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Effect of Different Fertilizers on Nitrogen Isotope Composition and Nitrate Content of *Brassica campestris*

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ABSTRACT: The effect of different fertilizers on the $\delta^{15}N$ value, nitrate concentration, and nitrate reductase activity of *Brassica campestris* and the $\delta^{15}N$ value of soil has been investigated through a pot experiment. The $\delta^{15}N$ mean value of *B. campestris* at the seedling stage observed in the composted chicken treatment (+8.65‰) was higher than that of chemical fertilizer treatment (+5.73‰), compost-chemical fertilizer (+7.53‰), and control check treatment (+7.86‰). There were significantly different $\delta^{15}N$ values ($p < 0.05$) between *B. campestris* cultivated with composted chicken manure treatment and with chemical fertilizer treatment. The similar results were also found at the middle stage and the terminal stage. The variation of $\delta^{15}N$ value in soil for different treatments was smaller than that of *B. campestris*, which was +6.71−+8.12‰, +6.83−+8.24‰, and +6.85−8.4‰, respectively, at seedling stage, middle stage, and terminal stage. With the growth of *B. campestris*, the nitrate content decreased in all treatments, and the nitrate reductase activity in *B. campestris* increased except for the CK. Results suggested that the $\delta^{15}N$ values of *B. campestris* and soil were more effected by the fertilizer than by the dose level, and the $\delta^{15}N$ value analysis could be used as a tool to discriminate the *B. campestris* cultivated with composted manure or chemical fertilizer.

KEYWORDS: *stable nitrogen isotope, fertilizer, nitrate, nitrate reductase, Brassica campestris*

INTRODUCTION

Consumers are becoming increasingly concerned about food quality and safety after some food incidents happened (e.g., mad cow disease, foot-and-mouth epidemic, Belgian dioxin scandal). The use of chemical fertilizers and pesticides is forbidden in organic food production, so consumers are prepared to pay more to purchase organic foods,[1,2] and there needs to be more research and technology to support consumer confidence in the growing organic food market.[3] Stable isotope analysis has been a useful tool to trace food origins and discriminate food authenticity.[4] Since the 1970s, there have been some reports about the $\delta^{15}N$ value of plants affected by different fertilizers.[5−9] The different fertilizers cause a change in the natural abundance nitrogen isotope signature in the plant and can be used to determine whether chemical fertilizer was added in organic production. However, most experiments were usually carried out with chemical fertilizer or organic fertilizer and seldom with chemical fertilizer combined with organic fertilizer. There was an overlap of $\delta^{15}N$ value between organic crops and conventional crops, and isotope analysis could only be used to determine whether chemical fertilizer had been singly applied.[11] Different fertilizer treatments had no significant influence on $\delta^{15}N$ values of cucumber and Chinese cabbage and affected the nitrate content as well as nitrate reductase activity.[12,13] Additionally, different fertilizer treatments could affect the nitrate content in crop. Nitrogen transfer, distribution, and fractionation were related to the activity of an enzyme like nitrate reductase, and the physiological mechanisms of plant could influence its nitrogen isotope composition.[14,15] The composition and fractionation of nitrogen in whole crop and its leaves were affected by the abundance of external nitrogen and physiological mechanisms, and the $\delta^{15}N$ value of whole crop had a positive correlation to the $\delta^{15}N$ value of nitrogen source when the nitrogen was limited during cultivation.[16] In China, it is common for conventional farmers to use organic manures combined with chemical fertilizer, and some farmers may claim their products are organic to get a higher price for their products. It may be more useful to find a tool to discriminate whether the two type fertilizers were used at the same time.

The current pot experiment was an attempt to study the effects of different fertilizer treatments on the $\delta^{15}N$ value, nitrate content, and nitrate reductase activity of *Brassica campestris* and the $\delta^{15}N$ value of soil. The ultimate goal of the experiment was to examine the correlation between the different fertilizer treatments and the $\delta^{15}N$ value, nitrate content, and nitrate reductase activity with a view to developing a tool to verify that organic cultivation practices had been followed.

MATERIALS AND METHODS

Soil and Fertilizers. A brown soil without any fertilization history was collected from an experimental farm of Qingdao Laoshan mountain (120°53′15″E, 36°22′10″N), China. The soil had a pH water (1:1) of 6.8, total N of 0.476 g kg^{-1}, available N of 100.1 mg kg^{-1}, available P of 38.8 mg kg^{-1}, available K of 90.0 mg kg^{-1}, and soil organic matter of 14.0 g kg^{-1}. The $\delta^{15}N$ value of total N was +6.91‰.

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Table 1. Fertilizer Treatments and Total Application Rate

treatments	rate		
	high dose (300 kg N ha^{-1})	middle dose (225 kg N ha^{-1})	low dose (150 kg N ha^{-1})
I composted chicken manure	71.3 g/pot chicken manure	53.5 g/pot chicken manure	35.6 g/pot chicken manure
II chemical fertilizer	2.90 g of urea, 21.2 g of $Ca(H_2PO_4)_2{\cdot}H_2O$, 1.97 g/pot K_2SO_4	2.17 g of urea, 15.9 g of $Ca(H_2PO_4)_2{\cdot}H_2O$, 1.48 g/pot K_2SO_4	1.45 g of urea, 10.6 g of $Ca(H_2PO_4)_2{\cdot}H_2O$, 0.98 g/pot K_2SO_4
III compost-chemical fertilizer (1:1)	35.6 g of chicken manure + 1.45 g of urea, 10.6 g of $Ca(H_2PO_4)_2{\cdot}H_2O$, 0.98 g/pot K_2SO_4	26.8 g of chicken manure + 1.08 g of urea, 7.95 g of $Ca(H_2PO_4)_2{\cdot}H_2O$, 0.74 g/pot K_2SO_4	17.8 g of chicken manure + 0.72 g of urea, 5.3 g of $Ca(H_2PO_4)_2{\cdot}H_2O$, 0.49 g/pot K_2SO_4
CK	no fertilizer application	no fertilizer application	no fertilizer application

Two types of fertilizers were chosen for the experiment. The organic fertilizer was chicken manure with high temperature compost treatment (total N of 12.2 g kg^{-1}, total P of 32.5 g kg^{-1}, total K of 11.1 g kg^{-1}, and total $\delta^{15}N$ of +13.58‰), and the chemical fertilizers were chosen with urea (total N of 460 g kg^{-1}, $\delta^{15}N$ of −1.34‰), calcium superphosphate (phosphorus P_2O_5 of 120 g kg^{-1}), and potassium sulfate (K_2O of 500 g kg^{-1}).

Pot Experiment. A pot experiment with *B. campestris* was conducted in a farm at Qingdao Academy of Agricultural Sciences, China. Three treatments were laid out in a completely randomized factorial design with three levels (300, 225, and 150 N ha^{-1}, respectively), each with three replications, which control check without fertilizer application (code: CK), composted chicken manure, fertilizer with urea application with calcium superphosphate and potassium sulfate, and compost-chemical fertilizer. The application rates were according to their content of chicken manure as shown in Table 1. The N, P, K as the basic manure was applied with the same rate on one occasion, except for CK, which had no application.

The soil was air-dried, then griddled through a 20 mm sieve, and placed into each pot (200 mm bottom diameter × 300 mm top diameter × 220 mm height) with 10 kg. The pots were prepared and watered on June 3, 2010, and the crop was planted with 20 in each pot after 3 days.

Sampling and Sample Preparation. Composted chicken manure samples, chemical fertilizer samples, and soil samples were sampled before the start of the experiment. During the experiment, *B. campestris* and soil were sampled at the seedling stage (30 days), middle stage (45 days), and terminal stage (60 days). The fresh samples of *B. campestris* were used to analyze the content of nitrate and the activity of nitrate reductase, and the dried soil sample and the dried *B. campestris* samples were used for $\delta^{15}N$ measurement. The chemical fertilizer sample was directly crushed into powder and sieved. The compost sample was first dried at 60 °C in an oven, then crushed, and sieved. Soil samples were air-dried, then passed through a 100 mesh sieve. The *B. campestris* samples for $\delta^{15}N$ were first dried in oven at 105 °C for 30 min, then air-dried at 70 °C, homogenized, passed through a 100 mesh sieve, and kept in a desiccator for testing.

Analytical Procedure. The content of nitrate was extracted in accordance with the Chinese National Standard Method GB/T5009.33-2003,[17] then measured by using ultraviolet radiation subtraction,[18] calculated, and expressed as μg of NO_3^- g^{-1} of fresh plant mass, and the procedure was as follows: fresh vegetable leaf sample (5.0 g) was ground and mixed with saturation borax solution (5.0 mL) and distilled water (100 mL) at volumetric flask and heated in boiling water, then the protein or organic matter of solution was deposited or absorbed after cooling and then filtered to test with UV + vis spectrophotometer (model UV-2500, Shimadzu), whose absorbance was read at 220 and 275 nm. The nitrate content was calculated with a standard curve established with $NaNO_3$ standard solution and expressed as μg of NO_3^-/g of fresh plant mass. The activity of nitrate reductase (NRA) was tested in vivo[19] as the following: about 0.5 g of sample was added to 25 mL tube containing 5 mL of KH_2PO_4 buffer (pH = 7.4) and 5 mL of KNO_3 (0.2 mol/L) and incubated at 30 °C for 30 min in dark. Afterward, 2 mL of reacting solution containing sulfanylamide and *N*-(1 naphthyl) ethylendiamine was added, and the formed NO_2 was measured using a UV/vis spectrophotometer (model UV-2500, Shimadzu) with the absorbance at 540 nm, expressed as μg of produced NO_2^- g^{-1} h^{-1}. About 180−280 μg of N contained in the samples of fertilizer, soil, or vegetable were weighed, wrapped with tin capsule, and then put into the autosampler with serial number for $\delta^{15}N$ measurement by Elemental Analyzer-Isotope Ratio mass Spectrometry (EA-IRMS, FLASH Elemental Analyzer 1112 combined with a CONFLO III, Thermo Finnigan DELTAplus XP, Thermo Fisher Scientific, United States). The EA-IRMS conditions were as follows: the temperature of the oxidation furnace and the reduction furnace set were at 1020 and 650 °C, respectively, and the rate of carrier gas flow was at 90 mL/min. The sample was first oxidized to N_2O, N_2O_2, and NO, which was subsequently reduced to N_2, then N_2 passed through water trap, and put into columns for separation. The separated N_2 was diluted with Conflo III and then detected for isotope ratio analysis by Thermo Finnigan DELTA^{plus} XP. The nitrogen isotope composition was calculated as

$$\delta^{15}N\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 1000 \qquad (1)$$

where *R* is the $^{15}N/^{14}N$ atom ratio of the sample or standard material. Ammonium sulfate, IAEA N_1 ($\delta^{15}N_{air}$ = 0.4‰), was used as the standard reference material. The analytical precision of the measurements was ±0.2‰, and reproducibility of the results was within ±0.2‰ for nitrogen.

Statistical Analysis. For statistical analysis, data from the experiment were first tested for homogeneity of variance and normality of distribution. Analysis of variance (ANOVA) was performed on all experimental variables using the general linear models procedure of the SPSS 17.0 package to assess treatment effects. When treatment effects were significant, means were separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

$\delta^{15}N$ Value of *B. campestris*. The mean value observed in the different composted chicken manure treatment (+8.65‰) at the seedling stage (30 days) was higher than that of chemical fertilizer treatment (+5.73‰), compost-chemical fertilizer treatment (+7.53‰), and CK (+7.86‰). For the high dose of 300 kg N ha^{-1} at 30 days, the $\delta^{15}N$ value of *B. campestris* observed in the composted chicken manure treatment (+8.86‰) was significantly different from that of the chemical fertilizer treatment (+6.07‰). There was a significant difference between the composted chicken manure treatment and the chemical fertilizer treatment at the middle dose and the low dose, respectively. However, the value observed in the compost-chemical fertilizer treatment was not significantly different from that of the composted chicken manure treatment and that of chemical fertilizer treatment at the high dose and the low dose. The differences in $\delta^{15}N$ value were not significant for all of the composted chicken manure treatment at different doses and also for the chemical fertilizer treatment at high dose and low dose. This indicated that different doses of the same fertilizer had less effect on the $\delta^{15}N$ value than different fertilizers did. However, the $\delta^{15}N$ values observed in the compost-chemical fertilizer treatment were significant at

Table 2. $\delta^{15}N$ Values of *B. campestris* for Different Treatments[a]

treatment		δ15N (‰) (mean ± SD)		
		seedling (30 days)	middle (45 days)	terminal (60 days)
CK	no fertilizer	7.86 ± 0.27 bB	8.38 ± 0.25 abB	11.67 ± 0.08 aA
composted chicken manure	high dose	8.86 ± 0.01 aA*	8.4 ± 0.31 abA	9.51 ± 0.61 bcA
	middle dose	8.77 ± 0.25 aB	8.81 ± 0.22 aB	10.77 ± 0.20 abA
	low dose	8.32 ± 0.11 aB	8.77 ± 0.20 aB	10.25 ± 0.15 bA
chemical fertilizer	high dose	6.07 ± 0.15 cB	6.8 ± 0.32 bAB	7.84 ± 0.24 cA
	middle dose	5.06 ± 0.40 dB	7.55 ± 0.17 bA	8.5 ± 0.07 cA
	low dose	6.07 ± 0.22 cB	7.33 ± 0.29 bB	8.91 ± 0.47 cA
compost-chemical fertilizer	high dose	8.66 ± 0.35 aAB	7.34 ± 0.15 bB	9.67 ± 0.37 bcA
	middle dose	7.69 ± 0.07 bB	8.33 ± 0.20 abB	10.15 ± 0.55 bA
	low dose	6.26 ± 0.13 cB	8.14 ± 0.88 abAB	9.5 ± 0.17 bcA

[a]Note that *lowercase letters indicate differences between different treatments at the same stage, and capital letters indicate differences of the same treatment between different stages.

Table 3. $\delta^{15}N$ Values of Soil for Different Treatments[a]

treatment		δ15N (‰) (mean ± SD)		
		seedling (30 days)	middle (45 days)	terminal (60 days)
CK	no fertilizer	6.91 ± 0.04 dA	6.83 ± 0.12 bA	6.85 ± 0.13 cA
composted chicken manure	high dose	8.12 ± 0.04 aA	8.19 ± 0.19 aA	8.36 ± 0.33 aA
	middle dose	7.89 ± 0.01 aA	7.8 ± 0.21 aA	8.4 ± 0.24 aA
	low dose	7.53 ± 0.08 bC	8.1 ± 0.0 aB	8.4 ± 0.08 aA
chemical fertilizer	high dose	6.76 ± 0.26 dA	7.35 ± 0.34 bA	7.27 ± 0.18 bcA
	middle dose	6.71 ± 0.02 dB	7.78 ± 0.35 aA	7.77 ± 0.06 aA
	low dose	7.43 ± 0.09 cA	7.28 ± 0.21 bA	7.81 ± 0.19 aA
compost-chemical fertilizer	high dose	7.57 ± 0.02 bB	8.01 ± 0.08 aA	7.8 ± 0.12 aA
	middle dose	7.7 ± 0.1 bA	8.24 ± 0.37 aA	7.69 ± 0.22 bA
	low dose	7.49 ± 0.02 bA	8.08 ± 0.18 aA	7.83 ± 0.33 aA

[a]Note that *lowercase letters indicate differences between different treatments at the same stage, and capital letters indicate differences of the same treatment between different stages.

different doses at 30 days. A possible cause for this result may be the introduction of compost-chemical fertilizer mixture, which influenced the nitrogen intake of *B. campestris*. The variation range (δ = value$_{max}$ − value$_{min}$) of $\delta^{15}N$ values for the composted chicken manure treatment and chemical fertilizer treatment was Δ0.54 and Δ1.01‰, respectively. However, for the compost-chemical fertilizer treatment, the variation range was wider (Δ2.4‰). So, the use of nitrogen isotope was more complex and difficult to test vegetables labeled as organic food when compost and chemical fertilizer were used simultaneously. There was a similar result that the average $\delta^{15}N$ values of *Brassica chinensis* was +19.72, +0.81, +12.78, and +6.55‰, respectively, for composted chicken manure treatment, chemical fertilizer treatment, compost-chemical fertilizer treatment, and CK.[20] The $\delta^{15}N$ values of pig compost and urea were +15.6 and −2.7‰, the $\delta^{15}N$ values of Chinese cabbage ranged from +9.4 to 14.9‰ and from +3.2 to +3.3‰, respectively, and the values for CK ranged from +6.8 to +7.7‰.[10]

The $\delta^{15}N$ values of *B. campestris* for different treatments had similar results at the middle stage (45 days) and the terminal stage (60 days) but significantly different between the composted chicken manure treatment and the chemical fertilizer treatment. However, the $\delta^{15}N$ values observed in the compost-chemical fertilizer treatment at different doses were significantly different at the seedling stage rather than the middle stage and the terminal stage. The $\delta^{15}N$ values of *B. campestris* were significantly different in the terminal stage when compared to that in the seedling stage and the middle stage as the values were increased with the crop growing. For the CK, the $\delta^{15}N$ values were +7.86, +8.38, and +11.67‰, respectively, for the three stages. We observed a 48.5% increase of $\delta^{15}N$ value for the CK, a 46.8% increase for the chemical fertilizer treatment, a 29.7% increase for the compost-chemical fertilizer treatment, and only a 17.6% increase for the composted chicken manure treatment from the seedling stage to the terminal stage. When our results are compared to those of previously published data by Choi et al., whose results show the $\delta^{15}N$ of different treatments for 30 days, corn plant was significantly different; however, the results also showed that difference was becoming smaller when the plant grew into the 70 day period.[21] The difference of different treatments was much significant at the seedling stage and became smaller at harvest.[10] That means the use of $\delta^{15}N$ testing to distinguish organic vegetable is much more suitable for crops with short growth periods.

Among the treatments of this pot experiment, the $\delta^{15}N$ value of *B. campestris* was significantly different only between chemical fertilizer treatment and compost treatment at seedling stage. There was also a report that the significant difference for $\delta^{15}N$ values of lettuce was between chemical fertilizer treatment and compost treatment with a similar pot experiment,[22] and these findings are consistent with our experimental results. However, the $\delta^{15}N$ value of lettuce changed slightly for the CK but tended to decrease for other treatments. So, the type of fertilizer other than doses had more influence on the $\delta^{15}N$ values; however, there is not a definitive threshold $\delta^{15}N$ value, which could be used to define the basis of a test to distinguish between organic and conventionally cultivated *B. campestris*.

Table 4. Nitrate Contents and NRA of *B. campestris* for Different Treatments[a]

treatment		nitrate content NO_3^- (mg kg^{-1}, mean ± SD)		nitrate reductase activity ($NaNO_2$ μg h^{-1} g^{-1}, mean ± SD)	
		seedling (30 days)	middle (45 days)	seedling (30 days)	middle (45 days)
CK	no fertilizer	4060 ± 485 bcd	2517 ± 28 ab	42.9 ± 11.0 a	22.9 ± 2.0 a
composted chicken manure	high dose	3774 ± 137 cd	3055 ± 196 ab	12.7 ± 11.0 bc	29.9 ± 12.0 a
	middle dose	3725 ± 264 cd	2656 ± 243 ab	3.7 ± 13.2 bc	19.5 ± 2.4 a
	low dose	3684 ± 78 cd	2499 ± 181 b	19.3 ± 3.5 b	27.1 ± 14.6 a
chemical fertilizer	high dose	4013 ± 200 bcd	2712 ± 739 ab	5.6 ± 5.0 bc	24.6 ± 8.4 a
	middle dose	4582 ± 557 ab	3139 ± 132 a	3.0 ± 1.1 bc	26.1 ± 11.3 a
	low dose	4468 ± 473 abc	2601 ± 228 ab	16.2 ± 13.0 bc	26.3 ± 4.5 a
compost-chemical fertilizer	high dose	4914 ± 530 a	2740 ± 342 ab	2.5 ± 1.6 c	30.7 ± 3.6 a
	middle dose	3305 ± 230 cd	2425 ± 284 b	6.7 ± 4.4 bc	23.1 ± 6.8 a
	low dose	4407 ± 711 abc	2462 ± 348 b	7.1 ± 8.7 bc	27.0 ± 10.4 a

[a]Note that *lowercase letters indicate differences between different treatments at the same stage.

$\delta^{15}N$ Value of soil. As the results showed in Table 3, the difference of $\delta^{15}N$ value in soil was quite insignificant ($P > 0.05$), which ranged from 6.71 to 8.12‰, 6.83 to 8.24‰, and 6.85 to 8.4‰, respectively, at seedling stage, middle stage, and terminal stage. At the seedling stage, the $\delta^{15}N$ value of soil for chemical fertilizer treatment was less than those of composted chicken manure treatment and compost-chemical fertilizer treatment. A similar trend was also found at other two stages. The variation range of $\delta^{15}N$ value for CK was the smallest (Δ0.08‰) among other treatments. We also observed the variation range for other treatments slightly increased, and the $\delta^{15}N$ value of planted crops and soil has a positive relationship. There was a report that the $\delta^{15}N$ value of total nitrogen in soil for compost treatment was higher than for chemical fertilizer treatment, +8.8 ± 2.0 and +5.9 ± 0.7%, respectively, and the $\delta^{15}N$ value of planted crops was +14.6 ± 3.3 and +4.1 ± 1.7%, respectively, with significant difference ($P < 0.05$), which possibly resulted from long-term compost treatment.[23] It has been observed in several studies that the $\delta^{15}N$ value of different types of nitrogen was changing with time, the $\delta^{15}N$ value of NH_4^+ in chemical fertilizer-treated soil increased from +2.9 to +18.9% due to nitrification after 6 weeks of water saturation; however, the $\delta^{15}N$ value of NO_3^- increased from +16.3 to +39.2% due to denitrification.[24]

At the early sampling stage, the $\delta^{15}N$ value of *B. campestris* was generally higher than that of the soil for CK treatment and compost treatment but lower for chemical fertilizer treatment. This trend was similar for the middle stage. At the terminal stage, however, the $\delta^{15}N$ value of *B. campestris* was higher than that of soil for all treatments, and the difference between the values of *B. campestris* and soil was Δ4.82‰ for CK, Δ1.79‰ for compost treatment, Δ2.0‰ for compost-chemical treatment, and only Δ0.8‰ for chemical treatment. Statistical analysis showed that the Pearson correlation coefficients (r) of $\delta^{15}N$ value between soil and crop for composted chicken manure treatment, chemical treatment, and compost-chemical fertilizer treatment were 0.991 (there is significant at the 0.01 level), 0.147, and −0.040, respectively, at the seedling stage, which were 0.424, −0.550, and −0.342 at the middle stage, respectively, and −0.809, −0.679, and −0.988 (this also is significant at the 0.05 level) at the terminal stage. The results did not show any strong correlation of $\delta^{15}N$ value between crop and soil for different treatments except for the composed chicken manure treatment at the seedling stage. The $\delta^{15}N$ value of vegetable was more affected by the exogenous chemical or compost fertilizer and less by the soil, due to a relatively small changing scope of $\delta^{15}N$ in the soil. There was a similar suggestion that exogenous nitrogen (from chemical fertilizer or compost) was absorbed by the plant as a priority as compared with the endogenous nitrogen in soil.[8] A conflicting argument is that the plant first absorbed the endogenous nitrogen in soil and then the exogenous nitrogen.[25]

Nitrate Content and Nitrate Reductase Activity of *B. campestris*. As shown in Table 4, the nitrate content of *B. campestris* varied according to different treatment doses at seedling stage (30 days). With high dose treatment, the nitrate content of *B. campestris* for compost-chemical fertilizer treatment was the highest (4914 mg kg^{-1}), higher than that of chemical fertilizer treatment (4013 mg kg^{-1}) and compost treatment (3773 mg kg^{-1}), whose nitrate contents had no significant difference, however. With middle dose treatment, the nitrate content of *B. campestris* for chemical fertilizer treatment (4582 mg kg^{-1}) was significantly higher than both compost treatment (2725 mg kg^{-1}) and compost-chemical fertilizer treatment (3305 mg kg^{-1}), and there was also no significant difference among other treatments. With the low dose treatment, the nitrate contents of *B. campestris* for chemical fertilizer treatment (4468 mg kg^{-1}) and compost-chemical fertilizer treatment (4407 mg kg^{-1}) were both higher than compost treatment (3684 mg kg^{-1}), and there was also no significant difference. The nitrate content of *B. campestris* at middle stage (45 days) has a similar trend for seedling stage but generally lower content than at seedling stage. The results proved that the nitrate content of *B. campestris* treated with chemical fertilizer or compost-chemical fertilizer treatment at seedling stage was higher than that with compost treatment due to the stronger nitrification process, which provided more nitrogen to the crop. As crop growing, the nitrate content decreased despite fertilizer treatment. The nitrate reductase activity of vegetable was obviously higher at the 45 days than at the 30 days but without significant difference between treatments.

The results showed that as the crop was growing, the nitrate content of the *B. campestris* gradually decreased while the nitrate reductase activity increased except for the CK. At the seedling stage, the nitrate contents had a positive correlation to the $\delta^{15}N$ value for the CK, compost, and chemical treatment ($R^2 = 0.7958$), and there was minor correlation at other stages and treatments. Previous studies have shown that there was a positive correlation between the $\delta^{15}N$ value with the nitrate content and nitrate reductase activity of cucumber.[12] As the our results were presented in Table 4, NRA in vegetable was significantly different for different treatments at the seedling stage. Nitrate reductase (NR) was a kind of inducible enzyme, whose activity suggested nutrition condition and nitrogen metabolization level of the crop, NRA was an endogenous factor affecting nitrate accumulation, but not the only one.[26] Therefore, the relationship between $\delta^{15}N$ value and nitrate reductase activity was more complex and needs to be further studied.

Summary. The results of pot experiment indicated different fertilizer treatments could effect the $\delta^{15}N$ value of *B. campestris* or soil and the nitrate content or nitrate reductase activity of *B. campestris*. There were significant differences between chemical fertilizer treatment and composted chicken manure treatment for the $\delta^{15}N$ value of *B. campestris*, and there was also an overlap of the value between the compost-chemical treatment and the composted chicken manure treatment or the chemical fertilizer treatment. The $\delta^{15}N$ values of *B. campestris* and soil were not significantly different at the different doses of the same fertilizer treatment. This suggested that the type of fertilizer other than the doses of fertilizer had more influence on the $\delta^{15}N$ values. Many researchers have suggested that nitrogen isotope could be used to determine whether chemical fertilizer had been used in organic production. However, there is an overlap of $\delta^{15}N$ value and no a threshold value for $\delta^{15}N$ that permits unequivocal differentiation between *B. campestris* cultivated under the organic, mixed, and conventional agricultural conditions described in these pot experiments.

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■ REFERENCES

(1) Batte, M. T.; Hooker, N. H.; Haab, T. C.; Beaverson, J. Putting their money where their mouths are: Consumer willingness to pay for multi-ingredient, processed organic food products. *Food Policy* **2007**, *32* (2), 145−149.

(2) Tranter, R. B.; Bennett, R. M.; Costa, L.; Cowan, C.; Holt, G. C.; Jones, P. J.; Miele, M.; Sottomayor, M.; Vestergaard, J. Consumers' willingness-to-pay for organic conversion-grade food: Evidence from five EU countries. *Food Policy* **2009**, *34* (3), 287−294.

(3) Siderer, Y.; Maquet, A.; Anklam, E. Need for research to support consumer confidence in the growing organic food market. *Trends Food Sci. Technol.* **2005**, *16* (8), 332−343.

(4) Ghidini, S.; Ianeri, A.; Zanardi, E.; Conter, M.; Boschetti, T.; Iacumin, P.; Bracchi, P. G. Stable isotopes determination in food authentication: A review. *Ann. Fac. Med. Vet. Di Parma* **2006**, *26*, 193−204.

(5) Kohl, D. H.; Shearer, G.; Commoner, B. Variation in ^{15}N in maize and soil following application of fertilizer nitrogen. *Soil Sci. Soc. Am. Proc.* **1973**, *37*, 888−892.

(6) Yoneyama, T.; Kouno, K.; Yazaki, J. Variation of natural ^{15}N abundance of crops and soils in Japan with special reference to the effect of soil conditions and fertilizer application. *Soil Sci. Plant Nutr.* **1990**, *36* (4), 667−675.

(7) Choi, W. J.; Ro, H. M.; Lee, S. M. Natural ^{15}N abundances of inorganic nitrogen in soil treated with fertilizer and compost under changing soil moisture regimes. *Soil Biol. Biochem.* **2003**, *35* (10), 1289−1298.

(8) Nakano, A.; Uehara, Y.; Yamauchi, A. Effect of organic and inorganic fertigation on yields, $\delta^{15}N$ values, and $\delta^{13}C$ values of tomato (Lycopersicon esculentum Mill. cv. Saturn). *Plant Soil* **2003**, *255* (1), 343−349.

(9) Nakano, A.; Uehara, Y. Effect of fertilizer and soil nitrogen on delta ^{15}N values of strawberry. *Bull. Natl. Inst. Veg. Tea Sci.* **2006**, *5*, 7−13.

(10) Lim, S. S.; Choi, W. J.; Kwak, J. H.; Jung, J. W.; Chang, S. X.; Kim, H. Y.; Yoon, K. S.; Choi, S. M. Nitrogen and carbon isotope responses of Chinese cabbage and chrysanthemum to the application of liquid pig manure. *Plant Soil* **2007**, *295* (1−2), 67−77.

(11) Bateman, A. S.; Kelly, S. D.; Woolfe, M. Nitrogen isotope composition of organically and conventionally grown crops. *J. Agric. Food Chem.* **2007**, *55* (7), 2664−2670.

(12) Yuan, Y. W.; Zhang, Z. H.; Zhao, M.; Xu, M. F.; Yang, G. L.; Zheng, J. C.; Wang, Q. Study on the effect of different fertilizer on the stable nitrogen isotope of soil, leaf and cucumber. *J. Nucl. Agric. Sci.* **2010**, *24* (1), 108−113 (in Chinese with English abstract).

(13) Yuan, Y. W.; Zhao, M.; Zhang, Z. H.; Yang, G. L.; Zheng, J. C.; Wang, Q. Effect of different fertilizer on the stable nitrogen isotope and nitrate of soil and Chinese cabbage. *J. Nucl. Agric. Sci.* **2011**, *25* (1), 138−143 (in Chinese with English abstract).

(14) Yoneyama, T.; Ito, O.; Engerlaar, W. M. H. G. Uptake, metabolism and distribution of nitrogen in crop plants traced by enriched and natural ^{15}N: Progress over the last 30 years. *Phytochem. Rev.* **2003**, *2* (1−2), 121−132.

(15) Evans, R. D. Physiological mechanisms influencing plant nitrogen isotope composition. *TRENDS Plant Sci.* **2001**, *6* (3), 121−126.

(16) Werner, R. A.; Schmidt, H. L. The in vivo nitrogen isotope discrimination among organic plant compounds. *Phytochemistry* **2002**, *61* (5), 465−484.

(17) GB/T5009.33-2010, China national food safety standard—Determination of nitrite and nitrate in foods.

(18) Luo, X. H.; Cai, X. J. Determination of Nitrate Content in Vegetables by UV-Spectrophotometric Method. *J. South China Univ. Trop. Agric.* **2004**, *10* (1), 13−16 (in Chinese with English abstract).

(19) Zhu, G. L.; Zhong, H. W.; Zhang, A. Q. *Plant Physiological Experiment*; Beijing University Press: Beijing, 1990; pp 123−125 (in Chinese).

(20) Liu, T. S.; Peng, T. R.; Wu, S. H.; Fan, J. H.; Lin, Y. W. Certification of organic vegetable by analyzing the nitrogen isotope composition of plant: A preliminary evaluation. *J. Taiwan Agric. Res.* **2009**, *58* (3), 169−175 (in Chinese with English abstract).

(21) Choi, W. J.; Lee, S. M.; Ro, H.-M.; Kim, K. C.; Yoo, S. H. Natural ^{15}N abundances of maize and soil amended with urea and composted pig manure. *Plant Soil* **2002**, *245* (2), 223−232.

(22) Šturm, M.; Maršić, N. K.; Lojen, S. Can $\delta^{15}N$ in lettuce tissues reveal the use of synthetic nitrogen fertiliser in organic production? *J. Sci. Food Agric.* **2010**, *91* (2), 262−267.

(23) Choi, W. J.; Ro, H. M.; Hobbie, A. Patterns of natural ^{15}N in soils and plants from chemically and organically fertilized uplands. *Soil Biol. Biochem.* **2003**, *35* (11), 1493−1500.

(24) Choi, W. J.; Ro, H. M.; Lee, S. M. Natural ^{15}N abundances of inorganic nitrogen in soil treated with fertilizer and compost under changing soil moisture regimes. *Soil Biol. Biochem.* **2003**, *35* (10), 1289−1298.

(25) Georgi, M.; Voerkelius, S.; Rossmann, A.; Graßmann, J.; Schnitzler, W. H. Multi-element Isotope Ratios of Vegetables from Integrated and Organic Production. *Plant Soil* **2005**, *275* (1−2), 93−100.

(26) Blom, Z. M. Nitrate accumulation in vegetables and its relationship to quality. *Ann. Appl. Biol.* **1989**, *115* (3), 553−561.