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Effects of Reduced Nitrogen with Bio-Organic Fertilizer on Soil Properties, Yield and Quality of Non-Heading Chinese Cabbage

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Citation: Qi, Y.; Jiang, F.; Zhou, R.; Wu, Y.; Hou, X.; Li, J.; Lin, W.; Wu, Z. Effects of Reduced Nitrogen with Bio-Organic Fertilizer on Soil Properties, Yield and Quality of Non-Heading Chinese Cabbage. *Agronomy* **2021**, *11*, 2196. https://doi.org/10.3390/agronomy11112196

Academic Editors: Othmane Merah, Purushothaman Chirakkuzhyil Abhilash, Magdi T. Abdelhamid, Hailin Zhang and Bachar ZEBIB

Received: 19 October 2021
Accepted: 28 October 2021
Published: 30 October 2021

Abstract: Fertilizer is extremely essential to increasing the yield of vegetables. However, excessively using fertilizers has had a negative impact on the yield and quality of vegetables as well as soil environment in recent years. Non-heading Chinese cabbage 'yellow rose' was applied to determine the influence of organic manure and inorganic fertilizer on the character of rhizosphere soil, the growth and quality of plants. There were five treatments: conventional fertilization (NF), a total nitrogen reduction of 20% (NF20), a total nitrogen reduction of 30% (NF30), a total nitrogen reduction of 20% with 100 kg·667 m^{-2} bio-organic fertilizer (BNF20) and a total nitrogen reduction of 30% with 200 kg·667 m^{-2} bio-organic fertilizer (BNF30). The results show that the content of nitrate nitrogen, organic matter in rhizosphere soil treated by BNF20 and BNF30, was significantly enhanced compared with NF. The yield, Vc and soluble protein of plants treated by BNF20 and BNF30 increased by 30.11%, 17.26%, 5.66% and 15.90%, 16.02%, 5.37%, respectively, compared with NF. On the contrary, the nitrate content significantly decreased in plants of BNF20 and BNF30 by 47.87% and 40.98% compared with NF. The significantly positive correlation was observed between nitrate nitrogen content in rhizosphere soil and the yield ($p < 0.05$). In conclusion, reduced nitrogen with bio-organic fertilizer can improve the yield and quality of 'yellow rose' cabbage by improving the quality of rhizosphere soil.

Keywords: nitrogen fertilizer reduction; bio-organic fertilizer; non-heading Chinese cabbage; yield; quality; rhizosphere soil

1. Introduction

The amount of chemical fertilizer (mineral fertilizer NPK) used in vegetable production in China is 3.3 times that which is used on other crops, and the total amount of nitrogen fertilizer applied for the open-field vegetables is 2.7 times the recommended amount. Excessive application of nitrogen fertilizer will not only reduce the yield and quality of vegetables [1] but also lead to a decrease in soil organic matter content, secondary salinization, acidification, accumulation of heavy metals and excessive nitrate content in edible parts [2–5]. Thereby, the replacement potential of nitrogen fertilizer reduction is enormous for the excessive use of chemical fertilizer [6].

Chemical fertilizer is the basis for a high yield of vegetables, while organic fertilizer is beneficial to the growth and quality of vegetables [7,8]. On the basis of properly reduced nitrogen fertilizer, the interaction of chemical and organic fertilizers could be strengthened, and the composition of plant nutrition structure was enhanced by using different types of organic fertilizers [9]. Bio-organic fertilizer is a new type of organic fertilizer formed by adding a variety of beneficial microbial communities, in which the unique microbial community can activate soil, enhance the physical and chemical properties of soil, increase soil biodiversity and improve soil enzyme activities [10,11]. The microorganisms could secrete substances to stimulate root and plant growth [12]. Moreover, microorganisms improve the quality of vegetables by increasing nitrate reductase activity to reduce nitrate content [13]. The rational application of fertilizer could also promote the accumulation of plant dry matter maintain or increase the yield of crops and reduce the dependence on fertilizers [14]. The combined application of chemical fertilizer with bio-organic fertilizer could be complementary to continuously increasing the yield and improving quality of crops.

Non-heading Chinese cabbage originated in China, which accounts for 30–40% of the vegetable multiple cropping area in the middle and lower reaches of the Yangtze River [15]. Nitrogen fertilizer is widely used in the production of non-heading Chinese cabbage. However, excessive application of nitrogen fertilizer gave rise to reduced yield and quality [16]. It also led to soil hardening, acidification and fertility decline, especially resulting in serious water pollution and eutrophication [17]. The rational application of fertilizers could improve the soil quality, yield and quality of non-heading Chinese cabbage. A non-heading Chinese cabbage variety, 'yellow rose' (*Brassica campestris* ssp. *chinensis* Makino), was used as the plant material. Our aim was to study the effects of reduced nitrogen with bio-organic fertilizer on plant growth, yield, quality of non-heading Chinese cabbage and rhizosphere soil characteristics. This study will provide theoretical support for scientific fertilization application of reduced nitrogen with bio-organic fertilizer in crop production.

2. Materials and Methods

2.1. Materials

The seeds of 'yellow rose' were provided by the key Chinese Cabbage Breeding Laboratory of Nanjing Agricultural University. The plants were cultivated in the field during 2019–2020 at the vegetable base of Agricultural Expo Garden at Jurong, Jiangsu, China (32° N, 119°12′ E). Chemical fertilizers (46% urea, 12% superphosphate and 52% potassium sulfate) were provided by Yuntianhua Co., LTD, Yunnan, China. A bio-organic fertilizer named 'No.1' (*Bacillus amyloliquefaciens* $2 \times 10^8 \cdot g^{-1}$ living bacteria count and 3-5-0.7 N-P-K) was provided by Lianye Biotechnology Co. LTD, Jiangsu, China.

The soil texture in the plowed layer (0–15 cm) was clay, with a pH of 4.83, an electric conductivity of 216.87 $\mu S \cdot cm^{-1}$, an organic matter content of 29.18 $g \cdot kg^{-1}$, an alkali-hydrolyzed nitrogen content of 178.50 $mg \cdot kg^{-1}$, a total nitrogen content of 1.50 $g \cdot kg^{-1}$, an available phosphorus 97.27 content of $mg \cdot kg^{-1}$ and an available potassium content of 190.00 $mg \cdot kg^{-1}$.

2.2. Experiment Design

Five treatments were set up: conventional fertilization (conventional fertilization was the average level of fertilization commonly used by farmers) (NF), a total nitrogen reduction of 20% (NF20), a total nitrogen reduction of 30% (NF30), a total nitrogen reduction of 20% with 1500 $kg \cdot ha^{-1}$ bio-organic fertilizer (BNF20) and a total nitrogen reduction of 30% with 2000 $kg \cdot ha^{-1}$ bio-organic fertilizer (BNF30). Each treatment was set up with three repetitions. There were fifteen plots at all. Each plots' area was 20 m $\times$ 1.5 m in a random block arrangement, and there were 480 plants in each repetition. 'Yellow rose' cabbage was transplanted with spacing of 25 cm $\times$ 25 cm. The specific fertilization situation of each plot is shown in Table 1. Fertilizers were applied once as a base fertilizer a week before transplanting. The second experiment was carried out with the rapid non-heading Chinese cabbage from April to May 2020, and the yield was determined.

Table 1. Fertilization situation in test plots.

Treatment	N kg·ha^{-1}	P_2O_5 kg·ha^{-1}	K_2O kg·ha^{-1}	No.1 kg·ha^{-1}
NF	193.2	45.0	45.0	-
NF20	154.5	45.0	45.0	-
NF30	135.2	45.0	45.0	-
BNF20	109.5	18.0	34.5	1500
BN30	45.2	18.0	34.5	3000

2.3. Determination Index and Method

2.3.1. Determination of Plant Rhizosphere Soil Characteristics

Rhizosphere soils were randomly collected from 5 to 10 cm depth with the top 5 cm soil layer removed to avoid exogenous disturbance. Fresh soils were stored at 4 °C for soil enzyme activity analyses. Air-dried and sieved (<2 mm) soils were used for chemical analyses. The growth stages of harvest were observed during early (30 days after colonization), middle (60 days after colonization) and late (90 days after colonization) harvest.

Electrical conductivity (EC) and pH of soil were determined by mixing soil with deionized water at 1:5 and 1:2.5 (*w*/*v*), respectively. Mixture was manually shaken for 30 min and the reading was taken using electrical conductivity meter (Leici DDS-307A, Shanghai, China) and pH meters (Spectrum pH-400, California, America) [18]. Soil organic carbon and total nitrogen content were determined by dry combustion at 900 °C using an elemental analyzer (Vario EL elemental analyzer, Hanau, Germany) [19].

Available P were extracted with sodium bicarbonate, and then determined using the molybdenum-blue method. Available K were extracted with ammonium acetate and then determined using a flame photometry [20]. Nitrate nitrogen content in soil was determined using continuous flow analyzer (BRAN + LUEBBE Auto Analyzer3, Hamburg, Germany) as described by Raigón (1992) [21].

The activity of soil urease (expressed as μg NH^{4+}-N g^{-1} soil 24 h^{-1}) and acid phosphatase (expressed as μg phenol g^{-1} soil 24 h^{-1}) were determined using the method descried by Sun [22]. Invertase (expressed as μg glucose g^{-1} soil 24 h^{-1}), was determined using the method descried by Guan (1986) [23]. FDA (fluorescein diacetate, expressed as μg fluorescein g^{-1} soil 24 h^{-1}) was determined using the method described by Taylor [24].

2.3.2. Growth, Quality, Photosynthetic Parameters and Yield Indexes

Six plants were randomly selected from uniform plants in each plot in order to measure plant height, maximum leaf length and width during three growing stages of 'yellow rose'. At the same period, three different plants' external green leaves were taken to determine the quality. At the harvest stage, external green, middle yellow and inner heart leaves were taken according to the different colors of the leaves (Figure S1) to determine the quality of leaves.

Photosynthesis was measured by Li-6400 (LI-COR Inc., Lincoln, NE, USA) from 9–11 am. The net photosynthetic rate (*P*n), stomatal conductance (*G*s), transpiration rate (*T*r) and intercellular CO_2 concentration (*C*i) were measured. The temperature was set at 25 ± 1 °C with 390 ± 10 μmol mol^{-1} CO_2 concentration and 1000 mmol·m^{-2} s^{-1} light intensity during the measurement.

Chlorophyll content was determined using the ethanol (95%) extraction colorimetric method [25]. Root vitality was determined using the TTC (triphenyltetrazolium chloride) method [26]. Nitrate content of leaves was determined using the salicylic acid colorimetric method [27]. Soluble protein content was determined using the Coomassie Brilliant Blue G-250 colorimetric method and VC (L-ascorbic acid, AsA) content was determined using the o-phenanthroline colorimetric method [28]. Total soluble sugar content was determined using anthrone colorimetry [29]. Economic benefit was calculated using the formula as total output value—production cost.

2.4. Statistical Analysis

Data analyses were performed using SPSS software (SPSS, Chicago, IL, USA) and Microsoft Excel 2010. The significance of differences between treatments was evaluated using Duncan's new multiple range test ($p < 0.05$). Duncan's new multiple range test (MRT) is a variant of the Student–Newman–Keuls method that uses increasing alpha levels to calculate the critical values in each step of the Newman–Keuls procedure. SPSS Pearson bivariate correlation analysis was used for the correlation analysis.

3. Results

3.1. Rhizosphere Soil Chemical Properties during the Whole Growing Stage

The chemical properties of rhizosphere soil under different treatments were different during the growing stages of 'yellow rose' cabbage (Table 2). In the early growth stage, the soil pH, conductivity, total nitrogen and nitrate nitrogen contents of BNF20 and BNF30 were significantly lower than NF. The available phosphorus and available potassium contents of BNF30 were 17.06% and 6.96% higher than NF, respectively. The conductivity and organic matter of NF20 and NF30 were significantly lower than NF. In the middle stage, the pH and total nitrogen contents of BNF20 were significantly higher than NF and NF20. The conductivity, nitrate nitrogen, available phosphorus and available potassium contents were significantly lower than NF and NF20. The total nitrogen content of BNF30 was significantly lower than NF and NF30. In the harvest stage, the conductivity, total nitrogen and nitrate nitrogen of BNF20 were significantly higher than NF and NF20.The pH, total nitrogen, organic matter and available phosphorus contents of the BNF30 treatment were all higher than NF and NF30. The contents of nitrate nitrogen and available potassium of NF20 and NF30 were significantly higher than NF.

Table 2. Effects of different treatments on chemical characteristics on rhizosphere soil of 'yellow rose' cabbage.

Sampling Time	Treatments	pH Value	Electric Conductivity $\mu s \cdot cm^{-1}$	Total Nitrogen $g \cdot kg^{-1}$	Nitrate Nitrogen $mg \cdot kg^{-1}$	Organic Matter $g \cdot kg^{-1}$	Available Phosphorus $mg \cdot kg^{-1}$	Available Potassium $mg \cdot kg^{-1}$
Early growth period	NF	5.17 ± 0.1 b	222.67 ± 2.08 a	1.49 ± 0.06 a	63.45 ± 2.82 b	30.40 ± 1.25 a	55.87 ± 0.42 c	263.33 ± 2.89 b
	NF20	5.01 ± 0.3 d	137.00 ± 2.65 d	1.62 ± 0.08 a	27.50 ± 0.90 e	27.80 ± 0.52 b	53.80 ± 1.78 c	235.00 ± 5.00 c
	NF30	5.24 ± 0.2 a	185.33 ± 3.51 c	0.44 ± 0.09 b	71.70 ± 1.31 a	27.71 ± 0.45 b	60.33 ± 0.95 b	263.33 ± 2.89 b
	BNF20	5.07 ± 0.2 c	186.33 ± 2.51 c	0.61 ± 0.13 b	48.15 ± 0.38 d	28.57 ± 0.09 b	59.73 ± 1.30 b	216.67 ± 2.89 d
	BNF30	4.99 ± 0.3 d	196.67 ± 2.89 b	0.69 ± 0.01 b	51.14 ± 1.02 c	28.84 ± 1.55 ab	65.40 ± 1.73 a	281.67 ± 2.89 a
Middle growth period	NF	4.87 ± 0.1 c	217.00 ± 1.73 a	1.20 ± 0.05 bc	49.44 ± 0.97 a	28.68 ± 0.86 a	64.40 ± 0.87 a	161.67 ± 2.89 c
	NF20	4.84 ± 0.1 c	167.00 ± 5.20 c	0.98 ± 0.06 cd	42.59 ± 0.82 b	28.07 ± 0.32 a	50.53 ± 0.81 d	223.33 ± 5.77 a
	NF30	4.94 ± 0.2 b	208.00 ± 1.73 b	1.38 ± 0.09 b	39.79 ± 0.75 c	28.04 ± 0.22 a	48.87 ± 1.50 d	218.33 ± 2.89 a
	BNF20	5.05 ± 0.4 a	120.00 ± 1.00 e	1.81 ± 0.14 a	24.27 ± 0.70 e	28.13 ± 1.09 a	52.80 ± 0.69 c	151.67 ± 2.89 d
	BNF30	4.82 ± 0.3 c	146.00 ± 1.73 d	0.77 ± 0.05 d	33.52 ± 0.54 d	28.79 ± 0.89 a	54.80 ± 0.72 b	185.00 ± 5.00 b
Harvest time period	NF	5.04 ± 0.2 bc	124.67 ± 2.89 d	0.96 ± 0.02 b	22.16 ± 0.27 d	29.39 ± 0.68 abc	52.33 ± 1.17 b	158.33 ± 2.89 e
	NF20	5.13 ± 0.3 a	119.00 ± 3.61 e	1.05 ± 0.09 b	25.17 ± 0.58 c	28.39 ± 1.11 c	50.07 ± 0.46 c	211.67 ± 2.89 b
	NF30	5.09 ± 0.4 ab	215.00 ± 3.00 b	0.90 ± 0.08 b	36.08 ± 0.65 b	30.29 ± 0.28 ab	44.60 ± 1.06 d	216.67 ± 2.89 a
	BNF20	4.98 ± 0.5 c	223.33 ± 2.89 a	1.54 ± 0.10 a	48.45 ± 0.44 a	29.01 ± 0.22 bc	50.00 ± 0.87 c	186.67 ± 2.89 d
	BNF30	5.16 ± 0.5 a	168.00 ± 1.00 c	1.78 ± 0.08 a	36.39 ± 0.99 b	30.67 ± 1.06 a	65.00 ± 0.87 a	195.00 ± 0.00 c

Note: NF is conventional fertilization, NF20 is 20% nitrogen reduction, NF30 is 30% nitrogen reduction, BNF20 is 20% nitrogen reduction + 100 kg No.1 bio-organic fertilizer, BNF30 is 30% nitrogen reduction + 200 kg No.1 bio-organic fertilizer. The values in the table are the average of three repetitions. Different small letters represent significant difference at 0.05 level by Duncan's test; the same below.

From the whole stage of growth, the pH of the soil made a trend of first decreasing and then increasing, except the BNF20. The content of organic matter of BNF20 and BNF30 decreased and then increased. The content of available potassium of NF, NF20 and NF30 showed a decreasing trend, while the content of available potassium of BNF20 and BNF30 decreased first and then increased.

BNF20 were significantly higher than NF and NF20.The pH, total nitrogen, organic matter and available phosphorus contents of BNF30 treatment were all higher than NF and NF30. The contents of nitrate nitrogen and available potassium of NF20 and NF30 were significantly higher than NF.

3.2. Soil Urease, Invertase, Acid Phosphatase, FDA Enzyme Activities of the Rhizosphere soil

The activities of rhizosphere soil invertase and acid phosphatase of the 'yellow rose' cabbage were improved by reduced nitrogen with bio-organic fertilizer (Figure 1). In the early growth period, the activities of sucrose and FAD enzymes with BNF20 and BNF30 were all significantly lower than NF. The activity of acid phosphatase was significantly higher than NF. The activities of urease of BNF20 were significantly higher than other treatments (Figure 1B,C). Activities of invertase, urease and FAD enzyme in NF20 and NF30 were all lower than NF (Figure 1B,D); in the middle growth period, the urease activity of BNF30 was significantly higher than NF30(Figure 1A); in harvest period, invertase and acid phosphatase activities of all the treatments were higher than NF. Invertase and acid phosphatase activity of BNF30 were the highest at 54.20% and 18.48% higher than NF, respectively, and invertase activity of BNF20 was higher than NF20 (Figure 1B,C).

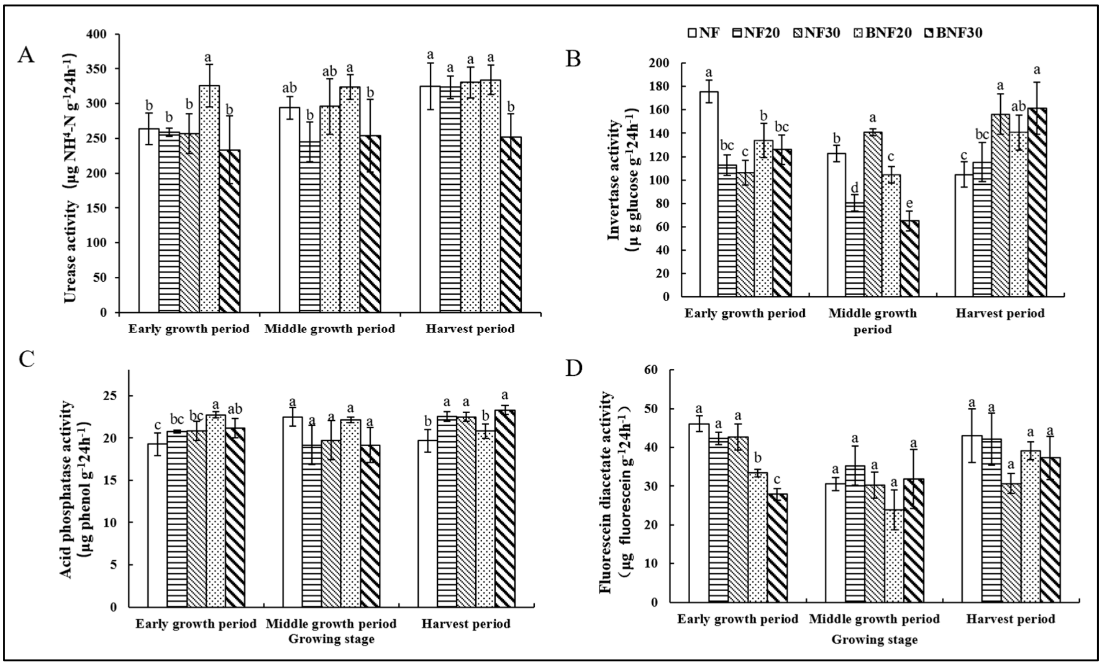

Figure 1. Soil-enzyme activities at the root soil of 'yellow rose' cabbage under different fertilization treatments; (**A**) urease activity; (**B**) invertase activity; (**C**) acid phosphatase activity; and (**D**) fluorescein diacetate activity. Note: At the same period, the results are the average of three different plant external green leaves; the same below.

From the whole growing stage of 'yellow rose' cabbage, overall, soil invertase and soil FDA activity showed decreased first and then increased.

3.3. Activity of the Root, Photosynthetic Pigment Content and Photosynthetic Characteristics of 'Yellow Rose' Cabbage

The root vitalities of BNF20 and BNF30 were higher than NF, and the root vitality of BNF20 was the highest, 13.82 $\mu g \cdot g^{-1} \cdot h^{-1} \cdot$ and 57.40% higher than NF (Figure S2). In the harvest period, compared with NF, BNF30 had the highest chlorophyll a, chlorophyll b, and the total chlorophyll content as significantly increased by 25.70%, 40.13% and 28.92%, respectively (Figure 2). The *P*n of all treatments were higher than NF, except NF30, while the Tr of NF20 were significantly higher than NF (Table S1).

3.4. Quality of 'Yellow Rose' Cabbage

In the middle growth period, the soluble protein content of BNF20 and BNF30 was significantly lower than NF20 and NF30 (Figure 3). The nitrate content of BNF30 was higher than other treatments, and the soluble protein content of NF20 and NF30 was significantly higher than NF.

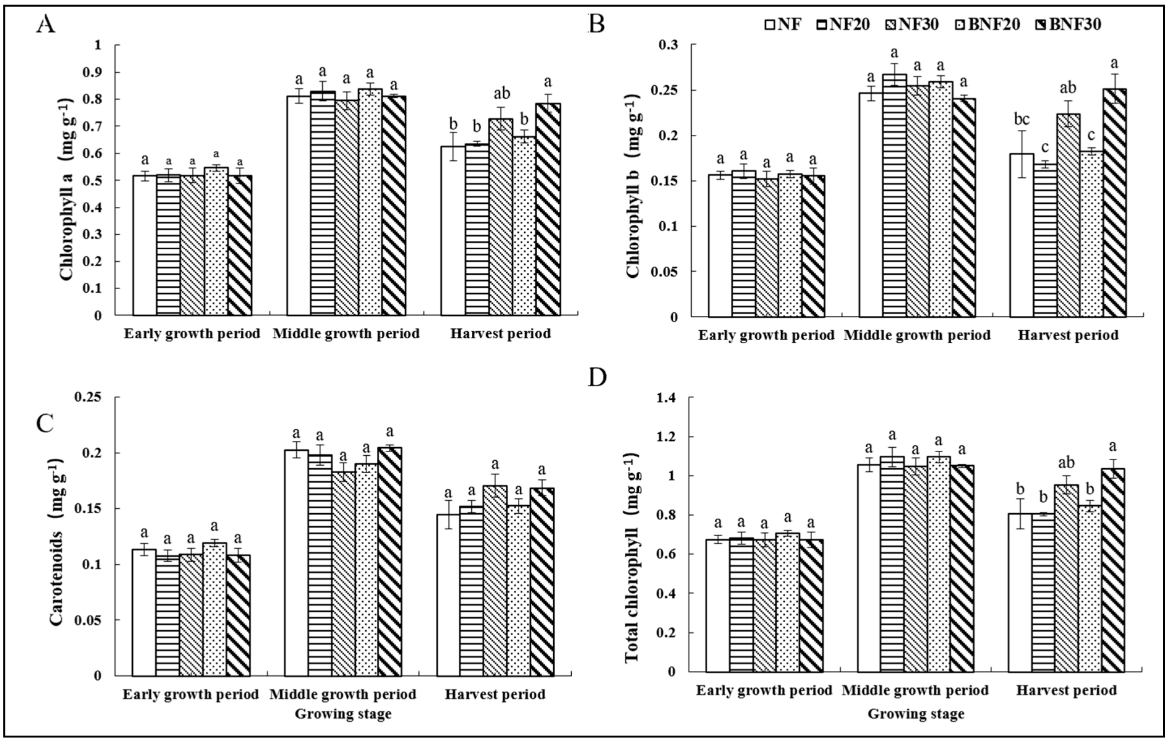

Figure 2. Effects of different fertilization treatments on leaf photosynthetic pigment content of 'yellow rose' cabbage at different growth stages: (**A**) chlorophyll a content; (**B**) chlorophyll b content; (**C**) carotenoids content; and (**D**) total chlorophyll content.

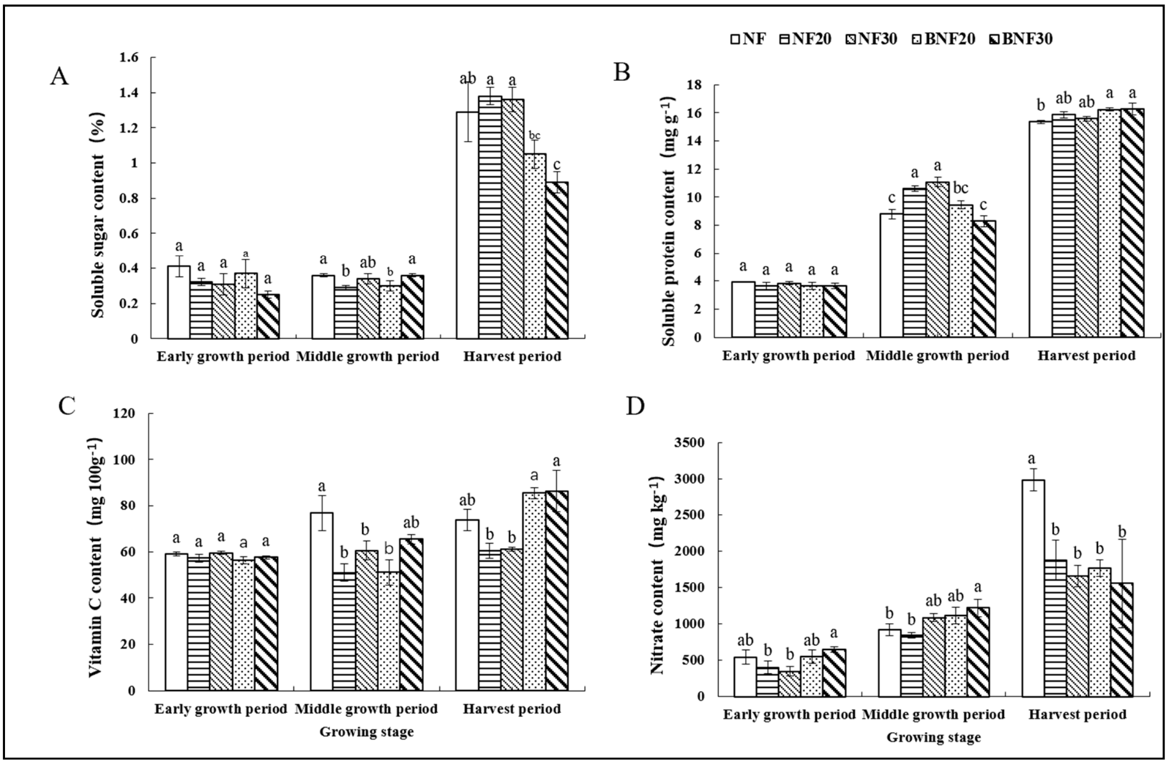

Figure 3. Effects of different fertilization treatments on leaf quality of non-heading Chinese cabbage at different growth stages: (**A**) soluble sugar content; (**B**) soluble protein content; (**C**) vitamin C content (**D**) nitrate content.

In the harvest growth period, the soluble sugar, soluble protein and VC content of BNF20 and BNF30 were higher than other treatments (Figure 3). On the contrary, the nitrate content of BNF20 and BNF30 was lower than other treatments (Figure 3). The content of soluble protein of BNF20 and BNF30 was significantly higher than NF (5.37% and 5.66%) (Figure 3B). The content VC of BNF20 and BNF30 was significantly higher than NF (16.02% and 17.26%) (Figure 3C). The nitrate content of BNF20 and BNF30 was 40.98% and 47.87% lower than NF, respectively (Figure 3D). From the whole growing stage, soluble sugar, soluble protein, VC and nitrate content showed an upward trend, while reduced nitrogen with bio-organic fertilizer could increase the content of soluble protein and VC and decrease the content of nitrate content of 'yellow rose' cabbage.

The harvest periods of 'yellow rose' cabbage under various fertilizer treatments, from external to internal quality, are shown in Table 3. The soluble sugar, soluble protein, VC and nitrate content of external green leaves was higher than the inner yellow leaves. The influence of external green leaves quality is shown in Figure 3. In the central yellow leaves, the soluble protein content of NF30 and BNF30 were significantly higher than NF, and the nitrate content was lower than NF with no significant difference. In the inner core leaf, the soluble protein content of BNF20 was significantly higher than other treatments, while the VC and soluble protein content of BNF30 were higher than NF. In addition, the contents of VC and nitrate in the leaves of 'yellow rose' cabbage decreased from outward to inward, while the soluble protein did not change significantly.

Table 3. Effects of different fertilization treatments on the quality of leaves in different positions of 'yellow roses' cabbage at harvesting stage.

Leaf Position	Treatment	Vitamin C Content (mg 100 g^{-1})	Soluble Protein Content (mg g^{-1})	Nitrate Content (mg kg^{-1})
External green leaves	NF	73.70 ± 4.46 ab	15.37 ± 0.11 b	2988.23 ± 151.22 a
	NF20	60.37 ± 3.21 b	15.87 ± 0.23 ab	1871.45 ± 276.04 b
	NF30	61.26 ± 0.86 b	15.58 ± 0.15 ab	1658.22 ± 150.50 b
	BNF20	85.51 ± 2.39 a	16.24 ± 0.11 a	1763.66 ± 114.10 b
	BNF30	86.42 ± 9.03 a	16.25 ± 0.42 a	1557.89 ± 610.00 b
Central yellow leaves	NF	46.35 ± 1.91 b	14.68 ± 0.16 c	1303.66 ± 331.16 a
	NF20	46.07 ± 2.78 b	14.74 ± 0.11 bc	1864.22 ± 445.65 a
	NF30	56.74 ± 0.68 a	15.64 ± 0.33 a	1223.95 ± 225.25 a
	BNF20	45.24 ± 2.33 b	15.16 ± 0.21 abc	1378.22 ± 433.89 a
	BNF30	45.3 ± 2.82 b	15.42 ± 0.16 ab	863.99 ± 24.35 ab
Inner heart leaves	NF	41.07 ± 1.25 b	15.11 ± 0.06 b	456.46 ± 33.26 bc
	NF20	40.99 ± 0.65 b	14.89 ± 0.12 b	761.92 ± 105.60 a
	NF30	50.57 ± 3.59 a	15.39 ± 0.20 b	629.59 ± 94.94 ab
	BNF20	38.64 ± 2.21 b	16.35 ± 0.48 a	490.03 ± 50.36 bc
	BNF30	42.47 ± 1.59 b	15.44 ± 0.03 b	324.32 ± 78.60 c

3.5. Growth, Yield and Economic Benefits of 'Yellow Rose' Cabbage

Compared with NF, in the early growth period, reduced nitrogen with bio-organic fertilizer (BNF20 and BNF30) significantly promoted the growth of 'yellow rose' (Table 4). NF20 and NF30 promoted the growth of 'yellow rose' in the early growth period. On the contrary, in middle growth period and harvest period the growth was restrained. In the harvesting period, the plant width length and width of BNF20 and BNF30 were significantly higher than NF. Compared with NF, the yield of BNF20, and BNF30 increased by 30.11% and 15.90%, respectively (Table 5), which was similar with the result on the yield of second year plant (Figure S3).

Table 4. Effects of different fertilization treatments on growth index of 'yellow rose' cabbage.

Period	Treatment	Plant Height (cm)	Leaf Length (cm)	Leaf Width (cm)	Crown Length (cm)	Crown Width (cm)
Early growth period	NF	9.44 ± 0.44 b	14.40 ± 0.42 c	13.49 ± 0.38 b	30.63 ± 0.72 b	27.69 ± 0.58 c
	NF20	9.50 ± 0.40 b	15.63 ± 0.49 ab	14.13 ± 0.40 ab	30.13 ± 0.58 b	28.88 ± 0.52 bc
	NF30	10.44 ± 0.50 ab	15.06 ± 0.39 bc	13.88 ± 0.41 ab	32.38 ± 0.46 b	30.00 ± 0.63 ab
	BNF20	11.06 ± 0.48 a	16.44 ± 0.27 a	15.00 ± 0.33 a	33.63 ± 0.32 a	31.75 ± 0.53 a
	BNF30	11.38 ± 0.42 a	16.50 ± 0.37 a	14.88 ± 0.49 a	33.38 ± 0.38 a	31.13 ± 0.58 a
Middle growth period	NF	16.44 ± 0.40 b	21.42 ± 1.01 a	18.49 ± 0.50 a	34.22 ± 0.99 b	33.69 ± 0.12 b
	NF20	16.50 ± 0.37 b	22.10 ± 1.04 ab	17.13 ± 0.37 b	32.06 ± 1.02 b	33.86 ± 0.21 b
	NF30	17.44 ± 0.90 ab	21.00 ± 0.88 b	17.87 ± 0.69 ab	33.42 ± 1.41 b	32.00 ± 1.21 b
	BNF20	18.06 ± 0.60 a	23.20 ± 0.74 a	18.00 ± 0.84 a	3604 ± 1.03 a	35.75 ± 1.60 a
	BNF30	18.38 ± 0.21 a	23.00 ± 0.89 a	18.40 ± 0.23 a	36.01 ± 0.98 a	34.65 ± 1.00 a
Harvest period	NF	19.75 ± 1.123 ab	22.17 ± 1.17 a	21.62 ± 1.30 a	37.58 ± 2.29 b	36.33 ± 2.25 c
	NF20	19.25 ± 0.52 b	19.00 ± 0.84 c	20.42 ± 1.11 a	38.00 ± 2.19 b	36.67 ± 1.50 bc
	NF30	18.67 ± 0.68 b	19.83 ± 1.03 bc	18.33 ± 1.33 b	37.00 ± 1.41 b	35.67 ± 1.37 c
	BNF20	20.67 ± 1.12 a	21.08 ± 1.28 ab	20.17 ± 0.98 a	40.83 ± 1.17 a	38.50 ± 1.38 ab
	BNF30	20.67 ± 1.33 a	22.33 ± 1.21 a	20.17 ± 0.93 a	42.17 ± 0.75 a	38.67 ± 1.21 a

Table 5. Effects of different fertilization treatments on economic benefit of 'yellow rose' cabbage.

Treatment	Yield (kg·hm^{-2})	Cost of Production ($·hm^{-2})	Total Production Value ($·hm^{-2})	Economic Benefit ($·hm^{-2})	Rank
NF	64,943.55 b	2921.53	50,737.15	47,815.62	4
NF20	65,275.88 b	2895.75	50,996.78	48,101.03	3
NF30	61,566.75 b	2813.72	48,099.02	45,285.30	5
BNF20	84,501.00 a	3298.13	66,016.41	62,718.28	1
BNF30	75,269.25 ab	3720.00	58,804.10	55,084.10	2

Due to the input of bio-organic fertilizers, the total production cost of BNF20 and BNF30 was higher than other treatments, but the total output value and the economic benefit of BNF20 and BNF30 was higher than others. BNF20 had an economic benefit increase of 30.17%, which was the highest rank.

3.6. Correlation between Rhizosphere Soil Chemical Properties with Yield, Quality of 'Yellow Rose' Cabbage by Reduced Nitrogen with Bio-Organic Fertilizer

The yield was positively correlated with soil nitrate nitrogen, and the correlation coefficient was 0.882 ($p < 0.05$) (Table 6). The soluble sugar content of 'yellow rose' was negatively correlated with the total nitrogen content of soil (correlation coefficient = −0.971, $p < 0.01$) (Table 6). The soluble protein content was positively correlated with the otal nitrogen ($p < 0.05$) (Table 6). The total chlorophyll content was positively correlated with soil organic matter and invertase activity with 0.911 and 0.903 correlation coefficients, respectively ($p < 0.05$) (Table 6).

Table 6. Correlation between rhizosphere soil nutrients with yield and quality of 'yellow rose' cabbage.

	Yield	Total Chloro-phyll	Soluble Sugar	Soluble Protein	Nitrate	VC	Root Activity	pH	EC	TN	NO_3^-	OM	AP	AK	Urea	Acid P	Invertase	FDA
Yield	1																	
Total chloro-phyll	0.177	1																
Soluble sugar	−0.617	−0.589	1															
Soluble protein	0.903 *	0.395	−0.786	1														
Nitrate	−0.666	−0.624	0.365	−0.714	1													
VC	0.572	0.329	−0.935 *	0.648	−0.085	1												
Root activity	0.776	−0.163	−0.438	0.82	−0.407	0.4	1											
pH	−0.282	0.544	−0.045	0.093	−0.366	−0.264	−0.04	1										
EC	0.617	0.416	−0.321	0.373	−0.575	0.295	−0.01	−0.413	1									
TN	0.731	0.528	−0.971 **	0.905 *	−0.488	0.873	0.625	0.102	0.288	1								
NO_3^-	0.882 *	0.371	−0.584	0.705	−0.65	0.556	0.388	−0.401	0.905 *	0.607	1							
OM	−0.104	0.911 *	−0.464	0.062	−0.29	0.302	−0.503	0.372	0.378	0.314	0.22	1						
AP	0.15	0.539	−0.8	0.52	−0.119	0.68	0.301	0.484	−0.258	0.774	0.002	0.437	1					
AK	0.231	0.359	0.22	0.245	−0.815	−0.507	0.126	0.503	0.297	−0.075	0.216	0.082	−0.263	1				
Urea	−0.102	−0.764	0.73	−0.493	0.335	−0.512	−0.156	−0.685	0.138	−0.707	−0.031	−0.633	−0.939 *	−0.021	1			
Acid P	0.481	0.903 *	−0.557	0.542	−0.819	0.322	−0.004	0.28	0.734	0.539	0.693	0.755	0.269	0.518	−0.5	1		
Invertase	0.221	0.705	−0.211	0.461	−0.835	−0.146	0.191	0.815	0.125	0.319	0.157	0.405	0.317	0.825	−0.581	0.675	1	
FDA	−0.116	−0.69	0.028	−0.035	0.618	0.157	0.431	−0.162	−0.742	0.025	−0.474	−0.679	0.236	−0.612	0.067	−0.824	−0.511	1
										−1	−0.5	0	0.5	1				

Note: * At the level of 0.05 (double-tailed), the correlation was significant. ** at 0.01 level (double-tailed), the correlation was significant. TN: soil total. nitrogen content, NO_3^-: Soil nitrate nitrogen content, OM: soil organic matter, AP: Available phosphorus, AK: soil available potassium, Acid P: Acid phosphatase activity.

4. Discussion

4.1. Effects of Reduced Nitrogen with Bio-Organic Fertilizer on the Chemical Properties of 'Yellow Rose' Cabbage Rhizosphere Soil

Reduced nitrogen with bio-organic fertilizer (BNF20 and BNF30) increased the chemical properties of rhizosphere soils (Table 2). A favorable soil environment was the prerequisite for a high yield and the quality of crops, and different nitrogen sources had significant effects on soil character [30–32]. At harvest period, the soil total nitrogen contents of NF20 and NF30 were significantly higher than others, while higher organic fertilizers substituted for BNF30 resulted in higher soil organic matter content (Table 2). Organic matter was essential as an overall indicator of soil quality [33]. Moreover, we found that reduced nitrogen with bio-organic fertilizer could improve the content of soil organic matter, available phosphorus and rapidly available potassium. Soil nutrients increased significantly with the increase of bio-organic fertilizer applied [14,34]. We suggested that a moderate amount of reduced nitrogen improved the soil carbon and nitrogen ratio, optimized the environment of the soil and increased the soil nitrogen effectively. We also found that the quality of 'yellow rose' was correlated with total nitrogen content of soil (Table 6).

At harvest period, reduced nitrogen with bio-organic fertilizer (BNF20 and BNF30) increased the activities of soil invertase and acid phosphatase (Figure 1), which was reported by Gou [35] and Wang [36]. Moreover, reduced nitrogen with bio-organic fertilizer could improve the root activity (Figure S2). The root activity of soybean and chili were improved by reduced nitrogen with bio-organic fertilizer [35,37]. The application of bio-organic fertilizer possibly stimulates enzyme secretion by roots [11].

However, the urease activity of BNF30 was significantly lower than NF. Using different fertilization had different effects on soil microenvironment and thus on soil enzyme activity [38]. We speculate that the difference between urease activity and other soil enzyme activities might be related to the amount of applied nitrogen fertilizer in Chinese cabbage.

4.2. Effects of Reduced Nitrogen with Bio-Organic Fertilizer on the Photosynthetic Characteristics and Quality of 'Yellow Rose' Cabbage

The soluble sugar and VC content of 'yellow rose' cabbage increased, while nitrate content decreased when the plants were applied by reduced nitrogen with bio-organic fertilizer (Table 3, Figure 3). Similar results were reported by Wang [39] on cabbages, by Brunetti [40] on tomato and by Yang [41] on cauliflower. The latest research shows that reducing nitrogen by 20–30% with bio-organic fertilizer had the best quality improvement effect on cabbage [42] and tomato [43]. Nitrogen was the key factor influencing the nitrate content in vegetables. The nitrate precursor was the synthesis of nitrosamines that endangered human body health by excessive intake, causing cancers of the digestive system [44]. The nitrate content of vegetables increased with the increase of nitrogen fertilizer used [45]. Maintaining quality and yield when reducing the application of nitrogen amount can directly reduce the risk of nitrate-related health problems.

We also found that the quality of 'yellow rose' cabbage increased overall during the whole growth period. The effect of reduced nitrogen with bio-organic fertilizer was optimal, which may be related to the fertility release potential of bio-organic fertilizers. Moreover, the VC and nitrate content of 'yellow rose' cabbage followed a downward trend from external leaves to the inner, soluble protein with no obvious change. Reduced nitrogen with bio-organic fertilizer had a better promotion on the quality of external leaves (Table 3). The difference may be due to the fact that the external leaves were mature leaves with a better accumulation effect of fertilizer than that of immature leaves.

Nutrient supply situation would affect the plant photosynthetic pigment content [46]. Insufficient or excessive nitrogen supply could also lead to a decrease in plant chlorophyll content. Nutrient suitable supply was beneficial to improve the efficiency of crop photosynthesis and increase the accumulation of dry matter basis in crop production [47]. Soil nutrients have certain effects on the plant photosynthetic pigment content; we found that the total chlorophyll content was positively correlated with soil organic matter and invertase activity (Table 6). At the harvest period, *P*n of BNF20 was the highest and the content of chlorophyll a, chlorophyll b and total chlorophyll of NF30 was the highest. Moreover, the photosynthetic pigment contents of BNF20, BNF30 were higher than those of NF20 and NF30. The results show that the application of bio-organic fertilizer was beneficial to increasing the photosynthetic pigment content of non-heading Chinese cabbage.

4.3. Effects of Reduced Nitrogen with Bio-Organic Fertilizer on the Growth and Yield of 'Yellow Rose' Cabbage

Nitrogen was involved in the formation and accumulation of plant dry matter and closely related to the growth, development and yield of crops [48]. The application of nitrogen fertilizer could improve the growth and development status of crops [49], thus laying a foundation for the realization of high yield. The application of bio-organic fertilizer could increase the yield of cabbage, cucumber, tomato, pepper, maize and so on [14,50–54].

However, neither excessive nor insufficient amount of nitrogen fertilizer was not conducive to the growth of non-heading Chinese cabbage [55], which led to the decrease of crop yield [56]. It was key to improve the quality and maintain yield under the application of reduced nitrogen with bio-organic fertilizer. In the experiment, the growth and yield of BNF20 and BNF30 were better than the other treatments (Tables 4 and 5), indicating that reduced nitrogen with bio-organic fertilizer was beneficial to improve the growth, yield and development of non-heading Chinese cabbage. *Bacillus amyloliquefaciens* consisted in BNF20 and BNF30. *Bacillus amyloliquefaciens* had satisfactory biocontrol effects on Fusarium wilt and growth-promoting abilities on pepper [51] and banana plant [50]. *Bacillus amyloliquefaciens* promoted the growth of cucumber seedings and reduced the rhizosphere bacterial diversity [57]. In the experiment, the effects of *Bacillus amyloliquefaciens* on soil microorganisms and the relationship between nitrogen fertilizer and *Bacillus amyloliquefaciens* need to be further studied.

5. Conclusions

Reduced nitrogen with bio-organic fertilizer (BNF20, BNF30) increased the content of total nitrogen, nitrate nitrogen, organic matter and available phosphorus. Meanwhile, it improved the activity of invertase and acid phosphatase of rhizosphere soil and enhanced the quality of rhizosphere soil. Reduced nitrogen with bio-organic fertilizer can promote plant growth and improve the photosynthetic characteristics of non-heading Chinese cabbage 'yellow rose'. At the harvest period, reduced nitrogen with bio-organic fertilizer significantly decreased nitrate content, increased soluble protein, VC content and the yield of 'yellow rose' cabbage. Overall, reduced nitrogen with bio-organic fertilizer increased the economic benefits with BNF20 as the optimal treatment.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11112196/s1, Figure S1: sampling location: the same part was taken respectively, the outer green leaves, the middle yellow leaves and the inner core leaves. Figure S2: effects of different fertilization treatments on activity of 'yellow rose' cabbage root. Figure S3: the yield of next year's plant. Table S1: effects of different fertilization treatments on the photosynthetic characteristics of 'yellow rose' cabbage (harvest period).

Author Contributions: Y.Q. and F.J. designed the experiments. Y.Q. and J.L. performed the experiments. Y.Q. and R.Z. analyzed the data. Y.Q. wrote the manuscript. F.J., Y.W., W.L., X.H. and Z.W. gave valuable comments on the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: We acknowledge The National Key Research and Development Program of China (2018YFD0201200, 2019YFD100190200), the Fundamental Research Funds for the Central Universities (KYZZ2021004), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), and the expert workstation of China Ministry of Science and Technology.

Institutional Review Board Statement: The study did not involve humans or animals.

Informed Consent Statement: Not applicable, for studies not involving humans.

Data Availability Statement: Data sharing not applicable.

Acknowledgments: We thank Mintao Sun and Xiaoting Wu for their valuable help.

Conflicts of Interest: The authors declare no conflict of interest.

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