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Effects of Artificial Soil Aeration Volume and Frequency on Soil Enzyme Activity and Microbial Abundance when Cultivating Greenhouse Tomato

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Artificial soil aeration can enhance soil enzyme activity, improve soil nutrient cycling, and increase crop growth and yield. We studied the response of soil microorganisms and soil enzyme activity to two levels of burial depths of subsurface tubing in combination with four levels of aeration volume and three frequency levels of supplemental soil aeration. The aeration volumes (*V*) were 0, 0.5, 1, and 1.5 times (CK, V_1, V_2, and V_3, respectively) the estimated porosity of the plot rhizosphere. Burial depths (*D*) of subsurface tubing were 15 and 40 cm (D_{15} and D_{40}). Aeration frequencies (*F*) levels were none and at 2- and 4-d intervals (CK, F_2, and F_4). The results demonstrated that aeration frequency and volume positively affected soil urease, phosphatase, and catalase activity and soil microbial abundance. The impact of aeration treatment on rhizosphere soil enzyme activity was greater than its impact on non-rhizosphere activity. When the drip irrigation tube depth was 15 cm, V_2 volume with 2-d aeration intervals led to an increase in the mean yield of first picking fruit of 75.1% compared with the unaerated control. When V_3 volume with 2-d aeration intervals was performed with a 40-cm irrigation tube, the mean yields of the first picking fruit increased by 135.5% compared with the unaerated control. These results suggest that artificial soil aeration can improve the plant root zone environment, increase microbial abundance and soil enzyme activity, and promote nutrient uptake, thus promoting plant growth and fruit output.

Soil microorganisms and soil enzymes are important components of agricultural ecosystems. Bacteria, fungi, and actinomycetes play important roles, including decomposing organic matter, degrading cellulose, and forming antibiotic substances. Nitrogen-fixing bacteria provide nitrogen sources for plants, whereas nitrobacteria prevent the accumulation of nitrite in the soil (Clarholm, 1985). Fungi are involved in the soil carbon cycle by decomposing cellulose, lignin, and pectin to release nutrients, and the development of the mycelium improves the physical structure of the soil (Tedersoo et al., 2014). Actinomycetes are the major producers of antibiotics, which have important biocontrol effect that is critical to

Core Ideas

- **Rhizosphere soil enzymes activity showed an initial increase followed by a decrease.**
- **Soil aeration can enhance the activities of three rhizosphere soil enzymes.**
- **Aeration frequency and volume can significantly affect rhizosphere enzyme activities.**
- **Aeration can enhance the activities of non-rhizosphere soil enzymes.**
- **Soil aeration can increase tomato yield.**

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soil phosphatase activity (Ghorbani-Nasrabadi et al., 2013). Soil enzymes drive the transformation of soil carbon, nitrogen, phosphorus, sulfur, and other elements. To a certain extent, soil enzymes reflect crop nutrient utilization, growth, and development (Bandick and Dick, 1999; van der Heijden et al., 2008; Yao and Huang, 2006). Soil urease is one of the most important hydrolases in the soil; it can hydrolyze urea applied to the soil to release ammonium, and it plays an important role in soil nitrogen circulation through determining the capacity of the soil to supply nitrogen (Klose and Tabatabai, 1999). Soil phosphatases can catalyze the hydrolysis of phospholipids and phosphoric anhydride, and phosphatase activity directly affects the transformation and utilization of soil organic phosphorus (Dodor and Tabatabai, 2003; Wang et al., 2013). Catalase prevents the formation of highly reactive hydroxyl radical from hydrogen peroxide (H_2O_2) by decomposing H_2O_2 into water and dioxygen; thus, catalase is considered an indicator of aerobic microbial activity and has been related to both the number of aerobic microorganisms and soil fertility (García and Hernández, 1997).

The rhizosphere is the micro-environment of plants and soil, the area where crops obtain the majority of their nutrients, and the primary location for the growth and development of roots and for nutrient absorption and metabolism. Root exudates are highly variable, with different quantities of compounds. These compounds include not only sugars, organic acids, amino acids, and other primary metabolites but also ketones, phenols, amines, and other secondary metabolites. Rhizosphere exudates primarily interact with the surrounding environment to form the rhizosphere (within 1 mm of the root–soil interface) and to produce rhizosphere benefits (Høgh-Jensen and Schjoerring, 2001; Liljeroth et al., 1990; Rovira, 1969). Rhizosphere microorganisms gather in the rhizosphere soil and use rhizosphere exudates as their major nutrient. Root exudates provide multiple nutrients and energy sources for soil microorganisms. Soil microorganisms, soil animals, crop roots, and their remains secrete enzymes into the soil, and the rhizosphere soil enzymes in turn affect the transformation of soil nutrients and the absorption and utilization of nutrients by crop roots.

The greenhouse environment is relatively closed, and different tillage practices can significantly affect the soil moisture and heat conditions, thereby affecting soil enzyme activity and eventually affecting the material and energy exchange between plants and the soil (Wang et al., 2015). Industrialized agriculture has developed rapidly and can lead to soil compaction. In addition to compaction, some unrelated factors, such as extraordinarily high groundwater tables, long-term rainfall, and tillage under clay or clay loam conditions, can often lead to reduced soil oxygen content, which limits crop yield and quality (Blokhina, 2003). Greenhouse cultivation differs from outdoor field planting because the soil trampling frequency in a greenhouse is much higher than that of field soil. The average bulk density of subsurface soil (16–30 cm deep) in a greenhouse increases with increasing time since tillage (Wang et al., 2004). In China, the soil in a greenhouse usually experiences shallow tillage once a season, which tends to cause compaction of the subsoil and topsoil layers and hence hypoxic stress in the root region (Liang et al., 2004; Wu et al., 2010). In compacted soil, the increase in soil bulk density and the accompanying decrease in porosity can hinder the exchange of oxygen, carbon dioxide, and other gases, thereby causing hypoxic stress in plant roots (Bhattarai et al., 2006).

Hypoxic stress can negatively affect crops in at least two ways: (i) the activities of soil animals and aerobic microorganisms are slowed and soil enzyme activity is reduced, thereby reducing soil fertility (Drew, 1992, 1997; Qiu et al., 2004), and (ii) the insufficient oxygen supply to plant roots negatively affects physiological functions (Niu et al., 2012a). Hypoxic stress causes an accumulation of leaf abscisic acid, which reduces stomatal density and opening and inhibits the growth of stems and leaves, resulting in a reduced net photosynthesis rate, slower plant growth, reduced accumulation of dry matter, and limited yield and quality improvement (Munns and Sharp, 1993). Buttery et al. (1998) studied soybeans and common beans under different soil bulk densities and found that, in highly compacted soil, both plant height and dry matter weight were lower than in less compacted soil.

Artificial soil aeration can enhance air permeability and improve soil oxygen content, effectively relieving hypoxic stress on roots (Bhattarai et al., 2006, 2008, 2010; Chen et al., 2011; Pendergast et al., 2013) while maintaining soil microorganism and enzyme activities (Niu et al., 2012b). Thus, soil moisture and soil aeration are coupled, and the soil water content influences soil aeration (Boone et al., 1986). Additionally, Meek and Stolzy (1978) showed that soil water content above or even below field capacity impairs air exchange between the soil and the atmosphere. Another study reported that the water use efficiency of cotton was greater with soil aeration in a heavy clay soil (Bhattarai and Midmore, 2009). Our previous studies have also shown that artificial soil aeration can improve the tomato (*Lycopersicon esculentum* Mill.) root zone environment, increase soil enzyme activity, and promote the uptake of nutrients, thus promoting plant growth and fruit output and improving soil quality (Li et al., 2015a; Niu et al., 2012a, 2012b).

Tomato is widely planted globally. The FAO estimates that total global tomato production was 148.74 t in 2013. China accounted for 31% of the total global production, and India, the United States, Turkey, and Egypt were also major producing countries (FAO, 2013). Because the tomato has a short growth period, is widely planted, and is easy to access, it is often used as a model plant for agricultural, physiological, and biochemical studies. Because the tomato is one of the most sensitive species to water and air in the soil (Bradford and Yang, 1981), the present study used greenhouse tomatoes as its study subject. Higher tomato yields are related to higher oxygen contents in the soil root zone (Meek et al., 1983; Niu et al., 2012a, 2012b). Observed changes in plant growth were mainly because of the changes in nutrient cycling and physiological status, and soil-available nutrients were affected by soil microorganisms and soil enzyme activity. Both soil microorganisms and soil enzyme activity were

constrained by the soil microenvironment. It was hypothesized that varying the aeration frequency, volume, and position would result in differing chemical and physical properties of the rhizosphere soil and could alter soil microbial abundance, enzyme activity, and nutrients. All of these changes ultimately influence tomato plant grow and fruit yield. Nevertheless, the best methods of aeration (including frequency and volume) and the optimal aeration position for roots (i.e., the burial depths of the aeration tubes) are unknown. In this study, we used an air compressor to examine the effects of different aeration quantities, aeration time intervals, and aeration positions on soil microbial abundance and soil enzyme activity during greenhouse tomato cultivation. The objective of the study was to determine the optimal combination of aeration parameters and burial depth and to provide a relevant theoretical basis for improving the rhizosphere oxygen environment of greenhouse tomatoes, thereby increasing soil aerobic microorganism abundance, enzyme activity, and nutrient utilization efficiency, ultimately improving crop yield and quality.

MATERIALS AND METHODS

General Description of Field Site and Materials

The experiments were conducted in a greenhouse at Yangling (108°2′ E, 34°17′ N), Shaanxi, between 18 Oct. 2014 and 20 May 2015. The tested cultivar of tomato (*Lycopersicon esculentum* Mill.) was FenyuYanggang (New Horizon Facilities Agricultural Development Co. Ltd., Northwest A&F University, China), and the preceding crop cultivated in the greenhouse was melon (*Cucumis melo* L.). Air was used for soil aeration, and the soil for the test was a silty clay loam (soil order Inceptisol based on USDA soil taxonomy). Bulk density of the soil was 1.34 g cm^{-3}, field capacity was 28.17% (moisture content of total mass), pH was 7.82, and soil porosity was 49.38%. The soil contained 9.4 g kg^{-1} organic matter, 1.3 g kg^{-1} total N, and 1.4 g kg^{-1} total P. Gravel (2–0.02 mm) accounted for 25.4% of the soil, silt (0.02–0.002 mm) accounted for 44.1%, and clay (<0.002 mm) accounted for 30.5%.

Experimental Design

The greenhouse was 108 m long and 8 m wide, and the cultivation area was 5.5 m long and 1.5 m wide, with a total planting area of 8.25 m^2. To maintain the interior temperature at night during the winter, straw mats were spread on the surface of the thermal polyethene plastic film; during the daytime, the interior temperature was controlled by a ventilation system on the roof (Fig. 1a). Two subsurface drip irrigation tubes with diameters of 16 mm were laid in each cultivation area; the spacing between the

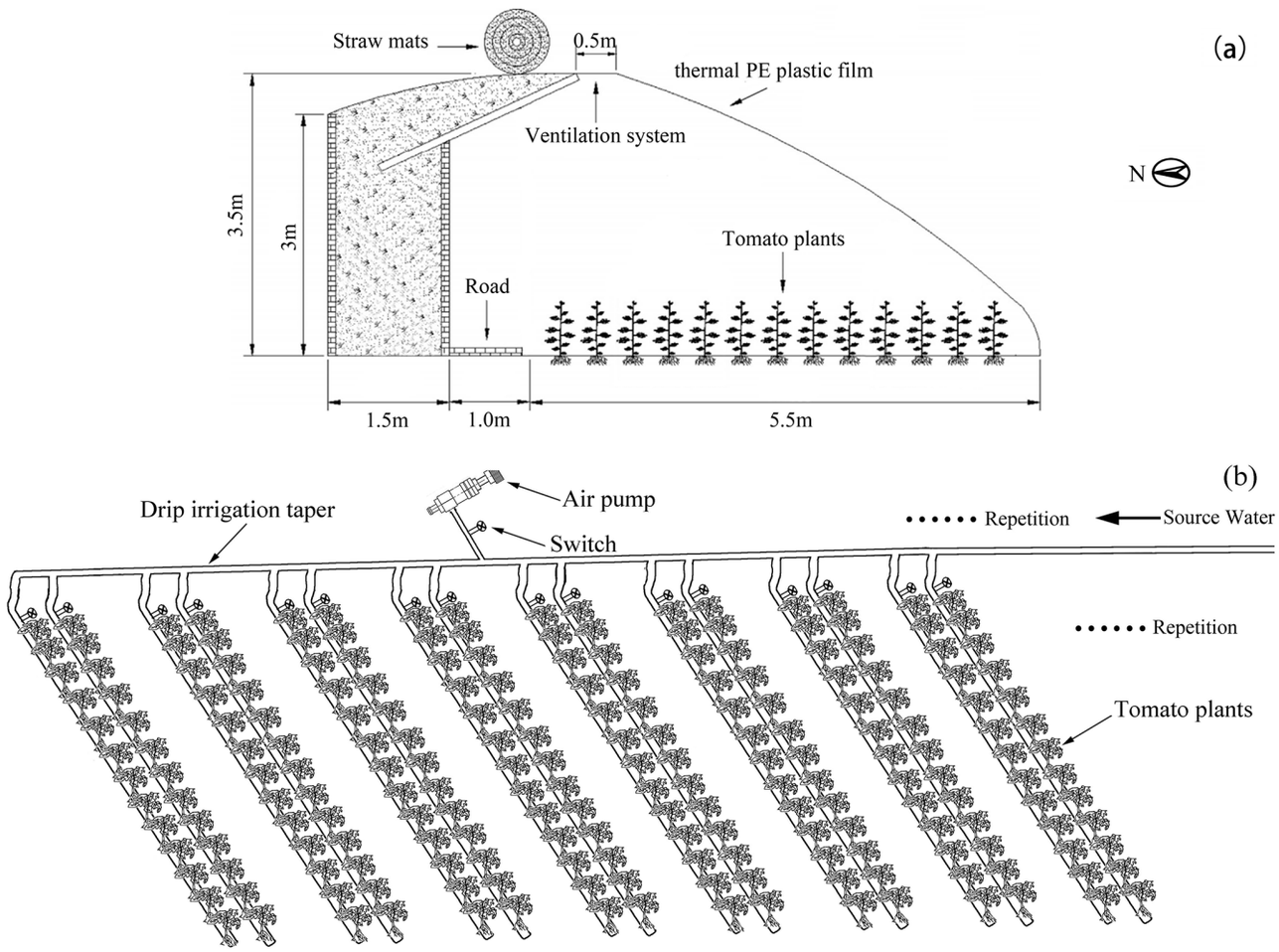

Fig. 1. (a) Cross-section of the greenhouse in northwestern China, and (b) experimental arrangement of an example block. Treatments were randomized within each block. PE, polyethene.

drippers was 30 cm, and the spacing between the drip irrigation tubes was 0.5 m. The main drip irrigation pipe was connected to an air pump, and water and air were supplied to the soil through the subsurface drip irrigation tubes (Fig. 1b). Seedlings (20 d old) were transplanted to beds and covered with film. The row spacing for planting was 0.5 m, and the plant spacing was 0.4 m. Two rows were planted in each area, with 13 plants in each row.

The experiment used two horizontal drip irrigation tube burial depths (D): D_{15} and D_{40} represent drip irrigation tube burial depths of 15 and 40 cm, respectively. The experiment used three aeration frequencies (F): CK, F_2, and F_4 represent no aeration, aeration once every 2 d, and aeration once every 4 d, respectively. In experiments that varied the aeration frequency, the aeration volume was the standard aeration volume. In experiments that varied the aeration volume, the aeration frequency was once every 2 d. The flow rate for each plot was 10.2 L min^{-1}. There were four aeration volumes (V); CK, V_1, V_2, and V_4 represent no aeration and 0.5, 1, and 1.5 times the standard aeration volume, respectively. The standard aeration volume was calculated as

$$V=\frac{1}{1000}SL\left(1-\frac{\rho_b}{\rho_s}\right)$$

where V is the volume (L) of each aeration, S is the cross-sectional area (1500 cm^2) of the plot, L is the plot length (550 cm), ρ_b is the soil bulk density (1.34 g cm^{-3}), and ρ_s is the soil density (2.65 g cm^{-3}) (Xie et al., 2010). Accordingly, the calculated standard aeration volume was 407.83 L.

Two separate factorial experiments were used: one testing drip irrigation tube burial depth plus aeration frequency (2 × 3) and the other testing drip irrigation tube burial depth plus aeration volume (2 × 4). The two experiments resulted in a total of 12 treatments, namely, D_{15}CK, $D_{15}F_4$, $D_{15}F_2$, $D_{15}V_1$, $D_{15}V_2$, $D_{15}V_3$, D_{40}CK, $D_{40}F_4$, $D_{40}F_2$, $D_{40}V_1$, $D_{40}V_2$, and $D_{40}V_3$ (Table 1), each of which was repeated three times. Before transplanting, the soil was rototilled, and 120 t ha^{-1} of decomposed organic manure (pig and sheep manure), 1500 kg ha^{-1} of diammonium phosphate (18% N and 46% P_2O_5), and 400 kg ha^{-1} of compound fertilizer (18% N, 15% P_2O_5, and 12% K_2O) were broadcast uniformly as the basal fertilizer in the soil. Irrigation water demand is mainly driven by farmers' perceptions and climatic conditions. Irrigation occurred four times, with the same irrigation quantity for each plot. The total amount of irrigation was 235 mm. During the experimental period, other management practices, such as pollination, branch stem pruning, and pest control, were the same for all treatments.

Table 1. Transposed design matrix for the 12 treatment combinations.

Treatment combination	Parameters†		
	Depth	Aeration frequency	Aeration volume
1	D_{15}	CK	CK
2	D_{15}	F_4	V_2
3	D_{15}	F_2	V_2
4	D_{15}	F_2	V_1
5	D_{15}	F_2	V_2
6	D_{15}	F_2	V_3
7	D_{40}	CK	CK
8	D_{40}	F_4	V_2
9	D_{40}	F_2	V_2
10	D_{40}	F_2	V_1
11	D_{40}	F_2	V_2
12	D_{40}	F_2	V_3

† CK, no aeration; D_{15}, subsurface tubing placement depth at 15 cm; D_{40}, subsurface tubing placement depth at 40 cm; F_2, 2-d aeration interval; F_4, 4-d aeration interval; V_1, tube aeration at 0.5 times the standard volume; V_2, tube aeration at the standard volume; V_3, tube aeration at 1.5 times the standard volume.

Measurement Time and Indicator Methods

Rhizosphere soils were collected during the seedling period (32 d after transplant), the blooming and setting period (63 d after transplant), and the maturation period (130 d after transplant) to measure soil enzyme activity. A shovel was used to excavate the plant roots, large pieces of soil were shaken off, and a soft bristle brush was used to brush and collect the soil that was closely associated with the roots. Non-rhizosphere soil enzyme activity at different depths was measured only during the fruit enlargement period (92 d after transplant). A soil auger was used to collect samples. Undisturbed soil samples were collected at depths of 10, 20, 30, 40, and 50 cm. Urease activity was measured by phenol-sodium hypochlorite colorimetry and was represented by the mass of NH_3–N generated from each gram of soil after 24 h [in units of mg g^{-1} (24 h)$^{-1}$] (Huang, 2012). Soil phosphatase activity was measured using a disodium phosphate–benzene colorimetric assay, and the mass of phenol released from each gram of soil after 24 h was used to represent phosphatase activity in units of μg g^{-1} (24 h)$^{-1}$. The titration method (0.1 mol L^{-1} standard $KMnO_4$ solution titration) was used to determine catalase activity, which was expressed by the number of milliliters of $KMnO_4$ solution consumed by each gram of dry soil (in units of mL g^{-1}) (Guan, 1986).

During the blooming and setting period (65 d after transplant), we collected and determined the abundance of rhizosphere soil microorganisms. The collected soil samples were temporarily stored in sealed sterile plastic bags at 4°C (for <24 h) until quantification of microorganisms. The serial dilution plate-smearing method was used to mix and inoculate the bacteria and fungi. The same soil sample (0.1-mL suspension) was inoculated at three consecutive dilutions, with each dilution replicated three times. Bacteria were cultured on a medium containing beef extract, peptone, and agar; fungi were cultured on Martin medium; and actinomycetes were cultured on improved Gao's no. 1 medium (Microbe Department of the Institute of Soil Science, Chinese Academy of Sciences, 1985). All soil samples were randomly collected in the cultivation area, and three repeated sample collections were conducted for each treatment.

The yield and root dry weight were measured during the full fruiting period, when the tomatoes were first harvested (150 d). The roots were removed and washed through a sieve, dried in the oven at 105°C for 15 min to deactivate enzymes, and then dried

at 75°C to constant mass before weighing. An electronic scale with 0.01-g increments was used to determine the fresh fruit yield and root dry weight for each plant.

Data Processing and Analysis

Experimental data were organized using Microsoft Excel. Statistical analysis was performed using SPSS22.0 (IBM Corp.). Rhizosphere soil enzyme activity and soil microorganism abundance were analyzed by two-way ANOVA with the factor aeration frequency treatment, aeration volume treatment, and burial depths of drip irrigation tubes and the interactions of aeration frequency treatment × burial depths and aeration volume treatment × burial depths. Multiple comparisons using Duncan's new multiple-range test were completed whenever the ANOVA indicated significant differences ($P \leq 0.05$). A *t* test was used to compare treatment marginal means for burial depths. Pearson correlation was performed between average soil enzyme activity, average microorganism abundance, and tomato yield, and the Pearson correlation coefficient was derived. Origin Pro 9.0 was used for plotting.

RESULTS

Rhizosphere Soil Enzyme Activity at Different Growth Stages

Soil Urease

Aeration frequency, aeration volume, and burial depth of drip irrigation tubes interacted to affect rhizosphere soil urease activity in the three measured growth stages. Throughout the growth period, the soil urease activity exhibited an initial increase followed by a decrease. Across all sampling times, urease activity was highest during the blooming and setting period when the drip irrigation tubes were buried 40 cm deep. The urease activity during the maturation period with a 40-cm burial of the drip irrigation tubes was greater than that observed during the seedling period, whereas the urease activity during the seedling period with a 15-cm burial of the drip irrigation tubes was greater than that observed during the maturation period. Across all sampling times, the effect of aeration on urease activity increased with increasing aeration frequency (Table 2). With a 40-cm burial of the drip irrigation tubes, soil urease activity increased with increasing aeration volume, whereas rhizosphere soil enzyme activity exhibited a decreasing trend with a 15-cm tube burial and V_3 aeration volume. Among all three periods, the soil urease activity was lowest with no aeration. During the seedling and the blooming and setting periods, the soil urease activity in the F_2 and V_2 treatments was significantly higher than in other treatments, and the activity with 40-cm burial and V_3 was also significantly higher than that of other treatments. During the maturation period, the rhizosphere soil urease activity was significantly higher than that of other treatments, with either a 15-cm burial of the drip irrigation tubes and F_2 or V_2 treatment or with a 40-cm burial and V_3 treatment. Among the single-factor analyses, all effects of the different aeration frequencies on soil enzyme activity were significant. Aeration volume had an extremely significant impact on rhizosphere soil urease activity during the seedling and the

Table 2. Rhizosphere soil urease activity under different rhizosphere aeration treatments.

	Seedling stage				Blooming and setting stage				Mature stage			
Treatment†	15 cm	40 cm	Mean	*t* test	15 cm	40 cm	Mean	*t* test	15 cm	40 cm	Mean	*t* test
	—— mg g^{-1} (24 h)$^{-1}$ ——				—— mg g^{-1} (24 h)$^{-1}$ ——				—— mg g^{-1} (24 h)$^{-1}$ ——			
					Aeration frequency treatment							
CK	0.182bB‡	0.188bB	0.230bAB	ns§	0.185b	0.258bA	0.179bB	ns	0.244c	0.170cB	ns	0.175c
F_4	0.250bAB	0.222bB	0.307bA	ns	0.236b	0.314bA	0.244bAB	ns	0.311b	0.278bAB	ns	0.261b
F_2	0.447aAB	0.331aC	0.542aA	ns	0.389a	0.547aA	0.328aC	ns	0.545a	0.379aBC	ns	0.354a
Mean	0.293	0.247	0.360			0.373	0.251			0.276		
					Aeration volume treatment							
CK	0.182bB	0.188bB	0.185b	ns	0.230cAB	0.258cA	0.244c	ns	0.179bB	0.170cB	0.175b	ns
V_1	0.373abA	0.199bB	0.286ab	ns	0.394bA	0.399bA	0.397b	ns	0.221abBC	0.307bAB	0.264ab	ns
V_2	0.445aAB	0.335aBC	0.390a	ns	0.546aA	0.551aA	0.549a	ns	0.311aC	0.380abBC	0.346a	ns
V_3	0.387abB	0.416aB	0.402a	ns	0.457abAB	0.559aA	0.508a	*	0.247aC	0.453aAB	0.350a	*
Mean	0.346	0.267			0.385	0.411			0.239	0.304		
					F-value							
Aeration frequency (F)	9.533**	6.110*			41.326**	59.130**			10.653*	40.508**		
Aeration volume (V)	0.304ns	16.087**			6.198*	10.998**			7.726*	8.408*		
F × D	ns				ns				ns			
V × D	ns				ns				*			

* Significant at the $P \leq 0.05$ level.

** Significant at the $P \leq 0.01$ level.

† CK, no aeration; F_2, 2-d aeration interval; F_4, 4-d aeration interval; V_1, tube aeration at 0.5 times the standard volume; V_2, tube aeration at the standard volume; V_3, tube aeration at 1.5 times the standard volume.

‡ Values followed by uppercase letters within rows and lowercase letters within columns are significantly different at $P < 0.05$.

§ Not significant (ANOVA *F*-value for main and interaction effects).

blooming and setting periods with a 40-cm burial of the drip irrigation tubes and a significant impact on rhizosphere soil urease activity during the blooming and setting period and the maturation period with a 15-cm tube burial. Interaction analysis found that only aeration frequency and the burial depth of drip irrigation tubes significantly affected the rhizosphere soil urease activity during the maturation period.

Soil Phosphatase

Table 3 shows that all of the combinations of different aeration frequencies, aeration volumes, and drip irrigation tube burial depths had significant impacts on rhizosphere soil phosphatase activity during the three growth stages. Throughout the growth period, the overall soil phosphatase activity exhibited a trend of initial increase followed by a decrease. Significance analysis of the same treatments with different burial depths and during different growth periods found that rhizosphere soil phosphatase had the highest activity under conditions of V_3 and a 40-cm burial of the drip irrigation tubes during the blooming and setting period. During the seedling period, soil phosphatase activity was higher with a 15-cm burial of the drip irrigation tubes than a 40-cm burial, whereas during the maturation period, the phosphatase activity was lower with a 15-cm burial than a 40-cm burial. During these three periods, soil phosphatase activity was lowest in the no-aeration treatment, and the benefit of aeration increased with increasing aeration frequency. With a 40-cm burial, rhizosphere soil phosphatase activity increased with increasing aeration volume, whereas with a 15-cm burial, rhizosphere soil enzyme activity reached a maximum in V_2. With a 15-cm burial of the drip irrigation tubes, soil phosphatase activity reached its maximal value in F_2; with a 40-cm burial of the drip irrigation tubes, rhizosphere soil phosphatase activity reached its maximal value in V_3. Among the single-factor analyses, all aeration frequencies had extremely significant impacts on soil phosphatase activity. Although aeration volume had a significant impact on rhizosphere soil phosphatase activity during the blooming and setting period and the maturation period with a 15-cm burial of the drip irrigation tubes, all other treatments had even more significant impacts. Interaction analysis found that combinations of aeration volume and the burial depth of the drip irrigation tubes had extremely significant impacts on phosphatase activity during the seedling and maturation periods.

Catalase

Table 4 shows that the soil catalase activity in the blooming and setting period was higher than that in the seedling and maturation periods. However, with a 15-cm burial of drip irrigation tubes and the V_1 treatment, the catalase activity was greater in the maturation period than in the seedling period. During the three growth periods, soil catalase activity was the lowest under the no-aeration treatment, and the catalase activity with a 15-cm burial was less than that with a 40-cm burial. Different burial depths had a significant effect on V_3 during the seedling period, a significant impact on the no aeration and F_2 treatments during the blooming and setting period and a significant impact on V_1 and V_3 during the maturation period. With a 15-cm burial of drip irrigation tubes, catalase activity

Table 3. Rhizosphere soil phosphatase activity under different rhizosphere aeration treatments.

	Seedling stage				Blooming and setting stage				Mature stage			
Treatment†	15 cm	40 cm	Mean	*t* test	15 cm	40 cm	Mean	*t* test	15 cm	40 cm	Mean	*t* test
	—— μg g^{-1} (24 h)$^{-1}$ ——				—— μg g^{-1} (24 h)$^{-1}$ ——				—— μg g^{-1} (24 h)$^{-1}$ ——			
					Aeration frequency treatment							
CK	35.3cB‡	28.6cC	32.0c	ns§	43.6bA	37.8cB	40.7c	ns	19.5cD	24.2cCD	21.9c	ns
F_4	48.3bB	40.8bC	44.5b	*	60.0aA	50.8bB	55.4b	*	33.0bD	38.8bC	35.9b	ns
F_2	61.9aAB	57.0aABC	59.5a	ns	70.0aA	67.0aAB	68.5a	ns	44.6aC	54.3aBC	49.5a	ns
Mean	48.5	42.1	45.3		57.9	51.9	54.9		32.4	39.1	35.8	
					Aeration volume treatment							
CK	35.3cB	28.6cC	32.0c	ns	43.6bA	37.8cB	40.7c	ns	19.5bD	24.2dCD	21.9b	ns
V_1	42.7cB	36.0bC	39.4b	*	51.9bA	46.0bB	49.0b	ns	26.5bD	33.0cC	29.7b	*
V_2	62.0aAB	55.6aBC	60.5a	ns	69.7aA	65.6aAB	67.6a	ns	44.1aC	55.2bBC	49.7a	ns
V_3	52.8bC	59.0aB	49.9a	ns	63.9aB	69.0aA	66.5a	ns	36.0aD	71.8aA	53.9a	**
Mean	48.2	44.8	46.5		57.3	54.6	55.9		31.5	46.1	38.8	
					F-value							
Aeration frequency (F)	22.016**	24.498**			17.157**	27.987**			22.944**	24.232**		
Aeration volume (V)	11.551**	28.006**			6.550*	31.330**			10.665*	60.013**		
F × D	ns				ns				ns			
V × D	**				ns				**			

* Significant at the $P \leq 0.05$ level.

** Significant at the $P \leq 0.01$ level.

† CK, no aeration; F_2, 2-d aeration interval; F_4, 4-d aeration interval; V_1, tube aeration at 0.5 times the standard volume; V_2, tube aeration at the standard volume; V_3, tube aeration at 1.5 times the standard volume.

‡ Values followed by uppercase letters within rows and lowercase letters within columns are significantly different at $P \leq 0.05$.

§ Not significant (ANOVA *F*-value for main and interaction effects).

Table 4. Rhizosphere soil catalase activity under different rhizosphere aeration treatments.

Treatment†	Seedling stage				Blooming and setting stage				Mature stage			
	15 cm	40 cm	Mean	*t* test	15 cm	40 cm	Mean	*t* test	15 cm	40 cm	Mean	*t* test
	—— mL g^{-1} ——				—— mL g^{-1} ——				—— mL g^{-1} ——			
Aeration frequency treatment												
CK	0.43bB‡	0.40bB	0.42c	ns§	0.52bB	0.73cA	0.63c	*	0.40bB	0.48bB	0.44b	ns
F_4	0.63aC	0.53bC	0.58b	ns	1.27aA	1.20bA	1.23b	ns	0.67abC	0.93aB	0.80a	ns
F_2	0.67aE	0.93aCD	0.80a	ns	1.37aB	1.63aA	1.50a	**	0.73aDE	1.13aC	0.93a	ns
Mean	0.58	0.62	0.60		1.05	1.19	1.12		0.60	0.85	0.73	
Aeration volume treatment												
CK	0.43cB	0.40bB	0.42b	ns	0.52cB	0.73cA	0.63b	*	0.40cB	0.48dB	0.44c	ns
V_1	0.97aC	0.73aCD	0.88a	ns	1.73aA	1.33bB	1.53a	ns	0.58bD	0.83cCD	0.71b	**
V_2	0.67bE	0.97aCD	0.82a	ns	1.40bB	1.67aA	1.53a	ns	0.83aDE	1.07bC	0.95a	ns
V_3	0.57bcC	0.80aB	0.65a	*	1.33bA	1.40abA	1.37a	ns	0.87aB	1.37aA	1.12a	*
Mean	0.66	0.73	0.69		1.25	1.28	1.26		0.67	0.94	0.80	
F-value												
Aeration frequency (F)	14.333**	18.909**			59.769**	109.400**			4.941ns	15.960**		
Aeration volume (V)	7.800*	2.294ns			24.800**	2.897ns			7.848*	21.444**		
F × D	**				*				ns			
V × D	**				**				ns			

* Significant at the $P \leq 0.05$ level.

** Significant at the $P \leq 0.01$ level.

† CK, no aeration; F_2, 2-d aeration interval; F_4, 4-d aeration interval; V_1, tube aeration at 0.5 times the standard volume; V_2, tube aeration at the standard volume; V_3, tube aeration at 1.5 times the standard volume.

‡ Values followed by uppercase letters within rows and lowercase letters within columns are significantly different at $P < 0.05$.

§ Not significant (ANOVA *F*-value for main and interaction effects).

decreased with increasing aeration volume during the seedling period and the blooming period, whereas catalase activity increased with increasing aeration volume during the maturation period. During each growth period at different burial depths, rhizosphere soil catalase activity increased with increasing aeration frequency. The benefit of aeration was greater with a 40-cm burial of the drip irrigation tubes than with a 15-cm burial. Among the single-factor analyses, with the exception of a 15-cm burial of the drip irrigation tubes during the maturation period, all aeration frequencies had a significant impact on rhizosphere soil catalase activity. Aeration volume had a significant impact on catalase activity during the seedling and maturation periods with a 15-cm burial of drip irrigation tubes and an extremely significant impact on rhizosphere soil catalase activity during the blooming and setting period with a 15-cm burial and during the maturation period with a 40-cm burial. The impact of interaction between aeration and burial on rhizosphere soil catalase activity gradually decreased with plant growth; the two-factor interaction had an extremely significant impact on rhizosphere soil catalase activity during the seedling period, but there was no significant impact during the maturation period.

Soil Enzyme Activity at Different Soil Depths

Urease

As soil depth increased, non-rhizosphere soil enzyme activity decreased (Fig. 2). The urease activity in non-rhizosphere soil under aeration treatment was greater than in those without aeration. Moreover, for both burial depths of the drip irrigation tube, soil urease activity increased with increases in aeration frequency and aeration volume. With a 15-cm burial of the drip irrigation tubes, urease activity in 20- to 30-cm deep soil was significantly higher with aeration than without aeration. Although aeration had an impact on the urease activity of soil deeper than 30 cm, the difference was not significant. With a 40-cm burial of the drip irrigation tubes, aeration increased soil urease activity at depths of 20 to 50 cm, but soil urease activity was similar in the F_2 and F_4 treatments.

Phosphatase

Across all depths, non-rhizosphere soil phosphatase activity was greater with aeration than without aeration (Fig. 3). With a 15-cm burial of the drip irrigation tubes, aeration had a significant impact on soil phosphatase activity at a depth of 40 cm. With increased aeration frequency and volume, the non-rhizosphere soil phosphatase activity increased, but the phosphatase activity at a depth of 30 cm in the F_4 treatment was slightly higher than in F_2. With a 40-cm tube burial, the phosphatase activity at a depth of 40 cm increased with increasing aeration frequency, but the phosphatase activity at a depth of 30 cm in the F_4 treatment was slightly higher than in F_2. Meanwhile, with a 40-cm tube burial, aeration volume had a significant impact on non-rhizosphere soil phosphatase activity at depths of 10 to 50 cm. Phosphatase activity at depths of 10 to 30 cm increased with increasing aeration frequency; for a depth of 40 to 50 cm, enzyme activity was lower in the V_3 treatment than in the other aeration treatments but was higher than in the treatment without aeration.

Catalase

Figure 4 shows that the non-rhizosphere soil catalase activity was greater with aeration than without aeration treatment. For both tube burial depths, soil catalase activity increased with increasing aeration frequency. Our investigation of aeration volume found that with a 15-cm burial of the drip irrigation tubes and at soil depths of 20, 30, or 40 cm, maximal values for non-rhizosphere soil catalase activity were obtained in the V_1, V_2, and V_3 treatments, respectively. With a 40-cm tube burial and at soil depths of 10, 20, or 50 cm, catalase activity increased with increasing aeration volume. However, soil enzyme activity at a soil depth of 40 cm decreased with increasing aeration volume, and enzyme activity in at a soil depth of 30 cm was greatest in the V_2 treatment.

Quantity of Rhizosphere Soil Microorganisms

Table 5 shows that all combinations of aeration frequencies, aeration volumes, and burial depths of the drip irrigation tubes had significant impacts on the abundance of rhizosphere soil microorganisms during the three growth periods. The abundance of rhizosphere soil bacteria in the F_2 and V_2 treatments was significantly greater than in the other treatments. With a 15-cm burial of the drip irrigation tubes, the abundance of rhizosphere soil bacteria in the V_3 treatment was significantly higher than in the other treatments. With a 15-cm burial of the drip irrigation tubes and the V_1 treatment or with a 40-cm burial and F_2, V_2, or V_3, the abundance of fungi was significantly higher than in the other treatments. With a 15-cm tube burial and F_4 or V_1 or with a 40-cm burial and V_3, the abundance of actinomycetes was significantly higher than in the other treatments. Among the single factor analyses, aeration frequency had a significant impact on bacterial abundance with a 40-cm burial and on fungal abundance with a 15-cm burial and an extremely significant impact on bacterial abundance with a 15-cm burial and on fungal and actinomycete abundance with a 40-cm burial. Aeration volume had an extremely significant impact on all three types of microorganisms with a 15-cm burial but no significant impact on any of the three microorganism types with a 40-cm burial. Among the interactions, aeration and the burial depth of drip irrigation tubes had an extremely significant impact on the abundances of fungi and actinomycetes.

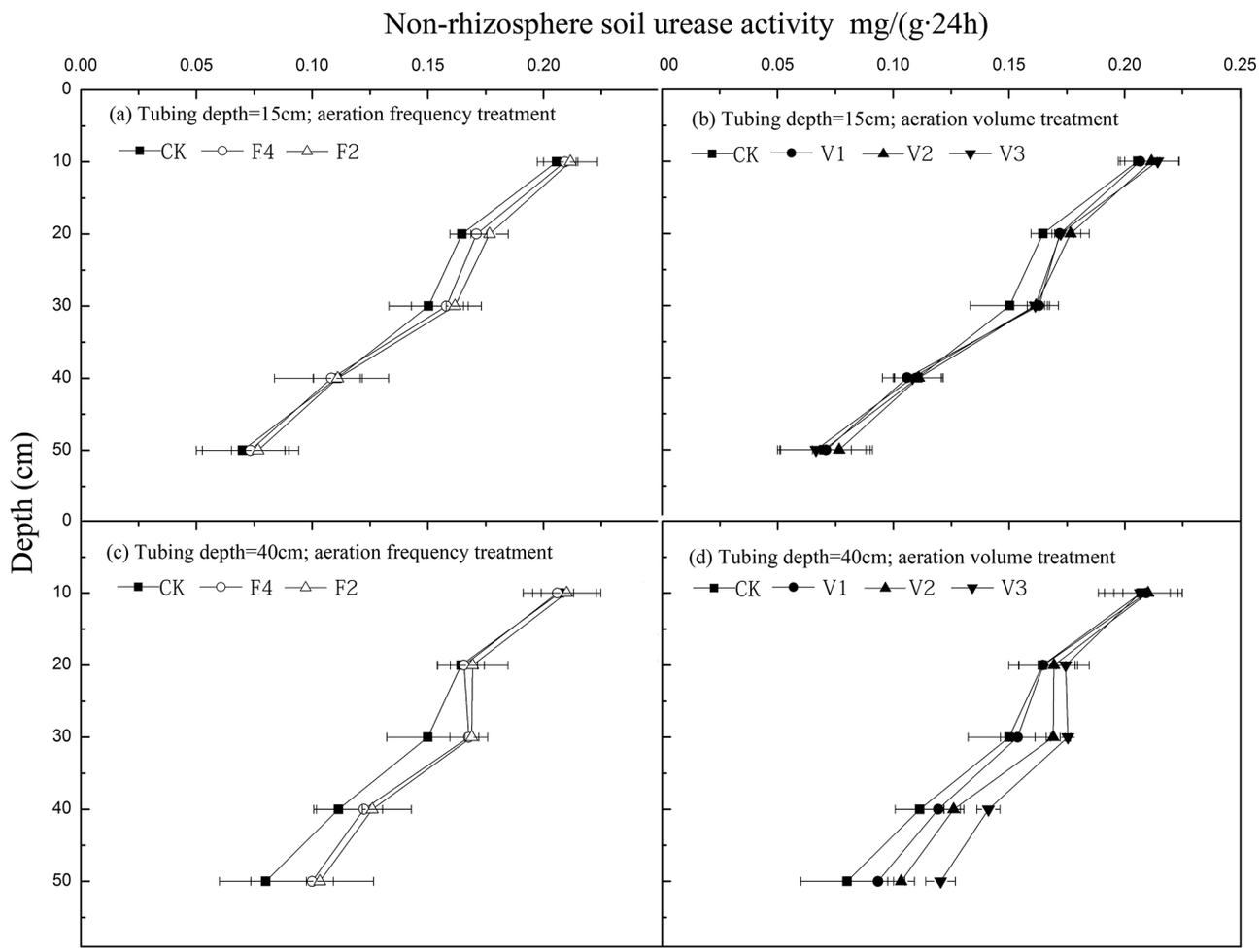

Fig. 2. Non-rhizosphere soil urease activity under different aeration treatments at (a,b) a tubing depth of 15 cm and (c,d) a tubing depth of 40 cm. CK, no aeration; F_2, 2-d aeration interval; F_4, 4-d aeration interval; V_1, tube aeration at 0.5 times the standard volume; V_2, tube aeration at the standard volume; V_3, tube aeration at 1.5 times the standard volume.

Root Dry Weight and Tomato Yield

Figure 5 shows the root dry weight results during the full fruiting period. For both tube burial depths, the overall root dry weight increased with increasing aeration frequency and volume, but the aeration frequency treatments had no significant effect on the root dry weight. The root dry weights for all of the aerated plants with a 40-cm tube burial were higher than for those with a 15-cm tube burial. Increased aeration frequency corresponded to increased yield (Fig. 6). The yield was the highest with a 15-cm tube burial and F_2, with a 75.1% increase in first-picked fruit compared with the no-aeration treatment. Yield was highest with the V_2 aeration volume, and the yield decreased with either increased or decreased aeration volume relative to V_2. With a 40-cm burial of the drip irrigation tubes, yield increased with increased aeration volume, and in V_3, yield of the first-picked fruit increased by 135.5% compared with the no-aeration treatment. With a 15-cm burial of the drip irrigation tubes, the no-aeration, F_4 and V_1 treatments increased the first-picked tomato yield by 10.7, 11.4, and 9.6%, respectively, compared with the yield from the same aeration treatment but with a 40-cm tube burial. With 40-cm burial of the drip irrigation tubes, F_2, V_2, and V_3 increased the first-picked fruit yield by 8.5, 4.4, and 53.6%, respectively, compared with the yield from the same aeration treatment but with a 15-cm tube burial. At both burial depths, the F_4, F_2, V_1, V_2, and V_3 treatments increased the yield by 27.7, 91.8, 41.1, 87.7, and 84.5%, respectively, compared with the no-aeration treatment.

DISCUSSION

Effect of Artificial Soil Aeration on the Abundance of Rhizosphere Soil Microorganisms

When soil temperature and moisture are suitable and aeration is sufficient, aerobic microorganisms in the soil are more active, and more nutrients can be released for plants to absorb (Gyaneshwar et al., 2002). The present study shows that high aeration frequencies can effectively relieve hypoxic stress in the root region and promote the proliferation of soil microorganisms. This conclusion is consistent with previous studies of soil microorganisms under varying aeration conditions, which demonstrated that aeration once every 2 d can yield a higher output–input ratio (Li et al., 2015b; Xie et al. (2010). The interaction between aeration volume and burial depth of drip irrigation tubes had an extremely significant effect on the abundances of fungi and actinomycetes. With a 15-cm burial and the V_1 treatment, the abundances of fun-

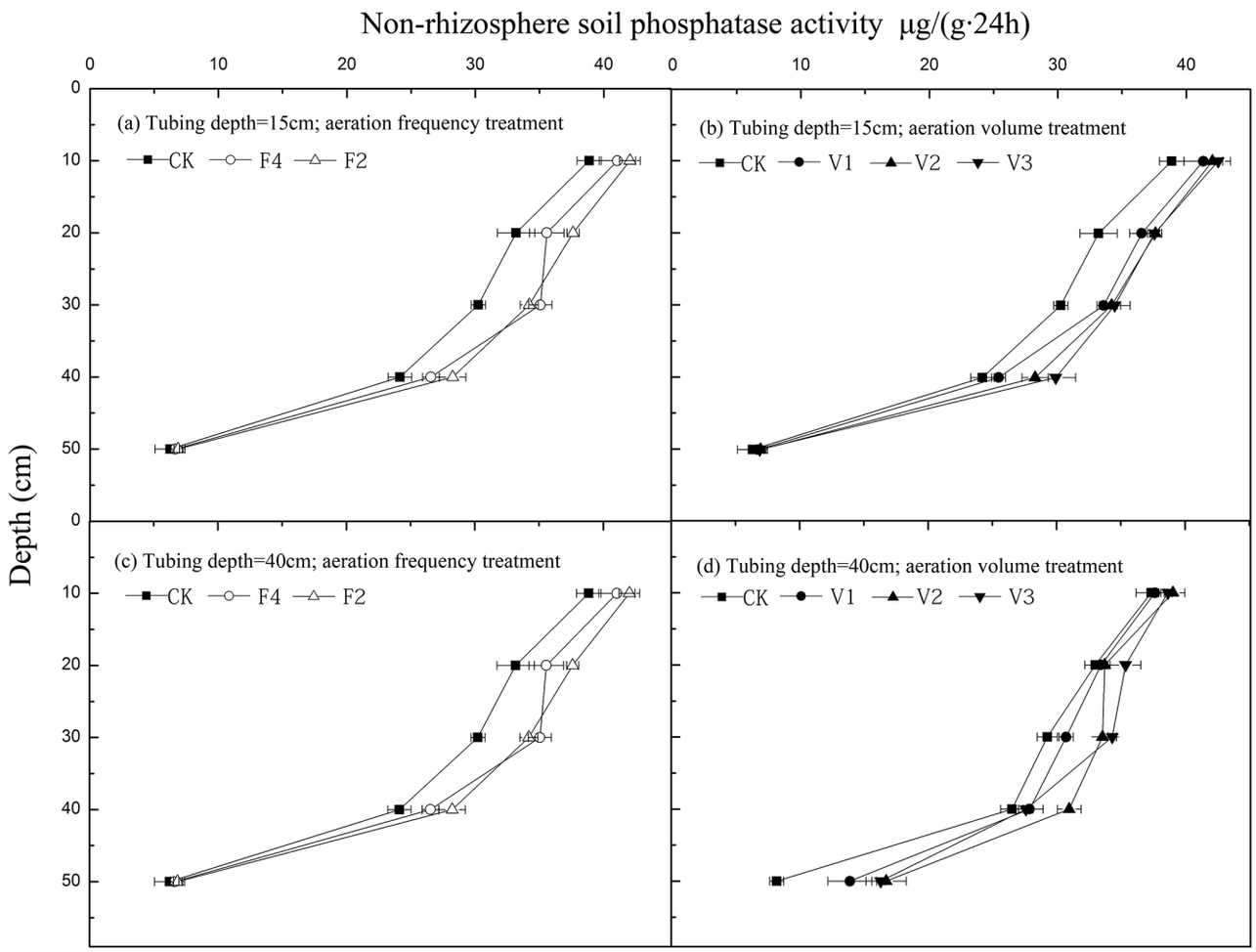

Fig. 3. Non-rhizosphere soil phosphatase activity under different aeration treatments at (a,b) a tubing depth of 15 cm and (c,d) a tubing depth of 40 cm. CK, no aeration; F_2, 2-d aeration interval; F_4, 4-d aeration interval; V_1, tube aeration at 0.5 times the standard volume; V_2, tube aeration at the standard volume; V_3, tube aeration at 1.5 times the standard volume.

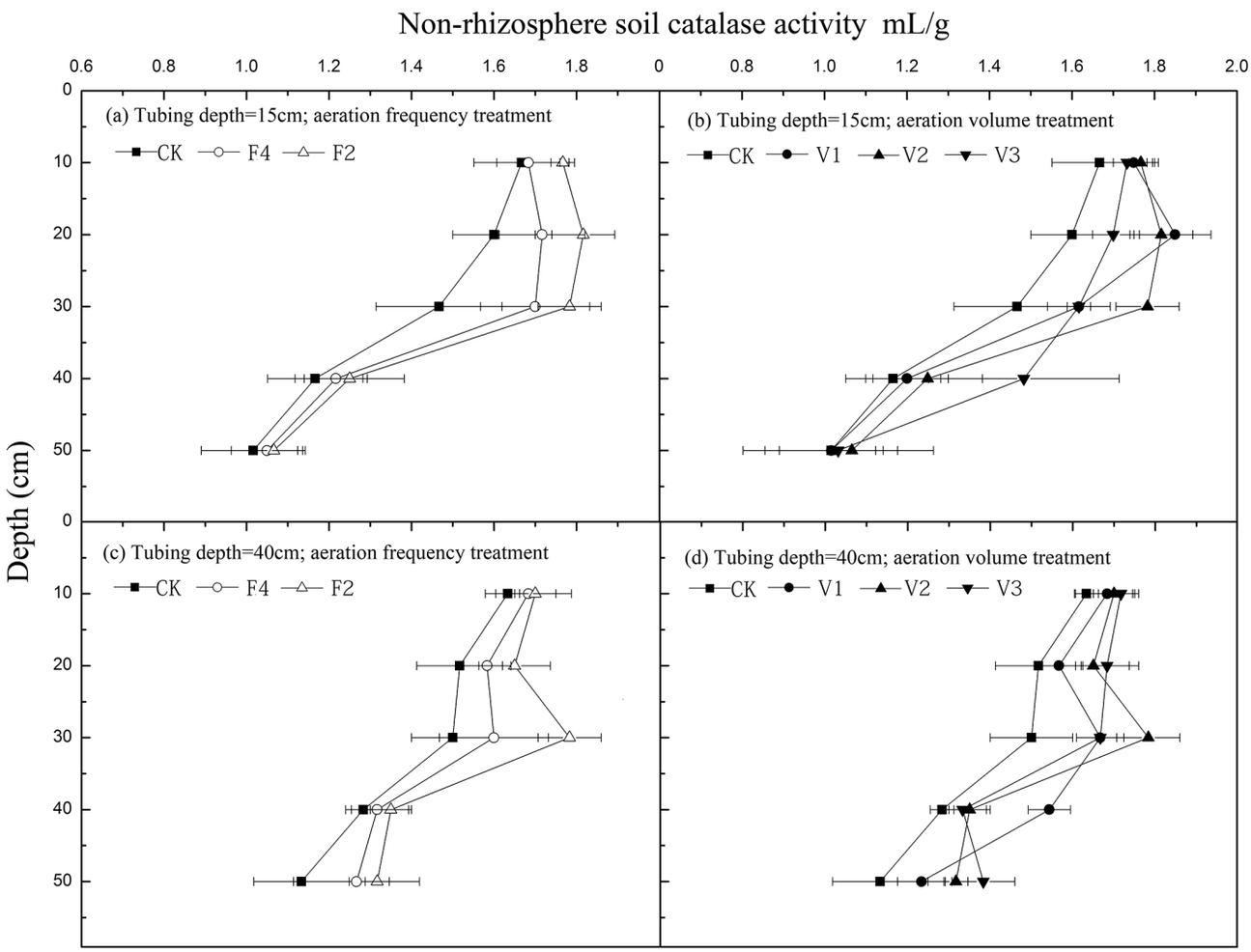

Fig. 4. Non-rhizosphere soil catalase activity under different aeration treatments at (a,b) a tubing depth of 15 cm and (c,d) a tubing depth of 40 cm. CK, no aeration; F_2, 2-d aeration interval; F_4, 4-d aeration interval; V_1, tube aeration at 0.5 times the standard volume; V_2, tube aeration at the standard volume; V_3, tube aeration at 1.5 times the standard volume.

gi and actinomycetes were highest. With a 40-cm burial, fungal abundance was highest in V_2 treatment, whereas the abundance of actinomycetes was highest in V_3. These different results occurred because with a 15-cm burial and high aeration volume, soil air is no longer the primary limiting factor for the abundance of soil microorganisms. The aeration treatment had already eliminated the hypoxic stress in the soil, but further increases in aeration volume increased the flow of air into the soil. High aeration increases soil disturbance and cavitation of the root area (a reduction in root-to-soil contact), which had a negative effect on the rhizosphere environment. Frequent airflow increases the disturbance of microorganisms, and the abundance of microorganisms therefore decreases. However, with a 40-cm burial of the drip irrigation tubes, the air supply location is below the major roots of the plant, and the effects of aeration on soil microorganisms will appear gradually and only with high aeration volumes.

Effect of Artificial Soil Aeration on Rhizosphere Soil Enzyme Activity at Different Growth Periods

Soil enzyme activity is influenced by soil texture, soil moisture, temperature, air availability, the variety of crops, tillage management, and other factors. Soil catalase can promote the breakdown of hydrogen peroxide into water and oxygen, thus reducing the toxicity of hydrogen peroxide to crops. This enzyme is also closely related to soil respiration intensity and microorganismal activities and is an important enzyme in the evaluation of soil fertility (Trasar-Cepeda et al., 1999). Throughout the growth period, the activities of the three enzymes exhibited an initial increase followed by a decrease, primarily because the rhizosphere soil enzyme activities were correlated with the growth stages, which is consistent with previous studies (Niu et al., 2012b). This phenomenon may occur because soil enzymes are secreted by crop roots and rhizosphere microorganisms and because the abundance of rhizosphere microorganisms is affected by root activity. Our previous research (Li et al., 2015a) indicates that tomato root activity exhibited an initial increase followed by a decrease, a similar pattern to that of the activities of the three enzymes in the present study. Moreover, throughout the growth period, at both tube burial depths, the activities of the three rhizosphere soil enzymes increased with increasing aeration frequency, suggesting the lessening of hypoxic stress. Although aeration can significantly increase soil enzyme activity,

Table 5. Rhizosphere soil microorganism abundance under different aeration treatments.

Treatment†	Bacteria				Fungi				Actinomycetes			
	15 cm	40 cm	Mean	*t* test	15 cm	40 cm	Mean	*t* test	15 cm	40 cm	Mean	*t* test
	×10^8 g^{-1}				×10^5 g^{-1}				×10^6 g^{-1}			
Aeration frequency treatment												
CK	3.75b‡	5.54b	4.65b	ns§	3.91b	4.31b	4.11b	ns	2.36b	3.92db	3.14b	*
F_4	5.51b	7.74b	6.63b	ns	5.79ab	6.74b	6.26b	ns	6.38a	8.66a	7.52a	ns
F_2	8.65a	12.16a	10.40a	ns	9.87a	11.49a	10.68a	ns	3.36b	6.67a	5.02b	**
Mean	5.97	8.48	7.22		6.52	7.51	7.02		4.03	6.42	5.23	
Aeration volume treatment												
CK	3.75c	5.54b	4.65b		3.91c	4.31c	4.11c	ns	2.36b	3.92dc	3.14b	*
V_1	6.43b	10.28ab	8.36a		14.81a	9.09b	11.95a	*	8.04a	6.56b	7.30a	ns
V_2	8.57a	12.26a	10.42a		10.22b	11.47a	10.85ab	ns	3.55b	6.65b	5.10ab	**
V_3	8.41a	10.06ab	9.24a		5.75c	9.80ab	7.77b	*	3.46b	8.96a	6.21a	**
Mean	6.81	9.51	8.16		8.59	8.67	8.63		4.31	6.53	5.42	
F-value												
Aeration frequency (F)	12.414**	8.968*			5.564*	13.597**			20.572**	13.250**		
Aeration volume (V)	18.964**	0.461ns			18.241**	2.976ns			14.398**	4.875ns		
F × D	ns				ns				ns			
V × D	ns				**				**			

* Significant at the $P \leq 0.05$ level.

** Significant at the $P \leq 0.01$ level.

† CK, no aeration; D_{15}, subsurface tubing placement depth at 15 cm; D_{40}, subsurface tubing placement depth at 40 cm; F_2, 2-d aeration interval; F_4, 4-d aeration interval; V_1, tube aeration at 0.5 times the standard volume; V_2, tube aeration at the standard volume; V_3, tube aeration at 1.5 times the standard volume.

‡ Values followed by lowercase letters within columns are significantly different at $P < 5\%$.

§ Not significant (ANOVA *F*-value for main and interaction effects).

hypoxic stress persists, even 2 d after aeration, and increasing the soil air content can thus effectively increase soil enzyme activity. However, soil enzyme activity did not increase with increased aeration volume. With a 15-cm tube burial and V_2 aeration volume or with a 40-cm burial and V_3 aeration volume, soil urease and phosphatase concentrations were greatest. The soil air requirements of catalase are somewhat different from those of soil urease and phosphatase. During the seedling and blooming and setting periods, catalase activity was greatest with a 15-cm burial and V_1 aeration volume. During the maturation period, catalase activity was greatest with a 40-cm burial and V_2 and with both burial depths and V_3. These results suggest that aeration volume is not the primary limiting factor for increases in rhizosphere soil enzyme activity. On the contrary, over-aeration can have a negative effect on soil enzyme activity.

Effect of Artificial Soil Aeration on Non-rhizosphere Soil Enzyme Activity at Different Soil Depths

Rhizosphere soil enzyme activity can indirectly affect non-rhizosphere soil enzyme activity. In addition, plant litter and soil parent materials affect non-rhizosphere soil enzyme activity (Xue et al., 2002). Aeration had a significant impact on rhizosphere soil enzyme activity, and the change in rhizosphere soil enzyme activity indirectly affected non-rhizosphere soil enzyme activity. Burial of the drip irrigation tubes at 15 cm increased non-rhizosphere urease activity at soil depths of 20 and 30 cm and non-rhizosphere phosphatase and catalase activities at soil depths of 20 to 40 cm. Tube burial at 40 cm and aeration treatment both increased the non-rhizosphere activities of the three enzymes at soil depths from 10 to 50 cm. This difference may have occurred because the change in aeration during the experiment stimulated plant roots, and the resulting root exudates directly affected rhizosphere soil enzyme activity; this change in rhizosphere soil enzyme activity indirectly affected non-rhizosphere soil enzyme activity. Moreover, although non-rhizosphere soil enzyme activity affects soil nutrient circulation, the increased distance from plant roots weakens their effect on crops.

Analysis found no significant correlation between rhizosphere and non-rhizosphere soil enzyme activities ($p > 0.05$; data not shown), likely because the experimental treatments changed the existing soil air environment and directly affected the physiological activities of crop roots rather than the surrounding soil. Because soil enzymes are secreted mainly by plant roots, the experimental treatments had a significant impact on rhizosphere soil enzyme activity. In contrast, the impact of experimental treatments on non-rhizosphere soil enzymes occurs mainly through the indirect effect of the changed rhizosphere soil enzymes in a process that is relatively slow. Therefore, the correlation between rhizosphere and non-rhizosphere soil enzyme activities was not significant.

Effect of Artificial Soil Aeration on Tomato Yield

In farmland ecosystems, soil enzymes can decompose organic substances in the soil into fast-acting fertilizers that can

be utilized by plants; such enzymes participate in almost all biochemical reactions in the soil and are important indicators for the evaluation of soil quality and fertility (Wang et al., 2008; Zhang and Wang 2006). In the present study, aeration volume and aeration frequency significantly increased tomato yield (Fig. 6), consistent with previous studies that reported that improving of the soil air condition can increase crop yield (Bonachela et al., 2010; Ityel et al., 2014; Niu et al., 2012a, 2012b, 2013; Shahien et al., 2014). Artificial soil aeration improved crop root growth environment and nutrient uptake in the roots, thus promoting plant growth and increasing yield. Fungi are important for plant acquisition of soil resources, especially soil nitrogen, phosphorous, and water, and inhibit pathogens, increasing overall plant growth and fitness (Burke et al., 2011; Smith and Read, 2008), and actinomycetes are important contributors to soil phosphatase activity (Ghorbani-Nasrabadi et al., 2013). The soil environment changes under artificial soil aeration treatment and oxygen and carbon dioxide concentration in soil, which further change the metabolic activity of microorganisms; thus, soil urease, phosphatase, and catalase activity increase, especially for rhizosphere soil enzymes. The present study also found that with a 15-cm tube burial, excessive aeration volumes decreased tomato yield, probably because higher aeration increased the airflow in the soil, thus increasing cavitation and other factors that have a negative effect on plant roots. When the irrigation tube burial is shallow, less aeration produces a higher yield; however, with deeper tube burial, more aeration produces a higher yield. At soil depth of 15 cm, tomato roots are abundant and air application is to the main root area of the plant; thus, aeration can alleviate hypoxic stress. However, a large aeration volume or an excessively high aeration frequency increases disturbance to the soil in the root area, which is not conducive to increasing tomato yield (Niu et al., 2012a). In contrast, the pattern is opposite when the drip irrigation tubes are buried at 40 cm. In this case, the soil is an open medium, and the main root area is above the aeration position; thus, the effect of the air on the roots and rhizosphere soil enzymes is not as direct as with a 15-cm tube burial. Therefore, a small amount of aeration can to a certain extent improve the oxygen environment in the root area, but the benefit is not as strong as with a 15-cm burial. With high aeration frequency or large aeration volume at a soil depth of 40 cm, because the air uses the soil medium as the buffer and the air diffuses from the 40-cm soil depth to the root area, the airflow in the root area is much slower than with a 15-cm tube burial. At the same time, aeration largely relieves the hypoxic stress in the root area. Thus, with a 40-cm burial, large-volume aeration or high-frequency aeration can more significantly increase the plant yield.

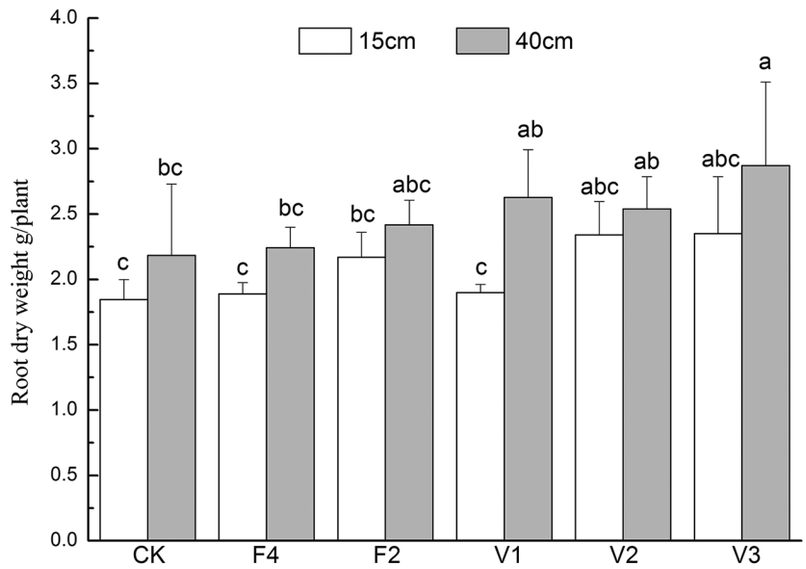

Fig. 5. Root dry weight for different aeration treatments. Bars with the same letter are not significantly different at $P \leq 0.05$.

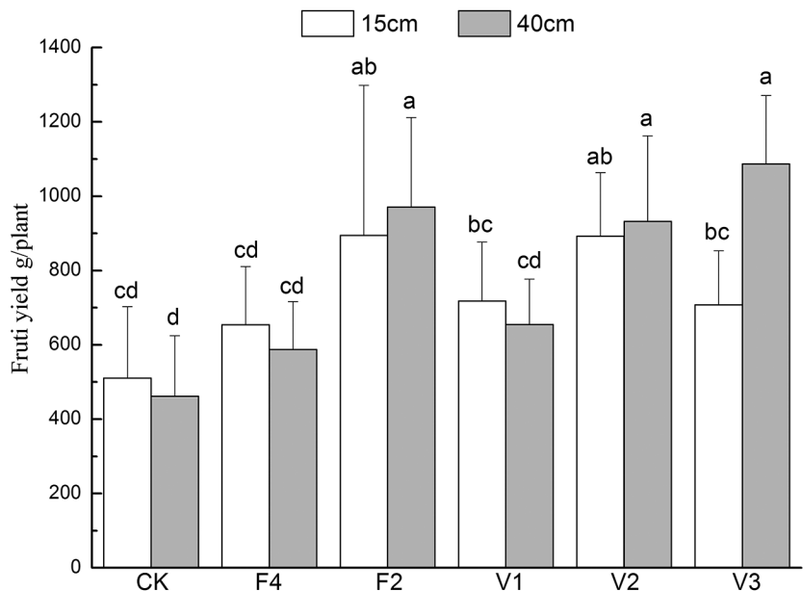

Fig. 6. Fruit yield under different aeration treatments. Bars with the same letter are not significantly different at $P \leq 0.05$.

CONCLUSIONS

Across the entire growth period, all three rhizosphere soil enzymes exhibited an initial increase in activity followed by a decrease. Soil aeration can enhance the activities of the three rhizosphere soil enzymes. Both the aeration frequency and aeration volume can significantly affect the activities of the three rhizosphere enzymes. Aeration treatment can also enhance the activities of non-rhizosphere soil enzymes, but it has a smaller effect on these activities than on rhizosphere soil enzyme activity.

Soil aeration can increase tomato yield. The first-picked fruit yield increased with increasing

aeration frequency, and one aeration event every 2 d increased the yield by 91.8% compared with the control. With a 40-cm burial of drip irrigation tubes, 1.5 times the standard aeration volume produced the highest first-picked fruit yield, which was increased by 135.5% relative to the control group. Meanwhile, with a 15-cm burial, the standard aeration volume produced the highest first-picked fruit yield, which was increased by 75.1% compared with the treatment without aeration.

Rhizosphere soil enzyme activities and the abundance of rhizosphere soil microorganisms have an extremely significant positive correlation with crop yield. Non-rhizosphere soil enzyme activities have a much weaker correlation with tomato yield.

ACKNOWLEDGMENTS

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