Subsurface aeration alters the fungal composition of rhizosphere soil and tomato plant performance in Northwest China

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Abstract: Rhizosphere hypoxia constrains plant growth, and numerous studies have shown that root zone aeration accelerates plant photosynthesis and growth and increases crop yields. Nevertheless, the mechanism by which soil microorganisms are involved in this process is not clear. The purpose of the present study was to examine the effects of aeration and irrigation depth on the composition and structure of rhizosphere soil fungal communities and tomato plant performance. The amount of aeration assayed was equal to 0 (CK), 0.5 (V1), 1 (V2), and 1.5 (V3) times the porosity of the soil. The two depths of subsurface drip irrigation used were 15 (D15) and 40 cm (D40). The results demonstrated that soil aeration not only increased tomato plant performance but also influenced fungal diversity and composition. Compared to the no-aeration treatment, the V3 soil aeration treatment increased the total dry weight and fruit yield by 39.9% and 65.6%, respectively. The results also showed that the abundance of the phylum Ascomycota and the family Lasiosphaeriaceae increased with increasing soil aeration, whereas those of members of the phylum Zygomycota and the order *Capnodiales* decreased with increasing soil aeration. Moreover, the variation in subsurface irrigation depth altered the rhizosphere soil fungal community. In general, the results of this study demonstrate that root zone aeration can ameliorate hypoxic conditions in Lou soils and is beneficial to soil fungal communities and tomato plant performance.

Keywords: tomato, soil aeration, rhizosphere soil fungal community, plant performance
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1 Introduction

Subsurface drip irrigation systems can be used to optimize soil water availability, accessibility, and use efficiency for maximum growth and marketable yields. The use of subsurface drip irrigation under protective structures to produce high-value vegetables for fresh food markets is increasing in the semiarid areas of Northwest China. Compared to spray-type and other widespread irrigation techniques, these production techniques significantly improve water-use efficiency (WUE)[1,2]. Agricultural producers can vary the depth of the subsurface drip irrigation line, the irrigation amount, and the irrigation frequency to manage the shape and size of the soil infiltration pattern and to optimize soil water availability for plant roots[3,4]. With respect to the requirements of precision irrigation, a subsurface drip irrigation system can efficiently improve WUE by reducing percolation, surface runoff, and evaporation.

Although irrigation provides water for crops, it displaces air from the soil and can result in water logging problems worldwide[5]. Most O_2 supplies are obtained directly by continuous diffusive air exchange between the atmosphere and soil[6]. A relatively high soil water content around the root zone increases the permeability of the soil, which leads to an unavoidable reduction in the diffusion rate of O_2[7]. Thus, in terms of the gas phase, irrigation inhibits plant growth. When roots are trapped in wet soils under various irrigation regimes, hypoxia is almost certain to occur, especially during periods after irrigation in clay/heavy soils, in soils with poor drainage, and in soils that are over-irrigated. Crop yields, shoot, and root growth, and the quality of products can be significantly reduced by rhizosphere hypoxic stress[8,9]. Moreover, rhizosphere hypoxia reduces the level and availability of O_2, which is continuously needed as an electron acceptor in the tricarboxylic acid metabolic cycle, for adenosine triphosphate (ATP) production, and for normal root cell activity[10-12], and reduces root activity, mineral element uptake, and absorption of water and nutrients[13]. Gao et al.[14] reported that rhizosphere hypoxia significantly increases enzymatic activity, such as that of glutamate synthase and nitrate. This phenomenon also affects changes in metabolite levels, such as ethanol, nitrate, ammonium, and H_2O_2. Kuzyakov and Cheng[15] reported that oxygen deficiency decreases root membrane exclusion, which could result in toxic levels of salt accumulation in plants. Oxygen deprivation leads to the destruction of mitochondria and proteins in root cells, leading to cytoplasmic acidosis and the inhibition of plant growth[16]. Studies have demonstrated the negative effects of O_2 deficit in the root zone on plant root and shoot growth, soil respiration, stomatal conductance, and nutrient uptake[17,18].

Multiple techniques can effectively ameliorate the soil

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environment by increasing the oxygen concentration in the root zone. The chief advantages of soil aeration techniques include being economical, easily implemented, and pollution free and the ability to exert marked effects, especially when injected air is applied via the same subsurface drip irrigation line. Recent studies have shown that the application of this technique can significantly improve crop and fruit yields, quality, and irrigation WUE[19,20]. Similar studies in semiarid regions in Northwest China have indicated that aerated irrigation or supplemental soil aeration can improve soil enzyme activities, plant root activity, plant physiological functions, WUE, yield, and quality of crops[21-24].

Soil microbial community structure is crucial to sustainable agroecosystems, in which soil fungi are especially important elements. Soil fungi are involved in a wide range of soil biochemical processes, including the soil carbon cycle, nitrification, denitrification, and pathogenesis[25-27]. Plant health and crop yield significantly depend on microbial composition and activity in the rhizosphere. Some fungi affect nutrient availability in soil, and plant-microbe partnerships can improve stress tolerance in the host plant. Many important agricultural crops, such as maize, potato, sunflower, wheat, and soybean, benefit from fungi, especially under nutrient-limiting conditions, since extensive hyphal networks in the soil improve the efficient uptake of orthophosphate and other minor nutrients[26]. In addition, soil microbes can also be used to predict the health of agroecosystems, including the performance of plants or entire crops[28,29]. Soil fungal composition and diversity play an important role in maintaining the balance of ecosystems via modulation of the biogeochemical cycle.

Horticultural plants are exposed to a variety of environmental stresses that negatively influence their growth, development, and performance. Moreover, plant growth and development can be restricted by the inhibition of several physiological and microbiological processes. Tomato (*Lycopersicon esculentum* Mill.) plants are an important source of food in traditional Chinese food dishes, with tomato fruits having been shown to be associated with a lowered risk of digestive system disease, angiocardiopathy, and so on. Extensive research published in the last decade alone demonstrates that the positive role of tomato fruits on human health is highly correlated with lycopene and vitamin C[30,31].

Many studies have focused on the effects of soil aeration on plant growth and yield. However, few studies have investigated the effects of soil aeration and the burial depth of subsurface lines on rhizosphere soil fungal communities and fungi. How might soil aeration influence soil fungal composition and structure? Unfortunately, there are no recent field investigations on these phenomena involving soil fungi. Furthermore, it remains unknown whether there is a correlation between soil fungi and tomato plant performance under different soil aeration practices. However, such information has important reference and practical value for producers, researchers, and consumers, especially in heavy soils with unfavorable drainage conditions. It was hypothesized that varying the depths of subsurface drip irrigation tubes and aeration volume might affect the fungal composition, abundance, and plant growth. Using high-throughput sequencing, this study investigated the effects of soil aeration volume and burial depth of subsurface lines on the soil fungal composition structure and diversity in the root zone of tomato plants and further analyzed the relationships between the soil fungal communities and tomato plant performance. It is expected that some negative growth effects from irrigation applications could be offset by soil aeration delivered via drip irrigation lines placed at a given depth in the soil.

2 Materials and methods

2.1 Site description and soil properties

The experiment was performed in Yangling (34°17'N, 108°02'E), Shaanxi Province, Northwest China, from October 2014 to May 2015. The tested tomato cultivar was "Fenyuyanggang", the seeds of which were obtained from New Horizon Facilities Agricultural Development Co., Ltd. The average annual cumulative sunshine duration was 2165 hours. The tested soil was a Lou silty clay loam, the texture classification of which was as follows: gravel (2.00-0.02 mm), 25.4%; silt (0.020-0.002 mm), 44.0%; and clay (<0.002 mm), 30.6%. The average values of the physical and chemical properties of the soil within a depth of 0-60 cm were as follows: bulk density of 1.35 g/cm^3; field capacity (mass water content) of 28.18%; soil porosity of 49.38%; pH 7.83; soil organic matter of 9.5 g/kg; total nitrogen of 1.3 g/kg; and total phosphorus of 1.4 g/kg.

2.2 Experimental design and treatments

The experimental setup of the tomato plants was designed such that the effects on soil fungal diversity and community composition under four levels of soil aeration volume in combination with two levels of subsurface drip irrigation depth could be investigated. The experiment was limited to 8 treatments arranged as a randomized complete block design with 2 factors, namely, D15V1, D15V12, D15V3, D15V4, D40V1, D40V2, D40V3 and D40V4 (Table 1). Detailed planting information is specified in Figure 1. The primary drip irrigation pipe was connected to an air pump, and water and air were supplied to the soil through the subsurface drip irrigation tubes (Figure 1a). There was only one subsurface drip irrigation line (φ16 subsurface drip irrigation pipe, Qinchuan Water-saving Irrigation Equipment Engineering Co., Ltd., Yangling, China) under each row of plants. The depths of the subsurface drip irrigation were 15 cm or 40 cm, defined as D15 or D40, respectively (Figure 1b). As subsurface drip irrigation delivers materials belowground, this practice can ensure that the same volume of water and air are delivered into the ground. Two subsurface drip irrigation lines were laid in each plot; the spacing between the drippers was 30 cm, and the spacing between the drip irrigation lines was 40 cm. Each plot was raised with 2 ridges and was 550 cm in length and 150 cm wide. The ridge's total width was 60 cm, the inside slope width was 20 cm, and the furrow width and depth were 30 cm and 20 cm, respectively. To prevent the lateral spread of air and water into adjacent treatments, the plots were separated from each other by a 1.5 m wide empty space. In addition, 0.8 m deep tarps were buried vertically between the plots. The tomato plants were transplanted onto the middle of the slope (Figure 1b). Traditional subsurface drip irrigation and aeration with 0.5, 1, and 1.5 times the standard aeration volume were defined as CK, A1, A2, and A4, respectively. The airflow rate for each plot was 10.2 L/min, regulated by a switch and flowmeter. The aeration frequency was once every two days. The standard volume of aeration was calculated as the volume of soil porosity[32].

Table 1 Transposed design matrix for the 8 treatment combinations

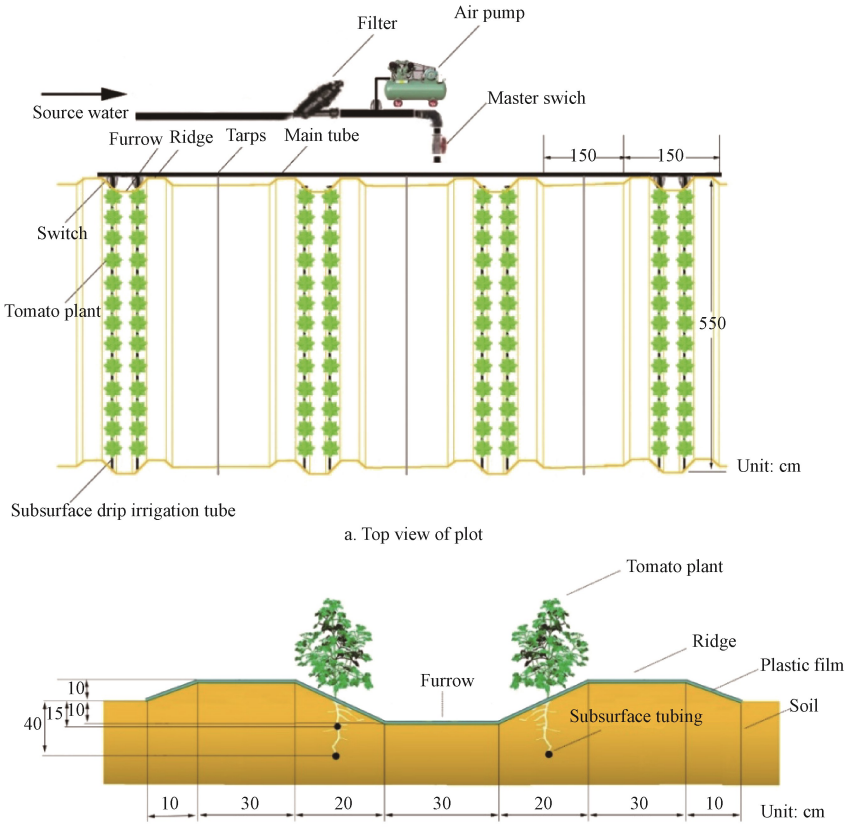
Treatment	Sub-surface tubing placement depth/cm	Soil aeration
D15 CK	15	no aeration
D15 V1	15	aeration at 0.5 times the standard volume
D15 V2	15	aeration at the standard volume
D15 V3	15	aeration at 1.5 times the standard volume
D40 CK	40	no aeration
D40 V1	40	aeration at 0.5 times the standard volume
D40 V2	40	aeration at the standard volume
D40 V3	40	aeration at 1.5 times the standard volume

The standard volume of aeration (V_a) was calculated according to Equation (1).

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

where, V_a is the calculated volume of standard aeration (407.8 L/plot), S is the cross-section of the targeted area (1500 cm^2), L is the length of the plot (550 cm), ρ_b is the local soil bulk density (1.34 g/cm^3), and ρ_s is the soil particle density (2.65 g/cm^3).

All the plants were treated with the same agronomic management practices before transplantation. The seed bed is composed of solid digestate, vermiculite, perlite, and activated carbon at a ratio of 16:1:2:1. Twenty days after the seeds were sown, 26 plugs of tomato plants were transplanted into the experimental plots, spaced 40 cm apart (each plant corresponded to a subsurface dripper). The field was direct rotary tillage at a depth of 25 cm before transplanting, and fertilizer was broadcast uniformly to the soil. The relevant fertilizer information is as follows: farmyard manure (pig manure), 120 t/hm^2; diammonium hydrogen phosphate (18% nitrogen and 46% P_2O_5), 1.5 t/hm^2; and nitrogen-phosphorus-potassium composite fertilizer (total nitrogen, 18%; total phosphorus, 6.5%; and total potassium, 8.4%), 0.4 t/hm^2. The amount and timing of irrigation were primarily based on specific climatic conditions, plant growth conditions, and experienced farmers' perceptions. In total, 235 mm of irrigation was provided during the entire growth period for each plot. The techniques of other conventional greenhouse management practices (such as agricultural spraying, crop pollination, branch pruning, and pest control) for all plots were consistent with local production practices. Each treatment was replicated 3 times.



Note: There is only one subsurface drip irrigation line under each row of plants. The depths of the subsurface drip irrigation line were 15 or 40 cm.

Figure 1 Example of an experimental arrangement of a block

2.3 Measurements

2.3.1 Plant measurements

Double row planting was adopted in each plot. Three plants were labelled in each plot, where the first labelled plant was located approximately at the middle of the row, and the other two labelled plants were at the nodes of one-third of another row. During the period of 154-214 d after transplanting (DAT), all marketable tomato fruits were harvested from each labelled plant and weighed. At 214 DAT (after harvest), the roots (those reaching a depth of 70 cm and extending 60 cm around the plant) were dug up from the ground, and any soil particles attached were removed via a sieve and water. All the aboveground parts (stems, leaves) and roots of the tomato plants were subsequently dried in an oven. Root, stem, and leaf dry weights were then measured for 3 labelled tomato plants from each plot.

2.3.2 Soil sample measurements

1) Sample collection

After the fruits were harvested, picks and shovels were used to excavate the tomato plant roots and soil near the roots with an approximately 10-20 cm depth of the soil. The soil near the roots was shaken off, and a soft brush was used to collect the rhizosphere soil. Three rhizosphere soil samples were selected in each plot at the labelled plants. Each soil sample weighed approximately 10 g.

2) DNA extraction and high-throughput sequencing

Total genomic DNA was extracted from a 0.25 g soil sample using a FastDNA® SPIN Kit for soil (MP Biomedicals, Solon, CA, USA). A NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, USA) was used to determine the concentration of DNA, and agarose gel electrophoresis (1%) was used to assess the quality of the DNA. The sequences of primers used to amplify the internal transcribed spacer (ITS) sequence were

as follows: forward primer ITS1 region, 5'-CTTGGTCATTTAGAGGAAGTAA-3', and ITS2-2043R, 5'-GCTGCGTTCTTCATCGATGC-3'[33]. Polymerase chain reaction (PCR) amplification was performed in 20 μL reactions comprising rTaq polymerase (0.2 μL), buffer (2 μL), bovine serum albumin (BSA) (0.2 μL), template DNA (10 ng), forward and reverse primers (0.8 μL each), dNTPs (2 μL) and ddH_2O to 20 μL. PCR amplification followed a specific thermal program: 94°C for 5 min; 35 cycles of 94°C for 40 s, 55°C for 30 s, and 72°C for 45 s; and a final extension at 72°C for 10 min. The resulting PCR products were excised from an agarose gel (2%), purified with an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and then quantified with a QuantiFluor®-ST Fluorometer (Promega, USA). The resultant amplicons were paired-end sequenced on an Illumina MiSeq™ System (Illumina Inc., San Diego, CA, USA) by Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China[34].

3) Sequencing data and analysis

Raw fastq files were spliced using FLASH. After they were compared to the data within the Genomes OnLine Database, valid tag data were obtained by removing any detected chimeric sequences[35]. Representative fungal operational taxonomic unit (OTU) sequences were clustered into OTUs using the UPARSE pipeline version 7.0 (with an identity threshold of 97%).

2.4 Statistical analysis

Statistical analysis was performed with SPSS 22.0 (IBM Corp., New York, NY, USA), and differences were compared by Duncan's test and considered significant at a level of 0.05. Fungal sequences and abundance, the diversity of the rhizosphere soil, and plant performance were analyzed by ANOVA, and the primary effects (aeration treatment and burial depth) and interactions were analyzed. Pearson correlations between plant performance and rhizosphere soil fungal communities were calculated at the phylum and genus levels, and rarefaction curves were calculated with Mothur[36]. Principal component analysis (PCA) plots, redundancy analysis (RDA) plots, and Venn diagrams were generated on the basis of the relative abundance data. The diversity was subsequently analyzed with the I-Sanger platform (https://www.i-sanger.com).

3 Results

3.1 Plant performance under different aeration and emitter depth treatments

The ANOVA results showed that soil aeration could significantly improve tomato plant performance by increasing leaf dry weight (Table 2). In addition, soil aeration had a significant effect on aboveground dry weight, total dry weight, and tomato yield. However, the results showed that the variation in aeration volume did not markedly affect the root dry weight, stem dry weight, or root/shoot ratio. The burial depth and interaction effects (soil aeration × different line depths) were not significant for any of these variables. It was also determined that at a subsurface drip irrigation depth of 15 cm, the variation in stem, leaf, and total dry weight and in fruit yield tended to initially increase but then decreased with increasing soil aeration volume (V2>V3>V1>CK). However, at the 40 cm subsurface drip irrigation depth, the results showed that the root, stem, and total dry weight and the fruit yield increased with increasing aeration volume and that the maximum root, stem, and total dry weight and the maximum fruit yield occurred in the V3 treatment. Fruit yields increased from 711.7 to 1461.5 g/plant when the aeration scheduling changed from CK to V3, with D15CK and D40CK presenting the lowest fruit yields and D40V3 presenting the greatest fruit yields. Without considering the subsurface drip irrigation depth, compared to the no-aeration treatment, the V3 treatment increased the total dry weight and fruit yield by 39.9% and 65.6%, respectively.

Table 2 Tomato plant performance under different aeration and emitter depth treatments

Treatments	Root dry weight /(g·plant^{-1})	Stem dry weight /(g·plant^{-1})	Leaf dry weight /(g·plant^{-1})	Total dry weight /(g·plant^{-1})	Root/shoot ratio	Aboveground dry weight	Fruit yield /(g·plant^{-1})
D15CK	1.8±0.2^{c}	38.1±6.6^{b}	21.0±7.5^{c}	61.0±13.6^{c}	0.033±0.010^{ab}	59.1±13.7^{c}	808.7±182.5^{cd}
D15V1	1.9±0.1^{bc}	43.1±7.1^{b}	22.3±5.2^{c}	67.3±2.4^{bc}	0.029±0.002^{ab}	65.4±2.5^{bc}	1038.9±255.5^{bc}
D15V2	2.3±0.3^{abc}	63.2±13.8^{a}	39.3±4.4^{a}	104.8±18.1^{a}	0.023±0.003^{b}	102.5±17.9^{a}	1286.1±307.9^{ab}
D15V3	2.4±0.4^{abc}	49.8±8.1^{ab}	36.2±11.0^{ab}	88.3±9.4^{ab}	0.028±0.006^{ab}	86.0±9.4^{ab}	1056.6±210.0^{bc}
D40CK	2.2±0.5^{abc}	37.1±6.4^{b}	21.5±5.2^{c}	60.8±10.9^{c}	0.039±0.014^{ab}	58.6±11.1^{c}	711.7±238.0^{d}
D40V1	2.6±0.4^{ab}	40.8±10.1^{b}	23.4±7.6^{c}	66.8±10.6^{bc}	0.042±0.012^{a}	64.1±11.0^{bc}	993.9±176.6^{c}
D40V2	2.5±0.2^{abc}	41.2±15.4^{b}	26.8±4.9^{bc}	70.5±18.9^{bc}	0.039±0.009^{ab}	67.9±18.9^{bc}	1366.8±466.7^{a}
D40V3	2.9±0.6^{a}	52.6±6.4^{ab}	26.6±3.1^{bc}	82.1±8.5^{abc}	0.036±0.005^{ab}	79.2±7.9^{bc}	1461.5±144.2^{a}
			F-value				
Aeration volume (*V*)	1.660^{ns}	2.261^{ns}	5.120*	4.298*	0.329^{ns}	4.175*	14.732**
Depth (*D*)	0.065^{ns}	0.060^{ns}	0.240^{ns}	0.156^{ns}	0.001^{ns}	0.144^{ns}	0.402^{ns}
V×*D*	0.036^{ns}	0.318^{ns}	3.096^{ns}	0.545^{ns}	0.332^{ns}	0.544^{ns}	0.488^{ns}

Note: The data are shown as the means±standard deviations. Aeration treatment means at each depth of the drip irrigation line not followed by the same letter within a column are significantly different at 0.05 level. The asterisk indicates a significant difference in irrigation means (*, $p \leq 0.05$, **, $p \leq 0.01$); otherwise, the differences between means are not significant (ns). ANOVA *F*-values for the main and interaction effects were not significant (ns) or significant at 0.05 (*) and 0.01 levels (**).

3.2 Composition and structure of soil fungal communities in the tomato root zone

All the soil DNA sequences were subsamples collected on the basis of the fewest number of DNA sequences. Figure 2 shows the rarefaction curves used to standardize the number and richness of detected OTUs among samples on the basis of the 97% similarity threshold for OTUs. The distribution of fungal phyla and genera in response to the four soil aeration volumes and two burial depths is shown in Figure 3. The dominant phylum in the rhizosphere soil samples under the combination of each aeration and subsurface line depth was Ascomycota (33.98%-88.37%, average 68.22%), followed by Zygomycota (4.35%-58.38%, average 20.54%) and unclassified_k_Fungi (4.2%-26.9%, average 9.9%), all of which accounted for >98% of the total fungal sequences. Moreover, it was observed that the abundance of the phylum Zygomycota decreased with increased soil aeration volume and that the abundance of the phylum Ascomycota increased with increasing soil aeration volume. As shown in Figure 3b, the relative abundances (>0.01% precision) of fungi (at the genus level) within the root zone soil of the tomato plants were analyzed in the various subsurface line depth and aeration volume treatments. The dominant genera were *Mortierella*, Penicillium, unclassified_k_Fungi,

and *Fusarium*, all of which accounted for >50% of the total fungal sequences. Among these most abundant genera, the relative abundance of *Mortierella* was low in the soil aeration treatment, while that of *Penicillium* and *Fusarium* was high.

Hierarchical heat map analysis of the dominant fungal genera also produced similar results (Figure 4). As shown in Figure 4, the fungal communities among the subsurface line depth and aeration volume treatments could be divided into three groups (Figure 4), with the first, second, and third groups comprising the D15CK and D40CK treatments, the D15V3, D40V2, and D40V3 treatments, and the D15V1, D15V2, and D40V1 treatments, respectively. These results indicate that both soil aeration and subsurface line placement depth treatments can alter the fungal community compositions.

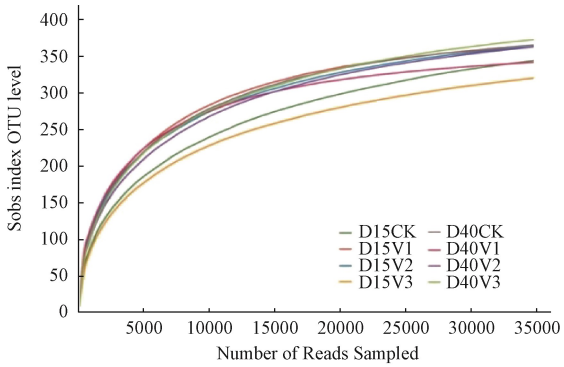
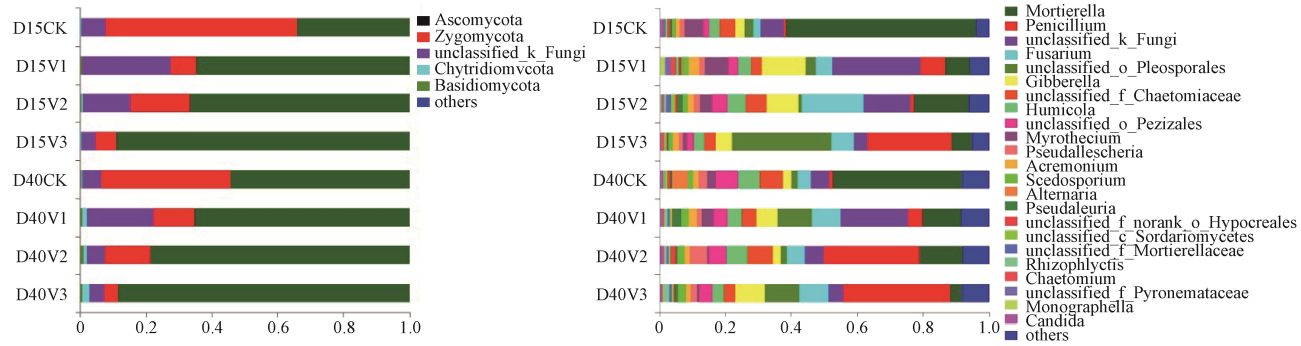
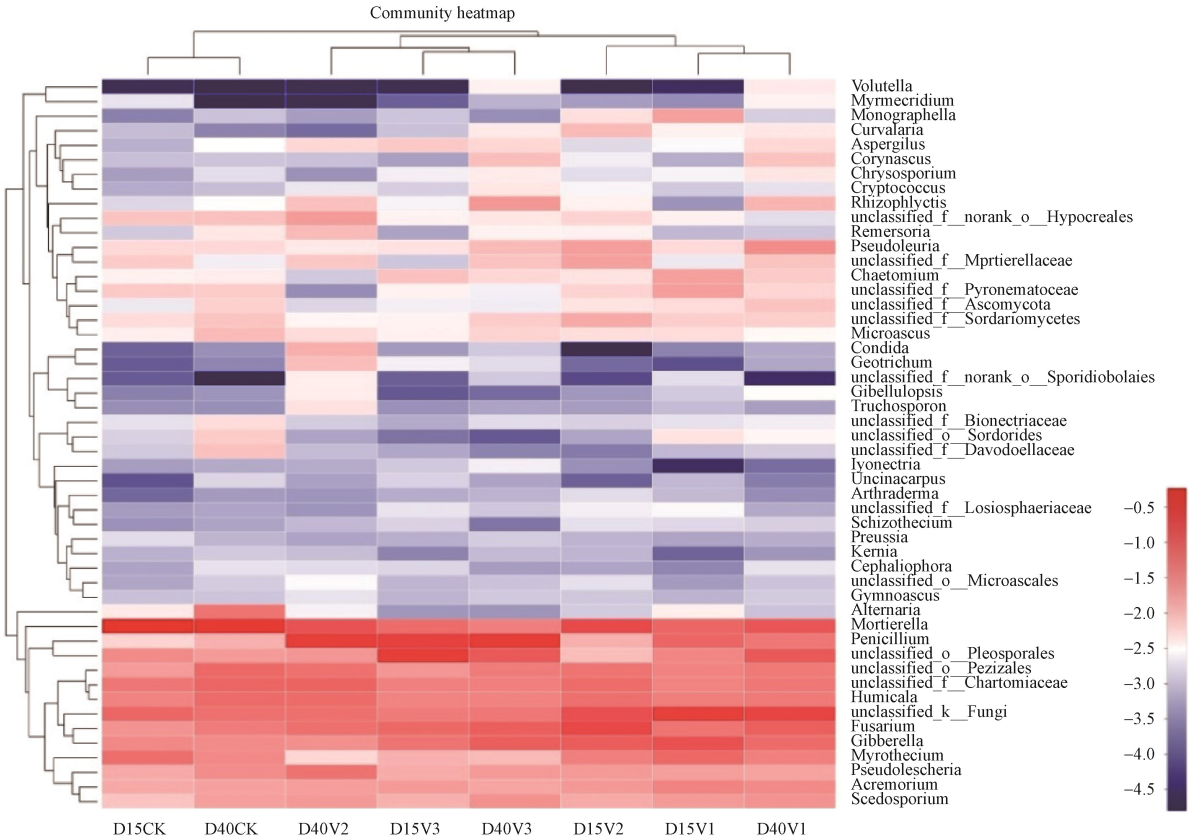


Figure 2 Rarefaction curves of the OTUs from the eight treatments at a 97% sequence similarity



a. Percent of community abundance on Phylum level b. Percent of community abundance on Genus Level

Figure 3 Fungal taxonomic classification at the phylum and genus levels in different soil treatments, i.e., soil aeration and subsurface line placement depth treatments



Note: The heatmap and clustering were computed and performed on the basis of the OTUs. The relative values are indicated by color intensity, with the legend shown in the bottom right corner.

Figure 4 Heatmap of the dominant genera of soil fungi and cluster analysis of fungal community compositions across the different soil aeration and subsurface line placement depth treatments

Four-way Venn diagrams were constructed to show the unique and shared genera and OTUs among the different aeration treatments (Figure 5). The Venn diagrams showed that the distribution of genera and OTUs among all the treatments consisted of 95 genera and 266 OTUs (Figure 5b). As such, all the aeration treatments shared 40.60% of the same genera and

30.30% of the same OTUs, suggesting that 40.60% of fungal genera were present in all treatments. In total, 23, 16, 23, and 44 genera were unique to CK, V1, V2, and V3, respectively, and 87, 69, 144, and 68 OTUs were unique to CK, V1, V2, and V3, respectively. The shared and unique fungal genera and OTUs in the subsurface line depth treatments are shown in Figure 5. The distribution of genera and OTUs among the different subsurface line depth treatments revealed 149 genera and 489 OTUs, constituting 63.67% and 55.69% of the total sequences, respectively. In total, 34 and 51 genera were unique to D15 and D40, respectively, and 163 and 226 OTUs were unique to D15 and D40, respectively.

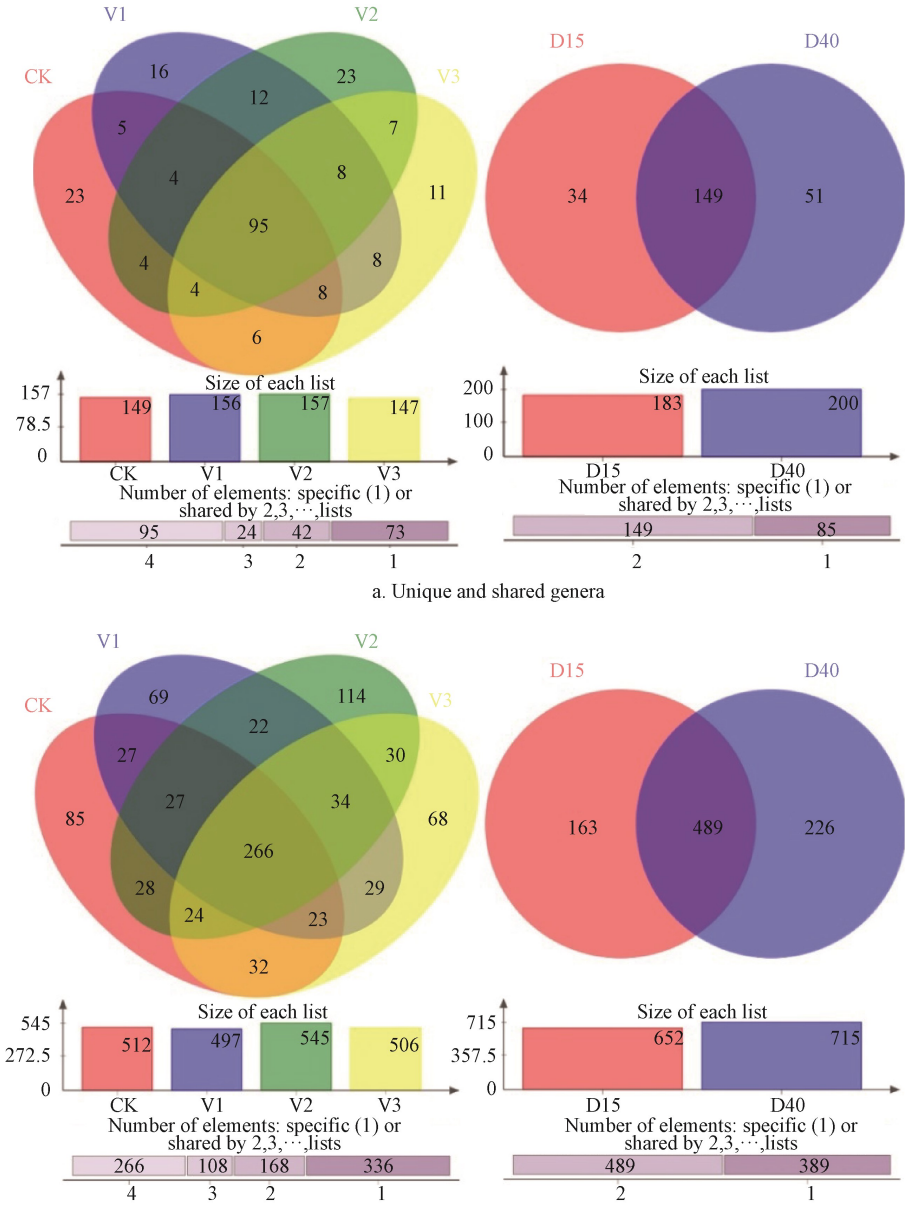
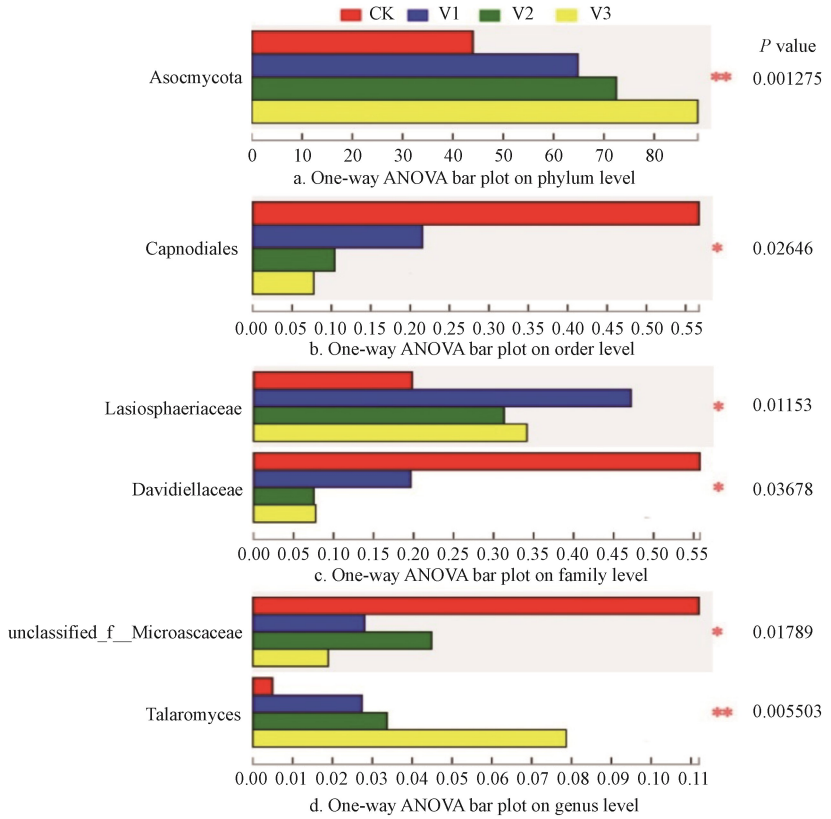


Figure 5 Venn diagrams showing the unique and shared genera and OTUs detected in the soil samples collected from different treatments, i.e., soil aeration and subsurface line placement depth treatments

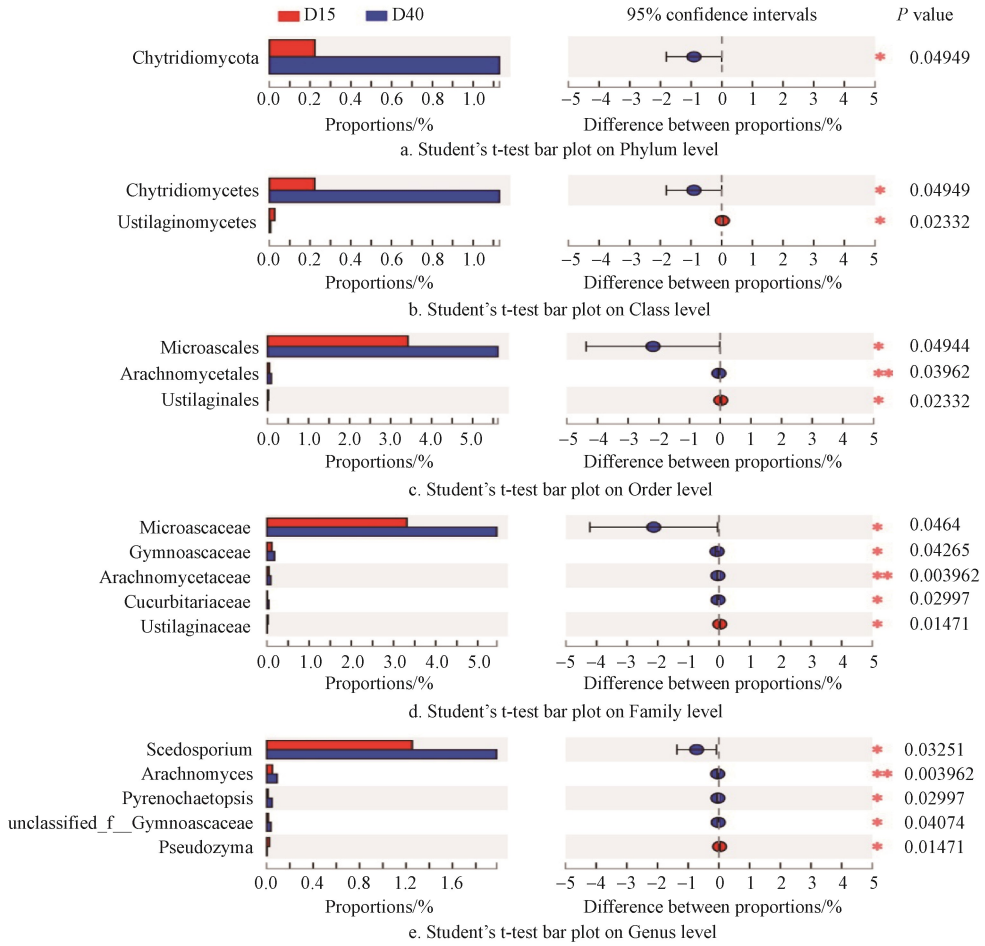
The significant differences in fungi at the phylum, order, family, and genus levels in the different soil aeration treatments are shown in Figure 6, which reveals that soil aeration obviously increased the abundance of Ascomycota. The results also showed that the abundance of Ascomycota increased with increasing aeration volume, and the maximum yield was obtained in the V3 treatment. Nevertheless, the proportion of *Capnodiales* decreased with increasing aeration volume. The proportion of Lasiosphaeriaceae in all aeration treatments was greater than that observed in the CK treatment, and the proportion of Davidiellaceae in all aeration treatments was lower than that observed in the CK treatment. The proportion of members of the genus Unclassified_f_microascaceae was lower in all aeration treatments than in the CK treatment, and members of the *Talaromyces* were more abundant in the aeration treatments than in the CK treatment.

As shown in Figure 7, compared to the 15 cm burial depth, the 40 cm burial depth presented increased numbers of Chytridiomycota at the phylum level; Chytridiomycetes at the class level; *Microascales* and *Arachnomycetales* at the order level; *Microascaceae*, Gymnoascaceae, and *Arachnomycetaceae* at the family level; and *Scedosporium*, *Arachnomyces*, and *Pyrenochaetopsis* at the genus level. The results also showed that the 40 cm treatment presented decreased numbers of *Ustilaginomycetes*, Ustilaginales, Ustilaginaceae, and *Pseudozyma*.



Note: the number of asterisks indicates significant differences between treatments according to one-way ANOVA: * $0.01<p\leq0.05$; ** $p\leq0.01$.

Figure 6 Significant differences in fungal abundance at the (a) phylum, (b) order, (c) family, and (d) genus levels in the different soil aeration treatments

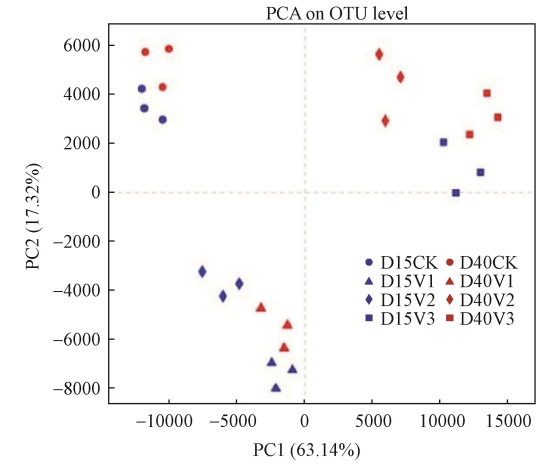


Note: the number of asterisks indicates significant differences between treatments according to one-way ANOVA: * $0.01<p\leq0.05$; ** $p\leq0.01$.

Figure 7 Significant differences in fungal abundance at the phylum, class, order, family, and genus species levels under emitter depths of 15 cm and 40 cm

3.3 PCA and RDA of rhizosphere soil fungal communities

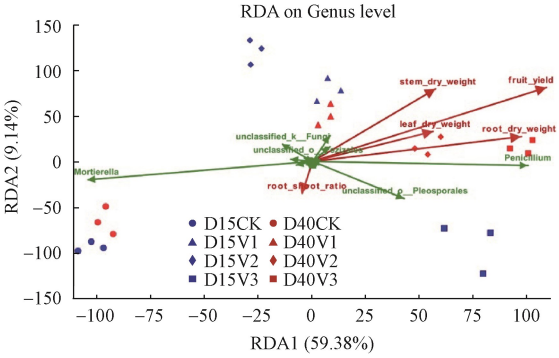
PCA revealed similarities between the fungal communities in the rhizosphere soil samples, as the effects of the different soil aeration and subsurface burial depth treatments were revealed by PCA at the OTU level (Figure 8). PCA revealed that the first and second axes explained 80.46% of the variance in the fungal community dataset and represented more information than the other principal components. The contributions of PC1 and PC2 to fungal community differences among the different aeration and emitter depth treatments were 63.14% and 17.32%, respectively. The effects of "D15CK and D40CK" were similar, as were the effects of "D15V1, D15V2, and D40V1" and of "D15V3 and D40V3".



Note: The points with the same color and shape indicate the replicate samples of each treatment.

Figure 8 PCA of the different soil fungal communities in the different soil aeration and subsurface line placement depth treatments

RDA was used to quantify the relative influence of plant performance (root, stem, and leaf dry weight; root/shoot ratio; and fruit yield) of the fungi at the genus level (Figure 9). The results showed that RDA1 and RDA2 explained 59.38% and 9.14% of the observed variation in weight, respectively. In addition, 5 combinational variables accounted for 68.52% of the observed variance. For the fungal community, a negative correlation between plant performance and the abundance of the genus *Mortierella* was detected. In contrast, a positive correlation between plant performance and the genus *Penicillium* was detected.



Notes: The red and green arrows represent plant performance and the genera of soil fungi, respectively.

Figure 9 RDA of fungi at the genus level, soil samples, and plant performance

4 Discussion

4.1 Effects of soil aeration on soil fungal communities and structure and tomato plant performance

All management practices to optimize the rhizosphere environment can only indirectly influence crop growth and yield via their direct effects on root systems, with limitations dictated by species genetics[37]. Tomato plants are particularly vulnerable to root zone hypoxic stress[14]. A study by Morard and Silvestre[38] showed that low oxygen concentrations within plant root zones can restrict nutrient absorption by and transpiration of plants, thereby inhibiting plant growth. Furthermore, hypoxic conditions inhibit plant growth, and numerous studies have concluded that the yields of various vegetable species as well as root weight, length, surface area, and volume are reduced by hypoxic stress[19,39,40]. From a plant physiology perspective, O_2 deficiency in the soil reduces ATP production, leading to reductions in the uptake and transport of nutrients and water between shoots and roots, causing leaf growth and photosynthesis to become inhibited[41]. The results of our present study suggest that soil aeration can significantly improve the total dry weight and fruit yield of tomato plants (Table 2), which is consistent with the results of other studies showing that soil aeration can restore plant growth[42,43].

Plant performance and crop yield are highly dependent on the composition and structure of rhizosphere soil fungal communities, and the relationships between the performance of plants and microorganisms are often highly mediated by chemical communication[44]. Soil microbial communities and structures have been shown to be primarily driven by limitations of available resources[45,46]. Soil fungi play an important role in biogeochemical, ecological, and cyclical processes in farmland ecosystems. Soil environmental conditions are generally not favorable for fungal growth because of relatively high/low temperatures or extremely poor conditions[47,48]. Nevertheless, there is little information available concerning soil fungal diversity and composition in response to different soil aeration treatments. The fungal phyla Ascomycota and Zygomycota are the most abundant phyla recorded in soil libraries[49,50]. Previous research has shown that the abundance of Ascomycota tends to increase in environments rich in soil nutrients[51]. This study discovered that the abundance of Ascomycota was greater in the aerated soils than in the CK soil. It was also observed that soil aeration reduced the abundance of Zygomycota, which was the second dominant phylum, with an average relative abundance of 20.54%.

Previous findings have confirmed that Lasiosphaeriaceae species function in breaking down organic matter (plant litter), which provides nutrients for plants and is beneficial for ecosystems[52]. It was observed that the relative abundance of Lasiosphaeriaceae increased in response to the soil aeration treatments (Figure 6). The family Lasiosphaeriaceae comprises aerobic fungi that are part of the phylum Ascomycota. Because Lasiosphaeriaceae family members can decompose hard-to-decompose organic matter, it was speculated that soil aeration may accelerate the soil nutrient cycle by increasing the abundance of this family. Moreover, our results suggest a relatively low abundance of *Capnodiales* in the aeration treatments (Figure 6). Previous studies confirmed that the relative abundance of the order *Capnodiales* was high under drought conditions[53], a finding that is consistent with the results of our present study. Therefore, the soils with good permeability restrict the abundance of *Capnodiales*. Members of the genus *Mortierella* are generally considered to be positively correlated with the heterotrophic nitrification rate[54]. In the present study, as soil aeration reduced the abundance of *Mortierella* (Figure 3), it was inferred that soil aeration can affect nitrogen cycling by altering the abundance of

this genus. In addition, it was also observed that the soil aeration increased the abundance of the fungal genera *Talaromyces*, *Penicillium*, and *Fusarium*, and the family Lasiosphaeriaceae (Figures 3 and 6).

In general, different groups of fungi have different O_2 requirements, and the soil environment changes in response to soil aeration. Therefore, soil aeration alters the rhizosphere soil fungal composition. In addition, the activities of soil enzymes and the performance of plant roots can be enhanced by soil aeration[20,55]. Rhizosphere microorganisms consume root exudates as their major nutrient. Therefore, soil aeration can indirectly affect soil enzyme activity, which in turn affects the soil nutrient cycle, while changes in soil nutrients have the opposite effect on soil fungi.

4.2 Effects of the burial depth of subsurface lines on soil fungal communities and structure and on tomato plant performance

The burial depth of subsurface drip irrigation lines determines the position (depth) of wetted soil and affects the oxygen environment of the soil around plant roots. The physical and hydraulic properties of the deep, loess-derived clay loam soil used in the present study were quite uniform with respect to depth. Therefore, it would be expected that the dimensions and shape of the wetted soil around each buried emitter would be similar for all line placement depths. The shape and dimensions of the wetted soil around the buried emitters are important with respect to explaining the effects of subsurface drip irrigation treatments for different line placement depths on plant growth.

Sefer et al.[56] reported that variation in the depth of drip tape had significant effects on green bean yield and that the maximum yield was obtained in response to a 10 cm subsurface drip irrigation depth in the greenhouse. Nevertheless, our results showed that burial depth had no significant influence on tomato plant performance (Table 2). It is interesting that at the early stage (20-60 d after transplanting), plants grew slowly with a subsurface tubing placement depth of 40 cm. Nevertheless, at later stages (60-214 d after transplanting), plants grew fast with a subsurface tubing placement depth of 40 cm. Tomato is a medium-depth-rooted plant species, and tomato roots are primarily distributed within a depth of 40 cm in farmland soils. It was speculated that water could not completely reach the primary root zone at the early stage due to the subsurface tubing placement depth of 40 cm. At later stages, the benefits of irrigation and aeration resulted from the subsurface tubing placement depth of 40 cm. In addition, it was observed that root growth at the 40 cm subsurface drip irrigation depth was much better than that at 15 cm when collecting rhizosphere soil samples. Compared to tomato plants, green bean plants have a shallower root system[57]. Therefore, the findings described by Sefer are inconsistent with the results of our study with respect to plant performance in response to different drip irrigation depths, which demonstrated that line depth has no significant influence on tomato plant performance. Interestingly, however, the soil fungal composition was affected by subsurface line depth (Figures 5 and 7).

Soil is an open medium, and if aeration lines are placed at a 15 cm (shallow) depth, the concentration of oxygen in the soil may not be significantly improved because of the chimney effect that occurs in soils. Nevertheless, as the plough layer restricts the diffusion of oxygen into deeper soil, the line depth of the 40 cm treatment was more effective than that of the 15 cm treatment for increasing the concentration of soil oxygen. In addition, variations in subsurface drip irrigation line depth can lead to variations in soil water distribution. Compared to the deep line depth (40 cm), the shallow (15 cm) line depth increased surface evaporation. Because the tested soil was a Lou silty clay loam and originating from the plough layer, it restricts the diffusion of both water and air into deeper layers. High soil moisture is primarily present at a depth of 30-50 cm (below the plough layer) after a short period of irrigation.

Figure 7 shows that the 40 cm line depth had a strong negative effect on the abundance of Chytridiomycota at the phylum level, Chytridiomycetes at the class level, and *Microascales* and *Arachnomycetales* at the order level. This result could indicate that the members of these fungal groups are sensitive to the environment created by the line depth in the 40 cm treatment, possibly because of the relatively high oxygen and soil water content in the soil.

4.3 Correlations between tomato plant performance and soil fungi in response to different soil aeration and subsurface line depth treatments

To the best of our knowledge, this is the first study addressing fungal composition in the field in response to various soil aeration and subsurface line depth treatments. Previous studies have shown that members of the phylum Ascomycota constitute the majority of soil fungal decomposers[58]. Zhang et al.[59] revealed that Ascomycota members could improve the growth of bamboo. In addition, it has been proven that Ascomycota can increase rice productivity by improving plant tolerance to adverse environmental conditions[60]. In our present study, positive relationships between Ascomycota and root dry weight and fruit yields were detected (Figure 10A). Based on these results, it can be concluded that soil aeration increased the relative abundance of Ascomycota, which is good for plant growth. Moreover, the relative abundance of Chytridiomycota was positively correlated with root dry weight (Figure 10a), and studies have shown that the relative abundance of Chytridiomycota increases in response to tillage farming[61]. Chytridiomycota plays an important role in the decomposition of refractory organic matter, such as pollen, cellulose, and keratin[62]. As a result, it was concluded that soil aeration can affect cyclical processes by affecting the relative abundance of Chytridiomycota.

The plant performance and the rhizosphere soil fungal communities were also analyzed at the genus level. Species of *Curvularia* and *Fusarium* are the most important plant pathogens[63,64], and variations in soil aeration can also lead to variations in pathogenic species in soil (Figure 10b). However, in the present study, the abundance of *Curvularia* and *Fusarium* was too low to cause plant disease. In addition, soil aeration has additional positive effects on plants. Therefore, the results showed that, interestingly, *Curvularia* and *Fusarium* were positively correlated with root dry weight (Figure 10b). Moreover, it was suggested that if soil aeration is to be implemented, the level of soil sterilization must also be improved.

In general, soil aeration influenced fungal diversity and composition. The relative abundances of Ascomycota and Chytridiomycota were both increased by soil aeration, leading us to speculate that soil aeration influences fungal diversity. This phenomenon may accelerate the decomposition of refractory organic matter in the soil and improve the efficient uptake of nutrients, all of which lead to increased tomato yields.

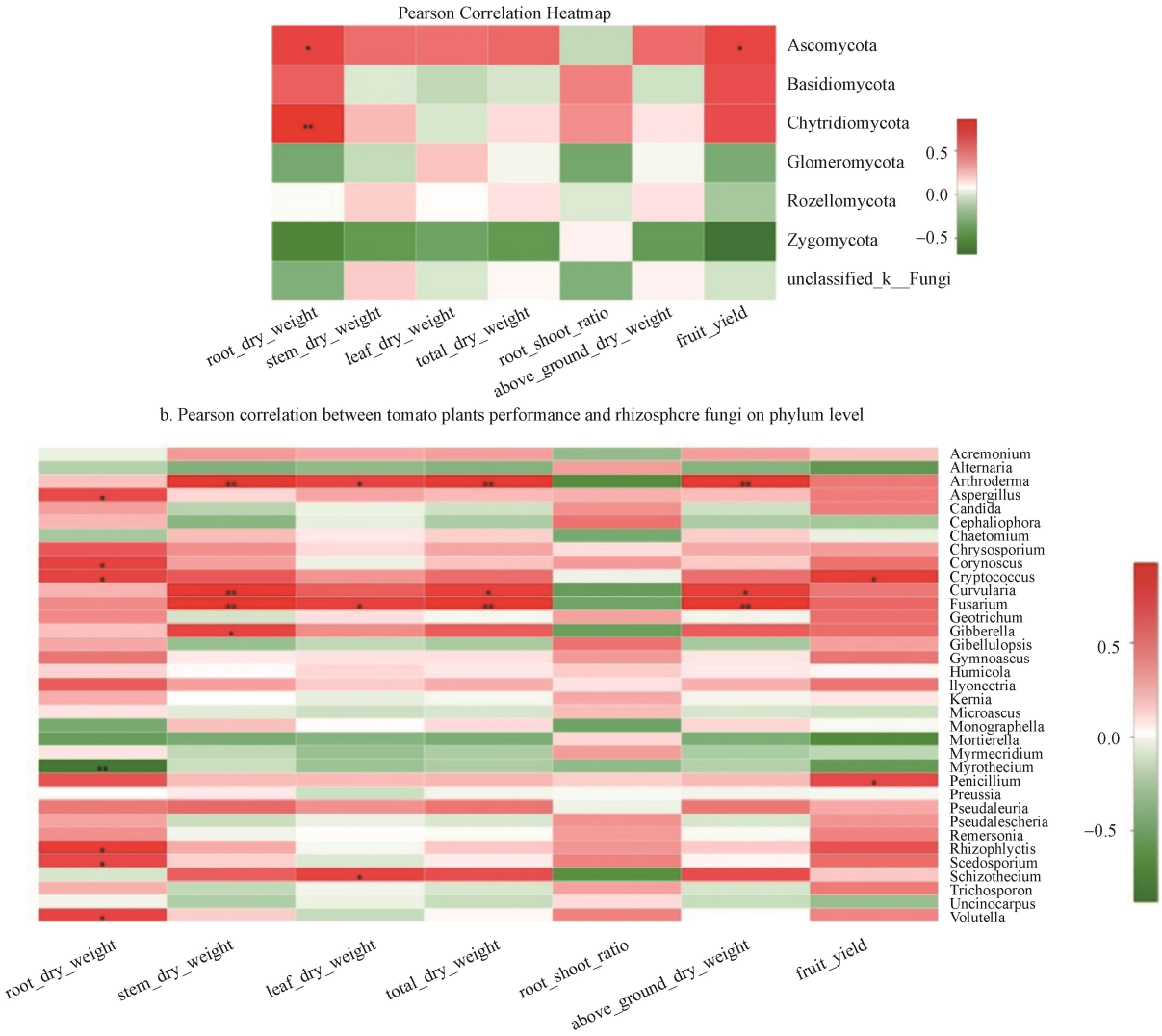


Figure 10 Pearson correlations between plant performance and rhizosphere soil fungal communities at the (a) phylum and (b) genus levels

5 Conclusions

To the best of our knowledge, this is the first study to investigate the impact of soil aeration volume and burial depth of subsurface irrigation on the soil fungal community and structure in a Lou silty clay loam in the cultivation of tomato plants in a greenhouse. The results of this study confirm that soil aeration not only increased tomato plant performance but also influenced fungal diversity and composition. Compared to the no-aeration treatment, the V3 treatment increased the total dry weight and fruit yield by 39.9% and 65.6%, respectively. The results also showed that the abundance of the members of the phylum Ascomycota and the family Lasiosphaeriaceae increased with increasing soil aeration volume, whereas the abundance of the members of the phylum Zygomycota and order *Capnodiales* decreased with increasing aeration volume. In addition, different burial depths of the subsurface line differentially altered the rhizosphere soil fungal community. It was also observed that the implementation of soil aeration may increase the spread of soil pathogens. In general, these results suggest that soil aeration can ameliorate hypoxic conditions associated with drip-irrigated tomato crops grown in Lou soils and can benefit soil fungal communities and tomato plant performance.

Acknowledgements

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