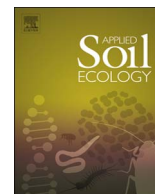




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# Effect of alternate partial root-zone drip irrigation on soil bacterial communities and tomato yield



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## ABSTRACT

The aim of this study was to assess the effect of alternate partial root-zone drip irrigation (ADI) on the soil microbial communities in the crop root zone and the relation between the soil microbial community changes and crop growth. We investigated the effect of ADI at different lower limits of irrigation (ILLs, 50%, 60%, and 70% of the field capacity (FC)) on soil bacterial diversity in the root zone of greenhouse tomato and analyzed the relation between the soil bacterial community changes and tomato growth. The soil bacterial community structure was markedly different under ADI compared with SDI (ground drip irrigation). It was closely related to the soil microenvironment. Among various environmental factors, the tomato root activity, root length, and soil CO<sub>2</sub> flux showed significant effects on the difference in soil bacterial communities. Environmental changes in the root-zone soil inevitably affected crop growth, and SDI and ADI resulted in significant differences in root growth, single fruit weight, number of fruits and fruit yield per tomato plant. Our results suggest that an ILL at 70% of the FC could significantly improve the root-zone soil environment, which was beneficial for the organic matter, cellulose, nitrogen and sulfur metabolism and increased the oxygen content in the root-zone soil. Therefore, the root areas, root forks, the fruit number and the yield per plant were better under an ILL at 70% of the FC.

## 1. Introduction

Alternate partial root-zone drip irrigation (ADI) is a technique that has been of increasing interest to researchers worldwide in recent years. The ADI technique artificially controls alternate wetting and drying in the partial root zone of crops, which can regulate stomatal conductance, reduce transpiration, and improve water use efficiency through crop root signals (Dodd et al., 2008; Kirda et al., 2007). ADI has been shown to significantly improve water use efficiency while also increasing crop photosynthesis (Wang et al., 2011) and preventing yield loss (Kang and Zhang, 2004). Additionally, alternate wetting and drying in the crop root-zone soil can stimulate root growth, enhance root activity (Yang et al., 2010), regulate assimilation distribution (Shao et al., 2008), and markedly increase the root-to-shoot ratio (Kang et al., 1998; Liang et al., 2000). Changes in root growth will inevitably affect nutrient use in the root-zone soil. ADI has been found to facilitate root growth and simultaneously increase leaf nitrogen concentrations in maize (Wang et al., 2012). Moreover, alternate wetting and drying of the soil under ADI has been found to promote nitrogen accumulation from the soil to the root surface and, thereby, enhance the capacity of

the roots and crops for nitrogen uptake, ultimately improving the nitrogen-use efficiency (Wang et al., 2013).

However, the cycling and use of soil nutrients are closely associated with soil microbes (Zhong et al., 2010). The underlying reason for ADI improvements to soil nutrient use may be that alternate wetting and drying changes in the root zone alter soil water and heat conditions among the various environmental factors, thereby changing the structure of the soil microbial communities and accelerating the rate of soil nutrient cycling. With the same amount of irrigation, alternate wetting and drying of the soil under ADI has been found to enhance the activity and metabolism of soil microbes, accelerate the rate of soil organic matter mineralization, change the carbon-to-nitrogen ratio in the soil, and promote nitrogen uptake and use by tomato (Wang et al., 2010). Moreover, alternate wetting and drying under ADI has been reported to accelerate the rate of carbon and nitrogen mineralization in the soil, which can lead to soil carbon and nitrogen loss and is unfavorable to the health of soil quality (Sun et al., 2013). Therefore, investigating the effect of ADI on soil microbial community changes is critical for obtaining a better understanding of the underlying mechanisms of ADI and the rational allocation of drip irrigation. However, relevant

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research is lacking, and existing studies have mainly used the conventional microbiological method of pure plate culture (Wang et al., 2006, 2008; Yu et al., 2008) and have found that ADI could improve soil microorganisms in the root-zone compared to SDI, and water deficits could improve soil permeability to promote microorganism growth. However, the culture-based method can only detect a very small proportion (0.1–1%) of the microbes in the soil environment, which does not truly reflect the soil microbial communities (Cheung et al., 2010). A popular technique in microbial ecology research is high-throughput sequencing (Lin et al., 2014), which obtains a large amount of microbiological information and involves an automated process that can compensate for the shortcomings of the conventional culture method.

In the present study, we believe that changes in soil bacterial (the most important soil microorganisms) communities underlie ADI improvements in soil nutrients and promote crop growth. In addition, obtaining abundant and accurate microbiological information is central. Therefore, we used high-throughput sequencing to assess the effect of ADI with plastic mulch on soil bacterial communities in the root zone of a greenhouse tomato and further analyzed the relation between the changes in the soil bacterial communities and the root growth and yield of tomato, thereby providing evidence to prove the hypotheses. In addition, this study provides evidence for rationally allocating various agronomic measures, facilitating crop growth, and improving the soil and water use efficiency in the production practice of an agriculture facility.

## 2. Materials and methods

### 2.1. Experimental site

The experiment was carried out in a plastic greenhouse in the Dazhai Village, Dazhai County, Yangling District, Shaanxi Province, China. The experimental site was located at longitude 108°08'E and latitude 34°16'N, with an elevation of 521 m. This site belongs to the warm temperate, semihumid, monsoon zone. The mean annual temperature is approximately 16.3 °C, the mean annual rainfall is 535.6 mm, the mean annual sunshine time is ~2163 h, and the mean annual frost-free period is 210 d. The experimental soil had a bulk density of 1.34 g cm<sup>-3</sup> and a field capacity (FC) of 28.17% (by mass water content). The soil contained 25.4% gravel (2–0.02 mm), 44.1% silt (0.02–0.002 mm), and 30.5% clay (< 0.002 mm). The soil porosity was 49.38%.

### 2.2. Experimental design

The experiment was performed from October 2014 to May 2015 using the tomato cultivar “Haidi” (widely cultivated locally). The greenhouse was 108-m long in the east-west direction and 8-m wide in the south-north direction. In the greenhouse, plots were divided from west to east. Each plot was raised with double ridges and was 6.0 m in length. The ridge width was 0.6 m, and the furrow width was 0.3 m, with a height of 0.2 m and an area of 3.4 m<sup>2</sup>. Thirty-four tomato seedlings per plot were planted in double rows, with a plant spacing of 0.35 m. Guard rows were set up at both ends of the experimental field.

The experimental design included two treatments: ground drip irrigation (SDI) under plastic mulch and ADI under plastic mulch. The SDI treatment served as the control: drip tapes were located in the middle of the row between the tomato plants; the lower limit of irrigation (ILL) was set at 70% of the FC, corresponding to an upper limit of irrigation at 75% of the FC. For the ADI treatments, the ILLs were set at 50%, 60%, and 70% of the FC, corresponding to the upper limits of irrigation at 55%, 65%, and 75% of the FC, respectively. At both ends of each plot, a drip tape was laid at a distance of 30 cm from the roots of the tomato plants. Two drip tapes were used for alternate irrigation over a period of 15–20 days. Each treatment had three replicates, and 12 plots were included in the experiment. The plastic mulch was a

white, translucent, high-pressure, low-density, polyethylene film (Xinfeng Plastic Factory, Jingjiang, Jiangsum, China), 0.014 mm in thickness. The embedded flat drip tapes (Dayu Water-Saving Group Co., Ltd., Gansu, China) were 16 mm in diameter and 0.3 mm in wall thickness, with an emitter spacing of 30 cm, working pressure of 0.1 Mpa, and emitter flow rate of 1.2 l/h.

Soil water content was measured and controlled using a Field TDR 200 soil moisture meter (Spectrum, Aurora, IL, USA). A 100-cm deep probe was installed in the center of each plot. Soil water content was measured at 10-cm intervals down to a depth of 60 cm. Meanwhile, soil samples were taken by boring, and the measurements of the soil water content were calibrated by the oven drying method. When the soil water content dropped to the lower limit of the soil water, the water was supplemented according to the depth of the wetting layer at 40 cm. The irrigation amount was calculated as follows:

$$M = s\rho_b p h \theta_f (q_1 - q_2) / \eta,$$

where  $M$  is the irrigation amount, m<sup>3</sup>;  $s$  is the planned wetting area, 4.6 m<sup>2</sup>;  $\rho_b$  is the soil bulk density, 1.35 g/m<sup>3</sup>;  $p$  is the wetting proportion, 0.8;  $h$  is the depth of wetting layer, 0.4 m;  $\theta_f$  is the maximum FC, 31.54%;  $q_1$  and  $q_2$  are the upper limit of irrigation and measured soil moisture content, respectively, %FC; and  $\eta$  is the coefficient of water use, 0.95.

### 2.3. Analytical methods

#### 2.3.1. Sample collection

There were 3 replicates in each treatment. When the fruit was nearly ripe, 3 tomato plants per replicate per treatment were randomly selected, numbered and marked. Fruit was harvested from March 18–May 3, 2015. The fruit was marked with the corresponding plant number and weighed using a 0.01-g precision electronic balance.

After the harvest of the fruit, plant samples were collected on May 5, 2015, for laboratory analysis. The aboveground part of each plant (3 tomato plants per replicate per treatment) was cut, collected, and numbered. At the same time, root samples were collected by whole-root excavation. The roots of each plant (3 tomato plants per replicate per treatment) were excavated in a rectangular area of 40 cm × 30 cm, with the midline between adjacent plants as the boundary; the depth of the excavation was consistent with the actual depth of the tomato roots (~50 cm). The overall root sample was taken from the soil, and large soil clumps between the roots were removed. The soil attached to the roots was vigorously shaken off onto clean filter paper that had been sterilized at high temperature, placed in sterile plastic tubes and taken to the laboratory for soil bacterial community analysis. The root samples were placed in mesh bags with a mesh diameter of 0.5 mm and delivered to the laboratory for subsequent tests.

#### 2.3.2. Soil bacterial community analysis

The root-zone soil from 3 replicates per treatment was sequenced to analyze the bacterial communities using high-throughput sequencing technique. The specific steps were as follows:

- a DNA extraction and analysis: Bacterial DNA was extracted from the soil samples using an E.Z.N.A.® soil DNA Kit and purified using a DNA purification kit. The concentration and quality (OD260/OD280 ratio) of the DNA were determined using a NanoDrop 2000 spectrophotometer. The DNA integrity was checked by electrophoresis on 1% agarose gels. After completion of electrophoresis, the gel was stained with ethidium bromide (0.5 mg/L, Sigma, USA) for 20 min, rinsed with clean water for 10 min, and then observed under UV light (Bio-Rad, Gel Doc™ XR+, Hercules, CA, USA). The purified DNA was stored in a freezer at -20 °C for subsequent polymerase chain reaction (PCR) and Illumina MiSeq sequencing.
- b PCR amplification: The V3-V4 region of the bacterial 16S rRNA was amplified by PCR using the primers 338F 5'-

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