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REGULAR ARTICLE

Nitrogen and carbon isotope responses of Chinese cabbage and chrysanthemum to the application of liquid pig manure

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Abstract The effects of the liquid pig manure (LM) used in organic farming on the natural abundance of ^{15}N and ^{13}C signatures in plant tissues have not been studied. We hypothesized that application of LM will (1) increase $\delta^{15}N$ of plant tissues due to the high $\delta^{15}N$ of N in LM as compared with soil N or inorganic fertilizer N, and (2) increase $\delta^{13}C$ of plant tissues as a result of high salt concentration in LM that decreases stomatal conductance of plants. To test these hypotheses, variations in the $\delta^{15}N$ and $\delta^{13}C$ of Chinese cabbage (*Brassica campestris* L.) and chrysanthemum (*Chrysanthemum morifolium* Ramatuelle) with two different LMs (with $\delta^{15}N$ of +15.6 and +18.2‰) applied at two rates (323 and 646 kg N ha^{-1} for cabbage and 150 and 300 kg N ha^{-1} for chrysanthemum), or urea ($\delta^{15}N$ = -2.7‰) applied at the lower rate above for the respective species, in addition to the control (no N input) were investigated through a 60-day pot experiment. Application of LM significantly increased plant tissue $\delta^{15}N$ (range +9.4 to +14.9‰) over the urea (+3.2 to +3.3‰) or control (+6.8 to 7.7‰) treatments regardless of plant species, strongly reflecting the $\delta^{15}N$ of the N source. Plant tissue $\delta^{13}C$ were not affected by the treatments for cabbage (range −30.8 to −30.2‰) or chrysanthemum (−27.3 to −26.8‰). However, cabbage dry matter production decreased while its $\delta^{13}C$ increased with increasing rate of LM application or increasing soil salinity ($P < 0.05$), suggesting that salinity stress caused by high rate of LM application likely decreased stomatal conductance and limited growth of cabbage. Our study expanded the use of the $\delta^{15}N$ technique in N source (organic vs. synthetic fertilizer) identification and suggested that plant tissue $\delta^{13}C$ maybe a sensitive indicator of plant response to salinity stress caused by high LM application rates.

Keywords $\delta^{13}C$ · $\delta^{15}N$ · Liquid livestock manure · Organic input · Salinity · Synthetic fertilizer

Abbreviations
LM Liquid manure
SM Solid manure

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EC Electrical conductivity of saturated soil extracts
$\delta^{15}N$ The abundance of ^{15}N in a sample relative to the standard with per mil as unit
$\delta^{13}C$ The abundance of ^{13}C in a sample relative to the standard with per mil as unit
TOC Total organic C
TON Total organic N

Introduction

Disposal of pig manure is one of the environmental issues that operators and regulators have to deal with in large scale feedlot operations (Møller et al. 2000). Pig manure consisted of solid (SM) and liquid manure (LM) fractions that can be separated through centrifugation or filtration (Møller et al. 2002). The SM fraction can be transported off-farm, composted with other organic material, and used as a soil amendment. The LM fraction has lower concentrations of nutrients and pollutants as compared with the SM fraction and can be discharged into water systems after a treatment process such as anaerobic digestion (Martinez-Almela and Barrera 2005). Since the LM fraction has a low C/N ratio and nutrients contained are more readily available as compared with the SM fraction, use of the LM fraction as a fertilizer is now being accepted as an alternative to wastewater treatment (Sánchez and González 2005; Sørensen and Thomsen 2005). Several aspects of the field application of whole livestock manure (such as pig manure) or its LM fraction have been studied including nutrient uptake and crop growth (McGonigle and Beauchamp 2004; Sørensen and Thomsen 2005), soil nutrient dynamics (Loria and Sawyer 2005), effects on soil properties (Diez et al. 2004), and environmental impacts (Diez et al. 2001, 2004).

The use of whole livestock manure or its LM fraction as a fertilizer is permitted in organic farming systems according to the 'Guidelines for the Production, Processing, Marketing and Labelling of Organically Produced Foods' (Codex Committee on Food Labelling 2001). According to the guidelines, the organic inputs need to be recognized by certification bodies or authorities if they are not originated from organic production systems. Recently, the potential use of crop tissue nitrogen isotope ratio ($^{15}N/^{14}N$, expressed as $\delta^{15}N$) for certifying organic produce has been extensively tested (Choi et al. 2002, 2003; Bateman et al. 2005; Georgi et al. 2005; Yun et al. 2006). This approach is mainly based on the fact that produce conventionally grown with synthetic fertilizers (that are ^{15}N depleted as compared with organic fertilizers) tends to have lower $\delta^{15}N$ than that grown with organic fertilizers. Nitrogen in synthetic fertilizers has low $\delta^{15}N$ values (−5 to +3‰) reflecting their original source of N, i.e., atmospheric N_2 (that has a $\delta^{15}N$ of 0‰), while organic fertilizers such as livestock manure have higher $\delta^{15}N$ values (+10 to +22‰) largely due to a greater volatilization loss of $^{14}NH_3$ relative to $^{15}NH_3$ from manure, causing the remaining N in the manure to be enriched in ^{15}N (Freyer and Aly 1974; Choi et al. 2003).

Organic fertilizers such as solid pig manure (Choi et al. 2002, 2003; Yun et al. 2006), chicken manure (Bateman et al. 2005), cattle manure (Choi et al. 2006), corn steep liquor (Nakano et al. 2003), hornmeal (Georgi et al. 2005), and whole pig manure (Choi et al. 2006) have been studied to show that their application increased ^{15}N enrichment in plant tissues when compared with synthetic fertilizer applications. However, we do not know if the application of LM fraction has the same effect on plant tissue $\delta^{15}N$ despite the wide use of LM fraction in organic cropping systems. In Korea, farmers usually apply LM fraction at high rates, and yet the $\delta^{15}N$ response of plant tissues to the application of LM fraction at various rates has not been studied.

In a similar fashion, the stable C isotope ratio ($^{13}C/^{12}C$, expressed as $\delta^{13}C$) of crop tissues in conventional and organic farming system has been explored by a few researchers (Nakano et al. 2003; Georgi et al. 2005; Schmidt et al. 2005). They hypothesized that plants under contrasting farming systems (conventional vs. organic) may have different $\delta^{13}C$ as plant $\delta^{13}C$ is an integrator of gas exchange response to different environmental conditions associated with water and nutrient stresses (Choi et al. 2005a). These stresses can affect leaf stomatal conductance and photosynthetic enzyme activity, leading to changes in plant tissue $\delta^{13}C$ values (Farquhar et al. 1989).

As the LM fraction usually has a high salt concentrations (Sánchez and González 2005), application of LM fraction may cause water stress to plants due to salinity, resulting in decreases in

biomass production, particularly under high application rates of LM fraction. According to the ^{13}C discrimination model of Farquhar et al. (1989), water stress reduces stomatal conductance forcing plants to use CO_2 more efficiently and thus results in less carbon isotope discrimination against $^{13}CO_2$ (i.e., less negative $\delta^{13}C$ values).

The objectives of this study were to investigate (1) whether the application of LM fraction leaves typical $\delta^{15}N$ signatures in plant tissues consistent with the use of organic fertilizers, (2) whether increased application rate of LM fraction increases plant tissue $\delta^{15}N$ values, and (3) whether plant tissue $\delta^{13}C$ reflects soil salinity changes caused by different application rates of LM fraction.

Materials and methods

Soil and the LM samples

A sandy loam soil (Typic Dystrudepts in Soil Taxonomy) was collected from an experimental farm (126°36′08″E, 35°10′21″N) of Chonnam National University, Korea. The soil was hand-mixed and used for a pot experiment. The soil had a $pH_{water\ (1:1)}$ of 6.3, electrical conductivity of saturated pastes (EC_e, see below for a description of the methods for chemical analyses) of 1.1 dS m^{-1}, total N of 1.4 g kg^{-1}, NH_4^+–N of 7.2 mg kg^{-1}, NO_3^-–N of 10.1 mg kg^{-1}, and organic C of 14.0 g kg^{-1}. The $\delta^{15}N$ of total N was +6.2‰.

The two LM samples (referred to as LM-A and LM-B) were collected from storage tanks of two local farmers in Chonnam, Korea. The LM fraction was separated from the SM fraction with a vibrating screen (with a 0.2 mm opening) on the farms. The LM samples were transported to the laboratory and used for the pot experiment. The chemical properties of the LM samples were very similar to each other, showing electrical conductivities over 20.0 dS m^{-1} and $\delta^{15}N$ values over +15.0‰ (Table 1).

Pot experiment

A pot experiment with Chinese cabbage (*Brassica campestris* L. cv. Sambok) and chrysanthemum (*Chrysanthemum morifolium* Ramatuelle cv. Shinma) was conducted in a greenhouse at Chonnam National University, Korea. Six treatments were laid out in a completely randomized factorial design with four replications; control without nutrient application (code: Control), urea (Urea; $\delta^{15}N$ was −2.7‰) at the standard application rate, LM-A at the standard and double N rate (LM-A1 and LM-A2, respectively), and LM-B at the standard and double N rate (LM-B1 and LM-B2, respectively). Standard N rates recommended by the Korean government for cabbage are 111 kg N ha^{-1} as basal and 212 kg N ha^{-1} as additional applications and those for chrysanthemum are 100 kg ha^{-1} and 50 kg ha^{-1}, respectively. In the LM treatments, no supplementary application of P and K fertilizers was necessary as the LM applied provided plants with sufficient amounts of P and K over the standard application rate (34.5 kg P ha^{-1} and 92.5 kg K ha^{-1} for Chinese cabbage and 65.5 kg P ha^{-1} and 83.3 kg K ha^{-1} for chrysanthemum) recommended by the Korean government. For example, in the LM-A1 treatment, the rates of P and K applied were 207 kg P ha^{-1} and 440 kg K ha^{-1} for Chinese cabbage and 96.9 kg P ha^{-1} and 205.8 kg K ha^{-1} for chrysanthemum.

A total of 48 pots were prepared (six treatments × two crop species × four replications). A 5 cm layer of gravel (<2 cm diameter) was placed at the bottom of each pot (20 cm bottom diame-

Table 1 Chemical properties of the two studied liquid pig manure (LM)

Parameters	LM-A	LM-B
pH	9.4 (0.1)	8.7 (0.1)
EC (dS m^{-1})	24.7 (0.7)	20.1 (0.4)
Total organic C (g l^{-1})	2.4 (0.1)	3.7 (0.1)
Total N (g l^{-1})	2.5 (0.1)	2.6 (0.1)
Total P (g l^{-1})	1.6 (0.1)	1.7 (0.1)
Total K (g l^{-1})	3.4 (0.3)	2.9 (0.4)
$\delta^{15}N$ of total N (‰)	+15.6 (0.2)	+18.2 (0.2)

Values are means with standard errors in parentheses (n = 3)

ter × 25 cm top diameter × 20 cm height) and the surface of the gravel layer was lined with nylon nets to minimize the loss of soil through the gravel layer. After passing through a 10-mm sieve, the soil (4.5 kg on oven dry weight basis) was placed into each pot to form a 15 cm layer, resulting in a bulk density of 0.92 Mg m^{-3}. One seedling was planted in each pot on June 14, 2005 and basal fertilizer was applied on June 24. The additional fertilizer was applied on July 15. The LM and urea (in solution) was applied on the surface of the soil as evenly as possible. Details of the treatments (such as the amount of nutrients applied) can be found in Table 2.

During the experiment, soil moisture content was measured daily using time domain reflectometry (TRIME-FM, IMKO Micromodultechnik GmbH, Ettlingen, Germany) and adjusted to 0.25 m^3 m^{-3} (approximately 35 kPa) by manual watering. The aboveground parts of the plants were harvested on August 14. After harvest, the soil in each pot was thoroughly mixed and about 1 kg of sample was collected for chemical analyses.

Plant growth and N uptake measurements

For cabbage, the initial dry weight (0.22 ± 0.02 g, mean ± SE, n = 12) and N content (6.2 ± 0.2 mg N $plant^{-1}$) of seedlings were measured on 12 randomly selected extra seedlings that were relatively uniform. The final dry weight and N content of seedlings in each pot were measured at the final harvest. The increments of dry matter and N content of cabbage were calculated by subtracting these initial values from the final values. For chrysanthemum, because the seedling height was variable, the initial dry weight and N content of the planted seedlings were estimated using equations (DW = 0.0301H and N = 0.908H) developed on the seedling height (H, cm) and dry weight (DW, g $plant^{-1}$) or N content (mg N $plant^{-1}$) data, collected from 12 extra seedlings randomly selected before planting. The height, dry weight, and N content of the extra seedlings averaged 27.8 ± 1.6 cm (range 22.0–36.0 cm), 0.8 ± 0.1 g $plant^{-1}$ (range 0.5–1.3 g $plant^{-1}$), and 24.6 ± 2.9 mg N $plant^{-1}$ (range 15.2–43.1 mg N $plant^{-1}$), respectively. Those regression equations were developed in this study and the statistics show that there were strong relationships between seedling dry weight and height (r^2 = 0.72, $P < 0.001$) and N content and height (r^2 = 0.74, $P < 0.001$). Seedling height of chrysanthemum was measured immediately after planting on June, 14. The final dry weight and N content of seedlings in each pot were determined at the final harvest. Growth and N content increments were calculated as the difference between the final and initial values.

Chemical analyses

The initial chemical properties of the soil samples were measured as follows: pH at a 1:1 (v:w) water-to-soil ratio using a pH meter (P25, EcoMet, Seoul,

Table 2 Summary of treatments applied to cabbage and chrysanthemum in the pot experiment

Treatment code	Gram per pot for inorganic fertilizers and liter per pot for the liquid pig manure			
	Cabbage		Chrysanthemum	
	Basal	Additional	Basal	Additional
Control	None	None	None	None
Urea	Urea: 1.07 (111)[a]	Urea: 2.04 (212)	Urea: 0.96 (100)	Urea: 0.48 (50)
	KH_2PO_4: 0.68	K_2SO_4: 2.07	KH_2PO_4: 1.29	K_2SO_4: 1.16
	K_2SO_4: 0.49		K_2SO_4: 0.006	
LM-A1	0.190 (111)	0.365 (212)	0.165 (100)	0.085 (50)
LM-A2	0.380 (222)	0.730 (424)	0.350 (200)	0.170 (100)
LM-B1	0.200 (111)	0.385 (212)	0.180 (100)	0.090 (50)
LM-B2	0.400 (222)	0.770 (424)	0.360 (200)	0.180 (100)

[a] Values in the parentheses are application rates expressed as kg N ha^{-1}

Korea), EC in saturated paste (Rhoades 1996) using a conductivity meter (30/10 FT, YSI, OH, USA), total N and total organic C using a combustion method on an elemental analyzer (NA Series 2, CE Instruments, Milan, Italy), and mineral N concentrations in 2 mol L^{-1} KCl extract at a 5:1 (v:w) extractant-to-soil ratio using a steam distillation system (Pronitro 1, J.P. Selecta, Barcelona, Spain). The $\delta^{15}N$ of total N was measured using a continuous-flow stable isotope ratio mass spectrometer (IsoPrime-EA, Micromass, Manchester, UK) linked to a CN analyzer (NA Series 2, CE Instruments, Milan, Italy).

The LM samples were analyzed for pH and EC with the above-mentioned pH and conductivity meters, respectively, total N and organic C with a TOC/TON analyzer (TOC-Vcsn, Shimadzu, Kyoto, Japan), and total P and K with an ICP (IRIS-AP, Thermo Jarrell Ash, MA, USA) following digestion with H_2SO_4–H_2O_2. To determine $\delta^{15}N$ of total N in the LM, the LM samples were digested using the Kjeldahl digestion method that converts all N to NH_4^+ and steam-distilled after addition of 20 mL of 10 mol L^{-1} NaOH (Bremner 1996). The liberated NH_3 was collected in 0.005 mol L^{-1} H_2SO_4 solutions, and the solutions that contain the NH_4^+ from steam distillation were adjusted to pH 3 using 0.05 mol L^{-1} H_2SO_4. The solution was evaporated to dryness at 65°C in an oven, and the powder (ammonium sulfate) was analyzed for $\delta^{15}N$ using the above-mentioned mass spectrometer (Feast and Dennis 1996).

After harvest, saturated soil extracts were obtained using around 500 g of fresh soil samples (Rhoades 1996), and were analyzed for EC in saturated pastes. The plant samples were washed with distilled water, oven-dried at 65°C, and weighed to determine yield. The dried samples were ground to fine powder with a ball mil (MM 200, Retsch GmbH & Co. KG, Hann, Germany) and analyzed for $\delta^{15}N$, %N, and $\delta^{13}C$ with the mass spectrometer described above.

Carbon and nitrogen isotope compositions were calculated as

$$\delta(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1{,}000 \quad (1)$$

where R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and the standards are the Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen. Pure CO_2 ($\delta^{13}C = -28.2 \pm 0.1$‰) and N_2 ($\delta^{15}N = -2.1 \pm 0.1$‰) gas served as reference gases. The accuracy and reproducibility of the measurements of $\delta^{13}C$ and $\delta^{15}N$ checked with an internal reference material, a cabbage sample (-28.3 ± 0.1‰ for $\delta^{13}C$ and $+3.4 \pm 0.1$‰ for $\delta^{15}N$), calibrated against NIST SRM 8542 (sucrose, $\delta^{13}C = -10.5$‰) and IAEA-N2 (ammonium sulfate, $\delta^{15}N = +20.3$‰), were better than 0.2 and 0.1‰ for $\delta^{13}C$ and 0.3 and 0.2‰ for $\delta^{15}N$, respectively.

Statistical analyses

For statistical analysis, data were first tested for homogeneity of variance and normality of distribution. No heterogeneity was detected in the data set and the distribution was normal. Analysis of variance (ANOVA) was performed on all experimental variables using the general linear models procedure of the SPSS 11.5 package (SPSS Inc., Chicago, IL, USA) to assess treatment effects. When treatment effects were significant, means were separated by Duncan's multiple range test. Regression analysis was performed to examine the relationships between soil EC_e and dry matter accumulation and between soil EC_e and $\delta^{13}C$ of plant tissues. The level of significance for all statistical tests was set at $\alpha = 0.05$.

Results

Soil EC_e

At the time of final harvest, electrical conductivity increased over more than 200% by the application of N as compared with the control in both cabbage and chrysanthemum grown soils (Table 3). Overall, soils applied with LM at the standard rate had EC_e levels comparable to those applied with urea; however, application of LM at double the standard rate led to a higher EC_e than in the urea treatment. As a result a wide range of EC_e was obtained with values ranging between 0.9 and 2.4 and between 1.1 and 3.0 dS m^{-1} in the cabbage and chrysanthemum grown soils, respectively.

Plant growth and N uptake

Application of urea or LM increased dry matter production (up to 44.8% for the LM-A1 treatment) and N uptake (by 65.2% for the urea treatment) of

Table 3 Electrical conductivity of saturated extract (EC_e, dS m^{-1}) of soils after the final harvest of plants

Treatment code	Cabbage grown soil	Chrysanthemum grown soil
Control	0.9 (0.1) a	1.1 (0.2) a
Urea	1.9 (0.1) b	2.2 (0.2) b
LM-A1	1.7 (0.1) b	2.3 (0.2) b
LM-A2	1.9 (0.1) b	2.8 (0.1) c
LM-B1	1.7 (0.1) b	2.1 (0.1) b
LM-B2	2.4 (0.1) c	3.0 (0.2) c
ANOVA (Probability > *F*)		
Treatment effect	0.001	0.001

Values are means with standard errors in parentheses ($n = 4$). Values in the same column followed by different lowercase letters are significantly different at $\alpha = 0.05$

Table 4 Dry matter production and N uptake, $\delta^{13}C$, and $\delta^{15}N$ of cabbage

Treatment code	Dry matter (g $plant^{-1}$)	N uptake (g $plant^{-1}$)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Control	10.5 (0.6) a	0.23 (0.02) a	+7.7 (0.5) b	−30.8 (0.1) a
Urea	13.8 (0.4) c	0.38 (0.01) c	+3.2 (0.2) a	−30.2 (0.1) a
LM-A1	15.2 (0.6) d	0.32 (0.01) b	+11.9 (0.6) c	−30.5 (0.2) a
LM-A2	12.7 (0.3) bc	0.37 (0.01) c	+13.2 (0.6) cd	−30.4 (0.1) a
LM-B1	13.2 (0.3) c	0.32 (0.02) b	+13.0 (0.3) cd	−30.5 (0.2) a
LM-B2	12.0 (0.5) b	0.34 (0.01) bc	+14.9 (0.8) d	−30.2 (0.2) a
ANOVA (Probability > *F*)				
Treatment effect	0.001	0.001	0.001	0.086

Values are means with standard errors in parentheses ($n = 4$). Values in the same column followed by different lowercase letters are significantly different at $\alpha = 0.05$

cabbage as compared with the control (Table 4). Application of LM at the standard N rate resulted in dry matter production of cabbage (15.2 for LM-A1 and 13.2 g $plant^{-1}$ for LM-B1) at a level comparable to that in the urea treatment (13.8 g $plant^{-1}$). However, LM application at the double N rate decreased dry matter production of cabbage by 16.4% for LM-A and 9.1% for LM-B as compared to the standard N rate. Overall, the accumulation of N in cabbage applied with LM (0.32–0.37 g N $plant^{-1}$) tended to be lower than that applied with urea (0.38 g N $plant^{-1}$).

Similar to cabbage, dry matter production and N accumulation of chrysanthemum were also greater in the treated than in the control pots (Table 5). However, dry matter production of chrysanthemum was not decreased by the increasing rate of LM application, a response different from that of cabbage.

Plant $\delta^{15}N$ and $\delta^{13}C$

Compared with the control, urea application lowered the $\delta^{15}N$ in plant tissues from +7.7 to +3.2‰ for cabbage and from +6.8 to +3.3‰ for chrysanthemum (Tables 4, 5). In contrast, LM application increased plant tissue $\delta^{15}N$ to as high as +14.9‰ for cabbage and +11.3‰ for chrysanthemum. Between LM-A and LM-B, application of the more ^{15}N-enriched LM-B resulted in higher plant tissue $\delta^{15}N$ values. Increased rate of LM application tended to reinforce the ^{15}N enrichment in plant tissues; e.g., the $\delta^{15}N$ of cabbage increased from +11.9 to +13.2‰ in the LM-A treatment and from +13.0 to +14.9‰ in the LM-B treatment.

The $\delta^{13}C$ in plant tissues was not statistically different among the treatments. Plant tissue $\delta^{13}C$ ranged between −30.8 and −30.2‰ for cabbage (Table 4) and between −27.3 and −26.8‰ for

Table 5 Dry matter production and N uptake, $\delta^{13}C$, and $\delta^{15}N$ of chrysanthemum

Treatment code	Dry matter (g plant^{-1})	N uptake (g plant^{-1})	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Control	8.2 (0.4) a	0.10 (0.01) a	+6.8 (0.3) b	−27.3 (0.1) a
Urea	9.8 (0.5) b	0.16 (0.01) bc	+3.3 (0.2) a	−27.1(0.1) a
LM-A1	9.8 (0.4) b	0.15 (0.01) b	+9.4 (0.3) c	−27.0 (0.1) a
LM-A2	9.9 (0.5) b	0.19 (0.01) cd	+9.6 (0.7) cd	−26.9 (0.1) a
LM-B1	9.7 (0.2) b	0.18 (0.01) bcd	+10.8 (0.6) de	−27.0 (0.1) a
LM-B2	9.6 (0.4) b	0.20 (0.01) d	+11.3 (0.3) e	−26.8 (0.1) a
ANOVA (Probability > *F*)				
Treatment effect	0.022	0.001	0.001	0.074

Values are means with standard errors in parentheses ($n = 4$). Values in the same column followed by different lowercase letters are significantly different at $\alpha = 0.05$

chrysanthemum (Table 5), although the application of urea or LM tended to decrease ^{13}C isotope discrimination (less negative $\delta^{13}C$) as compared with the control.

Discussion

Plant tissue $\delta^{15}N$ values

The variation of $\delta^{15}N$ in plant tissues as affected by synthetic and organic fertilizer applications has been tested extensively with different input types, crop species, and soil types since Yoneyama et al. (1990) reported difference of $\delta^{15}N$ in rice (*Oryza sativa*) plants between synthetic fertilizer and livestock manure applications. However, through the literature search, we found few reports that studied the effects of liquid livestock manure application on $\delta^{15}N$ variations in plant tissues in spite of the wide use of liquid livestock manure. The pattern of $\delta^{15}N$ in plants subjected to different kinds of N input observed in our study is consistent with other studies. For example, Choi et al. (2003) found that the $\delta^{15}N$ in crops (20 samples from nine different crops) collected from conventional plots (the $\delta^{15}N$ of the synthetic fertilizers used ranged from −3.9 to +0.5‰) averaged +4.1‰ (range +0.3 to +6.4‰) and that from organic (the $\delta^{15}N$ of the composted manure applied ranged from +15.4 to +19.4‰) plots averaged +14.6‰ (range +9.3 to +21.2‰) in the early growth stage, about 1 month after fertilization. Bateman et al. (2005) also reported that carrots (*Daucus carota*) grown with chicken manure (+5.4‰) had $\delta^{15}N$ values higher than that grown with ammonium nitrate (−0.4‰) by 3–4‰ in a pot experiment. Such patterns were also observed in fertigation systems; Nakano et al. (2003) compared $\delta^{15}N$ of tomato (*Lycopersicon esculentum*) with chemical (0.0‰) and organic fertilizers (corn steep liquor, a byproduct from the cornstarch manufacturing industry, with a mean $\delta^{15}N$ value of +8.5‰) and found higher $\delta^{15}N$ in the organic fertigation (+7.1‰) than in the inorganic fertigation (+0.3‰) treatment. In addition, our study showed that the higher rate of liquid organic fertilizer application led to a higher $\delta^{15}N$ in plant tissues.

However, applications of organic N do not always result in plant $\delta^{15}N$ signatures that are different from those with synthetic fertilizer applications. Georgi et al. (2005) reported that $\delta^{15}N$ in plants, cabbage (*B. oleracea*), onion (*Allium cepa*), lettuce (*Lactuca sativa*), and Chinese cabbage, grown with ammonium nitrate (+0.7‰) ranged between ca. +5 and +9‰, while those grown with hornmeal (+6.0‰) made of animal horn and hoofs had $\delta^{15}N$ values between +6 and +10‰. These overlapping values may be attributed to ^{15}N enrichment of N derived from synthetic fertilizer through N loss via NH_3 volatilization and denitrification (Schmidt et al. 2005). It has clearly been demonstrated that ^{15}N enrichment in plant tissues is directly related with N losses that increase $\delta^{15}N$ of the N remaining in the soil due to N isotopic fractionation (e.g., Johannisson and Högberg 1994). For example, Choi et al. (2002) observed that the $\delta^{15}N$ (+1.1‰) of maize (*Zea mays*) treated with urea (−2.3‰) was lower than that (+7.7‰) treated with composted pig manure (+13.9‰) after 30 days of growth; however, thereafter the $\delta^{15}N$ of maize in the

urea treatment progressively increased to as high as +6.0‰, resulting in similar δ^{15}N signatures in plant tissues between the treatments.

In our study, the differences of δ^{15}N values in plant tissues between the urea and LM treatments were greater than the results observed by others; the application of LM increased plant δ^{15}N by more than 8‰ for cabbage and by more than 6‰ for chrysanthemum over those in the urea treatment. However, the difference in plant δ^{15}N at harvest between the synthetic and organic fertilizers was <4‰ in Bateman et al. (2005), <2‰ in Georgi et al. (2005), and <2‰ in Choi et al. (2002). This pattern might have reflected differences in the availability of N in the organic input; i.e., when the organic input was in the liquid form, as was in our study and in the fertigation study of Nakano et al. (2003), the difference in plant δ^{15}N between organic and inorganic inputs became greater than when the organic input was in the solid form, which usually have lower N availabilities than the liquid type of organic fertilizers (Lupwayi et al. 2005). In a 4-year crop rotation (canola-barley-wheat-canola) experiment conducted in northwestern Alberta, Canada, Choi et al. (2006) also found that δ^{15}N in grain was greater after liquid hog manure application (δ^{15}N in grain ranged from +5.6 to +8.4‰) than after solid cattle manure application (δ^{15}N in grain ranged from +2.2 to +4.1‰) in spite of the higher δ^{15}N of N in cattle (+7.9‰) than in hog manure (+5.1‰). They attributed this to a higher N availability in hog manure than in cattle manure, as higher N availability allows plants to assimilate more N from manure and increases N loss that led to further ^{15}N-enrichment of hog manure-derived N in the soil. These results suggest that plant δ^{15}N is a reliable indicator for identifying organic vs. synthetic fertilizer when the organic fertilizer was applied in the liquid form.

Due to inconsistent tissue δ^{15}N patterns in plants grown with synthetic vs. organic fertilizers, some concluded that the absolute value of tissue δ^{15}N cannot be used for organic produce certification (Bateman et al. 2005; Georgi et al. 2005). Bateman et al. (2005) further argued that the δ^{15}N cannot be used for organic produce certification because the restriction on the use of synthetic fertilizer is only one of the requirements (other requirements are maintaining soil fertilities using crop rotations, minimizing environmental impact, avoidance of the usage of chemical fertilizers and pesticides, and so on) for the certification of organic produce. For example, as organic fertilizers can be used not only in organic but also in conventional farming systems, there is a possibility for false differentiation if certification is solely based on δ^{15}N. We suggest that the δ^{15}N technique can be used as one of the proofs for differentiating organic and conventional produce. Considering the increasing public concerns on whether organically-labeled produce is truly grown with only organic fertilizers, development of detection tools is urgent (Siderer et al. 2005). In this context, Gundersen et al. (2000) and Ryan et al. (2004) suggested that trace element profiles between organically and conventionally produced crops can be used as a potential criterion. Regarding the δ^{15}N technique, we suggest that it can be applied selectively to suspected agricultural produce where the main concern is about the type of fertilizer used during a conventional investigation process. Because in the current certification system the determination whether a product is organically produced is largely based on cultivation history provided by a producer, more reliable techniques for detecting the use of synthetic fertilizers in agricultural production will complement the current system. We expect that a combination of the δ^{15}N technique and the trace element profiling method may need to be employed to more accurately certify organic produce.

Plant δ^{13}C

In our study, tissue δ^{13}C values were not different between the urea and LM treatments for both plant species (Tables 4, 5), consistent with what Nakano et al. (2003) reported. On the other hand, Georgi et al. (2005) reported that tissue δ^{13}C were more negative in organically grown than in conventional grown plants. In the study of Georgi et al. (2005), the higher soil respiration rates in the organic than in the conventional fields was assumed to be the primary cause for the change in tissue δ^{13}C, because CO_2 respired from the soil is more depleted in ^{13}C (about -27‰) as compared with atmospheric CO_2 (about −8‰).

At the initiation of this study, we hypothesized that the application of LM with high salinity may reduce the stomatal conductance of plants, resulting in less negative plant δ^{13}C values as compared with the urea

treatment. However, both LM and urea applications increased soil EC_e (Table 3), which may explain the lack of difference in plant $\delta^{13}C$ in our study (Tables 4, 5). It is notable that at a LM application rate double that of the standard rate the production of dry matter of cabbage, but not chrysanthemum, was decreased (Tables 4, 5). The dry matter yield of cabbage in the LM treatments was negatively correlated with soil EC_e, suggesting salinity-induced reduction of biomass production (Fig. 1a). At the same time, the $\delta^{13}C$ of cabbage tended to increase with increased soil EC_e (Fig. 2a), suggesting that a high rate of LM application (and thus high EC_e) can decrease stomatal conductance, leading to less negative $\delta^{13}C$ values (Choi et al. 2005b). Increased N availability in treatments receiving high rate of LM application may also lead to less negative plant tissue $\delta^{13}C$ values by increasing carboxlyation rate which allows plants to use CO_2 more efficiently, resulting in less $^{13}CO_2$ discriminations (Choi et al. 2005a). However, in our study, the relationship between $\delta^{13}C$ and plant N content at harvest was not significant (r^2 = 0.16 and P = 0.12 for cabbage and r^2 = 0.06 and P = 0.36 for chrysanthemum, data not shown). Salinity-induced decreases in ^{13}C discrimination have been reported for both halophytes and non-halophytes (Brugnoli and Lauteri 1991; Isal et al. 1998; van Groenigen and van Kessel 2002). For chrysanthemum, however, the effect of soil EC_e on $\delta^{13}C$ (Fig. 2b) was not observed, consistent with the fact that the growth of chrysanthemum was not suppressed by the increasing rate of LM application (Table 5). We suggest that although plant $\delta^{13}C$ itself

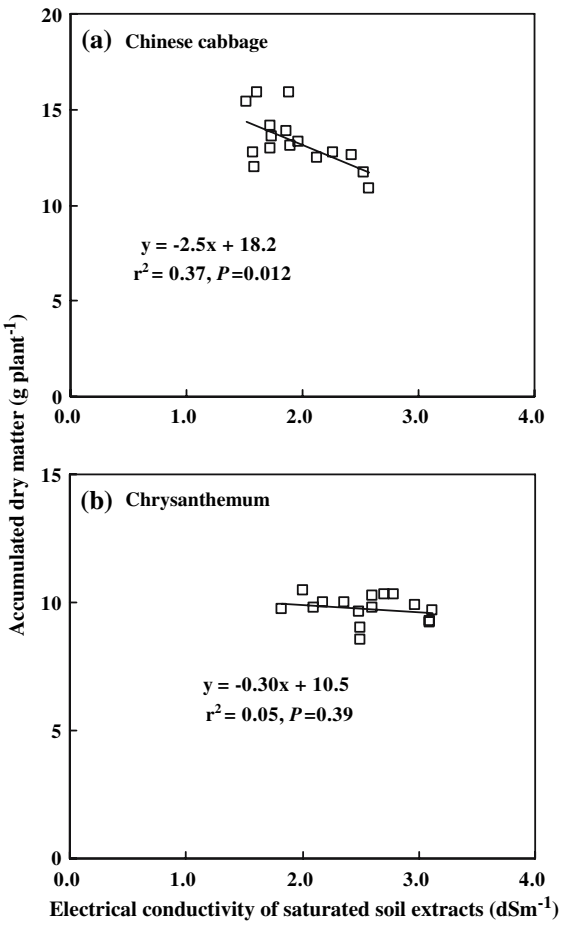

Fig. 1 Relationship between dry matter production of plants grown with liquid pig manure and electrical conductivity of saturated soil extracts after final harvest: (**a**) Chinese cabbage and (**b**) chrysanthemum

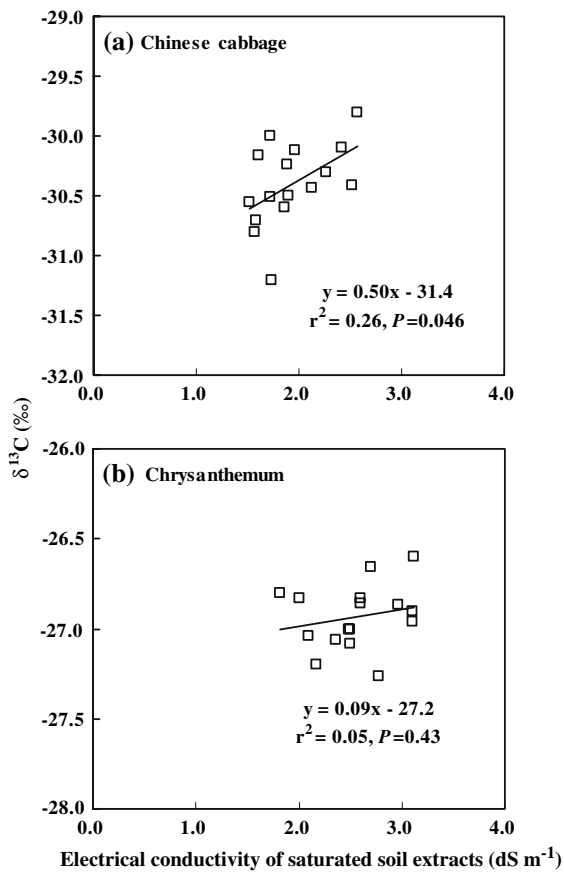

Fig. 2 Relationship between $\delta^{13}C$ of plants grown with liquid pig manure and electrical conductivity of saturated soil extracts after final harvest: (**a**) Chinese cabbage and (**b**) chrysanthemum

was not affected by the type of N applied, the relationship between plant $\delta^{13}C$ values, plant biomass production, and soil EC_e helps us understand physiological responses of plants to the treatments.

In conclusion, we found that the application of LM of liquid pig manure significantly increased plant $\delta^{15}N$ over that with the application of urea, particularly under the high rate of LM application. The difference in plant $\delta^{15}N$ between LM and urea applications was greater than that between solid organic and synthetic fertilizer applications reported in other studies. We conclude that the $\delta^{15}N$ technique can be employed as one of the measures for identifying the source of N (organic vs. inorganic N fertilizers) more reliably for crops grown with liquid type of organic inputs than those grown with solid type of organic inputs. The results from this study will contribute to the establishment of an organic-input-specific $\delta^{15}N$ database for crops, such that the $\delta^{15}N$ technique can be a more reliable tool for differentiating crops grown with synthetic vs. organic inputs. Plant $\delta^{13}C$ were not different between LM and urea applications, probably because both treatment increased soil salinity to a comparable level. However, growth of Chinese cabbage was significantly reduced by the increasing rate of LM application and their $\delta^{13}C$ values were positively related with soil EC_e, indicating that $\delta^{13}C$ values of plant species which are susceptible to salinity stress can reflect salinity limitation on C utilization by the plants.

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