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Variations in the Natural ^{15}N Abundance of *Brassica chinensis* Grown in Uncultivated Soil Affected by Different Nitrogen Fertilizers

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ABSTRACT: To further investigate the method of using $\delta^{15}N$ as a marker for organic vegetable discrimination, the effects of different fertilizers on the $\delta^{15}N$ in different growing stages of *Brassica chinensis* (*B. chinensis*) grown in uncultivated soil were investigated with a pot experiment. *B. chinensis* was planted with uncultivated soil and different fertilizer treatments and then harvested three times in three seasons consecutively. For the spring experiments in the years of 2011 and 2012, the $\delta^{15}N$ value of *B. chinensis*, which increased due to organic manure application and decreased due to chemical fertilizer application, was significantly different ($p < 0.05$) with manure treatment and chemical treatment. The $\delta^{15}N$ value of vegetables varied among three growing stages and ranged from +8.6‰ to +11.5‰ for the control, from +8.6‰ to +12.8‰ for the compost chicken manure treatment, from +2.8‰ to +7.7‰ for the chemical fertilizer urea treatment, and from +7.7‰ to +10.9‰ for the compost−chemical fertilizer treatment. However, the $\delta^{15}N$ values observed in the autumn experiment of 2011 without any fertilizer application increased ranging from +13.4‰ to +15.4‰, + 11.2‰ to +17.7‰, +10.7‰ to +17.1‰, and +10.6‰ to +19.1‰, respectively, for the same treatments mentioned above. This result was not significantly different between manure treatment and chemical treatment. The $\delta^{15}N$ values of soil obtained in the spring of 2011 during three growing stages were slightly affected by fertilizers and varied in the range of +1.6‰ to +2.5‰ for CK, +4.7‰ to +6.5‰ for compost treatment, +2.1‰ to +2.4‰ for chemical treatment, and +2.7‰ to +4.6‰ for chemical−compost treatment, respectively. High $\delta^{15}N$ values of *B. chinensis* were observed in these experiments, which would be useful to supplement a $\delta^{15}N$ database for discriminating organic vegetables. Although there was a significant difference between manure treatment and chemical treatment, it was still difficult to discriminate whether a labeled organic vegetable was really grown without chemical fertilizer just with a fixed high $\delta^{15}N$ value, especially for the vegetables planted simultaneously with chemical and compost fertilizer.

KEYWORDS: *$\delta^{15}N$, stable nitrogen isotope, chicken manure, chemical fertilizer*

INTRODUCTION

Organic agriculture is a farming system designed to achieve sustainability in agriculture; the global organic market is increasing, and the market values were estimated to be about \$1.58 billion in China in 2008 and about \$59 billion worldwide in 2010.[1] Chemical fertilizers and pesticides are widely used in conventional agriculture, but they are not permitted for use in organic food production.[2] Though concrete evidence is lacking, consumers regard that organically produced foods may be more health-promoting than conventional foods due to enhanced activation of plant defense mechanisms.[3] Because of this fact, the market prices of organic fresh fruits and vegetables in the United States are higher than those of conventional ones with an average difference between 15% and 60%.[4] The average retail price for organic fruits and vegetables in the world is 50%−200% higher than the conventional one.[5] Therefore, consumers are generally required to pay more to purchase organic foods and will feel cheated if conventional crops grown with chemical fertilizers were mislabeled as organic.[6] On the basis of the abundant natural resources and enormous market demand, China has the second largest organic cultivation area.[7] However, food scandals have occurred from time to time such that Chinese consumers' confidence has been severely damaged.[8] Meanwhile, fake labels have proved be a headache for organic suppliers, government agencies, and the industries. There is an urgent need to enhance the inspection and certification of organic food.[9] Therefore, additional research is needed to increase consumer confidence by developing methods to test organic products for their authenticity.[10]

The possible use of nitrogen isotope analysis to discriminate whether a labeled organic food was produced with synthetic nitrogen is based on the hypothesis that the application of synthetic N fertilizers, whose $\delta^{15}N$ values are similar to that of air, will result in lower $\delta^{15}N$ values than those in plants grown using organic manure.[11] Different nitrogen source fertilizers

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Table 1. Fertilizer Treatments and Application Rates

treatments (n = 3)	application rate	
	low dose (75 kg N ha^{-1})	high dose (150 kg N ha^{-1})
composted chicken manure	18.7 g/pot (coded L−M)	37.4 g/pot (coded H−M)
chemical fertilizer	urea 0.72 g, $Ca(H_2PO_4)_2 \cdot H_2O$ 1.39 g, K_2SO_4 0.33g/pot (coded L−C)	urea 1.45 g, $Ca(H_2PO_4)_2 \cdot H_2O$ 2.78 g, K_2SO_4 0.67 g/pot (coded H−C)
compost−chemical fertilizer (1:1)	chicken manure 9.35 g + urea 0.36 g, $Ca(H_2PO_4)_2 \cdot H_2O$ 0.70 g, K_2SO_4 0.16 g/pot (coded L−M+C)	chicken manure 18.7 g + urea 0.72 g, $Ca(H_2PO_4)_2 \cdot H_2O$ 1.39 g, K_2SO_4 0.33 g/pot (coded H−M+C)
control	no fertilizer application (coded CK)	

had different N isotope ratios, with a range from −3.9‰ to 0.5‰ for synthetic fertilizers, 5.3‰ to 7.2‰ for animal manure, and 9.3‰ to 20.9‰ for compost manure,[12] and the mean value of δ^{15}N was 0.2‰ for synthetic fertilizers and 8‰ for organic manure.[13] The δ^{15}N value of plants is affected by many factors such as the timing of application and the chemical form of synthetic fertilizer,[14] form of manure,[15,16] growth stages,[14,17] and irrigation water.[13]

The δ^{15}N values in different plants were significantly different between organic and conventional production and may vary with growth periods. The mean δ^{15}N values of products from organic and conventional agriculture vary with mean values 8.1‰ and −0.1‰ for tomatoes, 7.6‰ and 2.9‰ for lettuces, and 5.1‰ and 4.7‰ for carrots.[18] The δ^{15}N values of leaves and grains of maize grown with composted pig manure were also significantly (P < 0.05) higher than those grown with urea even after 70 days of growth,[14] and the δ^{15}N of maize increased for urea or decreased for composted manure with time, probably because of isotope fractionation accompanied by N losses and an increased uptake of soil-derived N by the maize.[14] Cabbage treated with compost had a higher δ^{15}N value (>9.0%) than that treated with urea (<1.0%). Further, specific δ^{15}N signals of basal N inputs were detected in the outer parts of cabbage formed in the early growth stage, while those of additional N inputs were detected in the inner parts of cabbage formed in the latter growth stage.[19] Different parts of the plant also had different δ^{15}N values, such as roots, stems, leaves, and grains for maize;[14] root, shoot, unpolished grain, and polished grain for rice;[20] and fruits and leaves for cucumber.[21] Thus, for these reasons it could be difficult to identify a certain value to discriminate organic food from conventional ones.

Although some reports indicated that the δ^{15}N of vegetables or crops differed between organic and conventional agriculture, the conclusions were drawn either from the results of a single cultivating experiment for carrots, tomatoes, lettuces,[13,22,23] maize,[14] Chinese cabbage,[19,22] cabbage, and onions,[22] or from statistical data on commercial products such as paprika,[22] tomatoes,[18,22] lettuces, and carrots,[18] or from a crop rotation experiment for canola, barley, and wheat for four years[15] and lettuces, onions, and cabbages for three years.[24] Particularly, as far as we know, no study has been published on investigating the variations of the δ^{15}N value of a single crop in different seasons.

The δ^{15}N values between organic crops and conventional crops overlapped, most probably due to ^{15}N enrichment of plant-available N derived from synthetic fertilizer through N loss via NH_3 volatilization and denitrification with time after fertilization in soil.[17,18,22,23] In our previous study, the overlapped δ^{15}N values were observed in *Brassica campestris* with combined application of organic and synthetic fertilizers.[17] Some reports drew a conclusion that ^{15}N isotope analysis could only be used to determine whether chemical fertilizer had been singly applied.[18,23] The chemical fertilizer combined with composted manure as the basal application was very common in practice, but there are relatively limited reports on the application of ^{15}N isotope analysis to identify the use of synthetic fertilizers in organic farming, so the ^{15}N technique is worth being further studied under the fact that there are currently no other better and reliable techniques available.

Because of the increasing demand on food in China, natural soils such as pasture and forest are converting to agricultural soils. Up to now, most of the relevant studies have been conducted with agricultural soils cultivated for a long time with chemical fertilization, which may have severe effects on the δ^{15}N of plants. It is very common for conventional agriculture to apply chemical fertilizer and composted manure as basal fertilizer, which could increase the δ^{15}N of plant.[17] It is difficult to discriminate if a vegetable with a high δ^{15}N value was really organic, because chemical fertilizer could be used during 2 years of organic conversion. Through a well-schemed experiment, the purpose of this study is to investigate the dynamics of the δ^{15}N value in a single crop with Chinese cabbage (*B. chinensis*) in different seasons with different fertilizer treatments in an uncultivated soil. This result would explore the potential of applying ^{15}N isotope analysis to discriminate the authenticity of organic food, and further contribute to the δ^{15}N database developed.

MATERIALS AND METHODS

Soil and Fertilizers. A brown soil without any fertilization history was collected from a site of Laoshan Mountain, China (120°53′13″ E, 36°22′10″ N), with temperate marine climate. The soil has a pH in distilled water (1:1) of 6.8, soil organic matter of 7.4 g kg^{-1}, total N of 0.676 g kg^{-1}, available N of 65.4 mg kg^{-1}, available P of 8.9 mg kg^{-1}, available K of 75.0 mg kg^{-1}, and a total δ^{15}N value of 6.9‰. Because there is no available standard for NPK content evaluation in organic soil in China, instead we referred to the Green food standard−Environmental quality for production area issued by Ministry of Agriculture, China, for the evaluation.[28] The result showed that the NPK content of the soil is low (high, median, and low).

Two types of fertilizer were chosen for this experiment. The organic fertilizer was chicken manure with high-temperature compost treatment (total N of 20.4 g kg^{-1}, P_2O_5 of 40.0 g kg^{-1}, K_2O of 24 g kg^{-1}, δ^{15}N of 13.0‰). The chemical fertilizers contained urea (total N of 460 g kg^{-1}, δ^{15}N of −1.2‰), calcium superphosphate (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. chinensis*, which has a short and fast growing period and can be planted for 2−3 seasons with continual harvest during its entire growing process, was successively conducted in the spring of 2011, the autumn of 2011,and the spring of 2012 at an erimental field of Qingdao Academy of Agricultural Sciences, China. Three treatments with two levels were conducted: for organic treatment, composted chicken manure (code L−M for low dose and H−M for high dose); for chemical treatment, urea application combined with calcium superphosphate and potassium sulfate (N/P/K = 2:1:1) (code L−C for low dose and H−C for high dose); for compost−chemical treatment, equal parts organic N, chemical N, and potassium sulfate (code L−M+C for low dose and H−M+C for high dose). The control was given no additional fertilizer (code CK). Fertilizer applications equivalent to 75 and 150 kg N ha^{-1} were made for each fertilizer type. Three treatments were employed in a completely randomized factorial design in triplicate.

The spring experiments of 2011 and 2012 were conducted according to the application rates shown in Table 1. After the spring experiment, the autumn experiment of 2011 was conducted in the same pot without any further application to explore the effect of previously added fertilizer in the soil. The soil was air-dried and griddled through a 50 mm sieve, and then 10 kg was thoroughly mixed with fertilizer as a basal application then placed into each plastic pot (200 mm bottom diameter × 300 mm top diameter × 220 mm height, ∼44 L in volume). Sixty-three pots were prepared 5 days before the spring and autumn experiments of 2011 and the spring experiment of 2012, and 20 seeds were planted in each pot on May 23 and August 25, 2011, and May 8, 2012. During the experiment, the vegetables were watered on demand with tap water containing NO_3^- of 33.0 mg L^{-1}.

Sample Collection and Preparation. Composted chicken manure sample and chemical fertilizer sample were taken before the start of the experiment. The above-ground parts of *B. chinensis* of each treatment were sampled from three pots at the seedling stage (∼20 plants per pot), middle stage (∼10 plants), and terminal stage (∼6 plants), which were conducted on the dates of June 14 (25 days after seeding, DAS 25), June 23 (DAS 31), and June 30 (DAS 38) for the spring experiment of 2011; September 19 (DAS 26), September 29 (DAS 36), and October 9 (DAS 46) for the autumn experiment of 2011; and June 4 (DAS 26), June 15 (DAS 37), and June 27 (DAS 49) for the spring experiment of 2012, respectively. To investigate the relationship between $\delta^{15}N$ of soil and plant, a portion of the soil sample was collected during the spring experiment of 2011 as the vegetable sampling time.

The urea fertilizer sample was directly crushed into powder and sieved. The compost sample was first dried at 60 °C in an oven and then crushed and sieved. The soil samples were air-dried and then homogenized and passed through a 100-mesh sieve. The *B. chinensis* samples were heated at 105 °C in an oven for 30 min to inactivate all enzymes and then dried at 70 °C, homogenized, and passed through a 100-mesh sieve. All the prepared samples were kept in desiccators before analysis.

Analytical Procedures. Samples of fertilizer or vegetable containing ∼200−400 μg of N were weighed into tin capsules and then automatically analyzed for their $\delta^{15}N$ and %N values using elemental analyzer−isotope ratio mass spectrometry (EA-IRMS, Elementar Vario PYRO cube equipped with Isoprime100, Isoprime Ltd., England). The EA-IRMS conditions were as follows: the temperature of the oxidation furnace and the reduction furnace were set at 1020 and 650 °C, respectively, and the rate of carrier gas flow was set at 230 mL min^{-1}. The nitrogen isotope composition was calculated as

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

where R is the ratio of $^{15}N/^{14}N$ and standard refers to the ratio for atmospheric N_2. Pure N_2 ($\delta^{15}N$ = 0‰) served as the reference gas. IAEA-N_1 (ammonium sulfate, $\delta^{15}N$ = 0.4‰ ± 0.2‰) was used as the standard reference material. The analytical precision and reproducibility of the measurements for $\delta^{15}N$ were better than ±0.2‰.

Statistical Analysis. For statistical analysis, data 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 10.0 package (SPSS Inc., Chicago, IL, USA) to assess treatment effects. When treatment effects were significant, means were separated by Duncan's multiple range test.

■ RESULTS AND DISCUSSION

Variations in δ^{15}N Value of *B. chinensis* with Different N Source and Rate in Spring of 2011. For the spring experiment of 2011 (as shown in Figure 1), the $\delta^{15}N$ values of

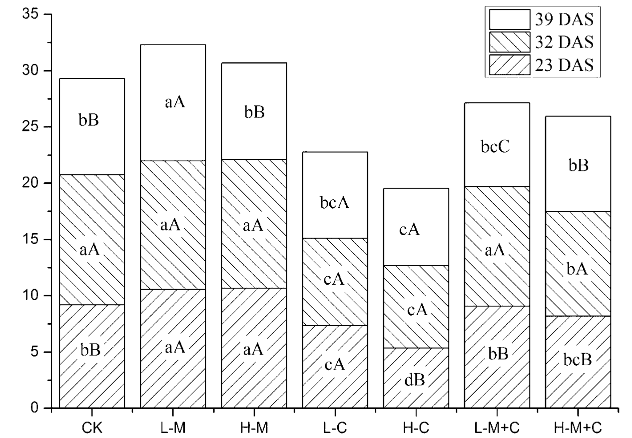

Figure 1. Variation in $\delta^{15}N$ of *B. chinensis* for the spring experiment of 2011. Treatment codes are described in Table 1. The same lowercase letter in the bars indicate no significant difference between different treatments at the same stage, and the same capital letters indicate no significant difference between different stages for the same treatment (α = 0.05 level).

B. chinensis receiving compost manure at 23 DAS were 10.6‰−10.7‰, significantly higher (p < 0.05) than that of the treatment L−C and H−C (5.4‰−7.4‰), L−M+C and H−M+C (8.2‰−9.1‰), and CK (9.2‰), which reflected the $\delta^{15}N$ of chicken compost manure (13.0‰), chemical fertilizer (−1.2‰), and soil N of CK (2.5‰). Temporal variations of $\delta^{15}N$ in the plant could be partially attributed to changes in the concentration and $\delta^{15}N$ value of soil inorganic N that is available for plant uptake. Figure 1 shows that the $\delta^{15}N$ values of *B. chinensis* became higher at DAS 32 for compost treatment (14.4‰), for chemical treatment (7.3‰−7.7‰), for compost−chemical treatment (9.3‰−10.6‰), and for CK (11.5‰). The $\delta^{15}N$ value of the vegetable increased during the later stage of growth, attributable to a decrease in the availability of the synthetic fertilizer over time and an increasing contribution of natural soil nitrogen to plant total nitrogen.[13] However, the value became lower at 39 DAS for compost treatment (8.6‰−10.3‰), for chemical treatment (6.9‰−7.7‰), for compost−chemical treatment (7.4‰−8.5‰), and for CK (8.6‰). There was also a similar report that the $\delta^{15}N$ value of *Brassica campestris* became lower at harvest time (60 DAT) for all treatments except for split additional manure.[19] A previous report also indicated the trend that the $\delta^{15}N$ of corn growing from 30 to 70 days varied from 7.7‰ to 6.7‰ for compost treatment, from 1.0 to 6.0‰ for chemical treatment, and from 4.5‰ to 6.1‰ for compost−chemical treatment.[14] A possible reason for this was that NO_3^- is the most abundant N for plant uptake when compost with enriched ^{15}N was added in upland soils,[25] and more ^{15}N-enriched NH_4^+ and NO_3^- produced by nitrification were assimilated with time.[19] The $\delta^{15}N$ values of *B. chinensis* for the same treatment generally did not significantly differ between the low and high doses. The possible reasons for this were that when excess compost or chemical fertilizers were added in the soil, the mineralized N would be the dominant source for plant-N. It is possible that this source exceeds the uptake ability of the plant, so the $\delta^{15}N$ would not be changed even though more N was still available for the plant.[26] However, we also observed exceptions for L−C (mean value of 7.4‰) and H−C (5.4‰) at 23 DAS, L−M+C (10.6‰) and H−M+C (9.3‰) at 32 DAS, and L−M (10.3‰) and H−M (8.6‰) at 39 DAS, and the $\delta^{15}N$ of *B. chinensis* for the compost−chemical treatment (8.2‰−9.1‰) was similar to that of CK. Reasons for this could be that the varied microbial immobilization ability of N leads to different mineralizations of fertilizers in soil, but this hypothesis needs to be further studied. There was a significant difference ($p < 0.05$) between the composted manure and the chemical fertilizer for three growth times with the exception of H−M and L−C at 39 DAS; the reasons could be that more N was lost and mineralization at 39 DAS resulted in the small difference of $\delta^{15}N$ between H−M and L−C. The difference of different treatments was large at 23 DAS but became smaller at 39 DAS, which is a trend that was similar to the previous reports.[14,19,20,23,26] The results of this experiment indicated that the $\delta^{15}N$ values of *B. chinensis* were affected by the addition of fertilizers, and that *B. chinesis* has a higher $\delta^{15}N$ value than that of the N source for chemical treatment and for CK but not for compost treatment.

There was an overlap value without a significant difference between H−M (11.4‰) or L−M (11.4‰) and L−M+C (10.6‰) at 32 DAS, and between H−M (8.6‰) and L−M+C (7.4‰) or L−M+C(8.5‰) at 39 DAS, which was also confirmed by the previous reports for vegetables of *Brassica campestris*,[17] tomatoes,[18] lettuces,[18,22,23] onions, cabbages, and Chinese cabbages.[22] Thus, $\delta^{15}N$ value could be used as a rough organic marker only in the case of single fertilizer application.[23] It is necessary to employ a statistical methodology to classify a randomly analyzed "off the shelf" sample as organic/conventional.[22]

Variations in $\delta^{15}N$ of *B. chinensis* without Fertilizer Application in Autumn of 2011. In the autumn experiment of 2011, no additional fertilizer was applied to any pot after the spring experiment. Although the $\delta^{15}N$ of the crop may be more affected by the presence of fertilizers, the value could also increase without the addition of fertilizer due to fractionation of N in previous fertilizers.[15] Compared with the results of the spring experiment of 2011, the $\delta^{15}N$ value of *B. chinensis* rose especially for CK, L−C, H−C, and H−M+C due to more N mineralized dominantly in soil with a similar amount of $\delta^{15}N$ for plant uptake. However, no significant difference was observed between the compost treatment and the chemical treatment (shown in Figure 2). For the autumn experiment of 2011, the $\delta^{15}N$ mean value for CK increased significantly to 13.4‰ at 26 DAS, which was higher than that of the spring experiment of 2011 with a 45.6% increase. The $\delta^{15}N$ mean values for the L−C (11.5‰), H−C (10.7‰), and H−M+C (10.6‰) were also higher than those of the same treatment in spring 2011 with increases of 55.4%, 98.1%, and 51.4%, respectively. However, the $\delta^{15}N$ mean values for L−M (11.2‰), H−M (13.4‰), and L−M+C (11.9‰) at 26 DAS increased slightly with increases of 6.7%, 25.2%, and 14.4%, respectively. Our result was in agreement with the previously reported canola case, where the $\delta^{15}N$ of canola was about 5.5‰ in the first year, 6.6‰ in the third year with hog manure, and 8.5‰ in the fourth year without hog manure application.[15] This could be explained by the fact that more ^{15}N were enriched due to N lost or fractionation in soil after the spring

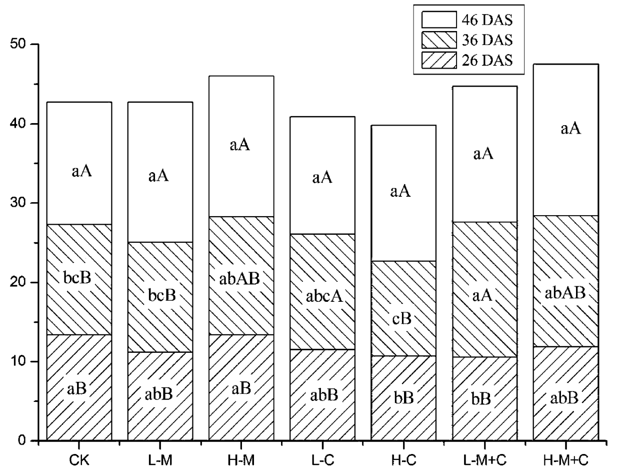

Figure 2. Variation in $\delta^{15}N$ of *B. chinensis* for the autumn experiment of 2011. Treatment codes are described in Table 1. The same lowercase letter in the bars indicate no significant difference between different treatments at the same stage, and the same capital letters indicate no significant difference between different stages for the same treatment (α = 0.05 level).

experiment and were absorbed by the vegetable in the autumn experiment.

With the growth of *B. chinensis*, the $\delta^{15}N$ mean value continued to be higher and was maintained at a high level with a range of 12.0−17.0‰ at 36 DAS and 14.8‰−19.1‰ at 46 DAS for all treatments (shown in Figure 2) due to high uptake of $\delta^{15}N$ with the help of microbial nitrification effects. The maximum value of $\delta^{15}N$ in *B. chinensis* was 17.7‰ for H−M, which was higher than that of organic tomato (16.6‰), lettuce (17.2‰), carrot (11.2‰),[12,17] cabbage (16.0‰),[18] and rice at harvest (<9.0‰),[19] as well as lettuce (<9.0‰),[22] and it extended the known database of the $\delta^{15}N$ value in organic food. The large variation of $\delta^{15}N$ in *B. chinensis* for most treatments between the terminal stage (46 DAS) and the seedling stage (26 DAS) was also observed, which was >6.0‰ for the treatment of L−M, H−C, L−M+C, and H−M+C. The mean values of $\delta^{15}N$ for the treatments L−C and H−C were lower than those of the treatments L−M and H−M at the terminal stage (46 DAS). However, there were no significant differences among the various treatments, except for H−C (12.0‰) compared with L−M+C (17.0‰) and H−M+C (16.5‰). These findings indicated that the $\delta^{15}N$ value of *B. chinensis* without chemical fertilizer application (such as in organic conversion) may also be higher than that of organic vegetable as other studies reported and proved that the vegetable with chemical fertilizer history within organic conversion would possibly be mislabeled as "organic food" if only judging on its high $\delta^{15}N$ value.

Variations in $\delta^{15}N$ Values of *B. chinensis* with Further Fertilizer Application in Spring of 2012. For the spring experiment of 2012, the same dose fertilizer as the 2011 spring experiment was employed to the corresponding treatment after the autumn experiment. As shown in Figure 3, the $\delta^{15}N$ values for *B. chinensis* at 26 DAS decreased compared with that of the autumn experiment. Compared with the 2011 autumn experiment, we observed a sharp decrease in chemical fertilizer treatment. The mean $\delta^{15}N$ values of L−C and H−C were significantly lower at 72.2% and 72.9%, respectively. This finding could result from additional urea-N application with more available ^{14}N to plants, and it is in good agreement with

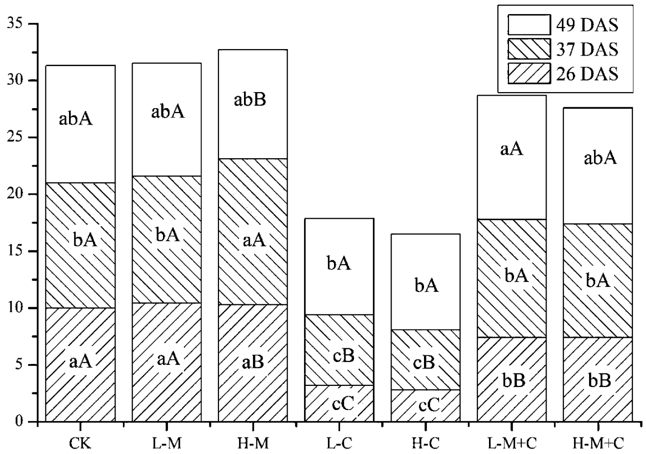

Figure 3. Variation in δ^{15}N of *B. chinensis* for the spring experiment of 2012. Treatment codes are described in Table 1. The same lowercase letter in the bars indicate no significant difference between different treatments at the same stage, and the same capital letters indicate no significant difference between different stages for the same treatment (α = 0.05 level).

previous reports depicting the fast response and sharp decline in δ^{15}N values with additional chemical fertilization in Chinese cabbage[19] and lettuce.[23] The results in the 2012 experiment at 26 DAS had a similar trend as those from the spring experiment of 2011, with the range of 10.0‰ for CK, 10.3‰−10.4 ‰ for L−M and H−M, 2.8‰−3.2‰ for L−C and H−C, and 7.4‰ for L−M+C and H−M+C. The values observed in this experiment for the treatment L−C and H−C were also lower than those of the 2011 spring experiment at 55.4% and 48.1%, respectively, which may result from two applications of urea-N. The δ^{15}N value of the vegetable reflected the isotopic composition and historical fertilization of N sources and changed remarkably when isotopically different N sources were added.[19] For treatments of L−M+C and H−M+C, the mean δ^{15}N values were much lower than those of the autumn experiment at 29.2% and 37.3%, respectively. The results were slightly lower than those from the 2011 spring experiment at 17.6% and 9.8%, respectively. However, results from treatments of CK, L−M, and H−M were similar to those of the spring experiment and lower than those of the autumn experiment in 2011. These results indicated that the types of fertilizers directly affected the δ^{15}N value of the vegetable as previously reported.[14] Different manures may have different temporal effect patterns, for instance, the grain δ^{15}N of barley in hog manure plots showed an increase tendency with time, but the phenomenon was not observed in the cattle manure treatment.[15] In our study, there was also a tendency observed that the δ^{15}N values of *B. chinensis* receiving chemical fertilizer treatment were significantly lower (p < 0.05) than those receiving compost manure at the early growth stages. The difference became narrower during the later stage, possibly due to a decrease in the availability of the chemical fertilizer over time (plant uptake, loss and immobilization, etc.) and contribution of natural soil nitrogen to plant total δ^{15}N.[13] At 49 DAS, the δ^{15}N value for L−M and H−M decreased compared with that at 37 DAS, from 11.2 to 9.9‰ and from 12.8‰ to +9.6‰, respectively, which may be explained by uptake of mineralized N from the organic pool decreasing the value during the later period.[14] The δ^{15}N value for treatment H−C (8.4‰) has significant differences (p < 0.05) with that of CK (10.3‰) and L−M+C (10.9‰), and there were no significant differences observed among other treatments. Although the δ^{15}N of *B. chinensis* may be increased by compost application or decreased by chemical application, the results of this season experiment also showed that no significant differences (p > 0.05) were observed between high dose and low dose for the same treatment, except for L−M and H−M at 37 DAS. This result corroborated that fertilizer quantity was not a key factor effecting δ^{15}N of carrot[13] and cabbage.[26] The δ^{15}N value of the vegetable may vary depending on the vegetable variety and the fertilizer type. The δ^{15}N values of *B. chinensis* observed in this experiment were different from those for cabbage that were 7.7‰ for CK, 3.2‰ for urea, and 11.9−13.2‰ for liquid manure-A and 13.0‰−14.9‰ for liquid manure-B.[16] Another example was that the δ^{15}N values for strawberry fruits grown in the presence of inorganic and organic fertilizers were −0.4 ± 1.5‰ and 9.2 ± 1.7‰, respectively.[27] This may be because of different manures with different δ^{15}N values and variations in the uptake rates of different vegetables.

As a summary of the pot experiments of three continuous seasons at middle stage, the δ^{15}N value of *B. chinensis* for the spring experiment of 2011 at 32 DAS has a similar value to that of 2012 at 37 DAS (shown in Figure 4), which was mostly affected by the types of fertilizers. Both experiments had a similar tendency, which is that there was a significant difference between the compost treatment and the chemical treatment. However, there was an apparent overlap in δ^{15}N value for the combined application of chemical and compost fertilizer. The δ^{15}N value of *B. chinensis* for the autumn of 2011 without any additional fertilizer at 36 DAS increased (shown in Figure 2), and was higher than those of the spring of 2011 and 2012, but there was no significant difference between the compost treatment and the chemical treatment.

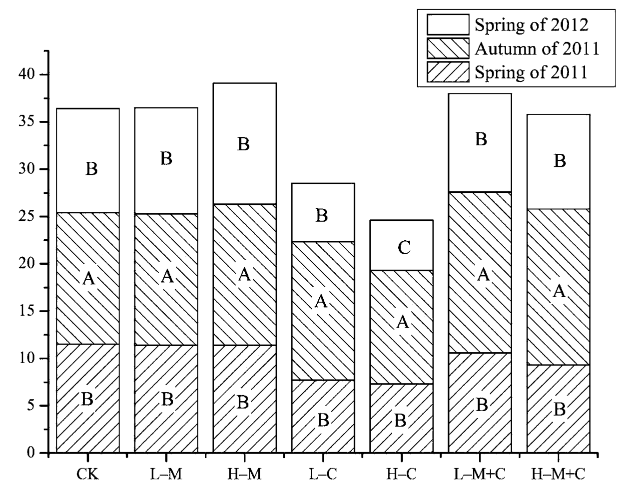

Figure 4. Variation in δ^{15}N of *B. chinensis* for the middle stage of three experiments. Treatment codes are described in Table 1. The same capital letter in the bars indicate no significant difference between different seasons at the middle stage (α = 0.05 level).

Variation in δ^{15}N Value of Soil with Different N Source and Rate. The total δ^{15}N of soil was determined only for the spring experiment of 2011 to investigate the variation in δ^{15}N of soil affected by different fertilizers and the relationship of δ^{15}N between soil and plant. The δ^{15}N mean value of soil (no fertilizer application) before this experiment was 6.9‰, which was very close to the previous report[20] with 6.4‰. After fertilization and uptake by vegetables, the $\delta^{15}N$ mean value range of soil at 23 DAS was 2.5‰ for CK, 5.3‰−6.3‰ for L−M and H−M, 2.2‰−2.4‰ for H−C and L−C, and 2.7‰−4.5‰ for L−M+C and H−M+C, as shown in Figure 5. The total $\delta^{15}N$ mean value of soil was lower for chemical treatment than that for compost treatment, which may be explained by the fact that the $\delta^{15}N$ of soil was more affected by the chemical fertilizer than by the compost fertilizer. There was a significant difference ($p < 0.05$) between the compost treatment (L−M, H−M) and CK as well as other treatments. There was also a report[25] that the total $\delta^{15}N$ value of soil was significantly higher for compost-applied (8.8‰) than for fertilizer-applied (5.9‰), which corroborated that the $\delta^{15}N$ value of upland-grown plants receiving compost would be higher than those treated with chemical fertilizer because NO_3^- is the most abundant N for plant uptake. However, another report showed that there was no difference between the organic production and the integrated production for total $\delta^{15}N$ of soil, because the quantity of fertilizer application added was only ∼1/100 of the total soil N-pool.[24] As shown in Figure 5, the $\delta^{15}N$ mean value of soil from 32 DAS to 39 DAS varied over time from 2.7‰ to 1.6‰ for CK, and also had a tendency to go from higher to lower for other treatments. Although the total $\delta^{15}N$ values slightly varied with the growing stage for all treatments, there were no significant differences ($p > 0.05$) among three stages. The total $\delta^{15}N$ values of the treatment L−M (5.3‰) and H−M (6.3‰) were higher than those of L−C (2.4‰) and H−C (2.2‰) at 23 DAS, which were also significantly different ($p < 0.05$) at 32 DAS and 39 DAS. The $\delta^{15}N$ values of soil were affected by the different fertilizer treatments found in this experiment, and the $\delta^{15}N$ values of *B. chinensis* were also affected by that of soil, as per the previous report that the $\delta^{15}N$ of upland-grown plants receiving compost would be higher than for those treated with chemical fertilizer.[16]

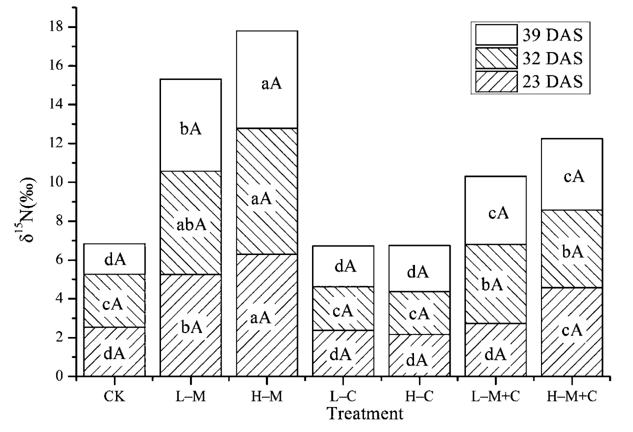

Figure 5. Variation in $\delta^{15}N$ of soil for the spring experiment of 2011. Treatment codes are described in Table 1. The same lowercase letter in the bars indicate no significant difference between different treatments at the same stage, and the same capital letters indicate no significant difference between different stages for the same treatment (α = 0.05 level).

Figure 6 shows that the $\delta^{15}N$ of *B. chinensis* were all higher than that of soil during the spring experiment of 2011, which has a positive correlation to that of soil at the seedling stage (R^2 = 0.537, p = 0.0656). As *B. chinensis* grew, the positive correlation became minor at the middle stage (R^2 = 0.485, p = 0.0848) and the terminal stage (R^2 = 0.305, p = 0.2102). The results also confirmed that total $\delta^{15}N$ value of *B. chinensis* was greatly affected by N derived from compost or fertilizer rather than indigenous soil-N during the early growth season as previously reported.[25] The results demonstrate that there was no strong correlation ($p > 0.05$) between *B. chinensis* and soil in terms of $\delta^{15}N$ value, which was in agreement with the previous study.[25]

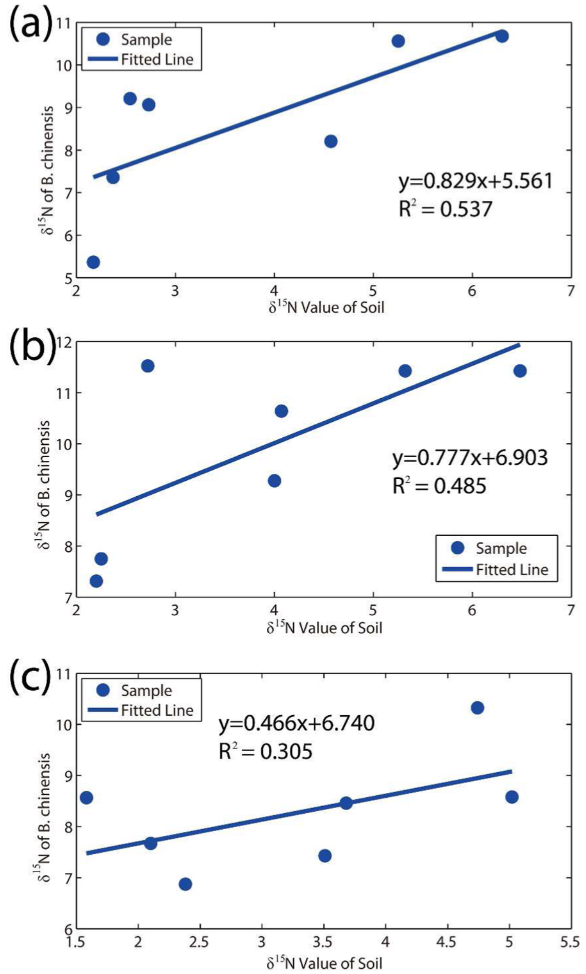

Figure 6. Relationship of $\delta^{15}N$ between *B. chinensis* and soil in the spring experiment of 2011 at the seedling stage (a), the middle stage (b), or the terminal stage (c).

Finally, the results of this experiment confirmed that the $\delta^{15}N$ value of *B. chinensis* increased when organic manure fertilizer was applied and was significantly different fromthose for manure treatment and chemical treatment. With the growth, the $\delta^{15}N$ value of *B. chinensis* varied depending on the fertilizer application. There was no fixed cutoff value for $\delta^{15}N$ in organic discrimination. The $\delta^{15}N$ value of *B. chinensis* in the autumn of 2011 without any fertilizer application was higher than those for the spring experiments of 2011 and 2012. This may be the result of the fact that more soil-^{15}N from N lost or fractionation in soil after the spring experiment was absorbed by the vegetable, which confirmed that using uncultivated soil in the pot experiment could eliminate the effect of background interference on the $\delta^{15}N$ of plant. The $\delta^{15}N$ value of soil was slightly affected by fertilizers and varied without significant difference among three growing stages of *B. chinensis*. Thus, the $\delta^{15}N$ value may be useful to discriminate whether a particular vegetable was grown in the presence of a chemical fertilizer (a low value, ~3.0‰) or in the presence of compost manure or a chemical−compost fertilizer (a high value, ~10.0‰). Once again, it was difficult to discriminate if a labeled organic vegetable was really grown without chemical fertilizer just using the $\delta^{15}N$ value, especially for the overlap value of those planted with chemical−compost fertilizer. However, the results also indicated that the $\delta^{15}N$ value of *B. chinensis* grown with compost manure or chemical−compost treatment has a higher value compared to vegetables previously reported, which could be useful for the development of the $\delta^{15}N$ value database for organic food discrimination.

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Notes

The authors declare no competing financial interest.

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