Effects of deficit irrigation on soil microorganisms and growth of Arabica coffee (*Coffea arabica* L.) under different shading cultivation modes

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Abstract: In the present research, the rational coupling mode of irrigation and shading cultivation for rapid growth and water saving of young Arabica coffee shrubs was investigated from 2016 to 2017. Taking full irrigation (FI, 1.2Ep) as the control, the effects of three deficit irrigation (DI) (DI_1, DI_2 and DI_3, with 1.0Ep, 0.8Ep and 0.6Ep) on soil water content, temperature, microorganism population density, photosynthetic characteristics, canopy structure and dry mass of Arabica coffee under three shading cultivation modes (S_0, monoculture coffee; S_1, mild shading cultivation, intercropping with one line of Arabica coffee and one line of castor (*Ricinus communis* L.); S_2, severe shading cultivation, intercropping with one line of Arabica coffee and two lines of castor) were investigated using plot experiments. Compared to FI, DI_1 not obviously changed the population density of soil bacteria and actinomycetes, but increased net photosynthetic rate (Pn), crown area and dry mass of Arabica coffee by 7.0%, 9.53% and 10.46%, respectively. In addition, DI_1 also decreased total radiation under canopy (TRUC) by 5.51%. DI_2 and DI_3 reduced the population density of soil bacteria, fungi and actinomycetes with a range of 8.94%-47.06%. Compared to S_0, S_1 increased the population density of soil fungi, bacteria and actinomycetes by 13.99%, 30.77% and 9.72%, respectively. S_1 also increased Pn, transpiration rate (Tr), leaf apparent radiation use efficiency (ARUE), leaf area index (LAI) and dry mass by 9.29%, 5.39%, 60.98%, 10.31% and 30.02%, respectively. DI_1S_1 obtained the highest Pn and dry mass and higher LAI and the lowest TRUC. DI_1S_1 increased Pn, ARUE and dry mass by 18.98%, 72.37% and 62.90% respectively but decreased TRUC by 21.77% when compared to FIS_0. Thus, DI_1S_1 was found to be the rational mode of irrigation and shading cultivation for young Arabica coffee.

Keywords: Arabica coffee, shading cultivation, *Ricinus communis* L., irrigation quantity, soil microorganism, canopy structure, light-water regulation

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1 Introduction

The People's Republic of China is one of the most important coffee-producing areas in Asia. Arabica coffee is also known as Arabian coffee (*Coffea arabica* L., Fam.: Rubiaceae) and is mainly cultivated in China under warm and humid climates. The planting area and yield of Arabica coffee in Yunnan Province were about 1.18×10^5 hm^2 and 1.39×10^8 kg, respectively in 2016 which occupy more than 98% of the total production of coffee in China. The qualitative characters of Arabica coffee in Yunnan are special. The extracted liquor is dense but not bitter, fragrant but not strong and has slight fruit acid taste. The regions of Yunnan with coffee plantation have abundant sunshine, warm winter, high rate of evaporation and longer duration of dry season. Associated with all these factors, lacking of scientific irrigation management actually restricts high and efficient production of Arabica coffee in this region[1].

Deficit irrigation (DI) is a water-saving irrigation technique aiming to solve water scarcity and low water use efficiency (WUE)[2]. Many studies have shown that DI can save irrigation water greatly, improve irrigation WUE, while maintaining or increasing crop yields[2,3]. Compared to full irrigation, DI significantly reduced the coffee root activity, number of flower and fruit, plant height, crown width, stem diameter and root density, but increased the content of chlorophyll, carotenoids, malondialdehyde, proline and soluble sugar in leaves[4-7]. Mild DI (irrigation amount: 80% of full irrigation) reduced the yield of Arabica coffee by only 6.4%, but significantly increased water use efficiency[5,8,9]. While moderate and strong DI (irrigation amount: 60% and 40% of full irrigation) significantly reduced yield and water use efficiency[10]. Compared to conventional irrigation, moderate DI increased the population density of soil microorganisms, C-biomass, catalase activity, urease activity and invertase activity[11,12]. However, until recently the effect of DI on the environment of soil microorganism and the promotion of Arabica coffee growth is unknown.

To see the effect of shading cultivation, coffee intercropping was carried out with maize[13], banana[14], macadamia[7], Tabebuia rosea and Simarouba glauca in a number of research works[15]. It has been observed that shading cultivation can create a suitable microclimatic environment for Arabica coffee production. The microclimatic environment can help to reduce leaf surface temperature[16], increase leaf area[17], change leaf photosynthetic

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characteristics[18,19] and control pests and diseases. Balance in nutrition and reproductive growth, increasing biomass accumulation[20], decreasing the phenomenon of biennial bearing[21] and increasing the bean size were also observed. All these factors help to improve the quality of drinks (aroma, taste and acidity)[20-22], and improve economic benefit and water-fertilizer-light use efficiencies[23]. Shading cultivation can also improve soil quality of coffee cultivation and increase soil microbial population[24-26], organic carbon storage[27] and infiltration capacity[28]. But improper shading cultivation can lead to yield reduction[29]. However, the effects of different shading cultivation modes on soil micro-ecological environment, canopy structure and growth and suitable shading mode of young Arabica coffee plant need further investigation.

Under shading cultivation, drip irrigation can promote coffee growth, yield and can bring economic benefits[30-32]. Shading at DI significantly reduced leaf transpiration but increased the apparent radiation use efficiency, and a combination of proper shading (50% natural light intensity) with mild DI (65%-75% field capacity) can also cause higher dry mass and water use efficiency[33]. As Arabica coffee sapling stage is longer, choosing proper shading crop can make full use of land resource and compensate economic gap, and create a suitable light environment for Arabica coffee growth. Castor (*Ricinus communis* L., Fam.: Euphorbiaceae) is a perennial shrubby plant having fast growth and good shading of crown. The cultivation method of this plant is also easy and the produced biomass has got high medicinal value. However, it is still unknown about the effective combination of DI and shading cultivation mode by castor plant to improve the soil environment particularly the soil microorganisms in order to promote the growth and production of Arabica coffee.

Therefore, the objective of this study was to investigate the impacts of DI on soil microecological environment, canopy structure, photosynthetic characteristics and dry mass of Arabica coffee under different shading cultivation modes by castor plants. By comparing the results with full irrigation and by selecting the appropriate coupling mode of DI and shading cultivation it will be possible to provide scientific basis for rational irrigation and light management of young Arabica coffee shrubs.

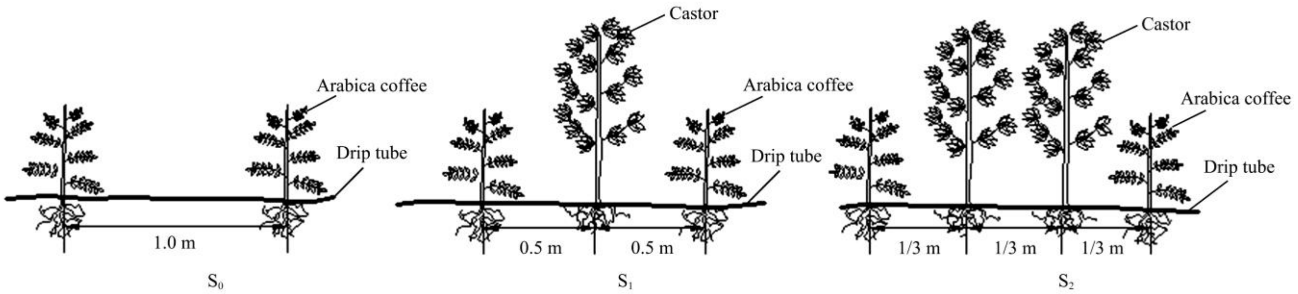
2 Materials and methods

2.1 Experimental site and materials

The experiment was conducted from June 2016 to December 2017 in a well-equipped greenhouse present in Kunming University of Science & Technology, Kunming, Yunnan, China (24°9'N and 102°79'E; 1978.9 m a.s.l.). The greenhouse was oriented from north to south, and the light intensity was approximately 90% of the natural light. The length, span, and ridge height were 100 m, 21 m, and 3 m, respectively. The temperature was 20°C-35°C, the air humidity was 45%-70%, and the CO_2 concentration was 365-395 μmol/mol. The soil of the experimental field was dry red (a Typic Hapludult, a Ferric Acrisol). The top soil contained total nitrogen 0.87 g/kg, total phosphorus 0.68 g/kg, total potassium 13.90 g/kg and organic matter content 5.05 g/kg. One-year-old Arabica coffee seedling (Catimor P7963), which is the major cultivar in Yunnan, China, was used as experimental plant. The seedlings were transplanted with planting spacing and row spacing of 1.0 m and 1.3 m, respectively on June 1, 2016. The shading crop used with Arabica coffee was castor plants having large canopy with an easy control and good symbiotic condition between them. After 69 d of nursery period, coffee seedlings were given water treatment on August 8, 2016.

2.2 Experimental design and method

Four irrigation levels and three shading modes were included in the experiment. This experimental plan yielded 12 treatments (i.e., 4×3). Each treatment was replicated four times so a total of 48 experimental plots were created. The area per plot was 40 m^2 (8 m×5 m). The four irrigation levels used in the experiment included full irrigation (FI, irrigation amount was 1.2Ep and Ep was evaporation from water surface in irrigation adjacent time[34] and three deficit irrigation (DI_1, DI_2 and DI_3, with 1.0Ep, 0.8Ep and 0.6Ep of irrigation amount, respectively). Irrigation frequency was seven days. Three shading cultivation modes included were S_0 (monoculture coffee), S_1 (light shading cultivation, intercropping with one line of Arabica coffee and one line of castor plant) and S_2 (severe shading cultivation, intercropping with one line of Arabica coffee and two lines of castor plants), respectively (Figure 1).



Note: S_0 no shading (monoculture), S_1 and S_2, mild and severe shading, respectively.

Figure 1 Schemtic diagram of three shading modes of Arabica coffee

Irrigation amount of FI during the experiment period (from August 8, 2016 to December 17, 2017) was 858.4 mm, and the cumulative amount of irrigation has been shown in Figure 2. Surface drip irrigation was used with system working pressure of 0.1 MPa and irrigation was measured by water meter. The equivalent compound fertilizer ($N:P_2O_5:K_2O$ = 15:15:15) of 40 g/plant was applied on September 10, 2016; March 13, 2017 and June 17, 2017. Fertilizer was uniformly spread under the crown of Arabica coffee plants and then turned over and raked soil. Standard evaporator was set in the center of the greenhouse and the height was always consistent with Arabica coffee canopy height. Water surface evaporation was measured every morning at 8:30.

2.3 Parameters and measuring methodology

Soil water content was determined by oven-drying method during typical irrigation period (April 11, 13, 15 and 16 in the Spring of 2017, August 15, 17, 19 and 20 in the Summer of 2017, October 7, 9, 11 and 12 in the Autumn of 2017). The process followed 5-point measurements 20 and 40 cm away from plant truck at 0.1 m intervals with maximal soil depth of 50 cm. Observation date and position of soil temperature were same as soil water content. Geothermal temperature in ploughing layer 0-50 cm (every 5 cm) was determined by digital thermometer (LCD-06058, China) every 2 h from 08:00-18:00.

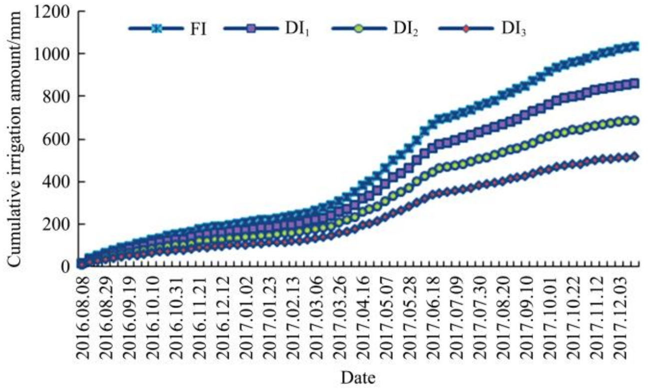


Figure 2 Changes of cumulative irrigation amount during the experimental period

Soil samples were collected by 5-point mixing method on 4th day after irrigation in a typical irrigation period (April 13th in spring, August 17th in summer and October 9th in August, 2017). The location and method of boring earth samples were same as soil water content. Soil microorganism population density was determined by dilution plate method. Soil bacteria, fungi and actinomycetes were cultured by beef extract + peptone + agar medium, Martin medium and improved Gauss No. 1 medium, respectively[11].

Photosynthetic characteristics of functional leaves in same direction were determined every 2 h using a portable photosynthesis system (LI-6400XT, USA). The measurements were carried out from 10:00-16:00 h on 4th day after irrigation in a typical irrigation period (April 6th and August 20th, 2017) in spring and summer with replicates in each.

Leaf water use efficiency is counted using Equation (1).

$$\mathrm{LWUE}=Pn/Tr \tag{1}$$

where, LWUE is leaf water use efficiency, mmol/mol; Pn is net photosynthesis rate, μmol/m^2 s; and Tr is transpiration rate, mmol/m^2 s.

Leaf apparent radiation use efficiency[35] was calculated using Equation (2).

$$\mathrm{ARUE}=Pn/\mathrm{PAR} \tag{2}$$

where, ARUE is leaf apparent radiation use efficiency, μmol/mmol; PAR is photosynthetic active radiation, mmol/m^2 s.

Leaf area index (LAI) and the total radiation under the crown (TRUC) of Arabica coffee were determined by canopy analyzer (Winscanopy, Canada) at the end of the experiment, the single leaf area index is the ratio of leaf area index and leaf number.

The crown area[36] was calculated using Equation (3).

$$\mathrm{CA}=0.25\pi\cdot\mathrm{CL}\cdot\mathrm{CW} \tag{3}$$

where, CA is crown area, cm; CL is crown length, cm; CW is crown width, cm.

At the end of the experiment, different organs were harvested separately for determining dry biomass accumulation. The plant material was firstly dried at 105℃ for 30 min, and then dried at 80℃ to constant weight. Root-shoot ratio has been defined as the amount of root dry mass per unit canopy dry mass.

2.4 Statistical analysis

The experimental data was collected and collated in Excel 2010 (Microsoft Corp., 2010). Analysis of variance (ANOVA) was performed using two-way ANOVA from SAS software. All treatment means were compared for any significant differences using the Duncan's multiple range tests at significant level of $P_{0.05}$ using the SAS8.2 for Windows software package (SAS Institute, USA).

3 Results

3.1 Soil water content

The effect of irrigation level and shading mode on soil water content was significant. Their interaction prominently affected it in spring and autumn (Table 1). Compared to FI, DI reduced the soil water content in spring, summer, autumn and the seasonal average by 16.23%-33.26%, 5.33%-11.88%, 8.86-32.15% and 9.33%-25.20%, respectively. In contrast with S_0, S_1 and S_2 did not apparently reduce soil water content except S_2 dramatically reduced it in spring and autumn. Compared to FIS_0 (CK), the other treatments reduced soil water content by 7.91%-36.90%, 5.12%-14.69% and 7.78%-35.17% in spring, summer and autumn, respectively. In addition, when the average value for three seasons was considered, the soil water content was also seen reduced by 5.50%-28.49%. However, the trend as mentioned before was not valid for FIS_1, DI_1S_0 and DI_1S_1, which did not show any reduction in the soil water content during summer. The treatment FIS_2 also did not reduce the soil water content obviously.

Table 1 Soil water content under deficit irrigation and shading mode

Irrigation level	Shading mode	Spring/%	Summer/%	Autumn/%	Three-season average/%
FI	S_0	24.39 ± 0.62^{a}	25.39 ± 0.32^{a}	21.72 ± 0.26^{a}	23.83 ± 1.10^{a}
	S_1	23.68 ± 0.1^{b}	25.2 ± 0.21^{ab}	21.32 ± 0.41^{a}	23.40 ± 1.13^{ab}
	S_2	22.46 ± 0.42^{c}	25.07 ± 0.48^{ab}	20.03 ± 0.25^{b}	22.52 ± 1.46^{ab}
DI_1	S_0	20.57 ± 0.28^{d}	24.72 ± 0.11^{bc}	19.44 ± 0.36^{c}	21.58 ± 1.61^{ab}
	S_1	20.09 ± 0.44^{d}	24.33 ± 0.32^{cd}	19.23±0.23cd	21.22 ± 1.58^{ab}
	S_2	18.42 ± 0.39^{e}	24.09 ± 0.22^{de}	18.81±0.11d	20.44 ± 1.83^{ab}
DI_2	S_0	18.05 ± 0.47^{e}	23.72 ± 0.18^{ef}	18.22 ± 0.13^{e}	20.00 ± 1.86^{ab}
	S_1	17.94 ± 0.39^{e}	23.56 ± 0.2^{f}	17.41 ± 0.43^{f}	19.64 ± 1.97^{ab}
	S_2	17.79 ± 0.18^{e}	23.54 ± 0.25^{f}	16.26 ± 0.13^{g}	19.20 ± 2.22^{ab}
DI_3	S_0	15.91 ± 0.38^{f}	22.88 ± 0.12^{g}	14.61 ± 0.24^{h}	17.80 ± 2.57^{ab}
	S_1	15.77 ± 0.24^{f}	22.13 ± 0.51^{h}	14.1 ± 0.29^{i}	17.33 ± 2.45^{b}
	S_2	15.39 ± 0.14^{f}	21.66 ± 0.32^{h}	14.08 ± 0.29^{i}	17.04 ± 2.34^{b}
Significance test (p values)					
Irrigation level		<0.001	<0.001	<0.001	<0.001
Shading mode		<0.001	<0.001	<0.001	0.043
Irrigation level* Shading mode		<0.001	0.129	<0.001	0.999

3.2 Soil temperature

The influence of irrigation level on soil temperature in autumn was remarkable, and shading mode had a significant effect on soil temperature (Table 2). Compared to FI, DI_1 did not conspicuously increase soil temperature in autumn, while DI_2 and DI_3 increased it by 6.84% and 7.95%, respectively. If compared to S_0, S_1 reduced the soil temperature in spring, autumn and seasonal average by 8.05%, 8.30% and 6.19%, respectively, while decreased it in summer was not obvious. S_2 declined the soil temperature in spring, summer and autumn and seasonal average by 10.10%, 8.35%, 10.60% and 9.56%, respectively.

3.3 The population density of soil microorganisms

The impact of irrigation level and shading mode on the population density of soil bacteria, fungi and actinomycetes was significant except the influence of irrigation level on actinomycetes in spring and shading mode on bacteria in autumn (Table 3). Soil bacteria population density was predominant in summer, followed in spring, and least in autumn. If compared to FI, DI_2 and DI_3 diminished the seasonal average by 17.41% and 47.06%, respectively, while DI_1 did not change the population density of bacteria in summer, autumn and seasonal average and DI_2 did not reduce it in summer obviously. Soil bacteria population density firstly increased and then decreased with increasing shade degree and S_1 had the highest soil bacteria population density. Compared to S_0, S_1 added the seasonal average by 13.99%, while S_2 reduced it by 11.11%.

Table 2 Soil temperature under deficit irrigation and shading mode

Irrigation level	Shading mode	Spring/ ℃	Summer/ ℃	Autumn/ ℃	Three-season average/ ℃
FI	S_0	21.64±1.90^{abc}	24.78±2.67^{a}	15.98±1.38^{bcd}	20.80±2.57^{a}
	S_1	20.11±1.33^{bc}	24.37±2.78^{a}	15.25±0.91^{d}	19.91±2.63^{a}
	S_2	19.83±1.28^{c}	22.87±3.16^{a}	14.67±0.68^{d}	19.12±2.39^{a}
DI_1	S_0	22.67±2.09^{ab}	24.85±2.00^{a}	17.07±1.73^{abc}	21.53±2.32^{a}
	S_1	20.54±1.41^{abc}	24.27±1.97^{a}	15.40±1.16^{d}	20.07±2.57^{a}
	S_2	20.21±1.39^{bc}	22.87±1.30^{a}	14.97±1.02^{d}	19.35±2.32^{a}
DI_2	S_0	23.07±3.09^{a}	25.53±2.52^{a}	17.78±1.82^{a}	22.13±2.29^{a}
	S_1	20.84±1.73^{abc}	23.90±2.15^{a}	15.78±0.86^{cd}	20.17±2.37^{a}
	S_2	20.62±1.67^{abc}	22.92±1.97^{a}	15.48±0.83^{cd}	19.67±2.20^{a}
DI_3	S_0	23.05±2.34^{a}	24.28±1.92^{a}	17.45±1.39^{ab}	21.59±2.10^{a}
	S_1	21.66±2.29^{abc}	23.87±2.26^{a}	16.18±1.21^{bcd}	20.57±2.29^{a}
	S_2	20.64±1.61^{abc}	22.48±1.65^{a}	15.92±1.03^{bcd}	19.68±1.95^{a}
Significance test (p values)					
Irrigation level		0.236	0.876	0.016	0.406
Shading mode		<0.001	0.008	<0.001	<0.001
Irrigation level* Shading mode		0.994	0.996	0.902	0.574

The population density of soil fungi decreased with the increase of water deficit while increased with the increase of shading degree. When compared with FI, the seasonal average of fungus population density in DI_1, DI_2, and DI_3 decreased by 9.72%, 18.62% and 34.12%, respectively. In contrast with S_0, the seasonal average in S_1 and S_2 increased by 30.77% and 63.99%, respectively.

Table 3a Population density of soil microorganisms (bacterium) under deficit irrigation and shading mode

/×10^{7} g^{-1}

Irrigation level	Shading mode	Spring	Summer	Autumn	Season average
FI	S_0	6.50±0.94^{abc}	7.93±1.09^{ab}	3.64±0.73^{abc}	6.02±0.92^{a}
	S_1	7.51±0.73^{a}	8.15±1.08^{ab}	4.14±0.12^{a}	6.6±0.98^{a}
	S_2	5.60±0.37^{cde}	7.91±0.58^{ab}	3.28±0.54^{abc}	5.6±0.83^{a}
DI_1	S_0	5.73±0.08^{cd}	8.18±0.85^{ab}	3.66±0.36^{abc}	5.86±0.43^{a}
	S_1	6.86±0.57^{ab}	8.74±0.29^{a}	4.06±0.63^{ab}	6.55±0.50^{a}
	S_2	4.74±0.68^{def}	7.77±0.31^{ab}	3.41±0.47^{abc}	5.3±0.69^{a}
DI_2	S_0	4.53±0.34^{ef}	7.75±0.81^{ab}	2.58±0.52^{bcde}	4.96±0.59^{a}
	S_1	5.86±0.75^{bcd}	8.05±0.60^{ab}	2.92±0.46^{abcd}	5.61±0.71^{a}
	S_2	3.74±0.86^{f}	7.39±0.53^{b}	2.33±0.26^{cde}	4.48±0.67^{a}
DI_3	S_0	3.77±0.47^{f}	4.38±0.36^{c}	1.40±0.23^{ef}	3.18±0.36^{a}
	S_1	5.06±0.68^{de}	5.35±0.38^{c}	1.76±0.26^{def}	4.06±0.44^{a}
	S_2	2.31±0.23^{g}	4.30±0.49^{c}	0.61±0.09^{f}	2.41±0.29^{a}
Significance test (p values)					
Irrigation level		<0.001	<0.001	<0.001	<0.001
Shading mode		<0.001	<0.001	0.056	0.004
Irrigation level* Shading mode		0.929	0.006	0.994	0.981

Table 3b Population density of soil microorganisms(fungus) under deficit irrigation and shading mode

/×10^{3} g^{-1}

Irrigation level	Shading mode	Spring	Summer	Autumn	Season average
FI	S_0	7.65±0.55^{b}c	6.52±0.50^{de}	11.29±1.15^{bc}	8.49±0.73^{abc}
	S_1	8.76±0.95^{ab}	7.92±0.53^{c}	12.08±0.89^{bc}	9.59±0.79^{abc}
	S_2	9.85±0.85^{a}	10.99±0.83^{a}	14.42±1.20^{a}	11.75±0.96^{a}
DI_1	S_0	6.95±1.28^{bcd}	5.89±0.49^{ef}	9.59±0.90^{de}	7.48±0.89^{abc}
	S_1	7.80±1.25^{abc}	7.37±0.49^{cd}	11.93±1.15^{bc}	9.03±0.97^{abc}
	S_2	8.96±1.28^{ab}	9.63±0.51^{b}	12.66±0.78^{b}	10.42±0.86^{ab}
DI_2	S_0	6.44±0.74^{cd}	4.63±0.51^{fg}	8.63±0.56^{ef}	6.56±0.60^{bc}
	S_1	7.15±1.72^{bcd}	7.32±0.85^{cd}	10.68±0.53^{cd}	8.38±1.03^{abc}
	S_2	7.92±2.14^{abc}	8.46±1.46^{bc}	11.60±0.50^{bc}	9.33±1.37^{abc}
DI_3	S_0	5.11±0.69^{d}	3.96±0.86^{g}	7.60±0.58^{f}	5.56±0.71^{c}
	S_1	5.94±0.68^{cd}	4.86±0.72^{fg}	9.12±0.43^{e}	6.64±0.61^{bc}
	S_2	6.39±0.46^{cd}	5.37±0.65^{ef}	10.61±0.65^{cd}	7.46±0.58^{abc}
Significance test (p values)					
Irrigation level		<0.001	<0.001	0.010	<0.001
Shading mode		0.004	<0.001	0.005	<0.001
Irrigation level* Shading mode		0.993	0.064	0.990	0.2635

Table 3c Population density of soil microorganisms (*actinomycete*) under deficit irrigation and shading mode

/×10^{5} g^{-1}

Irrigation level	Shading mode	Spring	Summer	Autumn	Season average
FI	S_0	11.45±2.63^{ab}	14.25±1.96^{bc}	10.80±0.65^{ab}	12.17±1.75^{abc}
	S_1	12.54±2.06^{a}	16.19±0.49^{ab}	12.16±0.87^{a}	13.63±1.14^{a}
	S_2	10.15±0.51^{ab}	11.15±0.51^{de}	9.08±0.61^{cd}	10.12±0.54^{abc}
DI_1	S_0	11.47±0.95^{ab}	14.86±0.51^{abc}	11.03±0.22^{ab}	12.45±0.56^{abc}
	S_1	10.40±0.55^{ab}	16.73±0.77^{a}	11.27±0.39^{ab}	12.80±0.57^{ab}
	S_2	9.85±2.25^{ab}	13.42±0.89^{c}	9.03±0.38^{cd}	10.76±1.17^{abc}
DI_2	S_0	10.01±1.65^{ab}	13.13±2.74^{cd}	9.17±1.32^{cd}	10.77±1.90^{abc}
	S_1	10.94±0.74^{ab}	15.79±0.72^{ab}	10.08±1.11^{bc}	12.27±0.86^{abc}
	S_2	9.03±2.18^{b}	10.99±0.98^{de}	9.00±1.23^{cd}	9.67±1.46^{abc}
DI_3	S_0	9.20±0.93^{b}	11.21±1.14^{de}	7.52±0.65^{e}	9.31±0.91^{bc}
	S_1	10.22±0.94^{ab}	12.83±0.44^{cd}	7.99±0.94^{de}	10.34±0.77^{abc}
	S_2	8.67±1.21^{b}	9.02±0.92^{e}	7.23±0.59^{e}	8.31±0.91^{c}
Significance test (p values)					
Irrigation level		0.064	0.010	<0.001	<0.001
Shading mode		0.024	<0.001	<0.001	<0.001
Irrigation level* Shading mode		0.999	0.995	0.161	0.7149

The population density of soil actinomycetes was predominating in summer, but the same population was little in spring and autumn. Compared to FI, DI decreased the population density of soil actinomycetes in different degrees except DI_1 increased it remarkably in summer and did not increase the seasonal average significantly. DI_2 and DI_3 decreased the seasonal average by 8.94% and 22.17%, respectively. The population density of soil actinomycetes increased first and then decreased with the increase of the degree of shading. The treatment S_1 had the highest soil actinomycetes population density, but S_2 had the minimum. Compared to S_0, the seasonal average population density of actinomycetes raised in S_1 by 9.72%, while reduced in S_2 by 13.05%.

The results of the present investigation show that there were two significant functional relationships between the population density of soil bacteria, fungi and actinomycetes and soil water content or temperature. The population of all these microorganisms increased first and then decreased with subsequent increase in the soil water content and the temperature (Table 4). This indicates that suitable soil moisture and temperature had positive effect on soil microorganism growth and reproduction.

Table 4 Results of regression analysis of soil temperature, water content and population density of different microorganisms under deficit irrigation and shading mode

Season	Microbial species	Regression equation	R^2
Spring	Bacterium	$Y=-0.028W^2-0.303T^2+1.541W+13.159T-156.423$	0.756*
	Fungus	$Y=-0.045W^2+0.302T^2+1.992W-13.754T+141.744$	0.944*
	Actinomycetes	$Y=-0.003W^2-0.282T^2+0.207W+12.259T-127.314$	0.689*
Summer	Bacterium	$Y=-0.404W^2-0.245T^2+20.270W+11.722T-105.774$	0.918*
	Fungus	$Y=-0.292W^2+0.222T^2+15.319W-12.429T-22.062$	0.958*
	Actinomycetes	$Y=-0.542W^2-0.829T^2+26.282W+40.759T-804.336$	0.721*
Autumn	Bacterium	$Y=-0.037W^2-0.065T^2+1.690W+2.138T-33.126$	0.919*
	Fungus	$Y=-0.020W^2+0.365T^2+0.874W-13.490T+123.852$	0.958*
	Actinomycetes	$Y=-0.025W^2-0.537T^2+1.473W+17.779T-155.353$	0.879*
Three-season average	Bacterium	$Y=-0.106W^2-0.332T^2+4.824W+13.793T-191.652$	0.961*
	Fungus	$Y=-0.062W^2+0.485T^2+2.907W-21.341T+208.111$	0.992*
	Actinomycetes	$Y=-0.117W^2-0.865T^2+5.301W+36.090T-423.118$	0.822*

Note: *W*, *T* and *Y* represent soil water content, temperature and population density of soil microorganism, respectively. R means correlation coefficient of regression equation, and the asterisk (*) means R was significant at the 0.05 probability level.

3.4 Photosynthetic characteristics of Arabica coffee

By analyzing the results of the present investigation, it has been seen that the irrigation level and shading mode had significant impact on the net photosynthesis rate (Pn), the transportation rate (Tr), the leaf water use efficiency (LWUE) and the apparent radiation use efficiency (ARUE). There were marked interaction effects of irrigation level and shading mode on Pn and ARUE in summer, and seasonal average of Pn and ARUE (Table 5).

In spring, compared to FI, DI_1 did not remarkably affect Pn, Tr, LWUE and ARUE in spring. However, DI_2 decreased Pn, Tr and ARUE by 7.99%, 5.37% and 7.39%, respectively. Meanwhile, DI_3 decreased Pn, Tr and ARUE by 13.84%, 10.95% and 13.55%, respectively. In contrast with S_0, S_1 did not add Tr and LWUE obviously, but increased Pn and ARUE by 5.88 and 56.98%, respectively. S_2 reduced Pn by 5.32%, decreased Tr and LWUE inconspicuously, but added ARUE by 104.88%.

Pn, Tr and ARUE raised first and then reduced with the increase of water deficit in summer. Compared to FI, DI_1 increased Pn and Tr by 9.70% and 6.90%, respectively with no obvious increase in ARUE. DI_2 and DI_3 did not change Pn significantly. DI_3 reduced Tr and ARUE by 7.35% and 8.68%, respectively. Compared to S_0, S_1 added Pn, Tr and ARUE by 11.93, 8.60 and 64.10%, while S_2 reduced Pn and Tr by 10.43% and 6.41%, respectively and increased ARUE by 94.22%. javascript:;javascript:;S_1 increased Pn, Tr and ARUE by 11.93%, 8.60% and 64.10%, respectively while S_2 decreased Pn and Tr by 10.43% and 6.41%, respectively and increased ARUE by 94.22%. DI_1S_1 got the largest Pn and the larger ARUE, which were 1.27 and 1.83 times of CK, respectively.

In seasonal average, compared to FI, DI_1 increased Pn by 7.00% while it did not increase Tr, LWUE and ARUE obviously. DI_2 did not prominently change photosynthetic characteristics. DI_3 reduced Pn, Tr and ARUE by 8.88%, 9.32% and 10.87%, respectively. Pn, Tr and LWUE first boosted and then decreased with the increase of shading degree, while ARUE continued to raise. S_1 increased Pn, Tr and ARUE by 9.29%, 5.39% and 60.98%, respectively, while did not increase LWUE obviously. S_2 reduced Pn by 8.20% and increased ARUE by 98.88% if compared to S_0. DI_1S_1 obtained highest Pn of 3.42 umol/m^2 s and raised it by 18.98% if compared to CK. Compared to CK, the other treatments increased ARUE by 2.76%-109.11% except for DI_3S_0 which reduced it by 7.83%, and DI_1S_1 added it by 72.37%.

Table 5a Photosynthetic characteristics of Arabica coffee under deficit irrigation and shading mode

Irrigation level	Shading mode	Spring			
		Pn/μmol m^{-2} s^{-1}	Tr/mmol m^{-2} s^{-1}	LWUE/μmol mmol^{-1}	ARUE/mmol umol^{-1}
FI	S_0	2.64±0.02c	2.15±0.02ab	1.19±0.10a	9.26±0.36e
	S_1	2.77±0.03b	2.21±0.09a	1.25±0.15a	14.36±1.10cd
	S_2	2.53±0.01de	2.13±0.06ab	1.16±0.12a	19.26±0.87a
DI_1	S_0	2.74±0.01b	2.11±0.05abc	1.27±0.11a	9.43±0.34e
	S_1	2.88±0.01a	2.19±0.05ab	1.29±0.11a	14.83±1.19cd
	S_2	2.61±0.00cd	2.11±0.08abc	1.21±0.14a	19.18±0.78a
DI_2	S_0	2.41±0.04f	2.04±0.04abc	1.15±0.13a	8.52±0.41e
	S_1	2.59±0.04cd	2.11±0.09abc	1.20±0.16a	13.52±1.11cd
	S_2	2.30±0.04g	2.00±0.10abc	1.12±0.16a	17.68±1.05ab
DI_3	S_0	2.29±0.03g	1.95±0.05bc	1.16±0.13a	8.11±0.37e
	S_1	2.43±0.04ef	1.95±0.09bc	1.22±0.17a	12.72±1.05d
	S_2	2.11±0.04 h	1.88±0.09c	1.11±0.16a	16.24±1.04bc
Significance test (*P* values)					
Irrigation level		<0.0001	<0.0001	0.001	<0.0001
Shading mode		<0.0001	0.002	0.000	<0.0001
Irrigation level*Shading mode		0.486	0.713	0.912	0.234

Table 5b Photosynthetic characteristics of Arabica coffee under deficit irrigation and shading mode

Irrigation level	Shading mode	Spring			
		Pn/μmol m^{-2} s^{-1}	Tr/mmol m^{-2} s^{-1}	LWUE/μmol mmol^{-1}	ARUE/mmol umol^{-1}
FI	S_0	3.11±0.06e	1.79±0.01de	1.77±0.05cd	10.91±0.48fg
	S_1	3.68±0.02b	1.94±0.04b	1.88±0.05abcd	18.97±0.42d
	S_2	2.84±0.02g	1.63±0.01g	1.73±0.00d	22.92±0.06a
DI_1	S_0	3.50±0.03bc	1.91±0.02bc	1.84±0.00abcd	11.86±0.59f
	S_1	3.96±0.07a	2.08±0.05a	1.91±0.08abc	19.93±0.15cd
	S_2	3.10±0.01e	1.75±0.03ef	1.78±0.01bcd	22.77±0.01a
DI_2	S_0	3.40±0.01cd	1.70±0.01fg	2.00±0.02a	12.21±0.82f
	S_1	3.66±0.04b	1.86±0.01cd	1.95±0.02ab	18.71±0.13d
	S_2	2.89±0.03fg	1.65±0.00g	1.77±0.03cd	21.83±0.04ab
DI_3	S_0	3.02±0.10ef	1.64±0.02g	1.83±0.09abcd	10.48±0.16g
	S_1	3.29±0.11d	1.77±0.01ef	1.85±0.07abcd	16.98±0.63e
	S_2	2.85±0.07fg	1.56±0.02h	1.82±0.07bcd	20.76±0.52bc
Significance test (*P* values)					
Irrigation level		0.018	0.016	0.021	0.002
Shading mode		0.004	0.042	0.017	<0.001
Irrigation level*Shading mode		0.025	0.125	0.151	0.0390

Table 5c Photosynthetic characteristics of Arabica coffee under deficit irrigation and shading mode

Irrigation level	Shading mode	Spring			
		Pn/μmol m^{-2} s^{-1}	Tr/mmol m^{-2} s^{-1}	LWUE/μmol mmol^{-1}	ARUE/mmol umol^{-1}
FI	S_0	2.88±0.04d	1.97±0.01bcd	1.48±0.07a	10.08±0.42f
	S_1	3.22±0.0 2b	2.07±0.06ab	1.57±0.10a	16.67±0.34d
	S_2	2.68±0.00e	1.88±0.03cdef	1.45±0.06a	21.09±0.40a
DI_1	S_0	3.12±0.01c	2.01±0.02bc	1.55±0.06a	10.64±0.47f
	S_1	3.42±0.03a	2.14±0.05a	1.60±0.09a	17.38±0.52cd
	S_2	2.85±0.00d	1.93±0.03cde	1.50±0.06a	20.97±0.39a
DI_2	S_0	2.90±0.02d	1.87±0.03def	1.58±0.07a	10.36±0.62f
	S_1	3.12±0.04c	1.98±0.05bcd	1.58±0.09a	16.11±0.62de
	S_2	2.59±0.04e	1.82±0.05efg	1.45±0.09a	19.76±0.54ab
DI_3	S_0	2.66±0.03e	1.79±0.01fg	1.49±0.02a	9.30±0.26f
	S_1	2.86±0.03d	1.86±0.04def	1.53±0.05a	14.85±0.21e
	S_2	2.48±0.01f	1.72±0.03g	1.46±0.04a	18.50±0.26bc
Significance test (*P* values)					
Irrigation level		<0.0001	<0.0001	0.045	<0.0001
Shading mode		<0.0001	<0.0001	0.000	<0.0001
Irrigation level*Shading mode		0.032	0.4314	0.310	0.0005

3.5 Arabica coffee growth

Irrigation level had significant effect on crown area, leaf area index (LAI) and total radiation under the crown (TRUC). The shading mode had also significant effect on LAI and TRUC. The interaction effects of irrigation level and shading mode on TRUC were prominent (Table 6). Compared to FI, DI_1 increased crown area by 9.53% and LAI unobvious, and decreased TRUC by 5.51%. DI_2 did not add crown area and TRUC and reduce LAI significantly. DI_3 cut down crown area and LAI by 14.57% and 18.49%, respectively but raised TRUC by 26.84%. LAI first increased and then decreased with the increasing degree of shade, but TRUC first reduced and then added. Compared to S_0, S_1 had biggest LAI and increased it by 10.31%. S_1 reduced TRUC by 8.20%, while S_2 increased it by 6.70%. The area index of single leaf rose with the increase of shade degree. DI_1S_1 had the minimum TRUC and reduced it by 21.77% in comparison with CK. DI_3 increased TRUC obviously under different shading modes.

The effect of irrigation level, shading mode and the interaction on dry mass and root-shoot ratio was significant (Figure 3). Aboveground, underground and total dry mass increased first and then decreased with the increase of irrigation deficit. Compared to FI, DI_1 increased aboveground, underground and total dry mass by 6.97%, 23.22% and 10.46%, respectively, while DI_2 and DI_3 reduced the total dry mass significantly. Compared to FI, the root-shoot ratio of DI_1, DI_2 and DI_3 increased 1.17, 1.13 and 1.08-fold, respectively. S_1 had the highest dry mass under different shade conditions, S_0 and S_2 were the second and the smallest. In contrast with S_0, S_1 raised aboveground, underground and total dry mass by 33.52%, 19.46% and 30.02%, respectively. The root-shoot ratio decreased with the increase of shade degree, whereby S_1 and S_2 reduced it by 9.20% and 22.02%, respectively. DI_1S_1 had the highest dry mass of raising 62.90% than CK. On the other hand, DI_3S_2 had the minimum dry mass which is 0.60 times of CK.

Table 6 Canopy configuration of Arabica coffee under deficit irrigation and shading mode

Irrigation level	Shading mode	Crown area/cm^2	LAI	Single LAI/×10^{-3}	TRUE/mol m^{-2} d^{-1}
FI	S_0	1551.65±224.56^{ab}	1.45±0.19^{bcd}	4.59±0.47^{c}	9.09±0.67^{bc}
	S_1	1613.98±316.88^{ab}	1.75±0.24^{ab}	7.27±0.67^{ab}	7.33±0.50^{e}
	S_2	1361.10±242.94^{ab}	1.45±0.14^{bcd}	7.36±0.45^{ab}	9.11±0.32^{bc}
DI_1	S_0	1726.46±207.91^{a}	1.60±0.21^{abc}	4.83±0.38^{c}	7.90±0.35^{de}
	S_1	1763.88±206.85^{a}	1.79±0.23^{a}	6.40±0.85^{abc}	7.11±0.57^{e}
	S_2	1467.76±171.33^{ab}	1.45±0.13^{bcd}	5.41±0.66^{bc}	9.11±0.62^{bc}
DI_2	S_0	1532.06±255.24^{ab}	1.50±0.17^{abcd}	4.89±0.25^{c}	8.83±0.42^{bc}
	S_1	1674.92±208.53^{ab}	1.52±0.30^{abcd}	5.71±0.85^{bc}	8.44±0.93^{cd}
	S_2	1387.72±294.66^{ab}	1.40±0.12^{cd}	8.42±0.97^{a}	9.47±0.37^{b}
DI_3	S_0	1300.06±179.42^{b}	1.25±0.05^{d}	5.79±0.27^{bc}	10.62±0.53^{a}
	S_1	1293.13±278.41^{b}	1.33±0.21^{cd}	7.25±0.65^{ab}	10.58±0.81^{a}
	S_2	1273.79±317.19^{b}	1.21±0.22^{d}	7.18±0.78^{ab}	11.19±0.57^{a}
Significance test (p values)					
Irrigation level		0.009	<0.001	0.1588	<0.001
Shading mode		0.052	0.001	0.0002	<0.001
Irrigation level*Shading mode		0.913	0.195	0.1005	0.037

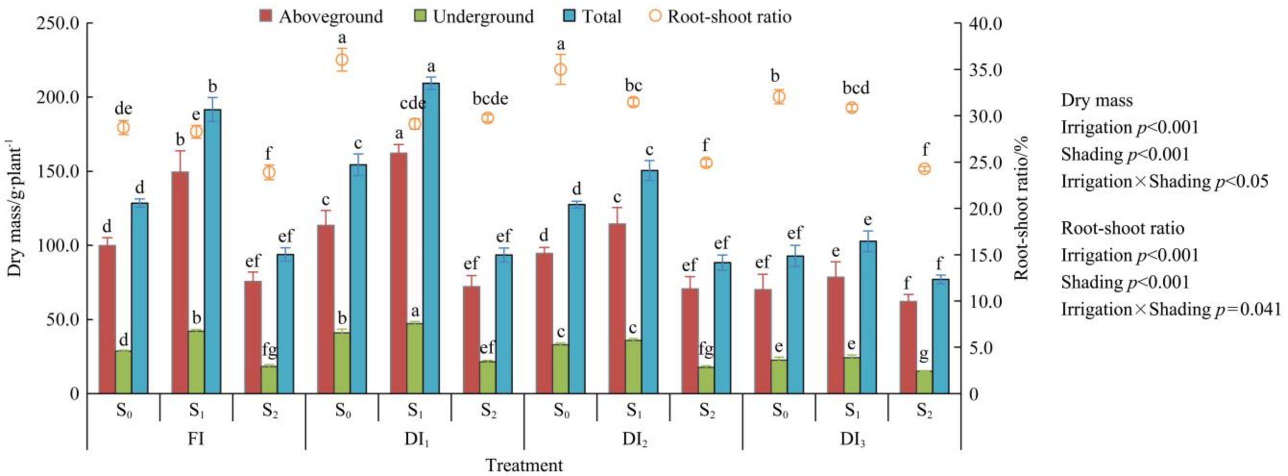


Figure 3 Dry mass distribution and root-shoot ratio of Arabica coffee under deficit irrigation and shading mode

4 Discussions

Soil microorganism is the main driver of nutrient cycling and substance transformation in soil. Rational irrigation regime could increase soil microbial biomass, promote microbial activity, meanwhile bring about corresponding changes in microflora population density and microbial community function diversity[37,38]. The results indicated that the irrigation level had a significant effect on the seasonal average density of soil bacteria, fungi and actinomycetes. It happened mainly because the irrigation level affected soil water content, temperature, organic matter composition, aeration and soil microbial activity. All these affected directly or indirectly the quality and density of soil microorganisms[11,39]. FI and DI_1 had higher population density of soil bacteria and actinomycetes, while DI_2 and DI_3 reduced the population density of soil microorganisms significantly. The possible explanation for this is that mild water deficit not only provided essential water for life activities, but also effectively improved soil temperature and aeration. All these increased available oxygen, and provided a good environment for microbial activities[11,12,40]. However, severe drought induced plants to produce some root exudates and inhibited microorganisms' growth. In addition, severe soil drought stress might lead to water potential unbalanced between soil microbial and soil solution, ultimately resulting in cell cytoplasmic separation and cell death[11,41]. The population density of rhizosphere fungi decreased with the increase of irrigation amount[42,43]. However, in this study, DI conspicuously reduced soil fungal population, which was different from previous results. The possible reason for this was the differences of water deficit degree, experimental soil type and the growth, metabolism and secretion of root systems[5].

Reasonable shading cultivation was favorable to improve soil structure, to enhance soil resistance to environmental changes and to maintain soil microbial diversity and activity[24-26]. Mainly, because shading crops affected environmental factors such as soil temperature and humidity, and increased the accumulation of root exudates, plant residues, vitamins, carbohydrates, amino acids and organic acids in soil to provide more nutrients for soil microorganisms[27,44]. All these facts are, consistent with the results obtained in S_1 treatment which showed the occurrence of highest population density of soil fungi, bacteria and microorganisms. The population density of soil microorganisms was prominently different under various shading modes, possibly because root distribution, root structure and root competition for water and fertilizer were obviously different under diverse shading cultivation modes. This however created different environments for reproduction and growth of soil microorganisms[41,45]. Irrigation and shading cultivation directly influenced soil moisture and temperature and then affected the environmental conditions of microbial growth activity. This study indicated that the population density of soil bacteria, fungi and actinomycetes had a quadratic function relationship with the soil water content and soil temperature. The results showed that the soil biological activity was the highest under moderate deficit irrigation and mild shading cultivation of castor, which was beneficial for maintaining root activity, slowing down the aging process, enhancing water and fertilizer absorption and promoting the growth of Arabica coffee.

Present study shows that DI_1 increased net photosynthesis rate (Pn), which was consistent with mild water deficit having no significant impacts on Pn (even increasing Pn)[46]. However, DI_3 remarkably decreased Pn of Arabica coffee, possibly because the resulting water stress led to decrease stomatal conductivity, obstruct CO_2 entrance into the leaves, or decreased photosynthetic activity of mesophyll cells[47]. In this study, DI_3 reduced apparent radiation use efficiency (ARUE) of leaves, mainly because severe soil water deficit brought about a decrease of the conversion absorptive capacity of the photosynthetic effective radiation. Shading mode had prominent effects on Pn, transpiration (Tr), water use efficiency (LWUE) and ARUE, which was consistent with the results of the related studies[18,20]. In this study, S_1 increased Pn, mainly because the mild shading reduced the damage of strong sunlight to photosynthetic mechanism and alleviated the phenomenon of “midday depression”. Meanwhile, shading improved the chlorophyll and PS II light conversion efficiency and reduced heat dissipation, making up the relative lack of light, thus enhancing photosynthetic efficiency[23,36,48]. However, S_0 could cause photoinhibition, inactivation or damage to the light system reaction center of leaves, simultaneously thicken the cell wall and restrict the gas exchange rate, which finally led to the decrease of photosynthetic rate[49]. Insufficient radiation capture by leaves in S_2 led to the decrease of photosynthetic electron transport and key enzymes, thus reducing Pn and WUE_L[50]. Shading mode significantly increased ARUE, possibly because shade leaves increased the distribution ratio of electron transport quantum in the PSII reaction center and reduced the quantum ratio for heat dissipation[51]. The results also showed that coffee leaves had a certain ability to regulate and adapt to weak light stress[35,52]. The interaction between irrigation and shading had significant effects on seasonal average ARUE of Arabica coffee, which was related to the regulation of soil water content by irrigation and the change of canopy microclimate environment by shading, thus changing the effective transformation of photosynthetically active radiation[33].

The canopy structure of crops directly affected radiation interception and conversion efficiency and thus reasonable and efficient canopy structure was the basis of high crop production[53,54]. This study showed that the irrigation level and shading cultivation mode notably affected leaf area index (LAI), which was consistent crop canopy structure not only was influenced by its own genetic characteristics and physiological and biochemical processes, but also by the constraints of cultivation measures and environmental conditions[53,55]. DI_1 and S_1 intercept more light radiation by increasing LAI, which was conducive to enhancing photosynthesis and promoting the growth of Arabica coffee. DI_1S_1 had smallest total radiation under the crown (TRUC), probably because this mode coordinated the competition of light resources between Arabica coffee and castor. As a result LAI of Arabica coffee increased and constructed more plausible canopy structure, which was beneficial to dry mass accumulation and the improvement of light energy use.

DI_1 increased dry mass of Arabica coffee, which probably because of mild deficit irrigation enhancing root’s absorption and synthesis capacity[33]. But DI_2 and DI_3 led to close of leaf stomata, weaken transpiration, inhibit photosynthetic rate and finally decrease total dry mass. S_1 had the highest dry mass, which was related to strong shade-bearing of Arabica coffee, and mild shading enhanced physiological activity, optimized photosynthetic characteristics, and improved the relative growth rate[1]. S_2 reduced dry mass, mainly because excessive shading reduced the solar radiation energy intercepted by the canopy, resulting in insufficient product synthesis and supply capacity of leaf photosynthesis[33]. DI increased the root-shoot ratio, which was related to DI regulating and optimizing the ratio and distribution of photosynthetic product between root and shoot[1]. The interaction between irrigation and shading mode had significant effects on the dry mass of Arabica coffee and DI_1 obtained the highest dry mass, which indicated that mild reduction irrigation could basically meet the growth demand of Arabica coffee under proper shading cultivation, mostly because proper shading cultivation increased Pn, decreased the ineffective evaporation of soil surface water, improved the soil environment, increased the population density of microorganisms and benefited nutrient transformation and absorption[20]. DI_1S_1 not only increased Pn and dry mass, but also improved ARUE, and received higher castor grain yield (only next to FIS_1, discussed in another article), which enhanced land use efficiency and saved irrigation water. Therefore, the combination of moderate deficit irrigation (1.0Ep) and mild shading cultivation could simultaneously achieve the rapid growth and water-saving and high efficiency of young Arabica coffee shrubs. The results could provide practical reference for the management of water and shading cultivation for Arabica coffee.

5 Conclusions

Based on seasonal average value, compared to full irrigation (FI) as control, deficit irrigation of 1.0Ep (DI_1) did not significantly change soil bacteria and actinomycetes population density, but reduced soil fungi prominently. DI_2 (0.8Ep) and DI_3 (0.6Ep) decreased the population density of soil microorganisms remarkably. The population density of soil bacteria and actinomycetes first increased and then decreased, while the population of fungi increased with the shading degree of increasing. Mild shading (S_1) obtained the uppermost population density of soil bacteria and actinomycetes, meanwhile added the population density of soil fungi when compared to S_0 (no shading, monoculture). The population density of soil bacteria, fungi and actinomycetes increased at first and then decreased with the increment of soil water content and soil temperature, showing a significant quadratic function relationship.

Compared to FI, DI_1 obtained the uppermost net photosynthetic rate (Pn), canopy area, leaf area index (LAI), root-shoot ratio and dry mass, while reducing total radiation under the canopy of Arabica coffee. The root-shoot ratio decreased with the increase of shading degree. S_1 obtained maximum Pn, transpiration rate (Tr), apparent radiation use efficiency (ARUE), water use efficiency (LWUE), LAI and dry matter of Arabica coffee.

DI_1S_1 (1.0Ep, mild shading) had the highest Pn and dry mass, the higher ARUE and the smallest total radiation under the canopy (TRUC). Thus DI_1S_1 was the suitable mode of irrigation and shading cultivation for young Arabica coffee shrubs.

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