Long-term irrigation reduces soil carbon sequestration by affecting soil microbial communities in agricultural ecosystems of northern China

Running title: Soil carbon sequestration under irrigation

Yongqing Qi[1†], Jian Li[2,3†], Shaojun Deng[2], Jun Wang[2], Yanju Zhang[2], Hongwei Pei[1], Yanjun Shen[1], Dafeng Hui[4], Hans Lambers[5,6], Jordi Sardans[7,8], Josep Peñuelas[7,8], Zhanfeng Liu[2*]

[1]Key Laboratory of Agricultural Water Resources, Center for Agriculture Resources Research, Institute of Genetics and Development Biology, Chinese Academy of Sciences, Shijiazhuang 050022, China;

[2]Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems & CAS Engineering Laboratory for Vegetation Ecosystem Restoration on Islands and Coastal Zones, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China;

[3]Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, 1799 Jimei Road, Xiamen, 361021, China

[4]Department of Biological Sciences, Tennessee State University, Nashville, TN 37209, USA;

[5]School of Biological Sciences, University of Western Australia, Perth, WA 6009, Australia;

[6]Department of Plant Nutrition, College of Resources and Environmental Sciences; National Academy of Agriculture Green Development; Key Laboratory of Plan-Soil Interactions, Ministry of Education; China Agricultural University, Beijing 100193, China;

[7]CSIC, Global Ecology Unit CREAF-CEAB-UAB, Cerdanyola del Valles, 08193 Catalonia, Spain;

[8]CREAF, Cerdanyola del Valles, 08193 Catalonia, Spain.

† These authors contributed equally to this work.

***Correspondence**:

Zhanfeng Liu Ph.D., Professor

Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China.

Oricd ID: 0000-0002-6602-6196

Tel: +86-20-37252592; **Fax**: +86-20-37252592; **E-mail**: liuzf@scbg.ac.cn

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ABSTRACT

Irrigation has become one of the main approaches to improve agricultural production in the arid area. The variation of soil moisture after irrigation has the potential to affect soil microbial community composition and soil organic carbon (SOC) storage, thus, the imbalances in the terrestrial ecosystem carbon cycle. However, the impact of long-term irrigation on the relationships between soil microbial community and SOC sequestration in semi-arid agroecosystems is still poorly understood. We took advantage of a 7-year irrigation experiment in a winter wheat-maize rotation system in Northern China, whereby the non-irrigation was subject to rain-fed conditions. We aimed to investigate the effects of long-term irrigation on soil microbial communities and their linkages with soil carbon sequestration. Seven years of irrigation significantly increased soil moisture content by 39% but decreased SOC concentration of topsoil (0-20 cm) by 4.2% on average across all sampling times. The responses of soil microbial communities to irrigation were highly taxa-dependent. Irrigation significantly decreased fungal biomass, fungi to bacteria ratio and Gram-positive to Gram-negative bacteria ratio, and did not affect the bacterial community biomass. The decreased SOC concentration under the long-term irrigation was mainly caused by the changes in the ratio of fungi to bacteria. Our findings highlight the important role of soil fungi: bacteria in mediating the response of SOC dynamics to a future drier climate in semiarid agricultural ecosystems.

KEYWORDS: Gram-positive bacteria, Gram-negative bacteria, Fungi, Soil moisture, Soil microbial community composition, Soil carbon, Semi-arid agricultural systems

HIGHLIGHTS:

• What is the main scientific question that your paper addresses?

How does irrigation affect the soil microbial community and carbon stock in semiarid agroecosystem?

• What is novel, important and timely about your work?

Highlight the role of fungi: bacteria in mediating the response of SOC dynamics to irrigation.

• What are your key findings?

Long-term irrigation decreased the soil carbon content by changing the microbial community.

• What is your main conclusion?

The reduced SOC storage after irrigation was due to the decreased ratio between fungi and bacteria.

1. INTRODUCTION

Long-term sustained severe drought can strongly decrease crop production, and thus affect the food supply and food security (Lesk *et al.*, 2016). The North China Plain is the main grain-producing area and the frequent occurrence of severe drought in China (Zhang *et al.*, 2019). Therefore, intensive irrigation has become the main practice to increase crop yield in this region. Irrigation not only influences soil physical processes (like water infiltration and leaching) but also affects biotic processes (like crop growth) in agroecological ecosystems (Du *et al.*, 2018). However, previous studies mainly focused on the effect of soil moisture variation after irrigation on aboveground carbon (C) processes, like leaf photosynthesis and aboveground net primary productivity (Jafarikouhini *et al.*, 2020; Wang *et al.*, 2021). In contrast, it is unclear the responses of belowground C processes to long-term irrigation, especially the effect of soil microbial community in this process.

The variation of soil moisture after irrigation will affect soil microbial community composition through their effect on microbial physiological tolerance and metabolic capacity (Kaisermann *et al.*, 2013). As a consequence, the adaptive microbial strategy will shift, resulting in changes in the composition and functioning of microbial communities (Evans *et al.*, 2014). Therefore, the variation of soil moisture may not only influence the physical characteristics of the soil and soil nutrient diffusion to microorganisms but also act as an environmental filter shaping the physiological response and composition of soil microbial communities.

Some bacterial strains adjust their osmotic pressure mechanisms in response to rapid changes in soil water potential (Sleator & Hill, 2002). Bacterial communities exhibit a highly conservative recovery strategy dependent on phylogeny and can rapidly synthesize proteins in

111 response to wetting events or seasonal rainfall patterns (Ruehr *et al.*, 2009). For instance,
112 Gram-positive (GP) bacteria generally grow more slowly than Gram-negative (GN) bacteria
113 and are considered *K*-strategists, since they show slower development, larger body size,
114 delayed reproduction, and longer lifespan (Punsalang *et al.*, 1989). A high abundance of GP
115 bacteria under lower moisture is associated with their thicker cell wall and greater drought
116 tolerance (Hueso *et al.*, 2012; Chodak *et al.*, 2015), while GN bacteria are more sensitive to
117 major changes in water potential (Nesci *et al.*, 2004). In addition, the ability of many GP
118 bacteria to sporulate allows them to quick recovery after disturbance(Drenovsky *et al.*, 2010).
119 Therefore, the resistance of the microbial community to disturbance may increase the ratio of
120 GP and GN bacterial biomass or the relative abundance of GP bacteria.
121
122 Another important group among soil microorganisms, soil fungi, are considered more tolerant
123 to the variation of soil moisture than bacteria because extensive fungal hyphal networks allow
124 them to internally transfer moisture and nutrients in a disturbance environment. Fungi generally
125 have a lower nutrient requirement than bacteria (de Boer *et al.*, 2005; Rousk *et al.*, 2010;
126 Strickland & Rousk, 2010). Further, fungal chitinous cell walls make them more resistant and
127 resilient than bacteria to variations in water availability (Guggenberger *et al.*, 1999). In
128 addition, mycorrhizal fungi can obtain assimilation products from plants and in return promote
129 water and nutrient supply since they are directly connected to roots (Jones *et al.*, 2009; de Deyn
130 *et al.*, 2011). Hence, the abundance of fungi tends to be greater than that of bacteria in lower
131 soil moisture, and the fungi-to-bacteria ratio increases when a greater resistance of microbial
132 communities against drought stress is required (Preece *et al.*, 2019). However, the effects of
133 soil moisture on the microbial community composition are inconsistent, and some studies
134 found a relatively higher ratio of fungi and GP bacteria in low soil moisture (Barnard *et al.*,

2013; Fuchslueger *et al.*, 2014; Preece *et al.*, 2019), while others showed limited or almost no influence on the microbial community composition (Rousk *et al.*, 2013; McHugh & Schwartz, 2015; Canarini *et al.*, 2016).

In terrestrial ecosystems, soil microorganisms play an important role in the soil carbon cycle because they can mineralize SOM, and convert soil C into CO_2 then releasing it into the atmosphere (Bardgett *et al.*, 2008; Bruun *et al.*, 2014). The relative contributions of fungal and bacterial functional groups to SOM decomposition differ (Wang *et al.*, 2014). In some cases, the C assimilation efficiency of bacteria is lower than that of fungi, and the fungal storage C is much greater than bacterial storage C (Suberkropp & Weyers, 1996). In addition, fungal-dominated soil microbial communities can improve soil C stability and generate more stable C (Bardgett *et al.*, 2008; Zhang *et al.*, 2019). Therefore, soil moisture can influence the soil C cycle through changes in soil microbial community composition (Borken & Matzner, 2009; Lei *et al.*, 2016; Su *et al.*, 2020).

In this study, we took advantage of a seven-year irrigation experiment in a winter wheat-maize rotation system in Northern China. The annual precipitation during the experimental period varied from 330 mm to 576 mm. A high crop yield in this region is mainly achieved by intensive irrigation (Xiao *et al.*, 2016). The field treatments included irrigation plots and non-irrigation plots (only rain-fed conditions). We aimed to investigate how long-term irrigation affects the soil microbial community composition and the soil C stock in the semiarid agroecosystem. We hypothesized that: 1) long-term irrigation would decrease the ratio of fungi and bacteria, as well as the ratio of GN and GP bacteria; 2) the variation of soil microbial community composition under long-term irrigation would decrease the soil C stock. The findings of this

study will advance our understanding of the relationship between microbial community composition and soil C cycling in future precipitation regimes.

2. MATERIALS AND METHODS

2.1 Study site and experimental design

This study was conducted at the Luancheng Agroecosystem Experimental Station (37°53′N, 114°41′E, 50.1 m a.s.l.), located in the high-yield zone of the North China Plain. The climate of this study area is a semi-arid warm temperate continental monsoon with a mean annual temperature of 12.3°C. About 75% of the annual precipitation occurs in the summer (June - September). The soils are classified as silt loam Haplic Cambisol according to the FAO soil taxonomy system. The basic information of top-soil (0 – 20 cm) were listed as follow: SOM, 12 – 13 g kg^{-1}; total N, 0.7 - 0.8 g kg^{-1}; soil available N, 60 – 80 mg kg^{-1}; soil available P, 15 - 20 mg kg^{-1}; soil available K, 150 - 170 mg kg^{-1}; saturated volumetric water content, 44.1%; water holding capacity, 35.4 %. The cropping system in this area is winter wheat-maize rotation with winter wheat grown from early October to mid-June and maize grown following wheat harvest.

Field irrigation experiments were set up at the research station in 2006 and were maintained since then. A completely randomized block design was used with four plots (5-m in width, 10-m in length and 1.5-m in depth) assigned to an irrigation treatment and three plots assigned to a non-irrigation (rainfed) treatment. The irrigated plots received sufficient water to maintain the soil moisture content of the 1-m soil profile near field capacity (~75% of water-holding capacity). Irrigation timing was mainly determined according to the key growth period of crops and was consistent with local farmers' irrigation practices. The non-irrigated plots were subject

183 to the rain-fed condition, and only received natural precipitation without extra irrigation during
184 the experimental period. Due to an unfortunate event, one of the non-irrigated plots was
185 destroyed, so there were only three replicates of the non-irrigated treatment. To avoid the
186 effects of plants and soil outside the plots, the plots were separated by concrete walls (1500
187 mm deep, 200 mm tall and 245 mm thick) according to Food and Agriculture Organization
188 standards. Based on local historical planting and fertilization regimes, the winter wheat cultivar
189 Kenong 199 was sown in early October at a seed rate of 150 kg ha^{-1} with a 20 cm row space
190 and harvested in mid-June in the following year. 600 kg ha^{-1} of nitrogen and phosphorus
191 compound fertilizer ($(NH_4)_2HPO_4$) and 150 kg ha^{-1} of urea fertilizer ($CO(NH_2)_2$) before wheat
192 sowing. 300 kg ha^{-1} of urea fertilizer at the jointing stage of winter wheat. The maize cultivar
193 Zhengdan 958 was sown at a density of 60,000 plants ha^{-1} in mid-June and harvested in early
194 October. 500 kg ha^{-1} of urea fertilizer in late July during the maize growing season. All field
195 management practices were uniformly applied to the irrigation treatment plots and non-
196 irrigation plots.
197
198 *2.2 Sample collection and analysis*
199 The inner 3 m × 8 m of each plot was used for sampling plant and soil, with the outer 2 m
200 serving as a buffer zone. Soil volumetric moisture content in a 1.8-m soil profile was measured
201 using a neutron probe (aboveground 20 cm and belowground 180 cm, Institute of Hydrology,
202 Wallingford, UK) by 200-mm intervals in the center of the plot every 7-15 days. Daily air
203 temperature and precipitation were monitored automatically by the weather station at the
204 research station. The choice of sampling time is based on the different plant growth periods
205 and included different seasons. The soil sampling time was usually before irrigation or at a
206 sufficiently long time after the last irrigation in order to exclude short-term effects of specific

irrigation events on soil microorganisms. Topsoil (0-200 mm) was sampled from each plot by using soil core (38 mm in diameter). Soil sampling in 2013 focused on the wheat-growing season, including the wheat jointing stage (April 4), grain-filling stage (May 15), seedling emergence stage (October 12) and initial winter stage (November 22). Soil sampling in 2014 focused on maize growth periods, including wheat jointing (April 9), maize seedling emergence (June 12) and maize jointing (July 8). In each plot, at least five soil samples were taken randomly, homogenized, and bulked into one composite sample. Each composite sample was split in two after the surface organic material and visible roots were carefully removed. One-half sample was air-dried for analysis of soil physicochemical properties and the other was stored at -20°C for microbial analysis after sieving to 2 mm.

Soil moisture content in the samples was determined gravimetrically after drying for 24 h at 105°C. Soil pH was determined in a 1: 2.5 ratio of soil and water slurry using a combination glass electrode. Soil organic carbon (SOC) was determined with the $K_2Cr_2O_7$ titration method after digestion (Lu, 1999). Available N was determined with an alkaline hydrolysis diffusion method (Lu, 1999). Available P was extracted with 0.5 M $NaHCO_3$ solution (pH = 8.5) and determined by the Mo-Sb colorimetric method (Lu, 1999). Soil microbial community composition was characterized using phospholipid fatty acids (PLFA) analysis, as described by Frostegård and Bååth (1996) with minor modifications. Briefly, 8 g freeze-dried soil samples were extracted in a chloroform-methanol-phosphate buffer (1:2:0.8 v/v/v), and the extracted lipids were fractionated into neutral lipids, glycolipids and polar lipids on silica acid columns by successive elution with chloroform, acetone, and methanol, respectively. The methanol fraction (containing phospholipids) was subjected to mild alkaline methanolysis to transform the fatty acids into free methyl esters and analyzed on a gas chromatograph (Agilent

231 Technologies 7890B, USA), equipped with a flame ionization detector. Peaks were identified
232 using bacterial fatty acid standards and MIDI peak identification software (MIDI, Inc., Newark,
233 DE, USA). The PLFAs i15:0, a15:0, i16:0, i17:0, a17:0 were used to indicate the Gram-positive
234 (GP) bacteria, and the PLFAs 16:1ω9c, 16:1ω7c, 18:1ω7c, cy17:0, cy19:0 were used to indicate
235 the Gram-negative (GN) bacteria (Frostegård & Bååth, 1996; Zelles, 1999). The sum of PLFA
236 markers chosen to represent GP and GN bacteria was used to determine total bacteria. The
237 PLFA 18:2ω6c was used as an indicator of fungi. The PLFAs were chosen to represent fungi
238 and the sum of the PLFA markers chosen to represent bacteria was used to calculate the fungi
239 to bacteria ratio. All PLFAs were expressed as nmol g^{-1} dry soil.
240
241 *2.3 Statistical analysis*
242 All data were checked for normal distribution and homogeneity of variance before statistical
243 analysis and all values were presented as means ± standard error (SE). The effects of treatment,
244 sampling time and their interactions on soil properties and microbial indices were tested using
245 two-way repeated-measures ANOVA. Multivariate dimensionality reduction analysis was
246 applied to quantify and test the effects of treatment and environmental factors on the variation
247 of soil microbial community composition. All the above analyses were performed in SPSS
248 Statistics 20.0 (IBM, Chicago, IL, USA), with a significantly different set with $P < 0.05$. A
249 structural equation model (SEM) was used to distinguish the direct and indirect influence of
250 soil moisture on SOC content. The fitness of the model was evaluated based on the following
251 criteria: $X/df < 2$, $P > 0.05$, root mean square error of approximation < 0.07 and goodness-of-
252 fit index (GFI) > 0.9 (Hooper *et al.*, 2008). SEM was conducted using the Amos 20.0 software
253 program (Amos Development Corporation, Crawfordville, FL, USA).
254

3. Results

3.1 Effects of irrigation on soil properties

The precipitation at our field station varied over seven experimental years (Fig.1). The precipitation was particularly low in 2010 and 2011, resulting in stronger low moisture in that period (Fig. 1A). Soil water content (SWC) in the non-irrigation treatments was generally lower than in the irrigation over the seven years (Fig. 1B, C).

In this experiment, irrigation significantly increased the soil moisture content by 38% and decreased the SOC concentration by 4.2% ($P < 0.05$; Table 1), while the available soil N and P were not affected by the irrigation ($P > 0.05$; Table 1). All abiotic soil properties measured in this study were strongly affected by sampling time, showing variations among sampling times ($P < 0.05$; Table 1). In addition, soil pH was significantly influenced by both the irrigation and sampling time ($P < 0.05$; Table 1).

3.2 Effects of irrigation on soil microbial community composition

The fungal biomass significantly decreased after irrigation and varied with sampling time ($P < 0.05$; Fig. 2A). Under non-irrigation treatments, the total microbial, bacterial, GP bacterial and GN bacterial biomass all stayed at a similar level as the non-irrigation across sampling times ($P > 0.05$; Fig. 2B, 2C, 2D and 2E). Sampling time significantly affected the biomass of different microbial groups ($P < 0.05$; Fig. 2). The ratio of fungal to bacterial PLFA abundance and the ratio of GP to GN bacterial PLFA abundances decreased by 30% and 10%, respectively, in the irrigation treatment compared with the non-irrigation across sampling time ($P < 0.001$; Fig. 3).

Results from the multivariate dimensionality reduction analysis showed that the relative abundance of PLFA biomarkers distinctly differed between the irrigation and non-irrigation treatments. The effect of soil properties explained 27.7% in axis 1 and 5.4% in axis 2 of the microbial PLFAs biomarker abundances, and soil moisture showed the strongest effect among all soil properties (Fig. 4).

3.3 The effect of irrigation on the relationship between soil microbial community and soil organic carbon content

We used a structural equation model (SEM) to assess the extent of the direct or indirect influence of soil moisture on SOC concentration (Fig. 5). The fitted models met the significance criteria (χ^2/df=0.24, p=0.88). Total microbial biomass, soil physicochemical index (including available N, available P and pH) and the ratio of microbial group changes explained 39% of the variation in SOC concentration. Soil moisture contributed most to the increased SOC concentration by influencing the ratio of soil fungi to bacteria PLFA abundance among all variables, while the ratio of GP to GN bacteria PLFA abundance showed no significant effect on SOC concentration (Fig.5). In addition, soil moisture, soil physicochemical index, and total microbial biomass all showed direct or indirect influences on SOC concentration (Fig. 5).

4. DISCUSSION

In our study area, the rainfall is not sufficient for rapid growth of the vegetation, especially during the dry, windy spring season. Therefore, intensive irrigation regimes have long been imposed to relieve drought stress in agriculture in this region (Xiao & Tao, 2014). Our results

demonstrated that seven-year irrigation strongly influenced soil microbial community composition, and hence affected the soil carbon stock in the semiarid agroecosystem.

4.1 The response of soil microbial community composition to irrigation

Soil microbial community composition can be strongly affected by soil moisture (Frindte *et al.*, 2019). Our results showed that irrigation strongly increased soil moisture during the seven years. Accordingly, we found that irrigation significantly affected the ratios of different microbial groups and the PLFA biomarker abundances. These results support our first hypothesis that the ratio between fungi and bacteria decreased after long-term irrigation, and dependent on sampling time in a semiarid agroecosystem.

Although the total microbial biomass was not affected by irrigation, both fungal biomass and the ratio of fungal to bacterial PLFA abundance was significantly decreased under irrigation. This was consistent with previous studies showing that fungi are more tolerant than bacteria to drought in both laboratory and field experiments (de Vries *et al.*, 2018; Preece *et al.*, 2019). Fungal chitinous cell walls make them more resistant to drought, and their hyphal networks may benefit the growth of fungi and allow them to acquire more water and nutrients over long distances in low-moisture environments (Guggenberger *et al.*, 1999; de Boer *et al.*, 2005). Furthermore, soil moisture can affect both the quantity and composition of root exudates, and these exudates may strongly influence the microbial community via labile C, signaling molecules, and phytohormones (Fuchslueger *et al.*, 2014; de Vries *et al.*, 2020; Lei *et al.*, 2020; Li *et al.*, 2020). Indeed, a field study has demonstrated that low soil moisture content reduces plant-assimilated C acquired by bacteria, but does not influence C transfer to fungi (Fuchslueger *et al.*, 2014).

In contrast to our hypothesis, both GP and GN bacterial biomass did not significantly differ between irrigation and non-irrigation treatments. However, we found that the microbial community differed after irrigation, and the ratio of GP to GN bacterial PLFA abundance in the non-irrigation treatment was higher than that in the irrigation treatment. The lower ratio of GP bacteria to GN bacteria in the irrigation treatment may be caused by differences in cell-wall structure and physiological functioning between the two bacterial groups (Canarini *et al.*, 2016). GP bacteria are generally more resistant to drought due to their cell wall composition and are classified as 'drought-adapted generalists' (Schimel *et al.*, 2007; Manzoni *et al.*, 2012). GP bacteria also a more complex substrates and potentially have a more advanced osmoregulatory strategy than GN bacteria (Harris, 1981). Moreover, the capacity of many GP bacteria to sporulate allows them to withstand a variety of disturbances, including lower soil moisture (Drenovsky *et al.*, 2010). In summary, the above microbial survival strategies offer a possible explanation for the variation in the ratio between different bacterial groups after irrigation. Similar results have been found in other studies showing that variation of soil moisture can strongly influence the bacterial community biomass or diversity (Sheik *et al.*, 2011; Alster *et al.*, 2013; Fuchslueger *et al.*, 2014).

4.2 Irrigation effect on the soil C storage and its linkage with soil microbial community composition

Cycling of soil C strongly depends on soil environmental factors, such as soil water content *(Condron et al., 2014; Lei et al., 2016; Lei et al., 2020).* In our experimental period, the non-irrigation treatment significantly increased SOC concentration compared with the irrigation treatment. Other studies also reported that a low soil moisture content reduces SOM

mineralization and increases soil C content (Borken & Matzner, 2009; Larsen *et al.*, 2011). Low water availability can limit soil organic matter decomposition processes by decreasing microbial activity (Hueso *et al.*, 2012; Zeglin *et al.*, 2013), as well as decreasing the mobility of nutrients and energy sources, in return, resulting in a low substrate supply to microbial decomposers and nutrient limitation (Suseela *et al.*, 2012). In addition, there was more crop biomass in the irrigation treatment than in the non-irrigation treatment. Greater crop biomass would increase the carbon input into the soil from plants (e.g., root residues and exudation). The greater C input from plants would also result in variation of the microbial community, and induce rhizosphere priming, which might increase soil organic matter decomposition rate by 380% (Cheng *et al.*, 2014; Li *et al.*, 2021).

Soil C content is directly and indirectly affected by the soil microbial community dynamics, microbial activity and microbial decomposition (Six *et al.*, 2006). We found that the ratio of soil fungal to bacterial PLFA abundance greatly contributed to the increased SOC concentration based on the SEM. Our result is consistent with reports that the fungal biomass showed a relative increase under low soil moisture content because fungi are better adapted to low-moisture conditions (Bapiri *et al.*, 2010; Barnard *et al.*, 2015; Preece *et al.*, 2019). The variation in soil physical conditions by moisture availability might lead to soil C stabilization when the microbial community shifts towards a fungi-dominated community (Bardgett *et al.*, 2005; Manzoni *et al.*, 2012). The soil fungal communities can sequester more C through a more recalcitrant cell-wall composition and higher carbon-use efficiency than those of bacteria (Bailey *et al.*, 2002; Six *et al.*, 2006). As observed in previous studies, a large population of fungi favors the formation of macroaggregates by the effect of their hyphae aggregating smaller aggregates into bigger ones, in which soil organic matter is more resistant to decomposition

than in microaggregates (Johnson *et al.*, 2016). Thus, soil C sequestration capacity would be more persistent when fungi dominate the microbial community, and the lower fungal biomass and ratio of fungal to bacterial abundance under irrigation would decrease soil C sequestration in the semi-arid agroecosystem in the study area.

The bacterial community is strongly affected by soil water content in terms of their physiological status and decomposition capacity of SOM, and different microbial groups generally show a different response to soil moisture content (Jansson & Hofmockel, 2020). Therefore, the bacterial community composition, e.g., the ratio of GP to GN bacterial abundance, after soil moisture changes influences soil C content. Although there was a lower ratio of GP bacteria and GN bacteria in the non-irrigation than that in the irrigation plots, our results show that the ratio of GP to GN bacterial biomass had no significant effect on the increased SOC concentration according to the SEM. This was probably due to neither the GP nor the GN bacterial biomass being significantly affected by the irrigation treatment. Moreover, the SEM analysis showed that the soil physicochemical index had a significant direct effect on the SOC concentration, but its residual value was high in the model and not caused by irrigation. Our results demonstrate that soil microbes are associated with higher soil C content through changes in microbial community composition in the semi-arid agricultural ecosystems.

5. CONCLUSIONS

Using a seven-year irrigation experiment, we found that irrigation significantly decreased soil C content of the topsoil (0-20 cm). Irrigation also, directly and indirectly, affected soil abiotic properties, influenced the soil microbial community composition, decreasing the fungal community and soil C content in the semi-arid agroecosystem. Overall, our experiment

demonstrates that (1) the responses of soil microbial communities to irrigation were highly taxa-dependent and the groups adapted to low availability of water such as the fungi-dominated group were not favored; (2) irrigation could influence soil C sequestration through its effect on the ratio between bacteria and fungi. These findings allow us to predict the response of soil C content in a semi-arid agroecosystem to future climate change.

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CONFLICT OF INTEREST

The authors declare to have no conflict of interest.

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561
562 **Table 1**. Soil properties in irrigation and non-irrigation plots during different sampling times. The effects of irrigation on soil properties
563 were tested by two-way repeated-measured ANOVA with sampling time and drought treatment as factors. Values represent means ±
564 standard errors.

Treatment	Time	Soil pH	Soil moister (%)	SOC ($g\ kg^{-1}$)	Soil available N ($mg\ kg^{-1}$)	Soil available P ($mg\ kg^{-1}$)
Irrigation	Apr-13	7.8±0.06	8.7±0.2	9.4±0.1	74.9±5.6	55±11
	May-13	8.1±0.03	18.5±0.2	9.1±0.1	72.9±3.4	39±4
	Oct-13	7.8±0.07	15.4±1.5	10.5±0.3	78.8±4.8	94±7
	Nov-13	7.8±0.05	13.1±0.5	9.5±0.4	71.0±2.6	91±6
	Apr-14	7.7±0.05	14.6±0.2	10.3±0.4	78.4±3.5	30±2
	Jun-14	8.1±0.03	5.5±0.2	8.8±0.4	63.2±6.1	43±7
	Jul-14	7.7±0.05	11.2±0.9	10.1±0.2	78.9±4.4	25±3
Non-irrigation	Apr-13	7.8±0.06	6.4±0.5	9.8±0.2	70.5±3.2	48±4
	May-13	8.2±0.03	4.3±0.1	9.8±0.4	66.6±0.8	46±4
	Oct-13	7.8±0.01	14.2±0.6	10.8±0.4	76.6±2.7	89±7
	Nov-13	7.8±0.04	11.0±0.2	10.4±0.2	73.8±3.6	83±6
	Apr-14	7.4±0.06	5.2±0.1	10.5±0.3	88.8±5.4	31±4
	Jun-14	7.8±0.03	4.7±0.3	9.3±0.3	69.4±0.9	42±2
	Jul-14	7.6±0.02	7.6±0.3	10.2±0.2	81.9±7.6	30±6
Treatment Effect	Irrigation	$P<0.01$	$P<0.001$	$P<0.05$	$P>0.05$	$P>0.05$
	Time	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.01$	$P<0.001$
	I×T	$P<0.01$	$P<0.001$	$P>0.05$	$P>0.05$	$P>0.05$

565

566 **Figure Legends**

567 **Figure 1.** (A) Precipitation and irrigation (mm d^{-1}), soil moisture content in (B)
568 irrigation and (C) non-irrigation plots at a different depth from 2007 to 2014.

569

570 **Figure 2.** Fungal (A), bacterial (B), Gram-positive (GP) bacterial (C), total microbial
571 (E) and Gram-negative (GN) bacterial (D) biomass (represented by phospholipid fatty
572 acid abundance) in irrigation and non-irrigation plots across different sampling times.

573

574 **Figure 3.** Fungal: bacterial phospholipid fatty acid (PLFA) ratio (A) and Gram-positive
575 (GP): Gram-negative(GN) bacterial phospholipid fatty acid ratio (B) in irrigation and
576 non-irrigation plots across different sampling times.

577

578 **Figure 4.** Multivariate dimensionality reduction analysis of the irrigation effect on
579 microbial phospholipid fatty acid (PLFA) biomarker abundance across different
580 sampling times.

581

582 **Figure 5.** Structural equation model showing the hypothesized causal relationships
583 among soil moisture, soil physicochemical index (soil availability N, P and soil pH),
584 total microbial biomass, fungi to bacteria ratio and Gram-positive (GP) to Gram-
585 negative (GN) bacteria ratio effect on the soil carbon content. The width of the arrow
586 represents the strength of the standardized path coefficient. Continuous arrows indicate
587 significant relationships, whereas dashed arrows indicate no significant relationship.
588 The e value indicates residual. Standardized total effects (direct plus indirect effects)
589 from the model (right panel) indicate the effect size of the relationship.

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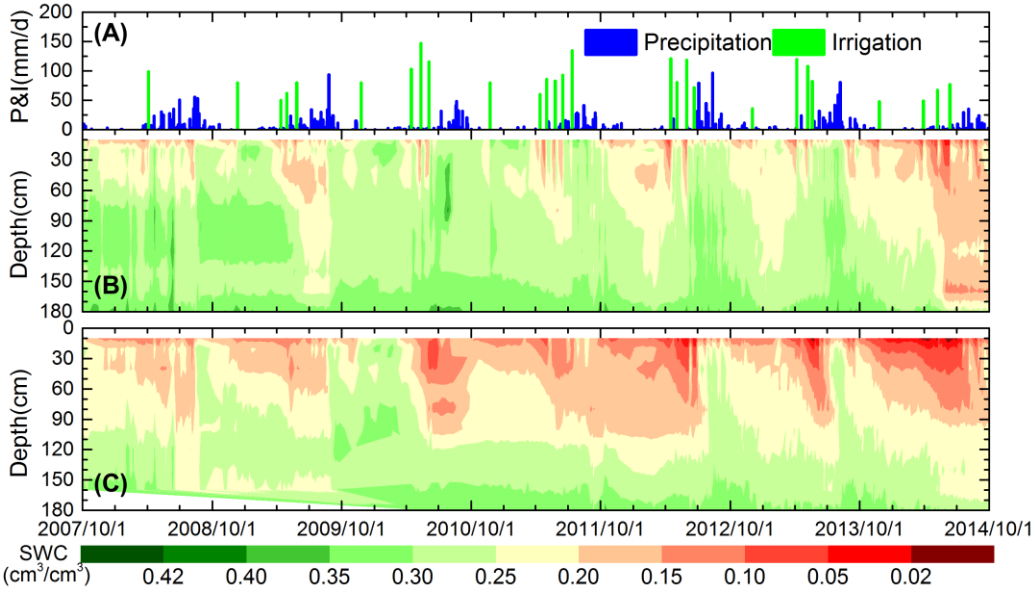

591

592 **Figure 1.**

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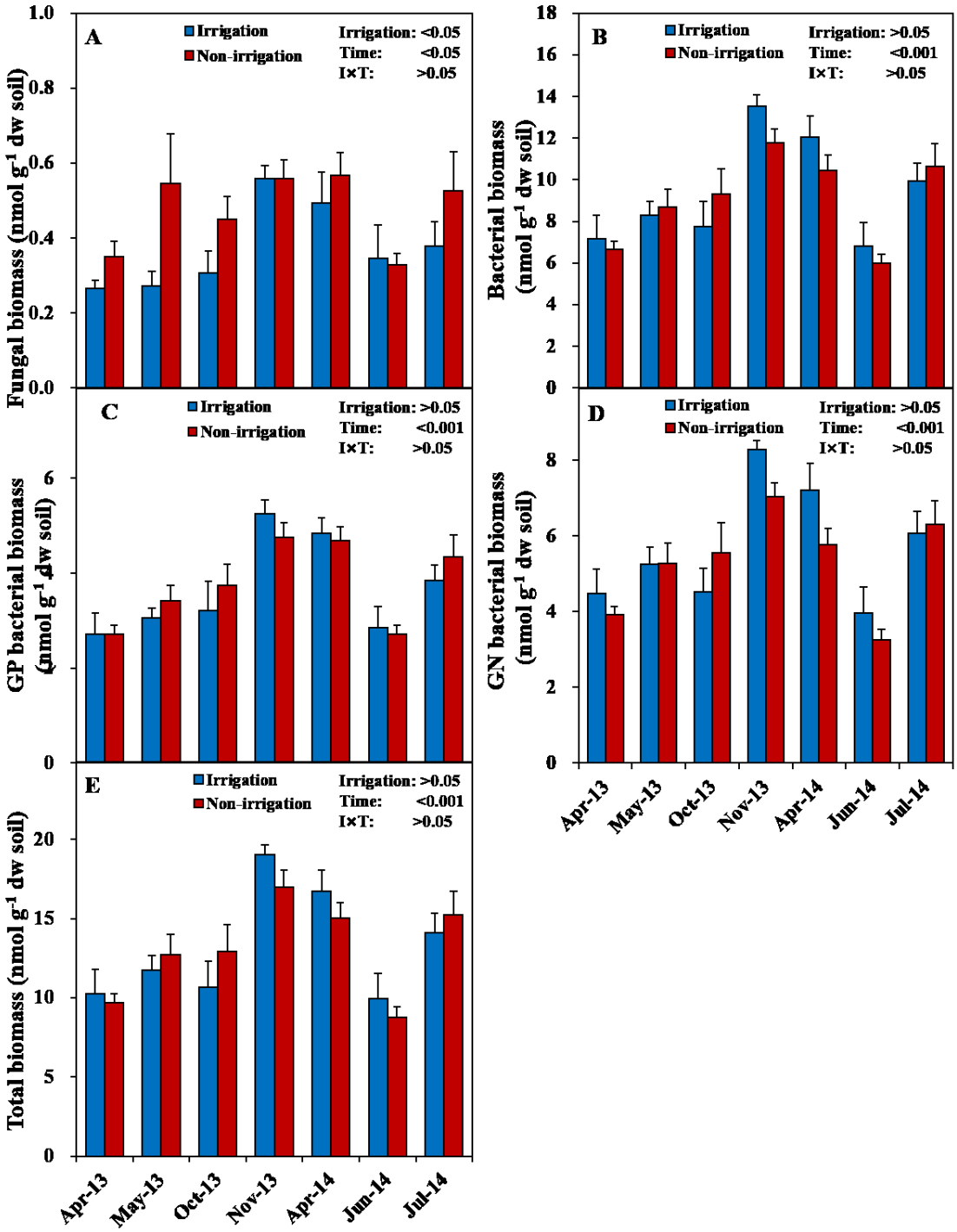

A
Irrigation
Non-irrigation
Irrigation: <0.05
Time: <0.05
I×T: >0.05
Fungal biomass (nmol g-1 dw soil)
B
Irrigation
Non-irrigation
Irrigation: >0.05
Time: <0.001
I×T: >0.05
Bacterial biomass (nmol g-1 dw soil)
C
Irrigation
Non-irrigation
Irrigation: >0.05
Time: <0.001
I×T: >0.05
GP bacterial biomass (nmol g-1 dw soil)
D
Irrigation
Non-irrigation
Irrigation: >0.05
Time: <0.001
I×T: >0.05
GN bacterial biomass (nmol g-1 dw soil)
E
Irrigation
Non-irrigation
Irrigation: >0.05
Time: <0.001
I×T: >0.05
Total biomass (nmol g-1 dw soil)
Apr-13
May-13
Oct-13
Nov-13
Apr-14
Jun-14
Jul-14

Figure 2.

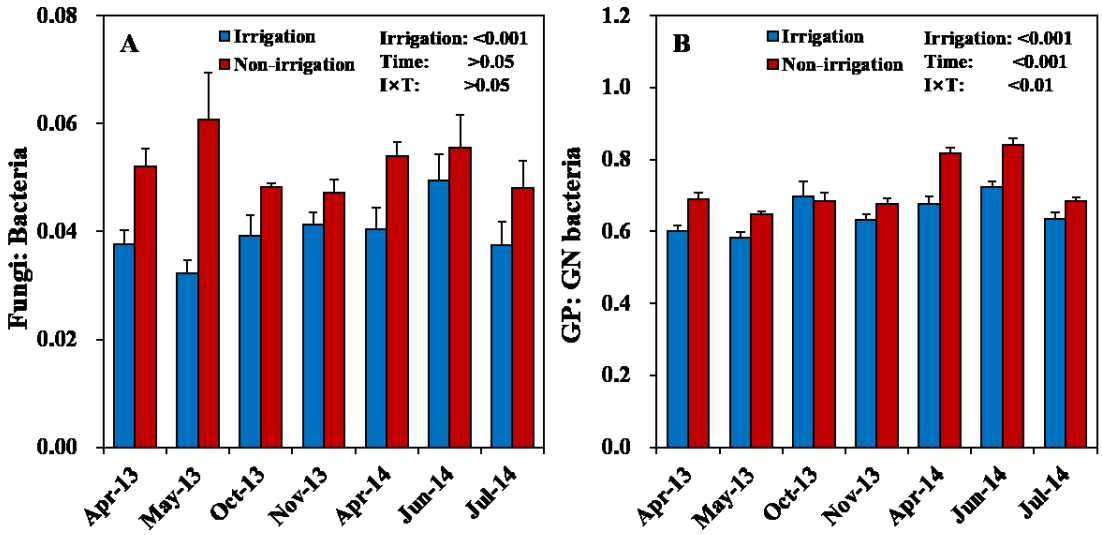

A
Irrigation
Non-irrigation
Irrigation: <0.001
Time: >0.05
I×T: >0.05
Fungi: Bacteria
0.08
0.06
0.04
0.02
0.00
Apr-13
May-13
Oct-13
Nov-13
Apr-14
Jun-14
Jul-14
B
Irrigation
Non-irrigation
Irrigation: <0.001
Time: <0.001
I×T: <0.01
GP: GN bacteria
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Apr-13
May-13
Oct-13
Nov-13
Apr-14
Jun-14
Jul-14

Figure 3.

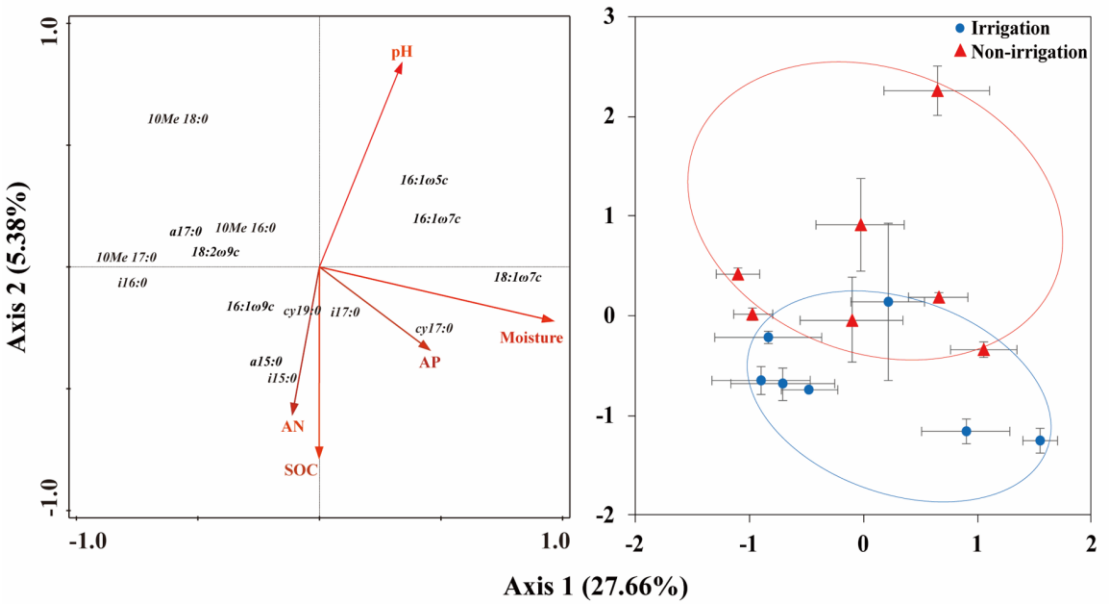
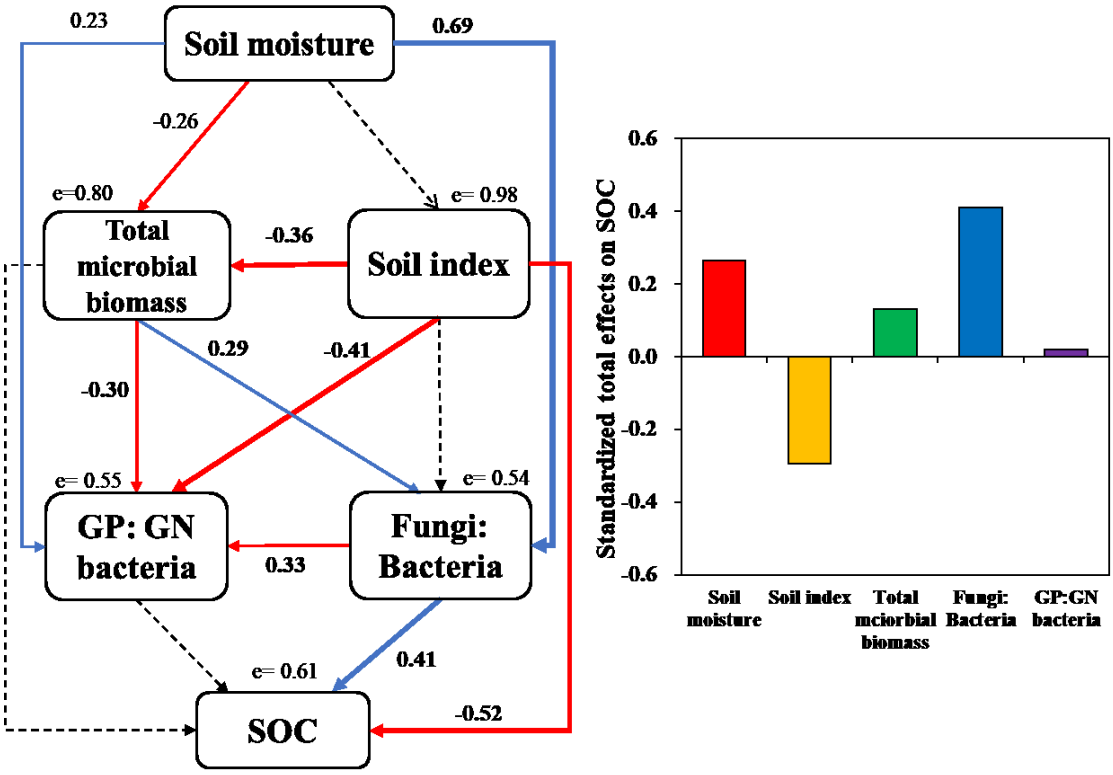

Axis 2 (5.38%)
Axis 1 (27.66%)
1.0
-1.0
3
2
1
0
-1
-2
pH
Moisture
AP
AN
SOC
10Me 18:0
16:1ω5c
16:1ω7c
a17:0
10Me 16:0
18:2ω9c
10Me 17:0
i16:0
18:1ω7c
16:1ω9c
cy19:0
i17:0
cy17:0
a15:0
i15:0
Irrigation
Non-irrigation

Figure 4.



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662 **Figure 5.**