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Improvement of Wolfberry (*Lycium barbarum* L.) Fruit Yield and Quality and Enhancement of Soil Fertility by Nitrogen Reduction Combined with Organic Fertilizers

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Abstract: Excessive amounts of nitrogen (N) fertilizers are applied during wolfberry production, resulting in some soil problems as well as potential environmental risks in the Qinghai-Tibet Plateau. In this study, organic fertilizers were used to replace part of the N fertilizer in wolfberry fields with different fertility levels. N fertilizer rates had 0, 50, 100, 150, 200, and 250 g N/plant. Organic fertilizer rates had 0, 2, 4, 6, 8, and 10 kg organic fertilizer/plant. The experimental treatments included 6 combinations of N0M10, N50M8, N100M6, N150M4, N200M2, and control was N250M0. The results showed that in the high-fertility soils, combinations of N150M4, N100M6 and N50M8 treatments were increased in yields, fruit shape index, flavonoid content, total phenol content, mineral nutrient content, and antioxidant activity of wolfberry fruits. Also they were improved in soil fertility and decreased in residual nitrate through the soil depth of 0-300 cm. In the soil with less fertility, fruit yield, amino acid contents, flavonoids, total phenols, mineral nutrients and antioxidant activity of fruits were increased by the N200M2, N150M4 and N100M6 treatments and soil fertility was improved as well. Also more residual nitrate was found in the depth of 0-100 cm of soil with both chemical and organic fertilizer compared with the control. Therefore, in the Qinghai-Tibet Plateau, combining decreased N fertilizer with organic fertilizer rather than chemical fertilizer alone could help farmers achieve satisfactory yields and quality of wolfberry fruits and reduce the risk of nitrate leaching. In conclusion, 50-150 g/plant of N fertilizer combined with 4-8 kg/plant of organic fertilizer in high-fertility gardens and 100-200 g/plant of N fertilizer combined with 2-6 kg/plant of organic fertilizer in low-fertility gardens are recommended for wolfberry cultivation.

Key words: Wolfberry, fruit quality, antioxidant activity, organic fertilizer, nitrogen fertilizer.

1. Introduction

In recent decades, the global food system has been under immense pressure, stemming from rapid population growth, thereby rendering agriculture a pivotal step in addressing the food problem [1]. Nitrogen (N) fertilizer has played a crucial role in boosting crop yields [2]. In 2019, the global application of N fertilizer reached 11 million tons, while China's application stood at a staggering 2.6 million tons, accounting for 24.94% of the global usage. However, excessive use of N fertilizer leads to a waste of resources and increases the risk of environmental pollution. Prior findings indicate that approximately 16% of N fertilizer is lost through leaching and runoff [3], which enter the water cycle via rainfall or irrigation, potentially causing eutrophication in water bodies. Part

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of the remaining N is lost through denitrification and ammonification, resulting in volatilization and entering the atmosphere, thereby aggravating the greenhouse effect and degrading air quality [4]. To mitigate the risk of environmental pollution, the Chinese government issued the “Action Plan for Zero Growth in Fertilizer Use by 2020” in 2015, aiming to achieve zero growth in fertilizer use for major crops by 2020. Considering various aspects, including environmental protection and food security, it is imperative to devise reasonable fertilization strategies.

Wolfberry (*Lycium barbarum* L.) is a perennial medicinal plant from Solanaceae family. It has a lengthy history of cultivation in China with records dating back 1,400 years ago [5]. As a medicinal material, its fruit is abundant in various nutrients, including flavonoids, phenols, polysaccharides, carotenoids, amino acids and alkaloids [6]. It boasts advantages of reducing cardiovascular disease risk, providing eye protection and antiaging effects, preventing cancer and delaying the onset of Alzheimer’s disease [7-11]. Since the 21st century, owing to its unique benefits, it has garnered favour among many western consumers [12], leading to a growing trend of usage in numerous European countries [13]. In recent years, the cultivation area of Chinese wolfberry has gradually expanded and it is now planted in provinces like Ningxia, Qinghai, Gansu, Xinjiang and Inner Mongolia [14]. Qinghai Province has the largest wolfberry planting area, with over 49,700 hectares by the end of 2019. Due to its high economic benefits, wolfberry has emerged as a “leading industry”, enabling local people to overcome poverty and improve their financial well-being, thus driving the comprehensive development of the rural economy [15, 16]. To enhance profits, farmers employ significant amount of chemical fertilizer and pesticides during the wolfberry cultivation process [17]. The wolfberry production area is located in the hinterland of the Qinghai-Tibet Plateau, often referred to as the “third pole” of the world. The ecological environment here is fragile, and the recovery rate is slow after damage [18]. Therefore, excessive use of chemical fertilizers poses a heightened risk of environmental pollution, making it a crucial issue that needs to be addressed urgently: determining the reasonable amount of fertilizer required for wolfberry cultivation [19].

Organic fertilizers not only provide a large amount of nutrients for plant growth [20] but can also improve the physical environment of soil and increase the number of soil microorganisms and communities [21]. When organic fertilizer replaces N fertilizer, it can reduce the N leaching from chemical fertilizers and the emission of greenhouse gases such as N_2O [22], thus achieving the goal of reducing environmental pollution [23]. When organic fertilizer is combined with N fertilizer, it reduces the loss of quick-acting nutrients in chemical fertilizers and helps to increase the utilization rate of N fertilizer [24]. In addition, the Qinghai-Tibet Plateau is one of the main animal husbandry areas in China, so a large amount of organic waste is generated every year. Therefore, using organic fertilizers to replace N fertilizer in the wolfberry planting area is expected to contribute to the effective utilization of animal manure as well as environment protection in the Qinghai-Tibet Plateau.

Research on replacing N fertilizers with organic fertilizers is currently focusing on major crops such as grains, vegetables and fruits [25]. Regarding food crops, the researches primarily focus on wheat, rice and corn. Previous findings have shown that replacing N fertilizer with organic fertilizer increased wheat yield 18% by average [26], increased rice yield, its economic benefits and improved soil fertility [27]. In terms of vegetables, studies have mainly included cabbage, peppers and tomatoes. Meta-analysis results show that when the N substitution rate is less than or equal to 70%, the vegetable yield can be increased, whereas when it is higher than 70%, the yield is significantly reduced [28]. Additionally, in the process of vegetable planting, using organic fertilizers instead of N fertilizers can significantly reduce greenhouse gas emissions such as N_2O [29]. Regarding fruits, apples, kiwis and grapes are often considered research species by scholars. When organic fertilizer is used to replace N fertilizer, the soluble solid content of apples is increased, the titratable acid content is reduced [30], and the quality of grapes and wine is also improved [31]. However, few studies have focused on crops that have both food and medicinal value such as wolfberry, and few studies have investigated functional properties such as the antioxidant activities of wolfberry.

Based on the crucial need for improved nutrient management of wolfberry in the Qaidam Basin and the pressing environmental protection requirement in the Qinghai-Tibet Plateau, a trial was conducted to assess the impact of organic fertilizer instead of N fertilizer in both high- and low-fertility fields. The objectives of this trial were to: (1) determine the appropriate rate of organic fertilizer substitution for N fertilizer in wolfberry cultivation; (2) evaluate this substitution on yield, quality and antioxidant activity of wolfberry in Qinghai-Tibet Plateau.

2. Materials and Methods

2.1. Site Description

The experiment was conducted in Dulan County, Haixi Mongolian-Tibetan Autonomous Prefecture, Qinghai Province, spanning from 2019 to 2020. The area has a plateau continental climate with an altitude of 2,790 m, an average annual sunshine duration of 2,514.7 h, an annual precipitation of 56.4 mm and average annual temperature of 6.1 ℃. The field trial was established in both Nuomuhong Farm (36°44′ N, 96°42′ E) and the wolfberry base of Qinghai Kunlun River Wolfberry Co., Ltd. (36°41′ N, 96°48′ E). The basic chemical properties of the test soil are presented in Table 1. Among them, the soil fertility of Nuomuhong farm is relatively high (hereinafter referred to as the high-fertility field) and soil type is sandy loam classified as grey-brown desert soil according to the Chinese Soil Taxonomy [32]. Conversely, the soil fertility at Kunlun River Wolfberry Co., Ltd. is low (hereinafter referred to as the low-fertility field), and the soil texture is sandy.

2.2 Experimental Materials

The wolfberry variety was Ningqi No. 7. In the high-fertility fields, the wolfberry trees were 8 years old, and the cultivation method involved a row spacing of 2 m and a plant spacing of 1.5 m, with approximately 3,300 trees per hectare. The wolfberry trees in low-fertility fields were 4 years old, with a row spacing of 3 m and a plant spacing of 1 m, also resulting in approximately 3,300 trees per hectare.

The chemical fertilizers used in the experiment were urea (46% N) and superphosphate (46% P_2O_5), and the organic fertilizer was a commercial product, containing 45% organic matter, 3.38% nitrogen, 0.34% phosphorus, and 1.33% potassium.

2.3 Experimental Design

The same experimental program was set up in both high-fertility and low-fertility fields, comprising 6 treatments, including 5 gradients of organic fertilizer replacing nitrogen fertilizer and a control with full application of N fertilizer (Table 2). Each treatment was replicated 3 times, with 20-30 wolfberry trees in each replicate.

Table 1 Basic soil properties in the experiments.

Test site	Soil depth (cm)	Organic matter (g/kg)	Total nitrogen (g/kg)	Total phosphorus (g/kg)	Mineral nitrogen (mg/kg)	Available phosphorus (mg/kg)	Available potassium (mg/kg)	pH
High fertility	0-20	17.60	1.09	1.24	228.47	95.84	255.92	7.84
	20-40	12.82	0.78	0.67	178.87	16.38	154.72	8.04
Low fertility	0-20	3.50	0.10	0.87	1.43	51.30	78.93	8.73
	20-40	2.37	0.05	0.45	0.83	21.92	64.72	8.77

Table 2 Experimental fertilization design plan.

Treatment	Organic fertilizer substitution rate	Nitrogen fertilizer application rate (g N /plant)	Organic fertilizer application rate (kg/plant)	Phosphorus application rate (g P_2O_5/plant)	
				High fertility	Low fertility
N250M0 (control)	0%	250	0	100	200
N200M2	20%	200	2	100	200
N150M4	40%	150	4	100	200
N100M6	60%	100	6	100	200
N50M8	80%	50	8	100	200
N0M10	100%	0	10	100	200

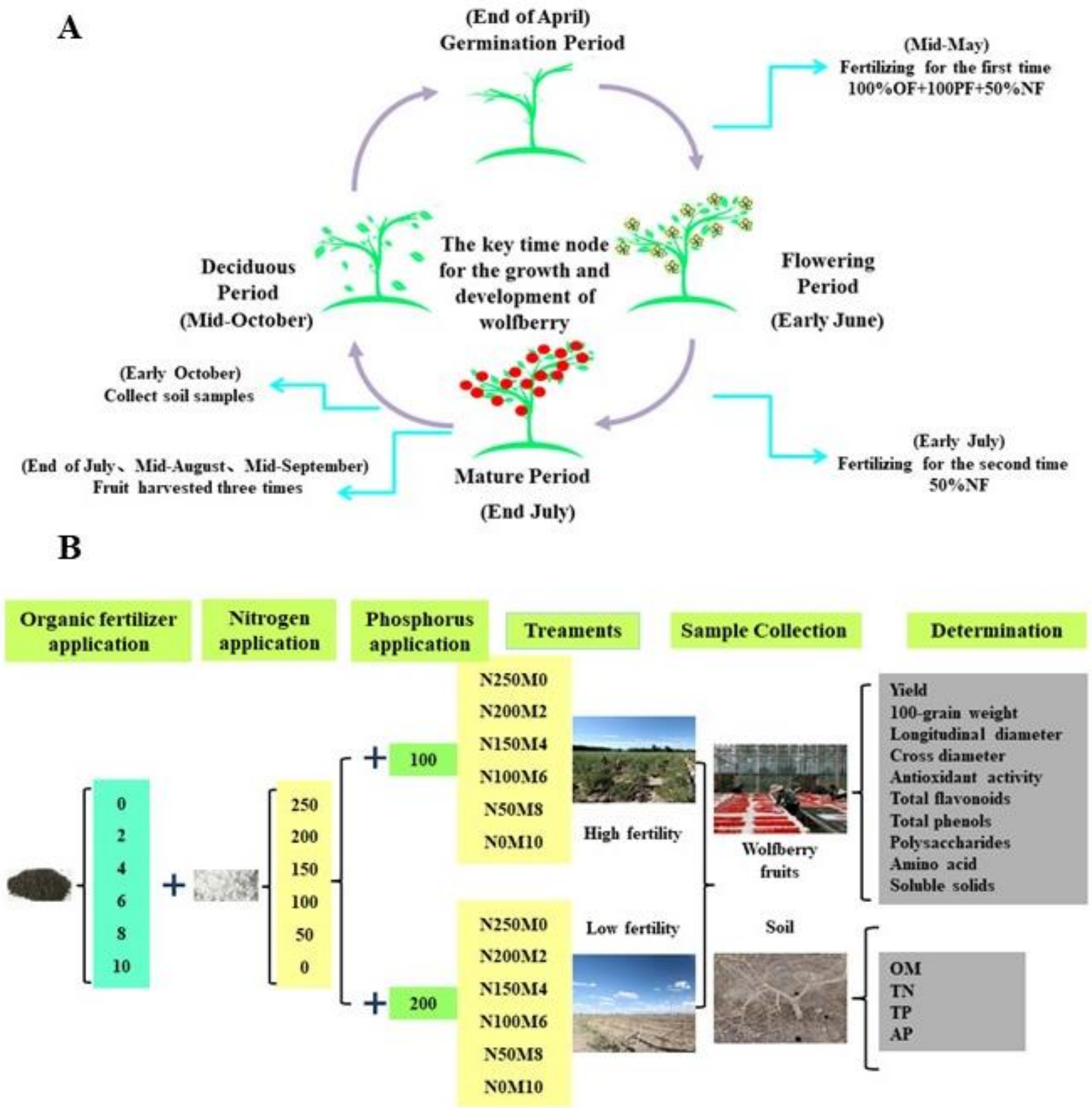

Fig. 1 The main phenological period of wolfberry and field management measures.

All organic fertilizer, all phosphate fertilizer, and 50% of the N fertilizer were applied as a base fertilizer in one application in mid-May while the remaining 50% of N fertilizer was applied as a top-dressing fertilizer in early July (Fig 1A). All the fertilizer was embedded into the ditches located on both sides of the wolfberry trees. Theses ditches were 20-30 cm deep, 20-30 cm wide, and situated 30-40 cm away from the tree trunk.

After harvesting the wolfberry fruit, an edible lye with a concentration of 5% (edible alkali:water = 1:20) was used to remove the waxy layer on the fruit's surface. Then the fruit spreads on a screen to dry naturally in the greenhouse (Fig. 1B). The dried fruits were washed with deionized water, placed in a blast drying box, dried to constant weight at 40 ℃, and crushed into powder using a high-throughput tissue grinder for fruit quality analysis.

In high-fertility fields, soils samples were collected from 0-300 cm depths using an earth boring auger at intervals of 20 cm. The soil nitrate content and water content were determined for these samples. Soil samples from 0-40 cm depths were also collected at intervals of 20 cm to determine the nutrient content (Fig. 1B). In low-fertility fields, soil samples from 0-100 cm were taken depths at intervals of 20 cm by digging a 1 m deep soil profile. The soil nitrate content and water content were determined for these samples as well. Soil samples from 0-40 cm depths were taken at intervals of 20 cm to determine the nutrient content (Fig. 1B).

2.4 Measurement and Methods

2.4.1 Yield and Appearance of Wolfberry

Fruits from all trees (in the high-fertility field) or 6 trees (in the low-fertility field) in each plot were picked and weighed. The yield per unit area was calculated after the fruit samples were dried, and 300 fresh fruits from each plot were weighed to determine the weight of hundred grains. Twenty fresh fruits from each plot were used to measure the horizontal and vertical diameters of the wolfberry fruits, and the fruit shape index was calculated as vertical diameter divided by the horizontal diameter. In the high-fertility field, fruits were harvested at the end of July, mid-August, and mid-September, while in the low-fertility field, fruits were harvested at the end of July and mid-August.

2.4.2 Determination of Intrinsic Quality of Wolfberry Fruit

The quality indicators of wolfberry fruit were measured as described by other researchers (Table 3).

Table 3 Determination indicators and methods of fruit quality.

Quality	Method
Flavonoid content	Peinado [33]
Polysaccharide content	Kushwaha [34]
Amino acid content	Wang [35]
Soluble solids	Zhang [36]
Total phenols	Khan [37]
DPPH scavenging effect	Yang [38]
ABTS scavenging effect	Apak [39]
Cupric reducing antioxidant capacity	Choi [40]
Potassium ferricyanide reducing antioxidant capacity	Cheng [41]

2.4.3 Determination of Mineral Elements in Wolfberry Fruit

The content of mineral elements in the fruit was determined using various methods: the Kjeldahl method for nitrogen, the vanadium molybdenum yellow colorimetric method for phosphorus, and the flame photometric method for potassium after H_2SO_4-H_2O_2 digestion. The contents of iron, manganese, copper and zinc in the fruit were measured using the atomic absorption method after HNO_3-$HClO_4$ digestion [42].

2.4.4 Determination of Soil Nutrients

The soil organic matter content was measured using the potassium dichromate external heating method. The soil total N content was measured using the H_2SO_4 digestion-Kjeldahl method. The soil total phosphorus content was measured using the H_2SO_4-$HClO_4$ digestion-molybdenum antimony colorimetric method. Soil available phosphorus was measured using the Olsen method. Soil mineral N was measured using the KCl extraction-flow analyser method [42].

The amount of residual nitrate in the soil profile was calculated according to the following formula: $RN = C_N \times d \times \rho \times 10^{-1}$, where C_N 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.

2.5 Data Processing and Analysis

SPSS 22.0 was used for statistical analysis of the experimental data, and the LSD method was applied for multiple comparisons of variance analysis ($p < 0.05$). Graphing was performed using Origin 8. Redundancy analysis (RDA) was conducted and visualized using the R language. Among the data analysed, the fruit quality metrics were obtained from fruit measurements in 2020, specifically the fruit quality data used in the RDA pertain to the second crop of fruit, and the amino acid data were also derived from the second crop of fruit.

3. Results

3.1 Fruit Yield and Appearance

A total of three crops of wolfberry were harvested in the high-fertility field, with the second crop having the highest yield and the first crop the lowest. The results from both 2019 and 2020 showed that treatments with organic fertilizers replacing N fertilizers yielded more fruits than treatments without organic fertilizer (N250M0) or nitrogen (N0M10). When organic fertilizer replaced 80% of the N fertilizer (N50M8), the total fruits output was the highest (Table 3).

Two crops of wolfberry were harvested in low-fertility fields. Data from both years indicated that treatments without any organic fertilizer (N250M0) and treatments with organic fertilizer replacing part of the N fertilizer yielded more fruits than treatments without any N (N0M10) (Table 4).

In the high-fertility field, the 100-fruit weight of wolfberry was inconsistent among different crops in both years, while in the low-fertility field, the 100-fruit weight of wolfberry decreased with the increase in organic fertilizer. The control (N250M0) had a significantly higher 100-fruit weight for the first crop than N0M10 in both years (Table 5).

In the high-fertility field, the longitudinal fruit diameter of treatments with organic fertilizer substituted for N fertilizer was higher than the control sample (N250M0) in 2020, while in 2019 there was no significant difference between the control sample and the other treatments (Table 1). The substitution of organic fertilizers for N fertilizers did not significantly alter the horizontal diameter of fresh wolfberry (Table 2). N200M2 had the highest fruit shape index among the three crops in both years. In 2020, treatments with organic fertilizer replacing nitrogen fertilizer had higher fruit shape index than that of the treatment without organic fertilizers (N250M0) (Table 3).

Table 4 The effect of different fertilization treatments on dry fruit yield.

Year	Treatment	High fertility				Low fertility		
		First crop (kg/ha)	Second crop (kg/ha)	Third crop (kg/ha)	Total yield (kg/ha)	First crop (kg/ha)	Second crop (kg/ha)	Total yield (kg/ha)
Y-2019	N250M0	269 ± 61^{c}	2,201 ± 317^{b}	1,541 ± 226^{b}	4,011 ± 588^{b}	331 ± 29^{ab}	694 ± 58^{a}	1,024 ± 29^{a}
	N200M2	413 ± 49^{ab}	2,694 ± 285^{ab}	1,743 ± 148^{ab}	4,850 ± 266^{ab}	264 ± 36^{ab}	604 ± 200^{a}	868 ± 204^{a}
	N150M4	342 ± 90^{bc}	2,786 ± 424^{ab}	1,953 ± 110^{ab}	5,082 ± 575^{a}	406 ± 153^{a}	623 ± 238^{a}	1,030 ± 390^{a}
	N100M6	384 ± 44^{ab}	2,726 ± 405^{ab}	1,842 ± 40^{ab}	4,952 ± 449^{ab}	292 ± 64^{ab}	521 ± 72^{ab}	814 ± 113^{ab}
	N50M8	455 ± 54^{a}	2,912 ± 545^{a}	1,974 ± 452^{a}	5,341 ± 886^{a}	338 ± 110^{ab}	593 ± 125^{a}	931 ± 230^{a}
	N0M10	403 ± 30^{ab}	2,684 ± 318^{ab}	1,807 ± 194^{ab}	4,893 ± 521^{ab}	234 ± 60^{b}	434 ± 86^{b}	667 ± 146^{b}
Y-2020	N250M0	660 ± 58^{b}	3,345 ± 64^{ab}	2,016 ± 414^{a}	6,021 ± 476^{ab}	444 ± 112^{ab}	506 ± 160^{ab}	950 ± 271^{abc}
	N200M2	874 ± 43^{a}	3,116 ± 295^{b}	2,070 ± 111^{a}	6,060 ± 225^{ab}	380 ± 48^{b}	402 ± 47^{bc}	782 ± 95^{bc}
	N150M4	903 ± 61^{a}	3,503 ± 239^{ab}	2,219 ± 195^{a}	6,625 ± 117^{ab}	531 ± 94^{ab}	409 ± 20^{bc}	939 ± 115^{abc}
	N100M6	839 ± 133^{a}	3,400 ± 384^{ab}	2,005 ± 186^{a}	6,244 ± 559^{ab}	573 ± 83^{a}	463 ± 81^{ab}	1,036 ± 164^{ab}
	N50M8	826 ± 155^{ab}	3,868 ± 341^{a}	2,150 ± 190^{a}	6,844 ± 91^{a}	543 ± 49^{ab}	570 ± 62^{a}	1,113 ± 111^{a}
	N0M10	829 ± 88^{ab}	3,198 ± 417^{b}	1,936 ± 328^{a}	5,963 ± 820^{b}	381 ± 163^{b}	303 ± 73^{c}	684 ± 236^{c}

Different lowercase letters in the same column indicate that the difference between different treatments reached a significant level ($p < 0.05$). The same applies below.

Table 5 The effect of different fertilization treatments on 100-fruit weight.

Year	Treatment	High fertility			Low fertility	
		First crop	Second crop	Third crop	First crop	Second crop
Y-2019	N250M0	98.39 ±8.76^{a}	95.16 ±9.30^{a}	79.82 ±4.47^{a}	76.93 ±2.37^{a}	61.69 ±12.94^{a}
	N200M2	101.27 ±4.46^{a}	101.68 ±3.81^{a}	75.55 ±1.34^{ab}	70.90 ±0.55^{ab}	57.42 ±2.61^{a}
	N150M4	94.61 ±2.94^{a}	94.11 ±3.86^{a}	75.25 ±2.74^{ab}	66.03 ±0.92^{b}	56.04 ±6.24^{a}
	N100M6	98.94 ±4.93^{a}	99.00 ±2.77^{a}	73.89 ±8.04^{ab}	72.87 ±3.07^{ab}	54.22 ±4.43^{a}
	N50M8	100.04 ±6.50^{a}	102.43 ±4.24^{a}	70.33 ±2.66^{b}	72.06 ±0.82^{ab}	54.23 ±7.01^{a}
	N0M10	97.51 ±2.02^{a}	101.69 ±5.36^{a}	69.68 ±2.85^{b}	67.57 ±3.81^{b}	51.98 ±5.72^{a}
Y-2020	N250M0	99.61 ±10.45^{b}	75.84 ±4.35^{b}	65.66 ±6.99^{a}	93.51 ±4.18^{a}	56.25 ±4.34^{a}
	N200M2	106.88 ±3.93^{ab}	85.27 ±6.90^{a}	64.34 ±4.29^{a}	92.37 ±1.72^{ab}	55.94 ±4.50^{a}
	N150M4	105.79 ±4.53^{ab}	83.00 ±1.89^{ab}	65.10 ±1.20^{a}	81.41 ±3.28^{c}	52.71 ±4.11^{a}
	N100M6	111.65 ±9.28^{a}	87.33 ±8.52^{a}	67.49 ±2.88^{a}	83.49 ±5.18^{bc}	54.34 ±3.95^{a}
	N50M8	110.71 ±5.43^{a}	87.72 ±4.72^{a}	64.26 ±1.78^{a}	82.24 ±7.62^{c}	52.63 ±1.63^{a}
	N0M10	111.45 ±5.49^{a}	80.00 ±3.10^{ab}	65.92 ±2.19^{a}	78.29 ±5.89^{c}	50.45 ±5.72^{a}

Generally, in the low-fertility field, substitution of organic fertilizer for N fertilizers did not lead to significant variations in the longitudinal diameter, horizontal diameter or fruit shape index of fresh wolfberry fruits. However, there were a few exceptions. Specifically, in 2019, the horizontal diameter of treatments with organic fertilizer substitution for N fertilizers was smaller than that of the control (N250M0). Additionally, for the first crop in 2019, the fruit shape index of treatments with organic fertilizer substitution of N fertilizers was more favourable compared to the control (N250M0) (Tables 1-3).

3.2 Intrinsic Quality of Wolfberry Fruit

In high-fertility fields, the substitution of organic fertilizer for N fertilizer did not significantly change the total phenol content of wolfberry fruit (Fig. 2A). However, it significantly reduced the flavonoid content (Fig. 2B). In general, the polysaccharide content, soluble solids content, ABTS scavenging power, and copper ion reducing power remained unaffected (Fig. 2C, 2D, 2F and 2G). The total amino acid content, essential amino acid content, semi-essential amino acid content and nonessential amino acid content were not significantly different among all treatments (Figs. 2I, 2J, 2K and 2L). Compared to the control (N250M0), the DPPH scavenging capacity of fruits decreased due to the replacement of N fertilizers with organic fertilizers (Fig. 2E).

In low-fertility fields, the substitution of organic fertilizer for N fertilizer did not affect the total phenol content of fruits in the first crop but reduced it in the second crop (Fig. 3A). There was a tendency for higher flavonoid content in fruits when organic fertilizer replaced N fertilizer compared to the control (N250M0) (Fig. 3B). Similarly, an increasing trend was observed in the polysaccharide content and soluble solid content of fruits when some of the N fertilizer was replaced by organic fertilizer, compared to the control (N250M0) (Figs. 3C and 3D). The DPPH scavenging ability of fruits did not change significantly due to the organic fertilizer replacement of N fertilizer (Fig. 3E). When a part of the N fertilizer was substituted by organic fertilizer, the ABTS scavenging power, copper ion reducing power and reducing power of potassium ferricyanide in fruits did not vary significantly. However, a large substitution of N fertilizer with organic fertilizer tended to reduce these parameters compared to the control (N250M0) (Figs. 3F, 3G and 3H). Similar results were also observed for the total amino acid, non-essential amino acid, essential amino acid content, and semi-essential amino acid content of fruits (Figs. 3I, 3J, 3K and 3L).

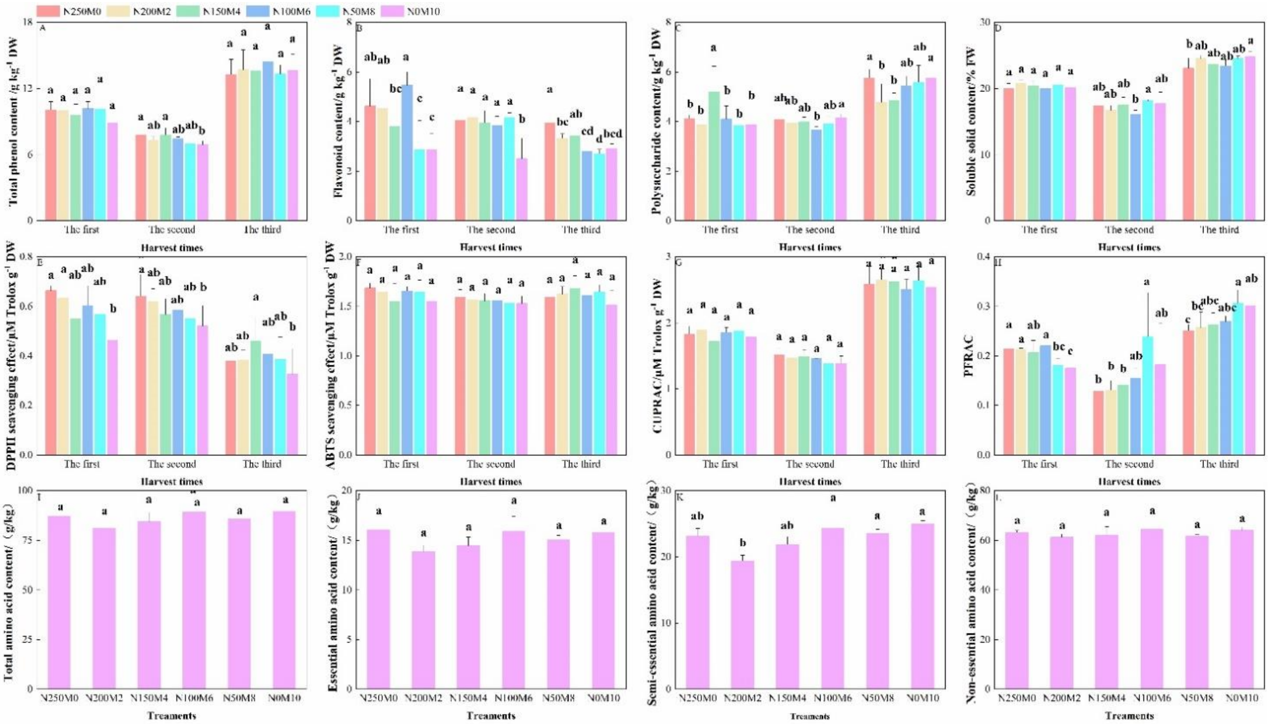

Fig. 2 The effect of different fertilization treatments on fruit quality in a high-fertility field.

A represents the total phenol content, B represents the flavonoid content, C is the polysaccharide content, D is the soluble solid content, E is the DPPH free radical scavenging power, F is the ABTS free radical scavenging power, G is the copper ion reducing power, H is the reducing power of potassium ferricyanide, I is the total amino acid content, J is the essential amino acid content, K is the semi-essential amino acid content, L is the nonessential amino acid content. Different lowercase letters indicate significant differences between treatments ($p < 0.05$). The same applies to subsequent figures.

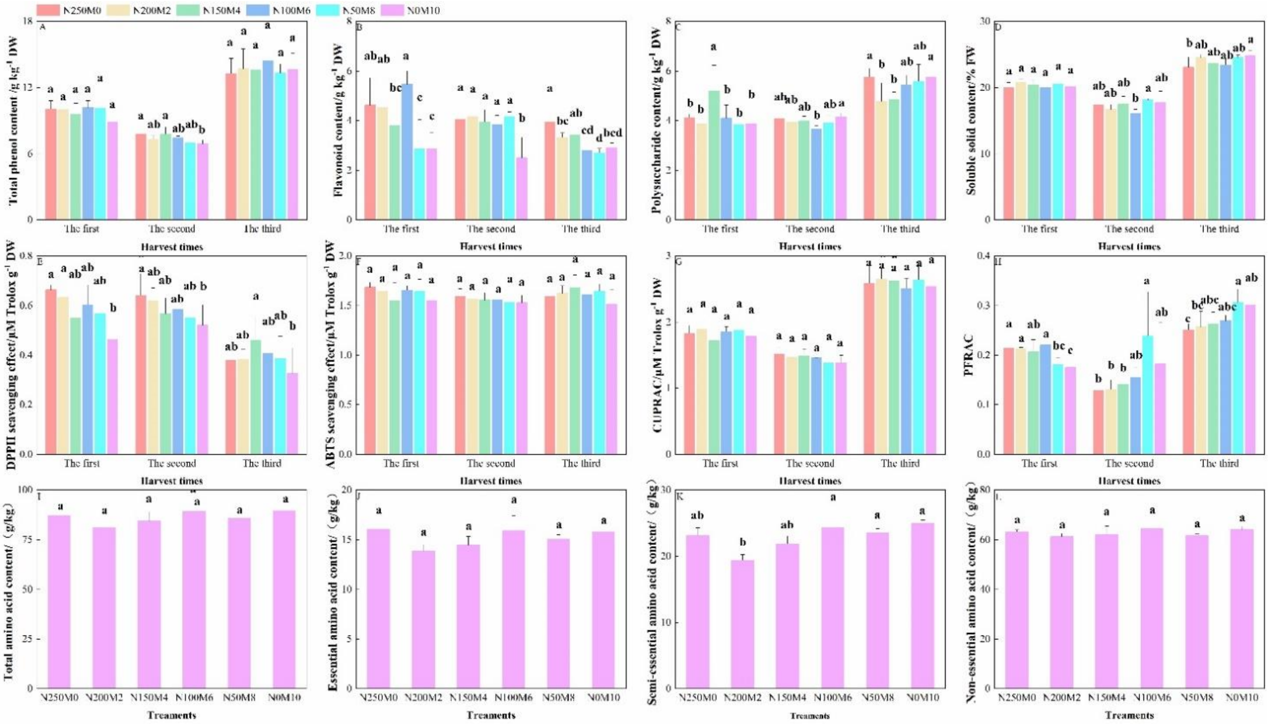

Fig. 3 The effect of different fertilization treatments on fruit quality in a low-fertility field.

3.3 Mineral Element Content in Fruit

In the high-fertility field, the fruit and phosphorus (P) contents of N200M2 treatment were higher than those of the other treatments in the first crop, while there was no significant difference between treatments in the second and third crops. The fruit potassium content of the N200M2 and N150M4 treatments was higher than that of the other treatments in the first and third crops. The fruit iron and manganese contents of the N150M4 treatment were higher than those of the other treatments in the first crops, while there was no significant difference among treatments in the second and third crops. The fruit copper content of the N100M6 treatment was higher than that of the other treatments in the first crops. However, the fruit copper content of the N0M10 treatment was lower than that of the other treatments in the second and third crops. The fruit zinc content in the N100M6 and N50M8 treatments was higher than that in the other treatments (Table 6).

In low-fertility fields, the replacement of N fertilizer with organic fertilizer decreased fruit N and potassium content while not affecting fruit P content. Specifically, the N0M10 treatment had the lowest N content and potassium content among all treatments. The fruit iron content of the N200M2 and N150M4 treatments was higher than that of the other treatments in the first crops. The fruit manganese content of the N150M4 treatment was higher than that of the other treatments in the first crop. For both iron and manganese content, no significant difference was found among treatments in the second crop. The replacement of N fertilizer with organic fertilizer significantly improved the fruit copper content. Furthermore, the fruit zinc content in the first crops also increased due to the organic fertilizer replacement for N fertilizer (Table 6).

Table 6 The effect of different fertilization treatments on the fruit contents of mineral elements.

Nutrient content			N250M0	N200M2	N150M4	N100M6	N50M8	N0M10
High fertility	N (g/kg)	First crop	18.50 ± 0.87^{b}	20.38 ± 1.85^{a}	19.00 ± 0.15^{ab}	18.67 ± 0.40^{b}	17.76 ± 0.33^{b}	18.51 ± 0.91^{b}
		Second crop	17.62 ± 1.10^{a}	16.32 ± 0.29^{a}	16.83 ± 1.17^{a}	16.78 ± 0.81^{a}	17.23 ± 1.19^{a}	16.08 ± 0.82^{a}
		Third crop	17.51 ± 0.46^{a}	17.37 ± 0.80^{a}	17.62 ± 1.05^{a}	17.34 ± 0.32^{a}	17.53 ± 0.24^{a}	17.25 ± 0.36^{a}
	P (g/kg)	First crop	2.41 ± 0.26^{ab}	2.48 ± 0.30^{a}	2.14 ± 0.09^{b}	2.22 ± 0.07^{ab}	2.12 ± 0.08^{b}	2.16 ± 0.12^{ab}
		Second crop	2.23 ± 0.11^{a}	2.12 ± 0.14^{a}	2.09 ± 0.20^{a}	2.09 ± 0.21^{a}	2.16 ± 0.13^{a}	2.00 ± 0.05^{a}
		Third crop	2.15 ± 0.05^{a}	2.10 ± 0.02^{a}	2.06 ± 0.12^{a}	2.13 ± 0.08^{a}	2.02 ± 0.02^{a}	2.04 ± 0.08^{a}
	K (g/kg)	First crop	16.09 ± 0.59^{b}	18.34 ± 1.64^{a}	16.51 ± 0.78^{b}	16.60 ± 0.55^{b}	15.54 ± 0.47^{b}	15.84 ± 0.56^{b}
		Second crop	16.97 ± 0.96^{a}	16.39 ± 0.36^{a}	16.96 ± 1.84^{a}	17.73 ± 0.98^{a}	17.38 ± 0.77^{a}	16.37 ± 0.77^{a}
		Third crop	16.97 ± 0.72^{ab}	16.68 ± 0.20^{ab}	17.53 ± 0.83^{a}	16.88 ± 1.44^{b}	15.74 ± 0.10^{bc}	15.09 ± 0.75^{c}
	Fe (mg/kg)	First crop	47.87 ± 15.16^{b}	39.65 ± 5.34^{b}	65.18 ± 7.32^{a}	39.84 ± 3.78^{b}	40.83 ± 2.01^{b}	37.50 ± 0.33^{b}
		Second crop	52.34 ± 5.92^{a}	46.09 ± 0.64^{a}	53.78 ± 25.45^{a}	36.41 ± 2.79^{a}	40.93 ± 4.32^{a}	39.01 ± 4.86^{a}
		Third crop	40.33 ± 13.42^{a}	32.12 ± 2.99^{a}	32.95 ± 3.26^{a}	31.25 ± 1.72^{a}	33.35 ± 0.94^{a}	31.33 ± 3.51^{a}
	Mn (mg/kg)	First crop	7.48 ± 1.44^{ab}	7.41 ± 0.44^{ab}	8.13 ± 0.68^{a}	8.05 ± 0.76^{a}	6.85 ± 0.33^{ab}	6.14 ± 0.34^{b}
		Second crop	10.22 ± 1.76^{a}	9.78 ± 0.34^{a}	8.86 ± 0.37^{a}	8.84 ± 0.66^{a}	8.92 ± 0.75^{a}	9.42 ± 0.83^{a}
		Third crop	7.74 ± 1.16^{a}	7.26 ± 0.74^{a}	7.47 ± 0.84^{a}	7.67 ± 0.32^{a}	7.88 ± 0.26^{a}	7.75 ± 0.88^{a}
	Cu (mg/kg)	First crop	7.70 ± 0.62^{b}	8.35 ± 0.44^{b}	8.58 ± 0.83^{b}	9.96 ± 0.26^{a}	8.01 ± 0.25^{b}	8.19 ± 0.28^{b}
		Second crop	7.82 ± 1.16^{a}	7.50 ± 0.43^{abc}	6.67 ± 0.44^{bc}	7.70 ± 0.48^{ab}	6.83 ± 0.42^{abc}	6.48 ± 0.54^{c}
		Third crop	7.78 ± 0.59^{a}	6.98 ± 0.20^{a}	6.65 ± 0.42^{ab}	7.58 ± 1.41^{a}	6.97 ± 0.72^{a}	5.63 ± 0.43^{b}
	Zn (mg/kg)	First crop	13.08 ± 3.95^{b}	12.54 ± 0.39^{b}	15.38 ± 1.75^{ab}	17.85 ± 1.85^{a}	15.73 ± 2.21^{ab}	16.46 ± 1.88^{ab}
		Second crop	16.86 ± 3.69^{a}	17.60 ± 1.64^{a}	13.70 ± 1.16^{a}	17.79 ± 2.26^{a}	16.25 ± 2.91^{a}	14.97 ± 2.43^{a}
		Third crop	8.54 ± 0.70^{b}	8.75 ± 1.97^{b}	10.22 ± 1.40^{ab}	10.81 ± 1.11^{ab}	12.40 ± 1.61^{a}	10.08 ± 2.46^{ab}

Table 6 to be contninued

Low fertility	N (g/kg)	First crop	19.17 ±0.71^{a}	18.60 ±2.05^{a}	18.75 ±1.42^{a}	18.21 ±0.58^{a}	16.88 ±1.03^{ab}	15.80 ±1.36^{b}
		Second crop	20.75 ±1.72^{ab}	21.13 ±2.33^{a}	20.12 ±1.65^{ab}	19.30 ±1.57^{ab}	18.16 ±0.65^{bc}	17.23 ±0.82^{c}
	P (g/kg)	First crop	2.90 ±0.13^{a}	2.98 ±0.06^{a}	2.95 ±0.10^{a}	2.87 ±0.14^{a}	2.84 ±0.07^{a}	2.85 ±0.05^{a}
		Second crop	2.89 ±0.10^{a}	3.12 ±0.02^{a}	2.99 ±0.18^{a}	2.92 ±0.03^{a}	2.86 ±0.17^{a}	2.89 ±0.26^{a}
	K (g/kg)	First crop	18.76 ±1.02^{a}	18.54 ±0.22^{a}	18.85 ±0.69^{a}	18.50 ±0.17^{a}	17.77 ±1.14^{ab}	16.93 ±1.15^{b}
		Second crop	19.03 ±0.68^{ab}	19.65 ±0.39^{a}	19.43 ±0.27^{ab}	18.57 ±0.49^{bc}	17.78 ±0.28^{c}	17.91 ±0.68^{c}
	Fe (mg/kg)	First crop	57.74 ±5.84^{b}	65.32 ±1.88^{a}	68.93 ±0.33^{a}	59.10 ±4.90^{b}	57.74 ±0.47^{b}	57.61 ±2.00^{b}
		Second crop	69.32 ±14.88^{a}	60.18 ±0.67^{a}	67.83 ±2.92^{a}	60.61 ±5.48^{a}	63.18 ±2.42^{a}	65.16 ±6.11^{a}
	Mn (mg/kg)	First crop	10.69 ±0.64^{ab}	11.05 ±0.39^{ab}	11.58 ±0.80^{a}	10.59 ±0.94^{ab}	10.17 ±0.82^{b}	10.51 ±0.41^{ab}
		Second crop	9.99 ±0.66^{a}	11.39 ±0.69^{a}	11.30 ±0.54^{a}	10.89 ±1.71^{a}	10.69 ±0.78^{a}	9.73 ±0.82^{a}
	Cu (mg/kg)	First crop	5.75 ±0.62^{b}	6.09 ±0.54^{ab}	6.70 ±0.53^{a}	6.35 ±0.23^{ab}	6.95 ±0.62^{a}	6.08 ±0.52^{ab}
		Second crop	4.76 ±0.61^{c}	5.42 ±0.61^{bc}	6.68 ±0.80^{a}	5.97 ±0.51^{ab}	6.35 ±0.61^{ab}	6.15 ±0.69^{ab}
	Zn (mg/kg)	First crop	13.80 ±1.47^{b}	16.48 ±0.61^{a}	15.69 ±0.68^{ab}	14.92 ±2.08^{ab}	15.52 ±1.67^{ab}	16.32 ±0.60^{a}
		Second crop	17.82 ±5.62^{ab}	14.07 ±1.82^{b}	14.59 ±1.33^{ab}	14.83 ±2.23^{ab}	19.92 ±3.98^{a}	19.26 ±0.73^{ab}

Different lowercase letters in the same peer indicate that the difference between different treatments has reached a significant level ($p < 0.05$).

3.3 Soil Nutrient Content

In high-fertility fields, soil organic matter (SOM), total nitrogen content (TN), and available phosphorus (AP) contents increased when part or all N fertilizer was replaced by organic fertilizer (Figs. 4A, 4B and 4D). The N150M4, N100M6 and N0M10 treatments had significantly higher SOM contents in the 0-20 cm layer, while the N50M8 and N0M10 treatments had significantly higher SOM contents in the 20-40 cm layer. The N100M6 and N0M10 treatments exhibited significantly higher soil TN contents in the 0-20 cm layer, whereas the N50M8 and N0M10 treatments had significantly higher soil TN contents in the 20-40 cm layer. The N150M4 treatment showed a significantly higher soil AP content in the 0-20 cm layer, and both the N150M4 and N0M10 treatments had significantly higher soil AP contents in the 20-40 cm layer. There were no significant differences in the soil total phosphorus (TP) content in the 0-20 cm layer or in the ratio of carbon (C) to N in the 0-20 cm and 20-40 cm layers among all treatments (Figs. 4C and 4E). Residual nitrate in the soil profile after fruit harvest was reduced due to the replacement of N fertilizer with organic fertilizer. The N150M4, N100M6, N50M8 and N0M10 treatments had significantly lower residual nitrate storage in the 0-300 cm soil profile compared to the control (N250M0). The N100M6 treatment exhibited the lowest nitrate reserves at the 0-100 cm, 100-200 cm and 0-300 cm soil depths (Fig. 4F).

In low-fertility fields, an increasing trend of SOM, TN and TP content was observed in the 0-20 cm and 20-40 cm soil depths when organic fertilizer was substituted for more N fertilizer (Fig. 5A, 5B, and 5C). The AP content in the 0-20 cm and 20-40 cm soil depths increased significantly due to the replacement of N fertilizer with organic fertilizer. Compared to the control (N250M0), all treatments that substituted organic fertilizer for N fertilizer had double or even triple the AP content in the 0-20 cm layer, and the N100M6, N50M8 and N0M10 treatments had almost double the AP content in the 20-40 cm layer (Fig. 5D). The ratio of soil carbon to N in the 0-20 cm and 20-40 cm layers remained unchanged due to the replacement of N fertilizer with organic fertilizer (Fig. 5E). Compared to the control (N250M0), treatment N0M10 had the similar residual nitrate storage in the 0-100 cm soil profile, while the other treatments had double or even six times the nitrate reserves after fruit harvest (Fig. 5F).

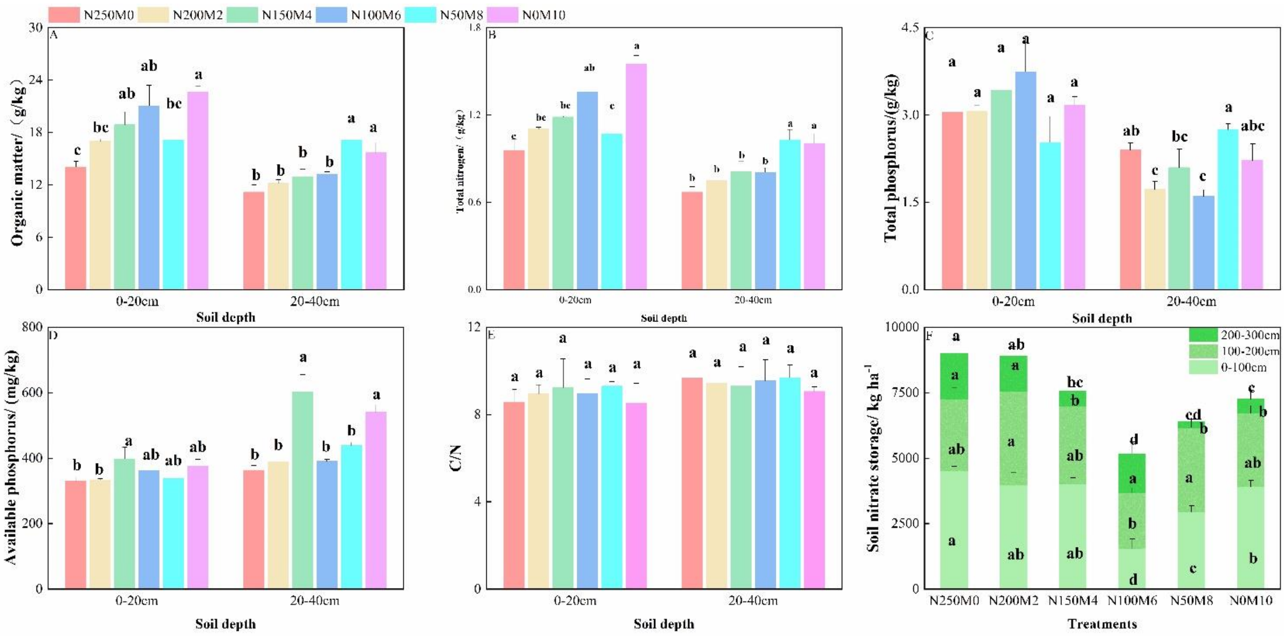

Fig. 4 Effects of different fertilization treatments on soil nutrient contents in a high-fertility field.

A is the organic matter content (OM), B is the total nitrogen content (TN), C is the total phosphorus content, D is the available phosphorus content, E is the carbon to nitrogen ratio, F is the 0-300 cm soil nitrate nitrogen storage. Different lowercase letters indicate significant differences between treatments ($p < 0.05$). The same applies to subsequent figures.

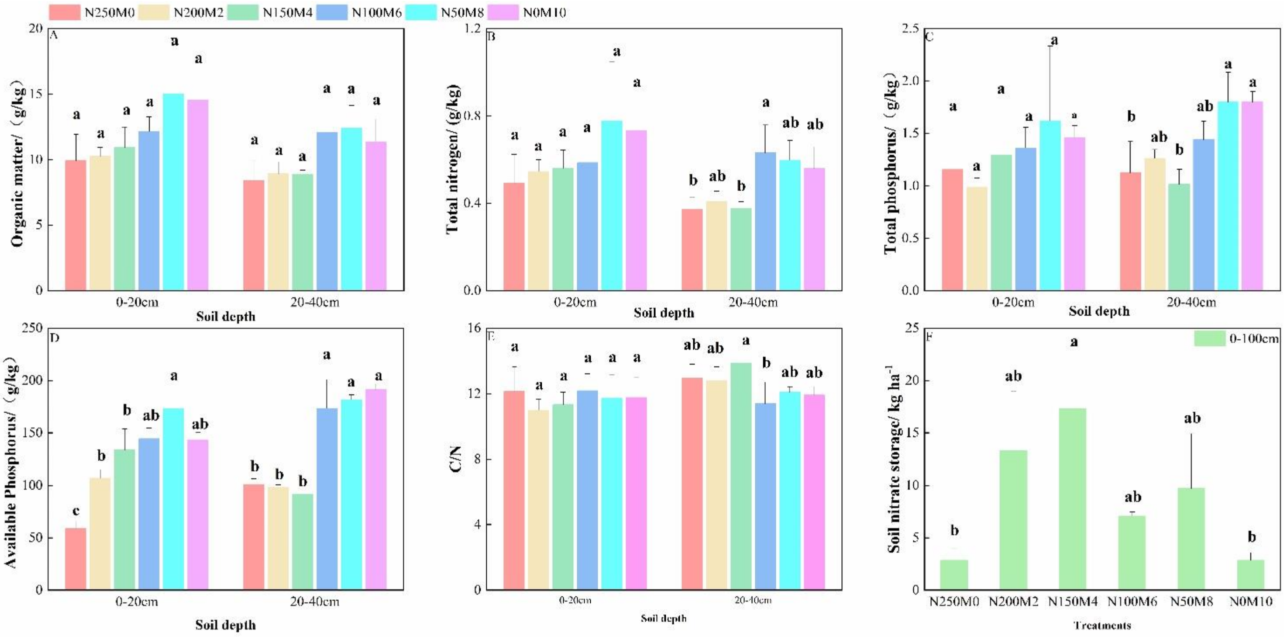

Fig. 5 Effects of different fertilization treatments on soil nutrient contents in low-fertility fields.

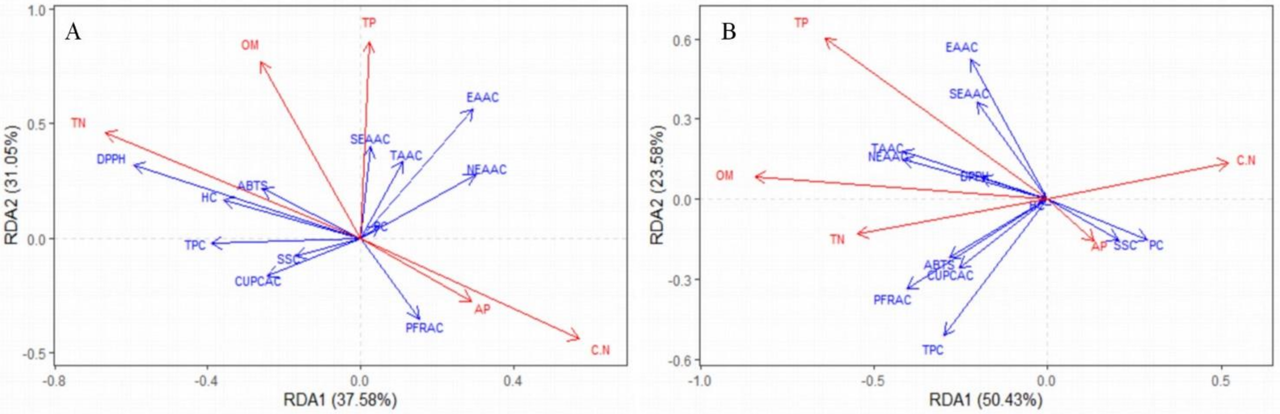

Fig. 6 Redundancy analysis (RDA) of the relationship between fruit quality and soil fertility.
A is the RDA of high-fertility soil; B is the RDA of low-fertility soil. OM is organic matter; TN is total nitrogen; TP is total phosphorus; AP is available phosphorus; C/N is carbon to nitrogen ratio; TPC is total phenols; HC is flavonoids; PC is polysaccharides; SSC is soluble solids; DPPH is DPPH scavenging power, ABTS is ABTS scavenging power; CUPRAC is copper ion reducing power; PFRAC is iron ion reducing power; TAAC is total amino acids; EAAC is essential amino acids; SEAAC is semi-essential amino acids; NEAAC is nonessential amino acids.

3.4 RDA of the Relationship between Fruit Quality and Soil Fertility

In the high-fertility field, the first two axes of RDA jointly explained 68.53% of the total variation in wolfberry fruit nutritional quality. Soil C/N, TP, OM and AP were positively correlated with amino acid content and PC content. Soil TP had a greater impact on the TAAC, SEAAC and EAAC contents, while C/N had a more significant impact on the NEAAC content. SOM and TN were positively correlated with antioxidant activity, HC, TPC and SSC content, whereas soil C/N and AP were negatively correlated with these indicators. Among them, soil TN exerted a more significant influence on antioxidant activity, HC, TPC and SSC content. This indicated that an increase in soil organic matter, total N and total P can improve wolfberry fruit quality and antioxidant activity (Fig. 6A).

In low-fertility fields, the first two axes of RDA jointly explained 74.01% of the total variation in wolfberry fruit nutritional quality. Soil TP and OM were positively correlated with the amino acid content of fruit. Soil TP had a larger effect on the contents of EAAC and SEAAC, while soil OM had a greater impact on the contents of TAAC and NEAAC. Soil OM and TN were positively correlated with the TPC content and antioxidant activity of the fruit, with soil OM having a greater impact. Soil C/N and AP were positively correlated with the PC and SSC contents of fruit, and C/N had a greater impact on them. This indicated that an increase in soil organic matter, total N, total phosphorus and C/N can enhance wolfberry fruit quality and antioxidant activity (Fig. 6B).

4. Discussion

4.1 Effects of Nitrogen Reduction Combined with Organic Fertilizer on Yield and Appearance

This study found parabolic relationships between wolfberry yield and organic fertilizer rate in treatments in high-fertility fields. Specifically, the N50M8 treatment had the highest yield, while treatment without any organic fertilizer and those without any N fertilizer produced fewer fruits (Table 4). These findings align with previous research [43, 44]. These results reveal the importance of applying organic fertilizer and combining it with N fertilizer. Organic fertilizer can significantly enhance the physical and chemical properties of the soil and increase the availability of soil nutrients [45]. Moreover, organic fertilizer not only contains a variety of nutrient elements essential for crop growth [46] providing long-term nourishment. When combined with chemical fertilizers, it can meet the nutrient needs of wolfberry throughout the growth and development stages. Liu et al. [47] discovered that during the rice planting period, applying organic fertilizer increases chlorophyll content, thus enhancing yield. However, relying solely on organic fertilizer may lead to yield reductions as a result of less available nutrients and slow-release nutrients, particularly in the cold Qinghai-Tibet Plateau region [48].

In the low-fertility field, treatments without any organic fertilizer (N250M0) and treatments where organic fertilizer replaced part of the N fertilizer yielded more fruits than treatments without any N (N0M10) (Table 4), implying that N fertilizer is essential for wolfberry growth in low-fertility soil and that organic fertilizer alone is insufficient to achieve a high yield of wolfberry fruits in such soil conditions.

4.2 Effects of Nitrogen Reduction Combined with Organic Fertilizer on the Internal Quality of the Fruit

Many studies have indeed revealed that the combined application of organic and chemical fertilizers maintains or improves crop yield and quality [43, 49]. For instance, Wang et al. [50] demonstrated that the combined application improved tomato’s Vc content, sugar-acid ratio, soluble solid content, and reduced fruit nitrate content. Additionally, meta-analysis results have shown that polyphenols and flavonoids in organic foods are beneficial for human health due to their strong antioxidant activity [51].

The findings of this experiment in both high-fertility and low-fertility fields also indicate that replacing N fertilizer with organic fertilizer can enhance the total phenol, flavonoid, and soluble solid contents in fruits. Similar results have been observed in calendula and black cumin where organic fertilizer substitution tests were conducted. The metabolic level of flavonoids in plants is mainly regulated by the content of soil organic carbon [52]. The results of the RDA in this experiment also indicate this phenomenon, which shows that to a certain extent, increasing the soil organic carbon content can promote the accumulation of flavonoids in plants. The application of organic fertilizer to improve the total phenol content in the fruit is similar to the results of the fertilizer experiments conducted by Tomo in apricot [53]. The main reason behind this might be that organic fertilizer increases the soil water-holding capacity, allowing more water to reach the leaves, thereby enhance the plant metabolic activity, promote the production of secondary metabolites, and ultimately leads to an increase in the fruit total phenol content [54]. Moreover, using organic fertilizer as a substitute for N fertilizer can increase the content of soluble solids in fruits. This is consistent with Stojanov’s [55] results in an organic fertilizer test on red raspberry, where fruit quality showed a decreasing trend with an increasing organic fertilizer replacement rate. The sugar accumulation in fruit is significantly influenced by organic fertilizer application, surpassing the impact of fertilization on the sugar content of the fruit themselves. Therefore, when organic fertilizer is applied, the polysaccharide content in the fruit is significantly increased.

The substitution of N fertilizers with organic fertilizers did not have significant effects on the content of various amino acids in fruits in high-fertility fields (Fig. 2), which is inconsistent with the results of Gurav on bananas [56]. Although the soil N significantly affects the amino acid content in fruits [57], the original TN content in the soil of high-fertility fields is higher. Even if the TN content of the soil is increased after applying organic fertilizer, the original nitrogen in the soil can meet the demand of the fruits, so it did not cause a significant change in the amino acid content in the fruits. The results of RDA can also explain this. There was a negative correlation between TN content in the soil and the amino acid content in the fruit, but the TP content in the soil was positively correlated with the amino acid content (Fig. 6A). This indicates that the TP content in the soil may be one of the factors affecting the changes in the amino acid content in fruits in the wolfberry fields with higher soil fertility. The substitution of organic fertilizer for nitrogen fertilizer increased the content of various amino acids in fruits on low-fertility fields (Fig. 3). The main reason may be that in low-fertility fields, the soil TN level is low. The application of organic fertilizers increased the soil total N content, which promotes an increase in amino acid content in fruits [57]. The results of RDA also support this view. In low-fertility fields, the soil organic matter and total nitrogen content are positively correlated with the amino acid content in the fruits.

The results regarding the mineral iron content in the fruit show that when organic fertilizer replaces nitrogen fertilizer, it can significantly increase the content of macronutrients such as nitrogen, phosphorus and potassium, as well as the content of trace elements like iron, manganese and zinc in the fruit (Table 6). This is similar to the findings of Lombardo et al. [58]. Applying organic fertilizer or planting cover crops can augment the mineral elements in fruits. The main reason for this phenomenon could be that organic fertilizer contains trace elements. These elements enter the soil with the application of organic fertilizer, thereby increasing trace elements content in the soil, and subsequently enhancing the content of trace elements in fruits [59]. On the other hand, organic fertilizer applied to the soil can activate the availability of soil nutrients, thus promoting the absorption of macronutrients and trace elements by plants, and consequently increasing the content of mineral irons in the fruit [60].

4.3 Effects of Nitrogen Reduction Combined with Organic Fertilizer on Antioxidant Activity

Oxidative stress, which is highly involved in numerous diseases, arises during cell metabolism and triggers the oxidation of multiple biomolecules, including DNA, lipids and proteins [61]. Existing research indicates that many diseases affect fruits or vegetables. Phenols are related to the antioxidant activity of fruits [62]. Consuming foods containing these substances is highly beneficial for human health. Studies conducted by Rostaei [63] and Fallah [64] on dill and soybean show that applying organic fertilizers can significantly improve the antioxidant activity of essential oils or fruits. The results of this experiment conducted on high-fertility and low-fertility fields are comparable. Substituting organic fertilizers for nitrogen fertilizers has a significant effect on the antioxidant activity of fruits. The main reason for this could be that the use of organic fertilizers increased the soil nutrient content, thereby promoting the levels of flavonoids and total phenols in the fruit [65]. The increase in phenols and flavonoids can stimulate an increase in the antioxidant activity of the fruit. Additionally, the results of RDA in this experiment showed that total nitrogen and organic matter contents in the soil positively correlate with antioxidant activity, and the content of total phenols and flavonoids is also positively correlated with antioxidant activity (Figs. 6A and 6B). Consequently, improving soil fertility under different soil conditions can promote an increase in the antioxidant activity of fruits.

5. Conclusions

In the wolfberry field with higher soil fertility, compared to control (N250M0), replacing part of the N fertilizer with organic fertilizer increased the yield, fruit shape index, flavonoids, total phenol content, and mineral nutrients in fruit while also improved soil fertility. In contrast, treatments with only chemical fertilizer or only organic fertilizer resulted in the lowest fruit yields. Additionally, the residual nitrate in the soil profile of 0-300 cm decreased due to the replacement of N fertilizer with organic fertilizer, suggesting that N reduction combined with organic fertilizer could mitigate the nitrate leaching risk. In the wolfberry field with lower soil fertility (Gobi Desert), treatments that utilized both chemical fertilizer and organic fertilizer resulted in higher yield, flavonoid, polysaccharide, soluble solid content and microelement content of fruits compared to control (N250M0). Furthermore, replacing part of nitrogen fertilizer with organic fertilizer improved soil fertility as well. In these treatments, more residual nitrate was found in the soil profile, indicating that N reduction combined with organic fertilizer could hold more nitrate within the soil 0-100 cm profile, preventing it from leaching into the deeper layer of the Gobi Desert. Therefore, in the Qinghai-Tibet Plateau, a new fertilization strategy should be implemented for wolfberry production. A decreased amount of N fertilizer combined with organic fertilizer rather than chemical fertilizer alone could help farmers in achieving satisfactory yields and fruits quality while reducing the risk of nitrate leaching. For wolfberry, it is recommended to apply 50-150 g/plant of N fertilizer combined with 4-8 kg/plant of organic fertilizer in high-fertility fields and 100-200 g/plant of nitrogen fertilizer combined with 2-6 kg/plant of organic fertilizer in low-fertility fields.

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