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Natural ^{15}N Abundance in Winter Wheat Amended with Urea and Compost: A Long-Term Experiment*[1]

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ABSTRACT

We investigated ^{15}N abundance (δ^{15}N) of winter wheat (*Triticum aestivum* cv. Jinmai 1) plants and soil at different growth stages in a field with a 13-year fertilization history of urea and compost, to determine whether or not the δ^{15}N of plant parts can be used as an indicator of organic amendment with compost. Plant parts (roots, leaves, stems and grains) and soil were sampled at re-greening, jointing, grain filling and mature growth stages of winter wheat. There were significant differences between the urea and compost treatments in δ^{15}N of whole plants, plant parts and soil over the whole growing season. Determination of the δ^{15}N of plant parts was more convenient than that of whole plant to distinguish between the application of organic amendment and synthetic N fertilizer.

Key Words: detection, growth stages, natural δ^{15}N, organic food, tissue

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INTRODUCTION

Recently, it has been stated that the ^{15}N abundance (δ^{15}N) of plants can help to identify organic amendments applied during organic farming production (Evans, 2001; Nakano *et al.*, 2003; Georgi *et al.*, 2005). Objections to this method center around a mass of uncertainty due to the changes of plant δ^{15}N values, especially during physiological processes in plants (Mariotti *et al.*, 1982; Yoneyama *et al.*, 1991; Choi, *et al.*, 2002; Ellis *et al.*, 2003). Variability of the plant δ^{15}N values in the soil-plant system is derived primarily from biogeochemical and physiological processes in the N cycle (Kahmen *et al.*, 2008). N isotope fractionation during N transformation, assimilation, metabolism, and reallocation in plants and N losses from the plants can affect plant δ^{15}N (Mariotti *et al.*, 1982; Shearer and Kohl, 1986; Herman and Rundel, 1989; Evans *et al.*, 1996; Högberg, 1997; Hopkins *et al.*, 1998; Werner and Schmidt, 2002). The δ^{15}N of soil is an additional factor that can affect the δ^{15}N of plants. Soil processes such as N minerlization, nitrification, denitrification, NH_3 volatilization and N leaching discriminate against ^{15}N and result in soil N pools with different δ^{15}N signatures (Mariotti *et al.*, 1981; Ugolini and Sletten, 1991; Handley and Raven, 1992; Nadelhoffer and Fry, 1994; Piccolo *et al.*, 1994; Robinson, 2001), which may further impact the δ^{15}N values of plants that interact with these soil N pools (Kahmen *et al.*, 2008). Large differences in δ^{15}N values in the forest soils were observed in different areas by Fry (1991) and Yoneyama *et al.* (1993).

Although the factors listed above complicate the interpretation of natural abundance δ^{15}N data in plants, positive arguments have been put forward in favour of this method. Choi *et al.* (2003) examined a field with 5 years of known fertilizer application history and found that the δ^{15}N in the plants treated with compost was 14.6‰±3.3‰ compared with 4.1‰±1.7‰ in those treated with urea. Nakano *et al.* (2003) found that the δ^{15}N of tomato under organic fertigation (7.09‰±0.68‰ was significantly higher than that of the plants treated with inorganic fertigation (0.30‰± 0.61‰). Bateman *et al.* (2005) found in a pot experi-

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ment that the δ^{15}N of carrot supplied with chicken manure was 3‰–5‰ higher than that of carrot grown with ammonium nitrate. It is worth mentioning that the previous studies have usually focused on the δ^{15}N of mature plant when trying to determine whether or not this method can be used as an indicator of fertilizer use. However, plants at different growth stages may have different δ^{15}N values because the effects of ^{15}N source on plant δ^{15}N may vary with growing stage and the contribution of applied N to total plant N may vary with time (Choi *et al.*, 2002). Choi *et al.* (2002) reported that the difference between the δ^{15}N values of maize treated with urea fertilizer and compost was observed to decrease with the increasing growth stage in a greenhouse experiment. In addition, different plant parts may have different δ^{15}N values (Handly and Scrimgeour, 1997; Dawson *et al.*, 2002). Evans *et al.* (1996) found that the δ^{15}N of tomato leaves was 5.8‰ higher than that of tomato roots in NO_3^- solution. In a greenhouse experiment, Choi *et al.* (2002) found that maize stems were the plant part with the highest δ^{15}N values.

In our earlier study on greenhouse tomato crops (Zhou *et al.*, 2012) we found that two amendments (urea and compost) influenced the final tissue δ^{15}N of the plants. Moreover, it was possible to distinguish the crops that urea or compost was applied to on the basis of δ^{15}N values of plant parts. In the present study we extended this work to a winter wheat field, with a known long-term history of chemical N fertilizer and organic compost application, to understand the physiological processes responsible for different δ^{15}N values among plant parts at different growth stages and to investigate whether or not the δ^{15}N value of specific plant parts can be used as an indicator for identification of compost application in crop production systems.

MATERIALS AND METHODS

The experiment was conducted in a calcareous alluvial soil (sandy loam, FAO classification) at Quzhou Research Station (36°46′ N, 114°56′ E), Hebei Province, North China. The soil had a bulk density of 1.33 g cm^{-3} and selected chemical properties are shown in Table I. The experimental site has a typical sub-humid temperate monsoon climate with an annual mean solar radiation of 485.6 kJ cm^{-2}, a mean temperature of 12.5 °C, a mean precipitation of 500 mm (70% during June–September), and a frost-free period of 200 d. The major cropping system in this region is irrigated winter wheat (October to May of the following year) and rain-fed summer maize (June to September).

Three N treatments were initiated in 1995: unfertilized control, 180 kg N ha^{-1} as urea (inorganic N) and 180 kg N ha^{-1} as compost (organic N). The compost was a mixture of chicken manure and plant straw (see Table I for selected chemical properties). The same amounts of P and K were applied as calcium superphosphate (270 kg P_2O_5 ha^{-1}) and potassium sulfate (250 kg K_2O ha^{-1}) to the urea treatment as basal fertilizers. Plots of all treatments were the same size (4 m × 8 m) and each treatment had three replicates in a randomized complete block design. The plant density was the same in all plots with 9 600 winter wheat plants. The δ^{15}N values of the urea and compost were −1.1‰±0.1‰ and 11.7‰±0.4‰, respectively, in 2007.

Ten plant samples and three soil samples were collected from each plot at the following crop growth sta-

TABLE I

Selected chemical properties of compost and soil (0–20 cm) in the experimental plots

Parameter	Soil under different fertilization treatments			Compost
	Control	Urea	Compost	
pH[a)]	8.6±0.2[b)]c[c)]	8.4±0.1a	8.3±0.1b	7.4±0.2
Total organic C (g kg^{-1})	8.68±0.06b	8.57±0.06b	17.43±0.14a	218.84±1.04
Total N (g kg^{-1})	0.92±0.00b	0.83±0.00c	1.71±0.01a	14.43±0.80
C/N ratio	9.6±0.6c	11.4±0.4a	10.4±0.1b	15.2±0.2
NH_4^+-N (mg kg^{-1})	1.48±0.46b	1.94±0.23b	2.87±1.26a	ND[d)]
NO_3^--N (mg kg^{-1})	4.45±0.11b	6.39±1.71a	7.16±1.85a	ND
^{15}N (‰)	5.14±0.51b	5.27±0.43b	7.44±0.15a	11.71±0.43
Olsen P (mg kg^{-1})	3.8±0.0c	12.8±0.1b	40.8±0.7a	(13.0±0.03) × 10^3
Exchangeable K (mg kg^{-1})	161.6±0.5b	130.9±0.5c	206.5±0.4a	(19.9±0.08) × 10^3

[a)] Distilled water:soil = 5:1 (v/w).
[b)] Means±standard errors ($n = 3$).
[c)] Means followed by the same letter within each row are not significantly different at $P < 0.05$.
[d)] Not determined.

ges in 2007: re-greening (March 13), jointing (April 12), grain filling (May 17) and maturity (June 4). Plants were dug out and more than 95% of each root system was collected. The plants were washed with distilled water, separated into leaves, stem, roots, and grains, immediately placed in an oven at 105 °C for 30 min, and then dried at 65 °C to a constant weight. Soil samples were collected between two lines of winter wheat at 0–10, 10–20, and 20–40 cm depth intervals by soil auger. Three cores per plot were collected and the samples in the same soil layers were uniformly mixed together by sieving with 4-mm sieves. Fresh soil samples (24 g) were extracted with 100 mL 2 mol L^{-1} KCl to determine NH_4^+ and NO_3^- contents and the remainder of each soil sample was air-dried. Both plant and air-dried soil samples were ground to pass a 0.15-mm sieve.

Nitrogen contents and δ^{15}N values in the plant and soil samples were determined with a continuous flow stable isotope ratio mass spectrometer (Delta plus XP, Thermo Finnigan, Bremen, Germany). The natural δ^{15}N (‰) is expressed as follows:

$$\delta^{15}\mathrm{N} = (\text{atom\% } {}^{15}\mathrm{N}_{\mathrm{sample}} - \text{atom\% } {}^{15}\mathrm{N}_{\mathrm{air}})/ \text{atom\% } {}^{15}\mathrm{N}_{\mathrm{air}} \times 1\,000‰ \quad (1)$$

where the standard is atmospheric N_2 (δ^{15}N = zero) by definition (Mariotti, 1983).

The whole plant δ^{15}N ($\delta^{15}\mathrm{N}_{\mathrm{plant}}$) was calculated according to Handley and Scrimgeour (1997):

$$\delta^{15}\mathrm{N}_{\mathrm{plant}} = (\delta^{15}\mathrm{N}_{\mathrm{root}} \times \mathrm{N}_{\mathrm{root}} + \delta^{15}\mathrm{N}_{\mathrm{stem}} \times \mathrm{N}_{\mathrm{stem}} + \delta^{15}\mathrm{N}_{\mathrm{leaf}} \times \mathrm{N}_{\mathrm{leaf}} + \delta^{15}\mathrm{N}_{\mathrm{grain}} \times \mathrm{N}_{\mathrm{grain}})/\mathrm{N}_{\mathrm{plant}} \quad (2)$$

where N_{root}, N_{stem}, N_{leaf}, N_{grain}, and N_{plant} are the N content in root, stem, leaf, grain, and whole plant (mg), respectively.

The soil δ^{15}N values ($\delta^{15}\mathrm{N}_{\mathrm{soil}}$) at 0–40 cm depth were calculated according to Handley and Scrimgeour (1997):

$$\delta^{15}\mathrm{N}_{\mathrm{soil}} = (\delta^{15}\mathrm{N}_{0-10} \times \rho_{0-10} \times V_{0-10} \times \mathrm{N}_{0-10} + \delta^{15}\mathrm{N}_{10-20} \times \rho_{10-20} \times V_{10-20} \times \mathrm{N}_{10-20} + \delta^{15}\mathrm{N}_{20-40} \times \rho_{20-40} \times V_{20-40} \times \mathrm{N}_{20-40})/\mathrm{N}_{\mathrm{soil}} \quad (3)$$

where $\delta^{15}N_{0-10}$, $\delta^{15}N_{10-20}$, and $\delta^{15}N_{20-40}$ are the soil δ^{15}N at depths of 0–10, 10–20, and 20–40 cm, respectively; ρ_{0-10}, ρ_{10-20}, and ρ_{20-40} are the soil bulk density at depths of 0–10, 10–20, and 20–40 cm (g cm^{-3}), respectively; V_{0-10}, V_{10-20}, and V_{20-40} are the soil bulk column at depths of 0–10, 10–20, and 20–40 cm (cm^3), respectively; N_{0-10}, N_{10-20}, and N_{20-40} are the soil N concentrations at depths of 0–10, 10–20, and 20–40 cm (mg g^{-1}), respectively; N_{soil} is the soil N content at 0–40 cm depth (mg).

The contribution of δ^{15}N of a plant tissue ($\delta^{15}\mathrm{N}_{\mathrm{tissue}}$) to the whole plant δ^{15}N was calculated as:

$$\text{Percentage} = (\delta^{15}\mathrm{N}_{\mathrm{tissue}} \times \mathrm{N}_{\mathrm{tissue}})/(\delta^{15}\mathrm{N}_{\mathrm{plant}} \times \mathrm{N}_{\mathrm{plant}}) \times 100\% \quad (4)$$

where N_{tissue} and N_{plant} are the N contents in the plant tissue and plant, respectively (mg).

The data were subjected to analysis of variance and the treatment means were compared using least significant difference at $P = 0.05$. Linear regression was used to examine the relationships between the δ^{15}N values of plants and soil using Microsoft Excel 2000 for Windows. All other statistical analysis was performed using the SPSS software package version 11.0 (SPSS Inc., 2001).

RESULTS

Soil δ^{15}N values

The δ^{15}N of soil under the compost treatment was significantly higher ($P \leq 0.05$) than that in the plots receiving urea fertilizer, but there was no significant difference between the control and urea treatments at different soil depths and over the growing season (Fig. 1). The soil δ^{15}N at each depth in each treatment remained stable throughout all the plant growth stages and the soil δ^{15}N in each treatment decreased with soil depth, especially in the compost treatment. Overall, the above results indicated that chemical fertilizer or compost application exerted a significant effect on the δ^{15}N of soil N.

Plant δ^{15}N values

The δ^{15}N values of whole plants and different plant tissues were significantly higher ($P \leq 0.05$) in the compost treatment than in the urea treatment at all four growth stages. Among the three fertilization treatments, the whole plant δ^{15}N values showed the following pattern of compost > control ≈ urea at the re-greening and jointing stages, and compost ≈ control > urea at both grain-filling and maturity stages (Fig. 2). The former pattern of compost > urea ≈ control was also found for the leaves and roots in the re-greening stage, and was generally observed in the leaves at jointing and in the roots at grain filling. The latter pattern of compost ≈ control > urea was also

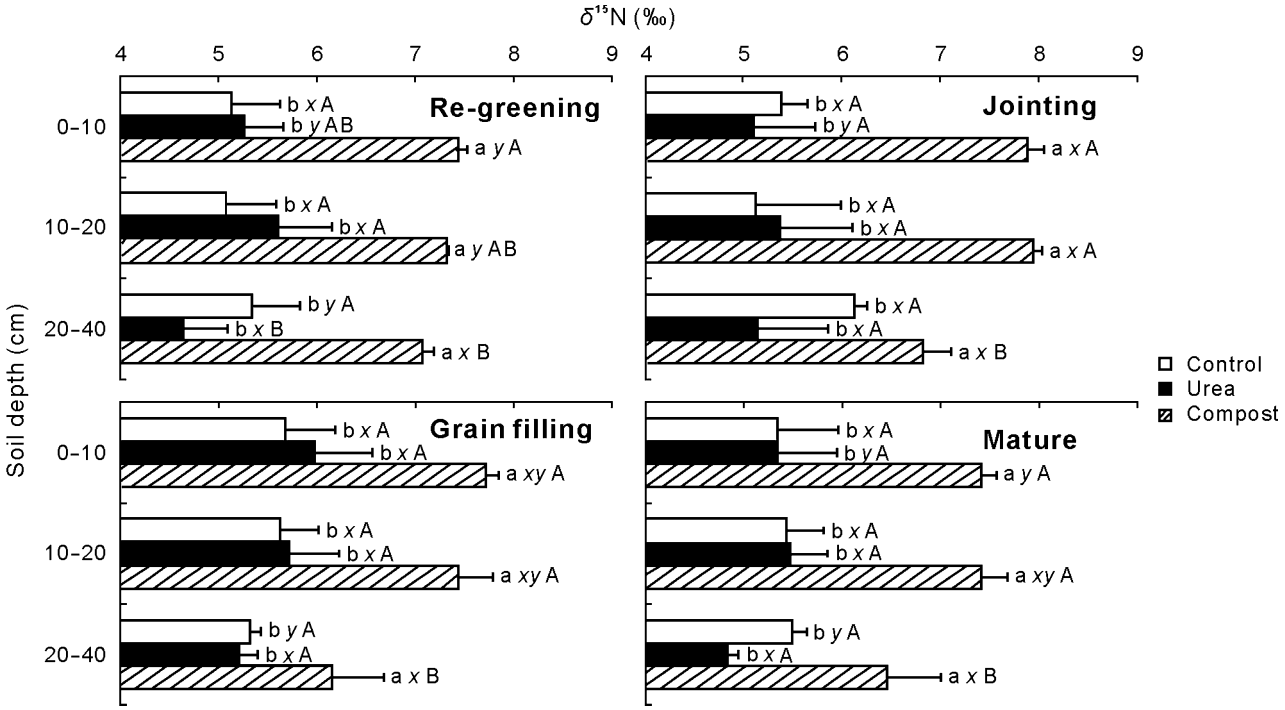

Fig. 1 Variations of soil ^{15}N abundance (δ^{15}N) values at different soil depths and plant growth stages after 13 years of urea fertilizer or compost application. Horizontal bars indicate standard errors of the means ($n = 3$). Bars with the same lowercase letter(s) are not significantly different at $P \leq 0.05$ among the different treatments at the same growth stage and the same soil depth. Bars with the same italic letter(s) are not significantly different at $P \leq 0.05$ among the growth stages under the same treatment and at the same soil depth. Bars with the same uppercase letter(s) are not significantly different at $P \leq 0.05$ among soil depths at the same growth stage and under the same treatment.

observed for the stems and grains at grain filling and for all plant tissues at maturity, and was generally found for the stems at the jointing stage.

The δ^{15}N values of whole plants and plant tissues generally tended to decrease with growth stage and generally showed the following pattern of re-greening $\geq$ jointing $\geq$ grain filling $\approx$ maturity in the whole plants except for the control. A pattern of jointing $\geq$ re-greening $\geq$ grain filling $\geq$ maturity occurred in the leaves and a pattern of re-greening $\geq$ jointing $\geq$ grain filling $\geq$ maturity in the roots, but there were no significant differences in either the stems or the grains.

The leaves showed the highest δ^{15}N values at the re-greening stage and the lowest at maturity, and the δ^{15}N in other tissues were maintained at constant values.

Relationships among whole plant, plant tissue, and soil $\delta^{15}N$ values

The whole plant δ^{15}N values were significantly correlated with the δ^{15}N values of the individual plant tissues during the experimental period. However, different plant tissues made different contributions to plant δ^{15}N at different growth stages and the leaves always made the largest contribution to plant δ^{15}N (Fig. 3) at the re-greening and jointing stages. At the grain filling and mature stages the contribution of the leaves to plant δ^{15}N decreased sharply and the grains became the plant part making the largest contribution to plant δ^{15}N.

In general, the relationship between soil δ^{15}N and plant δ^{15}N weakened as plant growth proceeded. The δ^{15}N of leaves and roots showed the most significant correlations with soil δ^{15}N (Fig. 4) and the relationships between soil and tissue δ^{15}N values decreased in the order of roots > leaves > stems > grains (Fig. 4).

DISCUSSION

Soil $\delta^{15}N$ values

After 13 years of urea or compost application, soil δ^{15}N values were significantly higher ($P \leq 0.05$) in the compost treatment than in the urea treatment, which is consistent with the previous reports (Choi *et al.*, 2003; Zhao and Zhang, 2003). However, in a 2-year field experiment, Georgi *et al.* (2005) did not find any significant difference in soil δ^{15}N between inorganic and organic fertilizers, possibly because of the short duration of the experimental amendment. Chemical fertilizer with a lower δ^{15}N ($-1.1‰ \pm 0.1‰$) should de-

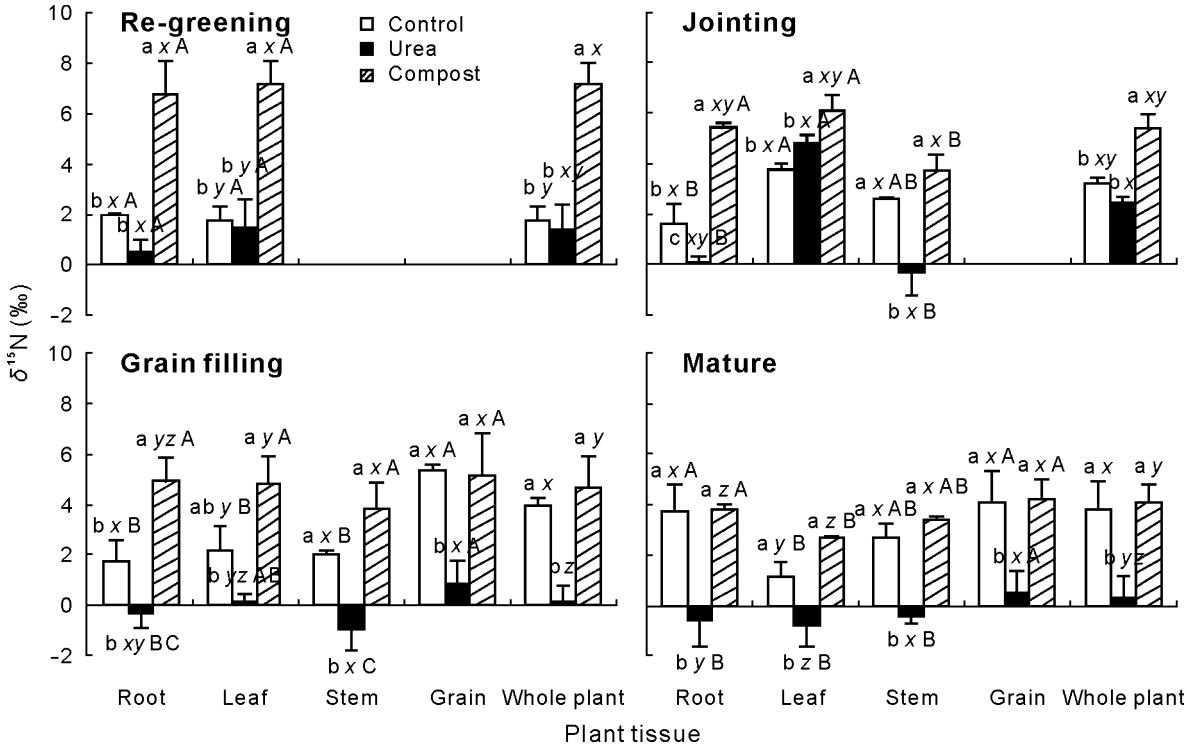

Fig. 2 Variations of ^{15}N abundance (δ^{15}N) values in plant tissues at different growth stages after 13 years of urea fertilizer or compost application. Vertical bars indicate standard errors of the means ($n = 3$). Bars with the same lowercase letter(s) are not significantly different at $P \leq 0.05$ among the different treatments at the same growth stage and the same soil depth. Bars with the same italic letter(s) are not significantly different at $P \leq 0.05$ among the growth stages under the same treatment and at the same soil depth. Bars with the same uppercase letter(s) are not significantly different at $P \leq 0.05$ among soil depths at the same growth stage and under the same treatment.

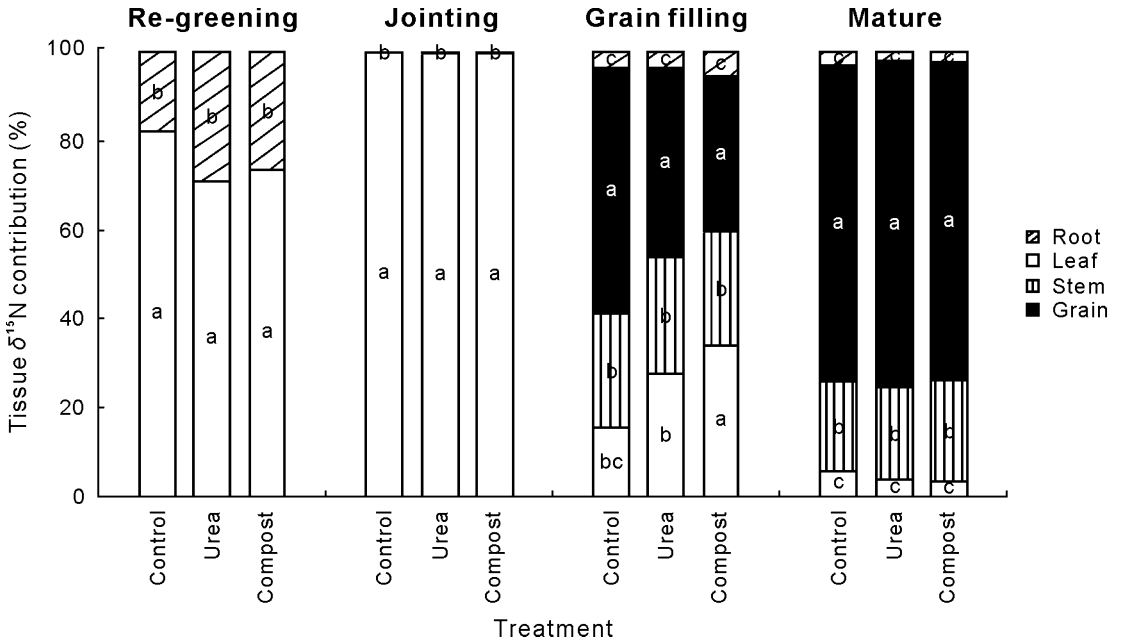

Fig. 3 Contribution of plant tissue ^{15}N abundance (δ^{15}N) to whole plant δ^{15}N at different growth stages after 13 years of urea fertilizer or compost application. Bars with the same letter(s) are not significantly different among different plant tissues at the same growth stage and under the same treatment at $P < 0.05$ ($n = 3$).

crease the δ^{15}N of surface soil, which did not change with long-term urea fertilization and increased with compost application (Table I, Fig. 1). This may be because soil pH of the winter wheat field was above 8

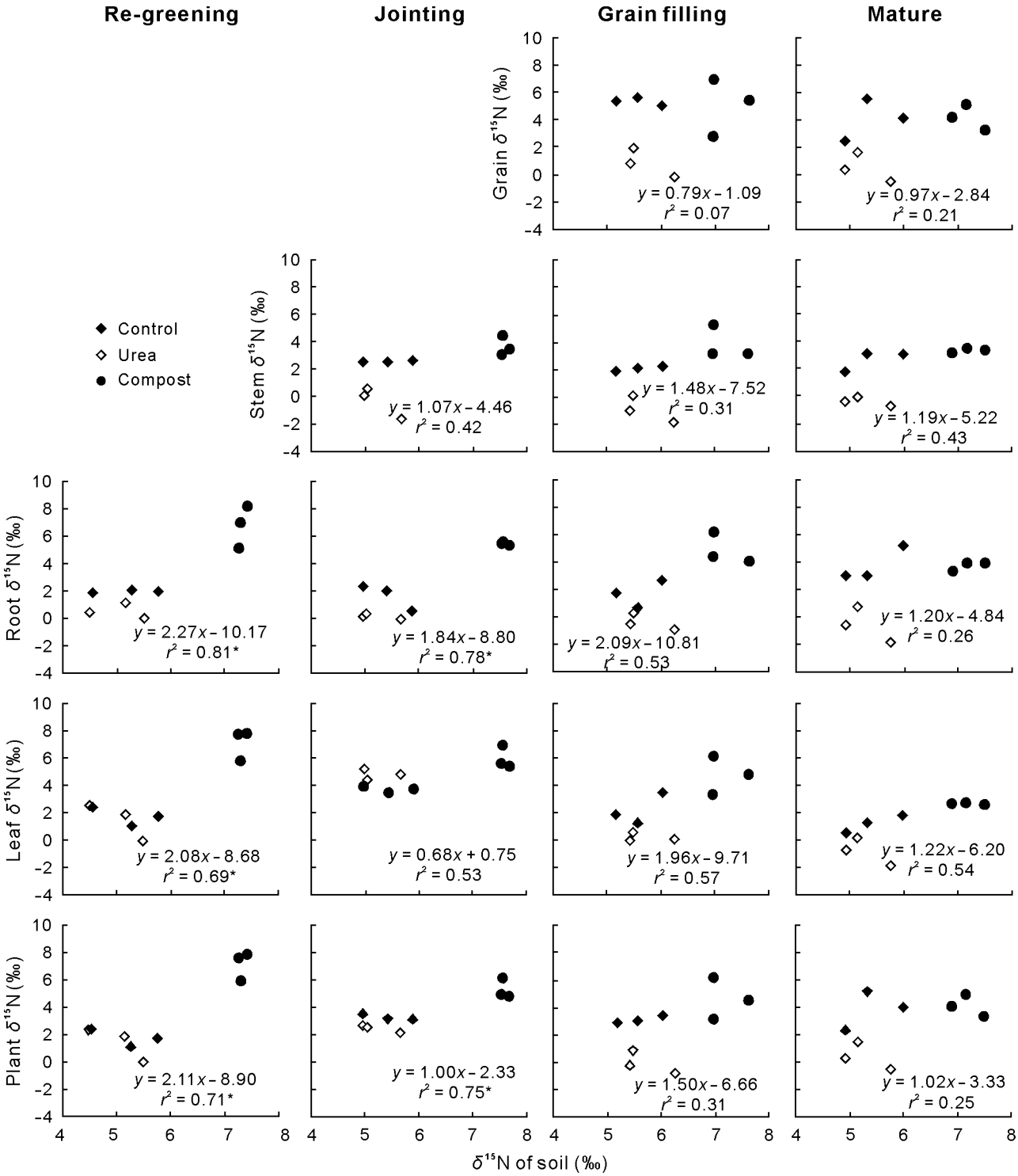

Fig. 4 Relationships between ^{15}N abundance ($\delta^{15}N$) values of soil (0–40 cm) and $\delta^{15}N$ values of plant tissues and whole plants at different growth stages after 13 years of urea fertilizer or compost application. The symbol * represents significance at $P = 0.05$.

(Table I), facilitating NH_3 volatilization from urea and leaving more ^{15}N in the surface soil. Nitrification is another factor that may have contributed to the higher $\delta^{15}N$ value of soil in the urea treatment, as nitrification is common and important in the soils of northern China (Ju *et al.*, 2004; Wang *et al.*, 2010). Yang *et al.* (2008) found the nitrification rate of cropland was 1.47 mg N kg^{-1} soil d^{-1}, which was much higher than that of forest or grassland because of the application of chemical fertilizer. More than 65% NH_4^+ as fertilizer could be nitrified to NO_3^- within 3 d (Wan *et al.*, 2007).

In our experiment the crop was harvested each year so that plant ^{15}N in the urea treatment was removed from the system, which may also have contributed to a decrease of soil δ^{15}N in the urea treatment. In the compost treatment, N would be much less readily released from the compost and more N would have remained in the surface soil (the N content in the compost treatment was significantly higher than that in the urea treatment), resulting in a higher surface soil δ^{15}N in the compost treatment than in the control plots.

δ^{15}N of plant tissues

The δ^{15}N values of plant tissues treated with compost were significantly higher ($P \leq 0.05$) than those receiving urea over the whole growing season (Fig. 1), indicating that the δ^{15}N of roots, leaves, stems or grains can be used as an indicator of fertilizer type instead of the δ^{15}N of whole plant, although the δ^{15}N values of plant tissues changed in different ways in the different treatments during plant growth stages. This confirms that different N sources and N assimilation, transformations and relocation can alter the δ^{15}N of plant tissues over the growing season.

The first step of plant uptake and assimilation of NH_4^+ and NO_3^- is *via* root uptake of N from the soil. Ammonium (NH_4^+) is the main form of N assimilated by glutamine synthetase in plant roots (Bloom, 1989), NO_3^- accounting for only 30% of total N assimilation in roots (Cramer and Lewis, 1993). Both forms of N discriminate against δ^{15}N during assimilation, leading to an increase in the δ^{15}N of the source (Mariotti *et al.*, 1982; Yoneyama and Kaneko, 1989; Hoch *et al.*, 1992). NH_4^+ assimilation by glutamine synthetase could increase 17‰ of δ^{15}N in roots (Yoneyama *et al.*, 1993), and NO_3^- assimilation by nitrate reductase could also increase 17‰ of δ^{15}N in roots (Handley and Raven 1992). NO_3^- is not assimilated in the roots is enriched in ^{15}N and later transported to the leaves and leaf δ^{15}N values increase at the re-greening growth stage (Yoneyama and Kaneko, 1989; Evans *et al.*, 1996). N content of plant (mg plant^{-1}) and δ^{15}N of leaves were significantly and positively correlated ($P \leq 0.05$) at the re-greening and jointing growth stages ($r^2 = 0.77$ and 0.91, respectively). At jointing stage some of the N in the leaves was transferred to stems and fractionation during reallocation of N gave the leaves the highest δ^{15}N values over the growing season. At the grain filling stage the N contents of stems and leaves decreased sharply and N transfer to the grains began. N content and δ^{15}N of the grain showed a negative relationship ($r^2 = 0.56$, $P \leq 0.05$), indicating that transformation of N also follows the principle of ^{14}N priority as observed by Serret *et al.* (2008).

Whole plant δ^{15}N values

The plants treated with compost always had significantly higher ($P \leq 0.05$) δ^{15}N values than those treated with urea at all growth stages (Fig. 2). This result is consistent with the previous studies (Yoneyama *et al.*, 1990; Choi *et al.*, 2002, 2003; Bateman *et al.*, 2005). Robinson (2001) also found that the differences in δ^{15}N can be used as a tracer for sources of N. The significant difference in the δ^{15}N of plants grown under different fertilizers implies that the δ^{15}N of plants can reflect different types of fertilization amendment (compost *vs.* urea) in our study.

The δ^{15}N of winter wheat grown under compost and urea treatments decreased as plant growth proceeded but increased in the control, indicating that plant δ^{15}N in each treatment varied with growth stage. In a study with a 1-year fertilizer history, Choi *et al.* (2002) found that, with 150 kg N ha^{-1} fertigation, the δ^{15}N of maize increased in the urea treatment and decreased in the compost treatment as plant growth proceeded. They attributed this to fertilizer N loss in the urea treatment (Recous *et al.*, 1988), low plant availability of compost N (Beauchamp, 1986; Paul and Beauchamp, 1993; Wen *et al.*, 1995), and an increase in the contribution of soil N to total plant N (Choi *et al.*, 2002). In our long-term urea treatment the amount of urea N in soil was sufficient over a number of years for winter wheat growth, therefore the δ^{15}N of winter wheat plants continued to decrease over the growing season.

The δ^{15}N of plants in the control treatment increased at the grain filling stage and showed no significant difference from the compost treatment at grain filling or maturity. This may be explained by the fact that the winter wheat in the control treatment did not receiving adequate N, P or K, and earlier and more thorough senescence of the plants compared with those receiving fertilizer or compost, perhaps leading to an increase of δ^{15}N through the loss of volatile amines or ammonia during protein hydrolysis (Kolb and Evans, 2002). Efflux N from the roots may have enriched the stems, leaves and roots in ^{15}N and further influenced the δ^{15}N of the grains during transfer of N to the grains.

Relationships among δ^{15}N values of plant tissues, whole plants and soil

There was a significant correlation between the δ^{15}N of plants and soil at early growth stages but

there was no significant relationship at later growth stages (Fig. 4). This may be explained by the fact that the grain was the last tissue produced by the plants and most of the N in the grains was reallocated from the leaves and stems. Numerous ^{15}N fractionation processes such as assimilation, reallocation and transformation occurred so that the grains became major tissue of the plants contributing to the whole plant $\delta^{15}N$ value at later growth stages. Fig. 4 obviously showed that there was no significant correlation between grain $\delta^{15}N$ and soil $\delta^{15}N$.

CONCLUSIONS

Both soil N source and physiological processes affected the $\delta^{15}N$ of winter wheat plants and their different plant parts during the growing season, but the N source (urea *vs.* compost) showed the major effects over the long term. The $\delta^{15}N$ values of winter wheat plant tissues and whole plants were significantly different between the urea and compost treatments at different growth stages, so it was possible by using $\delta^{15}N$ of the plant tissues (root, leaf, stem or grain) at any growth stage to distinguish between the two N sources, which would be much easier than using $\delta^{15}N$ of the whole plants.

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