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Evaluation of the Effects of Nitrogen, Phosphorus, and Potassium Applications on the Growth, Yield, and Quality of Lettuce (*Lactuca sativa* L.)

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Abstract: Lettuce (*Lactuca sativa* L.), a popular edible vegetable, is highly responsive to mineral fertilizers. A two-year field trial was carried out to determine how nitrogen (N), phosphorous (P), and potassium (K) application ratios affected lettuce growth, yield, and quality. Within a certain range of nutrient application rate, the plant height, stem diameter, yield, and the concentrations of vitamin C and soluble sugar were higher than those without fertilization. Compared with no fertilization condition, the yield increased approximately 13.56–22.03%, 15.15–42.42% and 7.14–10.32% under increased N, P, and K application conditions, respectively. Furthermore, substantial quadratic parabola relationships were observed between lettuce yield and fertilizer application amounts as well as most quality traits. The nitrate concentration was proportional to the N fertilization rate, while the concentrations of soluble sugar and vitamin C positively correlated with N and P application ratios, respectively. The lettuce leaves had the largest N (60.59%) and K (52.25%) accumulations, whereas the lettuce stem had the highest P (46.33%) accumulation. Above all, the optimal N, P, and K application amounts for lettuce were 315 kg N ha^{-1}, 210 kg P_2O_5 ha^{-1}, and 285 kg K_2O ha^{-1}, respectively.

Keywords: lettuce; field trial; balanced fertilization; regression relationship; nutrient accumulation

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1. Introduction

Lettuce (*Lactuca sativa* L.) is a member of the *Asteraceae* family and the only member of the genus *Lactuca* that is widely grown around the world [1]. With a short growing time and commercial availability all year long, lettuce is grown in open fields, greenhouses, or plant factories using artificial lighting systems [2–4]. Thus, lettuce is a widely consumed edible vegetable worldwide due to its great production and economic value [5,6]. Lettuce has been considered to be an important functional food as it has low calories, fat, and sodium but is rich in vitamins, folate, and fiber, as well as various minerals including phosphorus (P), calcium (Ca), magnesium (Mg), and iron (Fe) [7,8]. In addition, lettuce is an important source of phytochemicals that promote health, such as ascorbic acid, carotenoids, flavonoids, tocopherols, and so on. Hence, it is advantageous for human health to consume lettuce [9]. Field-grown lettuce accounts for the majority of commercial production with a high reliance on chemical fertilizers [2,10].

Due to intensive crop cultivation and poor soil management techniques, most of the agricultural soils around the world have suboptimal amounts of necessary nutrients for plants and/or have suffered significant degradation [11,12]. Many factors contribute to soil degradation, including depletion of nutrients, deterioration of soil structure, acidification, and inadequate application of organic and inorganic fertilizers to soil [12,13]. Poor fertility and deterioration of arable lands have been identified as major causes of yield reduction or stagnation in the extensive farming system around the world. Therefore, extra fertilizer inputs are necessary for agricultural soils to provide sufficient crop nutrition [14,15].

Vegetable productivity and quality are influenced by mineral fertilization [16–22]. However, unfavorable environmental consequences on water quality, leaching, and runoff may result from overusing fertilizers [23–25]. These negative factors have prompted a search for alternatives that would decrease the use of fertilizers and their detrimental effects. In addition, over fertilization may result in yield losses and a decline in product quality [26]. Therefore, it is important to identify optimal fertilizer management and make efficient use of nutrients [24,27]. Furthermore, application of balanced mineral fertilizers boosted crop productivity while also optimizing nutrient use efficiency [14,28].

Because of the wide range of morphological characteristics, the modern types of cultivated lettuce are primarily categorized as butterhead, crisphead, looseleaf, romaine, or stem lettuce [29]. However, large variability has been observed in biomass production, morphological traits, and nutrient absorption by different lettuce varieties. For example, romaine and crisphead types tend to accumulate higher amounts of nutrients than others as they generally produce appreciably more biomass [2]. Lettuce typically responds well to fertilizers [4,30,31]. For instance, high levels of nitrogen (N) application may have pronounced advantages for lettuce growth, yield, and quality [3]. The balanced supplies of N, P, and K provided the highest yield and best post-harvest quality of lettuce [27,31], but a high application amount of nutrients inhibited lettuce yield [32]. Thus, understanding lettuce responses to various nutrients, such as N, P, and potassium (K), allows us to fine-tune fertilizer use in order to optimize its performance and nutritional quality.

China produces the most lettuce in the world, accounting for nearly half of all lettuce widely cultivated and produced globally, particularly for stem lettuce products [33]. However, the best N, P, and K fertilizer combination for optimum lettuce yield and quality in Wuhan, China, is unclear. The objectives of the current research were (i) to evaluate the relative response of lettuce to different application rates of N, P, and K fertilizers, (ii) to make optimal fertilizer recommendations for obtaining high lettuce yield and quality, and (iii) to evaluate the nutrient accumulations in different parts of lettuce.

2. Materials and Method

2.1. Plant Materials and Experimental Design

A field used for growing agricultural crops was selected for this study in Wuhan city (30°42′40″ N, 114°27′45″ E), Hubei province, China, during the 2014–2016 lettuce growing season. The test area is characterized by a subtropical monsoon climate and is 27 m above sea level. This area has an average rainfall of 1219.5 mm annually, and the annual evaporation is about 1586 mm. The average annual temperature is 16.5 °C, while the effective accumulated temperature is 5222 °C. The frost-free period is 211–272 days. The experimental site has flat terrain and uniform fertility. The soil type is gray fluvial aquic soil which is convenient for irrigation. The previous crops are leafy vegetables. The soil chemical properties in the 0–20 cm layer are as follows: pH is 7.9, organic matter is 9.10 g kg^{-1}, total N is 1.05 g kg^{-1}, alkaline hydrolysis N is 50.08 mg kg^{-1}, Olsen-P is 12.7 mg kg^{-1}, and available K is 90.52 mg kg^{-1}.

A total of 15 treatments were set and denoted by N_1-N_5 in N fertilizer treatments, P_1-P_5 in P fertilizer treatments, and K_1-K_5 in K fertilizer treatments, respectively (Table 1). The individual plot area was 32 m^2 (length × width = 8.0 m × 4.0 m). The amount of normal N, P, and K fertilizers applied for each treatment was calculated according to the following nutrient rates: 315 kg N ha^{-1}, 210 kg P_2O_5 ha^{-1}, and 285 kg K_2O ha^{-1}. The fertilizer sources for N, P, and K were urea (46% N), calcium superphosphate (12% P_2O_5), and potassium sulfate (52% K_2O), respectively. P fertilizer was applied before sowing, whereas N and K fertilizers were applied three times. The distribution ratio of N fertilizer is 50% as basal fertilizer before sowing, 30% in the rosette stage, and 20% in the rapid growth stage. For K fertilizer, 40% as basal fertilizers were applied before sowing, 30% in the rosette stage, and 30% in the rapid growth stage.

Table 1. Nutrient application amounts for all the treatments in the field trials of *Lactuca sativa* L. during the growth season.

Treatments	Fertilizer Application Amount (kg ha^{-1})		
	N	P_2O_5	K_2O
N_1	0	210	285
N_2	157.5	210	285
N_3	315.0	210	285
N_4	472.5	210	285
N_5	630.0	210	285
P_1	315	0	285
P_2	315	105	285
P_3	315	210	285
P_4	315	315	285
P_5	315	420	285
K_1	315	210	0
K_2	315	210	142.5
K_3	315	210	285.0
K_4	315	210	427.5
K_5	315	210	570.0

A lettuce cultivar named "Huasun" provided by the vegetable research institute of the Wuhan Academy of Agricultural Sciences was used in this study. Lettuce plants were grown from seed in a nursery to the three-leaf stage. Then, the seedlings were transplanted to the field in the way of ridge cultivation. The width of the ridges was 90 cm, and the height of the ridges was 30 cm. There were two rows on the ridge, with 40 cm between each row. The distance between adjacent plants was 30 cm. Seeds were sown in August and transplanted by hand in September. Plants were harvested in May. The cultivation density of lettuce was 30,000 plants per hectare. Seeds were sown by hand, and the field management followed standard agricultural practice.

For assessing the effects of balanced fertilization on lettuce production, we performed a field trial with four treatments, including balanced fertilization (OPT, 315 kg N ha^{-1}, 210 kg P_2O_5 ha^{-1}, and 285 kg K_2O ha^{-1}), -N (0 kg N ha^{-1}, 210 kg P_2O_5 ha^{-1}, and 285 kg K_2O ha^{-1}), -P (315 kg N ha^{-1}, 0 kg P_2O_5 ha^{-1}, and 285 kg K_2O ha^{-1}), -K (315 kg N ha^{-1}, 210 kg P_2O_5 ha^{-1}, and 0 kg K_2O ha^{-1}), and CK (no N, P, or K were applied). At harvest time, the yield, plant height, and stem diameter of lettuce were investigated and recorded, as were the concentrations of nitrate, vitamin C, soluble protein, soluble sugar, amino acid, and three nutrients (N, P, and K).

2.2. Measurements of Agronomic and Yield Parameters

The whole lettuce plants in a quadrat of 8 m $\times$ 4 m were harvested 160 days after transplanting. Plant weight and root length were measured from a composite sample of ten plants. Leaf numbers were counted. Then, the stem yield was measured after separating the plants. Stem diameter was measured using a caliper in the middle part of the stem. In addition, the SPAD values of lettuce upper and lower leaves, which indicate the chlorophyll content, were measured using a portable SPAD-502 Plus chlorophyll meter (Konica Minolta, Japan) at both the rosette stage (47 days after transplanting) and the rapid growth stage (96 days after transplanting). In the dry matter accumulation analysis assay, the lettuce plants were sampled at 15 days, 43 days, 82 days, 112 days, and 128 days after transplanting. Then, the samples were washed with deionized water and divided into roots, stems, and leaves. To deactivate enzymes, samples were heated to 105 °C for 30 min before drying. Each fraction was oven dried at 65 °C to a constant weight. Then, the dry weight of each part was recorded.

2.3. Lettuce Quality Evaluations

After the harvest of the lettuce stem, the quality characteristics of three individuals from each plot were assessed. The vitamin C concentration was determined using the 2,6-dichloroindophenol staining approach [34]. The soluble sugar concentration was determined using the anthrone–sulfuric acid test technique [35]. The soluble protein content was determined by using Coomassie brilliant blue method [36]. The salicylic acid–sulfuric acid method was applied to assess the nitrate concentration [37]. Total free amino acids were determined using the previously mentioned ninhydrin colorimetric method [38].

2.4. Nutrient Concentration Analysis

The dried samples were digested with H_2SO_4-H_2O_2 [39]. In short, a sample of approximately 0.1 g was placed into the digestive tube after the samples had been dried to a consistent weight. The tube was then filled with 5 cm^3 of 98% H_2SO_4 and left overnight. With 5–10 drops of H_2O_2, the digestive tube was placed in the heating block and heated to 250 °C for 2 h. A flow injection analysis tool (FIAstar 5000 analyzer; FOSS, Hilleroed, Denmark) was used to examine the concentrations of N and P in the digested solution. Using a flame photometer (M-410, Cole-Parmer, Chicago, IL, USA), the K concentration in the digested solution was determined. The sum of the product of the dry matter and the total nutrient concentrations was used to calculate the nutrient accumulations in each part of the plants.

2.5. Statistical Analysis

A completely randomized block design was used, with four replications in each treatment. SPSS 21.0 (Chicago, IL, USA) software was used to conduct all statistical analyses. Duncan's tests ($p < 0.05$) were used to identify the significant differences across different treatments. The graphics were generated using GraphPad Prism 7.0. To examine their phenotypic association, Pearson's correlation coefficients were calculated between nutrient application amount and the yield and four quality traits.

3. Results

3.1. Effects of Different N, P, and K Application Rates on Lettuce Growth

The application of N fertilizer had a significant effect on lettuce plant height, with significant differences among the five N treatments (Figure 1A). With increasing N fertilizer application rate, lettuce plant height increased gradually. The plant height in the N_3 treatment was 42.87 cm which was the highest of all treatments. Furthermore, the plant height of lettuce treated with N was greater than that of lettuce not treated with N, indicating that N fertilizer can promote lettuce elongation. Furthermore, significant differences in lettuce stem diameter were observed under the five N fertilizer treatments (Figure 1D). The stem diameter increased first and then decreased as N application increased. When the N application rate was 315 kg ha^{-1}, the lettuce stem diameter reached its maximum of 5.55 cm. Under different N fertilizer treatments, the order of stem diameter was $N_3 > N_4 > N_5 > N_2 > N_1$.

With increasing P fertilizer application rate, lettuce plant height increased first and then decreased (Figure 1B). The shortest plant height was 39.77 cm when no P was applied (P_1), while the largest plant height was 47.8 cm under P_2 treatment. However, there was little difference between the four treatments (P_2–P_5) with varying P application rates. The application of P fertilizer can significantly increase the stem diameter of lettuce compared with those without P application (Figure 1E). The stem diameter increased with the increase in P application amount. The diameter of the lettuce stem increased as the amount of P applied increased. The smallest stem diameter was 4.57 cm when no P fertilizer was applied. In general, the order of stem diameter under different P fertilizer treatments was $P_5 > P_4 > P_2 > P_3 > P_1$.

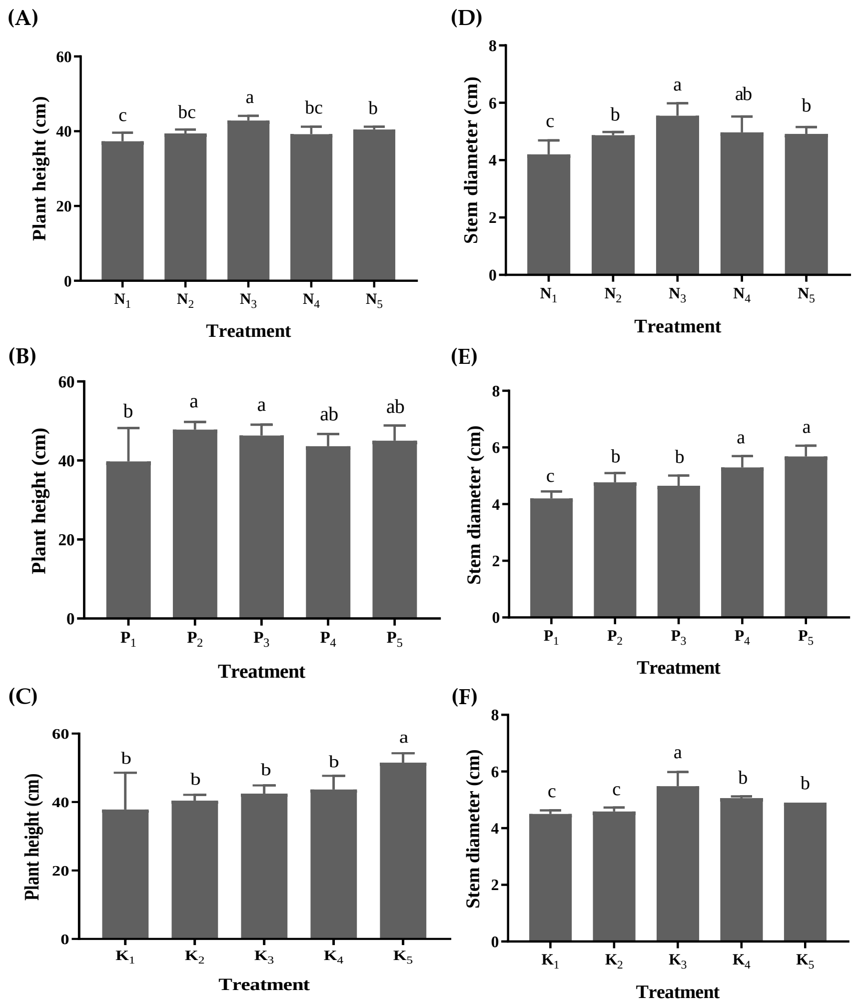

Figure 1. The plant height and stem diameter of lettuce under different application rates of N, P, and K. The lettuce plants were treated with five levels of N, P, and K fertilizers. Then, the plant height (**A**–**C**) and stem diameter (**D**–**F**) under N (**A**,**D**), P (**B**,**E**), and K (**C**,**F**) treatments were measured and recorded. Different letters indicate the distinct differences at $p < 0.05$.

Unlike the N and P treatments, applying K fertilizer did not significantly increase lettuce plant height, except for the K_5 treatment which increased plant height by 36.12% compared with the K_1 treatment. The use of K can significantly increase the diameter of lettuce stems (Figure 1F). The stem diameter increased first, then decreased as a result of the various treatments. The K_3 treatment produced the largest lettuce stem diameter (5.48 cm), while the K_1 treatment produced the smallest, at 4.50 cm. In general, the order of stem diameter under different P fertilizer treatments was $K_3 > K_4 > K_5 > K_2 > K_1$.

3.2. Effects of Different Nutrient Application Rates on Yield and Quality of Lettuce

N fertilizer application had a significant effect on lettuce stem yield. The N_3 treatment produced the highest stem yield (15,000 kg ha^{-1}), while the N_1 treatment produced the lowest stem yield (12,292 kg ha^{-1}). When compared with the N_1 treatment, the yield increase rates for the N_2 to N_5 treatments were 14.40%, 22.03%, 20.30%, and 13.56%, respectively. In general, as N application increased, both stem yield and yield increase rate increased first, then decreased (Table 2). Nitrate, vitamin C, soluble protein, and soluble sugar concentrations are important crop quality indicators. With increasing N application from N_1 to N_4, the nitrate concentration in lettuce increased gradually, reaching a maximum at N_4 treatment of 3.03 mg g^{-1}. However, the nitrate concentration in the N_5 treatment was significantly lower than in the N_4 treatment. Consistent with the nitrate concentration change trend, the concentration of soluble sugar reached a maximum of 1.16% at the N_4 treatment. In contrast to the nitrate and soluble sugar concentrations, the highest value of vitamin C concentration was observed at the N_3 treatment, which was 64.58 mg kg^{-1}. Furthermore, the concentration of soluble protein was not directly related to the amount of N fertilizer applied, and the highest value was observed with the most N applied (Table 2).

Table 2. Effect of different N application rates on yield and quality of lettuce.

Treatment	Yield (kg ha^{-1})	Increase Rate (%)	Nitrate Concentration (mg g^{-1} FW)	Vitamin C Concentration (mg kg^{-1} FW)	Soluble Protein Concentration (mg g^{-1} FW)	Soluble Sugar Concentration (% FW)
N_1	12,292 ± 786 ^{b}	-	1.48 ± 0.24 ^{c}	35.58 ± 3.59 ^{d}	0.46 ± 0.02 ^{c}	0.48 ± 0.01 ^{cd}
N_2	14,063 ± 312 ^{ab}	14.40	2.00 ± 0.15 ^{b}	45.30 ± 3.16 ^{bc}	0.58 ± 0.04 ^{b}	0.39 ± 0.07 ^{d}
N_3	15,000 ± 120 ^{a}	22.03	2.12 ± 0.24 ^{b}	64.58 ± 2.59 ^{a}	0.42 ± 0.04 ^{d}	0.85 ± 0.10 ^{b}
N_4	14,792 ± 1183 ^{ab}	20.34	3.03 ± 0.07 ^{a}	47.73 ± 3.22 ^{b}	0.49 ± 0.01 ^{c}	1.16 ± 0.11 ^{a}
N_5	13,959 ± 2345 ^{c}	13.56	1.89 ± 0.15 ^{b}	40.68 ± 4.27 ^{cd}	0.63 ± 0.03 ^{a}	0.57 ± 0.04 ^{c}

Note: Different letters indicate the distinct differences at $p < 0.05$. FW, fresh weight.

The application of P fertilizer had a significant effect on lettuce stem yield. The P_3 treatment had the highest stem yield (14,688 kg ha^{-1}), while the P_1 treatment had the lowest (10,312 kg ha^{-1}). The yield increases from P_2 to P_5 treatments were 23.23%, 42.42%, 27.27%, and 15.15%, respectively, when compared with no P application. Stem yield and yield increase rate increased first, then decreased as P application increased (Table 3). Except for the concentration of soluble protein, there were significant differences in the four quality traits under different P application rates. The lowest concentration of vitamin C (58.68 mg kg^{-1}) was found in the absence of P but was the highest in the P_4 treatment. When more than 315 kg ha^{-1} of P was applied, the vitamin C concentration was 80.28 mg kg^{-1}, a decrease of 24.65% when compared with the P_4 treatment. The concentrations of nitrate and soluble sugar changed in the same way under different P application rates. When compared with other treatments, the P_1 and P_5 treatments yielded a higher value. The nitrate concentration was 6.59 mg g^{-1} in the P_1 treatment and 6.92 mg g^{-1} in the P_5 treatment. Furthermore, when compared to the P_1 treatment, the nitrate concentrations in the P_2 to P_4 treatments decreased by 42.49%, 53.11%, and 59.33%, respectively. In comparison with the P_1 treatment, the soluble sugar concentration decreased by 33.33%, 50%, and 56.32% under the P_2 to P_4 treatments, respectively (Table 3).

Table 3. Effect of different P application rates on yield and quality of lettuce.

Treatment	Yield (kg ha^{-1})	Increase Rate (%)	Nitrate Concentration (mg g^{-1} FW)	Vitamin C Concentration (mg kg^{-1} FW)	Soluble Protein Concentration (mg g^{-1} FW)	Soluble Sugar Concentration (% FW)
P_1	10,312 ± 920 ^{c}	-	6.59 ± 1.09 ^{a}	58.68 ± 13.67 ^{c}	0.58 ± 0.13 ^{a}	1.74 ± 0.27 ^{a}
P_2	12,708 ± 70 ^{b}	23.23	2.68 ± 0.16 ^{b}	63.73 ± 9.15 ^{c}	0.47 ± 0.05 ^{a}	0.87 ± 0.13 ^{c}
P_3	14,688 ± 884 ^{a}	42.42	3.09 ± 0.82 ^{b}	97.33 ± 0.96 ^{a}	0.46 ± 0.02 ^{a}	0.76 ± 0.09 ^{c}
P_4	13,125 ± 92 ^{b}	27.27	3.79 ± 0.41 ^{b}	100.13 ±5.15 ^{a}	0.48 ± 0.03 ^{a}	1.16 ± 0.11 ^{b}
P_5	11,875 ± 1169 ^{b}	15.15	6.92 ± 0.82 ^{a}	80.28 ± 2.67 ^{b}	0.48 ± 0.09 ^{a}	1.85 ± 0.15 ^{a}

Note: Different letters indicate the distinct differences at $p < 0.05$. FW, fresh weight.

The application of K had a significant impact on lettuce stem yield (Table 4). It was highest (14,479 kg ha^{-1}) in the K_3 treatment and lowest (13,125 kg ha^{-1}) in the K_1 treatment. Compared with no K treatment, the yield under the K_2 to K_5 treatments increased by 8.73%, 10.32%, 9.52%, and 7.14%, respectively. With an increase in K application amount, lettuce stem yield and yield increase rate both increased initially and then decreased. Furthermore, different K treatments had a significant effect on lettuce quality, but this varied across quality traits. In particular, the concentration of vitamin C was lowest (63.25 mg kg^{-1}) without K application and increased as K application increased. The highest concentration of vitamin C (105.33 mg kg^{-1}) was found when 427.5 kg ha^{-1} K was applied in the field.

When more K was added, it decreased by 43.11% to 59.88 mg kg^{-1}. Furthermore, the K_4 treatment resulted in the highest nitrate concentration of 6.28 mg g^{-1}. The soluble sugar concentration of lettuce changed dramatically as K fertilizer application increased. The K_3 treatment had the highest soluble sugar concentration of 1.81%, while the K_5 treatment had the lowest soluble sugar concentration of 0.70%. The lettuce soluble protein concentration was highest (0.69 mg g^{-1}) in the K_3 treatment and lowest (0.57 mg g^{-1}) in the K_4 treatment (Table 4).

Table 4. Effect of different K application rates on yield and quality of lettuce.

Treatment	Yield (kg ha^{-1})	Increase Rate (%)	Nitrate Concentration (mg g^{-1} FW)	Vitamin C Concentration (mg kg^{-1} FW)	Soluble Protein Concentration (mg g^{-1} FW)	Soluble Sugar Concentration (% FW)
K_1	13,125 ± 1275 ^{c}	-	4.12 ± 0.73 ^{c}	63.25 ±2.60 ^{c}	0.58 ± 0.02 ^{b}	1.22 ± 0.14 ^{b}
K_2	14,271 ± 1178 ^{a}	8.73	5.11 ± 0.27 ^{b}	70.73 ± 3.23 ^{bc}	0.65 ± 0.04 ^{a}	1.47 ± 0.38 ^{b}
K_3	14,479 ± 1160 ^{a}	10.32	2.79 ± 0.48 ^{d}	85.34 ± 10.01 ^{b}	0.69 ± 0.05 ^{a}	1.81 ± 0.10 ^{a}
K_4	14,375 ± 765 ^{a}	9.52	6.28 ± 0.31 ^{a}	105.33 ± 17.88 ^{a}	0.57 ± 0.06 ^{b}	1.79 ± 0.20 ^{a}
K_5	14,062 ± 1276 ^{a}	7.14	2.68 ± 0.30 ^{d}	59.88 ± 2.33 ^{c}	0.66 ± 0.05 ^{a}	0.70 ± 0.05 ^{c}

Note: Different letters indicate the distinct differences at $p < 0.05$. FW, fresh weight.

In addition, Pearson's correlation coefficient was calculated to demonstrate the relationship between nutrient application level and lettuce quality (Figure 2). The results revealed a significant positive correlation between N application rate and soluble sugar concentration (0.47) but no significant correlation between N application rate and the other three quality traits, including the concentrations of nitrate, vitamin C, and soluble protein. However, the concentration of vitamin C was negatively correlated with the concentration of soluble protein, whereas the concentration of soluble sugar was positively correlated with the concentrations of nitrate and vitamin C under different N application rates (Figure 2A). P application rate was positively correlated with vitamin C concentration but not with the other three quality traits. Under different P application rates, vitamin C concentration was negatively correlated with soluble protein concentration, whereas soluble sugar concentration was positively correlated with nitrate concentration (Figure 2B). There was no strong relation between K fertilizer application rate and the four quality traits. Nonetheless, under different K application conditions, nitrate concentration was significantly positively correlated with vitamin C and soluble sugar concentrations but significantly negatively correlated with soluble protein concentration. Furthermore, soluble sugar concentration was significantly positively correlated with vitamin C concentration but negatively correlated with soluble protein concentration (Figure 2C).

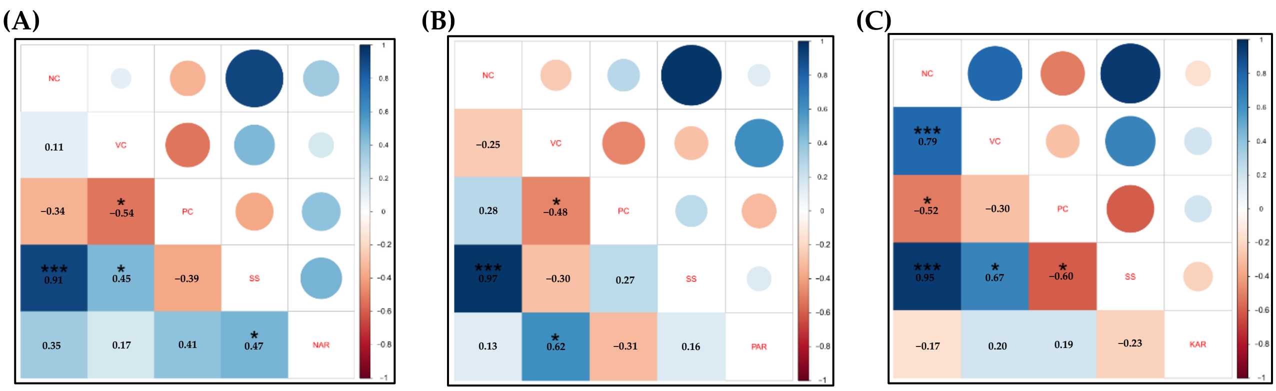

Figure 2. Correlation coefficient among N (**A**), P (**B**), and K (**C**) application rates and the four quality traits. NC, Nitrate concentration; VC, Vitamin C concentration; PC, Soluble protein concentration; SS, Soluble sugar concentration; NAR, N application rate; PAR, P application rate; and KAR, K application rate. * $p < 0.05$, *** $p < 0.001$.

3.3. The Functional Models of Fertilizing Effect between Nutrient Application and the Yield and Quality of Lettuce

The regression relationship between five lettuce traits and the fertilization rates of N, P, and K was established as the fertilizer effect models using SPSS 21.0 statistical software based on the survey results of stem yield and four quality traits of lettuce under different application rates of N, P, and K. (Figure 3). The results revealed a significant quadratic parabola relationship between lettuce yield, economic benefit, and nutrient application amounts. Additionally, with a few exceptions, there was a significant quadratic regression between most lettuce quality traits and nutrient application ratios.

According to the regression equation, the highest yield of 15,018 kg ha^{-1} was obtained with 378 kg ha^{-1} N fertilizer (Figure 3). When 315 kg ha^{-1} N is used, the stem yield is 14,928 kg ha^{-1}. In terms of quality traits, the concentrations of vitamin C and soluble sugar had a quadratic parabola relationship with the amount of N applied. The highest concentrations of vitamin C and soluble sugar were 56.85 mg kg^{-1} and 0.88% under the N application amount of 336 kg ha^{-1} and 399 kg ha^{-1}, respectively. When 315 kg ha^{-1} was applied, the concentrations of vitamin C and soluble sugar were 56.74 mg kg^{-1} and 0.85%, respectively. The recommended optimum application amount of N on lettuce for high-yield, high-quality, and high-efficiency agriculture was 315 kg ha^{-1}.

According to the regression equation, the highest yield of 14,128 kg ha^{-1} was obtained with a P fertilizer amount of 238 kg ha^{-1} (Figure 3). The yield was 14,089 kg ha^{-1} when 210 kg ha^{-1} was used, which was slightly less than the highest yield. In terms of quality traits, a significant quadratic parabola relationship was observed between P application rate and nitrate and soluble sugar concentrations. When 210 kg ha^{-1} was applied, the lowest nitrate concentration (2.56 mg g^{-1}) was detected. Taking yield, quality, and economic benefits into account, 210 kg ha^{-1} is the optimal P application amount.

In addition, the 342 kg ha^{-1} K application produced the highest lettuce yield (14,566 kg ha^{-1}) (Figure 3). The lettuce yield was 14,523 kg ha^{-1} when 285 kg ha^{-1} K was applied, which had no significant difference with the highest yield. A significant quadratic parabola relationship was discovered between K application rate and soluble sugar concentration. According to the regression equation, the highest soluble sugar (1.84%) was obtained with a K fertilizer amount of 266 kg ha^{-1}. The yield, vitamin C concentration, and soluble sugar concentration were 14,523 kg ha^{-1}, 91.26 mg kg^{-1}, and 1.83%, respectively, when 285 kg ha^{-1} K was applied in the field. Hence, for lettuce production, a K application rate of 285 kg ha^{-1} was recommended in the field.

3.4. Effect of N, P, and K Balanced Fertilization on Growth and Yield of Lettuce

Based on the recommended application amounts for N, P, and K, we further investigated the effects of the balanced fertilization on the phenotypic performance of lettuce. We measured the SPAD values at two growth stages. At the rosette stage of lettuce, the SPAD value of the upper leaves was greater than that of the lower leaves in all treatments, whereas at the rapid growth stage, the opposite results were observed. The SPAD values of both upper and lower leaves at both the rosette and rapid growth stages were higher in the OPT condition than in the other treatments. The lowest SPAD values were observed in the CK treatments at both growth stages (Figure 4).

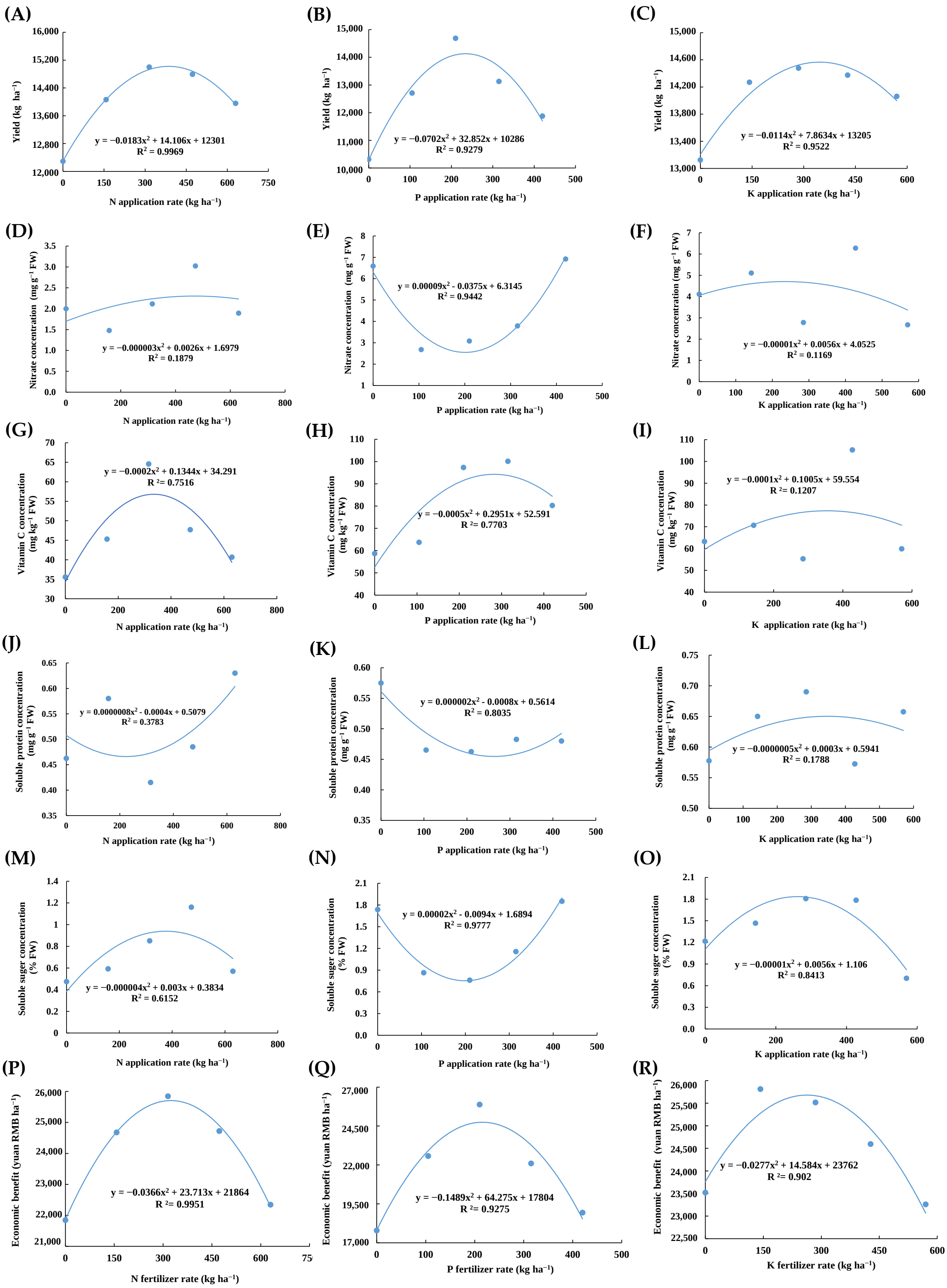

Figure 3. The models for fertilizer response of different lettuce traits under the varied nutrient application amounts. (**A**–**C**), yield; (**D**–**F**), nitrate concentration; (**G**–**I**), vitamin C concentration; (**J**–**L**), soluble protein concentration; (**M**–**O**), soluble sugar concentration; and (**P**–**R**), Economic benefit. FW, fresh weight.

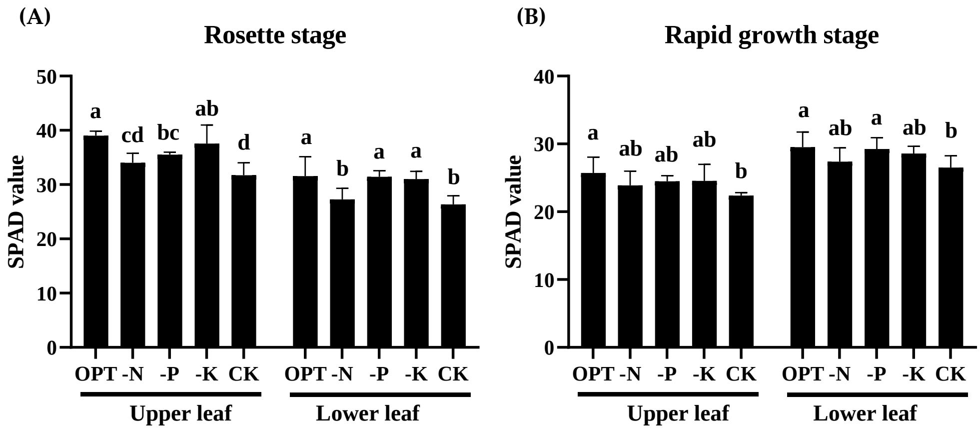

Figure 4. Effects of N, P, and K balanced fertilization on SPAD values of different leaves at two growth stages of lettuce. SPAD values of the upper and lower leaves were measured at both rosette stage (**A**) and rapid growth stage (**B**). Different letters indicate the distinct differences at $p < 0.05$.

Without fertilization, lettuce yield was significantly reduced (Figure 5A). The lettuce yield decreased by 17.26%, 28.99%, 10.00%, and 40.29%, respectively, when compared with the OPT condition. When compared with other treatments, OPT significantly improved lettuce growth performance in terms of plant height, leaf number, stem diameter, and stem length. Nutrient application, on the other hand, had no effect on root length (Figure 5B–F).

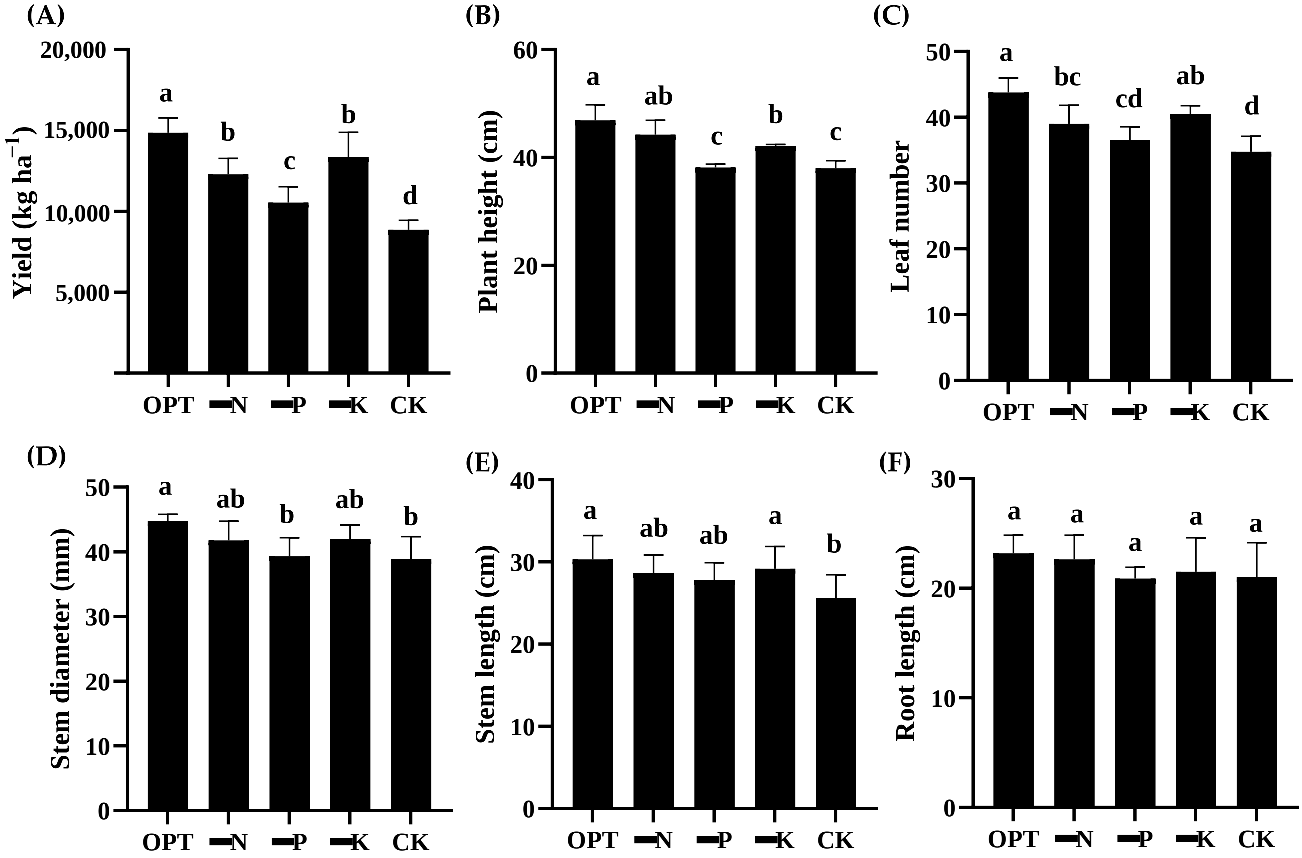

Figure 5. Effects of N, P, and K balanced fertilization on lettuce yield and other agronomic traits. (**A**) Yield; (**B**) Plant height; (**C**) Leaf number; (**D**) Stem diameter; (**E**) Stem length; and (**F**) Root length. Different letters indicate the distinct differences at $p < 0.05$.

We measured the concentrations of nitrate, vitamin C, soluble protein, soluble sugar, and amino acids in the five treatments to further investigate the effects of OPT on lettuce quality. In comparison with other nutrient stress conditions, OPT significantly increased the concentrations of vitamin C, soluble sugar, and soluble protein (Figure 6). However, nitrate concentration was more than twice as high under P stress than under other conditions (Figure 6A). There were no differences in amino acid concentration among the OPT, -P, and -K conditions. However, when compared with other treatments, N stress inhibits amino acid metabolism as well as the CK condition.

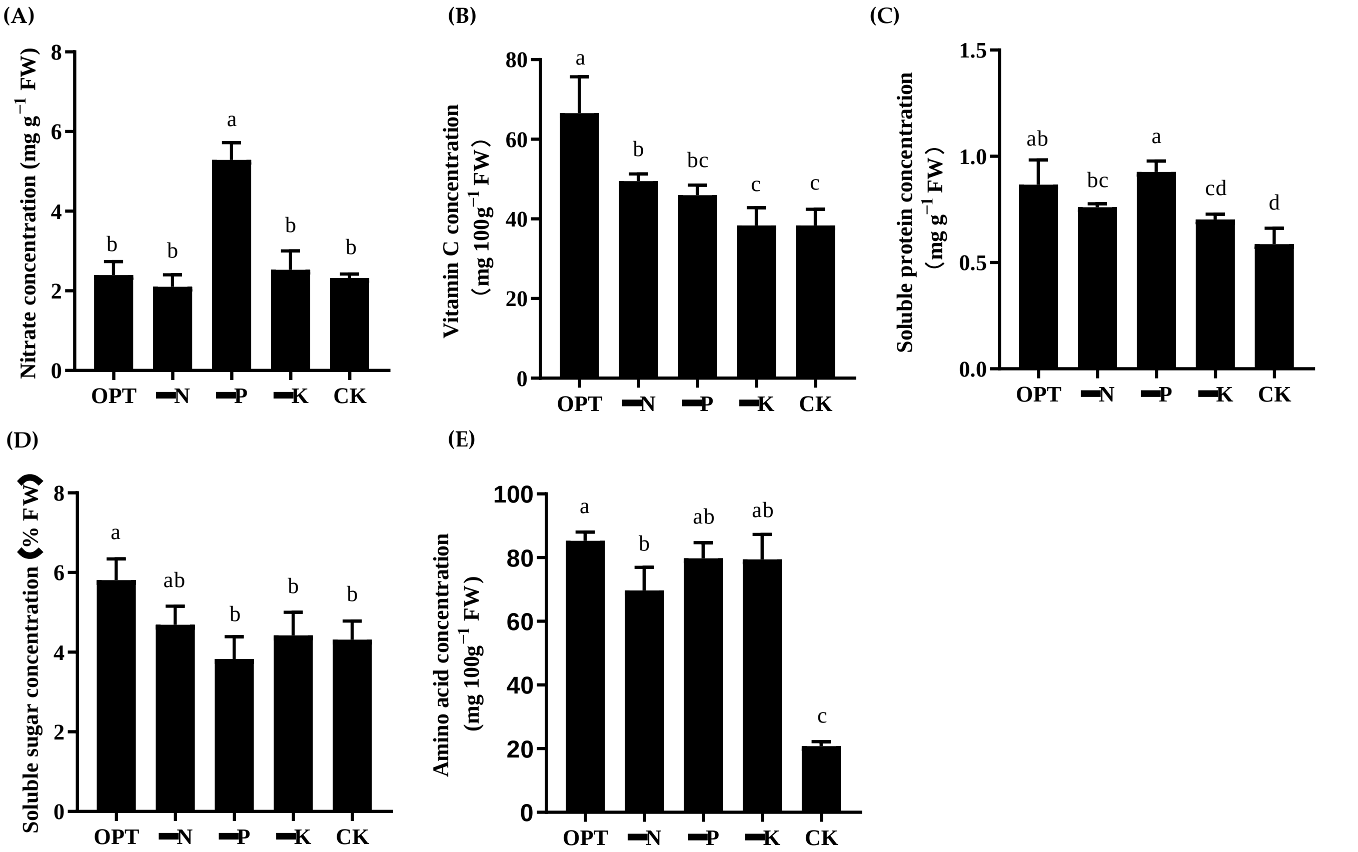

Figure 6. Effects of N, P, and K balanced fertilization on quality of lettuce. (**A**) Nitrate concentration; (**B**) Vitamin C concentration; (**C**) Soluble protein concentration; (**D**) Soluble sugar concentration; and (**E**) Amino acid concentration. Different letters indicate the distinct differences at $p < 0.05$.

3.5. Accumulation and Distribution of Dry Matter and Nutrients in Lettuce after Transplanting

The accumulation and distribution of dry matter in lettuce was also investigated. The results revealed that the dry weight of leaves accounted for the greatest proportion of the total dry matter, accounting for 55.26% of the total dry matter. The dry weight of lettuce stem accounted for 26.52% of the total dry matter, while the root dry weight accounted for 18.22% of the total dry matter (Figure 7A). The accumulation of dry matter in all organs was slow before 112 days after transplantation and accelerated after 112~128 days. Furthermore, the accumulation and distribution of nutrients including N, P, and K in lettuce were examined. Except for P accumulation in leaves, the accumulations of N, P, and K in the entire plant and three organs were similar to the accumulations of dry matter. They increased slowly before 112 days after transplanting and then became faster after 112~128 days (Figure 7B–D). However, P accumulation in leaves was still slow 112~128 days after transplanting (Figure 7C). The total N accumulation of lettuce was 36.18 kg ha^{-1}, with the highest N accumulation in leaves (21.92 kg ha^{-1}), accounting for 60.59% of the total N accumulation. The N accumulation in the stem was 9.21 kg ha^{-1}, accounting for 25.45% of the total. The root accumulated the least N, with only 5.05 kg ha^{-1}, accounting for 13.97%.

During the entire growth period of lettuce, the P accumulation was 5.07 kg ha^{-1}. In contrast with N accumulation, the highest P accumulation was observed in the stem, accounting for 46.33% (2.35 kg ha^{-1}). P accumulation in leaves was 2.01 kg ha^{-1}, accounting for 39.61% of the total. The lowest P accumulation was found in roots, with 0.72 kg ha^{-1}, accounting for 14.13%. The total K accumulation of lettuce was 55.41 kg ha^{-1}, with the leaf accumulating the most (28.95 kg ha^{-1}), accounting for 52.25%, followed by the stem, which accumulated 19.64 kg ha^{-1}, accounting for 35.44%. The root K accumulation was the lowest, at 6.82 kg ha^{-1}, accounting for 12.31% of the total.

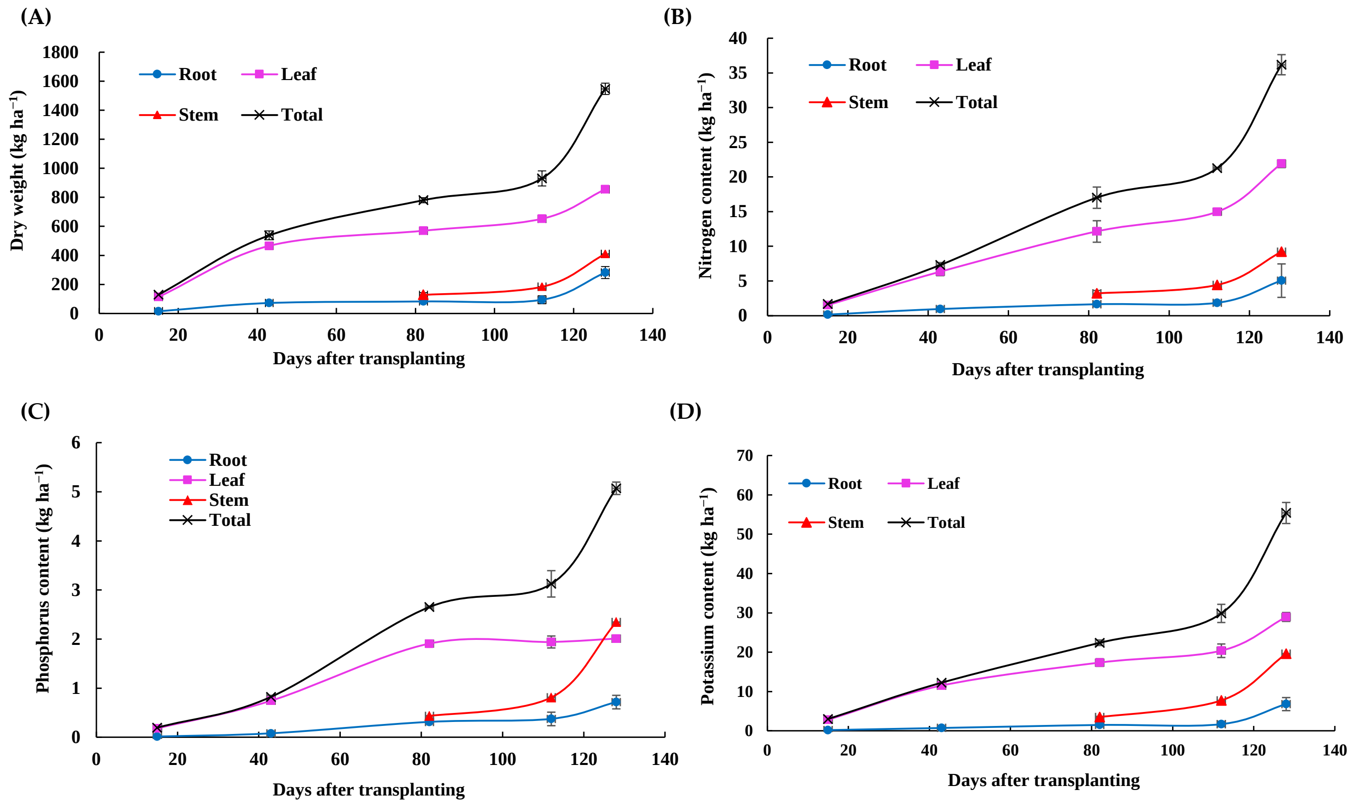

Figure 7. Accumulation and distribution of dry matter and nutrients after transplanting in lettuce. (**A**) Dry weight; (**B**) Nitrogen content; (**C**) Phosphorus content; and (**D**) Potassium content.

4. Discussion

N, P, and K fertilizers have long been important components of vegetable production. Recent research suggests that the lack of N and P greatly reduces the biomass and related composition of lettuce at the young leaf stage, while the lack of K only reduces the K content [40]. Similarly, the lack of N and P also has a strongly negative impact on yield at maturity. After adding different N and P application rates, the lettuce yield increased by 13.56–22.03% and 15.15–42.42%, respectively (Tables 2 and 3). However, the application of K fertilizer can significantly increase the yield and quality of lettuce at the mature stage, which is different from the effect of those on the lettuce seedling stage (Table 4). Applying K fertilizer to lettuce had a much lower yield increase rate than applying N and P fertilizer. However, the lettuce yield increased first and then decreased with increasing N, P, and K application rates, which indicates that an appropriate fertilizer application rate could maintain a high yield of lettuce with low nitrate concentration as well as high quality, including vitamin C and soluble sugar concentrations (Table 2). Additionally, the lettuce yield, vitamin C concentrations, and soluble sugar concentrations were reduced when the N, P, and K application rates exceeded the optimal level (Figure 3). Similar findings have been reported previously [3,19,41]. N fertilizer guarantees appropriate growing conditions for lettuce with optimum plant growth, which eventually promotes the best quality and production. Similar results were observed here under different P and K application ratios (Tables 3 and 4). These results indicated that nutrient application determines the yield of leafy vegetables, but an excessive concentration of nutrients may have negative effects on vegetable yield and quality [3,19]. Additionally, over fertilization reduces vegetable productivity, which has adverse short- and long-term consequences, including hazardous effects [26,32]. Based on the yield, quality and economic benefits of lettuce, the optimal N, P and K application amounts were recommended for the lettuce grown in Wuhan, China, which were 315 kg N ha^{-1}, 210 kg P_2O_5 ha^{-1}, and 285 kg K_2O ha^{-1}, respectively.

Sufficient nutrient availability for crop growth is required to maintain optimal crop yields. A substantial amount of research work has been done to determine the optimal fertilizer quantities for vegetables [16,42]. The majority of the work involved assessing crop response to various amounts of added fertilizer in order to record the likely range of response to a nutrient. For example, in comparison with imbalanced mineral fertilizer treatments, the long-term benefits of balanced fertilizer application enhanced the yields, nutrient use efficiency, and soil fertility of winter rye, oats, and potatoes [42]. Here, we conducted a field trial to assess how well-balanced fertilization affected lettuce productivity and quality. The findings demonstrated that balanced fertilization enhanced lettuce growth at both the rosette stage and the rapid growth stage, and it ultimately increased the final yield and other agronomic variables such as plant height, leaf number, stem diameter, and stem length (Figure 5). Additionally, when compared with the situation of nutritional imbalance, the quality of lettuce also improved under OPT conditions (Figure 6). Similar results were reported by other groups [27,31]. Thus, the key to effective fertilizer use for sustained high yields is balanced fertilization, which is the optimal application of fertilizers in the proper proportions to deliver the right ratio of plant nutrients to lettuce [28,42]. However, the nutrient application amounts recommended differed in each group, which may be due to the different lettuce varieties used in this research [2,10,29], the varied climate, and the growth media [16]. The uptake of nutrients by the crop, economic efficiency (i.e., nutrient inputs that optimize farm income), and environmental efficiency (i.e., minimizing nutrient losses to the environment) are all improved by balanced plant nutrition [42,43]. Therefore, our recommended application rates for N, P, and K fertilizers ensure the highest lettuce yield while also retaining great promise for increased economic gain and environmental efficiency. Certainly, how to guarantee a high lettuce yield while maintaining high quality will be a significant challenge.

In the current research, vitamin C, soluble protein, soluble sugar, and amino acids improved significantly under the OPT treatment, while nitrate remained at a low level (Figure 6). The nitrate concentration is a safety indicator for vegetables. Reducing nitrate content can add value to vegetable products, as nitrate causes serious health hazards when consumed by humans. Here, we found that it was proportional to the N fertilization rate but differed under P and K applications. These results agreed well with those of other studies [16,17,19], suggesting that it was a challenge to balance yield, quality, and economic benefits with the safety indices in vegetables. Interestingly, the nitrate concentration was significantly higher in the absence of P fertilizer than it was in the OPT treatment (Figure 6), demonstrating the critical role that balanced nutrient application plays in reducing health concerns in the production of lettuce.

5. Conclusions

Nutrients including N, P, and K significantly enhance the lettuce's growth performance, stem yield, and quality. The highest stem yield of lettuce (15,000 kg ha^{-1}, 14,688 kg ha^{-1}, and 14,479 kg ha^{-1}) was observed under N_3 (315 kg N ha^{-1}), P_3 (210 kg P_2O_5 ha^{-1}), and K_3 (285 kg K_2O ha^{-1}) treatments, respectively. However, the optimal treatments for the highest lettuce quality and best growth performance differed from those for the yield. Interestingly, nitrate concentration was significantly negatively correlated with soluble sugar concentration regardless of nutrient application ratios. In general, a significant quadratic parabola relationship exists between lettuce yield, economic benefit, and most of the quality traits and nutrient application ratios. In addition, the lettuce yield increased by 17.26%, 28.99%, 10.00%, and 40.29% under OPT conditions compared with that under -N, -P, -K, and no fertilization conditions, respectively. The highest nitrate content was found when no P was applied. In conclusion, the ideal quantities of N, P, and K to apply to lettuce in Wuhan, China, were 315 kg N ha^{-1}, 210 kg P_2O_5 ha^{-1}, and 285 kg K_2O ha^{-1}, respectively, taking into consideration lettuce agronomic performance, yield, quality, and economic benefits.

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References

1. Shi, M.; Gu, J.; Wu, H.; Rauf, A.; Emran, T.B.; Khan, Z.; Mitra, S.; Aljohani, A.S.M.; Alhumaydhi, F.A.; Al-Awthan, Y.S.; et al. Phytochemicals, nutrition, metabolism, bioavailability, and health benefits in lettuce-A comprehensive review. *Antioxidants* **2022**, *11*, 1158. [CrossRef] [PubMed]
2. Sanchez, C.A.; El-Hout, N.M. Response of diverse lettuce types to fertiliser phosphorus. *HortScience* **1995**, *30*, 528–531. [CrossRef]
3. Thapa, U.; Nandi, S.; Rai, R.; Upadhyay, A. Effect of nitrogen levels and harvest timing on growth, yield and quality of lettuce under floating hydroponic system. *J. Plant Nutr.* **2022**, *45*, 2563–2577. [CrossRef]
4. Zandvakili, O.R.; Barker, A.V.; Hashemi, M.; Autio, W.R.; Etemadi, F.; Sadeghpour, A. Influence of nitrogen source and rate on lettuce yield and quality. *Agron. J.* **2022**, *114*, 1401–1414. [CrossRef]
5. Křístková, E.; Doležalová, I.; Lebeda, A.; Vinter, V.; Novotná, A. Description of morphological characters of lettuce (*Lactuca sativa* L.) genetic resources. *Hort. Sci.* **2008**, *35*, 113–129. [CrossRef]
6. Mou, B. Nutrient content of lettuce and its improvement. *Curr. Nutr. Food Sci.* **2009**, *5*, 242–248. [CrossRef]
7. Kim, M.J.; Moon, Y.; Tou, J.C.; Mou, B.; Waterland, N.L. Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). *J. Food Compos. Anal.* **2016**, *49*, 19–34. [CrossRef]
8. Medina-Lozano, I.; Bertolín, J.R.; Díaz, A. Nutritional value of commercial and traditional lettuce (*Lactuca sativa* L.) and wild relatives: Vitamin C and anthocyanin content. *Food Chem.* **2021**, *359*, 129864. [CrossRef]
9. Yang, X.; Gil, M.I.; Yang, Q.; Tomás-Barberán, F.A. Bioactive compounds in lettuce: Highlighting the benefits to human health and impacts of preharvest and postharvest practices. *Compr. Rev. Food Sci. Food Saf.* **2022**, *21*, 4–45. [CrossRef]
10. Kreutz, G.F.; Bhadha, J.H.; Sandoya, G.V. Examining phosphorus use efficiency across different lettuce (*Lactuca sativa* L.) accessions. *Euphytica* **2022**, *218*, 28. [CrossRef]
11. Cakmak, I. Plant nutrition research: Priorities to meet human needs for food in sustainable ways. *Plant Soil.* **2002**, *247*, 3–24. [CrossRef]
12. Guo, J.H.; Liu, X.J.; Zhang, Y.; Shen, J.L.; Han, W.X.; Zhang, W.F.; Christie, P.; Goulding, K.W.; Vitousek, P.M.; Zhang, F.S. Significant acidification in major Chinese croplands. *Science* **2010**, *327*, 1008–1010. [CrossRef] [PubMed]
13. Lal, R. Restoring soil quality to mitigate soil degradation. *Sustainability* **2015**, *7*, 5875–5895. [CrossRef]
14. Mueller, N.D.; Gerber, J.S.; Johnston, M.; Ray, D.K.; Ramankutty, N.; Foley, J.A. Closing yield gaps through nutrient and water management. *Nature* **2012**, *490*, 254–257. [CrossRef] [PubMed]
15. Chen, X.; Cui, Z.; Fan, M.; Vitousek, P.; Zhao, M.; Ma, W.; Wang, Z.; Zhang, W.; Yan, X.; Yang, J.; et al. Producing more grain with lower environmental costs. *Nature* **2014**, *514*, 486–489. [CrossRef] [PubMed]
16. Hochmuth, G.J. Progress in mineral nutrition and nutrient management for vegetable crops in the last 25 years. *Hortscience* **2003**, *38*, 999–1003. [CrossRef]
17. Liu, Z.H.; Jiang, L.H.; Li, X.L.; Hardter, R.; Zhang, W.J.; Zhang, Y.L.; Zhang, D.F. Effect of N and K fertilizers on yield and quality of greenhouse vegetable crops. *Pedosphere* **2008**, *18*, 496–502. [CrossRef]
18. Wang, Z.H.; Li, S.; Malhi, S. Effects of fertilization and other agronomic measures on nutritional quality of crops. *J. Sci. Food Agric.* **2008**, *88*, 7–23. [CrossRef]

19. Xu, Y.; Liu, Z.H.; Gao, X.H.; Li, G.S.; Shi, J.; Wei, J.L.; Tan, D.S.; Wang, M.; Jiang, L.H. Effects of nitrogen levels on yield, quality and soil environment of Chinese cabbage in open field. *Acta Hortic.* **2018**, *1192*, 169–178. [CrossRef]
20. Hasnain, M.; Chen, J.; Ahmed, N.; Memon, S.; Wang, L.; Wang, Y.; Wang, P. The effects of fertilizer type and application time on soil properties, plant traits, yield and quality of tomato. *Sustainability* **2020**, *12*, 9065. [CrossRef]
21. Song, J.; Yang, J.; Jeong, B.R. Growth, quality, and nitrogen assimilation in response to high ammonium or nitrate supply in cabbage (*Brassica campestris* L.) and lettuce (*Lactuca sativa* L.). *Agronomy* **2021**, *11*, 2556. [CrossRef]
22. White, P.J.; Brown, P.H. Plant nutrition for sustainable development and global health. *Ann. Bot.* **2010**, *105*, 1073–1080. [CrossRef] [PubMed]
23. Fan, M.; Shen, J.; Yuan, L.; Jiang, R.; Chen, X.; Davies, W.J.; Zhang, F. Improving crop productivity and resource use efficiency to ensure food security and environmental quality in China. *J. Exp. Bot.* **2012**, *63*, 13–24. [CrossRef] [PubMed]
24. Dimkpa, C.O.; Fugice, J.; Singh, U.; Lewis, T.D. Development of fertilizers for enhanced nitrogen use efficiency—Trends and perspectives. Sci. *Total Environ.* **2020**, *731*, 139113. [CrossRef]
25. Conley, D.J.; Paerl, H.W.; Howarth, R.W.; Boesch, D.F.; Seitzinger, S.P.; Havens, K.E.; Lancelot, C.; Likens, G.E. Ecology. Controlling eutrophication: Nitrogen and phosphorus. *Science* **2009**, *323*, 1014–1015. [CrossRef]
26. Albornoz, F. Crop responses to nitrogen overfertilization: A review. *Sci. Hortic.* **2016**, *205*, 79–83. [CrossRef]
27. Hoque, M.M.; Ajwa, H.; Othman, M.; Smith, R.; Cahn, M. Yield and postharvest quality of lettuce in response to nitrogen, phosphorus, and potassium fertilizers. *HortScience* **2010**, *45*, 1539–1544. [CrossRef]
28. Jate, M. Long-term effect of balanced mineral fertilizer application on potato, winter rye, and oat yields; nutrient use efficiency; and soil fertility. *Arch. Agron. Soil Sci.* **2010**, *56*, 421–432. [CrossRef]
29. Zhang, L.; Su, W.; Tao, R.; Zhang, W.; Chen, J.; Wu, P.C.; Yan, Y.; Jia, R.; Larkin, D.; Lavelle, M.J.; et al. RNA sequencing provides insights into the evolution of lettuce and the regulation of flavonoid biosynthesis. *Nat. Commun.* **2017**, *8*, 2264. [CrossRef]
30. Coria-Cayupán, Y.S.; Sánchez de Pinto, M.a.I.; Nazareno, M.n.A. Variations in bioactive substance contents and crop yields of lettuce (*Lactuca sativa* L.) cultivated in soils with different fertilization treatments. *J. Agric. Food Chem.* **2009**, *57*, 10122–10129. [CrossRef]
31. Inkham, C.; Panjama, K.; Seehanam, P.; Ruamrungsri, S. Effect of nitrogen, potassium and calcium concentrations on growth, yield and nutritional quality of green oak lettuce. *Acta Hortic.* **2021**, *1312*, 409–416. [CrossRef]
32. Albornoz, F.; Heinrich Lieth, J. Over fertilization limits lettuce productivity because of osmotic stress. *Chil. J. Agr. Res.* **2015**, *75*, 284–290. [CrossRef]
33. Jin, N.; Jin, L.; Wang, S.; Li, J.; Liu, F.; Liu, Z.; Luo, S.; Wu, Y.; Lyu, J.; Yu, J. Reduced chemical fertilizer combined with bio-organic fertilizer affects the soil microbial community and yield and quality of lettuce. *Front. Microbiol.* **2022**, *13*, 863325. [CrossRef] [PubMed]
34. Arya, S.; Mahajan, M.; Jain, P. Non-spectrophotometric methods for the determination of Vitamin C. *Anal. Chim. Acta* **2000**, *417*, 1–14. [CrossRef]
35. Li, H.; Sun, Q.; Zhao, S.; Zhang, W. *Principles and Techniques of Plant Physiological Biochemical Experiment*; Higher Education Press: Beijing, China, 2000; pp. 195–197.
36. Sedmak, J.J.; Grossberg, S.E. A rapid, sensitive, and versatile assay for protein using Coomassie brilliant blue G250. *Anal. Biochem.* **1977**, *79*, 544–552. [CrossRef]
37. Cataldo, D.; Maroon, M.; Schrader, L.; Youngs, V. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Commun. Soil Sci. Plant Anal.* **1975**, *6*, 71–80. [CrossRef]
38. Lee, Y.P.; Takahashi, T. An improved colorimetric determination of amino acids with the use of ninhydrin. *Anal. Biochem.* **1966**, *14*, 71–77. [CrossRef]
39. Thomas, R.L.; Sheard, R.W.; Moyer, J.R. Comparison of conventional and automated procedures for nitrogen, phosphorus, and potassium analysis of plant material using a single digestion. *Agron. J.* **1967**, *59*, 240–243. [CrossRef]
40. Simko, L. Genetic variation in response to N, P, or K deprivation in baby leaf lettuce. *Horticulturae* **2020**, *6*, 15. [CrossRef]
41. Bottoms, T.G.; Smith, R.F.; Cahn, M.D.; Hartz, T.K. Nitrogen requirements and N status determination of lettuce. *HortScience* **2012**, *47*, 1768–1774. [CrossRef]
42. Bao, L. Significance of balanced fertilization based on long-term fertilizer experiments. *Better. Crops Int.* **1997**, *11*, 8–9.
43. Roberts, T.L. Improving nutrient use efficiency. *Turk. J. Agric. For.* **2008**, *32*, 177–182.