

EFFECTS OF ANTI-GRASS CLOTH COVERING ON SOIL AND CITRUS ROOT GROWTH IN ORCHARDS

Yun Zhong, Zebin Li, Yuanda Lv, Bo Jiang, Huaxue Yan, & Guangyan Zhong*

Institute of Fruit Tree Research, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China

*Corresponding author: gy_zhong@163.com

ABSTRACT

In order to reduce the use of chemical fertilizers and pesticides, while improving citrus production, it is important to study the effects of grass-proof cloth covering on soil environment, citrus root growth, and fruit yields in the orchard. The single factor experiment design was used to set up soil with the anti-grass cloth covering treatment and without covering (control) treatment. Soil hydrothermal conditions, nutrient levels, root growth, and fruit yield of both treatments were compared. Covering the ground surface with grass-proof cloth increased the soil water content and temperature; availability of phosphorus, potassium, zinc, and manganese, as well as the number of soil microbial population. Root growth and fruit yield also increased. The anti-grass covering can improve the soil hydrothermal condition and the overall nutrient level of a citrus orchard, which may be beneficial to the growth of citrus roots thus increasing productivity.

Keywords: Citrus, cover, soil environment, roots growth

1. INTRODUCTION

Surface mulching cultivation techniques have been widely used in different crop production. For the production of fruits such as apples, the use of surface mulching is also gradually becoming popular. Surface mulching can substantially reduce the occurrence of weeds in cropland and therefore reduce the use of herbicides and associated environmental pollution (Chen *et al.*, 2008). Moreover, it plays an important role in water holding capacity in soil, modification of soil environment, and improvement of fruit quality (Balwinder *et al.*, 2011; Cook *et al.*, 2006). However, studies on the effects of surface mulching on citrus cultivation and productivity are still insufficient, especially in the high-temperature zone of southern China. There is a research gap to understand the effects of surface mulching on soil physicochemical properties, microbial population, and root growth, which may limit the application of anti-grass cloth mulching techniques in citrus cultivation. This article reports the comparative study of anti-grass cloth mulching on citrus plant growth and development. It may provide some scientific evidence for the use of anti-grass cloth in the citrus industry.

2. MATERIALS AND METHODS

2.1. Study site

The experiment was conducted in a citrus orchard at the Lemon Modern Industrial Park (114°45'E, 24°6'N) of Zhongxing Lv Feng Development Co., Ltd., located in Shuntian Town, Dongyuan County, Heyuan City, Guangdong Province. The study region has an elevation of 70 m, with an average annual temperature of 20.7°C and average annual precipitation of 1567 mm–2142.6 mm. The soil type is classified as latosolic red soil, which contains 1% organic matter with a pH of 5.14.

2.2. Experimental materials and treatments

Two year old disease-free containerized citrus seedlings, (upper trunk diameter = 1.2 cm–1.5 cm, height = 1.5 m) which were scions of the cultivar 'W-Murcott' (*Citrus reticulata* Blanco × *Citrus sinensis* Osb.) grafted onto rootstock of the cultivar 'Ziyang Xiangcheng' (*Citrus junos* Sieb. ex Tanaka) were used in this experiment. Plants were spaced 3.0 m apart within rows and rows were 4.0 m apart. Ridge cultivation was adopted, with ridges of 0.4 m height and 2.0 m width. The plants showed normal growth and comparable growth potential, with a moderate management level.

A single-factor experimental design involving two treatments was used. One treatment used mulching cultivation with grass-proof cloth, whereas the other was open-field cultivation without mulching (control). Each treatment had three replica plots and a total of six plots were involved. There were 17 trees per plot, with 102 trees in total. For all plots of the mulching treatment, the ridges were completely mulched from March 2018 using black grass-proof cloth (an 80 g/m² degradable material manufactured by Taizhou Xiahui Trading Co., Ltd., Zhejiang Province, China).

2.3. Measurement parameters and methods

2.3.1. Measurement of soil water content

Soil samples were randomly taken at a depth of 20 cm using a soil sampler. The samples were collected from three points at the periphery of canopy dripline in each plot. Soil water content was measured gravimetrically after oven-drying (Institute of Nanjing Soil Science, 1978) and repeatedly every 30 days.

2.3.2. Measurement of soil temperature

In March 2018, three thermometers were inserted at approximately equal intervals along the vertical projection of the canopy periphery for each treatment. Diurnal variation (average soil temperature days for a year, based on data recorded from 8:00 to 18:00 at 2-hour intervals) and annual variation (average soil temperature of the observation days) in soil temperature of 20 cm depth were observed and recorded from March 15th, twice a month.

2.3.3. Measurement of soil mineral nutrient contents

To analyze soil mineral elements, orchard soil samples were collected in August 2018 (5 months after the start of the experiment for anti-grass cloth mulching). The soil samples were taken at a depth of 20 cm. The samples were kept in zipper bags and transported to the laboratory where they were air-dried, then crushed, ground, and passed through a 2 mm sieve. The sieved samples were stored under ventilated and dry conditions before testing.

The testing of soil nutrient elements was performed using the soil nutrient status systematic approach (PPI/PPIC, 1992). Alkali-hydrolyzable nitrogen content was analyzed using the alkali-hydrolysis and diffusion method. Available phosphorus content was quantified by anti-molybdenum-antimony colorimetry after extraction using a mixture of chloride acid (HCl) and ammonium fluoride (NH₄F). Available potassium, exchangeable calcium, and exchangeable magnesium contents were extracted using an ammonium acetate (CH₃CO₂NH₄) solution and then tested by atomic absorption spectrometry. Available zinc, available iron, and available

manganese contents were analyzed based on diet hylene triaminepenta acetic acid extraction-atomic absorption spectrometry, whereas available boron content was analyzed via boiling water extraction-curcumin colorimetry.

2.3.4. Enumeration of soil microbial population

The type and quantity of soil microbes were analyzed using the dilution plate method. Nutrient agar, modified Gause's No. 1 medium, and rose Bengal medium were used for the culture of bacteria, actinomycetes, and fungi, respectively (Li *et al.*, 2017).

2.3.5. Diversity analysis of soil microbial community

The diversity of the soil microbial community under different cultivation modes was analyzed using the Chao1, ACE, Shannon, and Simpson indices.

Surface soil samples (0–20 cm) were collected from the anti-grass cloth mulching and control treatments in November 2018. After recording their details, soil samples were immediately placed in sterile zipper bags and kept in a box with dry ice. Then the samples were sent to the Personal Biotechnology Co., Ltd. (Shanghai, China) for DNA extraction and high-throughput sequencing. The obtained sequences were clustered into operational taxonomic units (OTU) at $\geq 97\%$ similarity. For each OTU, the most abundant sequence was chosen as the representative sequence. The representative sequences of all OTUs were obtained against template sequences in the corresponding database (Silva, <https://www.arb-silva.de/>) to acquire the taxonomic information for each OTU.

2.3.6. Measurement of root morphology, physiology, and vigor

Three citrus trees representing average growth potential were chosen from each treatment in January 2019. The samples were prepared according to the protocols by Yang (2014). Briefly, a 40 cm cubic soil block was dug 60 cm from the trunk on the same side of the ridge.

The root samples were scanned using an EPSON root scanner (Expression 10000XL 1.0, Epson Inc., Japan) according to the manufacturer's instructions. The scan results were analyzed using WinRHIZO Pro(S) v.2004b (Regent Instrument Inc., Canada) to obtain root morphological parameters including total length, average diameter, and total surface area. After scanning was completed, the root samples were taken out and dried with absorbent paper, then weighed. Subsequently, the samples were dried in an oven at 80°C to constant weight and then weighed again.

Root vigor was determined using the triphenyl tetrazolium chloride method (Zhu *et al.*, 2018).

The following root parameters were calculated:

Specific root length = total root length (cm) \div root dry weight (g)

Specific root surface area = total root surface area (cm²) \div root dry weight (g)

2.3.7. Yield estimation

Seven fruit trees with normal growth were randomly selected for each treatment. The number

of fruit on each tree was counted by hand. Fruit yield per tree was calculated as: Yield (kg/tree) = single fruit weight × fruit number per tree.

2.3.8. Statistical analysis

All the experimental data were processed and analyzed using WPS Excel 2016 (Kingsoft Office Software Co., Ltd., Zhuhai, China) and SPSS 18.0 (SPSS Inc., Chicago, USA).

3. RESULTS

3.1. Effect of mulching on soil water content

Generally, soil water content exhibited consistent trends under the two different cultivation modes (Figure 1). The changes in soil water content were mainly affected by precipitation conditions. Higher soil water content occurred during the hot rainy period in summer, whereas soil water content decreased during the cold dry period in winter. However, the mulching treatment resulted in higher soil water content than the control across most of the experimental period. The difference in soil water content between the two treatments was especially evident during the hot rainy period in May, June, and July. In contrast, this difference gradually diminished in the winter, and soil water content was even higher under control than the mulching treatment on January 26th. The maximum difference in soil water content between the two treatments was found on May 20th (4.4%), and the maximum difference appeared on October 20th (0.7%).

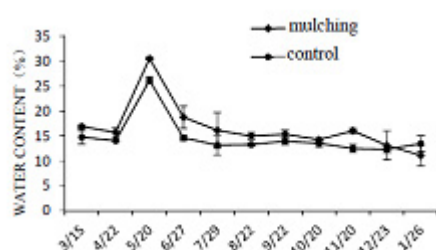


Figure 1. Effects of Covering on Dynamic Changes of Soil Water Content in Citrus Orchards

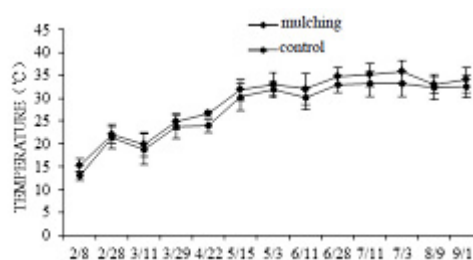


Figure 2. Effect of Coverage on Annual Change of Soil Temperature in Citrus Orchards

3.2. Effect of mulching on soil temperature

Generally, the annual variation in soil temperature exhibited consistent trends under the two cultivation modes (Figure 2). Mulching with anti-grass cloth showed an obvious warming effect, as the soil temperature of the mulching treatment was higher than that of the control across various growth periods of citrus. The maximum difference in soil temperature between the two treatments appeared on April 22th (2.7°C), with the minimum difference found on February 28th (0.7°C).

3.3. Effect of mulching on soil mineral nutrient contents

Soil mineral nutrient levels in the orchard were analyzed after five months of mulching with anti-grass cloth and the results are presented in Table 1. The use of anti-grass cloth had a significant effect on the contents of soil major elements. After mulching with anti-grass cloth, soil alkali-hydrolyzable nitrogen content decreased slightly, but not significantly, compared

with the control. However, both soil available phosphorus and potassium contents increased significantly after mulching. The available phosphorus content changed from 32.3 to 41.6 mg/kg, whereas the available potassium content increased by 5.0 mg/kg. These indicated that the cultivation mode of anti-grass cloth covering was beneficial to improve the contents of soil major elements in citrus orchard.

Table 1a. Effects of Mulching on Mineral Nutrient Content in Citrus Orchard Soil.

Treatment	Alkali-hydrolyzable N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	Exchangeable Ca (mg/kg)
Mulching	56.17±2.76a	41.63±2.40a	62.80±0.30a	1310.67±58.00b
Open-field	58.87±0.35a	32.33±0.80b	57.77±1.50b	1539.00±38.74a

Note: Different lowercase letters in the same column indicate significant difference at $P \leq 0.05$. The same as other tables.

Table 1b. Effects of Mulching on Mineral Nutrient Content in Citrus Orchard Soil.

Treatment	Exchangeable Mg (mg/kg)	Available Zn (mg/kg)	Available Mn (mg/kg)	Available B (mg/kg)
Mulching	52.97±2.77b	1.13±0.11a	7.37±0.09a	0.20±0.06a
Open-field	73.63±2.25a	0.53±0.06b	6.74±0.15b	0.23±0.05a

In the case of control, soil exchangeable calcium content was approximately 1539.0 mg/kg. After mulching with anti-grass cloth, the exchangeable calcium content changed to 1310.6 mg/kg, with a decrease of 14.9%. The exchangeable magnesium content was approximately 73.6 mg/kg for the control, whereas it decreased to 52.9 mg/kg (by 28.1%) after mulching. These results indicated that the cultivation mode of anti-grass cloth mulching could decrease the contents of medium elements in soil.

With regard to trace elements, both soil available zinc and available manganese contents increased after mulching with anti-grass cloth. The most increase was found in the available zinc content which changed from 0.53 to 1.13 mg/kg; the increase rate was 113.2% and the difference between treatments was highly significant. The available manganese content changed from 6.7 to 7.3 mg/kg, with an increase rate of 8.6%. Moreover, mulching with anti-grass cloth had no significant effect on soil available boron content.

3.4. Effect of mulching on soil microbial population

The effect of mulching on soil microbial population is shown in Figure 3. The number of soil

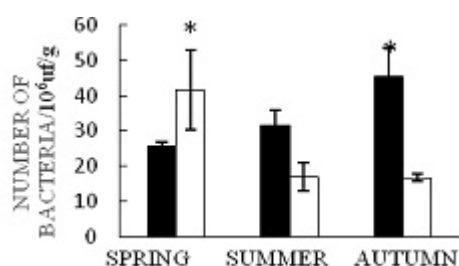


Figure 3a. Effects of Covering on Bacteria Population in Citrus Orchard (Note: Superscripts *indicate significant differences at the 0.05)

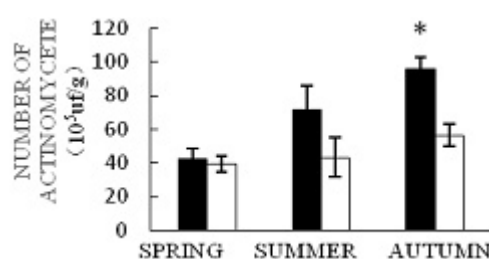


Figure 3b. Effects of Covering on actinomycetes Population in Citrus Orchard

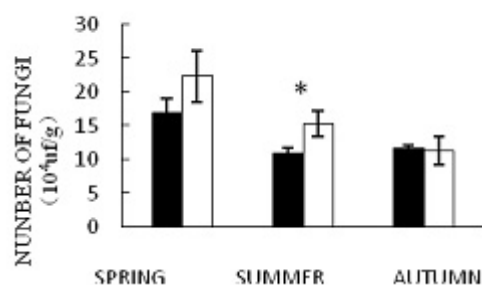


Figure 3c. Effects of Covering on Fungi Population in Citrus Orchard



Figure 4. A total of OTUs Venn map

microbes under both cultivation modes was highest for bacteria, followed by actinomycetes and fungi. The numbers of both soil bacteria and actinomycetes increased after mulching with anti-grass cloth, and in autumn values were significantly higher compared to the control. In summer, there was significantly poor number of soil fungi in the case of mulching cultivation compared to the control, whereas for the other seasons, no significant difference between the two treatments was found.

3.5. Effect of mulching on soil microbial community richness and diversity

For both mulching and control, the detected number of OTUs gradually decreased from phylum to species levels (Table 2). This is due to the high diversity of microbial species, which may not completely cover the commonly used databases, in addition to the limitation of sequencing read lengths. Therefore, in the actual analysis, the taxonomic information cannot be obtained at the genus or species level for all representative sequences of the OTUs. However, the number of OTUs at the phylum, class, order, and species levels was higher under the mulching cultivation than control cultivation, although this difference was not significant. But at the family and genus levels, mulching cultivation resulted in a significantly lower number of OTUs compared with control.

Table 2. OTU classification and classification status identification results statistics table

Treatment	Phylum	Class	Order	Family	Genus	Species	Unknown
Mulching	3038.0±183.5a	2939.2±182.6a	2511.6±122.9a	1664.0±121.8b	978.4±45.7b	121.0±17.9a	0.6±0.5a
Open-field	3012.0±176.6a	2948.2±174.0a	2428.2±160.7a	1867.6±68.2a	1117.4±49.3a	119.6±8.6a	0.6±0.5a

The common OTUs of soil microbes shared by the two different cultivation modes were also analyzed (Figure 4). A total of 6277 OTUs were detected under the mulching cultivation mode, 3583 of which were shared by the control mode. The remaining 2694 OTUs were unique, more than control by 78.

Both the Chao1 and ACE index values under mulching cultivation were higher than under control, whereas the corresponding Shannon and Simpson index values were lower than under control cultivation (Table 3). This result indicates that mulching cultivation resulted in higher richness but lower diversity of the soil microbial community compared with control cultivation.

Table 3. Soil microbial community diversity index table of two cultivation modes

Treatment	Simpson	Chao1	ACE	Shannon
Mulching	0.9975±0.0003b	3182.86±395.46a	3269.11±472.45a	10.4±0.1b
Open-field	0.9984±0.0005a	3012.85±176.45a	3019.61±183.44a	10.6±0.1a

3.6. Effect of mulching on citrus root growth

Mulching with anti-grass cloth had a significant positive effect on root growth and development of citrus trees (Table 4). Under mulching cultivation, the trees yielded larger total root length, total root surface area, total root volume, and average root diameter than under control cultivation. The increase rates of total root length, total root surface area, and total root volume were 37.7%, 46.0%, and 54.3%, respectively; these differences were significant compared with parameter values of the control. Moreover, mulching cultivation significantly increased the root dry weight of citrus trees, but no significant effect on specific root length or specific root surface area was observed (Table 5).

Table 4. Effect of coverage on citrus root length, surface area, volume and average diameter

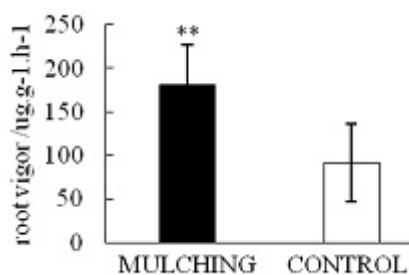
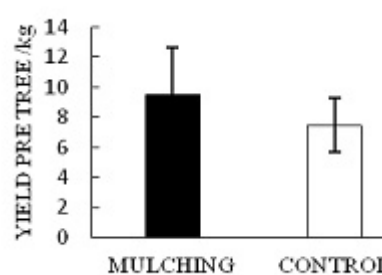
Treatment	Total root length (cm)	Total root surface area (cm ²)	Total root volume (cm ³)	Average root diameter (mm)
Mulching	5815.1±720.3a	1198.2±112.4a	19.9±1.4a	0.66±0.04a
Open-field	4224.0±300.9b	820.9±48.0b	12.9±0.9b	0.62±0.02a

Table 5. Effects of mulching on dry weight, specific root length and specific surface area of citrus roots

Treatment	Root dry weight (g)	Specific root length (cm/g)	Specific root surface area (cm ² /g)
Mulching	8.93±0.59a	649.82±39.48a	134.08±4.08a
Open-field	6.30±0.56b	671.78±36.61a	130.56±5.11a

3.7. Effect of mulching on root vigor of citrus

Under control cultivation, the root vigor of citrus trees was 91.7 µg/g/h. Mulching cultivation increased the root vigor of citrus trees to 181.5 µg/g/h, which was 89.8 µg/g/h higher than under control cultivation, with an increase rate of 97.9% (Figure 5). This result indicates that mulching cultivation improved the root vigor of citrus trees.

**Figure 5. Effect of coverage on citrus root vigor****Figure 6. The effect of coverage on the yield of citrus per plant**

3.8 Effect of mulching on the yield per plant of citrus

The effect of anti-grass cloth mulching on the fruit yield per plant of citrus is shown in Figure 6. The average fruit yield per plant of citrus under mulching cultivation was 12.3 kg, which was 3.8 kg higher than under control cultivation, with an increase rate of 44.7%. This result indicates that mulching with anti-grass cloth substantially increases the yield per plant of citrus.

4. DISCUSSION

Mulching cultivation is one of the most effective means to hold soil moisture. Additionally, mulching cultivation can reduce soil evaporation and improving crop transpiration and agronomic efficiency (Wang *et al.*, 2011). It increases the water reserves in the planting area via rainwater collection. During our experimental period, the soil water content under mulching cultivation was higher than under control cultivation through March to December, 2018. The difference in soil water content between the mulching and control treatments was significant in the hot and rainy summer, and the difference was reduced in the cold and dry winter. This result may be attributed to the fact that in the hot summer period, soil surface evaporation considerably increases, whereas the barrier effect of anti-grass cloth can effectively reduce the substantial soil water evaporation and drive the backflow of the evaporated water into the surface soil; these changes would stabilize the soil water content and eliminate the adverse effects of rapid soil water evaporation on the roots of fruit trees. Jiang *et al.* (2015) studied the water content and temperature in soils from mulched and open fields of orchard during summer, and they reported that mulching considerably reduced the ineffective evaporation of soil water, which is consistent with our findings.

Temperature exhibits an obvious effect on soil nitrogen mineralization and the rate of soil nitrogen mineralization increases with increasing soil temperature (Andersen & Jensen, 2001). The warming and water retention effects of anti-grass cloth can effectively raise soil temperature and thereby promote nitrogen mineralization in the soil, which in turn increases soil nitrogen content (Gen, 2010). Our results showed that soil exchangeable magnesium and exchangeable calcium contents both decreased significantly after mulching, which may be related to ionic antagonism. An increase in soil potassium content could lead to the decrease in the contents of antagonized elements including magnesium and calcium. Cui *et al.* (1998) found that mulching cultivation can increase the consumption of various zinc, copper, and manganese species in the soil. In the current study, mulching significantly increased soil available zinc and available manganese contents, along with a decrease in the available boron content. This result is inconsistent with the previous study.

Soil microbes are highly sensitive to human disturbances. In particular, agricultural management practices, such as mulching, tillage, and fertilization, can cause changes in the diversity and richness of the soil microbial community (Beauregard *et al.*, 2010; Huang *et al.*, 2019). Our experimental data showed that mulching with anti-grass cloth did not affect the number of soil fungi in citrus orchard, but it significantly increased the number of soil bacteria and actinomycetes and the total population of soil microbes. Moreover, the analysis microbial community diversity revealed that the OTUs of soil microbes detected under anti-grass cloth mulching was higher than under control cultivation. In terms of microbial community richness, the mulching treatment was also superior to the control treatment. These results are in agreement with other studies (Zhu *et al.*, 2018). The main reason is that mulching can inhibit soil water evaporation, increase surface soil temperature, and improve soil surface microenvironment.

Because it creates a root zone environment with favorable soil water content and temperature, mulching is conducive to root growth and proliferation, leading to significant increases in root morphological characteristics such as dry weight, length, surface area, and volume (Gu *et al.*, 2019; Sun *et al.*, 2018). However, other studies have indicated that mulching treatment can result in a decrease in the total biomass of fine roots and horizontal distribution of the roots in fruit trees, because the mulching material is not easily permeable for water and air, which affects the growth and activity of fine roots in the soil (Sun *et al.*, 2016).

In this study, the total root length, total root surface area, total root volume, and root dry weight of citrus trees respectively increased by 37.7%, 46.0%, 54.3%, and 41.7% under the mulching treatment compared with the control treatment. Additionally, the root vigor of citrus trees was significantly higher under mulching cultivation than under control cultivation. These results indicate that mulching has a significant effect at promoting the root growth of citrus. Since anti-grass cloth mulching can alter the soil environment, it also has a prominent effect on the growth of crop roots. On the contrary, past studies have also indicated that mulching can lead to a decline in root function during the later stages of crop growth (Zhao, 2013).

5. CONCLUSIONS

The anti-grass covering can improve the soil hydrothermal condition and the overall nutrient level of the citrus orchard, which is beneficial to the growth of citrus roots.

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