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Estimation of Carbon and Nitrogen Allocation during Stalk Elongation by ^{13}C and ^{15}N Tracing in *Zea mays* L.[1]

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ABSTRACT

***Zea mays* L. (cv Dea) plants grown to the stage of stalk elongation, were allowed to assimilate $^{13}CO_2$ and ^{15}N-nitrates from 45 to 53 days after sowing. Isotopic abundances in labeled nutrients were slightly enriched compared to natural abundances. The new C in plant was acropetally distributed and the new N was preferentially accumulated in the sheath and stalk in the medium region. C input was 25-fold higher than N input. The new C in total plant C was 20%, whereas it was 10% for N. The stalk acted as a major sink because it accumulated, respectively, 27.5 and 47.5% of the C and N inputs. The new C in soluble carbohydrates was 76% in growing organs (upper stalk) and only 39% in source leaves, whereas it was 43% and 13% in starch, respectively. New N in nitrates+amino-acids spanned in the range from 20% (leaf) to 50% (stalk). New C and N in soluble proteins were, respectively, 13.4 and 3.8% in leaves, 8.8 and 9.6% in stalk, and 8.7 and 14.3% in roots. In the middle stalk and leaves, the proteins and carbohydrates represent an equivalent C and N source for remobilization.**

Plant growth and seed production of grain crops are the result of the photosynthetic ability of source leaves and the integrated processes of allocation, accumulation and utilization of assimilated carbon at the whole plant level (14). Efficient translocation of photoassimilates to sink organs is one of the key factors in increasing production (14, 27). C and N metabolism are closely linked, so both should be considered when attempting to determine factors that limit efficient translocation. Recently, attention has been paid to relationships between C and N metabolism in the productivity of maize (1, 26, 28). However, only a few data are available on ^{13}C and ^{15}N redistribution in the Graminae (18), and little information is available for maize (8). Our interest was focused on C and N allocation in the stalk. This organ acts first as a sink during elongation and then as a source when ears develop (1, 23). We used here a CO_2 assimilation system designed for long-time incorporation of C at a constant ^{13}C abundance under steady state conditions: maize plants were supplied simultaneously with $^{13}CO_2$ and ^{15}N-nitrate in nutrient solution during stalk elongation (45th to 53rd d). Isotopic abundance in nutrients was only slightly enriched compared to the natural abundance. The isotopic enrichment was determined by mass spectrometry fitted for natural isotopic abundance measurements. The aim of the present paper is, first, to establish the C and N balance in the whole plant and the detailed partitioning between the different organs. Second, the fate of ^{13}C and ^{15}N in soluble metabolites (carbohydrates and amino acids+nitrates), in storage compounds (buffer-soluble and insoluble proteins, starch), and in cellulose was also examined to estimate the C and N availability in the intermediary reserves for ear development.

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MATERIALS AND METHODS

Plant Culture

Maize plants (*Zea mays* L., cv Dea) were grown in 7 L pots (one-third peat and two-thirds argile mixture) in a greenhouse until 11th leaf stage. Nutrient solution containing 12 meq NO_3^- and 2 meq NH_4^+ (6) was automatically supplied three times a day.

Plant Labeling

Plants were separated in two groups just at the beginning of stalk elongation (45 d). Four plants were chosen and sampled as control as indicated below. The other 16 plants were enclosed in a climatic chamber and fed with $^{13}CO_2$ and ^{15}N-nitrates for 8 d (45–53d). The climatic chamber (area, 1 m^2 and height, 1.5 m) was controlled at 26°C day and 20°C night, 70% R.H. A PAR of 280 mol quanta $m^{-2} \cdot s^{-1}$ (400 and 700 nm) was provided during the 15 h (0700–2200) from a combination of fluorescent and incandescent lamps. For each photoperiod, the $^{13}CO_2$ exposure was started at the beginning of the light and stopped 2 h before the light was switched off. As a result, plants had fixed all the CO_2 of the chamber before dark respiration started, plant carbon discrimination was avoided.

Natural ^{13}C abundance is about 1.1%. The experimental procedure was established to reach a constant ^{13}C value near 1.3% for CO_2 in the atmosphere of the chamber by continu-

ously mixing a small quantity of $^{13}CO_2$ diluted in N_2 (bottle F1: 99 volumes N_2 and one volume CO_2 with 100% ^{13}C) with the industrial CO_2 flow feeding plants (bottle F2: 76 volumes N_2, 19 volumes O_2, and 5 volumes CO_2 having ^{13}C natural abundance (1.07825%). The flow rate from bottle F1 was metered at 1/100 of the flow rate of the bottle F2 by means of two mass flow controllers, E1 and E2 (model Tylan FC260 and FC261). Through a master box a homemade controller operated the mass flow controllers in response to the output of the infrared CO_2 analyzer (ADC 225MK3) to maintain a constant ^{13}C abundance and a constant CO_2 concentration of 450 $\mu L\ L^{-1}$ in the chamber. The final ^{13}C abundance in CO_2 feeding plants was determined on duplicate samples of the atmosphere taken twice a day (1100 and 1800) through an exit valve using 5.5 dm^3 flasks previously evacuated. During the 8 d-exposure, each plant each day received 500 mL of nutrient solution containing 14 meq NO_3^- with 1.9643 atom% ^{15}N abundance.

Plant Sampling

Three plants only were harvested at the end of the photoperiod on d 8. The other plants will be further sampled in order to study redistribution during ear formation. Blades, sheaths, and stalk were divided into different batches according to their position in the plant: 'lower' for the organs which were below the sixth leaf from the base, 'middle' for organs including seventh to ninth nodes, and 'upper' organs which were above the rank 9. Lower and upper blades were further subdivided into: lower 1 (nodes 1–3) and lower 2 (4–6), and upper 1 (10–12) and upper 2 (13–15). Each sample was freeze-dried, weighed, and ground to a fine homogeneous powder by a grinder Tecator (Cyclotec sample mil, 1092). Samples were stored at −20°C.

Tissue Carbohydrates Fractionation and Content Determination

Five hundred mg of fine powder corresponding to the lyophilized organ were homogenized in chloroform/methanol/water (v/v/v). The chloroform extract was evaporated to dryness to obtain the total lipid fraction. The methanol/water extract was evaporated, redissolved in water, and then passed through a C18 column yielding the water soluble carbohydrates. Starch and cellulose in the residue was isolated by a procedure described previously in more detail (9). The cellulose preparation was dried and weighed. Glucose, fructose and sucrose were determined after extraction (from the lyophilized powder) in ethanol 80% using an enzymic method as described by Bergmeyer *et al.* (3). Starch was determined in the residue by the same enzymic method after solubilization in DMSO and hydrolysis to glucose by action of amyloglucosidase (3).

Tissue Nitrogen Fractionation and Content Determination

Five hundred mg of fine powder were ground in 50 mM sodium phosphate buffer (pH 7.4) in a mixer grinder (PCU-2 Polytron). The homogenate was centrifuged (10,000*g*, 20 min) and the buffer-soluble proteins were obtained from the supernatant by heat denaturation (60 min, 100°C) and collected by centrifugation (10,000*g*, 20 min). The residue contained only insoluble N. The total N content was determined in the powder by the Kjeldahl method using Nessler reagent after H_2SO_4 digestion. Buffer-soluble protein N was measured with bicinchoninic acid protein assay reagent (22). Amino acid N was determined by colorimetry following cyanide-ninhydrine treatment after extraction in salycilic acid (21). Nitrate N was determined with sulfosalycilic acid reagent after extraction in distilled water at 45°C for 60 min (5). Insoluble N equals: (total N)-(buffer-soluble N)-(aminoacid N)-(nitrate N).

Carbon and Nitrogen Isotope Analyses

The carbon and nitrogen isotope ratio of plant organic matter was measured on CO_2 and N_2 obtained by combustion of powdered plant tissues in a quartz sealed tube, with CuO in two steps, at 850°C and 600°C. The Cu appearing in the first step acts as a reductant in the second step for the N oxydized forms. After breaking the tube *in vacuo*, the evolved CO_2 and N_2 were cryogenically separated, purified, and analysed in a mass spectrometer fitted with a multiple ions collector and a dual inlet system equipped for rapid switching between reference and sample (VG sira 9). Results are expressed in ∂ units versus PDB (Belemnite from Pee Dee formation in South Carolina) for ^{13}C and atmospheric nitrogen for ^{15}N: $\partial‰ = (R\ \text{sample}/R\ \text{reference} - 1) \cdot 1000$ (10, 19).

$\partial^{15}N$ of the N-soluble fraction was calculated from the $\partial^{15}N$ value of the total organic matter, buffer-soluble and insoluble proteins, and their respective N quantities, by classical isotopic dilution calculations (9, 10).

Rationale of Plant Labeling Near the Natural Abundance

Plants exhibit a natural ^{13}C abundance according to their photosynthetic pathway (2). Maize (C_4 plant) is weakly depleted in ^{13}C compared to atmospheric CO_2 with an isotopic enrichment close to 4‰ (∂ units). $\partial^{13}C$ Control plant = $\partial^{13}C$ atmospheric CO_2 − 4‰. Because labeled CO_2 supply was stopped 2 h before the dark period, the CO_2 disappeared almost totally (checked with the infrared analyser) in the chamber atmosphere at the end of the light period. The respiratory CO_2 that evolved during the night progressively accumulated in the chamber until the beginning of the light period when it was reassimilated. As a consequence, all the CO_2 introduced in the chamber during the light period was assimilated and the fractionation of 4‰, which occurs when the reserve of CO_2 is infinite, is not expressed in the closed system with total metabolization of the substrate (4). Thus, $\partial^{13}C$ of new photosynthates $\approx \partial^{13}C$ air supplied.

The absolute content of heavy isotope per 100 atoms (A) is symbolized by $A\%t$ and $A\%c$ for treated and control plants, respectively, and $A\%l$ and $A\%n$ for label and natural nutrients, respectively: if H and L stand respectively for quantities of heavy and light isotope, then: $A\% = (H/H+L) \cdot 100$ as $R = H/L$, then $A\% = (R/R+1) \cdot 100$. After exposure, the theoretical $A\%$ value of an organ can span from $A\%$ control (no new incorporated C) to $A\%$ value of new assimilates, *i.e.* those of

Table I. *Changes in the $\partial^{13}C$ and A% Excess for ^{13}C of Atmospheric CO_2 in the Chamber during the 8 d Exposure to Labeled $^{13}CO_2$ (mean value of duplicate)*

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
$\partial^{13}C$ (11:00)	79	145	155	160	160	163	160	160
$\partial^{13}C$ (18:00)	115	151	155	166	166	168	166	166
A% excess (11:00)	0.0998	0.1721	0.1830	0.1885	0.1885	0.1918	0.1885	0.1885
A% excess (18:00)	0.1392	0.1786	0.1830	0.1950	0.1950	0.1972	0.1950	0.1950

Table II. *Dry Weight, Isotopic Composition Expressed as A% Excess and Relative Specific Allocations (RSA_C and RSA_N in %) in the Different Organs*

Organ	Dry Weight	A% excess		%N[a]	New C	New N	RSA_C[b]	RSA_N	$\%P_C$	$\%P_N$
		^{13}C	^{15}N							
	g plant^{-1}				*g*	*mg*				
Blade	12.1	0.0352	0.0582	3.04	0.89	13.2	18.5	3.7	57.1	22.4
Sheath	2.7	0.0369	0.1695	2.51	0.21	7.1	19.3	10.7	13.3	11.9
Stalk	3.4	0.0610	0.4160	3.44	0.44	28.6	32.0	26.2	27.5	47.5
Roots	2.2	0.0067	0.3363	2.24	0.03	10.3	3.5	21.1	2.1	18.2
Total	20.4	0.0368	0.1648	2.95	1.57	59.2	19.3	10.4	100	100

[a] Organ N content. [b] For RSA and %P calculations, see "Materials and Methods."

air supplied (100% new incorporated labeled *C*). So,

$$A\%t = X(A\%l) + Y(A\%n)$$

with $X + Y = 1$, X, and Y being, respectively, the proportions of new photosynthates and preexisting matter. In our experimental conditions, there was no fractionation between light and heavy isotopes during their incorporations. It implies that $A\%n = A\%c$.

Parameters of C and N Distribution after Plant Exposure

The RSA[2] is the proportion of newly incorporated atoms relative to total atoms in the sample. %P expresses the repartition of the total C and N inputs in the different fractions (organ or biochemical). The control ∂ values are the natural heavy isotope abundances in organs and in biochemical compounds determined on control plants treated by similar methods as treated plants.

Thus, using *A*% expression

$$\text{RSA} = \frac{A\%t - A\%c}{A\%l - A\%c}$$

The difference between absolute contents in heavy isotopes is defined as the *A*% excess due to labeling in treated plants: $A\%t$ excess $= A\%t - A\%c$. *A*% excess in the tissue represents the incorporation due to the heavy isotope excess in plant-fed nutrients ($A\%l$). This value is calculated similarly:

$$A\%1 \text{ excess} = A\%1 - A\%n = A\%1 - A\%c.$$

[2] Abbreviations: RSA, relative specific allocation in percent; %P, partitioning.

For carbon

$$\text{RSA}_C = \frac{A\%^{13}\text{C assay} - A\%^{13}\text{C control}}{A\%^{13}\text{C air supplied} - A\%^{13}\text{C control}}$$

$$\%P_C = \frac{(\text{RSA}_C \text{ fraction})\cdot\text{DW fraction}}{\text{RSA}_C \text{ plant}\cdot\text{DW plant}}$$

For nitrogen

$$\text{RSA}_N = \frac{A\%^{15}\text{N assay} - A\%^{15}\text{N control}}{A\%^{15}\text{N nutrient } NO_3^- - A\%^{15}\text{N control}}$$

$$\%P_N = \frac{(\text{RSA}_N \text{ fraction})\cdot(\text{DW fraction})\cdot(\text{N content})}{(\text{RSA}_N \text{ plant})\cdot(\text{DW plant})\cdot(\text{N plant})}$$

The quantity of new C or N in an organ compound was calculated using the organ dry weight (DWi), the C or N proportion (%C or %N), and its relative amount (Ai) in the organ:

$$Q_{C \text{ or } N} = (\text{DWi})\cdot(\text{Ai})\cdot(\%\text{ C or N})\cdot(\text{RSA}_{C \text{ or } N}).$$

It is assumed that C content is homogeneous in the different organs. In contrast, the N content in any organs is heterogeneous and was specifically determined (see above samplings). C and N proportion in the biochemical compound was deduced from its chemical formula. The C and N proportions in proteins were 40 and 16%, respectively, these values corresponding to a mean of 118 known proteins (7).

RESULTS

Accuracy of the Determination of the RSA Parameter

Accuracy of the determination of the RSA parameter is closely related to the maximum isotopic dilution range between control and labeled nutrient and the precision of isotopic measurements. Mean values of natural abundances of heavy isotopes in the control plants were −12‰ for carbon

and +12‰ for nitrogen. The long-term automatic CO_2 supply was successfully carried out at a constant $\partial^{13}C$ value of −162‰ ± 4‰ from the third day to the end of the exposure period (Table I). The first 2 d were probably the time necessary for the complete loading of all the surfaces of the chamber and all the exchange surfaces of plant tissues. The ^{13}C excess during the label was 0.1914 ± 0.0047 (Table I). The enrichment to 2% of ^{15}N nitrate led to a $\partial^{15}N$ value of 4,450‰, *i.e.* an excess of [1.5871]%. The standard error on $\partial^{13}C$ determination is 0.1‰ and those of ^{15}N is 2‰ for ∂ values near 4,500‰. As a result, the minimal RSA value that can be determined is 0.05% (0.1/174) for carbon and 0.05% (2/4438) for nitrogen.

C and N Allocations between Lower, Middle, and Upper Organs

The C and N inputs per plant were, respectively, 1570 mg C and 59.2 mg N for 8 d of development (Table II). The relative specific allocations in the plant were two-fold higher for carbon (19.3%) than for nitrogen (10.4%). Leaves that assimilate primarily carbon and roots that absorb primarily nitrates exhibited almost the same RSA values for their respective elements, RSA_C 18.5% in blades and RSA_N 21.1% in roots (Table II). Conversely, roots were the minor sinks for C (RSA_C, 3.5%) and leaves for N (RSA_N, 3.7%). Sheaths remained a high sink for N (RSA_N, 10.7%) compared to leaf blades. Stalks were the main sink for both elements since RSA_C and RSA_N had the highest values, 32.0 and 26.2%, respectively. New growth during this period was mainly located in the upper organs (upper stalk and sheath and upper two blades were not present before the labeling period). The total upper compartment (upper 1+2), representing 25.3% of the total dry weight, included young and just mature leaves; it received 50.3% and 13% of C and N input, respectively (Fig. 1, sum of upper organs). The growth was mainly located in blades, as sheath formation and internode elongation was just beginning. The middle compartment included the mature leaves and acted as C source organs; this compartment, which represented 39.4% of plant dry weight, was the main sink for C and N with partitioning values of 38.9 and 44.6% respectively, shared between the blades, sheaths, and stalks (Fig. 1, sum of middle organs). Growth in the lower compartment was restricted to the stalk, the leaves being senescent. This compartment and that of the roots which represented 35.3% of plant dry matter received only 10.8% of C input but a large share of N, 42.4% (Fig. 1, sum of lower organs).

RSA_C increased acropetally in blades (nil in lower 1–50% in upper 2), sheaths (nil in lower 2–75% in upper 1), and stalks (16% in lower 2–64% in upper 1), (Fig. 2). The RSA_N value did not show any special trend in blades (values below 10%), except in sheaths in which C and N RSA values showed parallel evolution (Fig. 2). The uppermost leaves, which were at the onset of their development, were strong sinks for newly incorporated C while the lower blades at the onset of their senescence period exported new C and rarely renewed their own structures. Of the new C, 35% went to the upper 1 leaves and 16% to the middle stalk (Fig. 1). Sheaths and stalks, which act as transitory reservoirs, exhibited a high capacity for N accumulation compared to blades. The main sink for

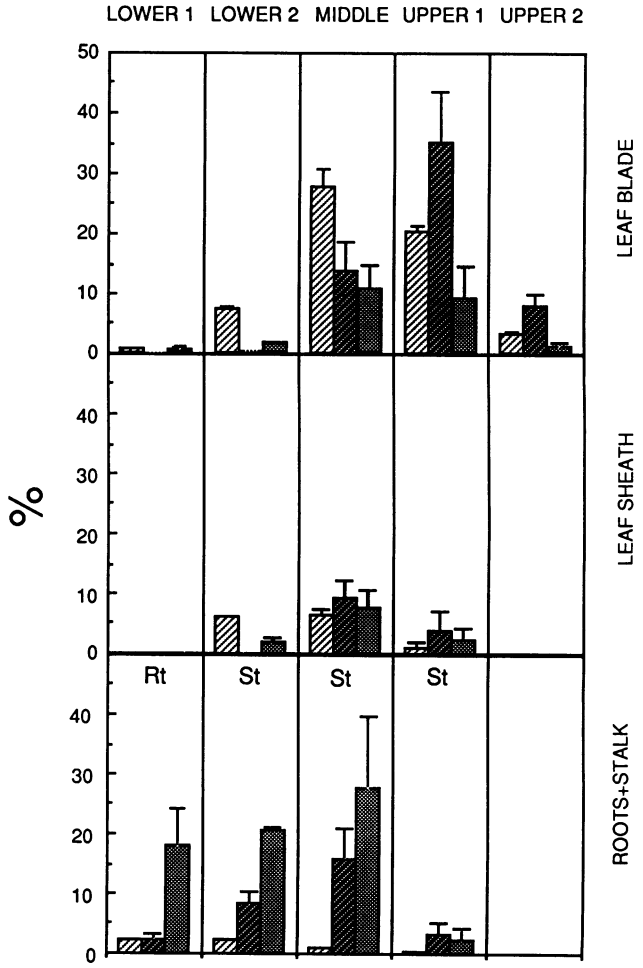

Figure 1. Organ repartition of dry matter (expressed in % total plant), and C and N partitioning in the different plant organs after 8 d exposure (expressed as % of total C and N inputs). Bars, left to right: dry weight, C, N.

N was the middle stalk with 28% of the new N, but the lower stalk contained 20% of the total input.

C and N Repartition in the Different Biochemical Compounds

Free Soluble Carbohydrates and Starch

Hexose content exceeded sucrose content in the middle stalk, the growing sink organ (66.0 and 17.8 mg per g dry weight, respectively, for hexoses and sucrose, Table III). Hexose contribution to total soluble carbohydrates decreased in middle blades (19.1 mg and 6.3 mg per g dry weight for sucrose and hexoses, respectively, Table III). RSA_C (Fig. 3) values for soluble carbohydrates and starch were analyzed with respect to the source-sink behavior of the organ. Middle leaves, which were totally source organs, exhibited an intermediary RSA value (39%) in soluble carbohydrates (Fig. 3). Surprisingly, the RSA_C value of starch in the same blades was notably lower, 13%. As plants were sampled at the end of the day, the starch pool was considered to be completely replenished. Importing organs exhibited the same discrepancy be-

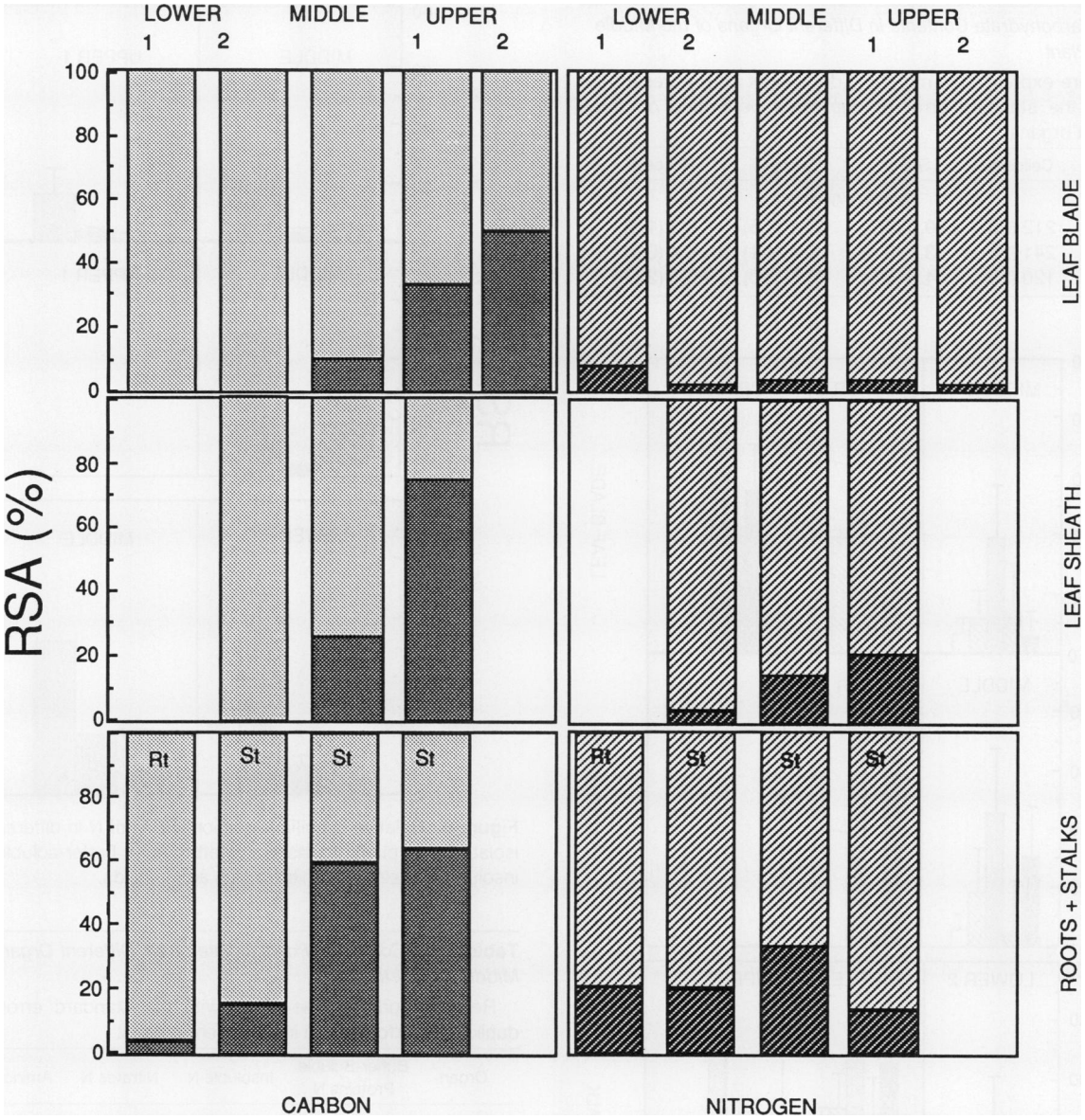

Figure 2. Relative specific allocation (RSA) of C and N in different plant organs after 8 d exposure. Bars, carbon: upper portion, ancient; lower portion, new. Bars, nitrogen: upper portion, ancient; lower portion, new.

tween RSA_C values of soluble carbohydrates and starch. Upper 1 sheath and stalk which were formed during the exposure period reached the highest RSA_C values of free soluble carbohydrates (82 and 76%, respectively), but RSA_C values of starch remained at a comparatively lower level (13 and 43%, respectively). The absolute quantity of starch in middle stalk (4.5 mg/g dry weight, Table III) was low and associated with a low labeling compared to soluble carbohydrates (Fig. 3). The reservoir organs, sheath and stalk, exhibited high contents of nitrates and amino acids compared to leaf blades, Table IV.

Nitrate and Aminoacid Pools

The RSA_N of this fraction in stalks was 50%, but the values in blades was notably lower, around 20% (Fig. 4). The preexisting N pool remained a great part of the soluble N compartment after the 8-d exposure.

Membrane Synthesis Markers

Both lipids and insoluble proteins can be considered as markers of membrane biosynthesis. The biochemical fraction which exhibited the smallest RSA_C value was the lipid whose RSA_C value remained roughly half of that of cellulose for each organ (Fig. 3). The insoluble proteins include mainly membraneous proteins (28). It was not possible to measure their RSA_C value because this fraction was recovered with added detergent. However, the $\partial^{15}N$ determination is correct: RSA_N values for insoluble proteins varied from 2.7% in the middle blades to 20.5% in middle stalk (Fig. 4). These values were especially low compared to those obtained in free soluble

Table III. *Carbohydrate Contents in Different Organs of the Middle Part of the Plant*

Results are expressed in mg per g dry weight and represent the mean with the standard error of duplicates performed on three independent organs.

Organ	Cellulose	Starch	Sucrose	Hexoses
		mg/g dry weight		
Blade	212 (28)	10.0 (3.7)	19.1 (3.5)	6.3 (1.5)
Sheath	241 (2)	3.1 (0.4)	9.4 (4.1)	5.4 (0.5)
Stalk	120 (29)	4.5 (0.6)	17.8 (2.8)	66.0 (22.9)

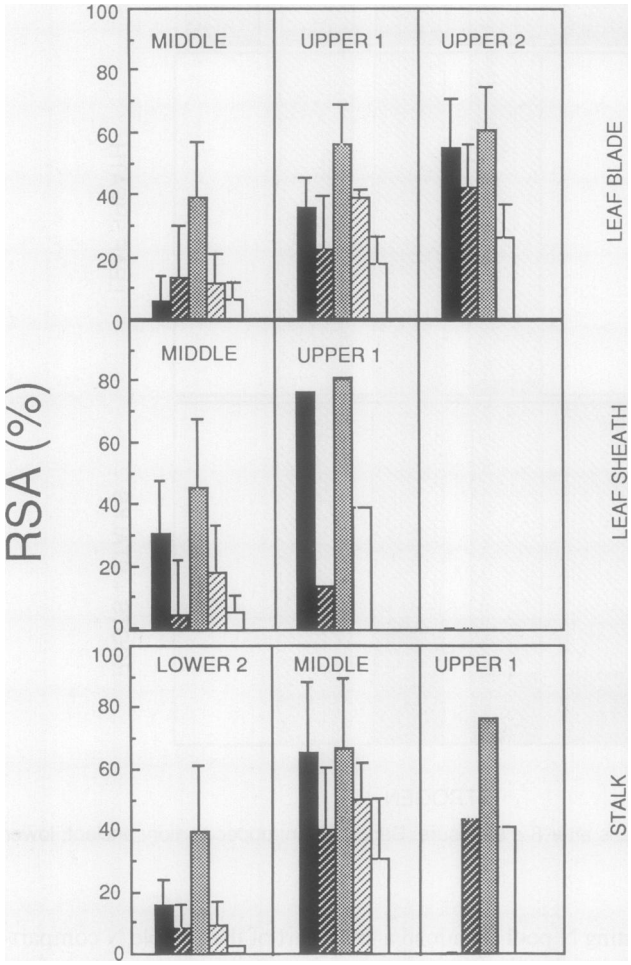

Figure 3. Relative specific allocation (RSA) of C in different biochemical compounds isolated from plant organs. Bars, left to right: cellulose, starch, soluble carbohydrates, soluble proteins, lipids. The cellulose and the buffer-soluble proteins from upper 1 stalk and the buffer-soluble proteins from upper 1 sheath and upper 2 blades were not analyzed.

N compounds: 20.1% in middle blades and 52.4% in middle stalk (Fig. 4).

Enzymatic Proteins

The buffer-soluble protein labeling with both ^{13}C and ^{15}N allows a direct comparison of the relative C and N renewal in these fractions (Tables IV and V). The RSA_C value was much higher than the RSA_N value in middle (13.4 and 3.8%, respectively) and upper 1 (29.0 and 2.3%) blades. In contrast, the RSA_C value in buffer-soluble proteins of roots was lower than RSA_N (8.7 and 14.3%, respectively). The RSA_C and RSA_N values were similar in lower stalk (8.8 and 9.6%, respectively) and middle sheath (25.1 and 18.0%) regions. In the middle stalk, the RSA_C was double that of RSA_N (50.4 and 22.1%, respectively). For all these organs, the RSA_C values of buffer-soluble proteins were highly correlated ($r = 0.96$) with the RSA_C value of the total C of the same tissue (Fig. 2). Similarly in the same organs, the RSA_N values of buffer-soluble proteins (Table IV) and total tissue (Fig. 2) were correlated ($r = 0.93$).

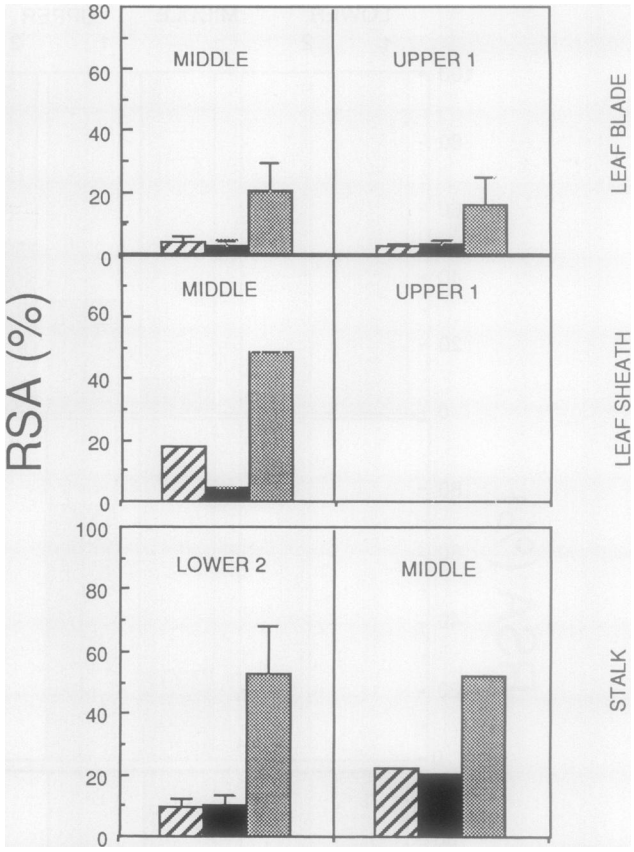

Figure 4. Relative specific allocation (RSA) of N in different N pools isolated from plant organs. Bars, left to right: buffer-soluble proteins, insoluble proteins, and nitrate plus amino acids.

Table IV. *N Content in Pools Isolated from Different Organs of the Middle Part of the Plant*

Results represent the mean with the standard error of three duplicates performed on independent organs.

Organ	Buffer-Soluble Proteins N	Insoluble N	Nitrates N	Amino Acids N
		mg/g dry weight		
Blade	10.6 (1.0)	17.4 (1.4)	0.2 (0.01)	2.1 (1.5)
Sheath	0.9 (0.1)	13.1 (3.3)	0.9 (0.08)	5.5 (2.2)
Stalk	12.8 (1.8)	28.9 (2.0)	1.6 (0.2)	3.3 (0.2)

Table V. *Relative Specific Allocation (RSA) for C and N in Buffer-Soluble Proteins Isolated from Different Plant Organs*

RSA values in buffer soluble proteins showed correlation with RSA values of total C in the organ.

Organ	RSA_C[a]	RSA_N[b]
	%	
Middle blade	13.4	3.8
Upper 1 blade	29.0	2.3
Middle sheath	25.1	18.0
Lower 1 stalk	8.8	9.6
Middle stalk	50.4	22.1
Roots	8.7	14.3

[a] RSA_C (proteins) = 0.6838 RSA_C (organ) + 3.1116 (R = 0.96). [b] RSA_N (proteins) = 0.5951 RSA_C (organ) + 0.2322 (R = 0.93).

Cell Wall Synthesis Markers

Cellulose is accumulated and only slightly renewed under normal conditions. Cellulose (Fig. 3) and total organic matter (Fig. 2) for each organ had closely related RSA_C values. Cellulose from upper organs which exhibited the highest growth rates, showed the higher RSA_C value (up to 77%).

Availability of C and N from Transitory Stored Reserves

The quantities of new C and N contained in the different biochemicals was detailed for the middle compartment (Table VI). The total new N was allocated to two close primary sinks, the nitrate and aminoacid pool (9.2 mg) and the insoluble proteins (11.2 mg) and a secondary sink, the buffer-soluble proteins (4.9 mg). The new N was especially accumulated in the insoluble proteins of the stalk (6.1 mg). A complete balance for C cannot be estimated (Table VI) because the biochemical fractionation of the plant organs was not exhaustive. Nevertheless, the main biochemical sink for C was cellulose especially in sheaths (42.4 mg) which were elongating during the labeling period. In stalk and in blade, the soluble carbohydrates (25.5 and 24.0 mg, respectively) were both large C sinks. The C reserve in the soluble proteins was estimated to be 26.7 mg in blades and 22.2 mg in the stalk. These values were of the same order of magnitude as values for the free soluble carbohydrates.

DISCUSSION

The total C and N renewal in an organ depends on growth, biochemical turnover, and the migration process. Only a few investigations have been carried out with the long time period required for isotopic saturation of the total plant elements. Literature reports either short-term, (2 h [30], 3 d [18]), or very long-term (8, 12) labeling nitrogen experiments, during all the preanthesis period. From these data, it is not possible to estimate the time necessary to saturate the total plant N. It was previously shown that the carbon isotope composition of young and mature leaves of *Sinapis alba* reached an almost constant value 4 d after the change in carbon isotope composition of the CO_2-fed plants (10). This saturation corresponds to 50% of new C for mature leaves and 75% for young leaves. The time scale measured on small *Sinapis* plants cannot be directly extended to 45-d old maize plants in which reservoir organs are well differentiated.

Compartmental analysis of newly incorporated C or N showed that the readily transported and temporarily stored pools of soluble carbohydrates and nitrates plus amino acids were renewed within a photoperiod (10 h of ^{13}C labeling [17, 29] and 2 h of ^{15}N labeling [30]). Rocher and Prioul (20) demonstrated by short time pulse ^{14}C-labeling that temporary storage sucrose (residence half-time near 2 h) is virtually renewed within 10 h in mature maize leaves. In the present long-time labeling experiment, the RSA values were related to initial biosynthesis of the compound (compared to the time of exposure) and to its turnover. It can be supposed that the isotopic saturation of translocates or of the pool of primary products of C and N fixation in source organs was achieved at the end of the 8-d exposure period. Indeed, the soluble pool (soluble carbohydrates, nitrates+amino acids) did not show isotopic saturation.

The soluble carbohydrates in sink organs (sheaths and stalks) exhibited high RSA_C values (from 60–80%) and showed high amounts of hexoses (Table II). However, the RSA_C of soluble carbohydrates in mature source leaves was only 39%. This means that the C skeletons of soluble carbohydrates originated in almost equal proportions from current photosynthesis and from preexisting material stored before the exposure. This fact is characteristic of the behavior of mature and senescing leaves which are strong exporters not only of new photosynthates but also of their own cellular material. In the stalk, the soluble carbohydrate pool had its

Table VI. *Quantities of C and N Assimilated during the Exposure Period Stored in Biochemical Compounds Isolated from Blades, Sheaths, and Stalk of the Middle Plant Area*

Results are expressed in mg of C and N in the compound from the different organ (see "Material and Methods").

Organ	Carbon				Nitrogen		
	Hexoses + sucrose	Cellulose	Starch	Soluble proteins	Soluble proteins	Insoluble proteins	Amino acids + nitrates
				mg			
Blade	24.0	29.7	3.4	26.7	2.0	2.1	2.5
Sheath	3.8	42.4	0.8	0.7	0.2	3.0	4.0
Stalk	25.5	36.0	0.8	22.2	2.9	6.1	2.7
Total	53.3	108.1	5.0	49.6	4.9	11.2	9.2

origins from many sources such as sucrose from the phloem and sucrose plus hexoses linked to *in situ* metabolism. However, the gradient of RSA_C value in soluble carbohydrates from lower to upper stalks could reflect the successive contribution to the translocated sucrose of leaves of decreasing age.

It is generally agreed that leaf starch is remobilized at night and represents about 20% of its maximal accumulation at the end of dark period (16). The RSA_C value in starch isolated from source leaves was considerably lower than the values obtained for soluble carbohydrates if daily photosynthates participate mainly to starch formation. In sink organs, the same discrepancy between RSA_C value of starch and soluble carbohydrates was noted. Moreover, the cellulose in sink organs always exhibited a higher RSA_C value (65%, in middle stalk) than the starch. Two hypotheses could explain the origin of starch in source and sink organs. First, amyloplasts constituted before the labeling could have remained in place especially in the bundle sheath and were not further degraded. Second, starch could be synthesised from carbon flow originating from remobilization of preexisting material *in situ* for source organs or from migration for sink organs. Sucrose fed to a sink organ is partly converted to starch as shown by Giaquinta (13). In source organs, the neoglucogenesis could be an active pathway involved in the replenishment of starch pools. In CAM plants, the replenishment of the starch pool was attributed in part to the use of C skeletons coming from translocated carbohydrates (11).

In contrast to Yoneyama *et al.* (30), we find a low turnover of N in nitrates and amino acids: the RSA_N values in the soluble-N pool were especially low and even lower in leaves (around 20%) than in stalks and sheaths (around 50%). Yoneyama *et al.* (30) also reported lower labeling in the metabolic nitrate pool of leaves than of petioles plus midribs in *Brassica campestris*. Preexisting amino acids, originating either from storage pools (having a low turnover) or from protein catabolism, formed a major part of the total pool.

The RSA_N value obtained in soluble proteins of the source leaves was low (around 3%). The turnover of major proteins (ribulose-1,5-biphosphate carboxylase/oxygenase, phospho*enol*pyruvate carboxylase, and pyruvate,Pi dikinase) was low (24, 25). The origin of N recycled in buffer-soluble or insoluble proteins could be attributed to either catabolism of preexisting proteins or the presence of a transitory stored N pool (nitrates + amino acids) associated with a low turnover acting as buffer reserves. Turnover of membranes must be low since the C and N inputs in both components (lipids and insoluble proteins) were small. Thus, the RSA_C values of lipids showed that they have a very low turnover or that they arise mainly from preexisting C skeletons. Golberg *et al.* (15) demonstrated that extension of the membrane involved mainly previously stored compounds.

Comparison of the RSA values for C and N in soluble proteins shows that the respective turnover of both elements is very different in source leaves and sink organs (Table V). The RSA_C value is much higher than the RSA_N value in C source organs (middle and upper blades). RSA_N value is higher than RSA_C value in N source organs, the roots. This fact can be explained if it is considered that leaves rapidly turn over the C skeletons and this C is then used for protein biosynthesis (because of a high photosynthetic rate). The internal N pool is thus used for protein rebuilding (due to a low N income in leaves). Conversely, roots, the initial source for N, rapidly turn over the N allocated to protein biosynthesis while cycling an internal pool of C skeletons. Both RSA_C and RSA_N values in soluble proteins (Table V) were similar in the stalk (8.8% for C and 9.6% for N) and sheaths (25.1 and 18.0%, respectively) indicating that these organs are large sinks for the amino acids originating from the leaves and the roots. C in buffer-soluble proteins turns over in a manner similar to the total C of plant organs. This fact emphasizes the role of these proteins as transitory buffer reserves for C. This role has previously been demonstrated for N (18).

The availability of C and N reserves for further remobilization depended on the stability of the structures in which they were incorporated. All the N pool can be remobilized as free amino acids and translocated to the ear. The C reserve in soluble carbohydrates and starch could be available as well for further C ear filling. In contrast, cellulose is considered to be a permanent structure not remobilizable in normal conditions. The high requirement of new C in cellulose implied that a large part of the label in plants will be sequestrated. The soluble and insoluble proteins usually considered as N sinks are also strong sinks for C. With only one-sixth of the dry weight of that of the corresponding leaves (blades+sheaths), the stalk exhibited almost the same potential source capacities for C (48.5 compared to 59.4 mg C) and N (11.7 compared to 13.8 mg N). This fact emphasizes the important role of the stalk as a transitory storage organ.

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