, 2010; Parker and Schimel 2011; Schaeffer et al., 2017), with reductions in bacterial and fungal abundance (Maestre et al., 2015). Similarly, depending on what limits productivity in an ecosystem, changes in soil moisture may increase or decrease the ratio of bacteria and fungi (Schimel et al., 1999, Williams, 2007), as bacteria tend to be more sensitive to drought than fungi (Evans and Wallenstein, 2012; Göransson et al., 2013).

With changes in soil moisture, changes in microbial activity are often observed. To avoid losing water to their environment, microbes must accumulate solutes to match the water potential of their surroundings. The osmolyte demand is often not met in dry soils (Boot et al., 2013; Kakumanu et al., 2013) and microbes may opt for switching into a dormant state, consequently slowing down metabolic activity. Multiple studies have found linear decreases in microbial activity and respiration with decreasing water availability (e.g., Davidson et al., 1998; Jones and Lennon, 2010; Carbone et al., 2011; Wu et al., 2011; Manzoni et al., 2012). However, the magnitude of the decreases in respiration vary, and in some cases, even substantial reductions in soil moisture have only produced muted effects on respiration (Lu et al., 2017; Nuñez, 2021). It could be that microbes opt for a more efficient alternative to dormancy. The depolymerization of organic compounds catalyzed by EEA results in increase C availability (Schimel, 2018) and soil microbes may be able to optimize EE production to maximize C uptake under water limitation (Vetter et al., 1998; Allison, 2012; Moorhead et al., 2012). However, just like respiration, responses of EEAs to moisture are inconsistent (Sardans et al., 2008; Alster et al., 2013).

Microbial access to soil C is the primary control on whether C is decomposed (and respired to CO_2) (Schimel and Schaeffer, 2012). With very few exceptions, the substrates used by microbes as energy sources are water soluble, often referred to as dissolved organic carbon (DOC) (Cleveland and Yavitt 1997; Schimel 2018). Because most substrates are water soluble, connected water-filled pores are vital to allow transport of resources to microbes (Skopp et al., 1990; Moldrup et al., 2001; Bailey et al 2017; Tecon and Or, 2017). Therefore, additional to moisture, substrate accessibility could limit soil microbial responses to irrigation retirement. While crop production and plant inputs are expected to decrease under dryland agriculture, studies have shown that drought increases plants' root to shoot ratio (Pallardy and Rhoads, 1993; Aspelmeier and Leuschner, 2004; Zhou et al., 2018), which could potentially indicate that substrate availability for microorganisms may be less affected than other inputs.

A transition experiment was established in 2016 to determine the effect of the ceasing of irrigation on SOC dynamics in a continuous maize agroecosystem in the High Plains of the United States (Nuñez et al., 2022). This experiment has focused on comparing biomass production and soil respiration between irrigated and non-irrigated (dryland) continuous maize. Fallow subplots were installed to partition soil respiration into its different components: a yearly fallow (YF) subplot where no new plants were allowed to grow but plant residue provided cover has served to represent R_h, and a long-term fallow (LTF) subplot to estimate respiration from native SOC. In each of the first three years following irrigation retirement, soil moisture and biomass production decreased significantly. Despite lower biomass—hence C returns into the soil— the effect on soil microbial community size and composition was limited. Lower extra-cellular enzyme activity (EEA) was observed in the third year following irrigation retirement suggesting that retirement reduced soil microbial activity. Soil C outputs via R_h (YF plots),

however, were affected by the change in irrigation regime only in the third year of accumulated differences in litter inputs. Respiration of native SOC from LTF plots was not affected by irrigation. These observed effects, or the lack thereof, suggested that the irrigation treatment affected microbial respiration indirectly, due to changes in substrate (labile C) availability, rather than changes in soil moisture alone.

Thus, this experiment was designed to explore the influence of different factors on C turnover under drought in the same agroecosystem by relating soil moisture, available substrates and microbial (heterotrophic) respiration (R_h). We define substrate as the dissolved organic carbon pool. We also explored how soil moisture and substrate availability—in conjunction—affected soil microbial EEA and community composition, and their consequent effects on R_h. We hypothesized that the soil microbiome is co-limited by moisture and plant residue inputs (as the main source of substrates), rather than available water alone (Fig 1). Therefore, we expected to see a reduced water-soluble C availability in plots under non-irrigated conditions that, in turn, would result in lower enzymatic activity and respiration. This shift in activity may also be associated with a shift in microbial community composition.

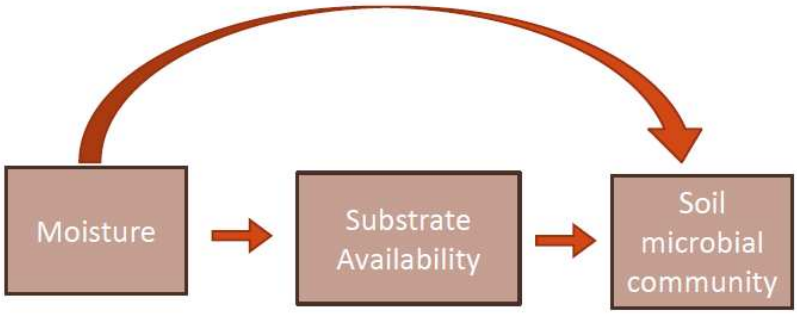

Figure 1. We hypothesized that soil microbial activity is co-limited by substrate and soil moisture rather than water availability alone.

2 Materials and Methods

2.1 Site description

Our research was conducted at a field experiment established in 2016 at Colorado State University's Agricultural Research Development and Education Center (ARDEC) near Fort Collins, CO (40°39'16"N, 104°59'55"W; 1555 m a.s.l). 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 a Fort Collins loam (Aridic Haplustalfs) (USDA NRCS, 2019). As described by Nuñez et al. (2021, 2022), the selected field was previously used for irrigated crop production and managed under conventional tillage but transitioned to a no-till system in 2016 when the present experiment was established. This factorial experiment has four replicate blocks of a continuous maize (*Zea maize*) system under two contrasting irrigation practices (full irrigation and no irrigation/dryland conditions) and two subplots within each main irrigation treatment plot representing a gradient of plant and plant residue inputs: long-term fallow subplot where all vegetation has been removed since the establishment of the experiment and a yearly fallow subplot where each year after planting a location is randomly selected and new vegetation is removed. Irrigated treatments are watered once per week from May to September. Weekly irrigation amounts were managed at the farm level and were defined based on moisture conditions, crop phenology, and weather forecast. As a long-term experimental site, our current analysis presented here covers the 2021 and 2022 growing seasons in continuation of results from previous seasons (2016-2019) that have already been reported (Nuñez et al., 2021, 2022).

2.2 *Aboveground biomass*

Aboveground maize biomass was measured in 2021 and 2022 at physiological maturity via hand harvest. To estimate C inputs for each treatment, we selected two representative areas (9.12 m^2) at the center of each plot and collected all plants, cutting about 8 cm from the soil

surface. We weighed the entire sample and separated ears and grain from stover. Dry matter was estimated by oven-drying a subsample of each portion at 55-60 °C for 72hrs.

2.3 *Soil sampling and analysis*

To evaluate the relationship between soil available C and water, and their collective importance in shaping an agroecosystem's microbiome, we measured soil gravimetric water content (GWC), available dissolved C (DOC), soil microbial activity and community composition across the growing season. Starting in August 2021, ten (August through October 2021 and May through October 2022) monthly surface soil samples (0-10 cm) were collected using a 2-cm diameter soil probe (Tate, 1979; Fang et al., 2005; Canizales-Paredes et al., 2012; Chen et. al., 2021). Triplicate soil cores were collected in linear transects from each subplot and main planted plot, homogenized using a 2mm soil sieve, and separated into subsamples for the various analyses within hours after collection. Soil subsamples for EEA and DOC/DON analyses were stored at 4°C for no more than 48hrs until ready to be analyzed and/or extracted. To measure GWC (lab GWC), approximately 10g of soil were weighed, dried in an oven at 105°C for 48hrs, and reweighed.

Soil microbial community composition was approximated via ester-linked FAME (EL-FAME) fingerprinting method. Microbial biomass and community composition can be estimated via fatty acid methyl ester (FAME) analyses since the abundance of individual fatty acids varies among soil microorganisms and membrane lipids have a quick turnover during metabolism (Balser et al., 2019). These measures, along with soil microbial extracellular enzyme activity (EEA), are often used as proxies for soil health as they are sensitive to changes in management systems (Cano et al., 2018; Lehmann et al., 2020). Following Shutter and Dick (2000), ester-linked fatty acids from field-moist soil samples were extracted and analyzed as described in Li et

al. (2020) by the Acosta-Martínez's soil microbiology lab at USDA-ARS in Lubbock, TX. Out of a total of 116 named FAME biomarkers, 46 selected fatty acids were used as microbial biomarkers according to previous research (Olsson et al., 1995; Zelles, 1999; Willers et al., 2015). These included gram positive (i13:0, a13:0, i14:0, a14:0, i15:0, a15:0, i15:1 ω6c, a15:1ω9c, i16:0, a16:0, i17:0, a17:0, i17:1ω9c, i18:0, i19:0), gram negative (cy17:0ω7c, i17:0 3OH, cy19:0ω7c, 14:1ω9c, 14:1ω8c, 15:1ω9c, 15:1ω8c, 15:1ω6c, 16:1ω9c, 16:1ω7c, 16:0 2OH, 17:1ω8c, cy17:0ω7c, 18:1ω7c, 18:1ω6c, 18:1ω5c, 19:1ω9c, 19:1ω8c, 19:1ω7c), Actinobacteria (10Me16:0, 10Me17:1ω7c, 10Me17:0, 10Me18:1ω7c, 10Me18:0, 10Me19:1ω7c) and Protozoa (20:4 ω6c). Fungal biomarkers included Saprophytic Fungi (SF) (18:3ω6c, 18:4ω3c, 18:2ω6c, 18:1ω9c), and Arbuscular mycorrhizal fungi (AMF) (16:1ω5c). Marker notations are represented as methyl group (Me), cyclopropane group (cy), and iso- (i) and anteiso- (a) branched fatty acids. Abundances were calculated to concentration (nmol g−1 soil). The bacterial sum was calculated based on gram-positive and gram-negative bacterial FAMEs, while the fungal sum was calculated by summation of SF and AMF biomarkers.

We quantified four soil EEAs related to glucose, cellulose, protein and chitin degradation (Table 1). Following the protocol by Saiya-Cork et al. (2002) we homogenized 1 g of fresh soil in 125 mL of 50 mM, pH 8.1, tris buffer with a blender. We then transferred 200 µL of each slurry into black, 96-well microplates and mixed with 50 µL of each 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 in microplates were incubated for 4 h at 25 °C and the developed fluorescence read on a microplate reader (Cytation 5, BioTek, Vermont, USA) at 365 nm excitation and 450 nm emission wavelengths.

Table 1. Enzyme name, abbreviation, function in the soil (nutrient cycle indicator), and final product for the four soil hydrolytic enzymes assayed.

Enzyme Name	Abbreviation	Function in Soil
β-1, 4-n-acetyl-glucosaminidase	NAG	Hydrolysis of chitin
Leucine amino peptidase	LAP	Hydrolysis of amino acid residues (N- terminus of peptides and proteins)
β-D-1,4-cellobiosidase	BCELL	Hydrolysis of cellulose and cellotetraose
β-1, 4-glucosidase	BGLUC	Hydrolysis of cellulose

To quantify water-soluble C (DOC) available in the soil, duplicate water extractions in a 1:5 fresh soil to water ratio were performed. Soil slurries were shaken for an hour, centrifuged at 3400 rpm for 15 min and filtered through 0.2 μm nylon syringe filters (J.T. Baker® syringe filter; VWR International, LLC, Radnor, PA, USA). Extracts were analyzed for total DOC content using a Shimadzu TOC/TN analyzer (TOC-L CPH/CPN; Shimadzu Scientific Instruments Inc., Columbia, MD, USA).

2.4 *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 the yearly and long-term fallow subplots weekly from May to November in 2021 and 2022. A 20-cm diameter PVC collar was installed at the center of each fallowed subplot at approximately 10-cm depth as attachments 20 cm diameter survey chamber (LI-COR, Nebraska, USA). Two collars were placed in the main plot planted area, one within a planted row and another one in-between rows. Soil CO_2 flux measurements were conducted the morning before weekly irrigations using a portable infrared gas analyzer connected to a chamber (LI-8100, LI-COR, Nebraska, USA). 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 3-minute measurement period.

Soil respiration measured in the fallow subplots was used to approximate heterotrophic respiration (R_h) (Subke et al., 2006). The measurements in the yearly fallow were assumed to include microbial respiration of native SOC and the decomposition of crop residues from previous seasons. We also assumed that soil respiration from the long-term fallow represented microbial respiration of native SOC, as these subplots received no new crop residues or living roots starting in 2016. Soil temperature and moisture (field VWC) to 10cm depth were also recorded during the 3-minute CO_2 measurement period at the collar location using the probe connected to the LI-COR chamber and analyzer.

2.5 *Data Analysis*

We analyzed data using both the factorial experimental design as well as exploring relationships between variables using correlation analyses and structural equation modeling. To assess the effect of irrigation and plant input treatments on response variables, the experimental data was analyzed as a replicated, randomized 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).

The effect of irrigation and fallow treatment on the microbial components (EEAs, FAME biomarker abundances), available substrate (DOC) and collective R_h CO_2 efflux was assessed by creating individual repeated measures model for each with irrigation, fallow treatment and their interaction as fixed categorical factors and block, sampling and interaction of block:sampling as random effects. Fixed effects were tested by anova with Kenward-Roger, and means were

compared using Tukey adjusted pairwise comparisons. All data analysis was done with R version 4.2.3 (R Core Team, 2023). Most data was log-transformed to meet normality assumptions (Shapiro-Wilk test), except for temperature and measures of water content (lab GWC, field VWC).

The response of soil respiration to soil temperature and moisture was analyzed across all sampling dates, with block, sampling date and their interactions as random effects. Relationships between individual variables were studied by creating linear regression models with block, sample ID and their interaction as random effects. Sample ID is a unique number assigned to each sample based on location in the field that remained constant across the duration of the experiment. For the temperature sensitivity of soil respiration, we used the van't Hoff equation (Lloyd and Taylor, 1994; Borken et al., 2006; Suseela et al., 2012; Nuñez et al., 2021):

$$R_s = ae^{bT} \quad (1)$$

where R_s is the measured CO_2 flux rate (μmol m^{-2} s^{-1}), T is soil temperature (°C) at 10 cm, a is the basal respiration, and b is the temperature sensitivity of R_s. The response to moisture was evaluated by fitting a linear equation between respiration and field volumetric water content (%). We used only the R_h measured on the week preceding the date of soil collection to relate R_h efflux to the monthly measured variables, including lab GWC.

We used structural equation modeling (SEM) to explore the direct and indirect factors regulating soil R_h efflux (Ding et al., 2016). An SEM tests hypothetical pathways of influence among multiple variables using covariance among them (Grace, 2006; Miao et al., 2009; Shipley, 2000). This technique allowed us to quantitatively test our hypothesized model of direct and indirect effects of moisture and substrate available on R_h as mediated by soil microbial variables. To obtain the final SEM (Fig 7), first we established a base model based on empirical knowledge

and proposed mechanisms (Fig 1 and Fig S8). We assumed a direct relationship between soil moisture and DOC, microbial community composition, EEAs, and soil R_h, since available water plays a key role in soil substrate availability and microbial processes. We assumed the microbial community would have a direct effect on soil R_h through community composition and an indirect effect through EE production. We created latent variables for each of these sets of microbial measures. Moreover, available substrates defined as DOC were theorized to play direct roles in soil CO_2 release and indirect roles through regulating microbial community composition and EEAs.

Once the base model was defined, we analyzed result statistics to optimize it. All data were tested for normality (Shapiro-Wilk) and non-normal data was log-transformed. First, we examined modification indices to ensure that no important paths were left out of the model. Non-significant paths were removed to improve model parsimony without removing pathways central to our core hypotheses. The iterative model optimization continued until the model predictions—chi-square (χ^2) statistic, whole model p-value, the root-mean-square error of approximation (RMSEA), comparative fit index (CFI) and Akaike information criterion (AIC) — fit well with the observed values (Grace, 2006; Ding et al., 2016). Low values of χ^2, RMSEA and AIC and high p-value and CFI suggest that there is a small difference between modeled and observed values (Grace, 2006; Shipley, 2000; Ding et al., 2016). The "lavaan" package in R version 4.2.3 was used to perform SEM analyses (R Core Team, 2015).

3 Results

3.1 Soil moisture

Soil moisture under dryland and irrigated water treatments followed similar temporal dynamics across both years (Fig 2). The irrigated treatment received nearly three times as much water (1160 mm vs 400 mm) than dryland across both 2021 and 2022 growing seasons, resulting in overall higher soil moisture when compared to dryland (Fig 2 and 3A, $p<0.01$). There was also an interactive effect of plant residue inputs and watering regime on soil moisture (Fig 3A, $p< 0.01$) as planted subplots had the lowest moisture across both watering regimes (Fig 3A, Table S2). However, there was no difference between planted, YF, and LTF subplots within the same irrigation treatment (Fig 3A, Table S2).

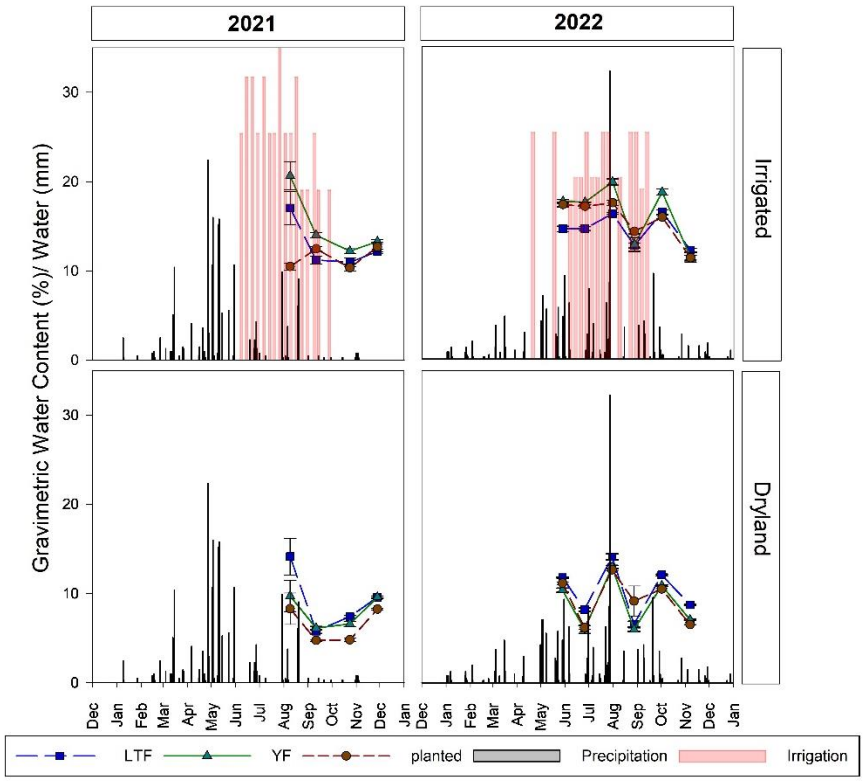

Figure 2. Monthly average (mean ± SE) soil (0-10 cm) gravimetric water content (shapes), weekly irrigation (red lines) and daily precipitation (black lines) dynamics of a continuous maize no-till agroecosystem under two irrigation regimes— irrigated and dryland agriculture—and three plant and residue input treatments— long-term fallow (LTF), yearly fallow (YF), and planted— in Northern Colorado.

3.2 C inputs

Our experimental design created a gradient of plant inputs across different water treatments. The LTF plots received no plant or plant residue inputs since the experiment initiation in 2016. The YF plots, on the other hand, received plant inputs each year except for the measurement year, thus included accumulated residues from harvest of the previous years. Overall, plots under irrigation, except for LTF, received plant residue inputs nearly three times larger in magnitude than their dryland counterparts (Table 2). Two years of crop failure resulted in no grain harvest from the dryland maize plots, but plant residue was retained (Table 2).

Table 2. Biomass inputs (Mg dry matter ha^{-1}) in irrigated and dryland maize during three growing seasons. Values represent the mean ± 1 SE with n = 4.

	Irrigated maize		Dryland maize	
Season	Aboveground Biomass (Grain + residue)	Plant residue	Aboveground Biomass (Grain + residue)	Plant residue
*2020	15.78 ± 1.50	7.46 ± 0.79	0.95 ± 0.3	0.95 ± 0.3
2021	17.93 ± 0.61	8.16 ± 1.44	2.60 ± 0.16	1.58 ± 0.25
*2022	13.44 ± 0.30	6.40 ± 0.52	4.49 ± 0.53	4.49 ± 0.53
Accumulated	47.15 ± 1.65	22.02 ± 1.72	8.04 ± 0.63	7.02 ± 0.66

* Years of dryland maize crop failure which resulted in total biomass being equivalent to plant residue as no grain was harvested.

3.3 Soil DOC

We observed a strong irrigation effect ($p < 0.01$) on soil water-extractable C (Fig 3B). Despite the larger C inputs in irrigated systems (Table 2), dryland plots had, on average, slightly higher concentrations of water-extractable C (Fig 3B, Table S2), with the long-term fallow plot under irrigation being the lowest average of all six unique combination treatments and dryland planted being the highest (Fig 3B, Table S2). Subplots under the same irrigation regime did not differ in water-extractable C (Fig 3B, Table S2).

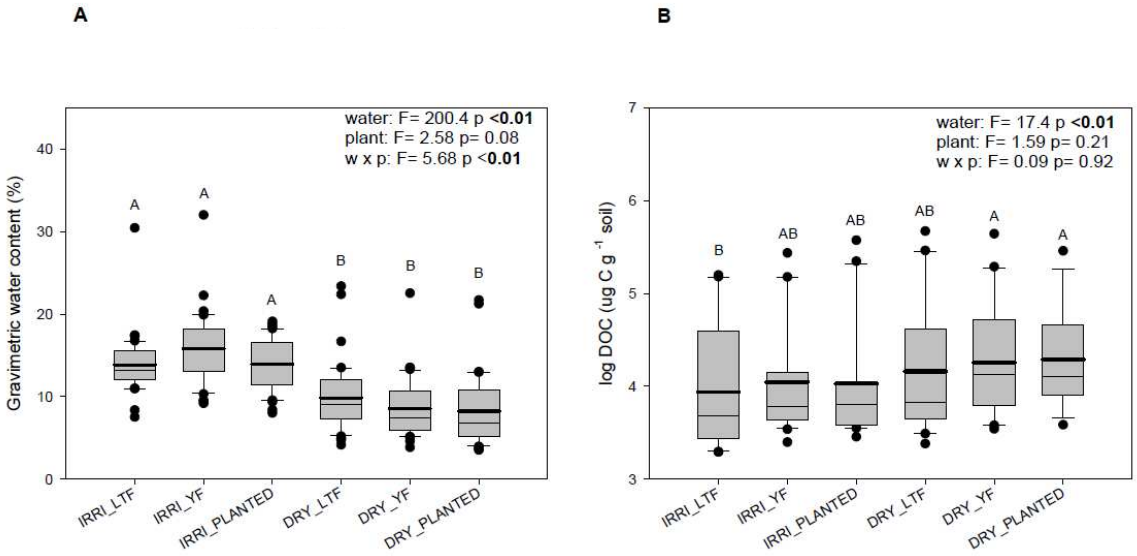

Figure 3. Gravimetric water content (A) and water-extractable C (B) from top-soil (0-10 cm) of irrigated (IRRI) and dryland (DRY) maize, under three plant and residue inputs— long-term fallow (LTF), yearly fallow (YF), and planted— in Northern Colorado. Overall significance for a two-way ANOVA with the main factors of watering regime (water) and plant residue input (plant) are shown ($\alpha = 0.05$). Different letters represent significant differences between unique treatments ($p < 0.05$). Horizontal lines represent mean (bold) and median (light).

3.4 Temperature and moisture as drivers of soil respiration

Soil respiration increased exponentially with increases in soil temperature (Fig 4), with the autotrophic component exhibiting greater differences between irrigation treatments than the heterotrophic component. Increases in soil moisture, on the other hand, resulted in almost negligible increases heterotrophic respiration from irrigated fallow plots (IRRI YF and LTF: slope= 0.01, p = 0.01 and 0.03, respectively) and decreases in dryland fallow plots (DRY YF: slope= -0.003, p = 0.35; LTF: slope= -0.002, p = 0.50). Autotrophic respiration decreased with increases in soil moisture under both irrigation treatments (IRRI: slope= - 0.006, p= 0.24; DRY: slope= -0.003, p = 0.27).

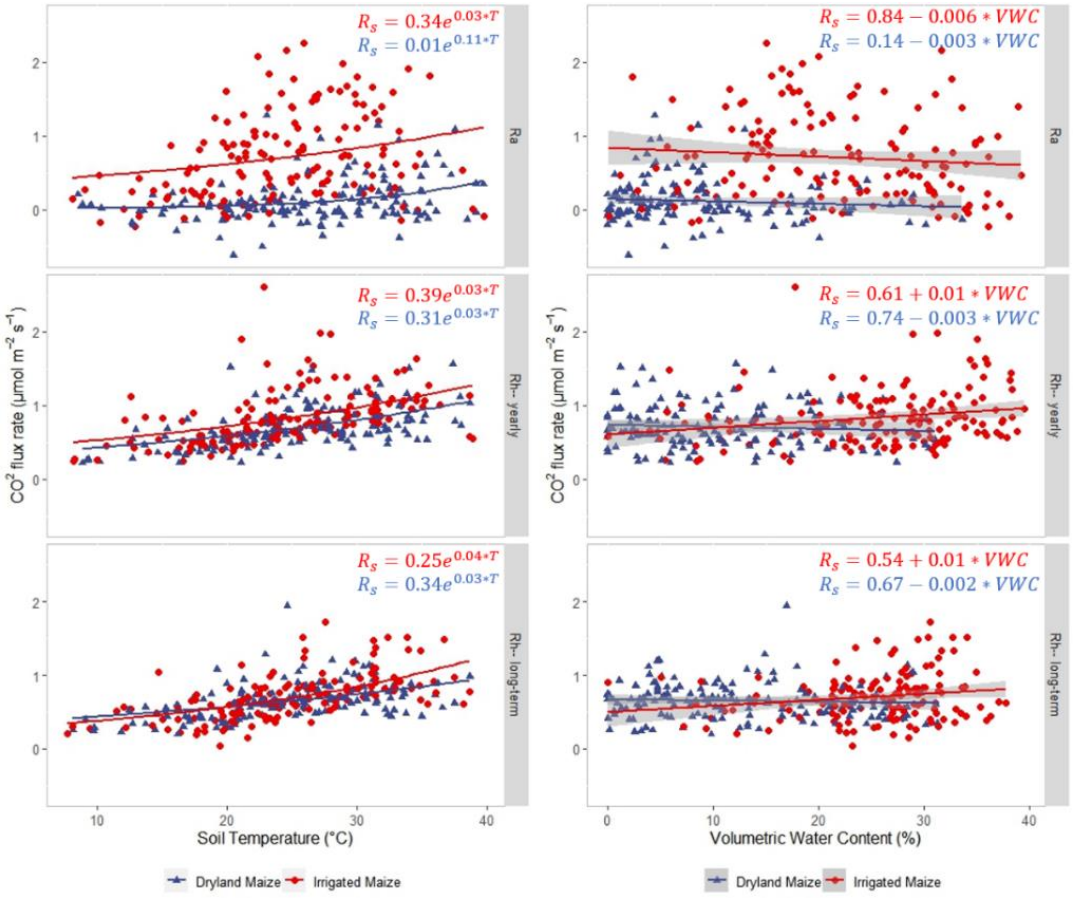

Figure 4. Response of individual components of soil respiration— autotrophic (R_a) and heterotrophic (R_h)— to field soil temperature (left) and moisture (right) in a Northern Colorado continuous, no-till maize agroecosystem under two irrigation regimes. Points represent individual measurements and lines represent fitted curves based on assumed exponential response to temperature and linear response to moisture.

Irrigation treatment and plant inputs —as well as their interactions— had strong effects on overall soil respiration (cumulative R_s data not shown, $p < 0.01$). Looking at just the heterotrophic component of soil respiration from the fallow subplots (Fig 5), we observed a response to irrigation ($p < 0.01$) and plant inputs ($p = 0.02$), and a weaker interactive effect of irrigation and plant inputs ($p = 0.05$), with the yearly fallow plot under regular irrigation exhibiting higher rates of CO_2 efflux than the other subplots (Fig 5, Table S2).

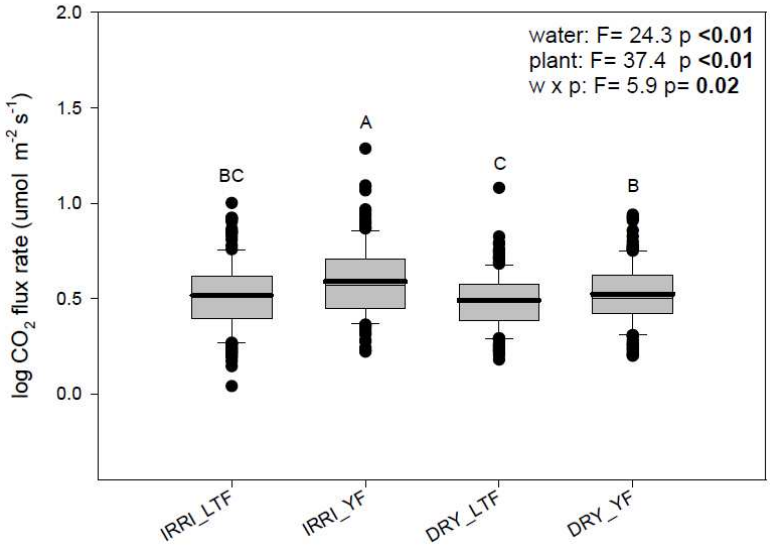

Figure 5. Soil R_h of irrigated (IRRI) and dryland (DRY) maize under two fallow treatments— long-term fallow (LTF), yearly fallow (YF), across two seasons. Overall significance for a two-way ANOVA with the main factors of watering regime (water) and plant residue input (plant) are shown ($\alpha = 0.05$). Letters represent significant difference between the four unique treatments ($p < 0.05$). Horizontal lines represent mean (bold) and median (light).

3.5 Microbial responses

Differences in plant inputs resulted in shifts in soil microbial community abundance with smaller effects of irrigation treatment. All selected FAME biomarkers for the microbial groups of interest showed strong responses to differences in plant inputs (Fig 5A-D; bacteria, fungi, protozoa and actinomycetes FAME: $p<0.01$), with planted plots generally showing the highest abundances in most microbial groups under both irrigation treatments, followed by YF and LTF, respectively (Fig 5; Table S2). Irrigation treatment, on the other hand, resulted in decreases in only three out of the four microbial groups for the dryland relative to irrigated plots (bacteria, fungi, protozoa FAME $p<0.01$; actinomycetes FAME $p= 0.93$). However, interactive effects of irrigation and plant input treatments were observed in all four microbial groups (bacteria, fungi, and protozoa FAME $p<0.01$; actinomycetes FAME $p= 0.01$). These interactions were driven by the joint effect of soil moisture and labile C in shaping the composition of the soil microbial community.

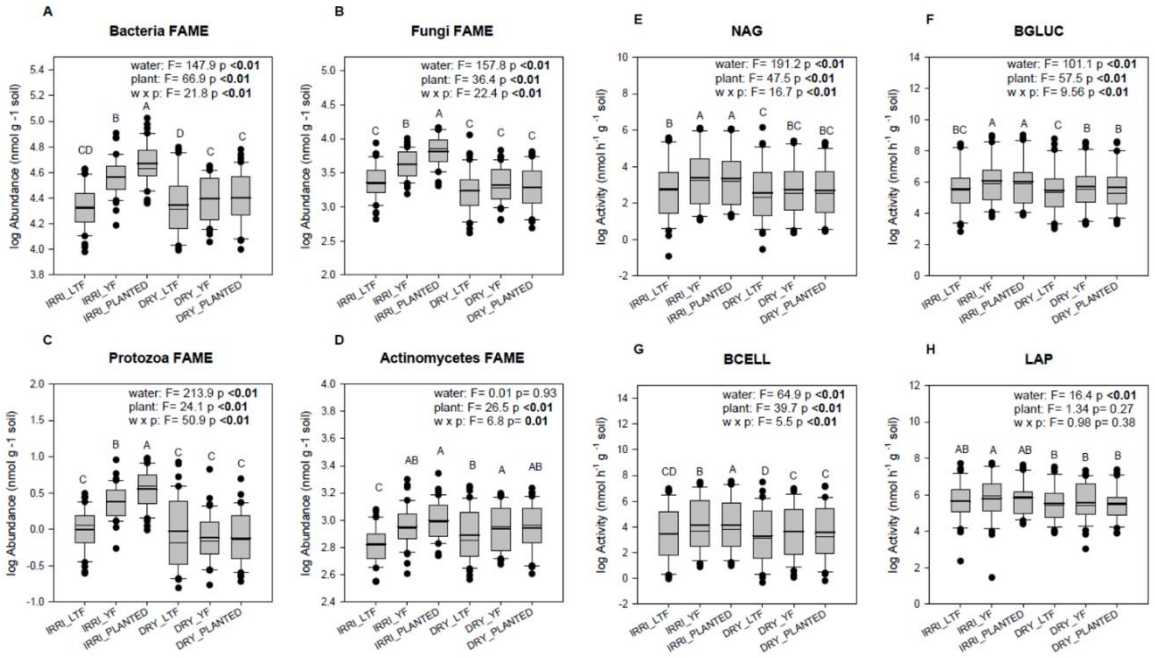

Figure 6. Fatty acid methyl ester biomarker abundances (A-D) and extracellular enzyme activities (E-H) from top-soil (0-10 cm) of irrigated (IRRI) and dryland (DRY) maize, under three plant and residue inputs— long-term fallow (LTF), yearly fallow (YF), and regular planted— in Northern Colorado. Overall significance for a two-way ANOVA with the main factors of watering regime (water) and plant residue input (plant) are shown ($\alpha = 0.05$). Letters represent significant differences between unique treatments ($p<0.05$). Horizontal lines represent mean (bold) and median (light).

Similarly, the irrigation treatments resulted in differences in activities of all four measured EEAs (Fig 3E-H; NAG, BGLUC, BCELL, and LAP = $p < 0.001$) and were generally higher in plots under irrigation treatment (Table S2). Three out of the four EEAs also showed strong positive responses to plant inputs (Fig 5E-G, NAG, BGLUC and BCELL $p < 0.01$) and the interaction between both water and plant input treatments ($p < 0.001$), except for LAP (Fig 5H, p=0.27, p= 0.38, respectively). Under the irrigated treatment, planted plots showed higher NAG (Fig 5E, $p < 0.01$), BGLUC (Fig 5F, $p < 0.01$) and BCELL (Fig 5G, $p < 0.01$) activities when compared to long-term fallow, but LAP activity did not differ by irrigated subplot (Fig 5H, p= 0.32). The yearly fallow plots had intermediary EEAs between planted and long-term fallow in the irrigated system (Fig 5H, Table 3). A similar trend was observed under dryland conditions: planted plots showed higher activities on three out of the four measured EEs when compared to LTF (Fig 5E-H, Table 3), but exhibited no difference from YF (Fig 5E-H, Table 3).

3.6 *Exploring relationships*

We explored simple, two-way correlations between soil moisture (GWC), substrate (DOC) and microbial properties (FAME, EEAs, soil R_h) using data across all ten sampling time points (Fig S4-S7; Fig 7). Soil moisture was not correlated with DOC ($r = 0.12$, $p = 0.17$) or the measured EEAs (NAG: $r = 0.05$, $p= 0.45$; BGLUC: $r = 0.01$, $p = 0.92$; BCELL: $r = 0.03$, $p = 0.61$) except for LAP ($r = 0.17$, $p = 0.01$). Soil moisture was positively correlated with some FAME marker abundances (fungi: $r = 0.35$, $p = <0.01$; bacteria: $r = 0.30$, $p = 0.04$), but not others (protozoa: $r = 0.25$, $p = 0.88$). Similar to soil moisture, soil DOC was positively correlated with LAP ($r = 0.58$, $p = <0.01$), and total FAME ($r = 0.29$, $p = <0.01$), bacteria ($r = 0.22$, $p = <0.01$), and fungi ($r = 0.27$, $p = <0.01$), as well as protozoa ($r = 0.31$, $p = <0.01$) biomarker abundances. While soil R_h was not strongly correlated with field-measured soil moisture relative to soil temperature as shown in Figure 4, R_h showed a stronger correlation with GWC ($r = 0.28$, $p = <0.01$) than DOC ($r = 0.25$, $p = 0.12$). Both DOC and GWC were more strongly correlated with R_h than with the selected FAME biomarker abundances and EEAs.

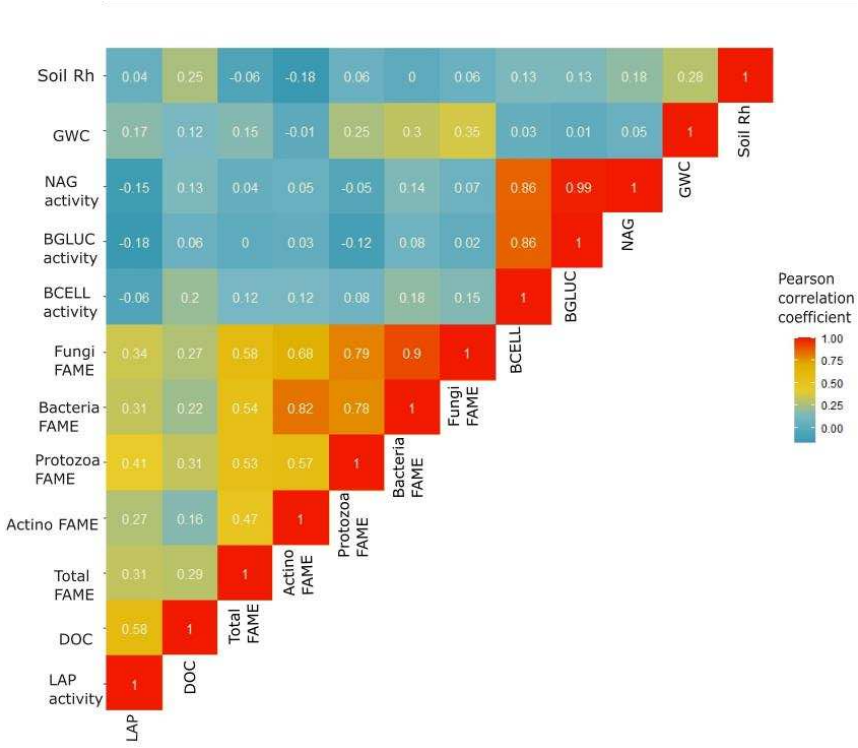

Figure 7. Pearson correlation coefficient heatmap exploring the relationship among all measured variables. All data (except GWC) has been log transformed for normality.

To test our initial theoretical framework (Fig 1), an SEM of soil heterotrophic CO_2 efflux rate was constructed, including all measured variables regardless of correlation strength with the other factors (Fig 8). The soil microbial community abundance was best represented by a combination of soil fungal and bacterial FAME biomarker abundances while soil EEAs were best represented by the single BGLUC activity due to the high degree of correlation amongst the EEAs (Fig 8). Soil GWC had direct effects on DOC, FAME biomarker abundance and was the only factor with direct influence on soil R_h. Soil moisture influenced soil EEA indirectly via its effect on FAME abundance, but EEA did not explain the variability in R_h. Together, these variables predicted only 24% of the variance in the soil heterotrophic respiration (Fig 8).

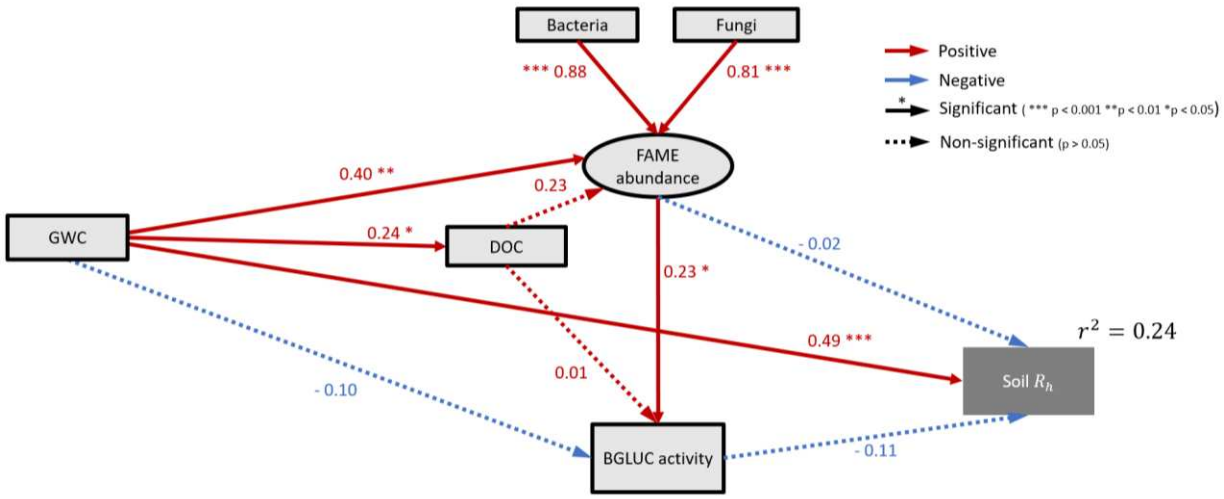

Figure 8. Structural equation model (SEM) with variables (boxes) and potential causal relationships (arrows) for soil heterotrophic CO_2 release rate (R_h) across a continuous maize system under two irrigation treatments. Arrows indicate the hypothesized direction of causation. The numbers are standardized path coefficients, which can reflect the importance of the variables within the model [Colman and Schimel, 2013]. The model had $\chi 2 = 5.62$, p-value = 0.23, CFI= 0.99, RMSEA = 0.07, and AIC = 1202.20.

4 Discussion

We followed the changes in SOC dynamics of a continuous maize agroecosystem, five years after its transition from irrigated to a non-irrigated system, across two growing seasons. We quantified C inputs in the form of crop residues as well as C outputs in the form of heterotrophic soil respiration. We hypothesized that soil moisture and plant residue inputs (as the main source of substrates), in combination, control the abundance and activity of the soil microbial community, rather than available water alone. Our findings indicate that R_h was seemingly regulated by both available substrates and soil moisture; differences in soil moisture were responsible for seasonal changes in R_h, while cumulative variability in R_h could be explained by available substrates and/or the interaction between both factors.

Relative to the irrigated treatments, non-irrigated plots had reduced soil moisture, aboveground biomass, microbial biomass, and microbial activity, but higher DOC and similar rates of heterotrophic respiration. Dryland plots had lower plant residue returns, and

correspondingly lower soil C inputs, than irrigated plots. We observed shifts in microbial community abundance with decreases in available bacteria, fungi, protozoa and actinomycetes FAME biomarkers under dryland conditions and with reduced C inputs. Similarly, decreases in microbial activity—quantified as activities of four extracellular enzymes—were observed in the dryland system. On the other hand, plots under dryland conditions showed higher DOC concentrations and soil R_h fluxes that are comparable to those from their irrigated counterparts.

We investigated all possible correlations between factors and used structural equation modeling to explore the direct and indirect influences on soil R_h in the system by building causal relationships between all measured variables. Across all 10 sampling points, soil moisture was weakly correlated with R_h. While soil moisture and DOC had stronger correlations with R_h than other soil variables measured, only soil moisture explained a significant proportion of the variability in our final model.

4.1 Effects on soil moisture and C inputs

Higher soil moisture content in irrigated plots than dryland was expected, given that the irrigated treatment received nearly three times as many water inputs than dryland across both growing seasons. Productivity under dryland conditions suffered a six-fold decrease from irrigated plots, and received only a third of estimated plant residue returns, which was again expected given maize's high irrigation requirements and high water-stress susceptibility (Sherrod et al., 2014; Cano et al., 2018). The transition to dryland agriculture likely has had cumulative effects on substrate availability and accessibility to microbes, as the difference in biomass and water inputs increased with each growing season.

4.2 Effects on the soil microbial community

After two seasons, the size of three major microbial groups (bacteria, fungi, protozoa) in the system, assessed as EL-FAME, was affected by irrigation, while actinomycetes were mostly affected by plant inputs, with strong interactive effects between these factors for all groups. Differences in plant inputs, and hence likely substrate availability, was much greater between planted and fallow plots under irrigation than those under dryland. Thus, differences in microbial group abundances was greater between irrigated subplots than dryland, further emphasizing the importance of both substrate availability and soil moisture in sustaining microbial development. A higher biomass production corresponded with increased litter and root inputs, which are an important microbial source of C (Finzi et al., 2015; Calderon et al., 2016). This remains consistent with what was observed in previous years of this study (Nuñez, 2021), where, under irrigation, soil moisture had greater effects in community size (measured as PLFAs) in the yearly fallow subplots, but now these differences have magnified given the cumulative differences in plant inputs.

Similarly, three out of four soil EEAs (BGLUC, NAG, BCELL) showed strong responses to soil moisture, plant inputs and their interaction, while LAP was mostly influenced by moisture. Long-term fallow plots under both treatments showed similar levels of potential enzymatic activity, and differences between irrigated planted and yearly fallow plots were muted, further emphasizing the importance of substrate availability in influencing microbial activity, not just water availability. Although measured under saturated substrate concentrations (German et al., 2011), microbial activity measured as EEAs in the lab was consistent with overall trends in measurements of *in situ* microbial activity (R_h).

4.4 Influences in soil CO_2 efflux and SOC turnover

Lower R_s fluxes were observed in non-irrigated plots. These differences were mostly driven by R_a, the component of respiration directly associated with plant growth (Hopkins et al., 2013). Variations in R_s related to plant productivity, even when R_h was the main component of R_s, have been reported (Xu and Shang, 2016; Gao et al., 2017). Plots differing only in soil moisture (LTF IRRI v DRY) showed negligible differences in R_h, and the magnitude of differences in R_h observed in yearly fallow plots (IRRI v DRY) do not reflect the magnitude of differences in water and plant residue inputs that the systems have received.

While plant inputs were used as a proxy for substrate availability at the cumulative annual scale, we used DOC as the proxy for monthly differences in substrate availability (Fang et al., 2005; Dannenmann et al., 2009), given that most substrates used by microbes as energy sources are water soluble (Cleveland and Yavitt, 1997; Schimel, 2018). Our monthly sampling aimed to account for the rapid changes in this pool. Consistent with other drought studies in agroecosystems (e.g., Bloor and Bardgett, 2012; Niboyet et al., 2017; Peng et al., 2023), our results indicate a greater DOC concentration in the dryland system, where we know C inputs are lower. This could be interpreted as strong evidence that microbial physical access to soil C is the primary control on whether that carbon is decomposed (and respired to CO_2) (Schimel and Schaeffer, 2012) and that the microbial community in these plots is water-limited and therefore unable to access substrates, thus allowing it to accumulate (Zeglin et al., 2013). This is supported by the slightly higher DOC in the planted and yearly fallow dry plots, and the observed lower potential enzymatic activity under dryland.

Using the conceptual framework proposed by previous models (Schimel and Weintraub, 2003; Manzoni et al., 2014), our initial theoretical framework depicted soil moisture and available substrates as co-limiting the microbial community and consequently soil R_h. The final

SEM revealed soil moisture as a direct driver of soil R_h, and a direct and indirect driver of soil DOC and microbial community abundance. Microbial effects on soil CO_2 release rates could be enhanced only when aridity is alleviated (Wu et al., 2014; Ding et al., 2016). Thus, the microbial community shows a stronger moisture limitation, given accumulation of labile C available to microbes under dryland conditions. Soil moisture also influenced EE production indirectly as mediated by soil microbial community abundance. This may indicate that the soil microbial community has opted for dormancy awaiting better moisture conditions.

4.5 Limitations in explaining soil CO_2 efflux variability

The final model created based on our theorized framework only explained 24% of the variability in soil R_h. We aimed to relate enzymatic activity and activity in the form of *in situ* heterotrophic respiration to soil moisture and DOC. For this, we included a combination of field and laboratory analyses in our experimental design, and thus some of the missing relationships could be attributed to the disconnection of field and laboratory conditions. Similarly, unlike other models linking soil CO_2 release rates to microbial properties (e.g., Allison, 2005; Davidson et al., 2012; Manzoni et al., 2014; Ding et al., 2016), we failed to include some of the same physical and environmental factors, such as pH and bulk density, into our initial framework. Another possible explanation to the low score in our model could lie in our choice of microbial group biomarker analysis. With lower cost and simpler methodology, EL-FAME has the potential to be a reliable method in agricultural management soil evaluations (Li et al., 2020). However, the EL-FAME method extracts all of the lipid groups present in the sample (Sharma and Buyer, 2015), this includes fatty acids originating from sources other than living microbes (Li et al., 2020). Thus, it is impossible to distinguish between active and inactive microbes just from EL-FAME analysis alone.

5 Conclusion

In summary, this study analyzed the effects of the transition into dryland agriculture on SOC dynamics and attempted to find links between soil moisture, soil DOC and the microbial community to explain variations in soil CO_2 fluxes. Our results revealed that soil moisture was a stronger driver of soil R_h than other measured variables, with direct effects on soil DOC and microbial FAME biomarker abundance. Soil DOC, microbial FAME biomarker abundance and EEAs were of minor importance in the tested SEM. However, this does not mean that they should be disregarded, as factorial analyses showed that the retirement of irrigation and the gradient of soil plant residue inputs created by our experimental design affected these ecosystem elements and R_h. Overall, our results help illustrate that changes in global C dynamics can be expected with transition of land areas from irrigated to dryland agriculture. However, focusing on resource-conserving practices and further exploring the resiliency of the soil microbiome can be the key to minimize the potential negative impacts of the transition into non-irrigated cropping systems.

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APPENDIX A

SUPPLEMENTAL MATERIALS

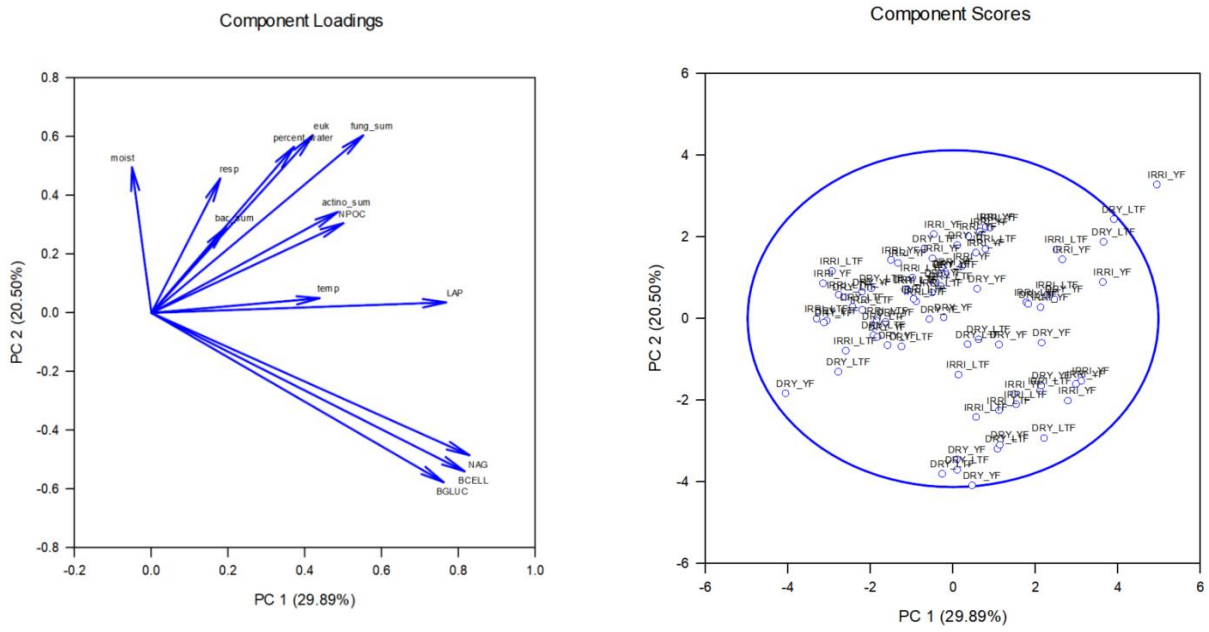

Figure S1. Results of Principal component analysis of ten soil samplings (0-10 cm) in 2021 and 2022 of a continuous maize agroecosystem in Northern Colorado. Field—resp: CO_2 flux, moist: field VWC, temp: field soil temperature; lab—percent_water: GWC, bac_sum: sum of bacteria FAME biomarkers, fung_sum: sum of fungi FAME biomarkers, euk: protozoa FAME biomarkers, actino_sum: sum of actinomycetes FAME biomarkers, NPOC: DOC, EEAs: NAG, BGLUC, BCELL, LAP. Most data was log transformed, except GWC, field VWC, and field soil temperature.

Table S1. Results of a permutational analysis of variance (PERMANOVA) between six unique irrigation and plant input treatment combinations across ten soil samplings (0-10 cm) in 2021 and 2022 of a continuous maize agroecosystem in Northern Colorado. *df:* irrigation= 1, plant input= 1, irri x plant= 1.

	irrigation			**plant input**			**irri x plant**		
	R2	*F*	*p*	*R2*	*F*	*p*	*R2*	*F*	*p*
GWC	0.38	99.5	**0.001**	0.003	0.88	0.37	0.023	5.93	**0.02**
DOC	-	-	-	-	-	-	-	-	-
soil Rh	0.01	1.90	0.155	0.008	1.26	0.27	0.005	0.85	0.38
FAME									
bacteria	0.72	15.28	**0.002**	0.16	35.07	**0.001**	0.03	6.94	**0.01**
fungi	0.13	25.74	**0.001**	0.09	18.11	**0.001**	0.02	4.56	**0.04**
protozoa	-0.67	-340.15	0.93	0.68	346.36	0.15	0.68	347.99	0.15
actino	0.006	1.09	0.28	0.07	12.61	**0.001**	0.02	2.54	0.11
EEAs									
BGLUC	-	-	-	-	-	-	-	-	-
NAG	-	-	-	-	-	-	-	-	-
BCELL	-	-	-	-	-	-	-	-	-
LAP	-	-	-	-	-	-	-	-	-
Together	0.20	40.74	**0.001**	0.01	2.81	0.05	0.02	3.37	**0.03**

Table S2. Means comparisons between six unique irrigation and plant input treatment combinations across ten soil samplings (0-10 cm) in 2021 and 2022 of a continuous maize agroecosystem in Northern Colorado, across eleven categories. *df* for: gravimetric water content (GWC): 195; dissolved organic carbon (DOC): 94.7; Soil R_h: 117; fatty acid methyl ester biomarkers (FAME): 195; extra-cellular enzyme activities (EEAs): 175.

	GWC		DOC		Soil Rh		Bacteria FAME		Fungi FAME		Protozoa FAME		Actino FAME	
Contrast	*t.ratio*	*p.value*	*t.ratio*	*p.value*	*t.ratio*	*p.value*	*t.ratio*	*p.value*	*t.ratio*	*p.value*	*t.ratio*	*p.value*	*t.ratio*	*p.value*
IRRI LTF - IRRI YF	-2.86	0.05	-1.13	0.87	-3.09	**0.01**	-8.42	**<0.01**	-6.22	**<0.01**	-7.97	**<0.01**	-5.53	**<0.01**
IRRI LTF - IRRI PLANTED	-0.17	1.00	-0.94	0.94			-11.82	**<0.01**	-10.63	**<0.01**	-11.79	**<0.01**	-7.46	**<0.01**
IRRI LTF - DRY LTF	-5.75	**<0.01**	2.34	0.19	-0.56	0.94	-1.16	0.85	-2.66	0.09	-0.51	1.00	2.91	**0.05**
IRRI LTF - DRY YF	-7.65	**<0.01**	3.29	**0.02**	-0.21	1.00	1.97	0.36	-0.77	0.97	-2.52	0.12	4.97	**<0.01**
IRRI LTF - DRY PLANTED	-8.09	**<0.01**	3.63	**0.01**			1.86	0.43	-1.49	0.67	-2.55	0.11	5.26	**<0.01**
IRRI YF - IRRI PLANTED	-2.70	0.08	-0.19	1.00			3.40	**0.01**	4.41	**<0.01**	3.82	**<0.01**	1.93	0.39
IRRI YF - DRY LTF	-8.62	**<0.01**	1.21	0.83	-3.66	**<0.01**	-9.59	**<0.01**	-8.87	**<0.01**	-8.47	**<0.01**	-2.62	0.10
IRRI YF - DRY YF	-10.52	**<0.01**	2.17	0.26	-3.31	**0.01**	-6.45	**<0.01**	-6.99	**<0.01**	-10.49	**<0.01**	-0.57	0.99
IRRI YF - DRY PLANTED	-10.95	**<0.01**	2.52	0.13			-6.56	**<0.01**	-7.71	**<0.01**	-10.52	**<0.01**	-0.27	1.00
IRRI PLANTED - DRY LTF	-5.92	**<0.01**	1.40	0.73			-12.98	**<0.01**	-13.28	**<0.01**	-12.29	**<0.01**	-4.55	**<0.01**
IRRI PLANTED - DRY YF	-7.82	**<0.01**	2.36	0.18			-9.85	**<0.01**	-11.39	**<0.01**	-14.31	**<0.01**	-2.49	0.13
IRRI PLANTED - DRY PLANTED	-8.26	**<0.01**	2.71	0.08			-9.96	**<0.01**	-12.12	**<0.01**	-14.34	**<0.01**	-2.20	0.24
DRY LTF - DRY YF	1.90	0.41	-0.95	0.93	-0.35	0.99	-3.13	**0.02**	-1.89	0.41	2.02	0.34	-2.06	0.32
DRY LTF - DRY PLANTED	2.34	0.18	-1.33	0.77			-3.02	**0.03**	-1.16	0.85	2.04	0.32	-2.35	0.18
DRY YF - DRY PLANTED	-0.44	1.00	0.39	1.00			-0.11	1.00	-0.73	0.98	-0.03	1.00	0.30	1.00

	NAG		BGLUC		BCELL		LAP	
Contrast	*t.ratio*	*p.value*	*t.ratio*	*p.value*	*t.ratio*	*p.value*	*t.ratio*	*p.value*
IRRI LTF - IRRI YF	-9.68	**<0.01**	-9.56	**<0.01**	-7.35	**<0.01**	-1.54	0.64
IRRI LTF - IRRI PLANTED	-9.25	**<0.01**	-8.82	**<0.01**	-7.53	**<0.01**	-2.05	0.32
IRRI LTF - DRY LTF	-3.28	**0.02**	-2.24	0.22	-2.00	0.35	-1.26	0.81
IRRI LTF - DRY YF	-0.36	1.00	2.16	0.26	1.86	0.43	-1.00	0.92
IRRI LTF - DRY PLANTED	-1.39	0.74	1.04	0.90	1.06	0.90	-1.16	0.86
IRRI YF - IRRI PLANTED	-0.43	1.00	-0.74	0.98	0.18	1.00	0.51	1.00
IRRI YF - DRY LTF	-12.95	**<0.01**	-11.80	**<0.01**	-9.35	**<0.01**	-2.80	0.06
IRRI YF - DRY YF	-10.04	**<0.01**	-7.40	**<0.01**	-5.50	**<0.01**	-2.54	0.12
IRRI YF - DRY PLANTED	-11.06	**<0.01**	-8.51	**<0.01**	-6.29	**<0.01**	-2.70	0.08
IRRI PLANTED - DRY LTF	-12.52	**<0.01**	-11.06	**<0.01**	-9.53	**<0.01**	-3.31	**0.01**
IRRI PLANTED - DRY YF	-9.61	**<0.01**	-6.66	**<0.01**	-5.68	**<0.01**	-3.05	**0.03**
IRRI PLANTED - DRY PLANTED	-10.63	**<0.01**	-7.78	**<0.01**	-6.47	**<0.01**	-3.21	**0.02**
DRY LTF - DRY YF	-2.92	**0.05**	-4.40	**0.00**	-3.85	**<0.01**	-0.26	1.00
DRY LTF - DRY PLANTED	-1.89	0.41	-3.29	**0.02**	-3.06	**0.03**	-0.10	1.00
DRY YF - DRY PLANTED	-1.03	0.91	-1.12	0.87	-0.79	0.97	-0.16	1.00

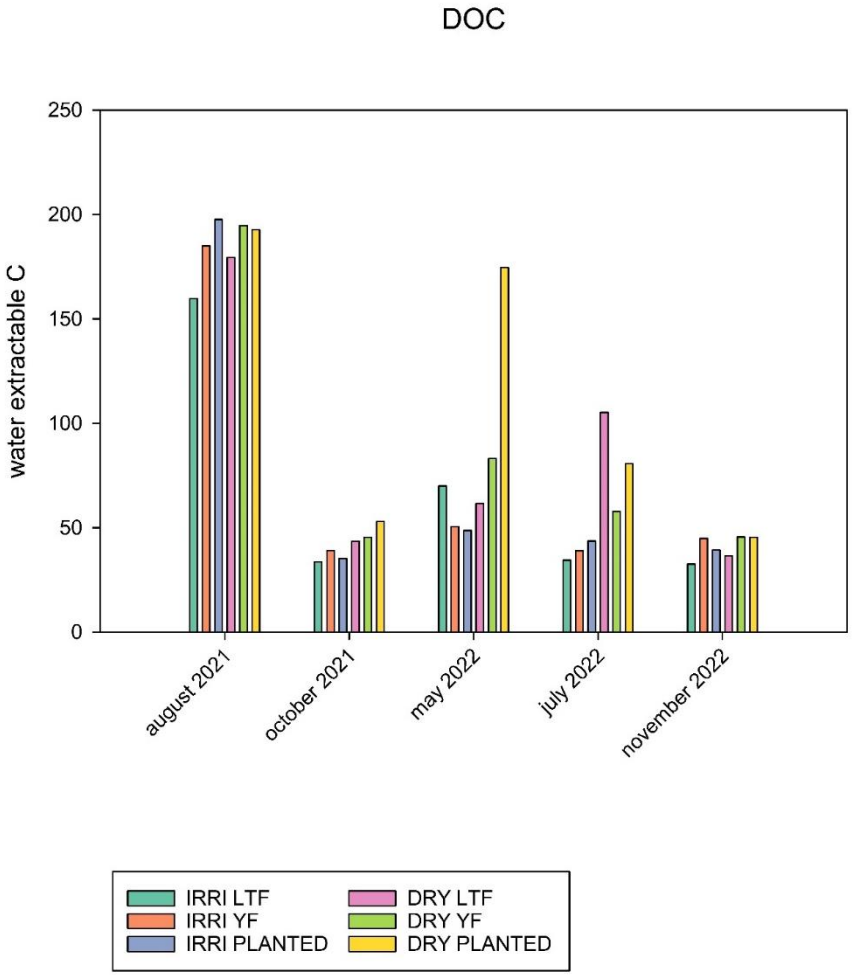

Figure S2. Temporal changes in dissolved organic carbon concentrations (DOC; ug C g -1 soil) from top-soil (0-10 cm) of irrigated (IRRI) and dryland (DRY) maize, under three plant and residue inputs— long-term fallow (LTF), yearly fallow (YF), and planted— in Northern Colorado.

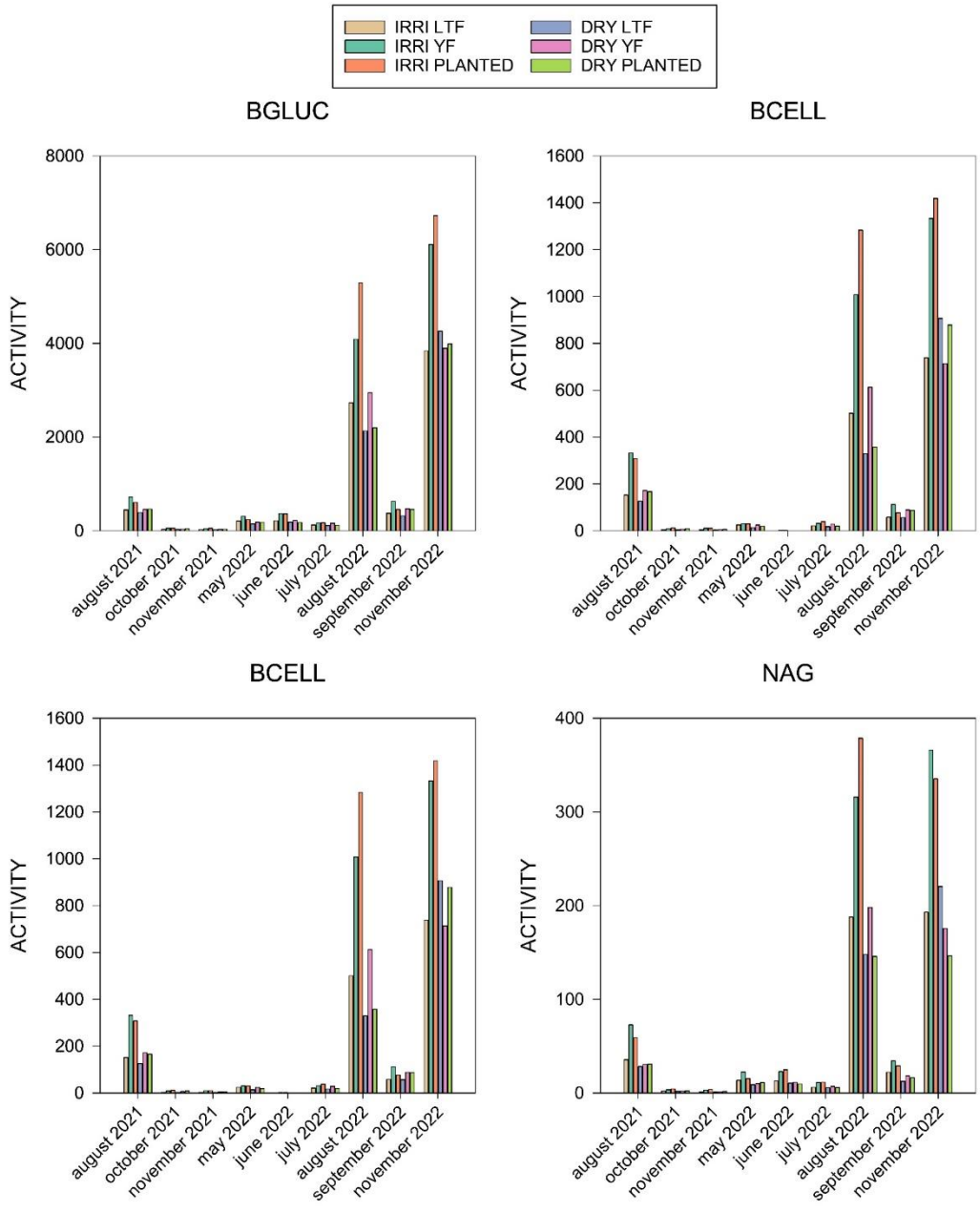

Figure S3. Temporal changes in four extra-cellular enzyme activities (EEAs; nmol h-1 g-1 soil) from top-soil (0-10 cm) of irrigated (IRRI) and dryland (DRY) maize, under three plant and residue inputs— long-term fallow (LTF), yearly fallow (YF), and planted— in Northern Colorado.

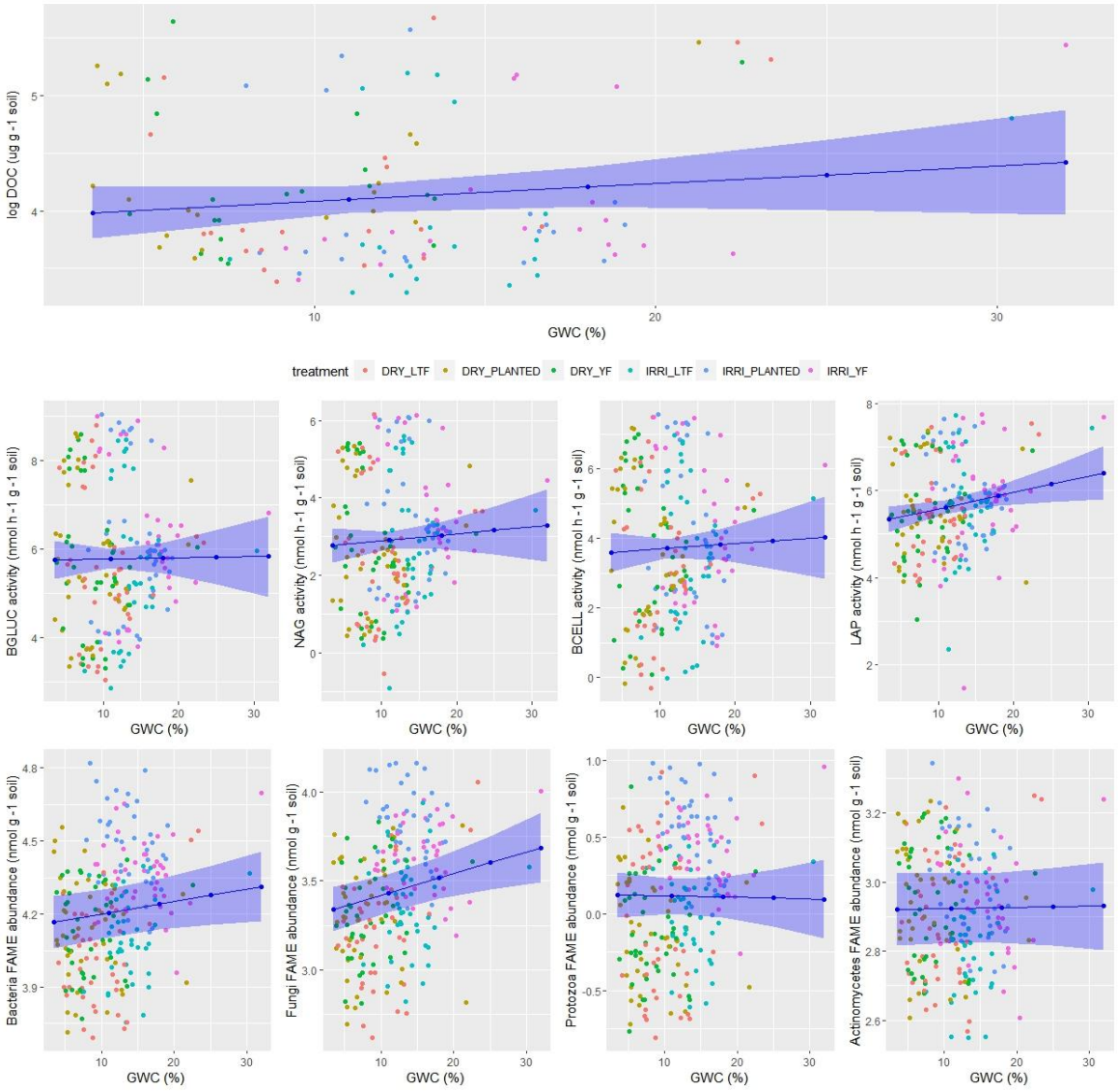

Figure S4. Relationship between soil moisture, represented by topsoil (0-10 cm) gravimetric water content of irrigated (IRRI) and dryland (DRY) maize, under three plant and residue inputs— long-term fallow (LTF), yearly fallow (YF), and regular planted— in Northern Colorado. All data has been log transformed (except GWC).

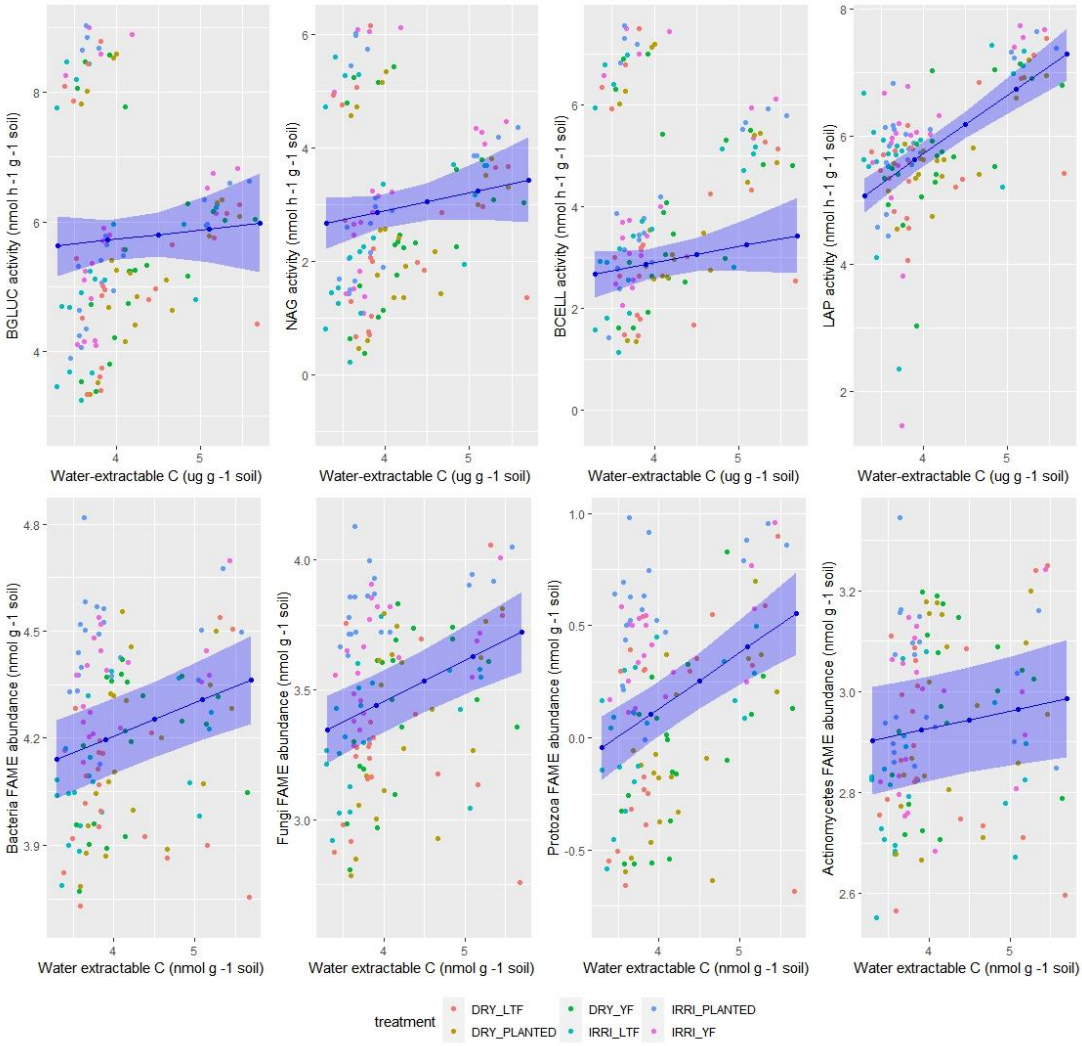

Figure S5. Relationship between available soil substrates, represented by topsoil (0-10 cm) water-extractable soil C content of irrigated (IRRI) and dryland (DRY) maize, under three plant and residue inputs— long-term fallow (LTF), yearly fallow (YF), and regular planted— in Northern Colorado. All data has been log transformed.

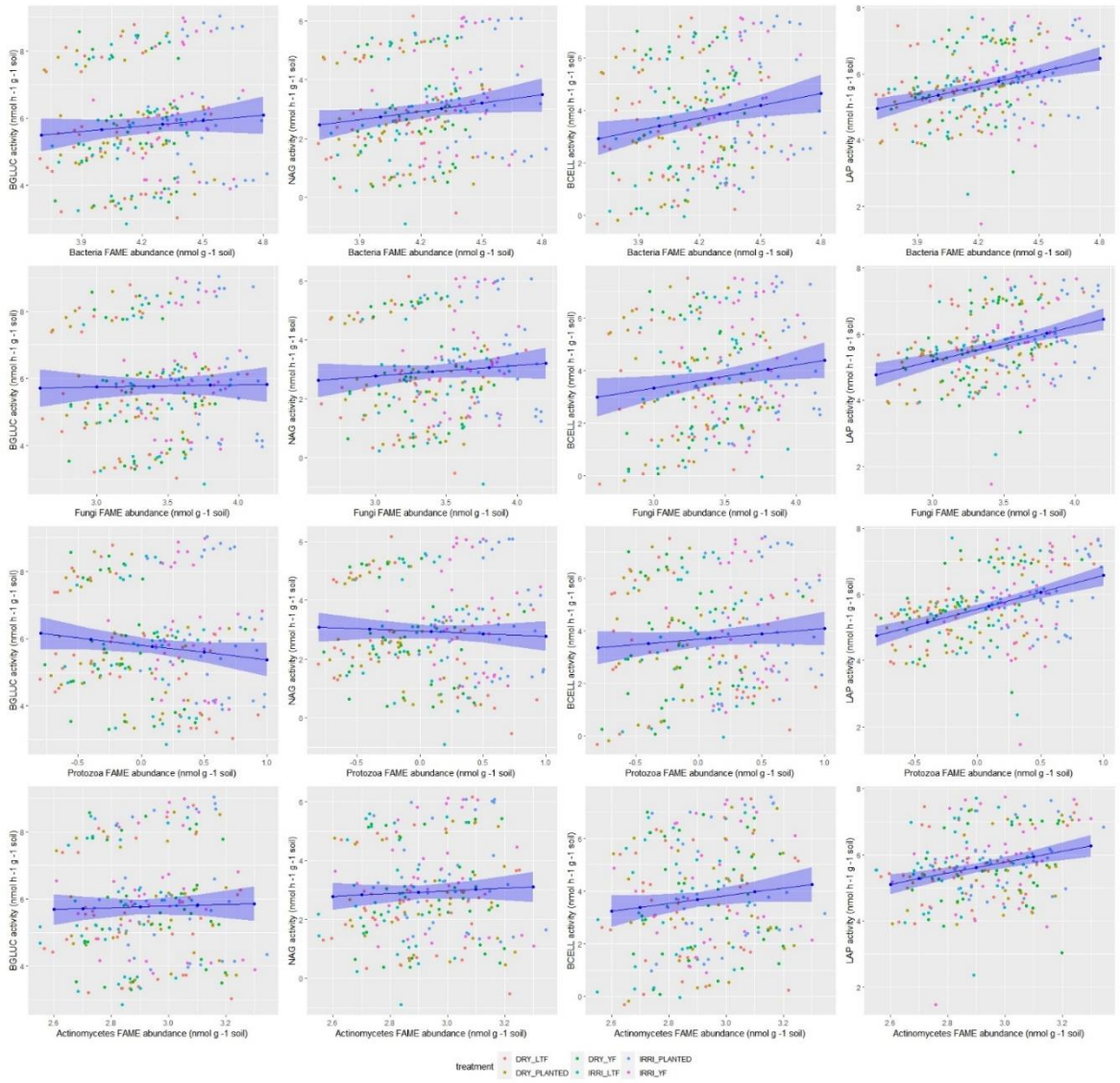

Figure S6. Relationship between bacteria, fungi, protozoa and actinomycetes (rows) fatty acid methyl-ester biomarkers and soil extracellular enzyme activities (columns), represented by β-1, 4-glucosidase (BGLUC), β-1, 4-n-acetyl-glucosaminidase (NAG), β-D-1,4-cellobiosidase (BCELL) and Leucine amino peptidase (LAP) activities in top soil (0-10 cm) collected from a maize agroecosystem undergoing irrigated (IRRI) and dryland (DRY) watering regimes, under three plant and residue inputs— long-term fallow (LTF), yearly fallow (YF), and regular planted— in Northern Colorado. All data has been log transformed.

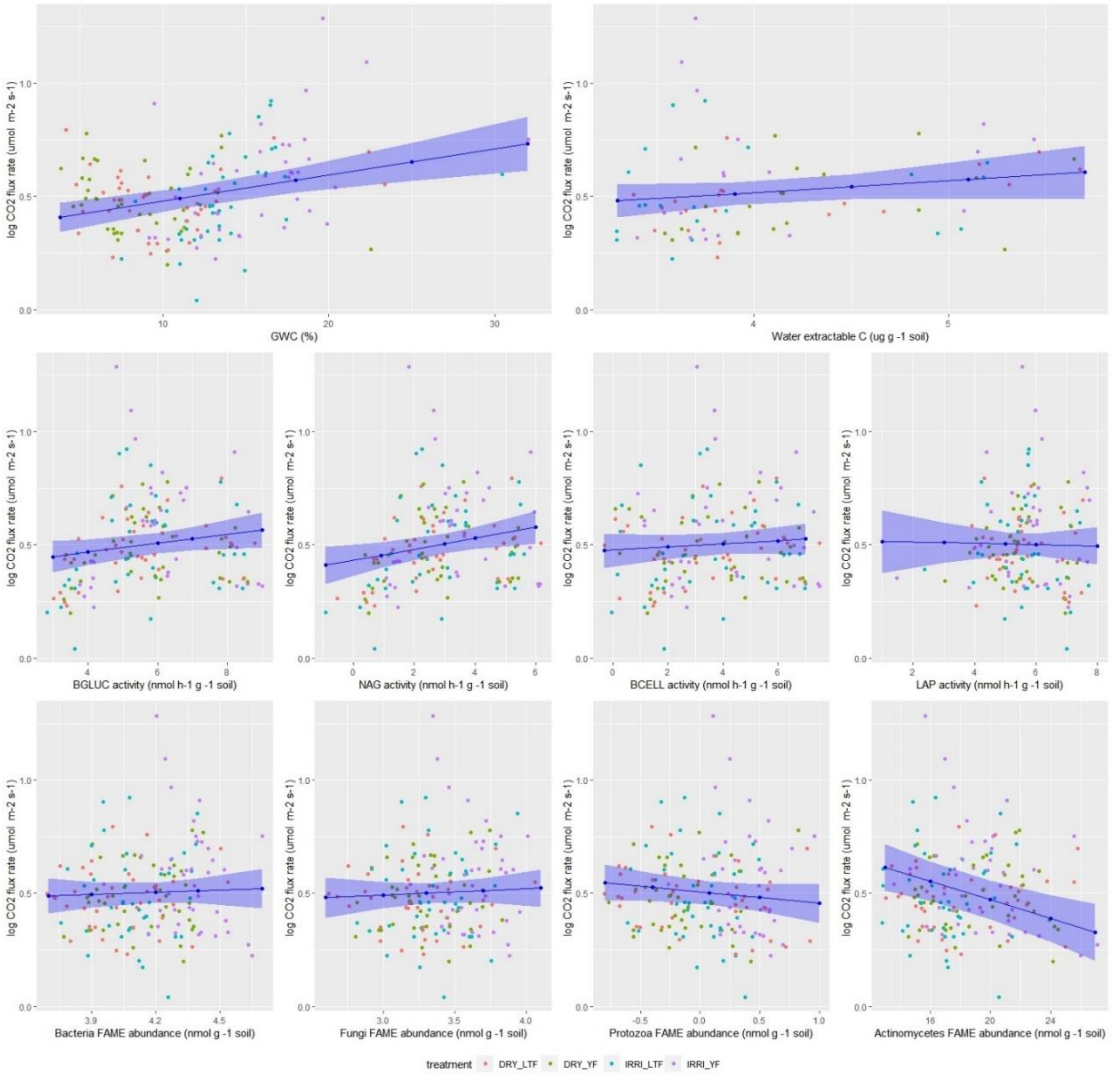

Figure S7. Relationship between soil heterotrophic respiration (soil Rh) and multiple soil characteristics (in order, top to bottom, left to right): gravimetric water content, water-extractable C (DOC), β-1, 4-glucosidase (BGLUC), β-1, 4-n-acetyl-glucosaminidase (NAG), β-D-1,4-cellobiosidase (BCELL) and Leucine amino peptidase (LAP) extracellular activities and bacteria, fungi, protozoa and actinomycetes (rows) fatty acid methyl-ester biomarkers and in top soil (0-10 cm) collected from a maize agroecosystem undergoing irrigated (IRRI) and dryland (DRY) watering regimes, under three plant and residue inputs— long-term fallow (LTF), yearly fallow (YF), and regular planted— in Northern Colorado. All data has been log transformed (except GWC).

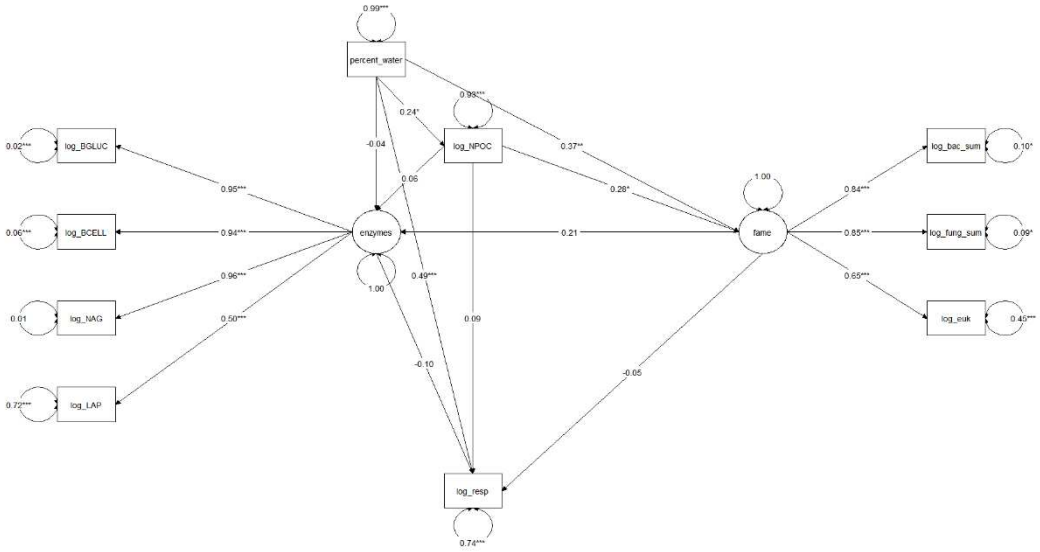

Figure S8. First iteration of the structural equation model (SEM) with variables (boxes) and potential causal relationships (arrows) for soil heterotrophic CO_2 release rate (R_h) across a continuous maize system under two irrigation treatments. FAME abundance and enzymes (ovals) are latent variables created from the combination of bacterial, fungal and protozoa FAME abundances and BGLUC, BCELL, NAG, LAP EEAs, respectively. Arrows indicate the hypothesized direction of causation. The numbers are standardized path coefficients, which can reflect the importance of the variables within the model [Colman and Schimel, 2013]. The model had $\chi 2 = 137.41$, p-value = 0.00, CFI= 0.87, RMSEA = 0.22, and AIC = 1550.15.

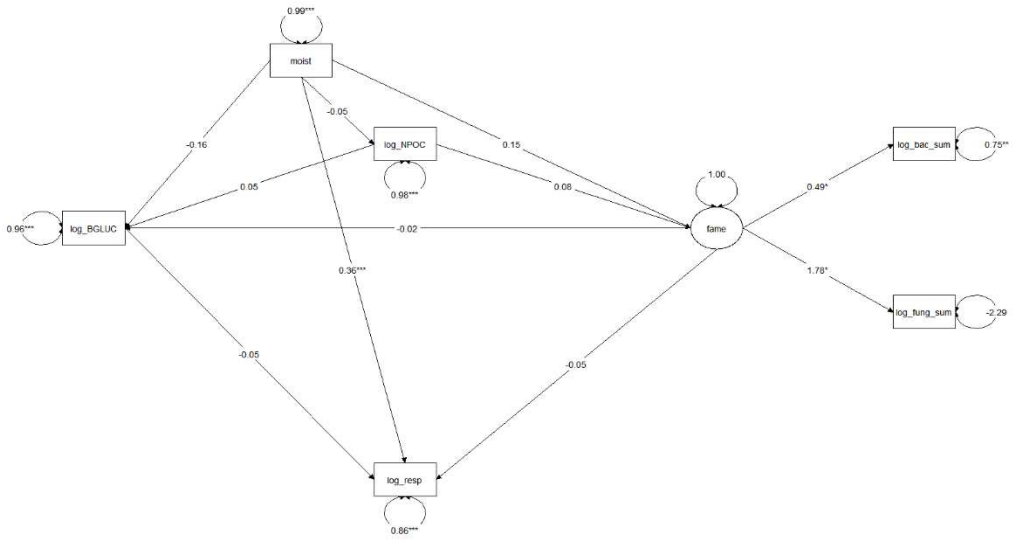

Figure S9. Iteration of the structural equation model (SEM) using field moisture measurements (LICOR soil probe) with variables (boxes) and potential causal relationships (arrows) for soil heterotrophic CO_2 release rate (R_h) across a continuous maize system under two irrigation treatments. FAME abundance and enzymes (ovals) are latent variables created from the combination of bacterial, fungal and protozoa FAME abundances and BGLUC, BCELL, NAG, LAP EEAs, respectively. Arrows indicate the hypothesized direction of causation. The numbers are standardized path coefficients, which can reflect the importance of the variables within the model [Colman and Schimel, 2013]. The model had $\chi 2 = 14.35$, p-value = 0.01, CFI= 0.94, RMSEA = 0.18, and AIC = 1211.61.

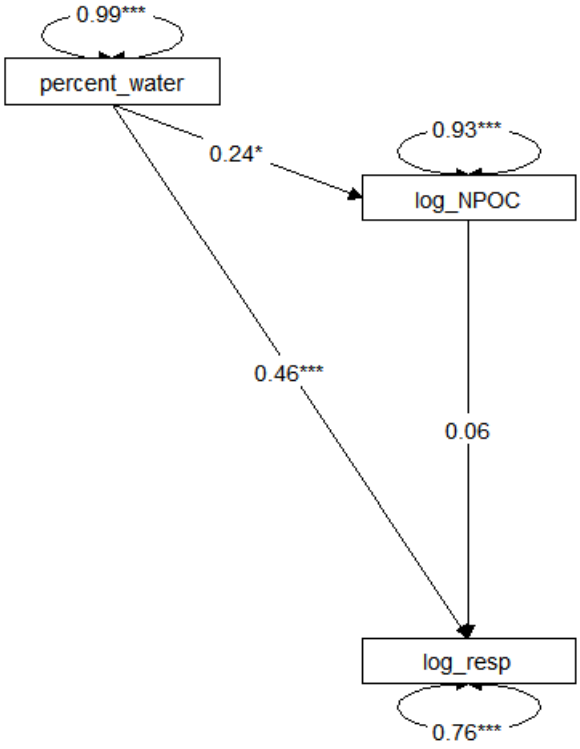

Figure S10. Iteration of the structural equation model (SEM) using factors with highest correlation coefficients related to R_h with variables (boxes) and potential causal relationships (arrows). Arrows indicate the hypothesized direction of causation. The numbers are standardized path coefficients, which can reflect the importance of the variables within the model [Colman and Schimel, 2013]. The model had $\chi 2 = 0.00$, p-value = 0.00, CFI= 1.00, RMSEA = 0.00, and AIC = 664.15.