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Annual Variation of Natural ^{15}N Abundance in Tea Leaves and Its Practicality as an Organic Tea Indicator

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ABSTRACT: To obtain basic knowledge about the relationship between the application of organic fertilizers and the $\delta^{15}N$ values of leaves of organically grown tea plants, annual variations in the $\delta^{15}N$ values of the tea leaves were investigated. Although variations did not immediately arise after the application of organic fertilizers, differences in the $\delta^{15}N$ values between organic and conventional cultivations appeared basically after three years from the beginning of the organic cultivation except when an organic fertilizer with a low $\delta^{15}N$ value was applied, and the variation depended on the $\delta^{15}N$ values of the fertilizers. In addition, the effectiveness of the $\delta^{15}N$ values as a practical indicator of organic teas was examined. The tea leaves collected from organic farms did not always have higher $\delta^{15}N$ values than the commercially available nonorganic teas. This result demonstrates that it is not easy to discriminate organic teas from nonorganic teas simply by their $\delta^{15}N$ values.

KEYWORDS: organic tea, organic fertilizer, $\delta^{15}N$ value

INTRODUCTION

Tea is an agricultural product made from the buds and young leaves of tea plants [*Camellia sinensis* (L.) O. Kuntze], and the infusion is a globally consumed beverage. Recently, amid increasing concern over food safety, there has been more interest in organic tea as well as other organic agricultural products,[1] because organic crops are cultivated without using chemical fertilizers and agricultural chemicals. In the past eight years, the amount of unrefined tea (tea leaves steamed and dried without fermenting) certified as organic has increased from 927 to 1873 tons in Japan, and the fraction of organic tea in total green tea production has approximately doubled (Ministry of Agriculture, Forestry and Fisheries of Japan, http://www.maff.go.jp/j/jas/jas_kikaku/yuuki.html). Meanwhile, there have been growing concerns that nonorganic tea is traded as organic tea in the market. Therefore, to prevent such subterfuge, it is necessary to develop a method that scientifically discriminates between organic and nonorganic teas.

Natural ^{15}N abundance, which is often expressed as a $\delta^{15}N$ value, has attracted attention as an indicator to discriminate organically grown crops from conventionally grown crops. The $\delta^{15}N$ value is calculated from the $^{15}N/^{14}N$ ratio of measurement samples and a standard sample (air) by the following equation: $\delta^{15}N\ (‰) = (R_{sample}/R_{air} - 1) \times 1000$, where R_{sample} and R_{air} are $^{15}N/^{14}N$ ratios of the measurement sample and the air, respectively. Generally, the $\delta^{15}N$ values of chemical fertilizers are about 0‰, because they are synthesized with nitrogen in the atmosphere.[2] On the other hand, the $\delta^{15}N$ values of organic fertilizers are higher than those of chemical fertilizers, because ^{15}N in organic fertilizers is concentrated by nitrogen isotope fractionation resulting from chemical reactions, decomposition, ammonia volatilization, and denitrification.[3] The discrimination method using the $\delta^{15}N$ value has been applied to rice[4,5] and various vegetables such as tomato,[1,6] lettuce,[1] cucumber,[7] eggplant,[7] sweet pepper,[7] pumpkin,[7] bell pepper (paprika),[8] strawberry,[9] and melon,[10] and it has been reported that organically grown crops had higher $\delta^{15}N$ values in comparison to the conventionally grown crops. Furthermore, there have been pioneering works concerning the $\delta^{15}N$ value of tea, in which organic teas had a tendency to show higher $\delta^{15}N$ values than nonorganic teas.[2,11] It is conjectured that the $\delta^{15}N$ values of tea leaves are influenced by the length of the organic cultivation because tea plants are a perennial crop unlike rice and vegetables. However, this hypothesis was not tested in the previous works.

In this study, to obtain basic knowledge about the relationship between the application of organic fertilizers and the $\delta^{15}N$ values of leaves of organically grown tea plants, annual variations in the $\delta^{15}N$ values of the tea leaves were investigated. In addition, the practical effectiveness of the $\delta^{15}N$ values as an organic tea indicator was examined by comparing tea leaf samples collected from organic farms with commercially available nonorganic green tea samples.

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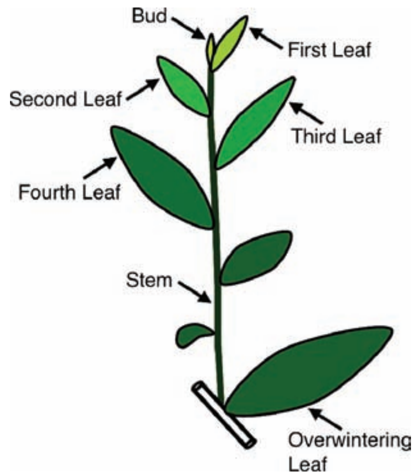

Figure 1. Young shoots of tea plants.

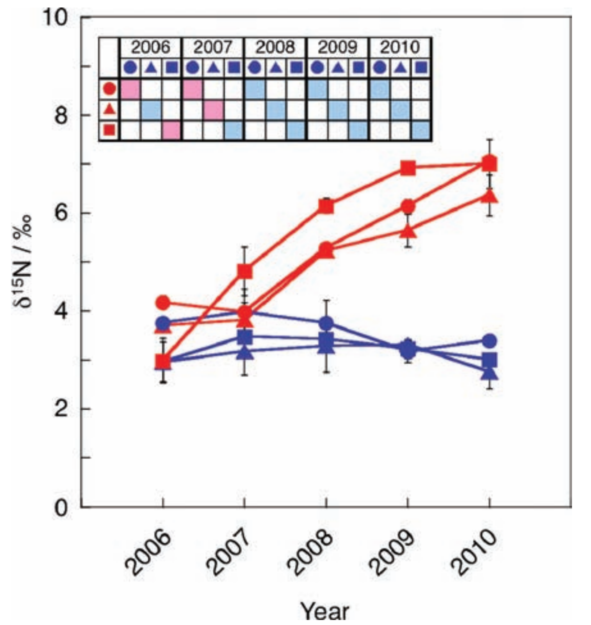

Figure 2. Annual variations in the $\delta^{15}N$ values of tea leaves: ●, Yabukita; ▲, Sayamakaori; ■, Hokumei. Red and blue indicate organic conditions and conventional conditions, respectively. The $\delta^{15}N$ values are the averages of the values obtained from duplicate samples, each of which was analyzed in triplicate. Vertical bars with the symbols indicate standard deviations. The grid diagram indicates the results of the Tukey multiple-comparison tests, blue (p < 0.01) and red (p > 0.05), where the comparisons between the different varieties are not shown.

MATERIALS AND METHODS

Experimental Field. The experimental fields of the Green Tea Laboratory, Saitama Prefectural Agriculture and Forestry Research Center (N 35° 48′, E 139° 21′, altitude 148.4 m) were used for our research concerning the annual variations in the $\delta^{15}N$ values of tea leaves. The tea plant varieties used were Yabukita, Sayamakaori, and Hokumei, which had been grown under conventional conditions since planting. At the beginning of the present experiments, the periods of the conventional cultivations were 14 years for Yabukita, 36 years for Sayamakaori, and 8 years for Hokumei.

Eight experimental plots were prepared: (1) Yabukita (conventional cultivation); (2) Yabukita (organic cultivation with fish meal/rapeseed meal =1:1); (3) Yabukita (organic cultivation with fish meal); (4) Yabukita (organic cultivation with rapeseed meal); (5) Sayamakaori (conventional cultivation); (6) Sayamakaori (organic cultivation with fish meal/rapeseed meal = 1:1); (7) Hokumei (conventional cultivation); (8) Hokumei (organic cultivation with fish meal/rapeseed meal = 1:1). Each experimental plot consisted of two nonadjacent ridges to collect samples in duplicate. The size of a ridge of tea plants was 2 m × 10 m. The adjacent ridges were separated by more than 4 m to avoid interference between ridges under different conditions.

Fertilization. Commercially available organic and chemical fertilizers were used in the experimental field. The fertilizers were applied on rain dropping lines of tea plants at the ends of March, June, and September. In the conventional cultivating plots, a fertilizer including both organic and chemical fertilizers was applied in March and September (nitrogen = 18 kg/1000 m^2, potassium = 9 kg/1000 m^2, phosphate = 9 kg/1000 m^2), and a chemical fertilizer was applied in June (nitrogen = 9 kg/1000 m^2, potassium = 4.5 kg/1000 m^2, phosphate = 4.5 kg/1000 m^2) in accordance with customary practice. In the organic cultivating plots, in addition to the organic fertilizers, potassium sulfate and fused phosphate fertilizer were applied to ensure the same elemental composition as the conventional cultivating plots.

Tea Samples. Tea leaves in the experimental field were plucked at a stage when 50−70% of new shoots temporarily stopped budding in mid to late May. Except for the samples for examinations relating to leaf positions, the bud and first, second, third, and fourth leaves (Figure 1) were collected together. The samples for examinations relating to leaf positions were taken in the following three parts: (1) the bud and first and second leaves; (2) the third and fourth leaves; and (3) the stem. The sampling positions were the center in the ridge of tea plants, the right side of the ridge edge, and the left side of the opposite edge. These tea leaves were gathered together. The amount of each sample was about 20 g as dry weight. Repeated sampling in the same plot was performed in the same manner.

The other 23 organic tea leaf samples (the bud and first, second, third, and fourth leaves) were obtained from 13 organic farms in Saitama (in mid to late May) and Miyazaki (in late April) prefectures. These samples were taken at three positions of each tea plant group. Thirty-one commercially available tea samples were purchased from sellers over the internet.

$\delta^{15}N$ Value Analysis. The tea leaves collected from the fields were immediately steamed for 2 min for the bud and first and second leaves, 2.5 min for the third and fourth leaves and for the mixture of the bud and first, second, third, and fourth leaves, and 3 min for the stem. Next, they were dried at 60 °C for 24 h and were powdered by a mill (Cyclone sample mill, CSM-F1, Shizuoka Seiki Co., Ltd.). These tea leaf and fertilizer samples were measured precisely within 2.50 ± 0.50 mg and were wrapped in capsules made of tin foil (∅ 5/l 9 mm, Lüdi Swiss AG Co.). Three tin capsules with the samples were prepared from each sample of the tea leaves and the fertilizers. The $\delta^{15}N$ values were measured using an IR mass spectrometer coupled with an elemental analyzer (DeltaXP, Finnigan Co.).

Statistical Analysis. To compare the $\delta^{15}N$ values, the Tukey multiple-comparison test or the Welch t test was employed by using Ekuseru-Toukei 2006 (Social Survey Research Information Co., Ltd.).

RESULTS AND DISCUSSION

Annual variations in the $\delta^{15}N$ values of tea leaves were investigated in the experimental fields for 5 years from 2006 to 2010 with regard to the three different tea varieties, leaf

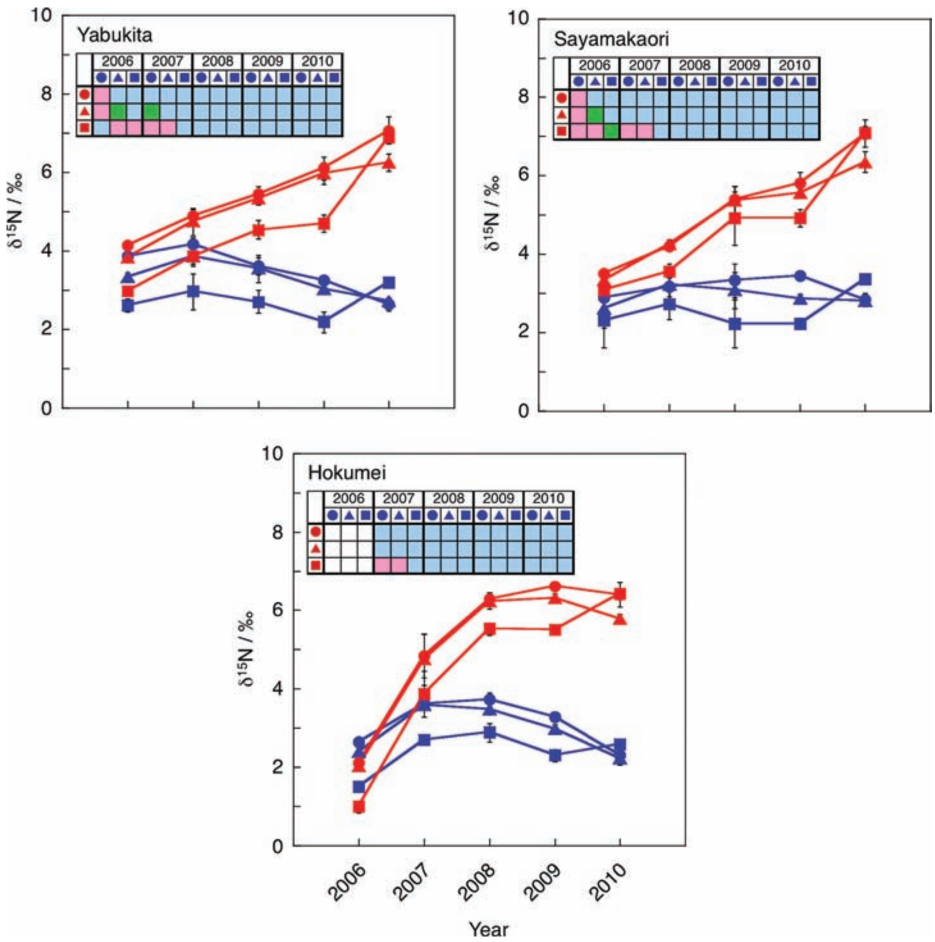

Figure 3. Annual variations in the δ^{15}N values of tea leaves and stems: ●, bud, first and second leaves; ▲, third and fourth leaves; ■, stem. Red and blue indicate organic conditions and conventional conditions, respectively. The δ^{15}N values are the averages of the values obtained from duplicate samples, each of which was analyzed in triplicate. Vertical bars with the symbols indicate standard deviations. The grid diagrams indicate the results of the Tukey multiple-comparison tests, blue ($p < 0.01$), green ($0.01 < p < 0.05$), and red ($p > 0.05$). The δ^{15}N values of Hokumei in 2006 were not tested because the organic plot values were lower than the corresponding conventional plot values.

positions, and fertilizers. In the conventionally cultivated experimental plots, a commercially available standard fertilizer including both organic and chemical fertilizers was used as in the actual conventional cultivations of tea plants. In the organically cultivated experimental plots, except in the case of the examination relating to the fertilizers, a mixture of fish meal and rapeseed meal (1:1) was applied as an organic fertilizer. Fish meal and rapeseed meal, which are typical organic fertilizers, were adopted because of the organic components in the fertilizer applied in the conventionally cultivated experimental plots.

Annual variations of the bud and first, second, third, and fourth leaves of Yabukita, Sayamakaori, and Hokumei are shown in Figure 2. In the first and second years of the experiments (2006 and 2007), only the δ^{15}N values of Sayamakaori and Hokumei showed significant differences between organic and conventional conditions, respectively. In the third year (2008), all of the varieties exhibited marked differences in the δ^{15}N values between organic and conventional conditions, with these differences becoming more apparent after the fourth year.

In the δ^{15}N values from the different positions of the tea leaves, compared between the same positions, significant differences between organic and conventional conditions were shown in the second year (Figure 3). However, all the significant differences including between the different positions appeared after the third year (2008). This tendency was common to the three varieties.

The influence of the difference in organic fertilizers on the δ^{15}N values of tea leaves was examined. Three different organic fertilizers, fish meal, rapeseed meal, and a mixture of fish meal and rapeseed meal (1:1), were applied to Yabukita. The δ^{15}N values of the fish meal and the rapeseed meal were 11.7 and 4.2‰, respectively. As illustrated in Figure 4, the leaves of the tea plants to which fish meal had been applied had the largest δ^{15}N value, whereas the δ^{15}N value of the leaves of tea plants to which rapeseed meal had been applied was the smallest. In the case of the mixture of fish meal and rapeseed meal (1:1), the tea leaves showed an intermediate δ^{15}N value. In the third year (2008), the δ^{15}N values of the tea leaves under organic cultivating conditions except rapeseed meal were higher compared to that under conventional cultivating conditions using fertilizers with a δ^{15}N value of 3.0‰. In the plot to which rapeseed meal was applied, the organic and conventional conditions made a difference after the fourth year (2009). These results revealed that the δ^{15}N values of tea

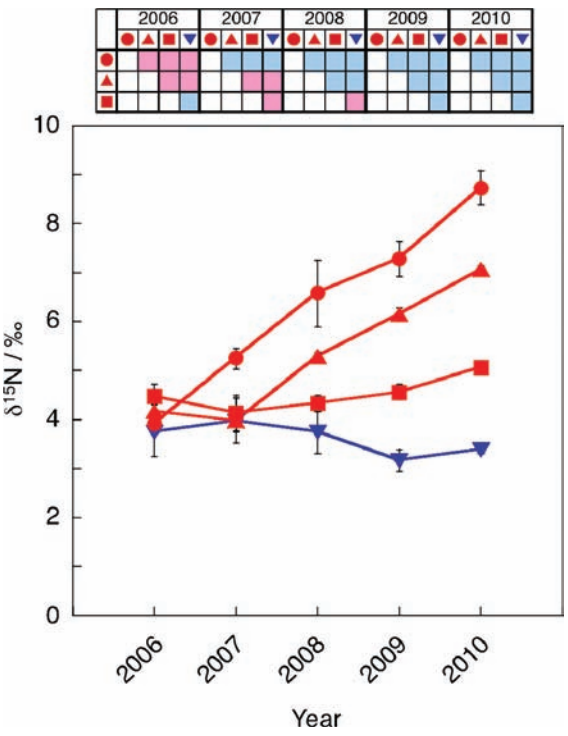

Figure 4. Annual variations in the $\delta^{15}N$ values of tea leaves according to fertilizer: ●, fish meal; ▲, fish meal and rapeseed meal (1:1); ■, rapeseed meal; ▼, conventional fertilization. Red and blue indicate organic conditions and conventional conditions, respectively. The $\delta^{15}N$ values are the averages of the values obtained from duplicate samples, each of which was analyzed in triplicate. Vertical bars with the symbols indicate standard deviations. The grid diagram indicates the results of the Tukey multiple-comparison tests, blue ($p < 0.01$) and red ($p > 0.05$).

leaves reflect those of the applied fertilizers, and apparent differences in the values between organic conditions and conventional conditions appear after the third year unless an organic fertilizer with a relatively low $\delta^{15}N$ value such as rapeseed meal was applied. Therefore, in the case of a perennial crop such as tea plants, also, the $\delta^{15}N$ value might be an indicator of organic cultivation, because a certain preparatory cultivation period is generally required for certification as an organic crop. For example, in Japan, three years or more are required for perennial crops before the first harvesting.

Thus, to evaluate the effectiveness of the $\delta^{15}N$ value as a practical indicator of organic tea, the $\delta^{15}N$ values of 23 tea leaf samples collected from 13 actual organic farms and 31 commercially available nonorganic green teas were investigated. The varieties of tea plants in the organic farms were as follows: Fukumidori (F), Kanayamidori (Ka), Komakage (Ko), Saemidori (Se), Toyoka (T), and Yutakamidori (Yu) in addition to Yabukita (Ya), Sayamakaori (Sy), and Hokumei (H) cultivated in the experimental field. The fertilizing conditions in the organic tea farms were too complicated for analysis of the relationship between the types of organic fertilizers and the $\delta^{15}N$ values of the tea leaves in detail. However, three groups (A, B, and C) in Figure 5 were organic sample sets cultivated by the same farmers under the same fertilizing conditions. In these groups, only group B showed a dependence on the magnitude of the $\delta^{15}N$ values on the years of organic cultivation. In group A, Ko30, which had been organically grown for the longest period (30 years), indicated the highest $\delta^{15}N$ value. However, despite organic cultivation for the same 30 years, Ka30 had a lower $\delta^{15}N$ value in comparison to F4 organically cultivated for only 4 years. In group C, Sy13, which had been organically cultivated for 13 years, had a much lower $\delta^{15}N$ value than the other samples cultivated for the same number of years or less. From these results, it appears that the period of organic cultivation is not necessarily a dominant factor influencing the $\delta^{15}N$ values of the tea leaves in the actual organic farms and that complicated cultivating conditions affect the values in unpredictable ways, unlike the experimental field in which cultivating conditions were rigorously controlled.

Next, $\delta^{15}N$ values of commercially available nonorganic teas were investigated. The lowest value was 3.0 ± 0.1‰ in all 31 samples, which was lower than the minimum value (Sy13, 3.4 ± 0.1‰) in the samples collected from the organic tea farms (the Welch *t* test, $p < 0.01$). However, overall, the $\delta^{15}N$ values of these nonorganic teas were not always lower in comparison to those of the organic tea leaves. Interestingly, the highest value in the nonorganic teas was 7.5 ± 0.0‰. Although there could be various reasons for this high value, one of them might be due to an application of organic fertilizers with a high $\delta^{15}N$ value and/or more application of organic fertilizers, because organic fertilizers are generally used in conventional tea cultivation as well. These results demonstrate that discrimination between organic teas and nonorganic teas only by the $\delta^{15}N$ value is not a simple task.

This study revealed that the variation in $\delta^{15}N$ values of tea leaves did not occur immediately after the application of organic fertilizers, but clearly arose after three years from the beginning of the organic cultivation except when the organic fertilizer with the low $\delta^{15}N$ value, rapeseed meal, was applied, and that the variation depended on the $\delta^{15}N$ values of the fertilizers. The leaves of tea plants grown by organic fertilizers with higher $\delta^{15}N$ values indicated higher $\delta^{15}N$ values in comparison to those of tea plants grown by fertilizers with lower $\delta^{15}N$ values. As described above, a certain preparatory cultivation period is required for certification of organic crops. From this perspective, the $\delta^{15}N$ value also seems to be effective as an indicator candidate of organically grown tea as well as other crops. However, it would be impossible to discriminate organic teas from nonorganic teas on the basis of only the $\delta^{15}N$ values, because, even if chemical fertilizers are applied, the $\delta^{15}N$ values can increase in principle by using organic fertilizers with high $\delta^{15}N$ values. In fact, this hypothesis is supported by the results that the commercially available nonorganic teas did not always have lower $\delta^{15}N$ values than the organic tea leaves. Conversely, in the case of using an organic fertilizer such as rapeseed meal with a lower $\delta^{15}N$ value, even under completely organic cultivation conditions, the $\delta^{15}N$ values of the tea leaves may not be particularly high. Moreover, in the case using agricultural chemicals despite cultivation with only organic fertilizers, discriminating organic teas from nonorganic teas may be impossible. However, these problems are undoubtedly not limited to teas. Therefore, to more accurately discriminate organic crops from nonorganic crops, there is a need to develop other effective discrimination methods that could be combined with the method using the $\delta^{15}N$ value.

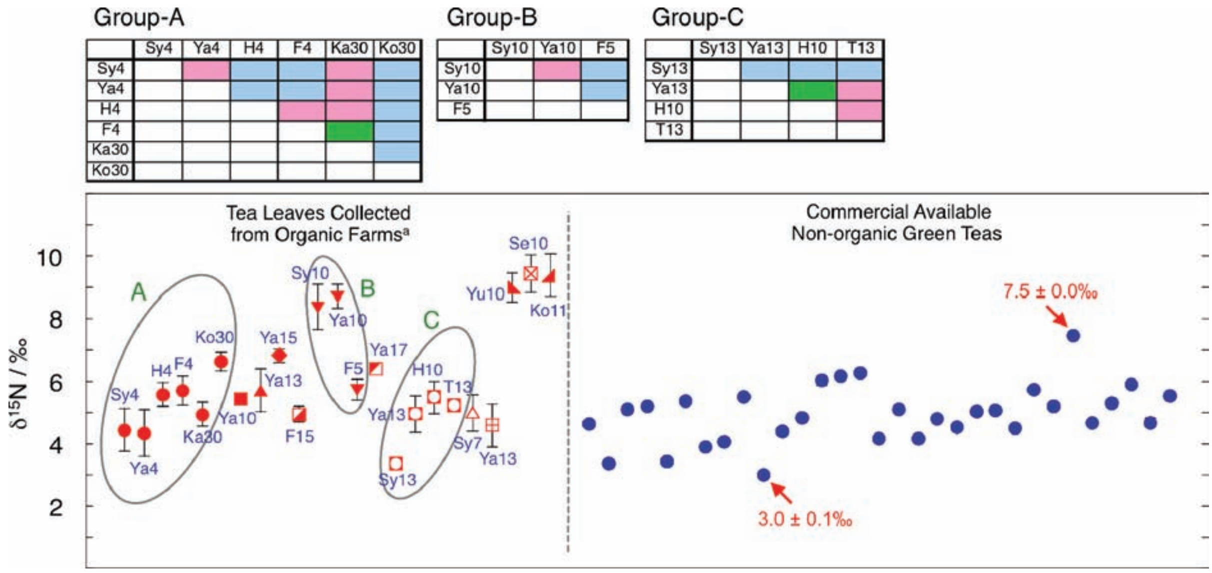

Figure 5. δ^{15}N values of tea leaves collected from organic farms and commercially available nonorganic green teas. Symbols in the graph of the organic tea samples are marked on the basis of fertilizing conditions (identical symbols indicate tea samples cultivated by the same farmers). The δ^{15}N values of the organic tea leaves are the averages of the values obtained from triplicate samples, each of which was analyzed in triplicate. Vertical bars with the symbols of the organic tea samples indicate standard deviations. The δ^{15}N values of the nonorganic teas are the averages of the values analyzed in triplicate. The standard deviation bars of the nonorganic teas are hidden under the symbols. The grid diagrams indicate the results of the Tukey multiple-comparison tests, blue ($p < 0.01$), green ($0.01 < p < 0.05$), and red ($p > 0.05$). [a]Varieties: F, Fukumidori; H, Hokumei; Ka, Kanayamidori; Ko, Komakage; Se, Saemidori; Sy, Sayamakaori; T, Toyoka; Ya, Yabukita; Yu, Yutakamidori. Numbers with abbreviations for the varieties stand for the years of organic cultivation. Applied organic matters: group A, rapeseed meal, poultry manure, compost (cattle manure), and compost (fallen leaf); group B, fermented organic matter compost (rice bran, oil cake, fish meal, crab shell, and calcined poultry manure); group C, fermented organic matter compost (oil cake, fish meal, bone meal, crab shell, soybean curd refuse, rice bran, poultry manure, and hog manure); Ya10 (■), crab shell and food residue; Ya13 (▲), soybean meal; Ya15, compost pellet and oil cake; F15, fish meal, rice bran, and oil cake; Ya17, rapeseed meal, fish meal, and fermented poultry manure; Yu10, compost (poultry manure and rice bran), compost (hog manure and rice bran), fermented organic matter compost (oil cake, rice bran, meat and bone meal, and offal), meat and bone meal, offal, and oil cake; Se10, compost (hog feces), compost (poultry manure) and fermented organic matter compost (bone meal, blood meal, offal, fish meal, rice bran, and rapeseed meal); Ko11, fermented organic matter compost (rice bran, offal, and rapeseed meal), compost (hog feces), and fish liquid fertilizer.

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