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Research article

Ginseng authenticity testing by measuring carbon, nitrogen, and sulfur stable isotope compositions that differ based on cultivation land and organic fertilizer type

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ABSTRACT

Background: The natural ratios of carbon (C), nitrogen (N), and sulfur (S) stable isotopes can be varied in some specific living organisms owing to various isotopic fractionation processes in nature. Therefore, the analysis of C, N, and S stable isotope ratios in ginseng can provide a feasible method for determining ginseng authenticity depending on the cultivation land and type of fertilizer.
Methods: C, N, and S stable isotope composition in 6-yr-old ginseng roots (Jagyeongjong variety) was measured by isotope ratio mass spectrometry.
Results: The type of cultivation land and organic fertilizers affected the C, N, and S stable isotope ratio in ginseng ($p < 0.05$). The $\delta^{15}N_{AIR}$ and $\delta^{34}S_{VCDT}$ values in ginseng roots more significantly discriminated the cultivation land and type of organic fertilizers in ginseng cultivation than the $\delta^{13}C_{VPDB}$ value. The combination of $\delta^{13}C_{VPDB}$, $\delta^{15}N_{AIR}$, or $\delta^{34}S_{VCDT}$ in ginseng, except the combination $\delta^{13}C_{VPDB}$–$^{34}S_{VCDT}$, showed a better discrimination depending on soil type or fertilizer type.
Conclusion: This case study provides preliminary results about the variation of C, N, and S isotope composition in ginseng according to the cultivation soil type and organic fertilizer type. Hence, our findings are potentially applicable to evaluate ginseng authenticity depending on cultivation conditions.

1. Introduction

Ginseng (*Panax ginseng* Meyer), a perennial plant, is well known as a representative specialty in Korea for its medicinal properties because of the presence of various bioactive compounds such as saponins, polyacetylenes, polysaccharides, phenolics, and volatile isoprenoids [1–3]. China, Korea, Canada, and USA are the major ginseng producers, comprising > 99% of the global ginseng production [4]. In general, ginseng production and quality are affected by various physical, chemical, and microbial properties of the soil. In particular, ginseng cultivation for 4–6 yrs usually decreases soil fertility. Thus, the continuous cropping of ginseng at the same place is not recommended because of a decrement of ginseng production and quality of the cultivation area caused by a continuous cropping injury associated with *Cylindrocarpon destructans*. Therefore, management of the soil used for ginseng cultivation is a crucial step in the production of high quality and high yield of ginseng [5,6].

In Korea, the total ginseng cultivation area and production decreased from 19,702 ha and 27,460 tons in 2009 to 14,652 ha and 20,978 tons, respectively, in 2014 because of the lack of new ginseng cultivation areas [7]. Thus, stable production of high quality ginseng is of interest to both ginseng farmers and consumers in Korea. Recently, paddy-converted fields have attracted attention in Korea as a potential solution to both the lack of new cultivation areas and appearance of various disorders caused by continuous ginseng cultivation. In general, paddy-converted fields decrease the levels of *Cylindrocarpon destructans* (a pathogen source of disorders in continuous ginseng cultivation) and toxins associated with the

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inhibition of ginseng growth [8,9]. Furthermore, the saponin content, yield, and quality of ginseng cultivated in paddy-converted fields were not different from those of ginseng cultivated in upland fields by using conventional ginseng cultivation methods. Therefore, the demand for paddy-converted fields is expected to increase in ginseng farming in Korea in the future [6,9].

Meanwhile, prolonged cultivation of ginseng in the same location requires a steady supply of nutrients to obtain a high yield of high quality ginseng. Certain organic materials (not meaning organically cultivated), including manure, food waste, and rice straw compost, have been usually applied as organic fertilizers, whereas the application of chemical fertilizers has been banned during the ginseng cultivation period by the Ginseng Industry Act [10]. Because of the difference in soil properties compared to those of upland fields, ginseng cultivation in paddy-converted fields requires careful application of organic fertilizers. In general, the application of slow-release organic fertilizer types at the managing stage of preplanting in a paddy-converted field is preferred to avoid the occurrence of various physiological disorders during the entire ginseng cultivation period [1,5].

Given the unique natural abundance of hydrogen (H), carbon (C), nitrogen (N), oxygen (O), or sulfur (S) in some living organisms, the analysis of its stable isotope composition has been applied to determine the authenticity of various foods. To date, this method has been successfully applied to determine the authenticity of various agricultural products (i.e., geographical origin: rice, vegetables, olive oil, juice, honey, wine, nut, tea; whether it is of organic origin: beef, milk; others: discrimination of cow milk vs. buffalo milk) [11–17]. In a prior study [18], the H isotope composition in ginseng was used to discriminate the geographical origin effectively between Korea and China. In particular, the negative value of N isotope composition in ginseng collected at Incheon, Korea was indicative of the application of synthetic fertilizer, possibly urea.

Few studies [18,19], to our knowledge, have investigated the authenticity of ginseng using the analysis of light element isotope composition. Therefore, in the present study, we have measured the difference and variation of C, N, and S stable isotope ratios in 6-yr-old ginseng root depending on ginseng cultivation conditions (i.e., cultivation soil type and fertilizer type/amount). The preliminary results reported from this case study can be potentially applicable to assessing ginseng authenticity with respect to cultivation environment.

2. Materials and methods

2.1. Ginseng materials

Six-yr-old ginseng roots (Jagyeongjong variety) were obtained from the Department of Herbal Crop Research, Rural Development Administration in Eumseong, Korea. The ginseng was cultivated in upland and paddy-converted fields from March 2009. The paddy-converted field had been used as a paddy field until 2006, when it was converted into the upland to manage the lack of new ginseng cultivation areas (upland) in Korea. Physical and chemical properties of the paddy-converted field were managed in the pre-planting preparation stage via the cultivation/decomposition of soilage crop such as sudangrass prior to ginseng cultivation [9]. Prior to ginseng seedling transplant, three types of organic fertilizers (cattle manure, food waste, and rice straw compost) were applied to the upland and the paddy-converted fields. Each organic fertilizer was applied at the level of 1 ton/1,000 m^2, 2 tons/1,000 m^2, and 4 tons/1,000 m^2. After the organic fertilizer application, 1-yr-old ginseng seedlings were transplanted to the upland and the paddy-converted fields in March 2009. The seedlings were planted with the planting density of 30 cm $\times$ 20 cm (the space between ginseng plants in a row $\times$ the space between the rows). The roots of the 6-yr-old plants were collected and stored at -70°C until required for the analysis. The land management, including applications of various agrochemicals (e.g., pesticide), was carried out using the standard ginseng farming method [20].

2.2. Sample preparation for the analysis of C, N, and S isotope composition

Collected ginseng roots were lyophilized at -45°C for > 3 d and pulverized before the isotope ratio mass spectrometry (IRMS) analysis. The pulverized ginseng was enclosed in a tin capsule (5 mm $\times$ 9 mm; Costech Analytical Technologies Inc., Valencia, CA, USA). About 5 mg of ginseng powder was used for simultaneous measurements of C and N stable isotope composition and about 20 mg for the measurement of S stable isotope composition. Finally, the encapsulated ginseng samples were placed in a desiccator before being used in the IRMS analysis.

2.3. Measurement of C, N, and S stable isotope compositions in ginseng by IRMS

The C and N stable isotope compositions ($\delta^{13}C_{VPDB}$ and $\delta^{15}N_{AIR}$) in ginseng ($n = 3$ per each treatment) were determined by using a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS; Sercon Ltd., Crewe Cheshire, UK) linked to a PDZ Europa ANCA-GSL elemental analyzer (Sercon Ltd.). The S stable isotope ratio ($\delta^{34}S_{VCDT}$) in ginseng ($n = 3$ per treatment) was measured by using a vario ISOTOPE cube (Elementar, Hanau, Germany) and a pre-concentration unit interfaced with a continuous-flow Sercon 20-22 IRMS (Sercon Ltd.). Detailed analytical conditions were described previously [16]. The samples' $\delta^{13}C_{VPDB}$, $\delta^{15}N_{AIR}$, and $\delta^{34}S_{VCDT}$ were calculated as follows:

$$\delta, ‰ = (r_{sample}/r_{standard}) - 1, \quad (1)$$

where r is the $^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$ ratio and r_{sample} and $r_{standard}$ from the samples of interest and the standard, respectively.

Thus, the ^{13}C, ^{15}N, and ^{34}S enrichment in ginseng were expressed against the international or established laboratory reference standards [Vienna PeeDee Belemnite (VPDB) for $\delta^{13}C_{VPDB}$; atmospheric (air) N_2 for $\delta\ ^{15}N_{AIR}$; Vienna Canyon Diablo Troilite (VCDT) for $\delta^{34}S_{VCDT}$].

For the quality control of the IRMS measurements, we simultaneously analyzed several replicates of our laboratory standards that were compositionally similar to our ginseng samples. These laboratory standards had been previously calibrated against the selected standard reference materials (IAEA-N1, IAEA-N2, IAEA-N3, USGS-40, and USGS-41 for $\delta^{13}C_{VPDB}$ and $\delta^{15}N_{AIR}$; IAEA-S-1, IAEA-S-2, or IAEA-S-3 for $\delta^{34}S_{VCDT}$) [21]. Based on the measurements of our laboratory standards (USGS41 for $\delta^{13}C_{VPDB}$ and $\delta^{15}N_{AIR}$; hair for $\delta^{34}S_{VCDT}$), the analytical precision was $\pm$ 0.1‰ for $\delta^{13}C_{VPDB}$, $\pm$ 0.1‰ for $\delta^{15}N_{AIR}$, and $\pm$ 0.2‰ for $\delta^{34}S_{VCDT}$. In addition, the long-term reproducibility ($\pm$ standard deviation) was $\pm$ 0.2‰ for $\delta^{13}C_{VPDB}$, $\pm$ 0.3‰ for $\delta^{15}N_{AIR}$, and $\pm$ 0.4‰ for $\delta^{34}S_{VCDT}$.

2.4. Statistical analysis

Statistical analysis was conducted using a general linear model procedure of the statistical analysis program (SAS version 9.3; SAS Institute Inc., Cary, NC, USA). The experimental design, including ginseng cultivation and sample collection, was a completely randomized design conducted in triplicate. The least significant difference test was based on a 0.05 probability level.

3. Results

Table 1 shows the differences in $\delta^{13}C_{VPDB}$, $\delta^{15}N_{AIR}$, and $\delta^{34}S_{VCDT}$ values in the 6-yr-old ginseng grown in the upland with three different types of organic fertilizers. We found that the type and the amount of organic fertilizers affected the C, N, and S stable isotope ratio in ginseng ($p < 0.05$). In particular, the type of organic fertilizer was discriminated more significantly by the $\delta^{15}N_{AIR}$ and $\delta^{34}S_{VCDT}$ values in ginseng than it was by the $\delta^{13}C_{VPDB}$ value. Thus, the mean $\delta^{13}C_{VPDB}$ value in ginseng cultivated by using rice straw compost was higher than that in the control (no organic fertilizer application); whereas the mean $\delta^{13}C_{VPDB}$ values in ginseng fertilized by cattle manure and food waste were lower than that in the control ($p < 0.05$). The mean $\delta^{15}N_{AIR}$ value of the ginseng grown in the upland field was ranked as follows: cattle manure (0.4 ± 0.7‰) > food waste (−0.6 ± 0.6‰) > rice straw (−1.3 ± 0.6‰) > control (−2.1 ± 0.5‰). The mean $\delta^{34}S_{VCDT}$ value of ginseng cultivated with rice straw compost (4.4 ± 0.4‰) was higher than those of ginseng cultivated with cattle manure (2.7 ± 0.3‰), food waste (3.0 ± 0.3‰), and control (3.2 ± 0.7‰; $p < 0.05$). The trend of isotope compositions in ginseng depending on fertilizer amounts applied at upland fields was not clear in this study; however, the $\delta^{15}N_{AIR}$ value of ginseng increased with increase in the amount of all organic fertilizers examined in this study. In addition, the $\delta^{34}S_{VCDT}$ value of ginseng tended to increase as the amounts of rice compost applied increased ($p < 0.05$, Table 1).

Table 2 shows the differences in $\delta^{13}C_{VPDB}$, $\delta^{15}N_{AIR}$, and $\delta^{34}S_{VCDT}$ values in the 6-yr-old ginseng grown in the paddy-converted field with three different types of organic fertilizer. The mean $\delta^{13}C_{VPDB}$ value in ginseng was differed slightly among organic fertilizers applied; however, the difference was very small ($p < 0.05$, Table 2). Similar to the upland field, the mean $\delta^{15}N_{AIR}$ value of ginseng grown in the paddy-converted field was also higher in the treatments with cattle manure and food waste than in the treatment with rice straw compost ($p < 0.05$). The mean $\delta^{34}S_{VCDT}$ value of ginseng cultivated in paddy-converted field with rice straw compost (3.0 ± 0.7‰) was higher than that of ginseng cultivated with cattle manure (2.6 ± 0.5‰) and food waste (2.3 ± 0.1‰), and it was similar to that in the upland field ($p < 0.05$, Table 2). In this study, there was no observed pattern in variation of C/N/S isotope composition in ginseng depending on fertilizer amounts applied in paddy-converted fields. However, the $\delta^{15}N_{AIR}$ value in ginseng was solely observed to reduce with increasing amounts of rice straw compost applied in paddy-converted fields ($p < 0.05$, Table 2).

In addition, the mean $\delta^{13}C_{VPDB}$ and $\delta^{15}N_{AIR}$ values in ginseng grown in paddy-converted fields were higher for all organic fertilizers than those in ginseng grown in upland fields, with the exception of $\delta^{13}C_{VPDB}$ in treatments with rice straw compost. By contrast, the mean $\delta^{34}S_{VCDT}$ value was higher in ginseng grown in upland field compared to that grown in paddy-converted fields ($p < 0.05$, Tables 1 and 2).

Figs. 1 and 2 show the variability of the combined $\delta^{13}C_{VPDB}$, $\delta^{15}N_{AIR}$, or $\delta^{34}S_{VCDT}$ in ginseng depending on the soil type and fertilizer type. The combinations $\delta^{13}C_{VPDB}$–$\delta^{15}N_{AIR}$ (Fig. 1) and $\delta^{15}N_{AIR}$–$\delta^{34}S_{VCDT}$ (Fig. 2) in ginseng have discriminated between upland and paddy-converted fields. The discrimination of fertilizer types applied with C/N/S isotope combination was not clear in both upland and paddy-converted fields. However, in the comparison to that in paddy-converted fields, the ginseng cultivated with rice straw compost in the upland fields relatively differed from the ginseng cultivated with cattle manure and food waste (Figs. 1 and 2).

4. Discussion

The measurement of light element stable isotope ratio can provide information such as geographical origin in various foods caused by various isotopic fractionation processes occurred in nature. For example, the analysis of H, O, S, or Sr isotope ratio provides information pertaining to geographical characteristics of various foods. By contrast, the analyses of C and N isotopic compositions in foods yield data regarding the dietary content (i.e., C3 vs. C4 plants) or agricultural practice (i.e., synthetic vs. organic fertilizer). In addition, N or S isotopic composition might be influenced by the local agricultural practice (fertilizer type, quantity, etc.) [16,22].

The C isotope composition ($\delta^{13}C_{VPDB}$) is mostly affected by the type of photosynthetic plant (i.e., C3, C4, or CAM). Discrimination of $\delta^{13}C_{VPDB}$ depending on plant type is the result of isotopic fractionation by enzymes and CO_2 diffusion during photosynthesis [23]. Accordingly, C4 plants show a higher $\delta^{13}C_{VPDB}$ value (from −9‰ to −20‰) than C3 plants (−21 to −35‰), and CAM plants are intermediate between C3 and C4 plants [24].

In the present study, the mean $\delta^{13}C_{VPDB}$ value of ginseng cultivated by the three organic fertilizers was in the range from −27.2‰ to −25.9‰ in the upland field and from −26.8‰ to −25.9‰ in the paddy-converted field, which is consistent with the $\delta^{13}C_{VPDB}$ values

Table 1
Differences of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in 6-yr-old ginseng roots cultivated in an upland field depending on the type and quantity of fertilizers

Fertilizer type	Quantity (ton/1,000 m^2)	$\delta^{13}C_{VPDB}$/‰, $n = 3$	Mean, $n = 9$	$\delta^{15}N_{AIR}$/‰, $n = 3$	Mean, $n = 9$	$\delta^{34}S_{VCDT}$/‰, $n = 3$	Mean, $n = 9$
Control			−26.5 ± 0.4^b		−2.1 ± 0.5^d		3.2 ± 0.7^b
Cattle manure	1	−27.0 ± 0.0^a	−27.2 ± 0.4^c	0.1 ± 0.2^b	0.4 ± 0.7^a	2.6 ± 0.3^a	2.7 ± 0.3^c
	2	−27.0 ± 0.1^a		0.0 ± 0.2^b		2.6 ± 0.1^a	
	4	−27.8 ± 0.0^b		1.5 ± 0.1^a		3.0 ± 0.1^a	
$LSD_{0.05}$ for fertilizer amounts		0.2	—	0.4	—	0.5	—
Food waste	1	−26.9 ± 0.1^a	−27.1 ± 0.2^c	−0.8 ± 0.2^b	−0.6 ± 0.6^b	2.9 ± 0.1^b	3.0 ± 0.3^{bc}
	2	−27.3 ± 0.1^c		−1.1 ± 0.2^b		2.7 ± 0.1^c	
	4	−27.1 ± 0.1^b		0.2 ± 0.0^a		3.3 ± 0.1^a	
$LSD_{0.05}$ for fertilizer amounts		0.2	—	0.3	—	0.2	—
Rice straw	1	−26.1 ± 0.2^b	−25.9 ± 0.4^a	−2.2 ± 0.2^b	−1.3 ± 0.6^c	4.0 ± 0.0^c	4.4 ± 0.4^a
	2	−26.2 ± 0.3^b		−1.2 ± 0.2^a		4.3 ± 0.1^b	
	4	−25.6 ± 0.1^a		−0.9 ± 0.3^a		4.8 ± 0.1^a	
$LSD_{0.05}$ for fertilizer amounts		0.5	—	0.5	—	0.2	—
$LSD_{0.05}$ for fertilizer type		—	0.4	—	0.6	—	0.4

Data are presented as mean ± standard deviation; $^{a-d}$ significant difference between the fertilizer amounts and/or fertilizer types.
LSD = least significant difference.

Table 2
Differences of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in 6-yr-old ginseng roots cultivated in a paddy-converted field depending on the type and quantity of fertilizers

Fertilizer type	Quantity (ton/1,000 m^2)	$\delta^{13}C_{VPDB}$/‰, $n = 3$	Mean, $n = 9$	$\delta^{15}N_{AIR}$/‰, $n = 3$	Mean, $n = 9$	$\delta^{34}S_{VCDT}$/‰, $n = 3$	Mean, $n = 9$
Control			-26.8 ± 0.2^{c}		4.4 ± 1.2^{a}		2.2 ± 0.5^{b}
Cattle manure	1	-26.8 ± 0.0^{c}	-25.9 ± 0.7^{a}	5.5 ± 0.1^{a}	4.4 ± 0.7^{a}	3.1 ± 0.4^{a}	2.6 ± 0.5^{ab}
	2	-26.1 ± 0.0^{b}		4.0 ± 0.1^{c}		2.0 ± 0.2^{b}	
	4	-25.1 ± 0.0^{a}		4.2 ± 0.1^{b}		2.8 ± 0.2^{a}	
$LSD_{0.05}$ for fertilizer amounts		0.1	—	0.2	—	0.6	—
Food waste	1	-26.3 ± 0.1^{a}	-26.6 ± 0.4^{bc}	5.5 ± 0.0^{a}	4.7 ± 0.7^{a}	2.3 ± 0.1^{b}	2.3 ± 0.1^{b}
	2	-26.4 ± 0.1^{a}		4.4 ± 0.0^{b}		2.2 ± 0.1^{b}	
	4	-27.0 ± 0.1^{b}		4.1 ± 0.0^{c}		2.5 ± 0.1^{a}	
$LSD_{0.05}$ for fertilizer amounts		0.2	—	0.0	—	0.1	—
Rice straw	1	-26.5 ± 0.0^{b}	-26.3 ± 0.3^{ab}	3.7 ± 0.0^{a}	3.0 ± 0.7^{b}	3.3 ± 0.1^{b}	3.0 ± 0.7^{a}
	2	-26.5 ± 0.1^{b}		3.3 ± 0.2^{b}		3.6 ± 0.1^{a}	
	4	-26.0 ± 0.0^{a}		2.1 ± 0.1^{c}		2.2 ± 0.1^{c}	
$LSD_{0.05}$ for fertilizer amounts		0.1	—	0.2	—	0.2	—
$LSD_{0.05}$ for fertilizer type		—	0.4	—	0.8	—	0.5

Data are presented as mean ± standard deviation; [a–c] significant difference between the fertilizer amounts and/or fertilizer types.
LSD = least significant difference.

detected in C3 plants [24]. Although the mean $\delta^{13}C_{VPDB}$ value of ginseng statistically differed between the two cultivation land types and organic fertilizer types applied in this study ($p < 0.05$), these differences were marginal. In fact, these differences might be associated with the variation of local (micro-) environmental conditions such as water deficit, vapor pressure deficit, light intensity, and temperature in the ginseng cultivation field. For example, the change of photosynthetic capability by the (micro-) environmental factors may discriminate against the heavier C isotope (^{13}C) between plant and atmosphere [24,25].

Meanwhile, the analysis of N isotope composition can be applicable to discriminate agricultural practice. Because atmospheric N_2 is the most crucial source of N in synthetic fertilizers, the $\delta^{15}N_{AIR}$ values of synthetic N fertilizers are ideally expected to be

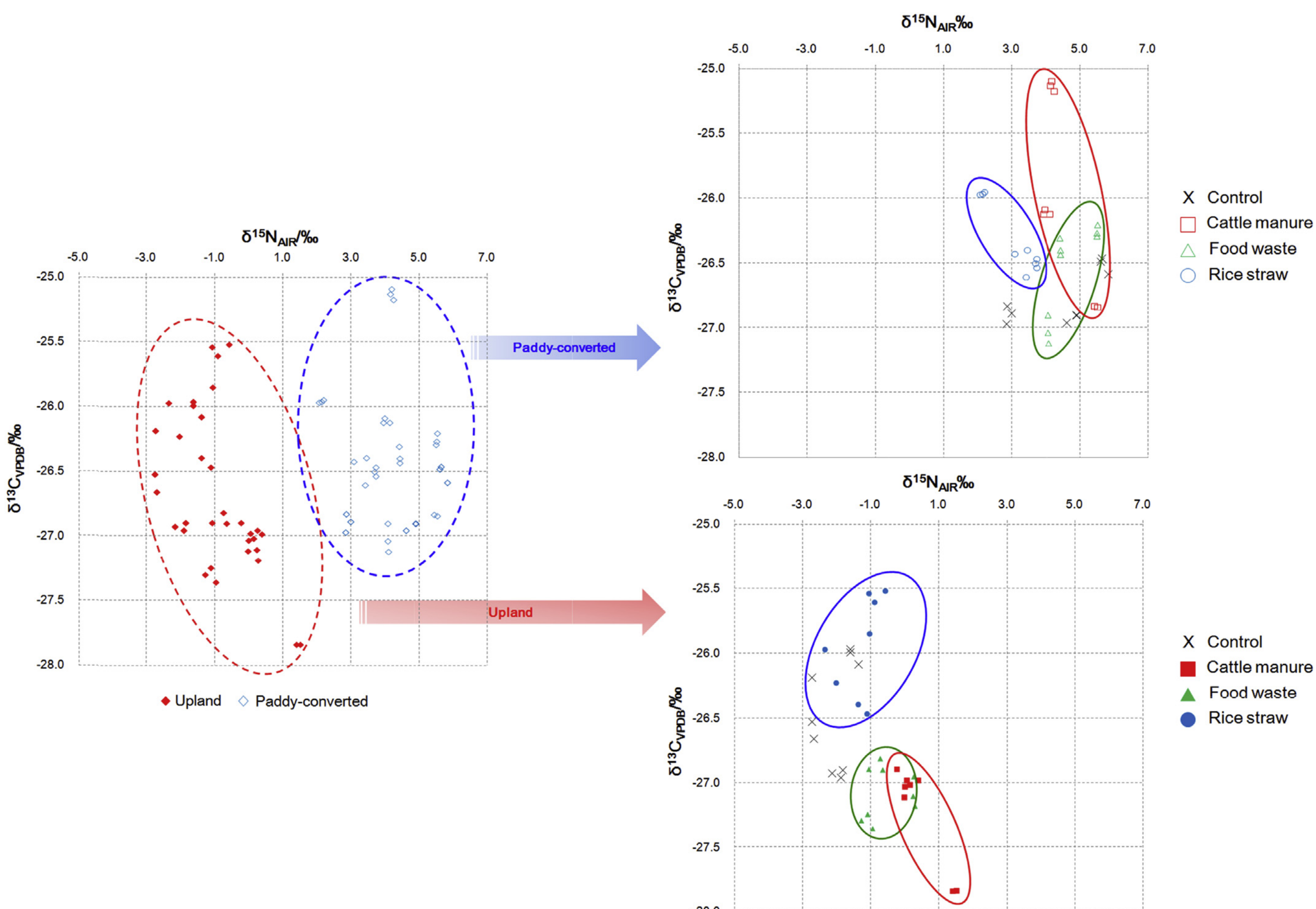

Fig. 1. Variability of the combined C and N stable isotope ratios in 6-yr-old ginseng roots depending on the type of cultivation land and organic fertilizer.

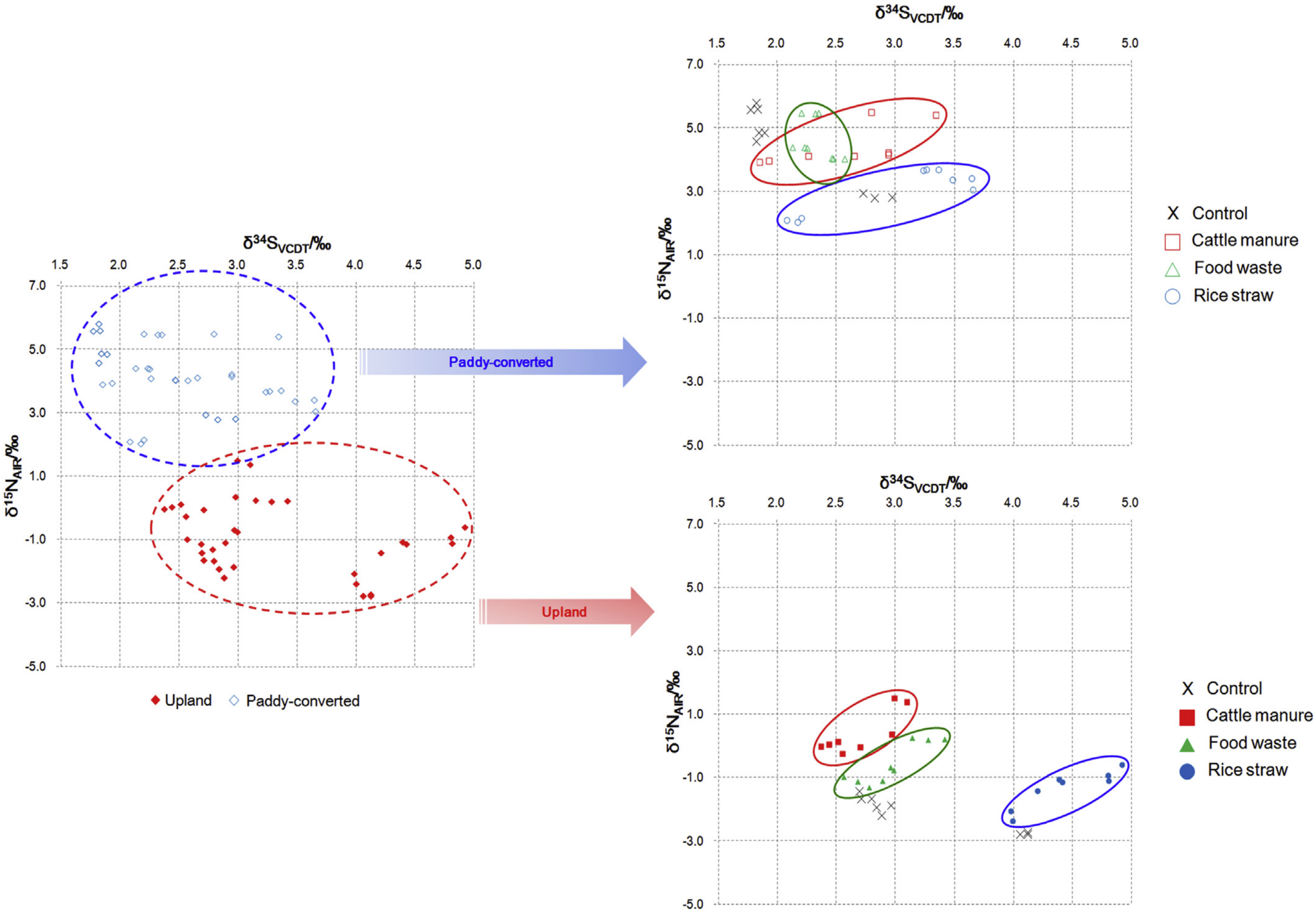

Fig. 2. Variability of the combined N and S stable isotope ratios in 6-yr-old ginseng roots depending on the type of cultivation land and organic fertilizer.

about 0‰; however, unlike synthetic N fertilizers, organic N fertilizers such as manure-based fertilizers have higher $\delta^{15}N_{AIR}$ value [26]. Thus, the $\delta^{15}N_{AIR}$ value of synthetic N fertilizers ranged from −4‰ to +4‰; whereas $\delta^{15}N_{AIR}$ of organic fertilizers (i.e., green manures, compost, and liquid/solid animal waste) had a more broad range, from 2‰ to 30‰, due to their diverse origin [26]. Hence, the different N isotope composition in crops reflects local agricultural practices (e.g., the amount and type of fertilizer) and soil type/condition (e.g., soil nutrition and isotopic fractionation by nitrification/denitrification) [22,27,28].

In this study, the $\delta^{15}N_{AIR}$ value of ginseng also varied with cultivation land type and organic fertilizer type ($p < 0.05$, Tables 1 and 2). With prior studies [1,9], unlike upland fields, the organic material is usually slowly decomposed in paddy-converted fields. Furthermore, the soil property of paddy-converted fields usually differs from that of upland fields, because of the repeated soil oxidation and reduction by the frequent flooding and drainage in paddy fields. In addition, because manures and composts usually contain low amounts of soluble nitrogen and organic nitrogen, they have to be transformed as plant-available forms (indicating mineralization) [26]. However, the N isotopic fractionation typically occurs during mineralization process, and it is also affected by soil type and conditions. In particular, manures are known to be susceptible against N isotopic fractionation [26].

Therefore, despite not measuring the N isotope composition in soil and fertilizer in this study, the variation of $\delta^{15}N_{AIR}$ in ginseng depending on soil type and fertilizer type may be explained with the N fertilizer uptake rate (or efficiency) of ginseng caused by different N fertilizer source, soil property, or combination of these two factors. Hence, the measurement of N isotope composition may be suggested as a potential chemical marker to evaluate ginseng authenticity (e.g., agricultural practice).

The variation in $\delta^{34}S_{VCDT}$ among agricultural products remains poorly understood [29]. In general, isotopic fractionation of S is known to be more likely to occur via physical processes than through biological processes [30]. The geology of a region (i.e., igneous vs. sediment or acidic vs. basic) can typically affect to the S isotope composition of soil, and subsequently influences to organic matters cultivated in these soil. Therefore, the analysis of S isotope composition can be connected with the authenticity of geographical regions [29,30]. Furthermore, the variation of $\delta^{34}S_{VCDT}$ is also associated with the use of the sulfur-containing fertilizers, SO_2 emission, as well as distance from the coast (called sea-spray effect) [22,31–33].

According to a prior study [34], the S isotopic composition of fertilizers is widely variable. For example, the $\delta^{34}S_{VCDT}$ value of a synthetic liquid fertilizer was 5.8‰, whereas in solid fertilizers it ranged from −0.5‰ to +19.9‰. Moreover, the $\delta^{34}S_{VCDT}$ range of animal manures was from 2.3‰ to 6.8‰, and in human septic sludge and sewage sludge, the mean $\delta^{34}S_{VCDT}$ value was 1.0‰ and 2.3‰, respectively. In particular, the reduction or oxidation is of the most important process to the S isotope composition in S-containing compounds, and the S isotopic fractionation is typically greater during the abiotic or bacterial dissimilatory reduction step (sulfate/sulfite → sulfide) than during the oxidation step [34].

Because the S isotope composition in soil and fertilizer did not measure in this study, the pattern of S isotopic fractionation between ginseng and soil (/or fertilizer) cannot be determined. However, we observed that the $\delta^{34}S_{VCDT}$ value in ginseng applied with the cattle manure was similar to that of the other animal manures [34]. Furthermore, the S isotope composition in ginseng also varies depending on soil type and fertilizer type, and it may indicate that the measurement of S isotope composition can be a potential isotope marker to assess ginseng authenticity.

In summary, this preliminary study describes the variation of C, N, and S isotope composition in 6-yr-old ginseng roots depending on the cultivation soil type and the applied organic fertilizer type. Although this study lacks $\delta^{15}N_{AIR}$ and $\delta^{34}S_{VCDT}$ information in the ginseng cultivation soil and fertilizers applied, the analyses of N and S isotope composition in ginseng can be discriminated with the cultivation soil types. Also, of N or S isotope composition in ginseng was found to be different depending on the fertilizer type applied. Hence, further analyses of isotope composition in soil and fertilizer is needed in order to confirm the reliable evidence about the isotopic fractionation pattern between ginseng and cultivation soil (or fertilizer type/amounts applied).

Conflicts of interest

All authors have no conflicts of interest to declare.

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