

Economy of Carbon and Nitrogen in a Nodulated and Nonnodulated (NO_3 -grown) Legume¹

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ABSTRACT

Partitioning and utilization of assimilated C and N were compared in nonnodulated, NO_3 -fed and nodulated, N_2 -fed plants of white lupin (*Lupinus albus* L.). The NO_3 regime used (5 millimolar NO_3) promoted closely similar rates of growth and N assimilation as in the symbiotic plants. Over 90% of the N absorbed by the NO_3 -fed plants was judged to be reduced in roots. Empirically based models of C and N flow demonstrated that patterns of incorporation of C and N into dry matter and exchange of C and N among plant parts were essentially similar in the two forms of nutrition. NO_3 -fed and N_2 -fed plants transported similar types and proportions of organic solutes in xylem and phloem. Withdrawal of NO_3 supply from NO_3 -fed plants led to substantial changes in assimilate partitioning, particularly in increased translocation of N from shoot to root. Nodulated plants showed a lower (57%) conversion of C or net photosynthate to dry matter than did NO_3 -fed plants (69%), and their stems were only half as effective as those of NO_3 -fed plants in xylem to phloem transfer of N supplied from the root. Below-ground parts of symbiotic plants consumed a larger share (58%) of the plants' net photosynthate than did NO_3 -fed roots (50%), thus reflecting a higher CO_2 loss per unit of N assimilated (10.2 milligrams C/milligram N) by the nodulated root than by the root of the NO_3 -fed plant (8.1 milligrams C/milligram N). Theoretical considerations indicated that the greater CO_2 output of the nodulated root involved a slightly greater expenditure for N_2 than for NO_3 assimilation, a small extra cost due to growth and maintenance of nodule tissue, and a considerably greater nonassimilatory component of respiration in root tissue of the symbiotic plant than in the root of the NO_3 -fed plant.

Several investigations of legumes have attempted to measure the cost of symbiotic N_2 fixation in terms of respired carbohydrate and to compare such costs with those of nonnodulated plants utilizing NO_3 . Some studies (6, 12) have reported little difference in C economy with the two forms of N assimilation; in others (11, 20, 21) a considerably lower output of respired CO_2 has been observed for NO_3 -reducing plants than for those fixing N_2 . Variation in results might be due to differences in pattern of NO_3 assimilation by the various species used, since if NO_3 reduction were accomplished in leaves from photosynthetically generated reductant (4) a lower respiratory requirement for NO_3 assimilation would be expected than if NO_3 were reduced heterotrophically in roots. Interpretation has also proved difficult due to lack of strict physiological comparability between nodulated and NO_3 -reducing plants (3, 6, 12), and to technical difficulties in measuring the separate respiratory outputs of shoots, root, and nodules of intact living plants (1, 10, 20). The present paper compares the functional

economy of nodulated and nonnodulated (NO_3 -fed) plants of white lupin (*Lupinus albus* L.) under conditions of closely matching rates of growth and N assimilation, using a nitrate level at which virtually all of the absorbed NO_3 is reduced by the root system (2). Advantage is taken of the capacity of white lupin to bleed from phloem and xylem to enable flow patterns of C and N to be modeled (16) for the two forms of nutrition.

MATERIALS AND METHODS

L. albus L. (cv. Ultra) plants were grown in lidded 11-liter containers (1) filled with heat-sterilized, organic matter-free quartz sand in a naturally lit glasshouse during July to November in Perth, Western Australia. One set of plants (nodulated, minus nitrate) was inoculated with sowing with *Rhizobium* WU 425, the effective bacterial strain previously used in a study of the carbon economy of nodules of *L. albus* (10). These plants received minus N culture solution (8) during growth. The other set of plants (nonnodulated, 5 mM NO_3) was not inoculated, but was fed from the 2nd week after sowing onward with culture solution containing 5 mM KNO_3 . This level of combined N had previously been shown to produce plants whose growth rate matched closely that of symbiotically effective plants. The population of NO_3 -fed, uninoculated plants remained virtually free of nodules and the occasional plant which had become nodulated was excluded from the harvested material. A third treatment (nonnodulated, 5 → 0 mM NO_3) consisted of plants fed with 5 mM NO_3 up to day 55 and then starved of NO_3 for the 10 days of the experiment. This treatment enabled a study of the effects of incipient N deficiency on plant C and N economy.

Plant Harvests. A sample of 20 plants was used for each harvest from a treatment. The 10-day intervals studied were 55 to 65 days after sowing from the nonnodulated, 5 mM NO_3 and nonnodulated 5 → 0 mM NO_3 treatments, and for the symbiotic treatments 52 to 62 days (nodulated A) and 55 to 65 days (nodulated B).

Dry Weight and C and N Analyses. Plants were separated into leaflets, stem + petioles, unexpanded apical regions of shoots (including inflorescence, if present), roots, and nodules (if present). Levels of C and N in dry matter were determined as described previously (7, 15). Salicylic acid was used in Kjeldahl digests of dry matter of NO_3 -grown plants so that NO_3 -N would be measured in determinations of total N(5).

Collection and Analysis of Xylem and Phloem Sap. Root bleeding exudate (xylem sap) was sampled over a 15-min period from root stumps of freshly decapitated plants, and phloem sap was collected from shallow incisions in petioles of mature leaves, or from base or top of shoots, at the locations described in an earlier study (16). Collections were made at least three times during the study interval of 10 days. Analyses of sap for sugars, organic acids, amides, and amino acids were as detailed elsewhere (14, 17–19). NO_3 levels were determined in xylem exudate of NO_3 -fed plants or in aqueous extracts of plant parts by reducing the NO_3 of a diluted sample of xylem sap or tissue extract on Cd:Cu columns

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and measuring the resulting NO_2 by an automated colorimetric technique (22). C:N weight ratios of solutes of xylem and phloem sap were determined from these analyses.

Respiration Measurements. The CO_2 efflux of the enclosed root systems of intact plants was monitored continuously during the 10-day study periods by attaching Pettenkoffer assemblies to the effluent gas stream from the plant containers. Details of technique were as described elsewhere (1), 10 plants (5 pots of 2 plants) being studied for each of the three treatments. The technique collected all CO_2 released to the rhizosphere so would have included respiratory products of rhizosphere organisms living on dead root tissue or on organic solutes excreted by the roots.

Modeling the Flow and Partitioning of C and N in Xylem and Phloem. Construction of models involved the primary data listed in Figure 1, and the modeling technique described recently (16). Data for C and N increments and CO_2 losses of plant parts were used to assess the plant's net uptake of C and N during the 10-day interval of a treatment. Then, using C:N weight ratios of appropriate transport channels, xylem and phloem fluxes of C and N within the system were determined, each flux matching precisely the consumption or production of C and N by a specific donor or receptor organ of the plant. The assumptions underlying this approach, the mathematical expressions used for calculating xylem and phloem fluxes, and the precision of the modeling techniques, were as detailed elsewhere (16).

RESULTS

Growth and N Accumulation in Plants. Initial and final dry weights and N contents of plant parts for the 10-day growth periods of the three treatments were as shown in Figure 2. The period 52 to 62 days for nodulated plants matched the NO_3 -fed more closely in initial dry matter content and dry weight incre-

ment. Withdrawal of nitrate supply from NO_3 -fed plants decreased N assimilation, but only slightly decreased dry matter accumulation in comparison with plants whose NO_3 supply was maintained.

C Economy of Plant Parts. The output of CO_2 from nodulated roots of the symbiotic plants was at comparable times of sampling greater (by 60% or more) than that of nonnodulated roots fed 5 mM NO_3 . Respiratory output of symbiotic and NO_3 -fed plants increased significantly with plant age, whereas that of NO_3 -starved plants remained fairly constant (Table I). Night respiration of shoots did not vary significantly between treatments in which N assimilation continued during the study period. The average rates were 20.8 mg C/plant·day for symbiotic plants 52 to 62 days old, 22.8 mg C/plant·day for nodulated plants 55 to 65 days old, and 21.7 for nonnodulated plants receiving 5 mM NO_3 . The treatment involving withdrawal of NO_3 showed a much lower average shoot night respiration (7.8 mg C/plant·day).

The net gain of C by plants of each treatment was computed as the sum of: (a) respiratory losses of C as CO_2 from underground parts (day and night); (b) CO_2 loss from shoots at night; and (c) the net increments of C in plant parts during the 10-day interval (7). A comparison of the proportioning of this net photosynthate in the three treatments (Fig. 3) showed respiration of below-ground parts to consume from 33 to 36% in nodulated plants, versus 20 to 22% for NO_3 -fed or NO_3 -starved nonnodulated plants. A lower input of net photosynthate was thus required in NO_3 -fed plants per unit weight dry matter synthesized than in the symbiotic plants. Withdrawal of NO_3 supply resulted in proportionately less C being incorporated into dry matter of apical regions than in other treatments, as might have been expected from the reduced growth rate of the starved plants.

Composition of Xylem and Phloem Sap. The principal constituents of xylem were Asn, Gln, and Asp. The preponderance of amides gave C:N weight ratios for xylem of from 1.9 to 2.5, the

MODELING THE C AND N ECONOMY OF LUPINUS ALBUS

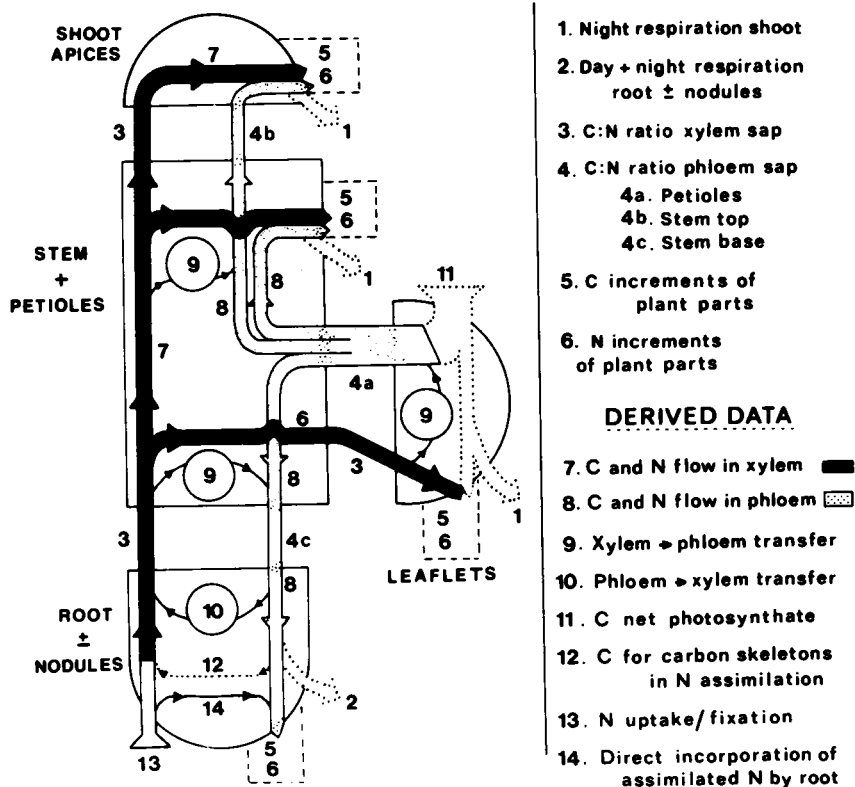


FIG. 1. Primary and derived data used in modeling the partitioning and utilization of assimilated C and N in white lupin.

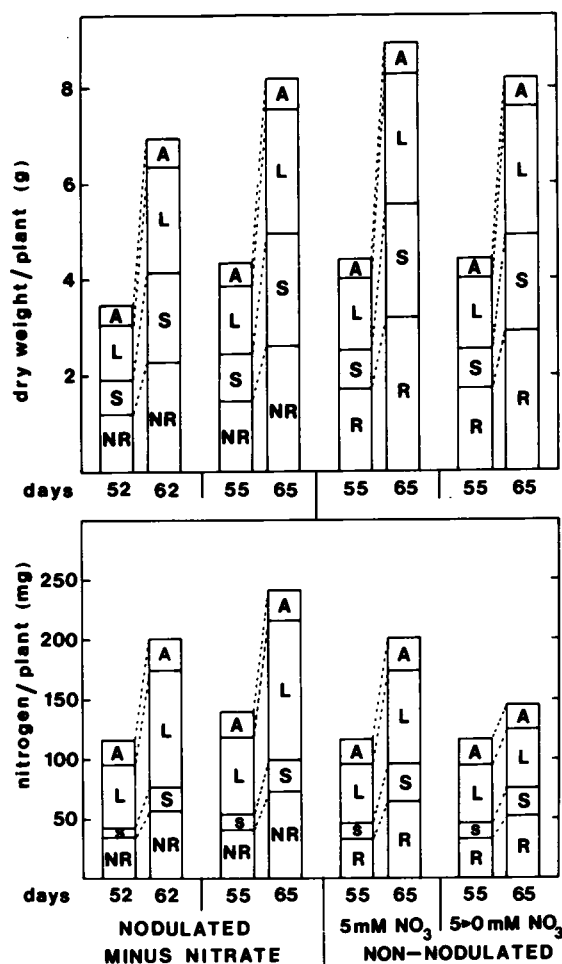


FIG. 2. Dry weight and N contents of plants at commencement and end of the 10-day study intervals used for comparing C and N economy of symbiotic, NO₃-fed and NO₃-starved white lupin. NR: nodulated root; R: root (not bearing nodules); S: stem + petioles; L: leaflets; A: unexpanded apical regions, including inflorescences, if present.

Table 1. Respiration Losses of Below-ground Parts of Intact Nodulated and Nonnodulated Plants of *L. albus*

Treatment	Plant Age		Respiration Loss as CO ₂
	days after anthesis	mg C/plant · day	
Nodulated (A.), minus NO ₃	52 to 55	66.8 ± 6.9 ^a	
	55 to 59	77.5 ± 11.8	
	59 to 62	106.4 ± 10.3	
Nodulated (B.), minus NO ₃	55 to 58	78.2 ± 10.1	
	58 to 62	99.7 ± 12.3	
	62 to 65	141.1 ± 12.6	
Nonnodulated, 5 mM NO ₃	55 to 58	48.4 ± 4.5	
	58 to 62	55.8 ± 9.0	
	62 to 65	85.4 ± 10.4	
Nonnodulated, 5 → 0 mM NO ₃	55 to 58	45.2 ± 5.2	
	58 to 62	39.8 ± 2.1	
	62 to 65	48.8 ± 2.6	

^a Mean ± SD (N = 5).

highest C:N value being for nonnodulated plants whose NO₃ supply had been withdrawn. Nitrate comprised from 6 to 10% of the xylem N of plants grown on 5 mM NO₃, so it was assumed that roots were responsible for reducing 90% or more of the

absorbed NO₃. Since no part of the NO₃-fed plants showed more than 0.5% of its total N as NO₃ (2) it was assumed that the total amount of NO₃ reduced during the 10-day interval was equal to the N increment of the plants during that period. Nitrate was not detected in xylem sap of the symbiotic plants or in the NO₃-starved plants.

Phloem sap composition varied markedly with site of collection on the plant, C:N ratios being highest (70–80) for petioles (see Table II), lowest (33–40) for stem tops, and of intermediate value (47–56) for stem base (16). There was little variation between treatments at the sampling sites (Table II). As reported earlier (15) sucrose was the only sugar present in quantity in phloem and the major nitrogenous compounds in phloem sap for all sites and treatments were Asn, Gln, Ser, Val, Asp, Glu, Thr, Ala, Gly, Leu, and Ile. Nitrate was not detected in phloem sap of symbiotic or NO₃-starved plants, and comprised less than 0.1% of the sap N in plants fed 5 mM NO₃ (2).

Flow Profiles for C and N. Models were drawn up for the three treatments depicting the partitioning of C and N in xylem and phloem, respiratory losses of C as CO₂, and the incorporation of assimilated C and N into dry matter during the 10-day intervals.

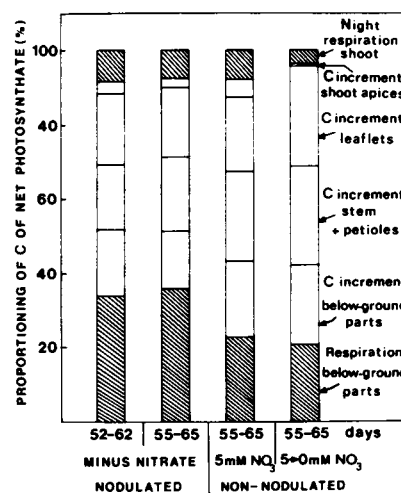


FIG. 3. Partitioning of C of net photosynthate for respiration and dry matter increments of plant parts of nodulated, NO₃-fed and NO₃-starved plants of white lupin.

Table II. C:N Weight Ratios of Solutes of Transport Fluids of Nodulated and Nonnodulated Plants of *L. albus*

The data are the means of series of samples collected during the 10-day study periods and include NO₃-N if present. Precision of measurement (SD) at any site was ±16% for phloem sap, ±10% for xylem sap (see ref. 16).

Treatment	Plant Age	Xylem Sap	Phloem Sap		
		Root	Stem base	Petioles*	Stem top
	<i>days after anthesis</i>		<i>C:N weight ratios</i>		
Nodulated (A) minus NO ₃	52 to 62	1.93	55.2	70.7	33.5
Nodulated (B), minus NO ₃	55 to 65	1.93	54.8	70.3	33.0
Nonnodulated, 5 mm NO ₃	55 to 65	2.13	47.2	81.8	34.2
Nonnodulated, 5 → 0 mm NO ₃	55 to 65	2.48	56.0	73.7	40.4

* Bulk sample from distal end of petioles of all fully expanded leaves.

To facilitate comparisons between treatments weights of C and N dispensed or consumed were expressed relative to an uptake by the plant of 100 units by weight of N as NO_3 or N_2 . Total amounts of C produced as net photosynthate and N absorbed as NO_3 or N_2 were also indicated in the legends to the models to provide comparative information on the assimilatory performances during the 10-day intervals (Fig. 4). A series of items of information (Table III) were derived from the modeling exercise to allow further comparisons between the treatments.

The major differences in partitioning of C and N between the nodulated and NO_3 -fed plants were the higher proportional loss of CO_2 from below-ground parts of symbiotic plants, the lower proportion of assimilated N abstracted from xylem by stems of symbiotic plants, the higher retention of N by leaves of symbiotic

plants, and the greater significance of xylem to phloem transfer in supplying phloem-borne N to roots of NO_3 -fed plants than in those fixing N (see Fig. 4 and items 1 to 4 of Table III). Points of close similarity between nodulated and NO_3 -fed plants included the extent of cycling of C through roots, the dependence of apical regions of the shoot on xylem for N supply, the degree of cycling of N through shoots back to roots, the C:N weight ratios of dry matter increments in plant shoots and roots, and consumption of net photosynthate for N assimilation (see items 5 to 9, Table III).

Plants whose NO_3 supply had been withdrawn became noticeably N-deficient, showing yellowing of lower leaves and curtailment of apical growth of shoots. Net gain of C by the NO_3 -starved plants was 78% of that achieved by plants still on full nitrate supply, but their N increment was only 35% of that of plants fed

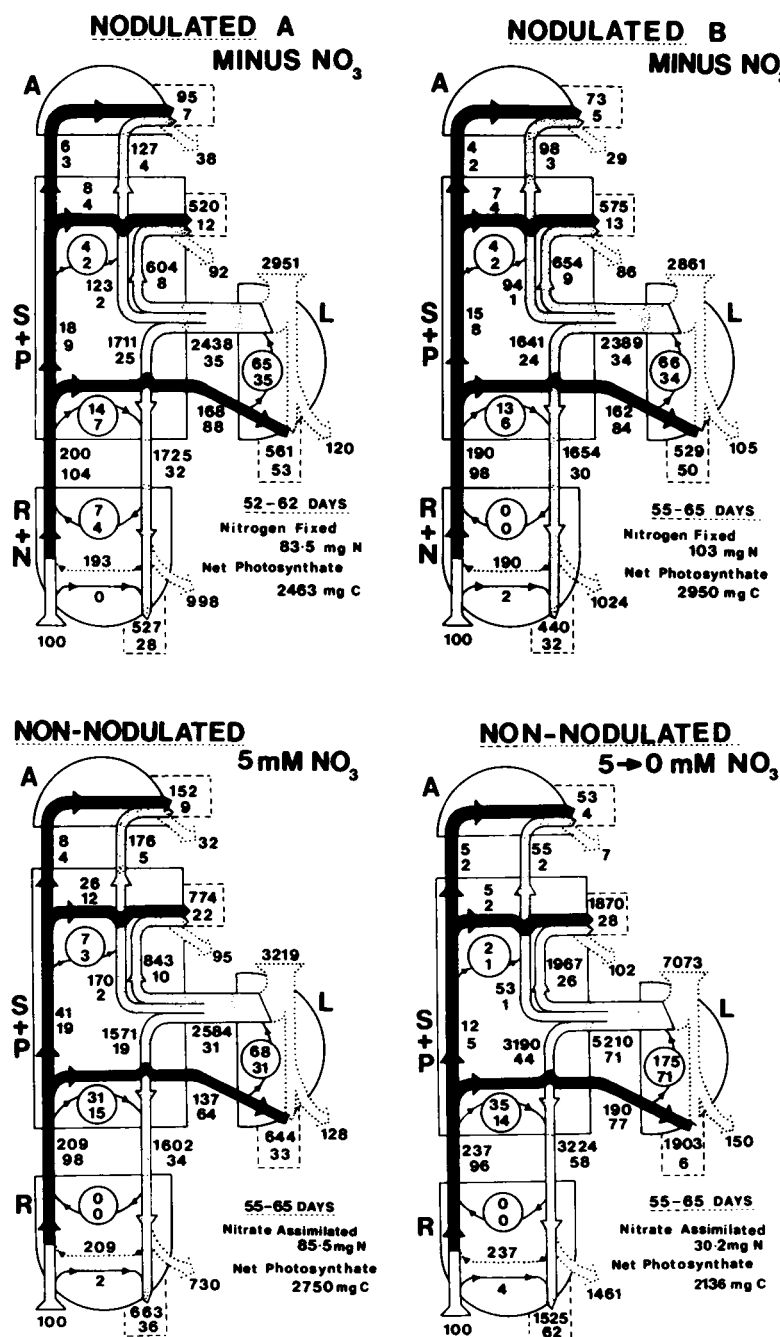


FIG. 4. Empirical models of partitioning and utilization of assimilated C and N in nodulated, NO_3 -fed and NO_3 -starved white lupin. Values for weights of C and N transported or consumed are expressed relative to a net intake by the plant of 100 units by weight of N. Actual amounts of C and N assimilated are given in the legends to the models. (See Figure 1, text, and ref. 16 for further details.)

nitrate. The N gain of the NO₃-starved plants was accounted for as absorption of residual NO₃ from the sand culture. There was, therefore, a large (2- to 3-fold) increase in the C:N ratio of the plants' dry matter increment in comparison with plants still receiving N (item 8, Table III). Other effects of the onset of N starvation were a high rate of mobilization and cycling of N through leaves (item 3, Table III), and a change in the partitioning of xylem derived N from one initially favoring the shoot to one in which roots received 62% of the N translocated in phloem (item 10, Table III). This latter would, in long term, lower the plant's shoot to root ratio, a feature typical of N-starved plants of many species. Nitrogen deficiency also resulted in increased photosynthate consumption per unit of N absorbed from the rooting medium (item 9, Table III).

DISCUSSION

White lupin, a legume reducing NO₃ actively in its roots (2, 13, 19), proved to be a useful species for studying the economy of below-ground organs in assimilating NO₃ and N₂ and for examining relevant costings in the context of empirically based models for C and N partitioning within the plants. Symbiotically nourished plants resembled comparable NO₃-fed plants in over-all patterns of exchange and utilization of C and N, and in the types and proportions of major organic solutes transported in their xylem and phloem, but differed from NO₃ plants in efficiency of conversion of photosynthate to dry matter, in respiratory output of below-ground parts, and in the relative extents to which their stem and leaves participated in abstraction, cycling and retention of N supplied from roots in the xylem. Differences of this nature, though not all of great magnitude, might have been responsible for generating the inequalities in growth and morphology observed

Table III. Comparative Features of C and N Partitioning and Utilization in Minus-N Nodulated, and Nonnodulated, NO₃-fed and NO₃-starved Plants of *L. albus*

Item	Nodulated		Nonnodulated	
	A (52 to 62 days)	B (55 to 65 days)	5 mM NO ₃ (55 to 65 days)	5 → 0 mM NO ₃ (55 to 65 days)
1. Proportion of C supplied to root lost as CO ₂ (%)	58	62	46	45
2. Proportion of plant's N increment abstracted from xylem by stem (%)	9	8	18	15
3. Proportion of plant's N increment retained by leaves (%)	53	50	33	6
4. Proportion of N translocated to root supplied by xylem to phloem transfer in stem (%)	22	20	44	24
5. Proportion of C supplied to below-ground parts returning to shoot with N assimilation products (%)	12	12	13	7
6. Proportion of N received by shoot apices supplied by xylem (%)	43	40	44	50
7. Proportion of N exported to shoot cycling back to below-ground parts (%)	32	30	34	58
8. C:N weight ratio of plant's dry matter increment	17.0	16.2	22.3	53.5
9. Ratio net photosynthate consumed to N uptake from rooting medium (mg C:mg N)	29.5	28.6	32.2	70.7
10. Proportion of plant's N increment retained by below-ground parts (%)	28	32	36	62

Table IV. Comparative Estimates of the Assimilatory and Nonassimilatory Components of Respiration of Below-ground Parts of Symbiotic and Nitrate-fed Plants of *L. albus* L.

Item	Nodulated		Nonnodulated
	A (52 to 62 days)	B (55 to 65 days)	5 mM NO ₃ (55 to 65 days)
1. Measured CO ₂ output of below-ground parts (mg C/plant)	833	1056	624
2. N assimilated by below-ground parts (mg N/plant) ^a	83.5	103.0	76.9
3. Estimated CO ₂ output due to nodules (mg C/plant) ^b	287	354	
4. Estimated CO ₂ output due to assimilation of N, to amino compounds in nodules (mg C/plant) ^c	242	299	
5. Estimated CO ₂ output due to NO ₃ reduction to amino compounds (mg C/plant) ^d			170
6. CO ₂ loss of supporting root not concerned with N assimilation (1-3 or 1-5) (mg C/plant)	546	702	454
7. CO ₂ loss of nodules not concerned with N assimilation (3-4) (mg C/plant)	45	55	
8. Proportion of CO ₂ loss of above-ground parts associated with N assimilation, 4 + 1 or 5 + 1 (%)	29.1	28.3	27.2
9. CO ₂ output of below-ground parts per unit N assimilated by these parts, 1 + 2 (mg C/mg N)	10.0	10.3	8.1
10. Proportion of plant's net photosynthate translocated to below-ground parts (%) ^e	58.5	57.8	49.8

^a Assumes that 90% of plant N increment is derived from NO₃ reduction in root (see text).

^b Uses data of Layzell *et al.* (10) of 3.44 mg C as CO₂ respired by nodules/mg N fixed.

^c Uses data of Layzell *et al.* (10) for costs of nitrogenase in H₂ evolution and N₂ fixation, and costs for NH₃ assimilation to amino compounds as derived by Atkins *et al.* (1) (total costs 2.90 mg C/mg N assimilated).

^d Assumes NO₃ → NH₃ requires 4 × 2e⁻, with associated CO₂ evolution calculated on basis of 3 ATP/2e⁻ and 6 ATP/CO₂. Cost of NH₃ assimilation to amino compounds as in nodulated plant (total cost 2.21 mg C/mg N assimilated).

^e See data of Figure 3 and models for C and N flow (Fig. 4).

between NO₃- and N₂-fed plants in other investigations (3, 20). There was no apparent reason for the differences between the symbiotic and NO₃-fed plants in degree of retention of N by stems, although differences in activity of tissues in absorbing N from xylem would be suspected. The dramatic changes in exchange of C and N in NO₃-fed plants after removal of their NO₃ supply demonstrated how the plants responded to stress, and how alterations in assimilate supply and demand fostered new patterns of growth within root and shoot system. The diversion of N from shoots to roots in incipient N deficiency suggested a strategy for mobilizing the limiting resource (N) to the organ (root) whose subsequent growth would be likely to lead to further acquisition of N from the rooting medium.

The substantial difference in CO₂ output from below-ground parts of NO₃- and N₂-fed plants (Table I) was analyzed further using data on photosynthate usage in nodules of white lupin (10), and theoretical estimates of the respiratory costs of nitrogenase functioning and NH₃ assimilation in the species (1). The comparison (Table IV) included estimates of the CO₂ output due to nodules (item 3, Table IV), to N assimilation of nodules (item 4),

and to the assimilation of NO_3 to amino compounds in the nonnodulated plants (item 5). It was then possible to estimate the components of CO_2 output of root (item 6) and nodules (item 7) not devoted to N assimilation.

The data obtained indicated that although similar proportions (27–29%) of the CO_2 output of NO_3 - and N_2 -fed roots were associated with N assimilation (see item 8, Table IV), the total CO_2 loss per unit of N assimilated (item 9) was higher in the nodulated root (10.0–10.3 mg C/mg N) than in the NO_3 -fed root (8.1 mg C/mg N), and, largely as a result of this, the nodulated root consumed a greater share of the plant's net photosynthate (item 10, Table IV) than in its counterpart fed NO_3 .

The greater respiratory output of the nodulated root reflected a greater respiratory cost in support of N assimilation (see items 4 and 5), an additional cost in growth and maintenance of nodule tissue (item 7), and, somewhat surprisingly, a significantly greater nonassimilatory component of respiration in the supporting roots of nodulated plants than in roots of NO_3 -fed plants (item 6). Because of the smaller average size of the root of nodulated plants (Fig. 2), this last difference involved a more than 2-fold greater output of CO_2 per unit dry matter of root of symbiotic plants (383–420 mg C/g dry weight root) than in roots of NO_3 -fed plants (185 mg C/g). This suggested the possibility of different patterns of carbon metabolism and electron flow in the two classes of root (e.g. see ref. 9), although it was equally possible that roots of nodulated plants excreted more carbohydrate and were therefore supporting a higher population of rhizosphere organisms.

It would be interesting to know whether the differences observed here between NO_3 -fed and N_2 -fed lupins apply generally to legumes, particularly to species which reduce NO_3 largely in their shoots. Nitrate-fed lupins, reducing NO_3 almost entirely in their roots, effected a conversion of 69% of the C of their net photosynthate into dry matter compared with only 56 to 58% in nodulated plants. In a species reducing NO_3 in leaves and directly utilizing photosynthetically generated reductant this discrepancy would be likely to be larger, leading possibly to even greater disparity in C economy of symbiotic and NO_3 -fed plants.

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