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Translocation of Carbon and Nitrogen from Mature Leaves to the Root System of Vegetative Wheat: Simultaneous Feeding of Gaseous $^{13}CO_2$ and $^{15}NH_3$ to Single Leaves

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Abstract

^{13}C-labelled CO_2 and ^{15}N-labelled NH_3 gases were simultaneously fed from the upper (5L) or lower (3L) leaves of the vegetative wheat, and the subsequent movements and fates of ^{13}C and ^{15}N in the root system were traced, with special attention to the nodal position of root. The ^{13}C and ^{15}N incorporated by the fed leaves were intensely translocated to the developing leaf (6L), tiller and the root system. Relative distribution of ^{13}C and ^{15}N from the fed leaf was compared among sink organs during the initial phase of translocation (within 1 day after isotopes feeding). The ^{13}C: ^{15}N weight ratio of the developing leaf was similar to that of the tiller, when isotopes were fed from the upper (5L) or lower (3L) leaf, while ^{13}C: ^{15}N weight ratio of the root system was 1.1 to 1.9 times higher than the developing leaf and tiller. The ^{13}C: ^{15}N weight ratio of the root system was 1.3 to 1.5 times higher when the plant was fed from the upper leaf than from the lower leaf. Within the root system ^{13}C: ^{15}N weight ratio was higher in the older roots (SR and LNR) than the younger roots (UNR). These results suggest that relative distribution of carbon and nitrogen from mature leaves to the roots is affected by the nodal position of source leaf as well as the physiological age of sink roots.

Introduction

Plant roots absorb inorganic nitrogen (N) from the ambient medium and supply shoots with N as inorganic and/or organic forms via xylem, and inversely receive photoassimilated carbon (C) from leaves through the phloem for their growth and maintenance. Recently, much attention has been focused on the movement of N between the shoots and the roots of graminaceous plants. Several studies have indicatd that roots are the organ importing N compounds from the shoots[1, 9, 11, 12, 16, 17]. In vegetative wheat, more than half of the N translocated to the shoots returns to the roots under low level of N status[15]. In rice plants mature leaves are the active center receiving N from roots via xylem, subsequently re-exporting it to the sink organs as the phloem assimilate[19]. Thus, the root system acts as the sink organ which imports not only C assimilates but also N compounds from mature leaves as the phloem translocate.

Our previous studies on the rice plants whose leaves were fed simultaneously with $^{13}CO_2$ and ^{15}N-labelled compounds indicated that the relative incorporation of ^{13}C and ^{15}N into the roots varied considerably by their nodal positions[17]. This suggests that the nodal roots of different ages receive the phloem assimilate which varies in the C:N weight ratio (C:N ratio). The variation of C:N ratio of the phloem translocate entering to sink organs has been reported for legume

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plants in relation to its significance for C and N nutrition of sink organs[12]. In nodulated root systems, nodules require solutes more rich in C relative to N as the phloem translocate from shoots than the other part of the root system[9]. Since metabolism responsible to C and N in the root changes as root age proceeds, the relative requirement of roots for C and N as the phloem translocate may be altered with aging. However, very few informations are available on the relative partitioning of C and N transferred from mature leaves to the root system particularly in relation to root growth. The relative distribution of C and N to the root system would also depend upon the distance and position in morphology between source leaves and sink roots, since relative contribution of mature leaves to the root system as C and N sources is different between upper and lower leaves[16,18].

In the present experiment ^{15}N-labelled NH_3 gas and ^{13}C-labelled CO_2 were simultaneously fed from the upper or lower leaves of the vegetative wheat, and the subsequent movements and fates of ^{13}C and ^{15}N in the root system were followed, with special attention to the nodal position of root. The aim of this experiment is (1) to investigate the relative partitioning of C and N delivered as the phloem translocate within the root system of vegetative wheat, and (2) to evaluate the effect of nodal position of the source leaf on the relative transfer of C and N to the root system. Gaseous ammonia is suitable for the simultaneous administration with CO_2 to leaves. Ammonia is readily absorbed by leaves, then its N is transferred from leaves as organic form within a short time[8,13].

Materials and Methods

Plant culture; Wheat seeds (*Triticum aestivum* L., cv. Norin 51) sterilized by 0.1% Benlate solution were germinated on the nylon net floating in a tap water with air bubbling. At the stage of the second leaf emergence seedlings were transplanted to the culture bed filled with 360 L of the solution (KNO_3, 27.7mg; $(NH_4)_2SO_4$, 12.0 mg; K_2SO_4, 31.8mg; $Ca(NO_3)_2 \cdot 4H_2O$, 15.0mg; $MgSO_4 \cdot 7H_2O$, 22.0mg; KH_2PO_4, 12.4mg; EDTA-Fe, 20.0mg per 1 L of tap water) in a naturally lit green house where the air temperature was set to 10 ℃ for the minimum and to 25 ℃ for the maximum. The culture solution was bubbled continuously with an air pump (5 L/min) and was renewed every week. The pH of the solution was adjusted to 5.0 with 1N HCl or 1N NaOH. On the 21th day after transplanting when plants began to emerge the 6th leaf, 40 plants were selected for the isotopes feeding. The feeding was done in the same green house and the fed plants were grown with the same solution until harvest.

Feeding of $^{13}CO_2$ and $^{15}NH_3$; Before feeding, leaves of the tiller were removed. Leaf blades of mature leaves (the 3rd or 5th leaf) were inserted into the feeding chamber made of transparent plexiglass through slits arranged on the side of the chamber (Fig. 1). Small gaps between slit edges and the fed leaf blade were sealed with wheat dough to prevent labelled gases from leaking. Two feeding chambers (one for the 5th leaves and another for the 3rd leaves) were connected parallel to each other with tube joints and further connected with silicon tubes to a chamber (20 L) having cooling jackets. The air was circulated through these chambers with air pumps at the rate of 15 L/min (Fig. 1).

Before the commencement of the isotopes feeding, CO_2 concentration in the system was reduced to 130 ppm by circulating the air through soda lime columns. The evolution of $^{13}CO_2$ was then started in the cooling chamber by adding a proper amount of 50% butyric acid to $Ba^{13}CO_3$ (90.7 atom% ^{13}C) by a syringe. The concentration of total CO_2 in the feeding system was monitored continuously with an infrared gas analyzer (model 315A, Beckman, USA), and was maintained 360 ± 50ppm by occasional addition of butyric acid to $Ba^{13}CO_3$.

^{15}N-labelled NH_3 gas was introduced by a syringe from a stock cylinder simultaneously with the generation of $^{13}CO_2$. $^{15}NH_3$ gas was

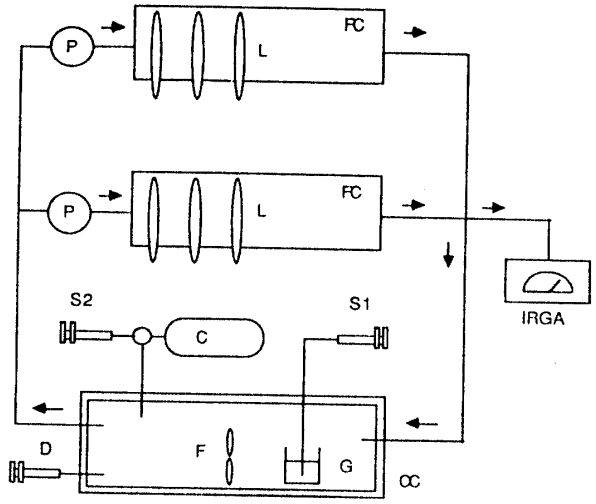


Fig. 1. The system for simultaneous feeding of gaseous $^{13}CO_2$ and $^{15}NH_3$ to single mature leaves of wheat. C, $^{15}NH_3$ gas cylinder; CC, cooling chamber with water jacket; D, NH_3 gas detector; F, fan; FC, feeding chamber; G, $^{13}CO_2$ generator; IRGA, infrared CO_2 analyzer; L, fed leaf; P, air pump; S1, liquid syringe for acid solution; S2, gas syringe.

generated previously in an evacuated Tumberg tube by the addition of conc. NaOH solution to $(^{15}NH_4)_2SO_4$ (97.5 atom% ^{15}N), and stored in a 300ml glass tube after desiccation by the CaO trap until feeding. The concentration of $^{15}NH_3$ in the feeding chambers was monitored in every 10 min with ammonia gas detector tubes (model Kitagawa-shiki, Komyou Rikagaku Co., Ltd., Kawasaki, Japan) and was maintained to 11 $\pm$ 6 ppm by an additional injection of labelled gas (Fig. 1).

The air temperature in the feeding chambers was maintained between 23.5℃ and 27.8 ℃, and the humidity between RH 62% and 70%. The light intensity above the feeding chamber changed between 30 and 60 Klx during the feeding period. The feeding was continued for 2 h started at 12:30 pm. At the end of the feeding, $^{13}CO_2$ and $^{15}NH_3$ remaining in the feeding system were reduced to below 29 ppm and 1 ppm respectively by circulating the air in the system through soda lime and 1N H_2SO_4 solution for 30 min, then the feeding chambers were removed. Thus 20 plants were fed with the isotopes from the 5th leaf (5L plot) and another set of 20 plants were fed from the 3rd leaf (3L plot).

Three plants for each plot were harvested after the end of the feeding (Day 0). Remaining plants were kept in the same green house, then sampled at 1 (Day 1), 3 (Day 3), 5 (Day 5) and 8 (Day 8) days after. After washing the root system of the harvested plants with tap water, the plant body was separated into the leaves, the root system, the culm, and the tiller. The leaves (blades + sheathes) were further divided according to the leaf position numbering from the bottom; the 1st and 2nd (1-2L), 3rd (3L), 4th (4L), 5th (5L), 6th (6L) leaves, and 7th (7L) and 8th (8L) leaves if emerged. The root system was separated into the seminal roots (SR) and the nodal roots. Nodal roots were further divided into the lower (LNR) and the upper (UNR) nodal roots by the method described elsewhere[17]. The LNR consists of the nodal roots which emerged 6 days prior to the isotopes feeding and UNR consists of the nodal roots newly emerging after the feeding. Separated plant parts were dried in an oven at 80℃ for 3 days, and ground to finer powder with a vibrating sample mill (model TI-200, Heiko Seisakusho Co., Ltd., Tokyo).

Determination of ^{13}C and ^{15}N; Total carbon content of the sample was determined with a CN analyzer (model MT-500, Yanagimoto Co., Ltd., Kyoto), and ^{13}C abundance was measured by infrared absorption spectrometry using a JASCO EX-130 $^{13}CO_2$ analyzer (Japan Spectroscopic Co., Ltd., Tokyo). Total nitrogen content of the sample was determined by the distillation method after Kjeldahl digestion where salicylic acid was used so that the nitrate nitrogen would be measured. ^{15}N abundance was determined by emission spectroscopy with a JASCO NIA-1 ^{15}N analyzer.

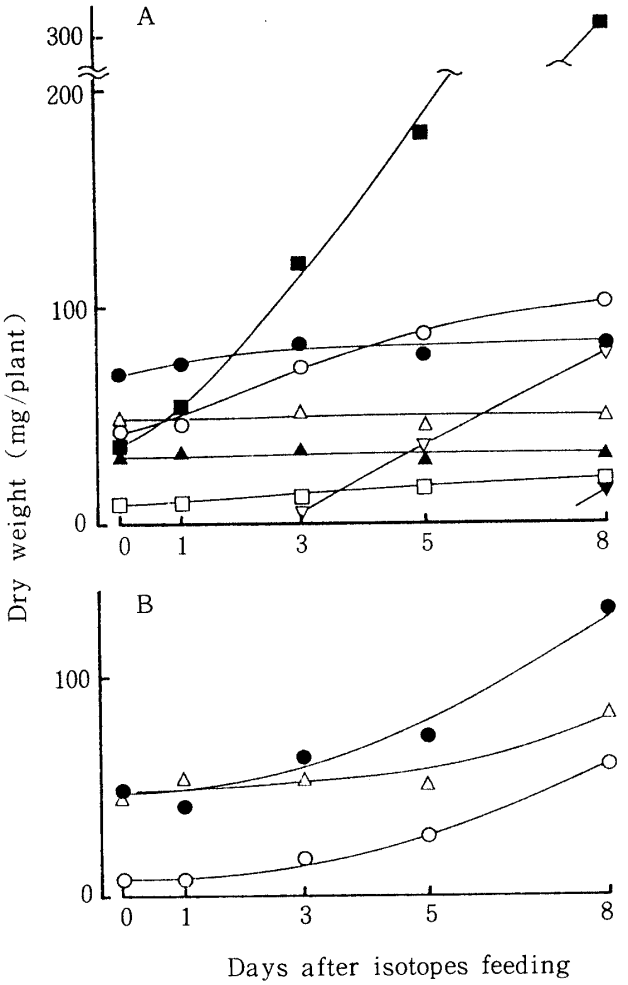
Results

Plant growth; Figure 2 shows the change in dry weight of the plant parts during the

isotopes chase period. The developing leaf (6L), the newly emerging leaves (7L and 8L) and the tiller rapidly increased dry weights. In the root system, UNR grew most rapidly, followed by LNR. The oldest SR grew most slowly. Changes in nitrogen content of the plant parts were similar to dry weight (data not shown), except that the mature leaves lower than 4L showed gradual decreases in nitrogen content during the chase period.

Incorporation of ^{13}C and ^{15}N by the plant; Table 1 shows the amounts of ^{13}C and ^{15}N recovered in the whole plant. Amount of ^{13}C recovered at Day 0 was 2.3 times greater in the 5L-feeding plants than in the 3L-feeding plants. This is due to the difference in the leaf area which is exposed to labelled gases (16.79 cm^2 in 5L and 7.04 cm^2 in 3L), since the photosynthetic rate of the fed leaf of 5L plot calculated from the total fixed ^{13}C at Day 0 is almost equal to that of 3L plot (16.8mg $CO_2/dm^2/h$ in 5L and 17.5mg $CO_2/dm^2/h$ in 3L). Rapid losses of ^{13}C by respiration took place in the whole plant of both feeding plots within the first 3 days, followed by slight but gradual losses. By Day 3 about 30% of the ^{13}C fixed initially was lost from the whole plant in both feeding plots, while 3 to 4% was lost subsequently.

Although considerable deviations were found in ^{15}N content, significant losses of ^{15}N incorporated initially by the plant were not found during the chase period in both feeding plots (Table 1). This indicates that losses of ^{15}N by volatilization from leaves and by excretion from roots are very small. Amount of ^{15}N recovered in the 5L plants was about 1.4 times greater than that of the 3L plants on the average during the isotope chase period. The rates of ^{15}N absorbed by the 5L and 3L during the feeding period are estimated to be 0.62mg $/dm^2/h$ and 1.02mg$/dm^2/h$, respectively. Presumably the higher rate of incorporation of ^{15}N by 3L may be due to its lower sensitivity of stomatal response to gaseous NH_3. In this experiment no visible injuries by the toxicity of excess ammonia were found in the fed leaves over the isotope chase period. Porter et al.[13] reported that the exposure of corn leaves to 20 ppm of gaseous NH_3 for 24 h caused no visual symptoms of toxicity.



Figs. 2A and 2B. Changes in dry weight (mg/plant) of plant parts of the shoots (2A) and roots (2B) during isotope-chase period.
Symbols in Fig. 2A are tiller (T, closed square), 1-2L (open square), 3L (closed triangle), 4L (open triangle), 5L (closed circle), 6L (open circle), 7L (open invert triangle) and 8L (closed invert triangle). Symbols in Fig. 2B are upper nodal roots (UNR, open circle), lower nodal roots (LNR, closed circle) and seminal roots (SR, triangle).

^{13}C partitioning; Table 2 shows the ^{13}C abundance in the plant parts. Just after the isotopes feeding (Day 0), among the sink organs, the tiller was most densely labelled with ^{13}C, followed by the developing leaf (6L) and the root system in both feeding plots. Figure 3 shows the changes

Table 1. Recoveries of ^{13}C and ^{15}N in the whole plant.

Isotopes	Isotopes were fed from									
	5th leaf					3rd leaf				
	Days after isotopes feeding									
	0	1	3	5	8	0	1	3	5	8
^{13}C(mg/plant)	1.62	1.37	1.15	1.11	1.11	0.71	0.62	0.52	0.54	0.49
(% of Day 0)	(100)	(84.6)	(71.0)	(68.5)	(68.5)	(100)	(87.3)	(73.2)	(76.1)	(69.0)
^{15}N(mg/plant)	0.18	0.23	0.20	0.21	0.22	0.13	0.15	0.14	0.15	0.15
(% of Day 0)	(100)	(128)	(111)	(116)	(122)	(100)	(115)	(108)	(115)	(115)

in the amount of ^{13}C in each plant part with the chase time. On Day 0 sink organs partook considerable percentage of ^{13}C recovered in the whole plant (37% in the 5L-feeding and 44% in the 3L-feeding plants), indicating that a substantial transfer of ^{13}C assimilate to sink organs occurred during 2 h of the feeding period before the first harvest (Day 0). Rapid loss of ^{13}C from the fed leaf continued subsequent 3 days, then gradual loss was followed. These features are similar in both feeding plots. Marked difference between the plants fed via 5L and 3L could be found in the pattern of ^{13}C allocation to the roots and the developing leaf on Day 1 when rapid ^{13}C efflux from the fed leaves took place, that when plants were fed from 5L ca. 13% of ^{13}C recovered by the whole plant was accumulated to the developing leaf (6L) and 7% to the root system, while when fed from 3L 10% was accumulated to the developing leaf and 13% to the root system.

^{15}N partitioning; Table 3 shows the abundance of ^{15}N in various plant parts. On Day 0 ^{15}N was detected in sink organs, indicating that gaseous $^{15}NH_3$ was readily absorbed and assimilated by the fed leaf and the transfer of ^{15}N to the other plant parts could occur within few hours. In both feeding plots, rapidly growing organs (6L, the tiller, culm and root system) were highly labelled with ^{15}N and their peak values were found between Day 1 and Day 3, then ^{15}N abundances decreased gradually. The changes in ^{15}N amount in plant parts are shown in Fig. 4. Rapid efflux of ^{15}N took place in the fed leaves during the first 3 days, then gradual efflux was followed. Although the initial transfer of ^{15}N to sink organs occurred somewhat slowly as compared with ^{13}C where considerable amount of transfer took place by Day 0, the allocation pattern of ^{15}N to sink organs was similar to that found in ^{13}C assimilate. The effect of nodal position of source leaf was also found in the ^{15}N partitioning.

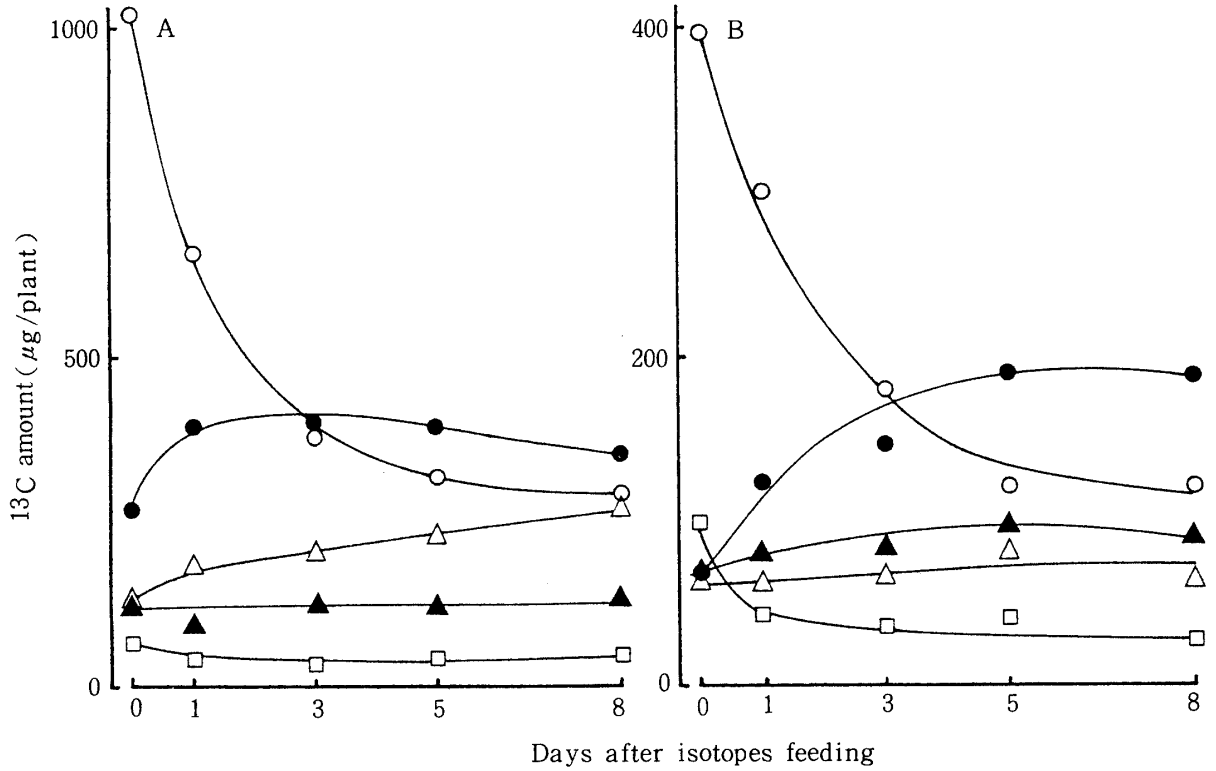
On Day 1 when the rapid transfer continuing, about 20% of ^{15}N absorbed by 5L is distributed to the developing leaf and 7% to the root system, while in the 3L-feeding plants ca. 8% of ^{15}N absorbed by 3L is translocated to the developing leaf and 11% to the root system.

Partitioning of ^{13}C and ^{15}N within the root system; The ^{13}C was detected on Day 0 in the roots of all categories in both feeding plots and their maximum abundances were found between Day 0 and Day 1, then ^{13}C abundance was decreased with the chase time (Table 2). The youngest UNR showed the highest ^{13}C abundance, followed by LNR. The oldest SR was the weakest sink among the roots. The ^{15}N was also detected on Day 0 in the roots of all categories (Table 3) and the maximum ^{15}N abundances were

Table 2. ^{13}C abundance (atom % excess) in various parts of the wheat fed $^{13}CO_2$ and $^{15}NH_3$ from single mature leaves.

Plant parts	Isotopes were fed from 5th leaf					Isotopes were fed from 3rd leaf				
	Days after isotopes feeding									
	0	1	3	5	8	0	1	3	5	8
Leaves[1]										
8L	–[2]	–	–	–	0.11	–	–	–	–	0.03
7L	–	–	0.75	0.39	0.22	–	–	0.22	0.14	0.04
6L	0.68	0.76	0.48	0.40	0.37	0.32	0.30	0.14	0.13	0.09
5L	3.35	1.84	1.01	0.89	0.75	0.14	0.06	0.04	0.05	0.03
4L	0.09	0.03	0.02	0.04	0.02	0.15	0.04	0.03	0.04	0.02
3L	0.13	0.03	0.02	0.03	0.01	3.11	2.06	1.19	0.98	1.00
1–2L	0.06	0.05	0.02	0.02	0.01	0.06	0.06	0.03	0.05	0.04
Culm	0.55	0.54	0.43	0.40	0.39	0.23	0.19	0.15	0.11	0.08
Tiller	1.42	1.50	0.71	0.50	0.25	0.45	0.47	0.24	0.20	0.10
Root system[3]										
UNR	0.45	0.69	0.42	0.27	0.12	0.41	0.51	0.23	0.17	0.07
LNR	0.32	0.23	0.28	0.21	0.13	0.16	0.19	0.13	0.13	0.07
SR	0.15	0.07	0.05	0.06	0.05	0.08	0.09	0.06	0.09	0.05

1) Leaves are devided by each nodal position and designated as the number from bottom to the top.
2) Leaves not emerged.
3) UNR, upper nodal roots; LNR, lower nodal roots; and SR, seminal roots.



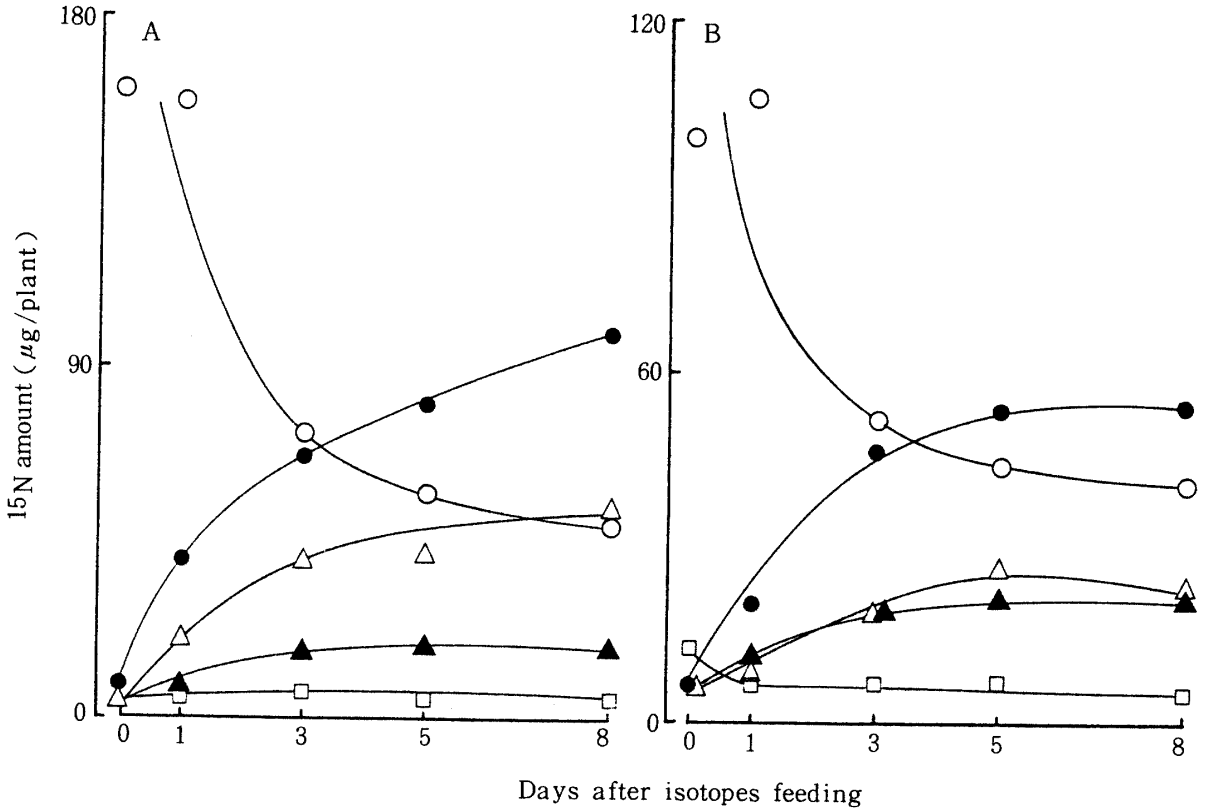
Figs. 3A and 3B. Changes in distribution of ^{13}C (μg/ plant) in various parts of the wheat fed $^{13}CO_2$ and $^{15}NH_3$ from the 5th (3A) or 3rd (3B) leaf. Symbols are the isotopes-fed leaf (FL, open circle), tiller (T, closed circle), developing leaves (DL, open triangle), root system (R, closed triangle), and other leaves + culm (C, square).

found around Day 1 and Day 2. Figures 5A and 5B show the changes in the ^{13}C amount in the root systems during the chase period in 5L and 3L plot, respectively. On Day 0 the LNR accumulated the largest amount of ^{13}C among the root categories, followed by SR in both feeding plots. Although UNR showed the highest ^{13}C abundance on Day 0

Table 3. ^{15}N abundance (atom % excess) in various parts of the wheat fed $^{13}CO_2$ and $^{15}NH_3$ from single mature leaves.

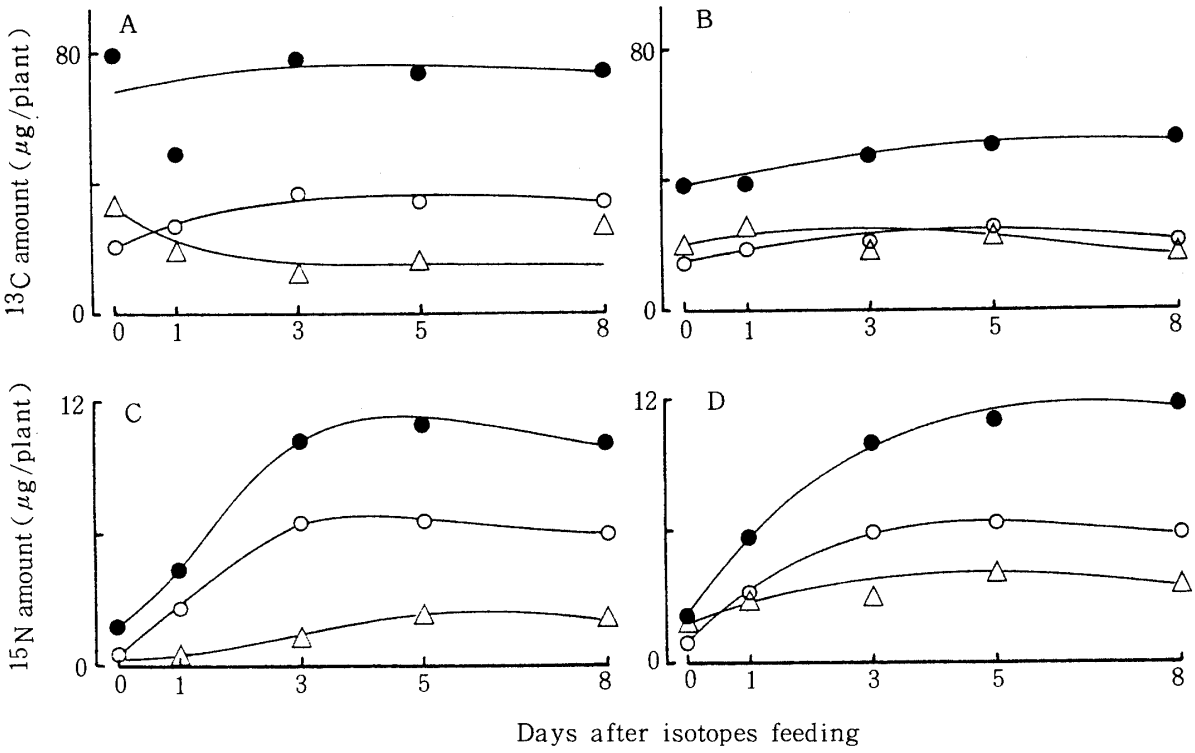
Plant parts	Isotopes were fed from 5th leaf					Isotopes were fed from 3rd leaf				
	Days after isotopes feeding									
	0	1	3	5	8	0	1	3	5	8
Leaves[1]										
8L	–[2]	–	–	–	0.36	–	–	–	–	0.15
7L	–	–	1.79	1.12	0.65	–	–	0.86	0.62	0.25
6L	0.20	0.75	0.57	0.52	0.41	0.26	0.44	0.31	0.31	0.23
5L	4.30	3.87	1.98	1.78	1.45	0.11	0.06	0.05	0.07	0.07
4L	0.04	0.01	0.05	0.02	0.04	0.10	0.03	0.06	0.06	0.04
3L	trace	0.05	0.06	0.03	0.03	6.80	5.97	3.42	3.17	3.61
1–2L	trace	0.04	0.03	0.02	0.03	0.39	0.15	0.06	0.14	0.09
Culm	0.23	0.69	0.78	0.66	0.40	0.27	0.42	0.41	0.29	0.18
Tiller	0.40	1.43	1.20	1.04	0.53	0.38	0.73	0.75	0.55	0.31
Root system[3]										
UNR	0.10	0.48	0.80	0.55	0.28	0.24	0.74	0.73	0.46	0.22
LNR	0.08	0.23	0.42	0.36	0.21	0.10	0.29	0.31	0.32	0.18
SR	0.01	0.01	0.06	0.10	0.05	0.09	0.13	0.12	0.20	0.13

1) Leaves are devided by each nodal position and designated as the number from bottom to the top.
2) Leaves not emerged.
3) UNR, upper nodal roots; LNR, lower nodal roots; and SR, seminal roots.



Figs. 4A and 4B. Changes in distribution of ^{15}N (μg/ plant) in various parts of the wheat fed $^{13}CO_2$ and $^{15}NH_3$ from the 5th (4A) or 3rd (4B) leaf. Symbols are the isotopes-fed leaf (FL, open circle), tiller (T, closed circle), developing leaves (DL, open triangle), root system (R, closed triangle), and other leaves + culm (C, square).

(Table 2), it accumulated the smallest amount of ^{13}C because of its smaller mass in dry weight. The ^{13}C amount of UNR increased gradually along its growth up to Day 3 or Day 5 then decreased with the chase time due to the loss of ^{13}C by



Figs. 5A, 5B, 5C, and 5D. Distribution of ^{13}C (5A, 5B) and ^{15}N (5C, 5D) in the root system of the wheat fed $^{13}CO_2$ and $^{15}NH_3$ from single mature leaves. Isotopes were fed from the 5th (5A and 5C) or 3rd (5B and 5D) leaf. Symbols are the upper nodal roots (UNR, open circle), lower nodal roots (LNR, closed circle), and seminal roots (SR, triangle).

respiration and the retranslocation of ^{13}C to other organs. The LNR showed slight change in ^{13}C amount throughout the chase period, while the oldest SR appeared to decrease its ^{13}C amount more promptly than UR.

Changes in ^{15}N amount in 5L and 3L plot are shown in Figs. 5C and 5D, respectively. The ^{15}N amount in the roots of all categories increased up to around Day 3 or Day 5, then decreased gradually with time. As compared with the pattern of ^{13}C, initial (Day 0) distribution of ^{15}N to the roots is relatively small. The largest proportion of ^{15}N was distributed to UNR, then LNR and SR followed in this order during the chase period except for Day 0 of 3L-feeding plants (Fig. 5D).

^{13}C : ^{15}N weight ratio in plant parts; The ^{13}C : ^{15}N weight ratio (^{13}C : ^{15}N ratio) in sink organs indicates the relative amount of ^{13}C and ^{15}N accumulated in the organ as the phloem solutes translocated from the fed leaf. As shown in Table 4, ^{13}C : ^{15}N ratio of the developing leaf (6L) in 5L-feeding plants was decreased rapidly up to Day 3 then small change was followed. The tiller and the root system also decreased ^{13}C : ^{15}N ratios during this period. These decrease in ^{13}C : ^{15}N ratio in the sink organs were associated with the rapid loss of ^{13}C by respiration in the whole plant (Table 1). Although the overall level of ^{13}C : ^{15}N ratios in the sink organs of 3L plants was lower than that in 5L plants, the pattern of time-course changes in ^{13}C : ^{15}N ratio in the sink organs of 3L-feeding plants were similar to that of 5L plants. The lower level of ^{13}C : ^{15}N ratio in 3L plants was due to that the fed leaf was initially more densely labelled with ^{15}N relative to ^{13}C as compared with 5L plants. The ^{13}C : ^{15}N ratio of the fed leaf was 1.6 times larger in 5L plants than in 3L plants.

On Day 0 and Day 1, the 6L and the tiller show very similar ^{13}C : ^{15}N ratio in each other, while the root system shows higher ^{13}C : ^{15}N ratio than these shoot organs

Table 4. $^{13}C : ^{15}N$ weight ratios in the fed leaves and sink organs of the wheat fed $^{13}CO_2$ and $^{15}NH_3$ from single mature leaves.

Plant parts	isotopes were fed from 5th leaf					3rd leaf				
	Days after isotopes feeding									
	0	1	3	5	8	0	1	3	5	8
Fed leaf	6.4	4.2	5.2	5.5	5.9	4.0	2.8	3.5	3.2	3.0
Tilller	32.7	9.7	6.0	4.9	3.6	11.0	6.1	3.2	3.6	3.5
Developing Leaves[1]										
6L	31.0	9.3	8.1	7.5	8.8	11.7	6.3	4.3	3.9	3.9
7L+8L	—	—	3.2	3.2	3.4	—	—	2.2	2.1	1.7
Root system[2]	60.0	13.2	7.1	6.3	7.3	14.8	7.0	4.6	4.6	4.3
UNR	40.4	10.3	5.6	5.3	5.6	15.5	6.1	3.5	4.0	3.6
LNR	46.4	11.2	7.6	6.7	7.3	18.0	6.8	4.7	4.6	4.5
SR	185.9	85.8	10.3	7.5	12.8	10.7	8.4	6.2	5.6	4.8
Whole plant	9.2	6.0	5.8	5.4	5.0	5.5	4.0	3.6	3.7	3.4

1) The 7th leaf emerged on Day 3, and the 8th leaf on Day 8.
2) UNR, upper nodal roots; LNR, lower nodal roots; and SR, seminal roots.

in both feeding plots. This indicates that the root system preferentially incorporates ^{13}C rather than ^{15}N as compared with the developing shoot organs. Comparing the $^{13}C : ^{15}N$ ratio of the root system with that of the developing leaf (6L) in the same feeding plants, the root system shows 1.9 and 1.4 times higher ratios than the developing leaf in 5L plants on Day 0 and Day 1 respectively, while in 3L plants the root system shows 1.3 and 1.1 times higher ratios on Day 0 and Day 1 respectively. These indicate that relative distribution of ^{13}C and ^{15}N from source leaves could be affected by the characteristic of sink organs as well as by the nodal position of source leaf.

The variation in the $^{13}C : ^{15}N$ ratio was found among the roots of different categories. On Day 1, the UNR showed the lowest ratio followed by LNR, while SR showed the highest ratio among the roots, whether isotopes were fed from 5L or 3L. Similarly on Day 0, the $^{13}C : ^{15}N$ ratio was lower in UNR than LNR in both feeding plots. On Day 0, SR in 3L plants shows exceptionally low $^{13}C : ^{15}N$ ratio, due to its high ^{15}N content shown in Fig. 5D, while in 5L plants $^{13}C : ^{15}N$ ratio of SR is calculated to be very large because of its trace amount of ^{15}N (Fig. 5C).

Discussion

Events in the fed leaf; In this experiment gaseous $^{15}NH_3$ was administrated to the leaves simultaneously with $^{13}CO_2$. Gaseous $^{15}NH_3$ would be rapidly absorbed by the mesophyll cell of the fed leaf and easily assimilated into amino acids through glutamine synthetase and glutamate synthase system and/or glutamine dehydrogenase system[10]. The patterns of ^{15}N transfer from assimilating leaves (5L and 3L) are similar to that previously reported for rice[11,17] and sunflower[20] where leaves are fed with ^{15}N-labelled gaseous NO_2. Feeding of ^{13}N-labelled NH_3 to sunflower and lupin leaves have indicated that ^{13}N is readily assimilated into organic N in the leaf then ^{13}N-labelled assimilate moved down in petioles as fast as ^{11}C-assimilate[4]. A part of ^{15}N incorporated into amino acids by the fed leaves in the present study could be rapidly loaded to the phloem then transferred to other sink organs. Changes in amounts of ^{13}C and ^{15}N in sink organs (Figs. 3 and 4) show that the increase of ^{13}C in sink organs takes place more rapidly than ^{15}N. This may be due to that the mass loading of ^{15}N assimilate into the phloem in the fed leaves takes place more gradually than that of ^{13}C

assimilate, although ^{15}N compounds would move together with ^{11}C assimilate in the same speed in the phloem[4]. The mass transfer of labelled amino acids applied to the leaves into the phloem takes place more slowly than photoassimilated C[5].

Partitioning of ^{13}C and ^{15}N; It was shown that ^{15}N exported from the fed leaves was intensely translocated downward to the root system while the roots absorbed inorganic N from medium (Fig. 4). This is consistent with the results obtained for rice[16,17], maize[13], and barley[14] whose leaves are fed with ^{15}N-labelled compounds. In addition, it was demonstrated that the lower leaf (3L) allocated larger proportion of its ^{15}N assimilate to the root system, as compared with the upper leaf (5L) did (Figs. 5C and 5D). This is consistent with the result for the rice plant fed ^{15}N-labelled urea from mature leaves[16]. Similar profile in translocation is also found for ^{13}C assimilate partitioning (Fig. 5A and 5B). In graminaceous plants, upper leaves preferentially supply developing leaves with their photoassimilated C while lower leaves feed mainly the root system[18]. Contribution of the lower leaves to the root system as N source might become more important particulary when plants encounter the N deficiency on their growth, since under such conditions senescence of lower leaves proceeds predominantly and in these leaves retrieval of N to other organs occurred in large extent due to the rapid degradation of leaf proteins.

^{13}C : ^{15}N weight ratio in the root system; The ^{13}C : ^{15}N ratio (Table 4) in the sink organs on Day 0 and Day 1 could be an indicator of the relative demand for C and N currently supplied from source leaves as well as the C : N weight ratio of the phloem solutes translocated to the sink organs, since the most rapid transfer of ^{13}C and ^{15}N from the fed leaves occurred during this period and the redistribution of isotopes among plant parts and the loss of ^{13}C by respiration may be restricted. On Day 0 and Day 1 the ^{13}C : ^{15}N ratio of the developing leaf (6L) is almost equal to that of the tiller, while the ^{13}C : ^{15}N ratio of the root system is higher than the developing leaf and tiller, when plants are fed from either the upper leaf (5L) or lower leaf (3L). This suggests that the root system receive the solute from mature leaves as the phloem translocate containing more rich in C relative to N than the developing shoot organs. This is consistent with the result obtained by the analysis of the phloem fluids for the nonnodulated white lupin, which indicates that C : N weight ratio of the solute delivered from leaves as phloem translocate is higher in the roots than the shoot apices[9].

Detailed comparison of ^{13}C : ^{15}N ratio of the sink organs of 5L- and 3L-feeding plants on Day 0 and Day 1 indicates that allocation pattern of C and N in the plant can be altered to some extent by the position of source leaf. As shown in Table 4, the magnitude in the lifting of ^{13}C : ^{15}N ratio of the root system relative to the developing leaf and the tiller is greater when the plant was fed from 5L than from 3L. This indicates that the upper leaf (5L) tends to allocate larger proportion of its ^{13}C relative to ^{15}N to the root system than to the developing shoots, as compared with the lower leaf (3L). These features suggest that the upper leaves contribute to alleviate the lowering of C : N weight ratio of the phloem solutes entering to the root systems of wheat, since the roots depend their phloem translocate largely upon the lower leaves (Figs. 3 and 4) which produce the phloem translocate of relatively lower C : N ratio as compared with the upper leaves[12].

It was demonstrated in Table 4 that the ^{13}C : ^{15}N ratio of the accumulate translocated from the fed leaves varied among the roots of different categories of wheat plants; i.e. the ^{13}C : ^{15}N ratio was low in the young roots (UNR) and progressively increase in the old roots (SR), whether the source leaf was 5L or 3L. This suggests that the younger roots receive solutes more rich in N relative to C as the phloem translocate from mature leaves than the older roots. The similar variation of C : N

ratio of the phloem solutes entering to the young and old roots is suggested in our previous study for rice plants fed ^{13}C and ^{15}N from leaves[17], although the changing pattern in relative distribution of $^{13}C : ^{15}N$ in the roots along with aging is quite opposite to that found in the present study for wheat root system; the upper nodal roots (younger roots) show the highest $^{13}C : ^{15}N$ ratio among the roots and the ratio decreases as the position of nodal roots lowered (older roots). The opposite trend in relative incorporation of C and N among the roots between wheat and rice suggests that C and N metabolism intimately depending on the phloem assimilate would be altered by aging in different extent between wheat and rice roots.

The form of inorganic N in the medium would also affect to the C and N metabolism in the absorbing roots, since ammonium N can be rapidly incorporated into amino acids in the roots then transferred to the xylem as the translocate of reduced N, while a large part of inorganic N absorbed as nitrate is not metabolized in the roots then transferred to the shoots as nitrate form[10]. The proportion of nitrate reduced in the absorbing roots may change as aging of the root. It was reported that the root respiration of the rice grown in the medium containing ammonium as a sole N source was higher than that of the rice grown in the nitrate medium[6]. Thus root demand for C as the phloem translocate also relates to the N nutrition of the roots. These results support the idea that in graminaceous plants C : N ratio of the solute entering to the nodal roots via phloem is regulated by the mechanism which allows nodal roots in different ages to receive the phloem translocate of various C : N ratios meeting to their current demands for C and N delivered from shoots.

The significance of relative distribution of C and N transferred from leaves as the phloem translocate should be further investigated in relation to the C and N economy of the root system as well as the C and N circulating system within the whole plant.

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栄養生長期のコムギ葉から根系への炭素と窒素の転流：単葉への$^{13}CO_2$と$^{15}NH_3$ガスの同時供与実験

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要　約

栄養生長期のコムギの第5葉（上位葉）あるいは第3葉（下位葉）に^{13}Cで標識したCO_2と^{15}Nで標識したNH_3ガスを同時に供与し、これらの葉からシンク器官へ転流する^{13}Cと^{15}Nを追跡した。この際とくに根系を構成する種子根および各節根に着目した。同化葉から吸収された^{13}Cと^{15}Nは伸長葉、分げつ、根系へと活発に転流した。同化葉からの^{13}Cと^{15}Nの一次分配の様相を$^{13}C/^{15}N$重量比で調べた結果、同化葉の葉位にかかわらず、伸長葉と分げつはよく似た$^{13}C/^{15}N$比を示した。一方根系の$^{13}C/^{15}N$比は1.1から1.9倍伸長葉と分げつよりも高かった。また根系の$^{13}C/^{15}N$比は上位葉が同化葉であった場合下位葉の場合と比較して1.5から1.3倍高かった。根系内で比較すると、$^{13}C/^{15}N$比はエイジの進んだ種子根と下位節根では低く、新根である上位節根では高かった。以上のことから、同化葉からの^{13}Cと^{15}Nの相対的分配の様相は、同化葉の葉位およびシンク根の齢によって影響されることが示された。