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The Effects of Water and Fertilizer Coupling on Plant and Soil Nitrogen Characteristics and Fruit Growth of Rabbiteye Blueberry Plants in a Semi-Arid Region in China

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ABSTRACT

To evaluate the effects of nitrogen (N) and irrigation coupling on the soil N distribution, plant N utilization, and fruit yield of rabbiteye blueberries (*Vaccinium virgatum*), a field experiment was designed using two factors (water and fertilizer application) with four levels of irrigation and three levels of fertilization, and a control. Under the different water and fertilizer combinations, N primarily accumulated in the leaves. Irrigation and N application within appropriate ranges (pure N ≤ 29 g/plant and irrigation volume ≤ 2.5 L/plant) significantly improved the blueberry fruit yield. Increases in water and N within these ranges promoted the effective accumulation of N in various organs and the absorption and utilization of N in the plants, which ultimately promoted blueberry yield. With increased N application rate, the nitrate N content of the 0–20 cm and 20–50 cm soil layers increased. With increased irrigation volume, the nitrate N content of the 0–20 cm soil layer decreased, while the nitrate content in the 20–50 cm soil layer increased. Low N and moderate water treatments resulted in high fruit yields and reduced nitrate N retention in the soil. Under these conditions, the economic input-output ratio was high and the soil N accumulation was low, and thus the economic and ecological benefits were maximized.

Abbreviations

BNC	Branch nitrogen content
WUE	Water use efficiency
LNAU	Leaf nitrogen absorption and utilization
LNC	Leaf nitrogen content
NPP	Nitrogen partial productivity
NUAU	Nitrogen uptake and utilization
NUE	Nitrogen use efficiency

RN   Residual nitrogen
SD   Standard deviation
AE   Agronomic utilization rate of N fertilizer

1 Introduction

The blueberry is an important economic fruit crop in China and has played a key role in increasing agricultural income. In 2020, the total blueberry cultivation area in China was 66,400 ha^{-1}, with an output of 347,200 t. The blueberry industry has contributed significantly to poverty alleviation and commercial uplift in China, and the blueberry cultivation area in Guizhou in Southwest China is 15,000 hm^2, with an output of 85,000 t [1]. The county of Majiang in Guizhou is an important blueberry production region, but water shortages and low fertilizer utilization rates hamper blueberry production [2]. Therefore, adopting appropriate irrigation and fertilization strategies could enhance water and nutrient use efficiency in blueberry cultivation.

Water shortages are a global issue, and the amount of water allocated for agricultural use is decreasing [3]. These shortages may prevent many fruit crops from obtaining sufficient water to meet their physiological requirements, especially in arid and semi-arid areas. In Southern China, blueberries are mainly cultivated in hilly and mountainous areas that are categorized by poor water availability and retention capacity, and are often threatened by local or intermittent droughts [4]. In addition, blueberries have shallow root systems, no root hairs, and are not tolerant to drought. Under limited summer precipitation, evaporation can cause varying degrees of soil water deficit [5]. Therefore, the efficient utilization of precipitation is key to improving the yield and water use efficiency (WUE) of blueberries in the region.

Fertilizer is another important factor that affects crop yield and nitrogen use efficiency (NUE) [6,7]. The application of nitrogen (N) fertilizer has an important impact on crop yield. Studies have shown that N application can increase crop yields [8]; however, excessive application of N fertilizer may reduce crop yields [9] and pollute the environment. This is because applications of N fertilizer in excess of crop requirements leads to the accumulation of nitrate in the soil. Nitrate accumulation degrades surface and groundwater resources, resulting in eutrophication and the contamination of potable water supplies [10].

In a previous study, it was found that N fertilizer application in non-irrigated ditches during drought years reduced N fertilizer absorption by 50% [11]. Meanwhile, other studies have shown that applied N fertilizer is not always fully absorbed by the crop, resulting in partial leaching into the deep soil and even the groundwater [12,13]. In one study, the accumulation of nitrate N in the soil increased with increasing N fertilizer application [14]. Furthermore, rates of residual fertilizer N in the soil have been found to reach 10 to 35% [15]. In an earlier study, water and N fertilizer were simultaneously transported to the roots of crops through drip irrigation tapes and a clear coupling was observed between water and N. N is a key nutrient in blueberry production [16]; unlike most plants, blueberries prefer ammonium N (NH$_4^+$-N) to nitrate N (NO$_3^-$-N) [17]. Therefore, it is important to explore the influence of water and N coupling on blueberry plants and their soil environment in order to develop efficient blueberry cultivation systems.

Most available research on the interaction between water and N has focused on wheat [18], tomatoes [19], red dates [20], apples [21], and other plants. Meanwhile, research on the coupling of water and fertilizer in blueberries has primarily concentrated on yield and quality. However, there are no reports available on the effect of water and N application on the soil N distribution, plant N utilization, and fruit yield of blueberries. Thus, in the present study, the soil N distribution, plant N distribution, and the fruit yield of rabbiteye "Brightwell" blueberries (*Vaccinium virgatum*) were analyzed under different water and N coupling treatments. The aim was to identify the most appropriate water and N input parameters under the local ecological conditions. The findings provide a scientific basis and practical guidance for using

appropriate water and N management to obtain high yields of blueberries in this region. The results also provide practical and technical guidance for minimizing environmental degradation and improving farmers' incomes.

2 Materials and Methods

2.1 Test Site

The research site was located in Xuanwei Town, Majiang County, Guizhou Province, China (26° 21′–26° 31′ N and 107° 33′–107° 47′ E; Fig. 1). The site was located in a subtropical monsoon humid zone with a warm and humid climate throughout the year. The annual average temperature, annual average rainfall, annual sunshine hours, and frost-free period were 15.7°C, 1266 mm, 1200 h, and 293 days, respectively. The base soil was acid yellow soil, which is suitable for the cultivation of rabbiteye blueberries in Guizhou. Some important soil parameters of the study site included a pH of 4.35–5.50, an organic matter content of 23.9 g/kg, a total N content of 0.78 g/kg, a total phosphorous (P) content of 0.19 g/kg, and a total potassium (K) content of 2.8 g/kg. Upon evaluation of the literature, it was discovered that the pH, annual average temperature, annual average rainfall, annual sunshine hours, frost-free period, and organic matter content values for blueberry production were within the natural ranges of the study site (4.35–5.50, 15.0–16.5°C, 1203–1329 mm, 1140–1260 h, 278–310 days, and 22.7–25.1 g/kg, respectively), and thus the test area was representative of the natural conditions [22,23].
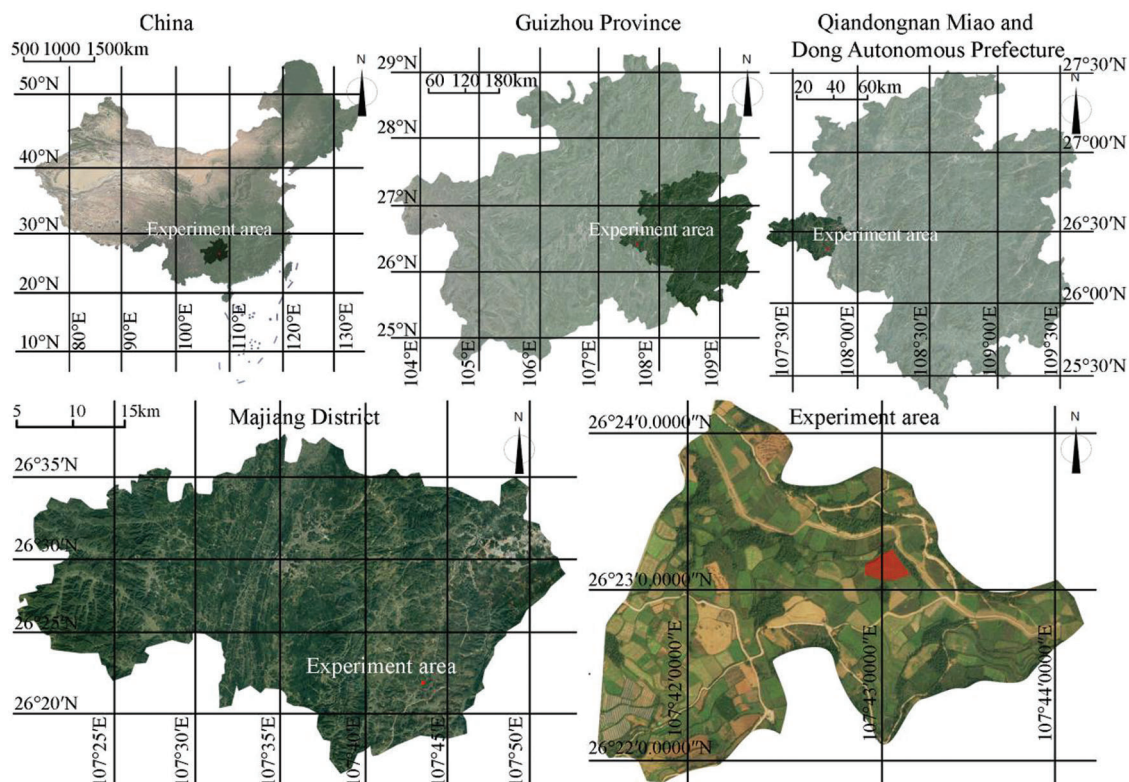
Figure 1: Schematic diagram of the geographical location of the experimental area

2.2 Randomized Block Design

Five-year-old plants of the rabbiteye blueberry cultivar "Brightwell" were used as the test materials under field conditions. Water (W) and fertilizer (F) were set as the two factors. The fertilizers used were

chemically pure and included ammonium sulfate (N content of 21.2%), superphosphate (P_2O_5 content of 60.6%), and potassium sulfate (K_2O content of 63.2%). There were 13 water and fertilizer treatment combinations, with three replicates per treatment and three plants per replicate, which resulted in a total of 117 plants. Among these 13 combinations, one combination consisted of the no fertilization and no irrigation control (CK).

Fertilizer was applied four times a year: in early March (before flowering), early May (before fruit production), late August to early September (post fruit production and in the flower bud differentiation period), and in early December (reduced fertilizer). Upon fertilization, a 50 cm × 20 cm × 20 cm trench was dug on the left and right sides of the outer periphery of the canopy projection. The fertilizer was dissolved in water (or after fully mixing it with water) according to the design plan (Table 1) and applied to the trench, which was then covered with soil. The other cultivation management practices were based on daily blueberry cultivation management methods [24]. The design scheme is shown in Table 1 and was created in accordance with the literature [25]. Preliminary tests confirmed that there were large differences in the growth of blueberries among the treatments.

Table 1: Experimental design for the fertilizer and irrigation used at the study site (single application)

Fertilization amount (g/plant)		Irrigation volume (L/plant)			
$(NH_4)_2SO_4$	0 (W0)	1.25 (W1)	2.50 (W2)	3.75 (W3)	5 (W4)
29 (F1, low)		F1W1	F1W2	F1W3	F1W4
59 (F2, medium)		F2W1	F2W2	F2W3	F2W4
118 (F3, high)		F3W1	F3W2	F3W3	F3W4
0 (CK)	0				

Note: The amount of fertilization and irrigation represents single application rates; F1 is low fertilizer, F2 is moderate fertilizer, F3 is high fertilizer; W1 is low water, W2 is moderate water, W3 is moderate high water, and W4 is high water. The same below.

2.3 Field Sample Collection

Samples were obtained on the 10th day after each treatment, and sampling that would have taken place on rainy days was postponed until the weather cleared. Soil samples were collected with soil augers, using the depth of the water and fertilizer application (20 cm) as the origin. Soil was collected from depths of 20–50 cm to assess the blueberry root distribution and the fertilizer utilization efficiency. Specifically, four soil samples were obtained vertically from the fertilization trench (at a depth of 20 cm) every 10 cm, corresponding to soil layer depths of 20, 30, 40, and 50 cm. Three replicate soil samples were collected for each treatment (Fig. 2). The soil samples were numbered and placed immediately into a Ziplock bag in an ice box. The samples were then brought back to the laboratory in the shortest possible time and placed in a refrigerator at −20°C for storage. To reduce the impact of time on the measurements, N was measured within one week. Similarly, according to the principle of symmetry, the soil on both sides of the fertilization point was collected and brought back to the laboratory for determination of the total N in the soil at the fertilization point. Mature, healthy, and pest-free leaves and annual branches were also collected from around the canopy, placed in a numbered Ziplock bag in an ice box, and transported back to the laboratory for processing.
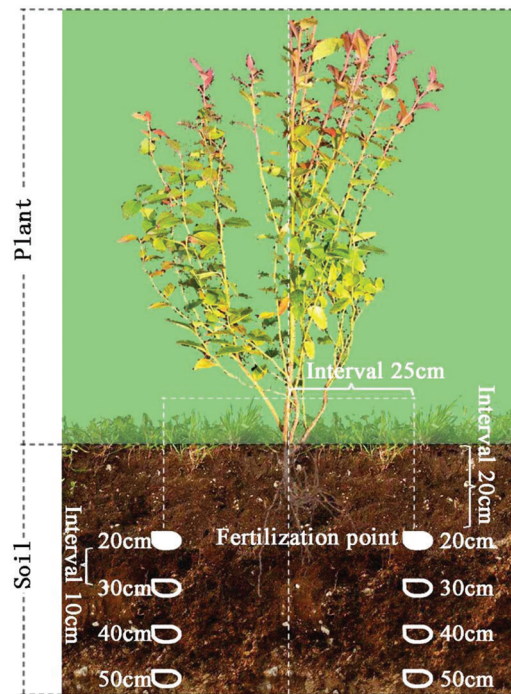
Figure 2: Sampling diagram

2.4 Determination of Plant Physiological Indexes

2.4.1 Determination of Aboveground Biomass and Total N Content of the Plants

Leaves and branches were collected in May (before fruit harvest) and September (after fruit harvest) from three plants in each treatment. Mature leaves and annual branches with normal growth and no pests and diseases were collected from the periphery of the canopy and packed in Ziplock bags. The leaves and branches were weighed and placed in a drying cabinet to dry the tissues at 105°C for 0.5 h. The tissues were then were dried at a temperature of 75°C until constant mass was reached. The dry matter was weighed using an electronic balance. All plant samples were dried, crushed, and then passed through a 0.5 mm sieve. The samples were kept sealed in a dry environment. To determine the total N content, plant samples (leaves and branches) were digested with concentrated H_2SO_4-H_2O_2 and were subjected to a Kjeldahl nitrogen analyzer [26].

2.4.2 Determination of Fruit Yield and the Utilization Rate of Water and Fertilizer

A one-hundredth decimal electronic balance was used to weigh the mature fruits, and the sum of the yield of plants from each treatment was recorded as the treatment yield. The average individual fruit weight was calculated and converted to g/plant. Various indexes were measured as follows:

$$AE(\%) = \frac{\text{fruit yield in N application area}}{\text{N application rate} \times 100\%} - \frac{\text{fruit yield in non} - \text{N application area}}{\text{N application rate} \times 100\%} \text{ [27]},$$

$$NPP(kg/kg) = \frac{\text{fruit yield in N application area}}{\text{N application amount}} \text{ [28]},$$

$$WUE(kg/hm^2) = \frac{Y}{Wi} \text{ [28]},$$

where Y is the fruit yield (g/plant) and Wi represents the irrigation amount per unit area (L/plant);

$$\text{NUE}(\%) = \frac{\text{N accumulation in plants in N application area}}{\text{N application amount} \times 100\%}$$
$$- \frac{\text{N accumulation in plants in non} - \text{N application area}}{\text{N application amount} \times 100\%} \quad [29],$$

$\text{RN}(\text{g/kg}) = \text{amount of N applied} - \text{amountofNapplied} \times \text{AE}.$

2.4.3 Determination of Soil Inorganic N

The fresh soil samples from each measuring point were air-dried, mixed, and passed through a 2 mm sieve. Weigh 5 g of soil sample, extract it with 50 mL of KCl solution with a concentration of 2 mol·L^{-1}, shake it for 0.5 h, centrifuge, stand and filter, and take the supernatant for determination [30].

2.4.4 Membership Function

In this study, 12 water-fertilizer coupling treatments were compared taking no application of water and fertilizer as control; thus making 13 treatments in total (Table 1). Each treatment had 3 replications ($3 \times 13 = 39$) and comprehensive evaluation with membership function by transforming the data related to physiological and growth responses in following Eqs. (1) and (2).

$$X = (x - x_{min})/(x_{max} - x_{min}) \tag{1}$$

$$X = 1 - (x - x_{min})/(x_{max} - x_{min}) \tag{2}$$

In the above equations, X and x represent the coded and the average calculated value of each treatment, respectively; x_{min} and x_{max} represent the minimum and maximum value, respectively, obtained from each parameter from different treatments. The membership function values and average value were accumulated and calculated. The larger average value represented the optimal treatment group [31].

2.4.5 Data Analysis

Excel 2010, SPSS 25 (IBM Corp., Armonk, NY, USA), and Graphpad (Prism x86) were used for data processing. One-way analysis of variance, two-way analysis of variance (ANOVA) and Tukey's significance test were performed on the experimental data.

3 Results

3.1 Effect of Water and N Coupling on the N Content of the Blueberry Plants

As shown in Table 2, the N content of the blueberry leaves and branches differed significantly ($P < 0.05$) under different water and N treatments in May and September. In all treatments, the N content of the shoots was significantly lower than that of the leaves. This indicated that the N demand of the leaves was higher than that of the shoots. Irrigation had a highly significant effect on leaf N content in May (before fruit production). At both low and high N application rates, the leaf N content decreased first and then increased with irrigation. However, under moderate N application, the leaf N content increased with irrigation. The leaf N absorption and utilization were highest in the F1W1, F1W3, and F1W4 treatments, and lowest in the F3W3 treatment. In May (before fruit production), the effect of water and N on the N content of the branches was highly significant; the branch N content was highest in F2W2 and F1W2 and lowest in F3W1 and F3W2. The interaction of water and N application on leaf N content was also significant in September (after fruit production); leaf N absorption and utilization was highest in F1W1 and F2W2, and lowest in F1W4. N application also had a significant impact on the N content of the branches in September. Under insufficient water conditions, the N content of the leaves decreased with increased fertilization. When water was sufficient, the N content increased first and then decreased with increasing fertilization, with higher leaf N content in F1W2 and lower in F3W3. These results show that the low N and low water, low N and moderate water, and moderate N and moderate water treatments were beneficial for the absorption and utilization of N in the leaves and branches of the blueberry plants.

Table 2: Effect of water and N coupling on the N of the blueberry leaves and branches

	May				September			
	LNC (g/kg)	LNAU (%)	BNC (g/kg)	NUAU (%)	LNC (g/kg)	LNAU (%)	BNC (g/kg)	NUAU (%)
F1W1	11.11 ± 0.72abc	8.10	5.33 ± 0.25b	1.48	11.69 ± 0.38ab	3.59	8.2 ± 0.22a	8.45
F1W2	9.22 ± 0.59bc	1.59	5.74 ± 0.09b	2.90	10.85 ± 0.59ab	0.69	7.78 ± 0.7ab	7.00
F1W3	10.64 ± 0.69abc	6.48	5.32 ± 0.24b	1.45	11.49 ± 1.41ab	2.90	6.78 ± 0.26abcd	3.55
F1W4	11.09 ± 0.71abc	8.03	5.05 ± 0.23b	0.52	9.69 ± 0.11b	−3.31	5.97 ± 0.11bcd	0.76
F2W1	10.5 ± 0.13abc	2.95	6.15 ± 0.25b	2.12	12.52 ± 1.2ab	3.17	6.55 ± 0.47abcd	1.36
F2W2	10.06 ± 0.84abc	2.20	7.43 ± 0.4a	4.29	13.17 ± 0.45a	4.27	8.02 ± 0.72a	3.85
F2W3	10.48 ± 0.09abc	2.92	5.14 ± 0.12b	0.41	10.5 ± 0.12ab	−0.25	8.21 ± 0.11a	4.17
F2W4	12.17 ± 0.37ab	5.78	5.66 ± 0.23b	1.29	12.06 ± 0.35ab	2.39	7.17 ± 0.57abc	2.41
F3W1	11.52 ± 0.36abc	2.34	5.12 ± 0.2b	0.19	9.66 ± 0.05b	−0.84	5.93 ± 0.12bcd	0.15
F3W2	10.7 ± 0.74abc	1.64	5.33 ± 0.01b	0.36	12.09 ± 0.14ab	1.22	5.97 ± 0.13bcd	0.19
F3W3	9.68 ± 0.36abc	0.78	5.85 ± 0.49b	0.81	12.31 ± 0.71ab	1.41	5.12 ± 0.12d	−0.53
F3W4	12.56 ± 0.13a	3.22	7.62 ± 0.33a	2.30	11.29 ± 0.85ab	0.54	6.78 ± 0.58abcd	0.87
CK	8.76 ± 1.12c	0.00	4.90 ± 0.05b	0.00	10.65 ± 0.47ab	0.00	5.75 ± 0.25cd	0.00
F-value								
F	1.22	–	8.78**	–	2.89	–	15.22**	–
W	7.77**	–	6.08**	–	1.22	–	1.32	–
F × W	1.47	–	13.38**	–	3.24*	–	5.25**	–

Note: * represents a significant difference at $P < 0.05$; ** represents a significant difference at $P < 0.01$. BNC, branch N content; LNAU, leaf N absorption and utilization; LNC, leaf N content; NUAU, N uptake and utilization. Different lowercase letters indicate significant differences within columns ($P < 0.05$). F, fertilizer effects; W, water effects, F × W, interaction effects.

3.2 Effect of Water and N Coupling on Blueberry Yield and the Utilization of Water and N

The effects of water and N on blueberry fruit yield were significant. F1W2 and F2W2 were associated with higher yields, and F1W4, F2W3, F3W4, and CK were associated with lower yields. This indicated that moderate amounts of water were beneficial for fruit yield (Table 3). The agronomic efficiency of N fertilizer decreased with increasing fertilization. F1W2, F1W3, and F2W2 plants displayed higher agronomic efficiencies, and F2W1 and F3W1 plants displayed lower efficiencies. Among these, the agronomic efficiency of N fertilizer was highest in the F1W2 and lowest in the F3W4 treatment. This indicated that the combination of high fertilization and high water reduced the NUE. The WUE of the leaves differed significantly among the treatments, and the irrigation treatment had a particularly significant effect on WUE. The irrigation efficiency decreased with increased irrigation. F1W1 and F3W1 displayed the highest irrigation efficiencies and F1W4 displayed the lowest. The soil residual N was the lowest under F1W2 and F1W3. These results indicate that optimal coupling of water and N can improve WUE and NUE, and consequently increase fruit yield.

Table 3: Effect of different water and N application combinations on blueberry fruit yield and N fertilizer utilization

	Yield (kg/plant)	AE (%)	NPP (%)	WUE (kg/hm^2)	RN (g/kg)
F1W1	1.88 ± 0.01a	27.12 ± 2.24bcde	65.06 ± 0.28b	1.51 ± 0.01a	21.14
F1W2	2.24 ± 0.13a	77.08 ± 4.62a	77.08 ± 4.62a	0.89 ± 0.05c	6.67

(Continued)

Table 3 (continued)

	Yield (kg/plant)	AE (%)	NPP (%)	WUE (kg/hm^2)	RN (g/kg)
F1W3	1.41 ± 0.03b	48.62 ± 0.9b	48.62 ± 0.9c	0.37 ± 0.01ef	14.79
F1W4	1.17 ± 0.07b	40.24 ± 2.65bcd	40.24 ± 2.65d	0.23 ± 0.01f	17.4
F2W1	1.43 ± 0.01b	6.91 ± 0.81e	24.22 ± 0.16e	1.14 ± 0.01b	54.87
F2W2	2.15 ± 0.14a	44.36 ± 18.19bc	36.42 ± 2.31d	0.86 ± 0.06c	33.04
F2W3	1.26 ± 0.08b	29.08 ± 8.19bcde	21.41 ± 1.36ef	0.34 ± 0.02f	41.89
F2W4	1.94 ± 0.06a	43.91 ± 10.82bc	32.81 ± 1.05d	0.39 ± 0.01def	33.04
F3W1	1.97 ± 0.14a	7.36 ± 1.65e	15.37 ± 1.14fg	1.57 ± 0.12a	109.74
F3W2	1.42 ± 0.07b	12.05 ± 0.57cde	12.55 ± 0.62g	0.57 ± 0.03d	103.84
F3W3	2.09 ± 0.04a	17.73 ± 0.34bcde	17.56 ± 0.22efg	0.56 ± 0.01de	96.76
F3W4	1.22 ± 0.03b	10.3 ± 0.30de	10.48 ± 0.34g	0.26 ± 0.02ef	106.20
CK	1.10 ± 0.06b	–	–	–	–
F-value					
F	0.08	28.78**	603.44*	3.20*	–
W	20.16**	10.36**	38.02**	408.38**	–
F × W	32.64**	3.24*	31.50**	18.50**	–

Note: * represents a significant difference at *P* < 0.05; ** represents a significant difference at *P* < 0.01. WUE, Water use efficiency; NPP, N partial productivity; AE, Agronomic utilization rate of N fertilizer; RN, residual N; F, fertilizer; W, irrigation.

3.3 Effect of Water and N Coupling on Blueberry Soil N Distribution

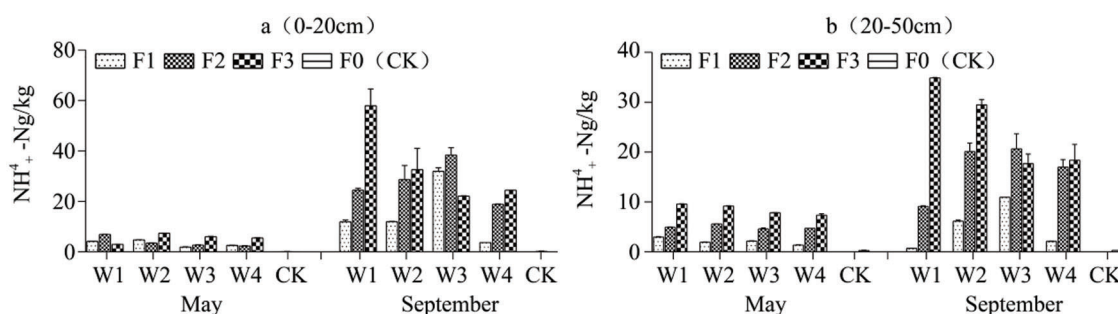#### 3.3.1 Effect of Water and N Coupling on Soil Ammonium N

As indicated in Fig. 3, ammonium N was mainly absorbed in the 0–20 cm soil layer. With the exception of CK, the 0–20 cm soil layer had the lowest ammonium N content in the F1W3 treatment in May, and in the F1W4 treatment in September. In the 20–50 cm soil layer, the F1W1, F1W2, F1W3, and F1W4 treatments resulted in lowest ammonium N contents in the two months. This indicated that the plants in these treatments were better at absorbing soil ammonium N. In the 0–20 cm soil layer, the ammonium N content showed an increasing trend with the season in the various treatments (Fig. 3a). The ammonium N content of the 0–20 cm soil layer in the two months ranged from 0.15 to 57.9 g/kg, while that of the 20–50 cm soil layer ranged from 0.29 to 34.9 g/kg. In September, the ammonium N in the soil layers showed an accumulating trend, with maxima of 57.9 g/kg in the 0–20 cm layer and 34.9 g/kg in the 20–50 cm layer. During the two months, under the same irrigation conditions, increased nitrogen application resulted in an overall increasing trend in the soil ammonium N content of the soil layers. Under the same N application conditions, increasing the irrigation led to a decrease in the ammonium N content of the soil layers in May; in September, the ammonium N content of the soil layers increased first and then decreased. These results demonstrated that the soil ammonium N was easily absorbed by the plants, and that the range of migration with water was small.

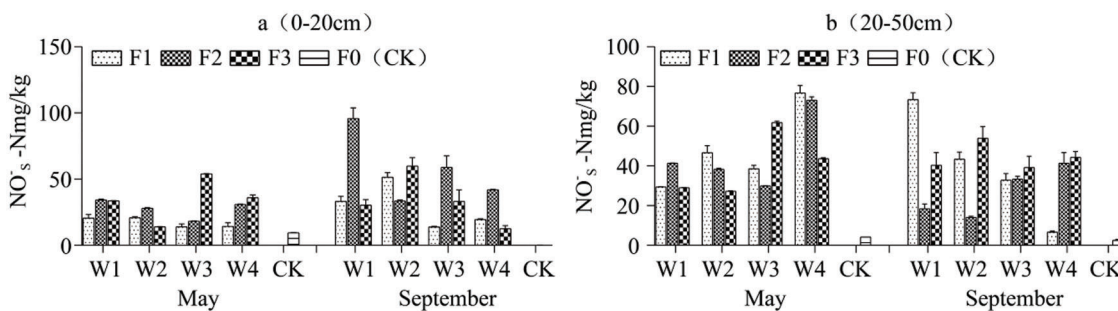#### 3.3.2 Effect of Water and N Coupling on Soil Nitrate N

As shown in Fig. 4, the F1W3 treatment was associated with the lowest nitrate N content in the 0–20 cm soil layer in the two seasons. However, in the 20–50 cm soil layer in September, the F2W2 treatment was associated with the lowest soil nitrate N content. This suggests that there is little threat of soil nitrate N pollution under these treatment conditions. The soil nitrate N content of the different treatments in the 0–20 cm layer (Fig. 4a) showed an upward trend with the season, while that of the 20–50 cm layer

(Fig. 4b) showed a downward trend with the season. The nitrate N content ranged from 0.11 to 97.5 g/kg in the 0–20 cm soil layer and from 2.55 to 79.7 g/kg in the 20–50 cm soil layer. Maximum nitrate N content values were reached in September. During the two months, under the same irrigation conditions, increased N application led to an overall increasing trend in the soil nitrate N content in the soil layers. Meanwhile, under the same N application conditions, increased irrigation led to a decrease in the total soil nitrate N content in the 0–20 cm soil layer and to an increase in total soil nitrate N in the 20–50 cm soil layer. Thus, the reduction of the soil nitrate N content in the 0–20 cm soil layer was related to both the nitrate content of the plants and the downward movement of soil nitrate N. This indicated that with increased irrigation and N application, there was significant nitrate N leaching in the soil.

Figure 3: Bar plot showing the effect of water and nitrogen coupling on blueberry soil ammonium nitrogen. a is the effect of water-nitrogen coupling on 0–20 cm soil ammonium nitrogen, b is the effect of water-nitrogen coupling on 20–50 cm soil ammonium nitrogen. The error bars show the standard deviation (SD)

Figure 4: Bar plot showing the effects of water and nitrogen coupling on nitrate nitrogen on the soil. a is the effect of water and nitrogen coupling on 0–20 cm soil nitrate nitrogen, b is the effect of water and nitrogen coupling on 20–50 cm soil nitrate nitrogen. The error bars show the standard deviation (SD)

3.3.3 Membership Function Analysis

As indicated in Tables 4 and 5, the average membership functions of each treatment in both seasons were higher than that of CK. This indicated that the indexes of N absorption and utilization of the blueberries in all water and N treatments were better than in CK. The average membership functions of the moderate N and water treatments (F1W2, F1W1, and F2W2) were significantly higher than those of the other treatments. This indicated that low N and moderate water (F1W2) was the optimal combination for producing high yields of blueberries with minimal environmental impact. The next best combinations were low N and low water (F1W1) and moderate N and moderate water (F2W2).

Table 4: Membership function values and rankings of the blueberry plant indexes in May

	Leaf	LNAU	Branch	NUAU	Yield	NUE	NPP	WUE	RN	NH_4^+-N	NO_3^--N	Average value	Total ranking
F1W1	0.62	0.05	0.16	0.34	0.68	0.35	0.84	0.96	0.19	0.91	0.32	0.49	3
F1W2	0.12	1.00	0.31	0.68	1.00	1.00	1.00	0.57	0.06	1.00	0.29	0.64	1
F1W3	0.49	0.04	0.15	0.34	0.27	0.63	0.63	0.24	0.13	0.84	0.76	0.41	7
F1W4	0.61	0.05	0.06	0.12	0.06	0.52	0.52	0.15	0.16	0.78	0.58	0.33	10
F2W1	0.46	0.02	0.46	0.49	0.29	0.09	0.31	0.73	0.50	0.61	0.48	0.40	8
F2W2	0.34	0.01	0.93	1.00	0.92	0.58	0.47	0.55	0.30	0.57	0.43	0.55	2
F2W3	0.45	0.02	0.09	0.10	0.14	0.38	0.28	0.22	0.38	0.46	0.32	0.26	12
F2W4	0.90	0.04	0.28	0.30	0.74	0.57	0.43	0.25	0.30	0.46	0.82	0.46	5
F3W1	0.73	0.01	0.08	0.04	0.76	0.10	0.20	1.00	1.00	0.36	0.32	0.42	6
F3W2	0.51	0.01	0.16	0.08	0.28	0.16	0.16	0.36	0.95	0.28	0.49	0.31	11
F3W3	0.24	0.00	0.35	0.19	0.87	0.23	0.23	0.36	0.88	0.22	0.43	0.36	9
F3W4	1.00	0.02	1.00	0.54	0.11	0.13	0.14	0.17	0.97	0.17	1.00	0.48	4
CK	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13

Note: WUE, Water use efficiency; LNAU, leaf N absorption and utilization; NPP, N partial productivity; NUAU, N uptake and utilization; NUE, nitrogen use efficiency; RN, residual N.

Table 5: Membership function values and rankings of the blueberry plant indexes in September

	Leaf	LNAU	Branch	NUAU	Yield	NUE	NPP	WUE	RN	NH_4^+-N	NO_3^--N	Average value	Total ranking
F1W1	0.58	0.44	1.00	1.00	0.68	0.35	0.84	0.96	0.19	1.00	0.58	0.69	2
F1W2	0.34	0.53	0.86	0.84	1.00	1.00	1.00	0.57	0.06	0.74	0.87	0.71	1
F1W3	0.52	0.82	0.54	0.45	0.27	0.63	0.63	0.24	0.13	0.46	0.58	0.48	5
F1W4	0.01	0.00	0.28	0.14	0.06	0.52	0.52	0.15	0.16	0.49	0.56	0.26	12
F2W1	0.81	0.85	0.46	0.21	0.29	0.09	0.31	0.73	0.50	0.31	0.58	0.47	6
F2W2	1.00	1.00	0.94	0.49	0.92	0.58	0.47	0.55	0.30	0.54	0.28	0.64	3
F2W3	0.24	0.40	1.00	0.52	0.14	0.38	0.28	0.22	0.38	0.61	0.61	0.44	7
F2W4	0.68	0.75	0.66	0.33	0.74	0.57	0.43	0.25	0.30	0.43	0.64	0.53	4
F3W1	0.00	0.33	0.26	0.08	0.76	0.10	0.20	1.00	1.00	0.08	1.00	0.44	8
F3W2	0.69	0.60	0.28	0.08	0.28	0.16	0.16	0.36	0.95	0.18	0.71	0.40	10
F3W3	0.75	0.62	0.00	0.00	0.87	0.23	0.23	0.36	0.88	0.39	0.43	0.43	9
F3W4	0.46	0.51	0.54	0.16	0.11	0.13	0.14	0.17	0.97	0.05	0.13	0.31	11
CK	0.28	0.44	0.20	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	13

Note: WUE, Water use efficiency; LNAU, leaf N absorption and utilization; NPP, N partial productivity; NUAU, N uptake and utilization; NUE, nitrogen use efficiency; RN, residual N.

4 Discussion

4.1 Blueberry Fruit Yield and Soil N Distribution under Water and N Coupling

Yield formation in the reproductive organs of plants is closely linked to nutrient accumulation and distribution in the soil. In the present study, it was shown that in May (before fruit production) and September (after fruit production), N accumulation was significantly higher in the leaves than in the branches. This is consistent with the results found for oil sunflower by Wang et al. [32]. These results imply that the N in the leaves may be transported to the fruit, thus promoting yield formation. The effects of different water and N applications on N accumulation, distribution, absorption, and utilization in the blueberry leaves and branches differed significantly. Under low and moderate irrigation conditions, the

application of additional N fertilizer within a certain range (pure N ≤ 29 g/plant) could promote fruit yield and increase Nitrogen levels. These results corroborate the findings of Pang et al. [33] for blueberry plants. However, the results are inconsistent with the findings of Xing et al. [34] for tomato plants. Thus, it can be deduced that appropriate amounts of N fertilizer are beneficial for N absorption by fruit crops and promote increased fruit yield, but different plants have different optimal N fertilization demands. Under low and moderate N fertilization conditions, increasing irrigation within a certain range (irrigation ≤ 2.5 L/plant) supported the accumulation of N in the blueberry leaves and branches and promoted yield. The variation in N accumulation and absorption trends of the blueberry leaves and branches reflect the biological impacts of the different water and fertilizer combinations.

The WUE results of the present study are consistent with those of Ertek et al. [35] and Zotarelli et al. [36]: under deficient irrigation, WUE is typically high, and a high irrigation volume often leads to low WUE. The findings of this study indicate that under higher levels of N application (F3), the agronomic utilization rate and partial productivity of N fertilizer decreased. This result is similar to that of the study of maize by Guo et al. [37]. Excessive N application thus reduced the agronomic utilization rate of N fertilizer. It was also found that the low N and moderate water (F1W2 and F1W3) treatments resulted in high fruit yields but also less nitrate N retention in the soil. Under these conditions, the economic input-output ratio was high and the soil N accumulation was low, and thus the economic and ecological benefits were maximized.

The main types of inorganic N absorbed and used by plants are ammonium N and nitrate N [38,39]. In the present study, it was found that the amount of ammonium N and nitrate N in the soil decreased with reduced N application and elevated irrigation over the two months. This is because nitrate N is highly mobile and thus easily lost with water. These results are consistent with the results of related studies in amaranth and spinach [40], and in a cucumber-tomato rotation system [41]. Controlling the irrigation and N application significantly slowed the migration of nitrate N in the soil. This indicates that water and N management can regulate the distribution of nitrate N and ammonium N in the soil and can also effectively reduce soil N, which has ecological and economic benefits. Studies have shown that different N application rates mainly influence the soil ammonium N content in the 0–20 cm soil layer [42]. These results demonstrate that soil particles and colloids have a strong adsorption effect on ammonium N. Moreover, due to the exchange reaction in the soil and the organification, nitrification, and denitrification of inorganic N, it is difficult for soil NH_4^+-N to migrate deeper. Ammonium N fertilizer provides a substrate for nitrification to ensure an effective N supply at a later period and avoids excessive ammonium N accumulation in the short term. Therefore, it is not surprising that in this study, there was no significant correlation between soil ammonium N content and N application rate. As the N application rate was increased, the nitrate N content of the 0–20 cm and 20–50 cm soil layers showed an increasing trend. With an increase in irrigation volume, the nitrate N content of the 0–20 cm soil layer decreased, and the nitrate content in the 20–50 cm soil layer increased. This increase in nitrate N content suggests that the reduction in soil nitrate N content in the 0–20 cm layer was not only related to the consumption of soil nitrate N by plants, but also to the downward movement of soil nitrate N. This result is similar to the conclusions of Zhang et al. [43] in their study of oats and of Li et al. [41] in their study of a cucumber-tomato rotation system. In these studies, increased irrigation and N application resulted in significant leaching of soil nitrate N.

4.2 Selection of a High-Yield Water and N Coupling Strategy for Blueberry Cultivation

The coupling of water and N significantly impacted the yield of rabbiteye blueberry fruit. As indicated in Tables 4 and 5, the top three treatments in terms of yield included the low N and moderate water (F1W2 and F1W3), and the low N and low water (F1W1) treatments. Thus, in the Guizhou blueberry production area, it

is recommended that farmers use a combination of low N and low water, or low N and moderate water for rabbiteye blueberry cultivation and management in order to maximize fruit yield.

Improving the yield and quality of the fruits is an important goal in blueberry cultivation. Bryla et al. showed that fertigation is a key factor affecting blueberry fruit yield, with a tight balance between ensuring sufficient water supply in the dry season and reducing N leaching [16]. Under sufficient irrigation, the fruit yield of the W2 treatments was still significantly higher than that of the W4 treatments. A comparison of the nitrate N content of the 0–20 cm and 20–50 cm soil layers in the W4 and W2 treatments in May indicated that less nitrate was leached into the soil below 50 cm when water was sufficient (as in W2). Therefore, without considering fruit yield or environmental friendliness, W2 irrigation conditions are the best choice. In terms of soil nutrients, the N application level of the F1 treatment was optimal throughout the growing season. Considering plant water requirements, the W2 irrigation parameters should be applied from May to September to ensure plant growth and development. As nitrate N accumulated in the 0–50 cm soil layer in September, the monitoring of ammonium N and nitrate N in the root zone below 50 cm should be strengthened.

The present analysis shows that the water-N ratio is of great significance for the precise cultivation of Guizhou rabbiteye blueberries and for the conservation of the soil. In particular, determining the best water-N ratios for the fruit farmers is necessary for improving fruit yield and quality, and for reducing environmental pollution, which have obvious economic and ecological benefits. In the present study, only the N and water requirements of rabbiteye blueberries in the Guizhou production area were evaluated. Other blueberry varieties, such as Blue Rain, North Jersey, and Jersey, have different ecological characteristics, and thus future research should focus on determining the optimal water and fertilization requirements for these varieties in Guizhou. The present results provide a theoretical and technical foundation for the establishment of a precise blueberry cultivation system in Guizhou.

5 Conclusion

In this study, N accumulation mainly occurred in the leaves of the blueberry plants, and was significantly higher in the leaves than in the branches in both seasons (May and September). Increasing water and N application within appropriate ranges (pure N ≤ 29 g/plant and irrigation ≤ 2.5 L/plant) could promote the effective accumulation of N in the blueberry leaves and branches, promote the absorption and utilization of N by the blueberries, and increase the fruit yield. For the same irrigation conditions, the inorganic N content of the soil increased with the increase in N application. With increased irrigation, the soil ammonium N content decreased, while the soil nitrate N content decreased in the 0–20 cm soil layer but increased in the 20–50 cm soil layer. With increased irrigation and N application, nitrate N began to leach significantly into the soil. Under the study conditions, the low N and low water, and low N and moderate water treatments improved rabbiteye blueberry production and supported the ecological soil environment. The findings of this study can be used to improve not only Guizhou rabbit-eye blueberry planting precision and effect, but also improve the soil environment and the revenue of local fruit growers.

Conflicts of Interest: The authors declare that they have no conflict of interest report regarding the present study.

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