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Different Soil Properties, Wolfberry Yields, and Quality Responses to Organic Fertilizer Levels in Two Fields with Varying Fertility Levels in Qaidam

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Abstract: (1) Background: This study aimed to evaluate the effects of organic fertilizer dose on soil nutrients, wolfberry fruit nutrient compositions, and fruit yields. (2) Methods: We conducted a two-year field trial in two typical fields with different fertility levels in the Qaidam area. Six treatments were applied to each field, including CK, M2 M4, M6, M8, and M10 (representing 0, 2, 4, 6, 8, and 10 kg organic fertilizer/plant, respectively) in the high-fertility field and CK, M3, M6, M9, M12, and M15 (representing 0, 3, 6, 9, 12, and 15 kg organic fertilizer/plant, respectively) in the low-fertility field. An ANOVA was used to determine the significant difference between treatments, and the LSD method was used for multiple comparisons of analysis of variance. (3) Results: In the high-fertility field, the application of organic fertilizer significantly affected the total nitrogen (N) content, mineral N storage, and soil organic matter content. The application of too much organic fertilizer significantly increased the soil's EC value. In the low-fertility field, the effect of organic fertilizer application on soil nutrient enhancement differed significantly among soil layers but significantly increased the contents of total phenols, flavonoids, and amino acids in wolfberry fruit, and there was a significant trend of increasing wolfberry yield with increasing organic fertilizer application. (4) Conclusions: In the Qaidam area of the Tibetan Plateau, it is recommended to apply 2–4 kg commercial organic fertilizer/plant in the high-fertility wolfberry orchards while 9–12 kg in the low-fertility wolfberry orchards is recommended.

Keywords: soil EC value; soil pH; soil organic matter; fruit antioxidant activity; fruit nutrition

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1. Introduction

Chinese wolfberry (*Lycium chinense* Miller) is an important plant resource with high medicinal value in China. It was recorded as early as in Li Shizhen's "Compendium of Materia medica" in the Ming Dynasty. Wolfberry plays a vital role in food and Chinese medicine and is commonly directly consumed and used for brewing, cooking and making

juice [1]. In Chinese medicine, it is generally decocted or ground into powder and mixed with other herbs. Studies have demonstrated that consuming berries such as wolfberry and pomegranate is conducive to human health [2]. With its well-developed root system and drought resistance, wolfberries can be planted in arid and saline areas, where the requirements of the tree species are high. In 2002, wolfberry was classified by the Ministry of Health of the People's Republic of China as a dual-use item for medicine and food, with the effects of nourishing yin and tonicity, nourishing the liver and eyes, delaying ageing, improving human immunity, and fighting cancer [3,4].

In recent years, the wolfberry industry in Qinghai Province has developed rapidly, and Qinghai Province has had the highest production of organic wolfberries in the country since 2015 [5]. However, with the rapid development of the Qaidam wolfberry industry, the application of large quantities of chemical fertilizers over many years has led to soil problems (such as increasing soil salinity), plant problems (such as wolfberry trees prone to premature death), and potential environmental problems (such as nitrate leaching) [6]. Organic farming of wolfberry is crucial to the ecological conservation of the Tibetan Plateau. Fertilizer application in the Qaidam area is generally performed with a single chemical fertilizer, and organic fertilizer application leaves severe deficiencies; therefore, the issue of suitable fertilization application in the Qaidam area needs to be addressed.

Organic fertilizers play a vital role in agricultural production. Organic fertilizers are long-lasting, rich in nutrients and nutrient elements contained in the soil, essential for global food security [7], can effectively maintain the stability of the soil's carbon pool, and can effectively regulate soil physicochemical properties and microbial community structure [8]. Organic fertilizers are effective in improving crop yields [9] and fruit quality [10]. In contrast, chemical fertilizers are characterized by fast fertilization, high nutrient content, and short duration, and the released nutrients are immediately available to plants, leading to rapid fertilization and high nutrient contents, thus rapidly promoting plant growth and improving crop yields. Agricultural production materials were previously used in large quantities. China has become the world's largest chemical fertilizer user [11], resulting in the emergence of problems such as soil degradation, surface pollution, and eutrophication of water bodies [12]. In 2018, the Chinese government proposed promoting organic fertilizer alternatives to chemical fertilizer technology, leading to the widespread use of commercial organic fertilizers, compost, bio-organic fertilizer, manure, and other kinds of organic fertilizers in recent years. China has abundant organic fertilizer resources, and the development of intensive organic fertilizer production and use of organic fertilizers has become an excellent approach to protect the ecological environment, fertilize the soil, and support other beneficial measures [13].

The research on wolfberries in recent years focused on intensive processing [14–19], medical effects [20–23], molecular mechanisms underlying physiological characteristics [24–29], identification of geographic origin [30–33] and mechanized picking technology [34,35], with less research involved in wolfberry cultivation [36,37]. The mechanism of the effect of organic fertilizer on wolfberries remains unclear. In this study, two wolfberry fields in the Qaidam area with large differences in fertility were selected for field trials to investigate the responses of wolfberry yield, quality and soil fertility to organic fertilizer application, with the aim of improving organic wolfberry production in this area.

2. Materials and Methods

2.1. Site Description

Field trials were conducted for two consecutive years, from 2020 to 2021, in Dulan County, Haixi Mongolian and Tibetan Autonomous Prefecture, Qinghai Province. The site has a plateau continental climate, with an elevation of 2790 m above sea level, annual

average sunshine hours of 2514.7 h, annual precipitation of 56.4 mm, and annual average temperature of 6.1 °C [38].

2.2. Experimental Design

The experiment was conducted in two fields: Nuomuhong Farm (96°20′ E, 36°25′ N), Dulan County, Haixi Mongolian and Tibetan Autonomous Prefecture, Qinghai Province, and Nuomuhong Wolfberry Base of the Kunlun River Wolfberry Company (96°48′ E, 36°42′ N). The soil on Nuomuhong Farm is more fertile (hereinafter referred to as the high-fertility field) than that on the Nuomuhong wolfberry base of the Kunlun River Wolfberry company (hereinafter referred to as the low-fertility field (Table 1).

Table 1. Basic physical and chemical properties of the soil.

Test Site	Soil Depth	Organic Carbon	Total Nitrogen	Total Phosphorus	Mineral Nitrogen	Rapidly Available Phosphorus	Rapidly Available Potassium	pH
	(cm)	(g/kg)	(g/kg)	(g/kg)	(mg/kg)	(mg/kg)	(mg/kg)	
High-fertility field	0–20	10.21	1.09	1.24	228.47	95.84	255.92	7.84
	20–40	7.44	0.78	0.67	178.87	16.38	154.72	8.04
Low-fertility field	0–20	2.03	0.1	0.87	1.43	51.3	78.93	8.73
	20–40	1.37	0.05	0.45	0.83	21.92	64.72	8.77

There were 6 treatments in the high-fertility field, including CK, M2, M4, M6, M8, and M10, representing 0, 2, 4, 6, 8, and 10 kg organic fertilizer per plant, respectively. The organic fertilizer was applied in mid-May every year. There were also 6 treatments in the low-fertility field, including CK, M3, M6, M9, M12, and M15. representing 0, 3, 6, 9, 12, and 15 kg organic fertilizer per plant, respectively. Each treatment was replicated 3 times, with 20–30 wolfberry trees in each replicate.

The organic fertilizer used in the high-fertility field is produced by Kunlun River Wolfberry Company, with an organic matter content of 45%, 1.27% nitrogen, 0.8% phosphorus, and 2.0% potassium. The organic fertilizer used in the low-fertility field was produced by Qinghai Enze Agricultural Technology Co., Ltd., with organic matter $\geq$ 40% and $N + P_2O_5 + K_2O \geq 5\%$.

The variety of wolfberries in the two experiments was NingQi 7. In the high-fertility field, eight-year-old plants were used, with a row spacing of 2 m and plant spacing of 1.5 m, while in the low-fertility field, four-year-old plants were used, with a row spacing of 3 m and plant spacing of 1 m. Organic fertilizer was applied twice, half in mid-November and half in mid-May. All organic fertilizer was embedded into the ditches located on both sides of the wolfberry trees. These ditches were 20–30 cm deep, 20–30 cm wide, and situated 30–40 cm away from the tree trunk. The experiments started in 2020.

2.3. Measurements and Methods

2.3.1. Collection and Determination of Soil Samples

After the harvest of wolfberry in October 2020 and 2021, soil samples were collected using an earth boring auger at intervals of 20 cm, from the 0–200 cm profile in the high-fertility field and from the 0–100 cm profile in the low-fertility field. Soils were taken between two wolfberry trees in each plot.

The nitrate nitrogen content of fresh soil throughout the 0–100 cm depth in the low-fertility field and 0–200 cm depth in the high-fertility field was determined using a continuous flow analyzer after leaching with 1 mol/L of KCl. The accumulated nitrate in kg/hm^2 (Nox.-N) was calculated using the following formula:

Nox.-N = $CN \times d \times \rho \times 10 - 1$, where CN represents the concentration of soil nitrate (mg/kg), d is the thickness of each soil layer (in this study, d = 20 cm), ρ is the bulk density (g/cm^3) of the corresponding soil layer, and 10^{-1} is a conversion coefficient.

The organic matter content of the soil at 0–20 cm and 20–40 cm depths in both of the two fields was determined by the potassium bichromate volumetric method. Soil total nitrogen was determined using the Kjeldahl method. Soil pH was determined using a DELTA 320 pH meter after shaking the 5:1 soil-to-water ratio for 3 min. Soil EC was determined using a conductivity meter after shaking the 10:1 soil-to-water ratio for 3 min [39].

2.3.2. Wolfberry Yield Determination and Plant Sample Collection

In the high-fertility fields, wolfberries were harvested in late July (the first harvest), mid-August (the second harvest), and mid-September (the third harvest) every year. Each time, all the mature fresh fruits in the plot were picked, the fresh weights were obtained, and fruit samples were collected. In the low-fertility fields, wolfberries were harvested in early August (the first harvest) and late August (the second harvest) every year. Each time, all the mature fresh fruits of 6 wolfberry trees in the plot were picked, the fresh weight was obtained, and fruit samples were collected. The wolfberry yield was calculated after the wolfberry samples were dried. The samples collected from the second harvest in 2021 were used to measure nutritional composition as well as antioxidant activity.

2.3.3. Determination of Nutrient Composition of Fruits

The total phenolic content (TPC) was determined using the Folin-phenol reagent method [40] according to the "Entry-Exit Inspection and Quarantine Industry Standard of the People's Republic of China". The flavonoid content (FC) was determined using the colorimetric method of aluminum chloride–acetic acid–sodium acetate, and the absorbance was recorded using a spectrophotometer at 415 nm [41]. Polysaccharide content (PC) was determined using the phenol–sulfuric acid method, as per Chinese National Standard (CNS) GB/T 18672-2014 Appendix A [42]. The soluble solid content (SSC) of wolfberries was determined using direct titration according to Chinese Agricultural Standards (CAS) NY/T 2637-2014. The amino acid content was determined using an automatic amino acid analyzer (A300 advanced03030901, Berlin, Germany, membraPure GmbH).

The scavenging effect of ABTS was determined using the method from Apak [43]. The cupric reducing antioxidant capacity (CUPCAC) was determined using the method from Güçlü [44]. The DPPH scavenging capacity was determined according to the method described by Cheng [45], as was the potassium ferricyanide-reducing antioxidant capacity (PFRAC) [45].

2.3.4. Data Processing and Analysis

All the experimental data were statistically analysed using the DPS data processing system, the LSD method was used for multiple comparisons of analysis of variance ($p < 0.05$), and analysis and graphing were performed using Excel 2019 software and the R language. Redundancy analysis (RDA) was used to determine the effects of soil properties on wolfberry qualities and yields.

3. Results

3.1. Evaluation of Different Organic Fertilizer Levels on the Fruit Yield and Appearance of Wolfberries

The berries were harvested three times in the high-fertility field in both years. The second harvest had the highest total berry yield among the three harvests (Figure 1). The results showed that the second, third, and total berry yields decreased with increased

organic fertilizer rates. A significant reduction in the total yield was found in the M8 treatment in 2020 and in the M6 treatment in 2021. There were no significant differences in the total yield among the CK, M2, M4, and M6 treatments or between the M8 and M10 treatments in 2020. There were no significant differences in the total yield among the CK, M2, and M4 treatments and no significant differences among the M6, M8, and M10 treatments in 2021.

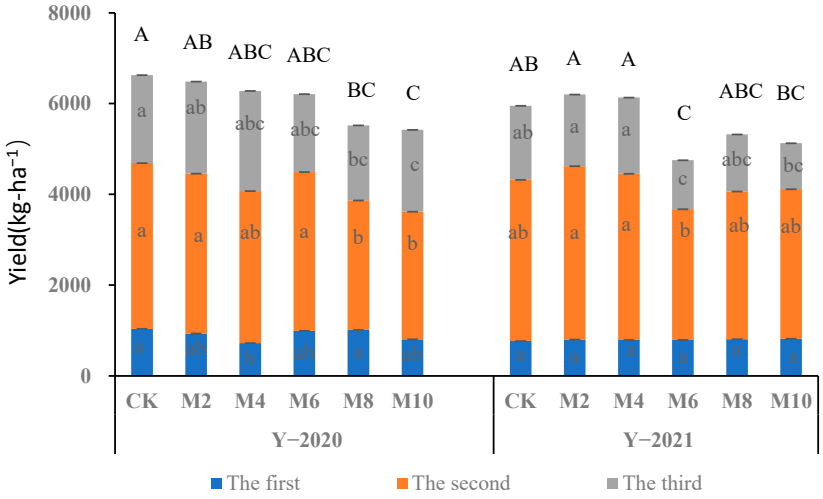


Figure 1. Effect of different organic fertilizer levels on dry fruit yield of wolfberries in high-fertility fields. The first means the first harvest (wolfberry fruits harvested in late July), the second means the second harvest (wolfberry fruits harvested in mid-August), and the third means the third harvest (wolfberry fruits harvested in mid-September). Different uppercase and lowercase letters represent significant differences between treatments ($p < 0.05$).

Berries were harvested twice in the low-fertility field in both years. The wolfberry yield increased with increasing organic fertilizer application from CK to M12, while no significant increase was found from M12 to M15. A linear curve plus a platform could be used to describe these trends (Figure 2).

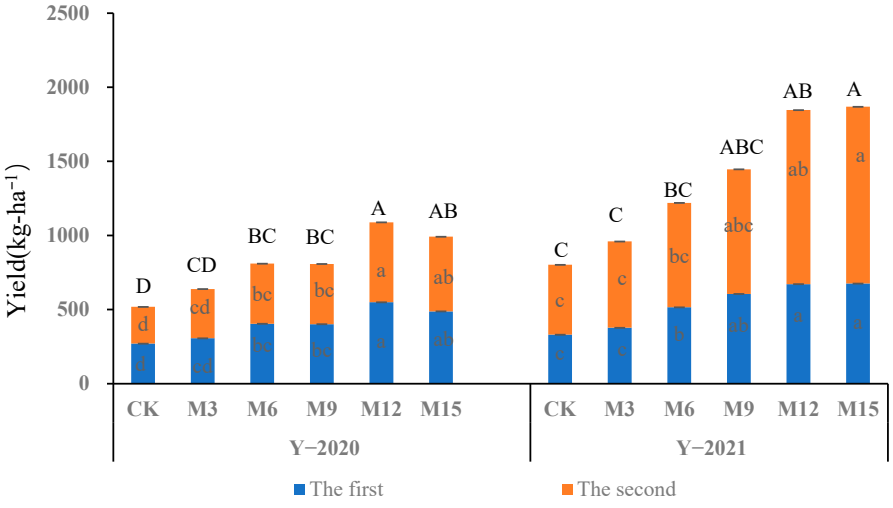


Figure 2. Effects of different organic fertilizer levels on dry fruit yield of wolfberry in low-fertility fields. The first means the first harvest (wolfberry fruits harvested in early August), and the second means the second harvest (wolfberry fruits harvested in late August). Different uppercase and lowercase letters represent significant differences between treatments ($p < 0.05$).

The results from the high-fertility field in both 2020 and 2021 showed that the responses of wolfberry 100 grain weight to organic fertilizer rates differed according to the harvest

time and harvest year. The M4 treatment had the highest 100 grain weight among the six treatments for the first harvest in both years; no significant differences were found among the six treatments for the third harvest in both years (Table 2).

Table 2. Effect of different organic fertilizer levels on the 100-grain weight of wolfberry (g).

Test Site		Year	CK	M2	M4	M6	M8	M10
High-fertility field	Y-2020	The first harvest	106.2 ab	98.5 bc	112.0 a	106.3 ab	92.8 c	100.8 bc
		The second harvest	87.2 ab	91.0 ab	87.5 ab	86.3 ab	79.7 b	95.6 a
		The third harvest	64.4 a	59.5 a	70.1 a	53.5 a	61.9 a	66.9 a
		Mean	85.5 ab	84.2 ab	89.7 a	85.8 ab	77.6 b	87.6 a
	Y-2021	The first harvest	102.8 ab	103.5 ab	105.5 a	101.4 ab	94.8 c	98.1 bc
		The second harvest	89.3 a	79.1 bc	79.1 bc	84.7 ab	74.6 c	73.3 c
		The third harvest	60.2 a	57.6 a	59.7 a	58.5 a	60.9 a	55.9 a
		Mean	84.1 a	80.1 ab	81.4 ab	81.5 ab	76.8 ab	75.8 b
			CK	**M3**	**M6**	**M9**	**M12**	**M15**
Low-fertility field	Y-2020	The first harvest	66.7 d	87.3 bc	87.2 bc	81.5 c	90.4 b	98.7 a
		The second harvest	45.5 c	54.2 b	54.1 b	45.2 c	51.0 bc	62.9 a
		Mean	56.1 d	70.8 b	70.7 b	63.4 c	70.7 b	80.8 a
	Y-2021	The first harvest	68.9 a	73.7 a	72.6 a	75.2 a	74.4 a	69.8 a
		The second harvest	60.7 a	55.7 a	59.0 a	61.1 a	60.8 a	57.5 a
		Mean	64.8 a	64.7 a	65.8 a	68.1 a	67.6 a	63.7 a

Note: The first harvest in the high-fertility field means wolfberry fruits were harvested in late July, the second harvest means wolfberry fruits were harvested in mid-August, and the third harvest means wolfberry fruits were harvested in mid-September. The first harvest in a low-fertility field means wolfberry fruits were harvested in early August, and the second harvest means wolfberry fruits were harvested in late August. Different lowercase letters represent significant differences between treatments ($p < 0.05$).

The results of the low-fertility field showed a significant increase in wolfberry 100 grain weight with the increase in organic fertilizer application for both harvests in 2020. No significant differences were found among the six treatments for both harvests in 2021 (Table 2).

In the high-fertility field, the results for both years showed that the fruit shape index was higher when 2 kg organic fertilizer (M2) was applied per plant. In 2020, the fruit shape index of the treatment that received 2 kg organic fertilizer (M2) per plant was significantly higher than that of the treatments that received 6–10 kg organic fertilizer (M6, M8, and M10) per plant. The fruit shape index of the third crop in 2021 was also significantly higher than that of the treatment that received 6 kg organic fertilizer (M6) per plant (Table 3). Compared with the control (CK), the M2 and M4 treatments had the best elevating effect.

For the first crop in 2020 in the low-fertility field, the application of organic fertilizer significantly improved the fruit shape index of fresh wolfberry, with the application of 3 kg organic fertilizer per plant (M3) resulting in an index that was 10.6% higher than that for the control (CK). For the second crop, each organic fertilizer treatment had a different amplitude of improvement compared with the control (CK). There was no significant difference between treatments in the fresh wolfberry fruit shape index in 2021 (Table 3).

3.2. Effect of Different Organic Fertilizer Levels on Soil Properties

In the high-fertility field, the soil organic matter and total nitrogen contents (TN) at depths of 0–20 cm and 20–40 cm showed an increasing trend with increasing organic fertilizer application. The application of organic fertilizer resulted in significant improvements of soil organic matter (SOM) and total nitrogen content in both years. A large amount of nitrate accumulated in the 0–200 cm soil profile, and the application of organic fertilizer enhanced nitrate accumulation in both years. There was no significant difference in soil pH values for either depth among treatments in 2020, while organic fertilizer application

significantly reduced soil pH values in 2021. The application of organic fertilizer also increased the EC value of the 0–20 cm and 20–40 cm soil depths in both years. The M10 treatment had the highest EC value among all the treatments (Table 4).

Table 3. Effects of different organic fertilizer levels on the fruit form index of wolfberry.

Test Site		Year	CK	M2	M4	M6	M8	M10
High-fertility field	Y-2020	The first harvest	2.4 ab	2.5 a	2.4 ab	2.4 ab	2.4 ab	2.3 b
		The second harvest	2.2 ab	2.3 a	2.2 ab	2.2 ab	2.2 b	2.2 ab
		The third harvest	2.1 b	2.2 a	2.1 ab	2.1 bc	2.0 c	2.0 bc
		Mean	2.2 ab	2.3 a	2.3 ab	2.2 b	2.2 b	2.2 b
	Y-2021	The first harvest	2.1 b	2.3 ab	2.3 a	2.2 ab	2.2 ab	2.2 ab
		The second harvest	2.0 a	2.0 a	2.0 a	1.9 a	2.0 a	1.9 a
		The third harvest	2.0 ab	2.1 a	2.1 a	2.0 b	2.0 ab	2.0 ab
		Mean	2.0 a	2.1 a	2.1 a	2.0 a	2.0 a	2.1 a
			CK	**M3**	**M6**	**M9**	**M12**	**M15**
Low-fertility field	Y-2020	The first harvest	1.9 d	2.1 a	2.0 bc	2.0 cd	2.1 abc	2.1 ab
		The second harvest	2.0 a	2.1 a	2.1 a	2.0 a	2.1 a	2.2 a
		Mean	2.0 b	2.1 ab	2.0 ab	2.01 ab	2.1 abc	2.1 a
	Y-2021	The first harvest	2.1 a	2.1 a	2.0 a	2.1 a	2.1 a	2.0 a
		The second harvest	1.8 a	1.8 a	1.8 a	1.8 a	1.8 a	1.8 a
		Mean	1.9 a	2.0 a	1.9 a	1.9 a	2.0 a	1.9 a

Note: The first harvest in a high-fertility field means wolfberry fruits were harvested in late July, the second harvest means wolfberry fruits were harvested in mid-August, and the third harvest means wolfberry fruits were harvested in mid-September. The first harvest in a low-fertility field means wolfberry fruits were harvested in early August, and the second harvest means wolfberry fruits were harvested in late August. Different lowercase letters represent significant differences between treatments ($p < 0.05$).

In the low-fertility field, the soil organic matter and total nitrogen contents at depths of 0–20 cm and 20–40 cm showed an increasing trend with increasing organic fertilizer application. The application of organic fertilizer resulted in significant improvements in soil organic matter and total nitrogen content in both years. A large amount of nitrate did not accumulate in the 0–200 cm soil profile, which was opposite to the effect in the high-fertility field. The application of much organic fertilizer enhanced nitrate accumulation in both years. Similarly to the high-fertility field, there was no significant difference in soil pH values for either depth among treatments in 2020, while the application of a large amount of organic fertilizer (the M15 treatment) significantly reduced the soil pH values in 2021. The application of high levels of organic fertilizer also increased the soil EC value in both years (Table 4).

3.3. Effects of Organic Fertilizer Level on Wolfberry Nutrient Composition

In the high-fertility field, the application of organic fertilizer increased wolfberry total phenol content (TPC) by 4.2% to 9.6% and increased polysaccharide content (PC) by 1.8% to 25.7%, except for the treatment with 8 kg organic fertilizer (M8). Compared with the treatment without organic fertilizer (CK), the wolfberries in the treatments with 8 and 10 kg organic fertilizer (M8 and M10) had higher flavonoid contents (FCs), soluble solids contents (SSCs), and amino acid contents (AACs) (Table 5).

In the low-fertility field, the application of organic fertilizer increased the total phenol contents, polysaccharide contents, flavonoid contents, and amino acid contents of the wolfberries while decreasing the soluble solid contents (Table 5).

Table 4. Effect of different organic fertilizer levels on soil properties.

Test Site	Years	Soil Properties	Soil Depth (cm)	CK	M2	M4	M6	M8	M10
High-fertility field	Y-2020	OM ($g \cdot kg^{-1}$)	0–20	13.4 b	17.9 a	18.9 a	19.2 a	19.2 a	19.99 a
			20–40	11.9 b	15.8 a	15.6 a	15.3 a	16.0 a	16.8 a
		TN ($g \cdot kg^{-1}$)	0–20	0.68 b	1.12 a	1.12 a	1.15 a	1.36 a	1.37 a
			20–40	0.58 b	0.60 b	0.73 ab	0.84 ab	0.88 a	0.92 a
		pH	0–20	7.72 a	7.52 a	7.71 a	7.66 a	7.69 a	7.50 a
			20–40	7.61 a	7.53 a	7.72 a	7.56 a	7.51 a	7.55 a
		Nox.-N (kg/hm^2)	0–200	3475 ab	6831 a	2545 b	3683 ab	4703 ab	4184 ab
		EC (μs/cm)	0–20	127.4 b	198.9 b	150.8 b	196.3 b	290.2 b	608.2 a
			20–40	245.5 b	311.3 b	262.5 b	271.9 b	342.3 b	435.7 a
	Y-2021	OM ($g \cdot kg^{-1}$)	0–20	12.3 b	16.1 a	16.2 a	17.2 a	18.4 a	18.4 a
			20–40	11.9 b	15.3 a	15.6 a	15.8 a	15.8 a	16.0 a
		TN ($g \cdot kg^{-1}$)	0–20	0.711 b	1.19 ab	1.21 ab	1.24 ab	1.38 ab	1.49 a
			20–40	0.684 b	1.09 a	1.11 a	1.16 a	1.19 a	1.20 a
		pH	0–20	8.38 a	7.95 b	7.94 b	8.03 ab	8.11 ab	7.75 b
			20–40	8.48 a	7.63 c	7.82 bc	7.75 bc	8.00 b	7.86 bc
		Nox.-N (kg/hm^2)	0–200	6554 a	9945 a	9427 a	9364 a	11,078 a	9778 a
		EC (μs/cm)	0–20	118.6 b	191.2 b	161.7 b	165.2 b	316.0 b	697.7 a
			20–40	115.8 c	310.3 b	211.8 bc	258.7 bc	284.3 b	727.5 a
				CK	**M3**	**M6**	**M9**	**M12**	**M15**
Low-fertility field	Y-2020	OM ($g \cdot kg^{-1}$)	0–20	8.1 c	9.0 bc	9.2 bc	9.8 bc	11.6 b	17.6 a
			20–40	7.8 d	9.8 cd	11.3 c	11.7 bc	13.9 ab	14.8 a
		TN ($g \cdot kg^{-1}$)	0–20	0.43 c	0.50 bc	0.51 bc	0.59 b	0.89 a	0.94 a
			20–40	0.29 b	0.42 ab	0.64 a	0.67 a	0.70 a	0.71 a
		pH	0–20	8.37 a	8.31 a	8.23 a	8.32 a	8.18 a	8.27 a
			20–40	8.38 a	8.50 a	8.42 a	8.32 a	8.24 a	8.31 a
		Nox.-N (kg/hm^2)	0–100	15.4 b	12.71 b	52.9 b	91.5 ab	192.6 a	130.4 ab
		EC (μs/cm)	0–20	124.9 c	262.1 ab	190.2 bc	277.5 ab	319.5 a	178.9 bc
			20–40	135.7 a	143.5 a	147.2 a	134.7 a	143.5 a	148.6 a
	Y-2021	OM ($g \cdot kg^{-1}$)	0–20	8.5 e	10.6 de	11.4 cd	13.8 bc	14.5 b	18.3 a
			20–40	5.9 c	9.2 bc	11.2 bc	11.6 abc	14.3 ab	17.5 a
		TN ($g \cdot kg^{-1}$)	0–20	0.33 c	0.52 bc	0.55 bc	0.64 bc	0.88 b	1.29 a
			20–40	0.25 c	0.31 c	0.47 bc	0.55 bc	0.69 b	1.13 a
		pH	0–20	8.24 cd	8.46 abc	8.56 ab	8.68 a	8.32 bc	7.96 d
			20–40	8.46 ab	8.70 a	8.72 a	8.62 a	8.34 ab	8.05 ± b
		Nox.-N (kg/hm^2)	0–100	386.1 b	123.6 c	25.0 c	109.7 c	102.3 c	600.1 a
		EC (μs/cm)	0–20	290.5 a	246.8 ab	200.1 bc	170.0 c	233.0 abc	208.0 bc
			20–40	220.4 ab	126.2 c	142.2 bc	145.3 bc	201.7 bc	312.3 a

Note: OM is the soil organic matter content; TN is the soil total nitrogen content, EC is the soil electrical conductivity value, pH is the soil pH, and Nox.-N is the soil-accumulated nitrate nitrogen at 0–200 cm depth for a high-fertility field or at 0–100 cm depth for a low-fertility yield. Different lowercase letters represent significant differences between treatments ($p < 0.05$).

Table 5. Effect of different organic fertilizer levels on the nutrient composition of wolfberries.

Test Site	Nutrient Content	CK	M2	M4	M6	M8	M10
High fertility field	TPC (mg GAE/g)	9.9 a	10.4 a	10.6 a	10.4 a	10.9 a	10.9 a
	PC (g/100 g)	2.5 ab	3.1 a	3.0 ab	2.5 ab	2.4 b	2.9 ab
	FC (g/kg)	5.0 a	4.2 b	4.9 ab	4.8 ab	5.4 a	5.1 a
	SSC (%)	21.2 a	21.6 a	21.2 a	22.1 a	22.9 a	22.5 a
	AAC (g/100 g)	8.1 a	8.3 a	8.0 a	8.0 a	8.6 a	8.2 a
		CK	**M3**	**M6**	**M9**	**M12**	**M15**
Low Fertility field	TPC (mg GAE/g)	9.8 b	10.0 ab	10.3 ab	10.7 ab	11.3 a	10.6 ab
	PC (g/100 g)	2.2 a	2.3 a	2.4 a	2.1 a	2.4 a	2.0 a
	FC (g/kg)	3.1 b	3.9 ab	3.6 ab	3.5 ab	4.4 a	3.9 ab
	SSC (%)	23.1 a	23.0 a	22.3 ab	22.6 ab	22.0 ab	21.5 b
	AAC (g/100 g)	8.2 b	9.5 a	8.7 ab	9.0 ab	9.1 ab	9.1 a

Note: TPC is total phenol content, PC is polysaccharide content, FC is flavonoid content, SSC is soluble solids content, and AAC is amino acid content. Different lowercase letters represent significant differences between treatments ($p < 0.05$).

3.4. Effects of Organic Fertilizer Level on Wolfberry Antioxidant Activity

In high-fertility fields, the application of organic fertilizer increased the ABTS and DPPH free radical scavenging power, copper ion reducing power, and ferricyanide potassium reducing power of wolfberries. Compared with the treatment without any organic fertilizer (CK), the treatments with organic fertilizer had 1.0–3.5% higher ABTS free radical scavenging power, 2.4–3.9% higher DPPH free radical scavenging power, 1.2–10.5% higher copper ion reducing power, and 3.0–12.1% higher ferricyanide potassium reducing power (Table 6).

Table 6. Effects of different organic fertilizer levels on the antioxidant activity of wolfberries in high-fertility and low-fertility fields.

Test Site	Antioxidant Activity	CK	M2	M4	M6	M8	M10
High fertility field	ABTS (μM Trolox/g)	1.890 b	1.909 ab	1.919 ab	1.932 ab	1.957 a	1.933 ab
	DPPH (μM Trolox/g)	1.401 a	1.378 a	1.435 a	1.436 a	1.455 a	1.373 a
	CUPCAC (μM Trolox/g)	0.765 b	0.774 b	0.812 ab	0.807 ab	0.845 a	0.824 ab
	PFRAC	0.066 b	0.069 ab	0.068 ab	0.07 ab	0.074 a	0.071 ab
		CK	**M3**	**M6**	**M9**	**M12**	**M15**
Low Fertility field	ABTS (μM Trolox/g)	1.996 a	1.993 a	1.994 a	1.994 a	2.021 a	1.987 a
	DPPH (μM Trolox/g)	1.393 b	1.533 a	1.559 a	1.554 a	1.527 a	1.461 ab
	CUPCAC (μM Trolox/g)	0.792 a	0.852 a	0.885 a	0.831 a	0.898 a	0.858 a
	PFRAC	0.085 a	0.075 a	0.078 a	0.075 a	0.080 a	0.078 a

Note: ABTS is ABTS free radical scavenging power, DPPH is DPPH free radical scavenging power, CUPRAC is copper ion reducing power, PFRAC is iron ion reducing capacity. Different lowercase letters represent significant differences between treatments ($p < 0.05$).

In the low-fertility field, the application of organic fertilizer increased the DPPH free radical scavenging power and copper ion reducing power of wolfberries. In contrast, decreased ferricyanide potassium lowered the reducing power of wolfberries. The ABTS free radical scavenging power of wolfberries did not respond to the application of organic fertilizer (Table 6).

3.5. Redundancy Analysis of the Effects of Soil Properties on Wolfberry Qualities and Yield

In the high-fertility fields, the two RDA axes together explained 97.62% of the total variation in berry quality (Figure 3). The soil's EC value and TN content were positively correlated with the TPC, SSC, and AAC. Soil EC and TN had a greater effect on SSC and AAC of fruits. Accumulated NO_3^--N (Nox.-N) and SOM in the soil also had a more significant effect on AAC in the fruit. Additionally, soil pH was positively correlated with the fruit's antioxidant activity and TPC content. SOM and Nox.-N were negatively correlated with each other. Soil EC and TN were also negatively correlated with the PC of the fruit. Overall, the soil TN content had a positive effect on fruit quality.

In the low-fertility fields, the two RDA axes for the low-fertility fields together explained 95.4% of the total variation in wolfberry fruit quality (Figure 4). Soil TN, OM, and Nox.-N were positively correlated with the DPPH, ABTS, CUPCAC, FC, AAC and TPC contents in wolfberry fruit. In contrast, pH was negatively correlated with these factors. In addition, soil pH had a greater effect on and was positively correlated with PC. Apart from pH, the other soil physical and chemical properties were negatively correlated with PC. In general, increasing the total nitrogen content, organic matter content and nitrate nitrogen reserves of the soil positively affected the quality of the wolfberry. It was found that soil total N content was a key factor in affecting the wolfberry fruit quality regardless of soil fertility.

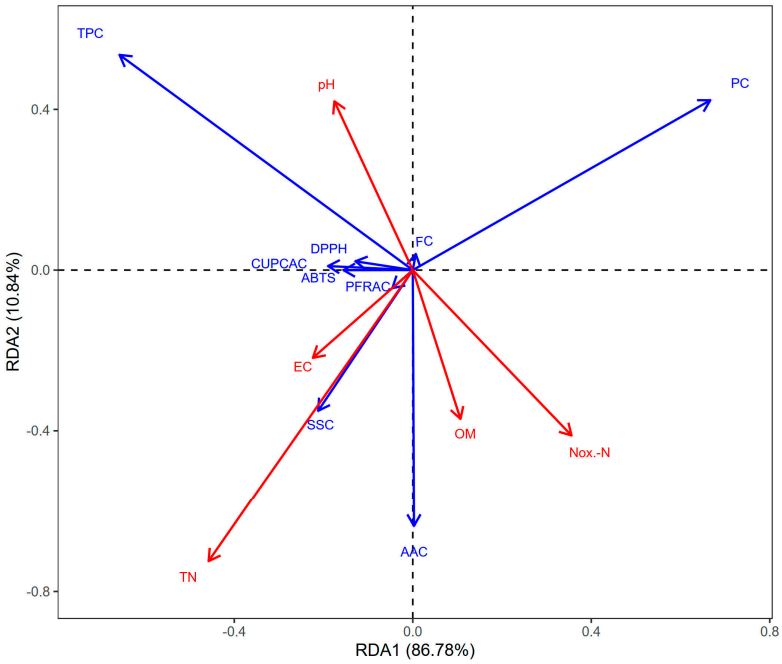


Figure 3. Redundancy analysis (RDA) of wolfberry fruit quality and soil properties in the high-fertility fields. Soil physical and chemical properties: TN, total soil nitrogen; OM, soil organic matter; pH, soil pH; EC, soil EC; Nox.-N, soil nitrate nitrogen storage; Fruit quality: FC, flavonoid; TPC, total phenols; PC, polysaccharide; SSC, soluble solid; ABTS, ABTS radical scavenging power; CUPRAC, copper ion reducing power; DPPH, DPPH scavenging power; PFRAC, potassium ferricyanide reducing power; AAC, total amino acids. The red lines refer to soil properties, the blue line refer to wolfberry fruit quality index.

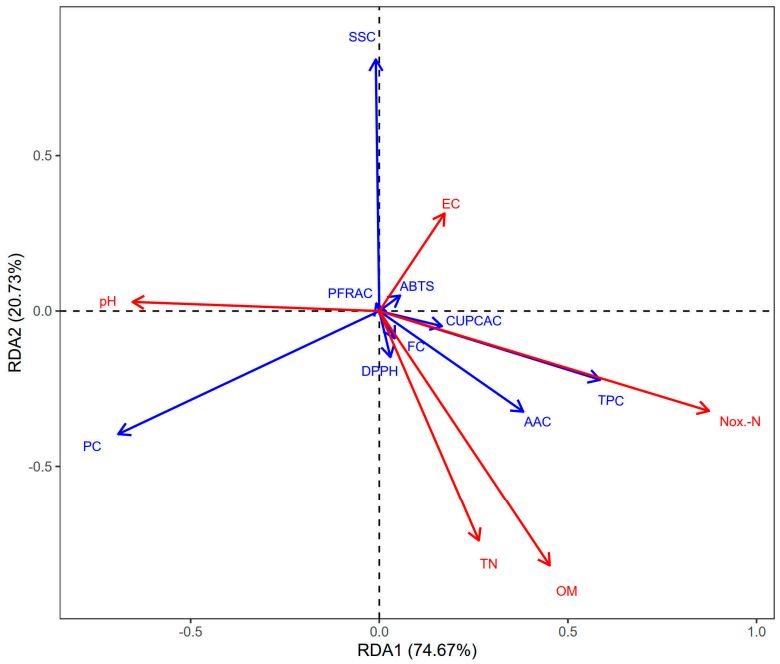


Figure 4. Redundancy analysis (RDA) of wolfberry fruit quality and soil properties in the low-fertility fields. Soil physical and chemical properties: TN, total soil nitrogen; OM, soil organic matter; pH, soil pH; EC, soil EC; Nox.-N, soil nitrate nitrogen storage; Fruit quality: FC, flavonoid; TPC, total phenol; PC, polysaccharide; SSC, soluble solid; ABTS, ABTS radical scavenging power; CUPRAC, copper ion reducing power; DPPH, DPPH scavenging power; PFRAC, potassium ferricyanide reducing power; AAC, total amino acids. The red lines refer to soil properties, the blue line refer to wolfberry fruit quality index.

3.6. Analysis of the Relative Importance of Wolfberry Fruit Yield, Appearance and Soil Physicochemical Properties

In the high-fertility fields, analysis of the relative importance of fruit yield, fruit appearance and soil physical and chemical properties of wolfberry showed that Nox.-N had the greatest influence on the yield and fruit shape index of wolfberries (Figure 5). SOM had the greatest influence on the 100-grain weight of fresh wolfberry fruit. SOM was also the second most important index affecting wolfberry yield and the fruit shape index. EC had the smallest effect on fruit yield, appearance, and shape index. In general, increasing the storage of nitrate nitrogen in soil is conducive to improving the fruit shape index of wolfberry yield, and increasing the content of organic matter in soil is conducive to increasing the hundred grain weight of wolfberry yield.

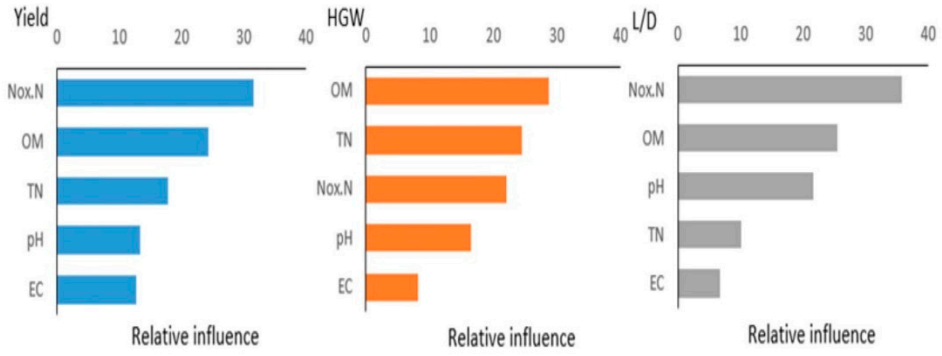


Figure 5. Analysis of the relative importance of fruit yield, appearance and soil physical and chemical properties in high-fertility fields. Yield, dried wolfberry fruit yield; HGW, hundred grain weight; L/D, fruit shape index.

In low-fertility fields, analysis of the relative importance of fruit yield, appearance and soil physical and chemical properties showed that TN had the greatest influence on the yield of wolfberries, followed by Nox.-N (Figure 6). SOM had the greatest influence on fruit 100-kernel weight, followed by EC. EC had the greatest influence on the fruit shape index, followed by SOM. pH had the smallest effect on fruit yield, 100 grain weight, and fruit shape index. In general, the increase in soil organic matter and EC value is conducive to the increase in fruit 100-grain weight and fruit shape index. The increase in soil total nitrogen content was beneficial to increasing fruit yield.

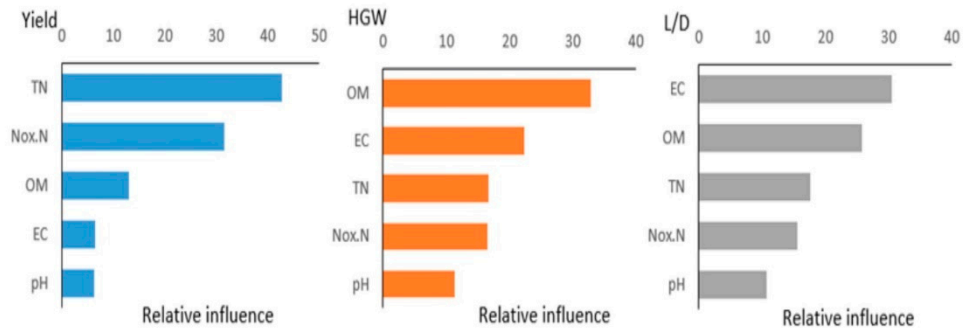


Figure 6. Analysis of the relative importance of fruit yield, appearance and soil physical and chemical properties in the low-fertility fields. Yield, dried wolfberry fruit yield; HGW, hundred grain weight; L/D, fruit shape index.

4. Discussion

4.1. Effect of Organic Fertilizer Application on High-Fertility Fields

The experiment in the high-fertility field showed that the fruit yield, 100-seed weight, and fruit shape index of wolfberries tended to decrease with the increase in the application rate of organic fertilizer, (Figure 1, Tables 2 and 3). However, the other research on grapes has shown that both grape yield and fruit weight increase linearly with increasing compost application [46]. In other research, Cataldo et al. found that grape harvest production data increased from the treatment with no organic fertilizer to treatment with 2.5 t/hm^2 and 15 t/hm^2 organic fertilizer and then decreased from the treatment with 15 t/hm^2 organic fertilizer to treatment with 40 t/hm^2 organic fertilizer [47]. The possible reason for the decrease in fruit yield and 100-grain weight in this study is the increase in soil salinity caused by the excessive application of organic fertilizer. Excessive soil salinity will lead to plant root rot, which will affect the nutrient absorption of crops and eventually lead to a decrease in crop yield [48–50]. In this study, organic fertilizers significantly increased soil EC (Table 4). Some scholars believe that excessive application of organic fertilizer will lead to the accumulation and even leaching of nitrate in soil, resulting in an unbalanced ratio of nitrogen, phosphorus and potassium in soil, crop nutrition imbalance, quality decline and other problems [51–54]. Thus, the nutrient resources of organic fertilizer are transformed into a potential pollution source. In this study, the relative importance analysis of the yield and appearance of soil physicochemical properties of wolfberries also showed that nitrate nitrogen reserves in soil had the greatest influence on wolfberry fruit yield and shape index (Figure 4).

The polysaccharides, flavonoids, total phenols, amino acids and other nutrients contained in wolfberry fruits improve human immunity and anticancer activity, delay human ageing, relieve visual fatigue, and so on [3,4,20–23]. In our study, the contents of total phenols, polysaccharides, flavonoids, soluble solids and amino acids in wolfberry fruits were significantly increased by applying organic fertilizers in high-fertility fields (Table 5). This result is similar to other research [55]. Some studies have shown that organic matter can improve fruit quality [56]. In this study, organic fertilizer application significantly increased the soil organic matter content, and the RDA results also showed that the organic matter content in soil was positively correlated with the amino acid content and soluble solids in fruits (Table 4 and Figure 3). Flavonoids, polysaccharides, vitamins, and other substances in wolfberries have antioxidant properties, have a bidirectional regulatory effect on the human body, and can regulate hormone levels in the human body to maintain normal health [57]. In this study, the analysis of polysaccharide content showed that the treatment with a low amount of organic fertilizer had better results. In contrast, the analysis of flavonoid content showed that the treatment with high amounts of organic fertilizer had a better result. The application of organic fertilizer had a significant effect on the antioxidant activity of fruits compared with CK, but the difference between organic fertilizer treatments was generally not significant (Tables 5 and 6). This result may be due to the ability of wolfberries to co-regulate polysaccharides and flavonoids. A study has shown that the antioxidant activity of tomato treated with organic fertilizer is higher than that of tomato treated with conventional fertilization, but the quality and antioxidant activity of tomato fruit is not affected by the increase in the application amount of organic fertilizer [58]. Other research has shown that the contents of SOC and wolfberry yield had the greatest response to cover cropping with medium manure rate rather than high manure rate [59]. These results together imply that good yield and quality of wolfberries need an appropriate organic fertilizer dose and over fertilization has to be avoided based on the high-fertility of soil.

In summary, compared with the CK treatment, applying organic fertilizer increased the contents of total nitrogen and organic matter in the soil, improving the soil pH in the high-fertility wolfberry fields. Moreover, the polysaccharide content and antioxidant activity of Wolfberry fruit were increased. Among all the organic fertilizer treatments, M2 and M4 had better performances. The yield in the second year of the high-fertility field decreased compared with that in the first year, while the decreases in the M2 and M4 treatments were smaller and higher than that in the CK treatment. Considering the decrease in yield in the second year of the study, it is suggested to apply 2–4 kg of commercial organic fertilizer/plant in the high-fertility field together with chemical fertilizer to ensure the yield and improve the soil quality and fruit quality.

4.2. Effect of the Application of Organic Fertilizer on Low-Fertility Fields

In the high-fertility field, the significant difference of soil total nitrogen and organic matter contents was only found between the control (CK) and treatments with organic fertilizer application, while there was no significant difference between treatments with organic fertilizer application. However, soil TN and SOM content was improved significantly with the increase in organic fertilizer rate from 0 to 15 kg/plant in the low-fertility field (Table 4). The possible reason is the higher basic soil nutrients content and lower organic fertilizer input in the high-fertility field than that in the low-fertility field. Yan et al. also reported that SOM content significantly increased with the organic fertilizer from 0 to 15 t/hm^2 in a wolfberry field with low-fertility in Ningxia province, China [60]. Wan et al. found similar results in an acid Ultisol with moderate fertility [61]. These together imply that the application of organic fertilizer observably enhances soil fertility regardless of soil basic fertility, while the maximum potential for fertility improvement depends on soil basic fertility as well as organic fertilizer rate. Soil fertility is the foundation for crop yield and quality. In this study, the results of the relative importance analysis showed that soil TN had the greatest effect on the yield of wolfberries, while SOM had the highest significant effect on the fruit weight of wolfberries (Figure 4). These results are consistent with the reports by Garratt et al. [62] and Oldfield et al. [63], which state that SOM content often relates positively to crop production. The yield of wolfberries in the second year increased significantly compared with that in the first year, possibly due to the slow effect of organic fertilizer. The decomposition of organic fertilizer in the first year and the application of organic fertilizer in the second year would have led to more rapidly available nutrients for the growth of wolfberries in the soil.

The results of the low-fertility field showed that the quality of wolfberry fruits was significantly affected by soil properties via the application of organic fertilizer. This is proved by the other reports. The results from Ozdemir et al. showed that the application of organic fertilizer can increase the flavonoid content of grapes [64]. Serri et al. found that antioxidant capacity of coriander increased due to the application of organic fertilizer [65]. RDA results also showed a positive correlation between soil TN content and fruit amino acid content (Figure 3), which was in accordance with the research by Shi et al. [66]. Results from research in six vineyards with low-fertility showed that the soluble solid content, reducing sugar and flavanol content of grape berry was positively correlated with soil TN while titrate acid and tannin content was negatively correlated with TN [67]. These imply the importance of soil nutrients on berry quality.

In summary, compared with the CK treatment, the application of organic fertilizer increased the contents of soil TN and SOM in the wolfberry fields with low-fertility. The total phenol, flavonoid, and amino acid contents of the wolfberry fruit were also increased, and the antioxidant activity of the fruit was enhanced. The yield of wolfberries showed an upward trend with the increase in the application amount of organic fertilizer (0–12 kg

commercial organic fertilizer/plant). When more than 12 kg of organic fertilizer was applied to each plant, the yield of wolfberries no longer increased. Therefore, applying 9–12 kg of commercial organic fertilizer/plant in low-fertility fields is recommended.

5. Conclusions

In the Qaidam area of the Tibetan Plateau, organic fertilizer improved soil properties and enhanced wolfberry growth and fruit quality. However, the appropriate organic fertilizer dose was different in a field with varied soil fertility. It is suggested to apply 2–4 kg of commercial organic fertilizer/plant together with chemical fertilizer to ensure the yield and improve the soil quality and fruit quality in the high-fertility wolfberry orchards, while 9–12 kg of commercial organic fertilizer/plant in the low-fertility wolfberry orchards is recommended. The results of this study provide a guideline for organic fertilizer application for wolfberry farmers in the Qaidam area of the Tibetan Plateau and other areas with similar conditions.

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