

Article

# Irrigation Combined with Aeration Promoted Soil Respiration through Increasing Soil Microbes, Enzymes, and Crop Growth in Tomato Fields

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**Abstract:** Soil respiration ( $R_s$ ) is one of the major components controlling the carbon budget of terrestrial ecosystems. Aerated irrigation has been proven to increase  $R_s$  compared with the control, but the mechanisms of  $CO_2$  release remain poorly understood. The objective of this study was (1) to test the effects of irrigation, aeration, and their interaction on  $R_s$ , soil physical and biotic properties (soil water-filled pore space, temperature, bacteria, fungi, actinomycetes, microbial biomass carbon, cellulose activity, dehydrogenase activity, root morphology, and dry biomass of tomato), and (2) to assess how soil physical and biotic variables control  $R_s$ . Therefore, three irrigation levels were included (60%, 80%, and 100% of full irrigation). Each irrigation level contained aeration and control. A total of six treatments were included. The results showed that aeration significantly increased total root length, dry biomass of leaf, stem, and fruit compared with the control ( $p < 0.05$ ). The positive effect of irrigation on dry biomass of leaf, fruit, and root was significant ( $p < 0.05$ ). With respect to the control, greater  $R_s$  under aeration (averaging 6.2% increase) was mainly driven by soil water-filled pore space, soil bacteria, and soil fungi. The results of this study are helpful for understanding the mechanisms of soil  $CO_2$  release under aerated subsurface drip irrigation.

**Keywords:** aerated irrigation; soil enzyme activity; soil microbial biomass; soil respiration

## 1. Introduction

Subsurface drip irrigation (SDI) has been largely applied in arid and semi-arid regions to supply water due to greater yield production and water-saving characteristics [1,2]. Nevertheless, a large number of wetting fronts are generated near emitters, producing ethylene and  $CO_2$ , which are harmful for crop growth [3]. Aerated irrigation (AI), a modified irrigation technique that involves injecting air into soils based on SDI, has been extensively proven to improve soil aeration, thus increasing crop yields and fruit quality [4–6]. Even so, the effect of AI on soil environmental pollution is relatively sparse.

Soil respiration, originating primarily from heterotrophic respiration and autotrophic respiration [7,8], is a principal component in the global carbon cycle. A few studies have reported an increase of soil respiration under AI [6,9,10], while the cause of  $CO_2$  release needs to be further explored. Previously, studies on drivers of soil respiration have been largely conducted on soil water content, temperature, and the interaction of these two parameters [9,11–14]. For AI treatment, a close correlation between soil  $CO_2$  fluxes with soil water content and temperature has been confirmed [9,10]. Soil microbes and enzymes as biocatalysts for all biochemical reactions in the soil would decompose and

oxidize soil organic matter [15] and intrinsically affect heterotrophic respiration, while the effect of AI on soil microbes and enzymes has been less tested [16]. Additionally, the properties of root morphology (total length, surface area, and volume) not only determine the ability of water and nutrient absorption but also determine the intensity of autotrophic respiration. Studies of root morphology under AI have been conducted in multiple crops [1,17–19], but the effect of AI on roots of greenhouse vegetables is still scarce. In recent years, researchers began to focus on the effects of soil microorganisms and plant growth on soil respiration [14,20,21]. However, the relationship between soil respiration and biotic components (microbes and plants) under AI remains unknown. Hence, studies of soil physical and biotic properties under AI are of critical significance to improve our mechanistic understanding of processes that release CO<sub>2</sub> to the atmosphere.

To better understand the mechanism of soil respiration change under different irrigation levels with and without aeration, soil respiration from greenhouse tomato fields, as well as soil physical and biotic components (soil water-filled pore space, temperature, abundance of soil bacteria, fungi, and actinomycetes, soil microbial biomass carbon, soil cellulase and dehydrogenase activity, tomato root morphology, and plant dry biomass) were investigated in the present study. We hypothesized that irrigation in combination with AI would increase soil respiration, soil microbes, soil enzyme activity, and plant growth. We also hypothesized that soil respiration would be closely related to soil physical and biotic components. Our results were used to manage irrigation measures under AI for CO<sub>2</sub> mitigation and to reveal the mechanism of soil respiration.

## 2. Results and Discussion

### 2.1. Environmental Variables

#### 2.1.1. Soil Water-Filled Pore Space and Temperature

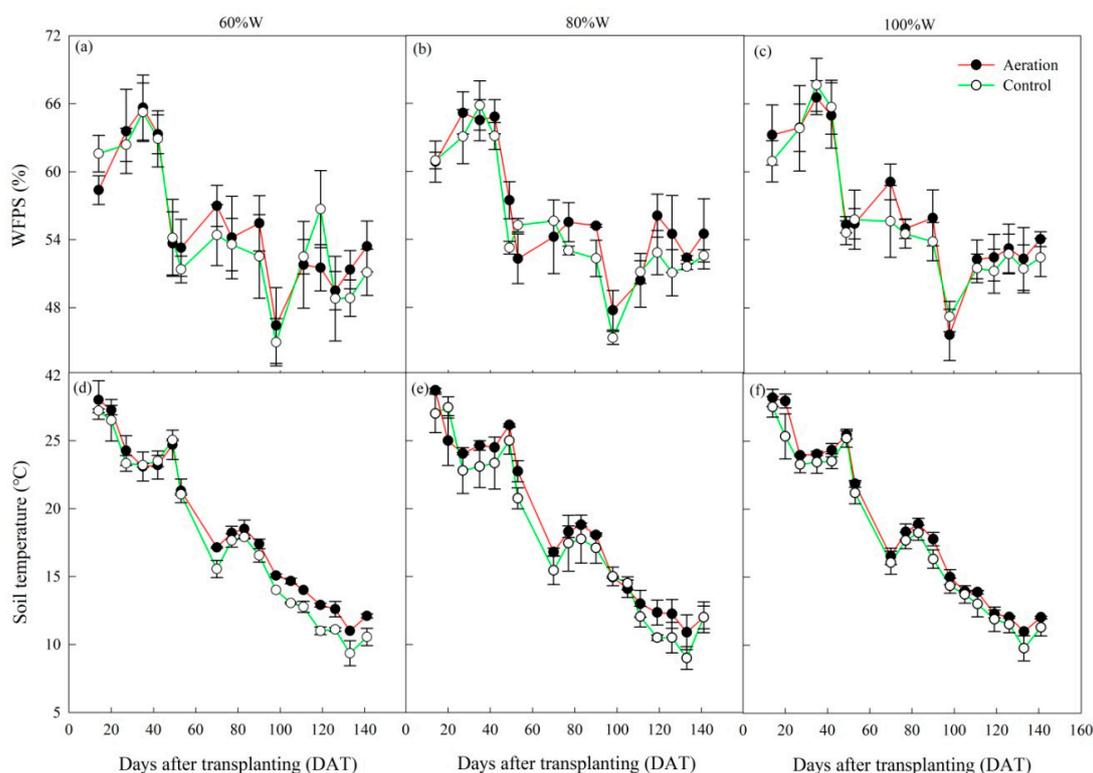
Soil water availability influences organic carbon decomposition, and soil temperature affects microbial growth and activity. They are considered as two major factors driving the variation of soil respiration [14].

A distinct seasonal difference of soil water-filled pore space (WFPS) and soil temperature can be observed (Figure 1). A sharp increase of WFPS occurred before 35 days after transplanting (DAT), and a decrease pattern was shown between 35 to 53 DAT. WFPS presented a total increase then decrease trend from 53 to 98 DAT. There was an upward trend of WFPS since 98 DAT (Figure 1a–c). As for soil temperature, a total decreasing trend was found throughout the whole tomato growing period except for a general increase between 35 to 49 DAT, between 70 to 83 DAT, as well as between 133 to 141 DAT (Figure 1d–f), which coincided with the seasonal patterns of air temperature. WFPS and soil temperature under aeration and high irrigation level were higher than the control and low irrigation level most of the time, which were in accordance with the findings of a previous study [9]. However, analysis of variance indicated that the effects of irrigation, aeration, and their interaction on mean WFPS and soil temperature were not significant (Table 1,  $p > 0.05$ ).

**Table 1.** The effects of irrigation, aeration, and their interaction on mean values of soil water-filled pore space (WFPS), temperature, the abundance of soil bacteria (*cfub*), fungi (*cfuf*) and actinomycetes (*cfua*), soil microbial biomass carbon (MBC), soil cellulase activity (CA), dehydrogenase activity (DHA), and soil respiration (Rs) using a two-way ANOVA.

Factor	Analysis of Variance ( <i>p</i> -Value)								
	WFPS	Temperature	<i>cfub</i>	<i>cfuf</i>	<i>cfua</i>	MBC	CA	DHA	Rs
Irrigation	ns	ns	ns	ns	ns	ns	ns	ns	ns
Aeration	ns	ns	ns	ns	ns	ns	ns	ns	ns
Irrigation × Aeration	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: ns—significance at  $p > 0.05$ .



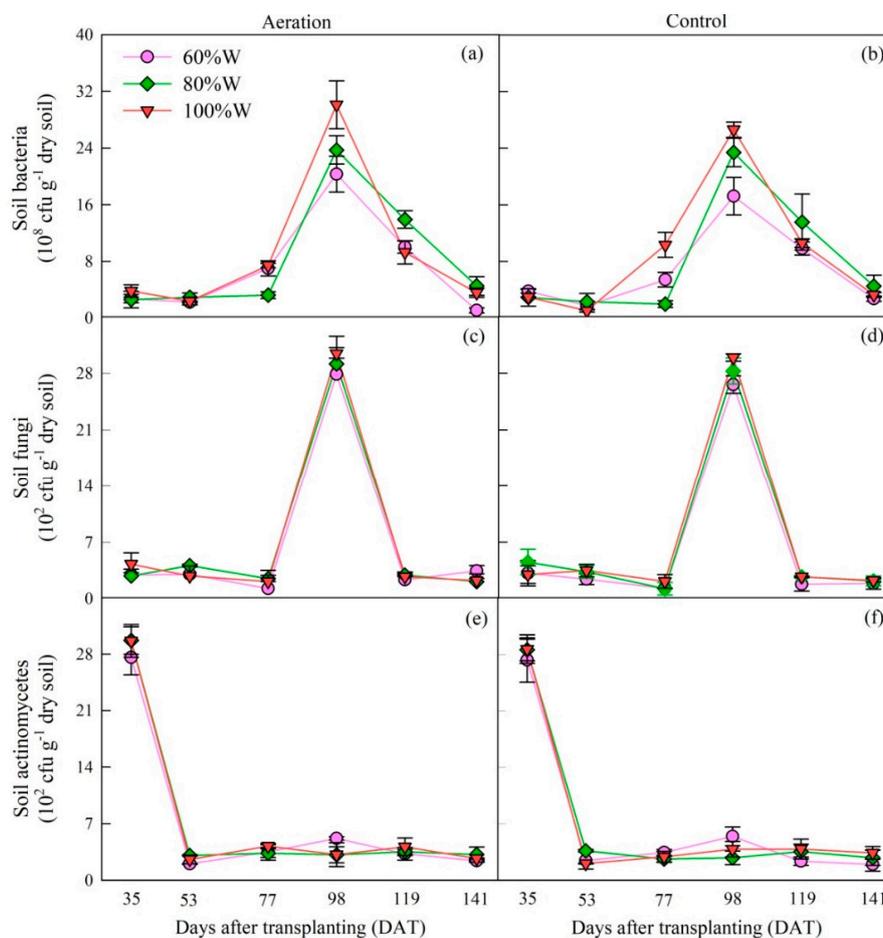
**Figure 1.** Soil water-filled pore space (WFPS) (a,b,c) and soil temperature (d,e,f) with and without aeration under the irrigation level of 60%W (a,d), 80%W (b,e), and 100%W (c,f) (mean  $\pm$  SD,  $n = 3$ ). W refers to full irrigation.

### 2.1.2. Soil Microbe and Enzyme Activity

Heterotrophic respiration, as a primary contributor to the soil respiration, is impacted by soil carbon-use efficiency which varies based on soil microbial abundance and richness [22]. A previous study demonstrated that the abundance of bacteria (*cfub*), fungi (*cfuf*), and actinomycetes (*cfua*) are involved in the soil carbon cycle by decomposing organic matter, degrading cellulose, and forming antibiotic substances [16]. Soil microbial biomass carbon (MBC), which affects the transformation of all organic matter entering the soil, is the key and driving force of the nutrient and energy cycle in the whole ecosystem and is also an important source and reservoir of soil nutrient transformation. Soil cellulose activity (CA), which participates in the decomposition and release of CO<sub>2</sub> from soil organic substances, is the main enzyme activity in soil carbon cycle. Soil dehydrogenase activity (DHA), which catalyzes dehydrogenation of organic substances and plays an intermediate role in hydrogen transformation and transfer, can be used as an indicator of the microbial redox system and is considered to be a global indicator of microbial metabolic activity in soil. However, soil biological activity can be limited by many factors, i.e., soil water and soil aeration conditions [23,24]. Very few pieces of literature were concerned with the soil microbes under AI [23,25], and the effect of AI on MBC, CA, and DHA have rarely been reported. Hence, study of the soil microbe and enzyme activity (*cfub*, *cfuf*, *cfua*, MBC, CA, and DHA) has great significance to reveal the mechanism of CO<sub>2</sub> release under AI.

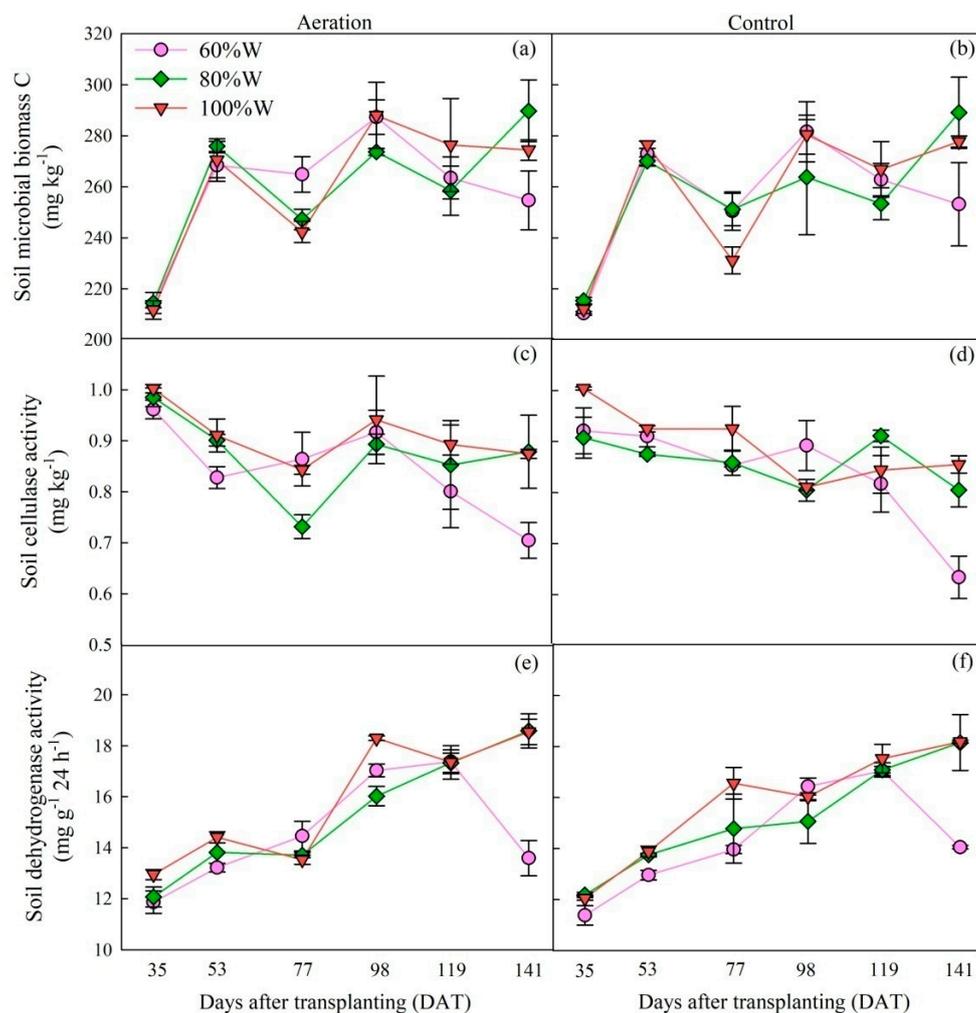
As seen in Figure 2, *cfub* made up the majority of soil microbes, followed by *cfua* and *cfuf*, which was generally supported by the results of Li et al. [16] and Zhu et al. [25]. Nevertheless, the microorganism abundance in the study of Zhu et al. [25] were greater than the values of the current research (Figure 2), which was influenced by higher soil temperature in their study (their study vs. our study = 18–32 °C vs. 9–29 °C). Furthermore, there were different results about the changing trends of soil microbes in the tomato growing period. Zhu et al. [25] pointed out that *cfub*, *cfuf*, and *cfua* integrally presented an increase pattern. Chen et al. [26] concluded that *cfuf* and *cfua* showed an initial increase then decrease

trend, and peaks were observed on 50 d. In our study (Figure 2), the number of *cfub* as a function of the days after transplanting was normally distributed, with the highest values observed on 98 DAT (Figure 2a,b). The number of *cfuf* peaked on 98 DAT, and the values during other periods were relatively stable (Figure 2c,d). The number of *cfua* peaked on 35 DAT, but remained at a relatively constant level during other periods (Figure 2e,f). The differences of changing patterns could have resulted from the combined effects of the availability of different rhizosphere secretions and substrates, changes of soil moisture, temperature, and fertility, as well as plant growth. Soil microbial abundance (especially for *cfub* and *cfuf*, Figure 2) peaked when soil hydrothermal conditions were good (Figure 1) and crops were growing vigorously on 98 DAT. Peaks of *cfua* during the early tomato growing period (on 35 DAT) were probably ascribed to the highest WFPS (64.5%–67.7%) and greater soil temperature (23.1–24.7 °C), as well as greater soil substrates resulted from base fertilizer application [9]. Compared with the control, aeration under each irrigation level slightly increased mean values of *cfub*, *cfuf*, and *cfua* (Table 1,  $p > 0.05$ ), with average increases of 4.6%, 5.5%, and 3.4%, respectively. Similar results were also reported by Li et al. [16], Du et al. [23], and Zhu et al. [25]. The increases of soil microbes under the aeration were likely due to the frequent alternation of soil dry and wet zones, thereby enhancing soil nutrient mineralization to improve microbial growth. Additionally, in line with previous researches [24,25], *cfub*, *cfuf*, and *cfua* in this study increased as irrigation amount increased (Figure 2), which was in order of 60% full irrigation (W) level without aeration (S) ( $W_{0,6S}$ ) < 80%W irrigation level without aeration ( $W_{0,8S}$ ) < 100%W irrigation level without aeration ( $W_{1,0S}$ ). The enhancement of soil microbes under aeration or high irrigation level was also probably ascribed to greater temperature (Figure 1d–f), which stimulated more microbial growth and activity [14].

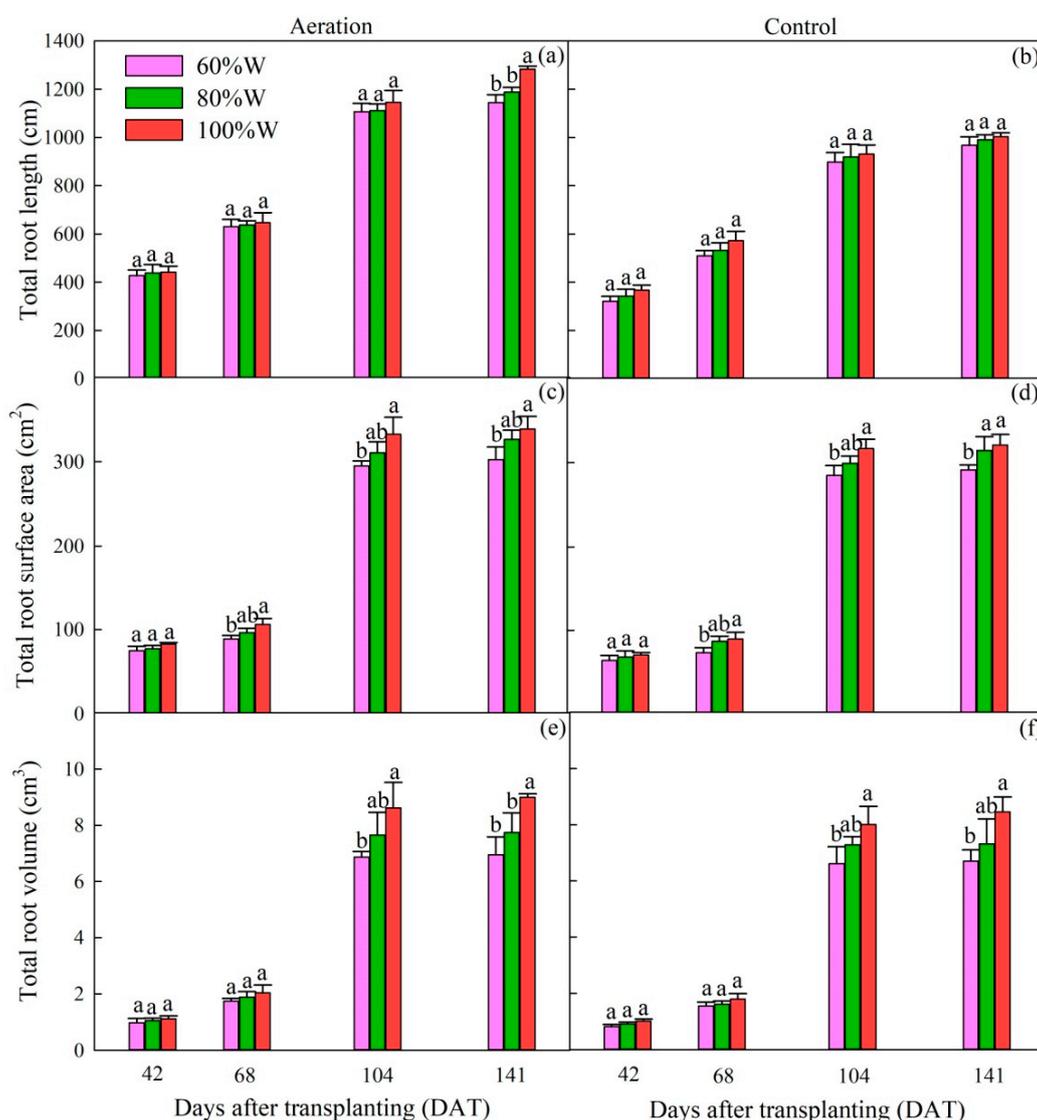


**Figure 2.** The abundance of soil bacteria (a,b), fungi (c,d), and actinomycetes (e,f) with the irrigation level of 60%W, 80%W, and 100% W under the aeration (a,c,e) and control (b,d,f) (mean  $\pm$  SD, n = 3).

MBC generally exhibited an initial increase followed by a volatility within the range of 210.43 to 289.75 mg·kg<sup>-1</sup> throughout the whole tomato growing period (Figure 3a,b). Across all sampling periods, CA among treatments varied from 0.63 to 1.00 mg·kg<sup>-1</sup> and peaked on 35 DAT except for W<sub>0.8</sub>S treatment on 119 DAT (Figure 3c,d). Contrary to the changing rule of CA, DHA generally increased throughout the tomato growing period (Figure 3e,f). The changing patterns of soil enzyme activities were primarily because soil enzymes were correlated with the growth stages, soil texture, soil water content, soil temperature, air availability, and other factors [16]. Compared with the control, mean MBC, CA, and DHA under the aeration were slightly greater (Table 1,  $p > 0.05$ ). As noted by Li et al. [16], soil enzymes are secreted by crop roots and rhizosphere microorganisms, as well as the decomposition of plant residues and microbial cells. Under the aeration, enhanced tomato root (Figure 4) and increased soil microbes (Figure 2) could immobilize and release nutrients into the soil and ameliorate soil fertility [23,27], which ultimately improved the CA and DHA (Figure 3). Additionally, soil water availability affects substrate availability, O<sub>2</sub> concentrations, osmotic potential, gas diffusion, and cellular metabolism [24,28], thus impacting soil microbes. Difference in mean MBC, CA, and DHA values among treatments in this experiment was not significant (Table 1,  $p > 0.05$ ).



**Figure 3.** Soil microbial biomass carbon (a,b), soil cellulase activity (c,d), and soil dehydrogenase activity (e,f) with the irrigation level of 60%W, 80%W, and 100%W under the aeration (a,c,e) and control (b,d,f) (mean  $\pm$  SD,  $n = 3$ ).



**Figure 4.** Total root length (a,b), surface area (c,d), and volume (e,f) with the irrigation level of 60%W, 80%W, and 100%W under the aeration (a,c,e) and control (b,d,f) (mean  $\pm$  SD, n = 3).

In our study, the highest mean values of soil microbe and enzyme activity were obtained when 100%W was applied coupled with AI. This indicated that in a way the effect of irrigation on soil microbe and enzyme activity was enhanced under AI and that the soil biological environment was improved.

### 2.1.3. Root Morphology

The root system plays a decisive role in water and nutrient absorption. The size of crop roots also determines autotrophic respiration. Hence, studies on root morphology are of great practical significance to the study of plant growth and root respiration.

Aeration has been determined to increase root dry biomass and root morphology in cucumber [17,18], soybean [1], and even in the conventional staple grain crop [19]. However, there have been few studies regarding tomato root morphology under AI. Our results showed that total root length, surface area, and volume on 104 and 141 DAT were significantly greater than those on 42 and 68 DAT (Figure 4,  $p < 0.05$ ). Compared with the control, total root length was significantly increased by 22.2% on average under aeration (Table 2,  $p < 0.05$ ). Meanwhile, total root surface area and volume under the aeration was 6.6% and 6.7% higher than that of the control, respectively ( $p > 0.05$ ). Li et al. [18] also showed that root morphology (root length, surface area, and volume) increased with

increasing frequency of aeration. Root length of greenhouse muskmelon was 7076, 5839, 5207, and 3864 cm, and root surface area was 1217, 1023, 998, and 746 cm<sup>2</sup>, while root volume was 31.0, 26.1, 25.7, and 20.1 cm<sup>3</sup> for daily, 2-day, 4-day, and no aeration, respectively ( $p < 0.05$ ) [18]. These increases of root morphology under the aeration were attributed to elongation, branching, and curving, influenced by the shape and dimensions of the wetted soil volume [18]. The injected air changed the soil structure owing to the shrinking and movement of soil particles, and it also pushed the water downwards [29]. All these characters in conjunction with higher soil moisture under aeration (Figure 1) were conducive to elongation of roots due to hydro-tropism. With respect to W<sub>1.0</sub>S, W<sub>0.6</sub>S significantly decreased the total root volume by 18.6% ( $p < 0.05$ ), while the effects of other irrigation levels on root morphology were not significant ( $p > 0.05$ ). Contrary to the results of the current study, Li et al. [18] stated that high irrigation levels has a negative effect on total root length, surface area, and volume with root length of 5981, 5364, and 5145 cm, surface area of 1114, 947, and 927 cm<sup>2</sup>, and volume of 30.8, 22.7, and 23.6 cm<sup>3</sup> for the 70%, 80%, and 90% of field capacity level, respectively. Xu et al. [30] demonstrated that root length and surface area presented an increasing then decreasing trend as soil changes from dry to moist. Differences among literature were likely due to different hydrophily of crops controlled by the genes and tropic response to stimuli [18].

**Table 2.** The effects of irrigation, aeration, and their interaction on mean root morphology and dry biomass using a two-way ANOVA.

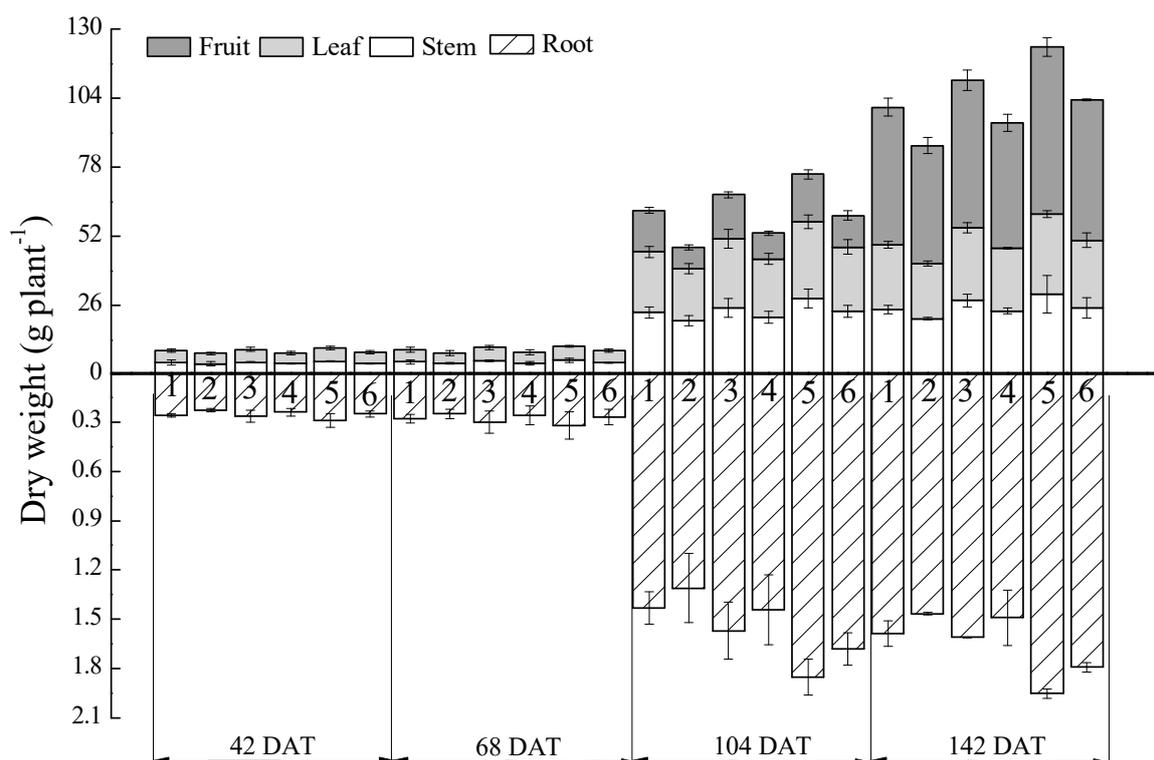
Factor	Analysis of Variance ( <i>p</i> -Value)						
	Root Morphology			Dry Biomass			
	Length	Surface Area	Volume	Leaf	Stem	Fruit	Root
Irrigation	ns	ns	ns	*	ns	**	**
Aeration	*	ns	ns	*	*	*	ns
Irrigation × Aeration	ns	ns	ns	ns	ns	ns	ns

Note: ns, \*, and \*\*—significance at  $p > 0.05$ ,  $p < 0.05$ , and  $p < 0.01$ , respectively.

#### 2.1.4. Dry Biomass

Soil respiration was influenced by not only soil physical environment (i.e., soil temperature and moisture), but also plant growth [14]. Study of dry biomass throughout the whole tomato growing period was an effective way to analyze the changes of soil respiration, especially for the autotrophic component.

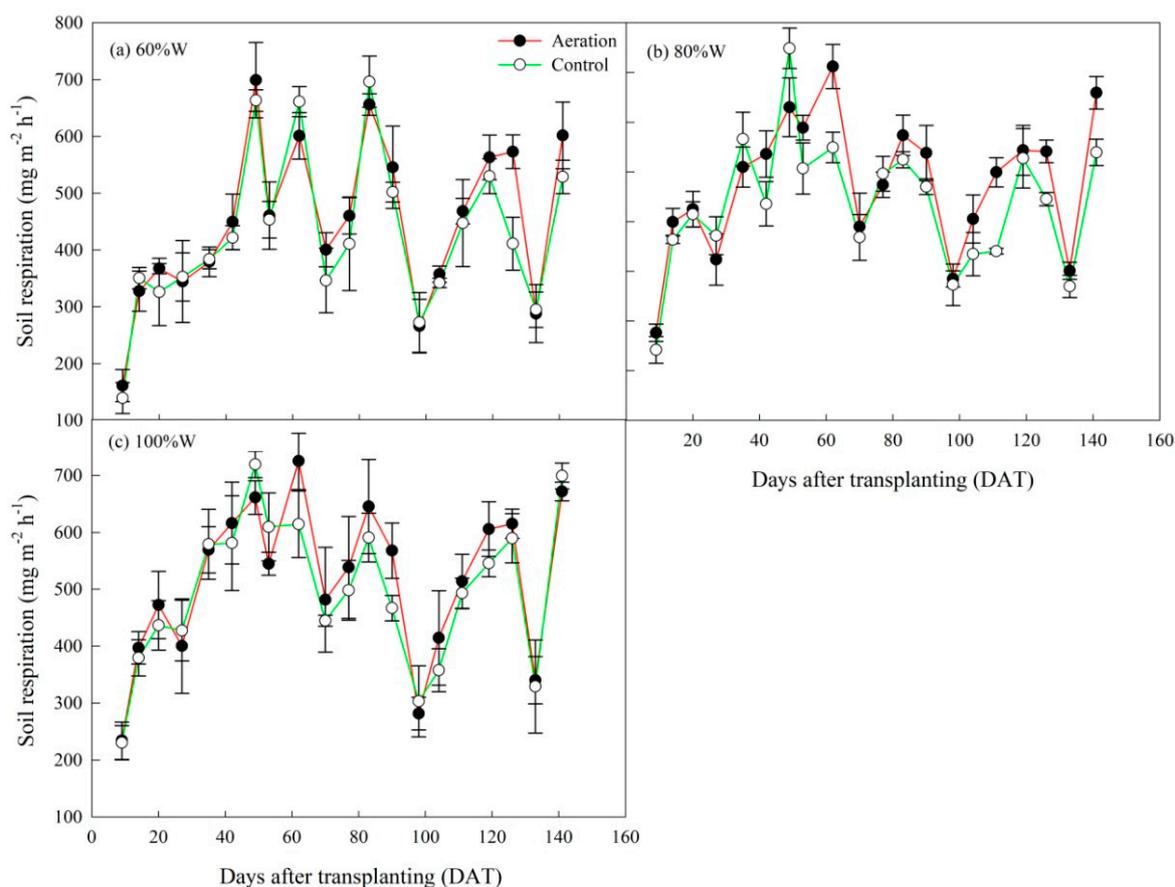
An increasing trend of dry biomass was observed throughout the whole tomato growing period, and dry biomass on 42, 68, 104, and 142 DAT showed a similar changing pattern among treatments (Figure 5). Taking dry biomass at harvest (142 DAT) as an example, dry biomass of tomato leaf, stem, fruit, and root under aeration were higher than the control (Figure 5). As reported previously [31], the average increases of each part were 17.8%, 17.7%, 17.8%, and 8.4%, respectively, and the effect of aeration on leaf, stem, and fruit was significant (Table 2,  $p < 0.05$ ). These improvements of dry biomass were in agreement with the results of former research [4,32], which were beneficial from increased soil aeration and reduced phytohormones under AI [31]. Dry biomass of tomato leaf, stem, fruit, and root increased as irrigation amount increased, and the effect was significant on leaf, fruit, and root (Table 2,  $p < 0.05$ ). As noted previously [31], dry weight of root, stem, leaf, and fruit under 100%W was increased by 22.2%, 19.3%, 22.5%, and 19.0%, and by 20.1%, 5.4%, 7.0%, and 12.1% than that under 60%W and 80%W treatment, respectively. Zhu et al. [4] demonstrated that with crop-pan coefficient increasing from 0.6 to 1.0, dry biomass of root, stem, and leaf was increased by 24.0%, 17.2%, and 22.8%, respectively. The enhancement of dry biomass as irrigation amount increased was primarily ascribed to the greater canopy and leaf area index [4], as well as increased assimilation rate under high irrigation level [33].



**Figure 5.** Dry biomass of tomato fruit, leaf, stem, and root among treatments on 42, 68, 104, and 142 days after transplanting (DAT). The number 1, 2, 3, 4, 5, and 6 represented treatment of 60%W with aeration, 60%W without aeration, 80%W with aeration, 80%W without aeration, 100%W with aeration, and 100%W without aeration, respectively.

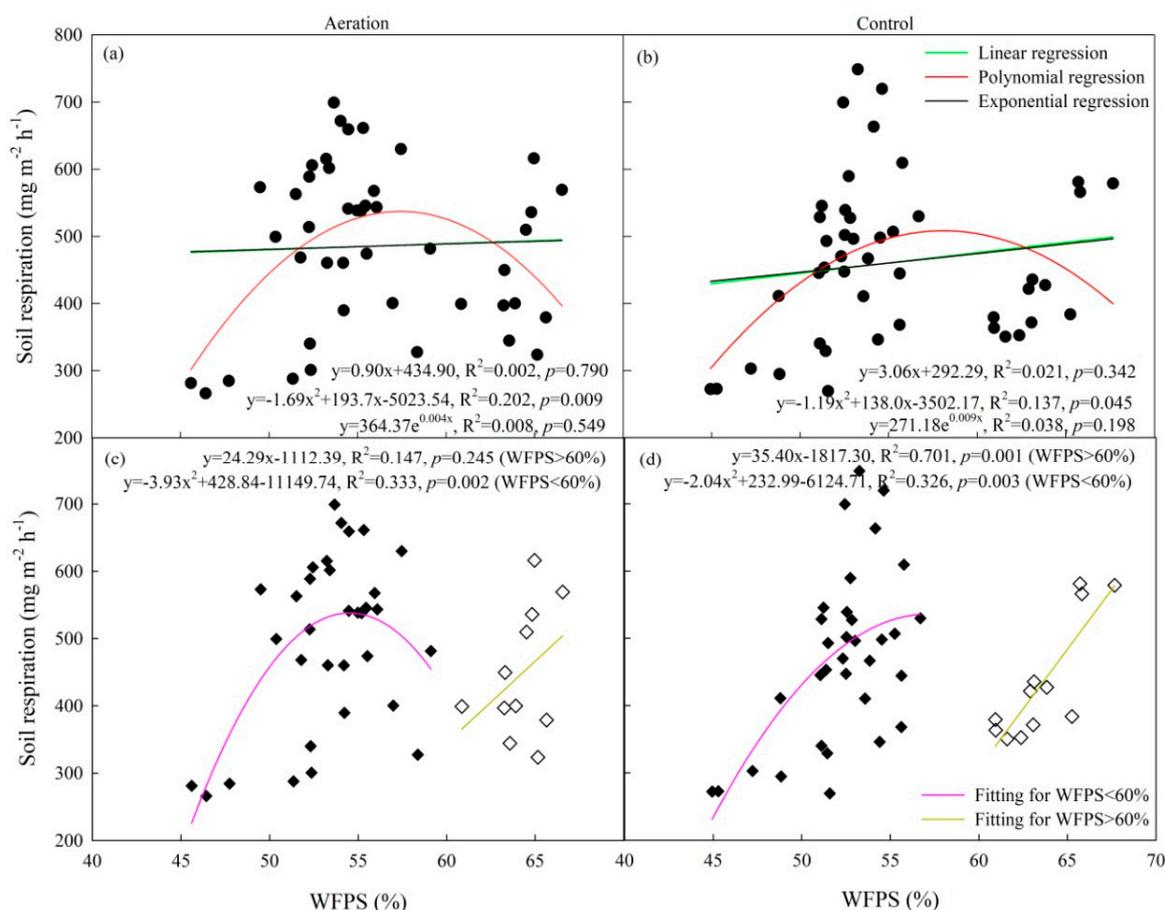
## 2.2. Soil Respiration

As presented in Figure 6, soil respiration showed fluctuated patterns during the whole tomato growing period, which varied from 139.19 to 748.64  $\text{mg}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$  among treatments. Ranges of soil respiration in the present study was similar to the results of Hou et al. [10] but was higher than the research of the same tomato cultivations [9]. Differences might be the results of different irrigation amount and weather condition based on the year of cultivation. The changing patterns of soil respiration could be explained mostly by the abiotic and biotic factors (Figures 1–5). The lowest values on 9 DAT were mainly due to lower soil microbes (especially for *cfub* and *cfuf*, Figure 2) and undeveloped tomato roots (Figures 4 and 5) at the onset of transplantation. As days after transplanting increased, *cfub* and DHA increased gradually (Figures 2 and 3), and the root growth enhanced slightly (Figures 4 and 5), inducing larger emissions on 83 DAT. Relatively lower WFPS and obvious increases of soil temperature on 49 DAT (Figure 1) resulted in the peaks of soil respiration under  $W_{0.6}O$ ,  $W_{0.8}S$ , and  $W_{1.0}S$  treatment. Higher soil respiration on 62 DAT was attributed to increased WFPS, resulting in peaks under  $W_{0.8}O$  and  $W_{1.0}O$  treatment. Lower soil respiration on 98 and 133 DAT was primarily ascribed to a sharp decline of WFPS (Figure 1). An increasing trend of soil respiration was detected since 133 DAT, which was probably due to the increase of WFPS and soil temperature.



**Figure 6.** Soil respiration with and without aeration under the irrigation level of 60%W (a), 80%W (b), and 100%W (c) (mean  $\pm$  SD,  $n = 3$ ).

Previous research has shown a good correlation between soil respiration and soil temperature, oxygen concentration, and air-filled porosity [34]. Nevertheless, the correlation between soil respiration and soil microbe and enzyme activity, as well as plant growth under the aeration and irrigation treatments has not yet been well studied. In our study, regression analysis (linear, polynomial, and exponential) between soil respiration and WFPS was conducted, and a significant polynomial function was observed (Figure 7a,b,  $p < 0.05$ ), similar to previous studies [11,12]. Further analysis found that a polynomial correlation was detected between soil respiration and WFPS when WFPS was below 60% ( $p < 0.01$ ), while a linear positive correlation was observed when WFPS was above 60% (Figure 7c,d,  $p = 0.245$  and  $0.001$  for the aeration and control, respectively). Moreover, there were significant negative correlations between soil respiration and *cfub*, as well as between soil respiration and *cfuf* (Table 3,  $p < 0.01$ ), which was different from the result of Zhu et al. [25] where soil respiration showed strong positive correlations with *cfub*, *cfuf*, and *cfua*. The reason for the inconsistent conclusions was probably due to the different growing seasons. Zhu et al. [25] conducted the experiment in the spring–summer period where the weather was gradually raised, while the present experiment was finished in the autumn–winter period where the weather was gradually reduced. Different variation of soil temperature would lead to different changing rules of soil respiration, microbial activity, and water content. In the present study, the interactive effect of WFPS, *cfub*, and *cfuf* on soil respiration was extremely significant (Table 3,  $p < 0.01$ ), which collectively accounted for 70.2% and 61.6% of changes in soil respiration under aeration and control, respectively. Unfortunately, correlations between soil respiration and other soil physical and biotic components (soil temperature, *cfua*, MBC, CA, DHA, tomato root morphology, and plant dry biomass) were not significant ( $p > 0.05$ , data not shown), which required further study.



**Figure 7.** Correlation between soil respiration with soil water-filled pore space (WFPS) for the all WFPS data (a,b) and data with WFPS piecewise analysis (c,d). (a)(c) and (b)(d) represented correlation under the aeration and control, respectively.

**Table 3.** Relationships of soil respiration (Rs) with soil water-filled pore space (WFPS), the abundance of soil bacteria (*cfub*) and fungi (*cfuf*) under the aeration and control treatments.

Treatment	Model	R <sup>2</sup>	p
Aeration	$Rs = -10.579cfub + 588.685$	0.503	0.001
	$Rs = -9.997cfuf + 571.845$	0.660	0.000
	$Rs = 0.257WFPS^2 - 36.187WFPS - 0.943cfub - 12.586cfuf + 1799$	0.702	0.001
Control	$Rs = -10.576cfub + 569.316$	0.491	0.001
	$Rs = -9.142cfuf + 546.848$	0.608	0.000
	$Rs = -0.351WFPS^2 + 41.536WFPS - 2.142cfub - 5.858cfuf - 664.275$	0.616	0.003

Similar to previous results [6,9,10,34], soil respiration under the aeration in our study was typically and on average 6.2% greater but no significant different to that under the control according to ANOVA (Figure 6, Table 1,  $p > 0.05$ ). Chen et al. [6] found that soil respiration increased by 42%–100% for oxygenation compared to control. Hou et al. [10] stated that aeration increased soil CO<sub>2</sub> emissions by 11.8% ( $p = 0.394$ ) compared to the control. Zhu et al. [34] revealed that mean soil respiration under the aeration was 22.5% higher than the control. Potential reasons explaining the enhancement of soil respiration under aeration include: (1) aeration increased soil microbes (Figure 2,3) and root growth (Figures 4 and 5), which essentially controlled heterotrophic respiration and autotrophic respiration [22,35]; (2) greater CA and DHA under AI (Figure 3), which were involved in the decomposition and release of CO<sub>2</sub> from soil organic substances, in turn promoted soil respiration; and (3) as a result of the enhanced aboveground dry biomass under AI, the increased demand for

nutrients stimulated belowground C allocation and root growth (Figures 4 and 5), which increased substrates to soil organisms and stimulated organic matter turnover [36,37], leading to higher biomass and/or activity that might stimulate the decomposition of soil organic matter [38,39]. All of these factors increased soil respiration conclusively. Consistent with previous research [9,40], soil respiration increased in the order of  $W_{0.6}S < W_{0.8}S < W_{1.0}S$  (Figure 6), which resulted from increased soil microbial biomass (Figure 2), soil enzyme activity (Figure 3), root biomass (Figure 5), mineralization and decomposition rate of soil organic matter, as well as the diffusion rate of gases in soil pores [41]. Soil respiration under  $W_{1.0}S$  was 16.0% and 13.9% higher than that under  $W_{0.6}S$  and  $W_{0.8}S$ , respectively. Nevertheless, the effect of irrigation on soil respiration was not significant (Table 1,  $p > 0.05$ ).

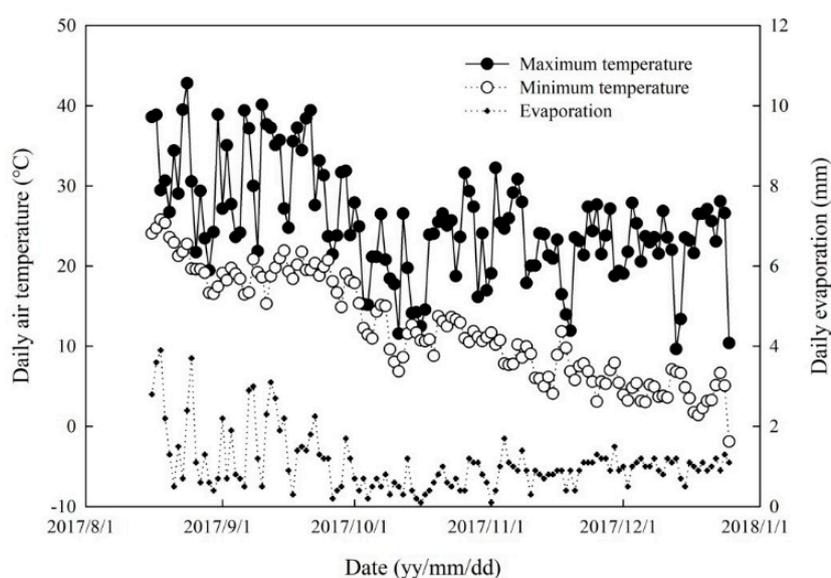
Although this paper analyzed the response of soil respiration to soil physical and biotic variables, we do not know the proportion of root or microbial respiration to soil respiration as no measurement was made in this study, which was the deficiency of this study, and needs to be further carried out in future experiments.

### 3. Materials and Methods

#### 3.1. Experimental Site

The experiment was conducted from August to December 2017 in a solar greenhouse located at 34°20' N, 108°04' E, at the Key Laboratory of Agricultural Soil and Water Engineering in Arid and Semi-Arid Areas of the Ministry of Education, Northwest A and F University, Yangling, Shaanxi Province, China. The site is in a semi-arid climate zone with an annual mean sunshine duration of 2163.8 h and frost-free period of 210 days. Lou soil was used in the experimental site. The texture was a silt clay loam (sand 26.0%; silt 33.0%; clay 41.0%). Soil properties of the top 20 cm were: field capacity 23.8% by weight; dry bulk density  $1.35 \text{ g}\cdot\text{cm}^{-3}$ ; organic matter  $14.62 \text{ g}\cdot\text{kg}^{-1}$ ; total N  $1.88 \text{ g}\cdot\text{kg}^{-1}$ ; total P  $1.37 \text{ g}\cdot\text{kg}^{-1}$ ; total K  $20.21 \text{ g}\cdot\text{kg}^{-1}$ ; and pH 7.82.

Daily maximum and minimum temperatures inside the greenhouse during the experimental period, collected by an Automatic Meteorological Observing Station (Hobo event logger, Onset Computer Corporation, Bourne, MA, USA), are shown in Figure 8. The weather station, which was placed 2 m above the ground, recorded meteorological data at an interval of 15 min [31]. Higher temperatures were observed in August, while lower temperatures were recorded in December (Figure 8).



**Figure 8.** Daily evaporation, maximum and minimum temperature during the growing season at the experimental site.

### 3.2. Experimental Design

Based on the irrigation amount of full irrigation ( $W$ ) calculated as Equation (1), three irrigation levels were set: 60%, 80%, and 100% of  $W$ . Non-aerated SDI ( $S$ ) was used as a control for aeration ( $O$ ). Therefore, six treatments were designed ( $W_{0.6}O$ ,  $W_{0.6}S$ ,  $W_{0.8}O$ ,  $W_{0.8}S$ ,  $W_{1.0}O$ , and  $W_{1.0}S$ ). Three replicates of each treatment were used (18 total plots), and the experiment was arranged using a randomized block [31]. Each plot with one row was  $4 \times 0.8$  m in size, with eleven tomato plants of cultivar “JINGPENG SEED” planted on 6 August 2017. The plants were spaced 35 cm apart. All plots were mulched with a layer of low-density polyethylene to minimize surface evaporation [42]. SDI was applied in the experiment, which was buried at a depth of 15 cm below the soil surface with a dripper interval of 35 cm [9,10]. Additionally, a Mazzei air injector Model 287 (Mazzei Injector Company, LLC, Bakersfield, CA, USA) was installed at the head of each irrigation line for AI (inlet pressure: 0.1 MPa; outlet pressure: 0.02 MPa) [42]. Definitively, the air injectors were set to inject 17% volumetric air concentration in the water [25].

Daily evaporation measured by an E601 evaporation pan is shown in Figure 8. In all growth stages, 20 irrigation events were applied every seven days, with a total irrigation amount for  $W$  of 19.80 L per plot [31]. Irrigation amount was determined following the Equation (1) [9,10,42]:

$$W = k_{cp} \times E_{pan} \times A \quad (1)$$

where  $k_{cp}$  is the crop-pan coefficient, being 1.0;  $E_{pan}$  is the total evaporation quantity following the last irrigation event (mm); and  $A$  is the area controlled by one irrigation dripper in this experiment, being  $0.14 \text{ m}^2$  ( $0.35 \text{ m} \times 0.4 \text{ m}$ ).

Only basal fertilizer, including organic fertilizer ( $\text{N-P}_2\text{O}_5\text{-K}_2\text{O} \geq 10\%$ , organic matter  $\geq 45\%$ ) and compound fertilizer (total nutrients  $\geq 45\%$ , N,  $\text{P}_2\text{O}_5$  and  $\text{K}_2\text{O}$  each at 15%), was applied for all plots. The application was achieved at a rate of 1875 and 1250  $\text{kg}\cdot\text{ha}^{-1}$  on 3 August 2017 for organic and compound fertilizer, respectively. Other agronomic managements were consistent with local production practices [42]. The experiment ended on 25 December 2017 with a total growth period of 142 days.

### 3.3. Measurement Index and Methods

Soil samples from 0 to 10 cm depth were collected when gas samples were collected except on 9, 20, 62, 83, and 104 DAT. Soil samples were taken through a diameter gauge with the three-point sampling method to measure soil water content via oven drying at  $105 \text{ }^\circ\text{C}$  for 12 h, and then converted to WFPS by the following equation:

$$\text{WFPS}(\%) = \frac{\text{gravimetric water content}}{\text{total soil porosity}} \times \text{soil bulk density} \times 100 \quad (2)$$

where total soil porosity =  $1 - \text{soil bulk density}/2.65$ , with  $1.35 \text{ g}\cdot\text{cm}^{-3}$  as the assumed particle density of the soil.

Soil temperature at a depth of 10 cm was recorded using a geothermometer (RM-004, Hengshui, China) when gas samples were collected, excluding on 9 and 62 DAT.

Soil samples of top-soil (0–20 cm) were collected to measure soil microbe and enzyme activity on 35, 53, 77, 98, 119, and 141 DAT. The  $cfub$ ,  $cfuf$ , and  $cfua$  were estimated using the plate dilution counting method in beef extract and peptone medium, Martin’s medium, and the improved Gao’s No. 1 medium, respectively. Media plates were incubated at 37 and  $25 \text{ }^\circ\text{C}$ , and the number of colonies after approximately 3 to 5 d was counted [23]. MBC was measured by the chloroform fumigation– $\text{K}_2\text{SO}_4$  extraction method. MBC in the extracts was determined by the  $\text{K}_2\text{Cr}_2\text{O}_7\text{-FeSO}_4$  additional heating method. Detailed measurement steps regarding CA and DHA are described by Хазиев[43].

On 42, 68, 104, and 142 DAT, one plant from per plot was sampled to measure dry biomass and root morphology (total root length, surface area, and volume). All plant samples were first separated into leaves, stems, fruits, and roots. The roots collected from soil by digging were gently washed, scanned (Epson Perfection V700 photo, Seiko Epson Corp., Nagano-ken, Japan) to obtain a gray-scale JPG image, and then analyzed with the WinRHIZO Pro image processing system (Regent Instrument Inc., 2672 Chemin Sainte-Foy, Quebec City, Quebec G1V 1V4, Canada) to obtain root morphology [18]. After that, every part of the tomato plant including root was put into a 105 °C oven for 1 h to deactivate enzymes and then dried at 75 °C until the parts reached a constant weight. The dry biomass of each part was weighed on an electronic scale [31].

Gas samples of soil respiration was measured using the static closed chamber method described by Hou et al. [10]. All chambers, which were made of polyvinyl chloride (PVC) materials and wrapped with sponge and aluminum foil, were 25 × 25 × 25 cm in dimension. The bases of the chambers were installed between two plants in the middle of each plot on the day of transplanting and remained there until the end of the experiment. There was a 3-cm-deep groove on the top edge of the bottom layer and on the base of the chamber that was filled with water to seal the rim of the chamber. A mercury thermometer (WNG-01, Hengshui, China) at the top of each chamber was equipped to measure air temperature when gas sampling for calculating gas emission flux. Gas samples at an average interval of seven days were collected at 10:00, 10:10, 10:20 and 10:30 a.m. of each sampling time. A 30-mL air sample was drawn each time with a syringe. Gas samples in the syringes were analyzed for CO<sub>2</sub> concentrations using a gas chromatograph (7890A GC System, Agilent Technologies, Santa Clara, USA) within a few hours. Sample sets were discarded unless they yielded an R<sup>2</sup> linear regression value higher than 0.90. Then, soil CO<sub>2</sub> fluxes (soil respiration), which were the sum of autotrophic and heterotrophic respiration, were calculated following the equation given by Hou et al. [10]:

$$F = \rho \cdot h \cdot \frac{273}{273 + T} \cdot \frac{dc}{dt} \quad (3)$$

where  $F$  is the soil respiration ( $\text{mg} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ );  $\rho$  is the gas density at standard state ( $1.964 \text{ kg} \cdot \text{m}^{-3}$ );  $h$  is the height of chamber above the water surface (m);  $\frac{dc}{dt}$  is the gas mixing ratio concentration ( $\mu\text{L} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ ); and  $T$  is the mean air temperature inside the chamber during sampling (°C).

### 3.4. Statistical Analysis

A two-way analysis of variance (ANOVA) followed by an LSD test (95% confidence level,  $p < 0.05$ ) was used to test for the effects of irrigation, aeration, and their interaction on soil respiration, soil physical and biotic properties (WFPS, temperature,  $cfub$ ,  $cfuf$ ,  $cfua$ , MBC, CA, DHA, root morphology, dry biomass). Regression analysis of soil respiration with soil physical and biotic variables was conducted. All statistical and regression analysis were performed using the software SPSS Statistics 22.0 (SPSS Inc., Chicago, IL, USA), and figures were generated using SigmaPlot 12.5 (Systat Software, Inc., Chicago, IL, USA).

## 4. Conclusions

This study investigated the variation of soil respiration and its influencing factors under different irrigation levels with and without aeration in a greenhouse tomato system. Aeration had a significant effect on tomato root length, as well as dry biomass of leaf, stem, and fruit, while no significant differences on other parameters were observed. As irrigation amount applied into soils increased, soil respiration increased in conjunction with its influencing factors, and the effect was significant on the dry biomass of leaf, fruit, and root. Soil respiration was significantly correlated with soil water-filled pore space, the abundance of soil bacteria and fungi. These results indicate that irrigation combined with aeration would increase soil physical and biotic variables, which stimulate more CO<sub>2</sub> release. The application of reduced irrigation and aeration has potential for alleviating CO<sub>2</sub> emissions.

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## References

1. Bhattarai, S.P.; Midmore, D.J.; Pendergast, L. Yield, water-use efficiencies and root distribution of soybean, chickpea and pumpkin under different subsurface drip irrigation depths and oxygation treatments in vertisols. *Irrig. Sci.* **2008**, *26*, 439–450. [[CrossRef](#)]
2. Mo, Y.; Li, G.; Wang, D. A sowing method for subsurface drip irrigation that increases the emergence rate, yield, and water use efficiency in spring corn. *Agric. Water Manag.* **2017**, *179*, 288–295. [[CrossRef](#)]
3. Růžička, K.; Ljung, K.; Vanneste, S.; Podhorská, R.; Beeckman, T.; Friml, J.; Benková, E. Ethylene Regulates Root Growth through Effects on Auxin Biosynthesis and Transport-Dependent Auxin Distribution. *Plant Cell* **2007**, *19*, 2197–2212. [[CrossRef](#)] [[PubMed](#)]
4. Zhu, Y.; Cai, H.J.; Song, L.B.; Chen, H. Impacts of oxygation on plant growth, yield and fruit quality of tomato. *Trans. Chin. Soc. Agric. Mach.* **2017**, *48*, 199–211. (In Chinese)
5. Bhattarai, S.P.; Pendergast, L.; Midmore, D.J. Root aeration improves yield and water use efficiency of tomato in heavy clay and saline soils. *Sci. Hortic.* **2006**, *108*, 278–288. [[CrossRef](#)]
6. Dhungel, J.; Torabi, M.; Pendergast, L.; Chen, X.; Bhattarai, S.P.; Midmore, D.J. Impact of oxygation on soil respiration, yield and water use efficiency of three crop species. *J. Plant Ecol.* **2010**, *4*, 236–248.
7. Hanson, P.J.; Edwards, N.; Garten, C.; Andrews, J. Separating root and soil microbial contributions to soil respiration: A review of methods and observations. *Biogeochemistry* **2000**, *48*, 115–146. [[CrossRef](#)]
8. Kuzyakov, Y. Sources of CO<sub>2</sub> efflux from soil and review of partitioning methods. *Soil Biol. Biochem.* **2006**, *38*, 425–448. [[CrossRef](#)]
9. Chen, H.; Hou, H.-J.; Wang, X.-Y.; Zhu, Y.; Saddique, Q.; Wang, Y.-F.; Cai, H. The effects of aeration and irrigation regimes on soil CO<sub>2</sub> and N<sub>2</sub>O emissions in a greenhouse tomato production system. *J. Integr. Agric.* **2018**, *17*, 449–460. [[CrossRef](#)]
10. Hou, H.; Chen, H.; Cai, H.; Yang, F.; Li, D.; Wang, F. CO<sub>2</sub> and N<sub>2</sub>O emissions from Lou soils of greenhouse tomato fields under aerated irrigation. *Atmos. Environ.* **2016**, *132*, 69–76. [[CrossRef](#)]
11. Qi, Y.; Xu, M. Separating the effects of moisture and temperature on soil CO<sub>2</sub> efflux in a coniferous forest in the Sierra Nevada mountains. *Plant Soil* **2001**, *237*, 15–23. [[CrossRef](#)]
12. Shi, W.-Y.; Du, S.; Morina, J.C.; Guan, J.-H.; Wang, K.-B.; Ma, M.-G.; Yamanaka, N.; Tateno, R. Physical and biogeochemical controls on soil respiration along a topographical gradient in a semiarid forest. *Agric. For. Meteorol.* **2017**, *247*, 1–11. [[CrossRef](#)]
13. Jia, S.; Wang, Z.; Li, X.; Sun, Y.; Zhang, X.; Liang, A. N fertilization affects on soil respiration, microbial biomass and root respiration in *Larix gmelinii* and *Fraxinus mandshurica* plantations in China. *Plant Soil* **2010**, *333*, 325–336. [[CrossRef](#)]
14. Yu, C.-L.; Hui, D.; Deng, Q.; Dzantor, E.K.; Fay, P.A.; Shen, W.; Luo, Y. Responses of switchgrass soil respiration and its components to precipitation gradient in a mesocosm study. *Plant Soil* **2017**, *420*, 105–117. [[CrossRef](#)]
15. Makoi, J.; Ndadikemi, P. Selected soil enzymes: Examples of their potential roles in the ecosystem. *Afr. J. Biotechnol.* **2008**, *7*, 181–191.
16. Li, Y.; Niu, W.; Wang, J.; Liu, L.; Zhang, M.; Xu, J. Effects of Artificial Soil Aeration Volume and Frequency on Soil Enzyme Activity and Microbial Abundance when Cultivating Greenhouse Tomato. *Soil Sci. Soc. Am. J.* **2016**, *80*, 1208. [[CrossRef](#)]
17. Li, J.; Sun, J.; Yang, Y.; Guo, S.; Glick, B.R. Identification of hypoxic-responsive proteins in cucumber roots using a proteomic approach. *Plant Physiol. Biochem.* **2012**, *51*, 74–80. [[CrossRef](#)]
18. Li, Y.; Niu, W.; Xu, J.; Wang, J.; Zhang, M.; Lv, W. Root morphology of greenhouse produced muskmelon under sub-surface drip irrigation with supplemental soil aeration. *Sci. Hortic.* **2016**, *201*, 287–294. [[CrossRef](#)]

19. Xu, C.-M.; Wang, D.-Y.; Chen, S.; Chen, L.-P.; Zhang, X.-F. Effects of Aeration on Root Physiology and Nitrogen Metabolism in Rice. *Rice Sci.* **2013**, *20*, 148–153. [[CrossRef](#)]
20. Jia, B.; Zhou, G.; Wang, F.; Wang, Y.; Yuan, W.; Zhou, L. Partitioning root and microbial contributions to soil respiration in *Leymus chinensis* populations. *Soil Boil. Biochem.* **2006**, *38*, 653–660. [[CrossRef](#)]
21. Iqbal, J.; Hu, R.; Feng, M.; Lin, S.; Malghani, S.; Ali, I.M. Microbial biomass, and dissolved organic carbon and nitrogen strongly affect soil respiration in different land uses: A case study at Three Gorges Reservoir Area, South China. *Agric. Ecosyst. Environ.* **2010**, *137*, 294–307. [[CrossRef](#)]
22. Lange, M.; Eisenhauer, N.; Sierra, C.A.; Bessler, H.; Engels, C.; Griffiths, R.I.; Mellado-Vázquez, P.G.; Malik, A.A.; Roy, J.; Scheu, S.; et al. Plant diversity increases soil microbial activity and soil carbon storage. *Nat. Commun.* **2015**, *6*, 6707. [[CrossRef](#)] [[PubMed](#)]
23. Du, Y.; Niu, W.; Zhang, Q.; Cui, B.; Gu, X.; Guo, L.; Liang, B. Effects of Nitrogen on Soil Microbial Abundance, Enzyme Activity, and Nitrogen Use Efficiency in Greenhouse Celery under Aerated Irrigation. *Soil Sci. Soc. Am. J.* **2018**, *82*, 606. [[CrossRef](#)]
24. Manzoni, S.; Schimel, J.P.; Porporato, A. Responses of soil microbial communities to water stress: Results from a meta-analysis. *Ecology* **2012**, *93*, 930–938. [[CrossRef](#)] [[PubMed](#)]
25. Zhu, Y.; Cai, H.; Song, L.; Chen, H. Aerated Irrigation Promotes Soil Respiration and Microorganism Abundance around Tomato Rhizosphere. *Soil Sci. Soc. Am. J.* **2019**, *83*, 1343. [[CrossRef](#)]
26. Chen, S.; Liu, A.; He, C.; Zou, Z. Microbial and enzyme activities in tomato rhizosphere with organic soil cultivation in solar greenhouse. *Chin. J. Soil Sci.* **2010**, *41*, 815–818. (In Chinese)
27. Chaparro, J.M.; Sheflin, A.M.; Manter, D.K.; Vivanco, J.M. Manipulating the soil microbiome to increase soil health and plant fertility. *Boil. Fertil. Soils* **2012**, *48*, 489–499. [[CrossRef](#)]
28. Drenovsky, R.; Vo, D.; Graham, K.; Scow, K. Soil Water Content and Organic Carbon Availability Are Major Determinants of Soil Microbial Community Composition. *Microb. Ecol.* **2004**, *48*, 424–430. [[CrossRef](#)]
29. Ben-Noah, I.; Friedman, S. Aeration of clayey soils by injecting air through subsurface drippers: Lysimetric and field experiments. *Agric. Water Manag.* **2016**, *176*, 222–233. [[CrossRef](#)]
30. Xu, G.-W.; Lu, D.-K.; Wang, H.-Z.; Li, Y. Morphological and physiological traits of rice roots and their relationships to yield and nitrogen utilization as influenced by irrigation regime and nitrogen rate. *Agric. Water Manag.* **2018**, *203*, 385–394. [[CrossRef](#)]
31. Chen, H.; Shang, Z.-H.; Cai, H.-J.; Zhu, Y. An Optimum Irrigation Schedule with Aeration for Greenhouse Tomato Cultivations Based on Entropy Evaluation Method. *Sustainability* **2019**, *11*, 4490. [[CrossRef](#)]
32. Bai, T.; Li, C.; Li, C.; Liang, D.; Ma, F. Contrasting hypoxia tolerance and adaptation in *Malus* species is linked to differences in stomatal behavior and photosynthesis. *Physiol. Plantarum.* **2013**, *147*, 514–523. [[CrossRef](#)] [[PubMed](#)]
33. Intrigliolo, D.S.; Bonet, L.; Nortes, P.A.; Puerto, H.; Nicolas, E.; Bartual, J. Pomegranate trees performance under sustained and regulated deficit irrigation. *Irrig. Sci.* **2013**, *31*, 959–970. [[CrossRef](#)]
34. Zhu, Y.; Cai, H.; Song, L.; Chen, H. Oxygenation improving soil aeration around tomato root zone in greenhouse. *Trans. Chin. Soc. Agric. Eng.* **2017**, *33*, 163–172. (In Chinese)
35. Borkhuu, B.; Peckham, S.; Ewers, B.; Norton, U.; Pendall, E.; Ewers, B. Does soil respiration decline following bark beetle induced forest mortality? Evidence from a lodgepole pine forest. *Agric. For. Meteorol.* **2015**, *214*, 201–207. [[CrossRef](#)]
36. Dieleman, W.I.J.; Luyssaert, S.; Rey, A.; de Angelis, P.; Barton, C.V.M.; Broadmeadow, M.S.J.; Broadmeadow, S.B.; Chigwerewe, K.S.; Crookshanks, M.; Dufrêne, E.; et al. Soil [N] modulates soil C cycling in CO<sub>2</sub>-fumigated tree stands: A meta-analysis. *Plant Cell Environ.* **2010**, *33*, 2001–2011. [[CrossRef](#)]
37. Cheng, W. Rhizosphere priming effect: Its functional relationships with microbial turnover, evapotranspiration, and C–N budgets. *Soil Boil. Biochem.* **2009**, *41*, 1795–1801. [[CrossRef](#)]
38. Dijkstra, F.A.; Cheng, W. Interactions between soil and tree roots accelerate long-term soil carbon decomposition. *Ecol. Lett.* **2007**, *10*, 1046–1053. [[CrossRef](#)]
39. Zak, D.R.; Pregitzer, K.S.; King, J.S.; Holmes, W.E. Elevated atmospheric CO<sub>2</sub>, fine roots and the response of soil microorganisms: A review and hypothesis. *New Phytol.* **2000**, *147*, 201–222. [[CrossRef](#)]
40. Scheer, C.; Grace, P.R.; Rowlings, D.W.; Payero, J. Soil N<sub>2</sub>O and CO<sub>2</sub> emissions from cotton in Australia under varying irrigation management. *Nutr. Cycl. Agroecosys.* **2013**, *95*, 43–56. [[CrossRef](#)]
41. Reth, S.; Göckede, M.; Falge, E. CO<sub>2</sub> efflux from agricultural soils in Eastern Germany-comparison of a closed chamber system with eddy covariance measurements. *Theor. Appl. Climatol.* **2005**, *80*, 105–120. [[CrossRef](#)]

42. Chen, H.; Hou, H.; Hu, H.; Shang, Z.; Zhu, Y.; Cai, H.; Qaisar, S. Aeration of different irrigation levels affects net global warming potential and carbon footprint for greenhouse tomato systems. *Sci. Hortic.* **2018**, *242*, 10–19. [[CrossRef](#)]
43. Хазиев, ф.Х. *Soil Enzymes Activities*; Science Press: Beijing, China, 1980. (In Chinese)



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