

Economy of Carbon and Nitrogen in Nodulated and Non-nodulated (NO₃-grown) Cowpea [*Vigna unguiculata* (L.) Walp.]

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ABSTRACT

The response of non-nodulated cowpea (*Vigna unguiculata* (L.) Walp. cv Caloona) to a wide range of NO₃ levels in the rooting medium was studied 40 days after sowing by *in vitro* assays of plant organs for NO₃ reductase (EC 1.6.6.1) and analyses of root bleeding (xylem) sap for nitrogenous solutes. Plants fed 1, 5, 10, 20, and 40 millimolar NO₃ showed, respectively, 64, 92, 94, and 91% of their total reductase activity in shoots and 34, 30, 66, 62, and 58% of the total N of their xylem sap as NO₃. These data, and the absence in the plants of significant pools of stored NO₃, indicated that shoots were major organs of NO₃ assimilation, especially at levels of NO₃ (10 to 40 millimolar) that maintained plant growth at near maximum rates. Partitioning and utilization of C and N were studied in nodulated, minus NO₃ plants and non-nodulated plants fed 10 or 20 millimolar NO₃, the levels of NO₃ which gave rates of growth and N assimilation closest to those of the symbiotic plants. The conversion of the C of net photosynthate to dry matter was similar in nodulated plants (67%) and NO₃-grown plants (64%), but greater proportions of photosynthate were translocated to below ground parts of nodulated plants (37%) than of NO₃-fed plants (23 to 26%). Greater photosynthate consumption by nodulated roots was associated with proportionately greater root growth and respiration and 2-fold greater export of C in xylem than in the NO₃-fed plants. Theoretical considerations suggest that the elevated CO₂ output of nodulated roots was due not only to CO₂ loss associated with nodule function, but also to a much greater nonassimilatory component of respiration in the supporting root of the nodulated plant compared to roots of the NO₃-fed plants. Data are compared with previously published information from other legumes.

A series of recent studies (9, 10, 15-19) on a range of legumes has indicated that nodulated plants deriving N solely from N₂ fixation lose significantly more C as respired CO₂ than do non-nodulated plants utilizing NO₃. In several instances, this difference has been shown to be due to a greater loss of CO₂ from below-ground parts of nodulated than of NO₃-fed plants, a result which has been variously explained in terms of extra costs in the functioning of nodules (9, 15, 18), higher nonassimilatory components of respiration of roots of N₂-fixing compared to NO₃-fed plants (14), and lower metabolic expenditure if a significant proportion of the NO₃ is being reduced photosynthetically in the shoot system.

The present study extends the comparison between nodulated and non-nodulated, NO₃-fed legumes to the tropical species, cowpea [*Vigna unguiculata* (L.) Walp.]. This species produces ureides rather than amides as major products of N₂ fixation (1, 6), exhibits an efficiency of carbon use by nodules significantly higher than that of other legumes, such as *Lupinus albus* and *Pisum*

sativum (1, 8, 11), and has a limited capacity for nitrate reduction in its root system.

MATERIALS AND METHODS

Plant Material. Plants of *V. unguiculata* (L.) Walp. cv Caloona were grown in lidded 11-liter containers (1) filled with heat-sterilized, organic matter-free quartz sand in a naturally lit glasshouse during January to April in Perth, Western Australia. A balanced culture solution (7) containing 0, 1, 5, 10, 20, or 40 mM NO₃ as sole nitrogen salt was used with frequent application to maintain fairly constant NO₃ levels around the roots. Plants receiving the N-free culture solution were inoculated with *Rhizobium* CB756 and were effectively nodulated. The root systems of most NO₃-grown plants showed negligible nodulation, and the occasional plant which developed nodules was excluded from the harvested material.

Plant Harvest and Analysis for C and N. Plant material was harvested 40, 46, or 56 days after sowing. Samples of 16 to 20 plants were collected from each treatment, the root systems washed with deionized H₂O and the plants were divided into roots, stem plus petioles, and leaflets. Nodules were separated from the supporting roots of nodulated plants. The material was dried to constant weight at 80 C and milled, and samples (75-100 mg) were taken for Kjeldahl analysis. The digestion technique used employed the modification by Eastin (5) to include NO₃. The C content of tissues was determined by dichromate digestion (21).

Collection and Analysis of Xylem Sap. Root bleeding exudate (xylem sap) was sampled over a 15-min period between 1000 and 1200 h from the stumps of 12 to 16 freshly decapitated plants in each treatment. Sap samples were taken from plants at 40, 46, and 56 days after sowing. For each treatment, equal volumes of sap from each sampling time were pooled for analysis. Measurement of amino acids and amides in sap was as detailed elsewhere (16). Allantoin and allantoic acid were assayed together as the phenylhydrazone of glyoxylate (22) and NO₃ as NO₂ following reduction with Cd/Cu (23). Estimates of the C/N weight ratios of xylem were derived from quantitative analyses of the above nitrogenous solutes. Total N in sap, including NO₃, was measured following a modified Kjeldahl digestion (5). Although there was a diurnal variation in the volume, total N, and solute composition of sap samples collected at 2-3-h intervals over the day and night during the photoperiod, solute composition did not vary greatly. As a consequence, the proportions of total N as NO₃ and the C/N ratios of sap during the period of transpiration were relatively constant.

Respiration Measurement. The CO₂ output of the enclosed root systems of intact plants was collected continuously from 46 to 56 days after sowing using attached Pettenkoffer assemblies (1). Measurements were made using 10 plants (five pots of two plants) in each treatment. The technique collected all CO₂ released to the rhizosphere. Shoot respiration of intact plants was measured for 6 nights during the 10-day study. Potted plants were enclosed in

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170-liter Plexiglas cuvettes with the lidded container closed to prevent gas exchange between the root system and the atmosphere of the cuvette. Release of CO₂ to the cuvettes was measured using an IR gas analyzer sampling technique (2).

NO₃ Reductase (EC 1.6.6.1) Assay. Groups of six plants were harvested between 1000 and 1200 h and 46 days after sowing from each treatment and separated into component organs. Duplicate 5-g samples from the bulked material were homogenized at 2 C with 15 to 20 ml of a breaking medium containing 100 mM (pH 8) Tris-HCl, 1 mM disodium-EDTA, 5 mM DTT, and 0.5% (w/v) BSA (Cohn Fraction V). Following centrifugation (20 min, 20,000g, 2 C), the clear supernatant fraction was assayed for NO₃ reductase activity at 30 C using the method of Neyra and Hageman (12). All assays were performed within 40 min of homogenizing the tissue and activities were calculated as the mean of four assays for each organ sample extracted.

RESULTS AND DISCUSSION

Xylem Sap Analyses, NO₃ Reductase, NO₃ Storage, and Sites of NO₃ Assimilation. The principal nitrogenous solutes in xylem sap of nodulated plants were the ureides, allantoin, and allantoic acid. Xylem exudates of non-nodulated plants contained primarily the amides (Asn, Gln) and only minor amounts (1-4% of the total N) of ureides (Fig. 1). Nitrate accounted for 34, 30, 66, 62, and

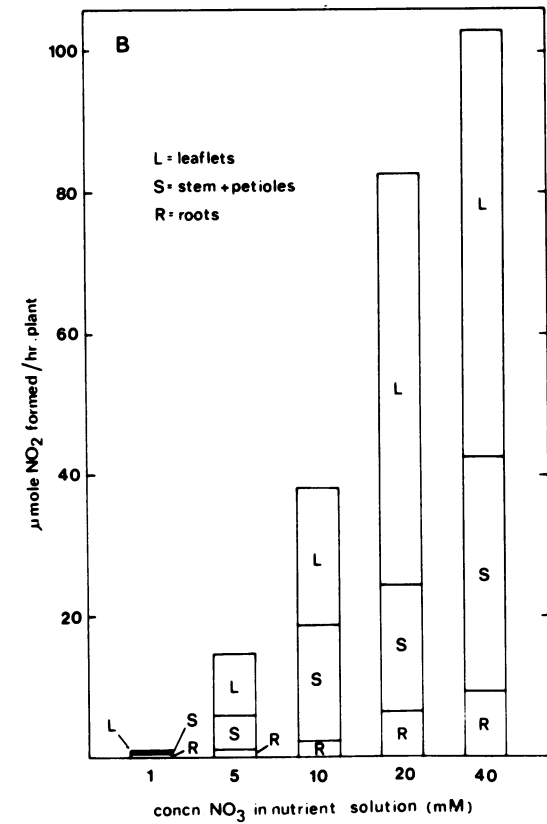
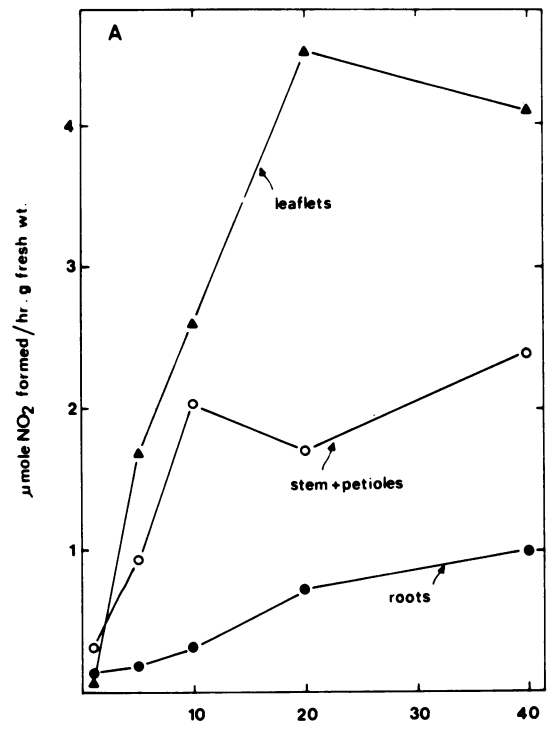


FIG. 2. Distribution of NO₃-reductase activity between different organs of 40-day old non-nodulated *V. unguiculata* plants grown with a number of NO₃ levels. A: enzyme activity on a unit tissue mass basis; B: total enzyme activity on a plant basis.

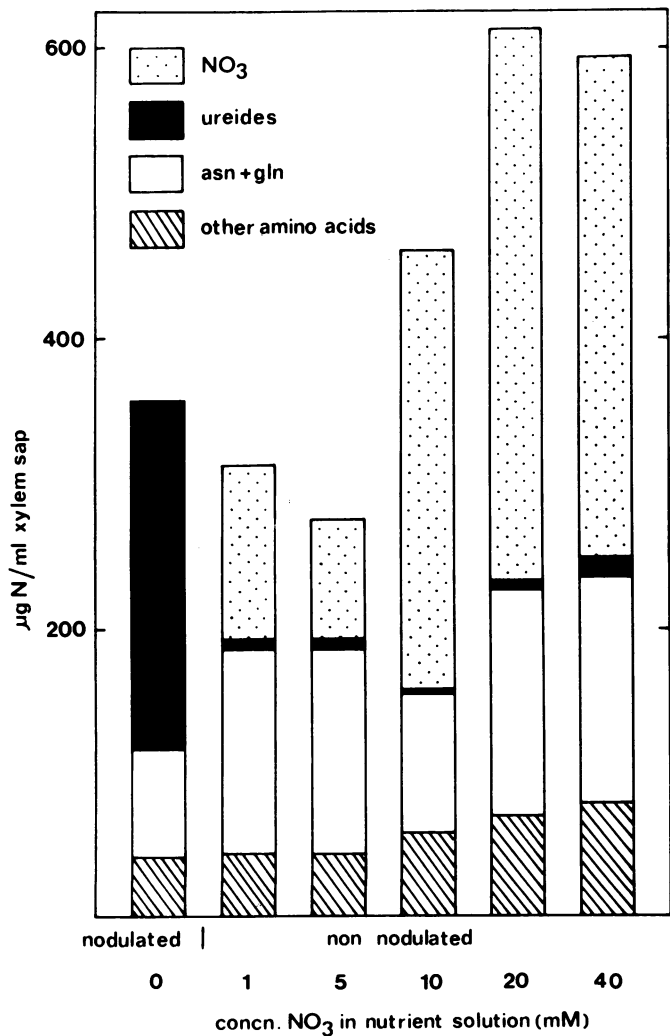


FIG. 1. Distribution of N between solutes in root bleeding (xylem) sap of nodulated and non-nodulated, NO₃-grown plants of *V. unguiculata* 40 days after sowing.

58% of the N in measured solutes of xylem sap in plants fed 1, 5, 10, 20, and 40 mM NO₃, respectively (Fig. 1). The compounds shown in Fig. 1 accounted for 89.5 ± 9.5% (SE \bar{x} , n = 8) of the sap total N, as measured by Kjeldahl analysis, so that forms of N

other than those assayed were not present at high levels in the exudates. The data corroborate earlier studies on xylem sap composition of the species (6, 14).

Specific activities (Fig. 2A) and total activity (Fig. 2B) of NO_3^- reductase in *in vitro* assay of plant organs demonstrated a general trend of increasing enzymic activity in organs with increasing levels of NO_3^- in the rooting medium. Roots contained 36% of the total reductase activity in plants fed 1 mM NO_3^- but only 8, 6, 8, and 9% of total plant activity when fed 5, 10, 20, and 40 mM NO_3^- , respectively. The larger proportion of the NO_3^- reductase activity of shoots was recovered from leaflets as opposed to stem plus petioles. Although the level of NO_3^- reductase does not firmly establish the flow of NO_3^- to the shoot, it indicates that NO_3^- was distributed principally to photosynthesizing tissues where it was likely to have been assimilated using photosynthetically generated reductant (4).

The above data for cowpea differ considerably from those described recently for *L. albus* (15), in which, over a comparable range of NO_3^- treatments, only 4 to 17% of xylem N consisted of NO_3^- and 31 to 78% of the *in vitro* NO_3^- reductase activity was recovered from roots.

Recently published data on cowpea (14), using comparable plants, showed that NO_3^- represented less than 4% of the total N of plant organs, suggesting that, as with *L. albus* (3), the species was most effective in reducing NO_3^- immediately on uptake by roots or following transfer to the shoot system. A recent study (20) has demonstrated rates of growth and N accumulation in cowpea cultured with NO_3^- equal to or better than those of nodulated, N_2 -fixing plants. The proportion of free NO_3^- in the xylem sap N leaving the root was taken as a measure of the extent of the shoot's involvement in NO_3^- reduction. Conversely, the proportion of the xylem sap N in organic form was taken to be indicative of the root's activity in reducing NO_3^- . It was assumed that cowpea derived only small amounts of the organic nitrogenous solutes of xylem from sources not associated with current assimilation in the root or from N returned to the xylem after translocation from the shoot in phloem. This latter assumption is supported by a ^{15}N study in nodulated peas (13) where negligible N cycled through roots and by the results of an empirical modeling study of N_2 - and NO_3^- -grown lupins (15). On this basis, about 60% of NO_3^- reduction was indicated in the shoots at the higher NO_3^- regimes (10–40 mM).

Growth, N Assimilation, and Partitioning of the C of Net Photosynthate in N_2 - and NO_3^- -fed Plants. These comparisons involved nodulated, minus NO_3^- plants, and non-nodulated plants fed 10 or 20 mM NO_3^- , the two levels of NO_3^- giving rates of growth and N accumulation most similar to those of the symbiotic plants. The study interval was 46 to 56 days after sowing, the period just before anthesis when plants showed maximum rates of dry matter gain and accumulation of N. Initial and final contents of dry matter and total N were as in Figure 3. Neither NO_3^- treatment gave plants that exactly matched the growth and accumulation of

N by the N_2 -fixing treatment. The match with symbiotic plants was closest to the 10 mM NO_3^- plants in terms of initial N content and final dry matter content, and closest to the 20 mM NO_3^- treatment in final N content and increment of dry matter. Despite lower initial N and dry matter, the symbiotic plants assimilated 50% more N during the 10-day period than did the NO_3^- -fed plants.

There were relatively minor differences between treatments in distribution of N and dry matter among plant parts. Symbiotic plants had a significantly higher percentage N in their final dry matter than in either of the NO_3^- treatment, and it was subsequently found that NO_3^- -fed plants tended to flower later and fruit less prolifically than the nodulated plants.

Total net photosynthate produced by the treatments over the 10-day period was computed, as defined earlier (1, 15), as the sum of the C gained in dry matter and the losses of C as CO_2 from the shoots at night and from below-ground organs day and night (Table I). Nodulated plants generated 5.0 g C as net photosynthate, plants of the 20 mM NO_3^- treatment 4.5 g C and of the 10 mM NO_3^- treatment 3.6 g C. Proportioning of this photosynthate was as shown in Figure 4. Nodulated plants converted 67% of the C of their net photosynthate to dry matter, NO_3^- -fed plants a closely similar 64%. However, in nodulated plants a greater proportion of net photosynthate was utilized for growth and respiration by below-ground organs (29%) than in either the 20 mM (18%) or the 10 mM NO_3^- (21%) plants. This difference was due principally, but not totally, to the significantly higher loss of CO_2 from root and nodules of the symbiotic plants (Table I).

A feature of the plant's C economy not shown in Figure 4 was the amount of C delivered to the root as phloem translocate and returned to the shoot as organic N compounds in the xylem. This was estimated (14) by multiplying the N increment of the shoot, over the study period, by the C/N weight ratio of xylem sap, the average value for this ratio being higher for symbiotic plants (1.2) than for 10 mM (0.8) and 20 mM (0.9) NO_3^- -fed plants because of the substantial amounts of NO_3^- -N in the xylem of the latter treatments.

The data of Table II compare the C economy of below-ground parts of plants of the three treatments, giving total amounts of C and proportions of total net photosynthate utilized as C exported from roots in xylem (item 1, Table II, calculated as above), C increments in root (plus nodule) dry matter (item 2, Table II), and C loss as CO_2 in respiration (item 3, Table II; see also Table I). The sum of these three items estimated the total C translocated to the roots (item 4) during the study period. The data show that nodulated plants diverted a substantially higher proportion (36.9%) of their net photosynthate as phloem translocate to roots than did either NO_3^- treatment (22.6 and 25.7%) and that the proportionately larger expenditure of the nodulated plants was due to greater photosynthate utilization on an absolute and percentage basis than in NO_3^- -fed plants for all three items of the C

Table I. Carbon Economy of Non-nodulated, NO_3^- -fed and Nodulated, N_2 -fed Plants of Cowpea (*V. unguiculata*) for the Period 46 to 56 Days after Sowing

Item	Non-nodulated ^a		Nodulated minus NO_3^- ^a
	10 mM NO_3^-	20 mM NO_3^-	
1. C increment in plant dry matter (mg C/plant)	2135 ± 399	3034 ± 450	3184 ± 529
2. CO_2 loss in shoot night respiration (mg C/plant)	930 ± 91	983 ± 95	952 ± 119
3. CO_2 loss of below-ground parts, day + night (mg C/plant)	515 ± 61	496 ± 113	882 ± 69
4. Total net photosynthate produced (1+2+3) (mg C/plant)	3580 ± 551	4513 ± 658	5018 ± 717

^a Values given are means ± SE.

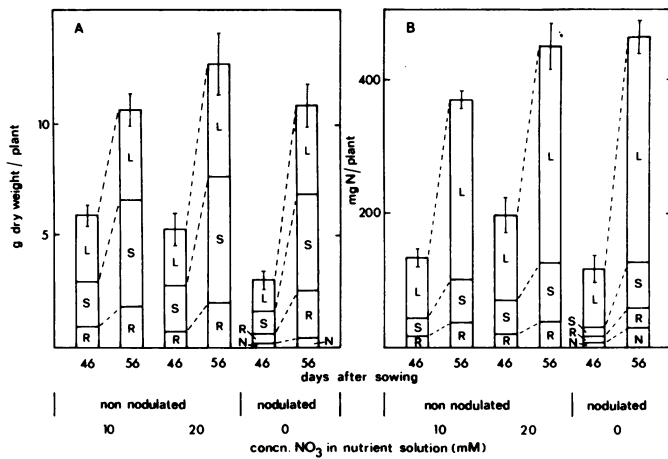


FIG. 3. Dry weight and N contents of nodulated and non-nodulated *V. unguiculata* plants at commencement and end of the 10-day period from 46 to 56 days after sowing. N: nodules; R: root; S: stem + petioles; L: leaflets. Error bars are the SE of the mean of the total dry weight or total N from 16 to 20 plants.

budget of below-ground parts.

The above data suggest substantial differences in allocation of photosynthate to roots of cowpea and *L. albus*, a species recently studied in relation to C and N economy of NO₃-fed and symbiotically dependent plants (3, 15). In the lupin, nodulated plants translocated 58% of their net photosynthate to the nodulated root versus 50% to roots of NO₃-fed plants, the relatively greater diversion to roots of lupin than of cowpea reflecting the tap-rooted habit of lupin, the use of amides (C/N ratio, 1.7–2.1) versus ureides (C/N ratio, 0.9) for translocation of fixed N, and the reduction in lupin roots of a much greater proportion of absorbed NO₃ than in roots of cowpea. With differences between species of this order of magnitude, it would obviously be unwise to generalize for legumes as a whole, without further information on other species.

Components of Respiratory Losses of CO₂ from Below-ground Parts of N₂- and NO₃-fed Plants. The substantial and significant differences in CO₂ output from below-ground organs of nodulated and non-nodulated plants were further resolved (Table III) using data from an earlier study (8) on CO₂ loss from intact, attached nodules of the cowpea/CB756 association, and theoretically based estimates of the CO₂ loss from NO₃-fed plants attributable to the generation of ATP and reductant for NO₃ assimilation (15). We assumed that the proportion of N present in organic form in xylem sap of the NO₃-fed plants indicated the extent to which their roots were involved in NO₃ reduction.

It then proved possible to estimate the amounts of CO₂ and the proportions of the total respiratory output of below-ground parts due to nodules, to NO₃ reduction, and, by difference, to the nonassimilatory activity of the NO₃-fed root and of the supporting roots of the nodulated plants (items 4 to 6, respectively, Table III). The comparison indicated that more than half (58.5%) of the CO₂ loss from N₂-fixing plants was due to nodules, whereas almost as great a proportion (42.5 and 51.4%) of the CO₂ efflux of NO₃-fed roots was due to NO₃ assimilation. The remaining CO₂ output, attributed to the nonassimilatory components of root tissue respiration, was equivalent to 57.5 and 48.6% of the root's output of CO₂ of NO₃-fed plants, 41.5% in the case of nodulated plants. When expressed as specific activity of respiration, these components of respiration not associated with N assimilation were higher in symbiotic plants (28.9 mg C/g dry weight of root·day) than in the non-nodulated plants fed 10 and 20 mM NO₃ (21.7 and 17.8 mg C/g·day, respectively). A similar result was obtained (15) in a comparison of N₂- and NO₃-fed *L. albus*, for which CO₂ release by supporting roots of nodulated plants (38.3 to 42.0 mg C/g dry

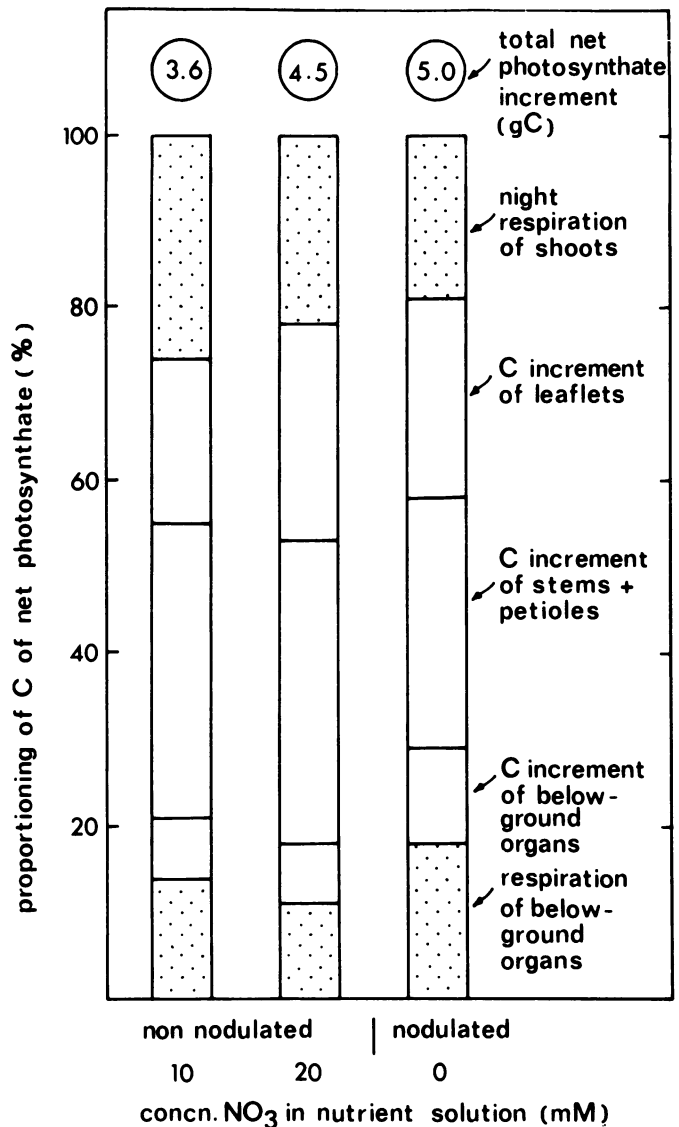


FIG. 4. Partitioning of the carbon of net photosynthate between respiration and dry matter increments of plant parts of nodulated and non-nodulated (NO₃-grown) plants of *V. unguiculata* for the 10-day period from 46 to 56 days after sowing.

weight root·day) was more than twice that of NO₃-fed roots (18.5 C/g·day). Possibly a more active rhizosphere flora was maintained on the root systems of the nodulated plants through greater exudation of oxidizable metabolites.

Finally, the symbiotic and NO₃-fed treatments were compared on the basis of respiratory output/unit N assimilated by nodules or root systems. The nodulated roots respired only 2.6 mg C (as CO₂)/mg N fixed (item 1 - item 3, Table III) versus values of 5.2 and 4.3 (same units) for plants fed 10 and 20 mM NO₃, respectively.

The lower return of assimilated N/unit C respired in NO₃-fed plants was due to the relatively low activity of the root in NO₃ assimilation. The high efficiency of N₂ fixation in terms of CO₂ output from root and nodules reflected the high economy of C in nodules of the species (8) and the relatively low respiratory output from its slender root system. The results contrast markedly with data for lupin (15) in which losses of CO₂/unit N assimilated were substantially higher, 10.2 mg C/mg N, for nodulated roots and 8.1 mg C/mg N for NO₃-fed roots. In lupin, the large tap root provided nonassimilatory components of respiration equivalent to 71% of the total CO₂ evolved from nodulated roots, 73% in the

Table II. Carbon Economy of Below-ground Parts of Non-nodulated, NO_3 -fed and Nodulated, N_2 -fed Cowpea (*V. unguiculata*) for the Period 46 to 56 Days after Sowing

Item	Non-nodulated ^a		Nodulated minus NO_3 ^a
	10 mM NO_3	20 mM NO_3	
1. N exported from roots to shoots in xylem (mg N/plant)	216 ± 12	234 ± 27	304 ± 19
2. C/N weight ratio of xylem sap	0.78	0.86	1.21
3. C transported to shoots with N solutes in xylem (1 × 2) (mg C/plant)	168 (4.7) ^b	201 (4.5)	368 (7.3)
4. C increment of root as dry matter (mg C/plant)	238 (6.7)	319 (7.1)	566 (11.3)
5. C lost as CO_2 from root system (mg C/plant)	515 (14.4)	496 (11.0)	882 (17.6)
6. Total C translocated to roots (3+4+5) (mg C/plant)	921 (25.8)	1016 (22.6)	1816 (36.2)

^a Values given are means ± SE.

^b Values in parentheses express items of C consumption as percentages of total net photosynthate produced (see Table I).

Table III. Assimilatory and Nonassimilatory Components of CO_2 Efflux from Below-ground Organs of Non-nodulated, NO_3 -fed and nodulated, N_2 -fed Cowpea (*V. unguiculata*) for the Period 46 to 56 Days after Sowing

Item	Non-nodulated		Nodulated, Minus NO_3
	10 mM NO_3	20 mM NO_3	
1. Total CO_2 output of below-ground organs (mg C/plant)	515	496	882
2. Total N assimilated by plant (mg N/plant)	235	253	344
3. Estimated N assimilated by below-ground parts (mg N/plant)	99 ^a	116 ^a	344
4. Estimated CO_2 output due to nodules (mg C/plant) ^c			516 (58.5)
5. Estimated CO_2 output due to NO_3 assimilation (mg C/plant) ^d	219 (42.5) ^b	255 (51.4)	
6. Estimated CO_2 output of root tissues not associated with N assimilation. (1-4 or 1-5) (mg C/plant)	296 (57.5)	241 (48.6)	366 (41.5)

^a Based on 42% of xylem sap being in organic form in 10 mM NO_3 treatment and 46% in 20 mM NO_3 treatment and assuming that these percentages reflect the proportions of absorbed NO_3 being reduced by roots.

^b Values in parentheses express items of CO_2 output as percentages of total CO_2 loss from below-ground parts.

^c Uses value obtained earlier (8) of 1.5 mg C as CO_2 /mg N_2 fixed by cowpea/CB756 nodules.

^d Uses value of 2.2 mg C as CO_2 /mg NO_3 -N assimilated (see ref. 15).

case of NO_3 -fed roots, showing how important the extent and pattern of root development of a legume may be in determining the efficiency with which its below-ground parts convert translocate to organic solutes of N.

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