A QUANTITATIVE TECHNIQUE FOR THE MEASUREMENT OF THE NITROGEN LOSS FROM THE ROOT SYSTEM OF FIELD PEAS (*PISUM AVENSE* L.) DURING THE GROWTH CYCLE

by

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Abstract

A modified split root technique was developed in order to investigate the nitrogen loss from the root system of field peas (Pisum avense L.) during active plant growth. The root systems of 4 day old pea seedlings were split between two different soil chambers. By labelling one soil chamber with a ^{15}N enriched nitrogen source, the nitrogen loss from the portion of the root system growing in the second soil chamber could be monitored. It was found using this technique that significant amounts of root derived nitrogen remained in the soil after the root material was removed from the soil chamber. The technique allowed for the determination of the amount of the nitrogen lost from the root system of field peas as a function of the concentration of nitrogen in the root system over the entire plant life cycle. The amount of nitrogenous material remaining in the soil was dependant on the time of harvest, and ranged from 20% to 85% of the nitrogen found in the root material from 16 to 83 days after planting.

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I. Introduction

In assessing the growth and nitrogen fixation potential of an annual legume crop, it is important to be able to accurately determine the total nitrogen budget of that crop during the growth cycle. The ^{15}N isotopic dilution analysis, commonly utilized to determine the total nitrogen fixation of an annual legume crop, requires an accurate determination of the total amount of nitrogen used by the crop from planting until harvest. Modelling of the nitrogen cycle within an annual legume crop requires a relatively accurate assessment of the strength of sources and sinks for the nitrogen pool within the crop at any time during the growth cycle.

It has generally been assumed that losses of nitrogen from an annual crop during active plant growth represent a relatively small portion of the total nitrogen budget of the plant. Thus, estimates of the total usage of nitrogen by an annual crop are based on the amount of nitrogen found in the plant material at the time of harvest. This assumption, however, has been brought into question by recent estimates of the amount of organic carbon lost from the root system during active growth. It has become evident that losses of root material during active plant growth due to lysis of root material and active exudation of carbonaceous material by plant roots represent a significant sink for photosynthetically fixed carbon in an annual crop. Since this loss of organic matter may represent a significant sink for plant nitrogen in an annual legume crop, it is important to be able to quantify the loss of organic nitrogen from the root system of an annual legume crop

during the growth cycle.

The loss of nitrogen from the plant roots is difficult to detect under natural soil conditions. The high ratio of total soil nitrogen to plant nitrogen under natural soil conditions makes it impossible to directly measure short term changes in the soil nitrogen content due to plant effects. The use of an ^{15}N tracer as a tag for a given nitrogen source in the soil can greatly improve measurements but the sensitivity still remains relatively low due to the natural abundance of ^{15}N in the soil.

A study was therefore conducted in order to develop a technique to measure the rate of nitrogen loss from the root system of field peas grown in the soil. The purpose of the study was to:

1. Develop a method sensitive enough to measure losses of nitrogen from the root system equivalent to 10% or less of the total root nitrogen content.
2. Develop a method which would uniformly measure the nitrogen loss during the entire plant root growth cycle
3. Measure the amount of nitrogen lost from the root system of field peas over the entire plant life cycle.
4. Compare the nitrogen lost from the root system of field peas with the nitrogen content of the root at any time, "t" during the growth cycle.

II. Literature Review

A. Carbon Loss From the Root System Under Natural Soil Conditions

Attempts to quantify the amount of organic carbon lost from the root system of naturally grown plants have become increasingly frequent in recent years. Based on data available on the volume of the root mucilage material, Sametsevitch (1965) estimated that the amount of material present in the root mucilage was as great as that found in the seed. Shamoot et al. (1968) labelled the photosynthetically fixed plant carbon by growing plants in a $^{14}CO_2$ atmosphere in order to study the rhizo-deposition of carbon into the soil from actively growing plant roots. The study was conducted over a 50 day period for several annual crops, and for 212 days for several perennial grasses and legumes. By assuming the carbon to organic matter ratios were similar for both the root material and the material deposited into the rhizosphere, it was calculated that the amount of organic debris remaining in the soil at the end of the study ranged from 20.2 to 49.4 g per 100 g of extracted root material. The differences in the amount of ^{14}C remaining in the soil at the end of the study were found to be primarily influenced by the amount of ^{14}C which was translocated to the roots rather than plant species or the length of the study. After adjusting for differences in root yields, it was found that the rhizodeposition among plant species was not statistically different.

The study conducted by Shamoot et al. failed to account for ^{14}C which was lost due to microbiological decomposition of plant material.

In an incubation study of the residual ^{14}C remaining in the soil after the roots were extracted, Shamoot et al. (1968) found that between 30% and 48% of the ^{14}C label was lost from the soil during a 14 week study. The majority of the loss of recently incorporated carbon occurred within the first 2 weeks of the study.

Similar results were found by Martin (1975). After 60 days in which ^{14}C was constantly supplied to plant shoots in the form of CO_2, Martin measured the amount of ^{14}C remaining in the soil and water leachates extracted from the soil at 7 day intervals. He found that the rhizodeposited ^{14}C collected was equivalent to 10.4%, 27.4%, and 38.4% of the total amount of ^{14}C found in the root material of ryegrass, wheat, and clover respectively.

Barber and Gunn (1974) measured the effect of mechanical impedance on the amount of carbon lost from the root system by comparing the total amount of water soluble and insoluble root exudate material lost by barley and maize plant roots grown for 3 weeks in sterile solution culture with roots grown in glass ballotini. The presence of 1 mm glass ballotini in the rooting zone was found to increase the measured amount of exuded material from 5% of the organic matter found in the root system to 9% of the root organic matter content. Since the pressure restriction of glass ballotini on the root system is small compared to that of a natural soil environment, Barber and Gunn suggested that the actual concentration of material lost due to mechanical impedance under natural conditions may be much greater.

The use of a constant $^{14}CO_2$ labelled atmosphere enabled investigators to study the rate of photosynthetically fixed carbon

transfer into the shoot, root, soil, and soil CO_2 from annual and perennial crops. In a short term study, in which plants were maintained in an environment enriched with $^{14}CO_2$ for 3.5 days, followed by 4 days growth under normal atmospheric conditions, Warembourg and Paul (1973) found that 23% and 17% of the ^{14}C labelled plant material was expired as CO_2 from the soil of wheat plants at heading and the soft dough stage respectively. Carbon 14 in the root material after 8 days accounted for only 10% of the total ^{14}C labelled material incorporated into the plant at heading and 8% of the total label at the soft dough stage. No measurable amounts of ^{14}C were found remaining in the soil after the 8 day study period. In a similar field study conducted on native grassland vegetation, it was found that the soil respired CO_2 accounted for between 9.8% and 14.8% of the incorporated plant label, depending on the time of harvest (Warembourg and Paul, 1973, Warembourg and Paul, 1977). The concentration of ^{14}C in the roots at the time of harvest ranged from 19.7% to 28.8% of the total ^{14}C activity measured. No attempts were made to separate $^{14}CO_2$ activity in the soil which was due to microbial decomposition of root material from that of root respiration. By determining the rate of change of the concentration of ^{14}C with time in the root system after the labelling period had terminated, it was found that the rate of turnover of old carbon from the root system followed an exponential decay pattern. By this method, it was estimated that the half life of the root material was approximately 109 days.

Barber and Martin (1976) found that the use of a non-sterile environment increased the amount of ^{14}C lost from the roots of 3 week

old wheat and barley plants from 5% to 10% of the total ^{14}C found in the roots, shoots and soil rhizosphere of the plants in the sterile environment to 12% to 18% in the non-sterile environment. The majority of the increase in the rhizodeposited concentration of ^{14}C measured in the non-sterile environment was found to be in the form of $^{14}CO_2$ released from the soil. Barber and Martin attributed this increase in the soil $^{14}CO_2$ soil respiration rate to the microbial decomposition of root material released into the soil. By assuming that the percent of the ^{14}C lost from the roots due to respiration remained relatively constant in the sterile and non-sterile environments, they estimated that the amount of organic material lost from the roots in the 3 week period was equivalent to between 18 and 25% of the observed dry matter increment of the plant.

Similar results were found by Martin and Kemp (1980) in a study to compare organic matter losses from the roots of different wheat cultivars. They found that in a 4 week study, the amount of organic matter lost from the 6 cultivars studied seemed to be independent of the cultivar grown. During the 4 weeks, between 17% and 21% of the total ^{14}C fixed by the plants grown at 15°c was lost from the root system. This was equivalent to between 35% and 47% of the total amount of carbon translocated to the root system. In a similar study conducted at 10°c, between 25% and 27% of the total amount of photosynthetically fixed ^{14}C was lost from roots of 6 different wheat cultivars during 24 days of growth.

Plant age will have an effect on the relative ratio of the amount of ^{14}C lost from plant roots as a function of that translocated to the

roots. Martin (1977b) found that wheat plants grown for 26 and 60 days at 18°c under a constant label of $^{14}CO_2$ lost 35.9% and 44.8%, respectively, of the total amount of ^{14}C translocated to the roots. No attempt was made to quantify the amount of ^{14}C loss from the roots which was due to root respiration. Although the results suggest little difference up to 60 days after transplanting, this study failed to consider losses which occurred after the heading stage. Although there would be expected to be little increase in the dry matter content of wheat roots after heading, Warembourg and Paul (1973) and Martin and Kemp (1986) demonstrated that newly fixed CO_2 was still being incorporated into the root and the surrounding soil at the soft dough stage. Sauerbeck and Johnen (1977) found in a long term ^{14}C labelling experiment conducted on wheat that during and after ear formation there was no net increase in the carbon content of the root system. No significant reduction in the rate of carbon loss from the root system was recorded after ear formation. By maturity, 153 days after planting, only 20% of the ^{14}C translocated to the root system during the course of the study was accounted for by mechanical isolation of the root system. The amount of ^{14}C lost from the soil as CO_2 gas was 3.8 times greater than the total amount of ^{14}C found in the root material. Only 20% of this value could be accounted for by actual root respiration. The remainder was determined to be due to the microbiological decomposition of root material. The concentration of ^{14}C remaining in the soil at harvest was equivalent to 20% of the carbon present in the recovered root material.

When mustard plants were grown in two different soil types until

flowering, it was found that after 73 days of growth, the $^{14}CO_2$ respiration from the soil accounted for 57% and 52% of the total ^{14}C translocated to the root system (Sauerbeck and Johnen, 1977). No direct attempt was made to measure the rate of root respiration. The amount of ^{14}C remaining in the soil at harvest was equivalent to between 30% and 60% of the carbon content of the extracted root material. Although there was a decline in the net increase in root dry matter after flowering, a similar decline in the rate of ^{14}C loss from the root system and $^{14}CO_2$ loss from the soil were not recorded. It is likely, therefore, that if the plants were taken to maturity, the total amount of ^{14}C lost from the root system would have been similar to that recorded for the wheat plants which were grown until maturity (Sauerbeck and Johnen, 1977).

B. Environmental Factors Affecting the Loss of Organic Carbon

Very little information is available on the effects of environmental conditions on the amount of carbon which is lost from actively growing roots under natural soil conditions. Since, however, exudation is closely associated with active root growth, it would be expected that environmental factors which affect the rate of growth of the root system would have a similar effect on the amount of organic material lost from the root system.

Lower soil temperatures seem to increase the percent ^{14}C lost from the root system as a function of the amount translocated to the root, although the results have been variable. Martin (1977b) compared wheat

seedlings grown at 18 and 10C for 26 days under a constant $^{14}CO_2$ atmosphere. He found that 35.9% and 44.4% of the total amount of ^{14}C translocated to the roots was lost from wheat seedlings grown at 18 and 10C respectively. The difference was too small to conclude that the temperature significantly influenced the loss of carbon from the wheat roots. Alternatively, Martin and Kemp (1980) found significant increases in the percent of the ^{14}C label which was lost from Condor wheat during a 24 day period when the temperature of the growth chamber was decreased from 15 to 10C. Whereas 53% of the total ^{14}C label translocated to the root system found in the soil or lost as $^{14}CO_2$ respiration from the soil at 10°c, only 35% of the translocated ^{14}C was lost from the wheat roots grown at 15°c during the 24 day period. Both studies reported significantly lower dry matter yields and incorporation of ^{14}C into the plant tissue at the lower growth temperatures. Whereas Martin (1977b) found that there was a reduction in rhizosphere $^{14}CO_2$ production when the temperature was reduced, which corresponded to the reduction in dry matter accumulation of the root tissue, little or no reduction in the soil $^{14}CO_2$ respiration was observed by Martin and Kemp (1980) when the growth temperature was decreased. No attempt was made to measure the change in root CO_2 respiration at the two temperatures.

Whipps (1984) measured the carbon loss from the roots of wheat and barley using a day/night temperature of 18/14C at two different photoperiods. A reduction in the photoperiod from 16 to 12 hours resulted in a decrease in the percent carbon lost from the root system from 68% to 50% of the carbon which was translocated to the root during

21 days of growth. No reduction in carbon loss from barley roots was recorded for the same growth period. Conversely, Barber and Martin (1976) reported a reduction in the carbon loss from wheat roots grown at 18C when the photoperiod was increased from 12 to 16 hours. With a 12 hour photoperiod, 42% of the ^{14}C label translocated to the roots during a 21 day period was lost from the roots whereas only 33% was lost using a 16 hour photoperiod. The difference between the studies is likely due to the changes in actual dry matter accumulation reported in the two studies. Whereas Whipps reported an increase in the dry matter accumulation of root material with the reduced photoperiod, the use of the 16 hour photoperiod caused nearly a 2 fold increase in the root and total dry matter production in the study by Barber and Martin. In both instances, the percent ^{14}C lost from the root system was greater under reduced root growth. Much of the difference in the percent ^{14}C lost from the root system may be due to changes in root respiration. If the predicted values for the amount of ^{14}C respired by the roots are considered in the study by Barber and Martin, the actual amount of organic carbon lost from the roots during the 16 and 12 hour photoperiod was 32% and 37%, respectively, of the carbon translocated to the roots.

Extremely adverse growth conditions, which affect the root metabolism would likely influence the percent of the translocated carbon lost from the root system. Smucker (1984) has reported on some of the material presented in the M.Sc. thesis by M.M. Shadan on the response of bean roots (Phaseolus vulgaris) to anaerobic stress. The root systems of two different bean cultivars were found to exude 19%

and 25% of the ^{14}C label added during a 17 day period when roots were grown in an environmental mist chamber. When O_2 gas in the chamber was replaced with N_2 during two 3 day periods from 6 to 9 and 13 to 15 days, 33% and 34%, respectively, of the ^{14}C label was lost from the root system. Smucker and Adler (1980) found that the majority of ethanol produced by the roots under anaerobic stress was lost to the surrounding medium. It is likely that other metabolites produced due to the inefficient use of photosynthates during anaerobic periods are similarly lost from the root system.

C. Nitrogen Loss From the Root System under Natural Soil Conditions

Few attempts have been made to quantify the amount of nitrogen which is lost from the root system of an annual crop during active growth. Heiter et al. (1986) labeled maize plants with both ^{14}C and ^{15}N in an attempt to investigate the carbon and nitrogen sources and sinks from a cropped soil. For this study, pots 1.4 m in depth were used and the ^{15}N source was applied as a dissolved nitrate salt in the irrigation water. Because the hydritic profiles indicated that the irrigation solution had not reached the lower layers of the soil column, it was assumed that any ^{15}N excess found in the lower soil depths was due to the rhizodeposition of nitrogen material from the root. From this assumption, it was calculated that the rhizodeposition of nitrogen was equivalent to 30% of the root nitrogen content. No data was presented, however, to indicate that the nitrogen and ^{15}N content of the root material measured at the single, final harvest is

indicative of the total nitrogen and ^{15}N present in the root system throughout the life cycle of the plant. Additionally, the ^{15}N isotopic excess in the lower soil profile, on which the calculation was based, was reported in some cases to be less than 0.1% of the natural abundance of ^{15}N measured in the soil. Thus, measurements would need to be accurate to the part per thousand level. Small downward movements of the added $^{15}NO_3^-$ fertilizer source, either through diffusion or mass flow, would be sufficient to cause considerable error in the results.

Poth et al (1986) utilized a soil which had been enriched with ^{15}N in the form of $(NH_4)_2SO_4$ during a 2 year period in order to quantify the input of nitrogen into the soil by N_2 fixation for Cajanus cajan (pigeon pea). It was assumed that any dilution in the ^{15}N enrichment of the soil greater than that found in a control in which no crop was grown would be due to the incorporation of fixed N_2 from the atmosphere into the soil. Using this method, it was estimated that the soil N originating from N_2 fixation was greater than the fixed nitrogen which was present in the above ground portion of the crop at harvest. No attempt was made to quantify the amount of nitrogen present in the root system.

No evidence was given in this study to validate the assumption which was used to calculate the quantity of nitrogen which was lost from the root nodules. The effect of free living soil microorganisms on the dilution of the soil ^{15}N pool was not determined. It is likely that the residual ^{15}N in the soil was more available for plant uptake and denitrification than the general soil N pool due to its short

residence time in the soil. Although it was mentioned that the soil demonstrated a very low denitrification potential, no attempt was made to account for the change in ^{15}N levels due to preferential denitrification or plant uptake of soil ^{15}N.

D. Site of Exudation

It is apparent that the area of root extension and active growth at or near the root cap will have the greatest rate of exudation. Schroth and Snyder (1961) measured the amount of sugar and amino acid exudates from bean roots grown along filter paper in solution culture. They found active exudation of organic material along the apical meristem, the region of elongation and of developing root hairs. Only traces of material were left on the filter paper from older roots. McDougall and Rovira (1970) used ^{14}C pulse labelling in order to demonstrate that the majority of ^{14}C exudation occurred along the zone of active root extension during the time period in which ^{14}C labelling took place. Low levels of exudate material, however, were observed along the entire length of the root. McCully and Canny (1985) demonstrated using ^{14}C labelling of field-grown maize that all parts of the maize roots, including the sections of the root cortex which were heavily lignified were equally active in the uptake of new carbon material. Exudation of carbonaceous material was observed along the entire root length. Only the root tip region which was actively growing during the labelling period demonstrated a higher rate of exudation of carbon compounds. It is likely that the majority of material exuded by the roots near the

root tip region is not water soluble, whereas the older root regions will tend to exude a higher proportion of water diffusible compounds (Rovira, 1969b).

E. Mechanism of Rhizodeposition

Because the exact origin of the rhizodeposited material is unknown, it is difficult to predict the nature of the root material deposited during active root growth. The water soluble fraction of the root exuded material, collected under aseptic conditions has been well studied since this material was easy to collect and analyze in an unaltered form. Several studies have also been conducted on root mucilage or mucigel, which likely represents the majority of the insoluble fraction of the rhizodeposited material before microbial decomposition. Root mucilage is generally defined to be an insoluble layer of mucilagenous material excreted from the root cap which closely adheres to the root surface. Root mucigel generally refers to the entire mucilaginous layer, which will also include insoluble excretion products from cell lysis and microbiological decomposition. The work conducted on root mucilage and root exudates prior to 1978 has been extensively reviewed (Rovira, 1969a; Hale et al, 1971; Rovira and Daveys, 1974; Hale et al 1978; Oades, 1978; Hale and Moore, 1979).

It is possible that autolysis of root cells and the degradation of cortical cell tissue represents a significant sink for carbonaceous material in the active root. Holden (1975) indicated that as much as 70% of the cortical cells of 3 week old wheat roots were anucleate.

Several electron microscopy studies have demonstrated the breakdown of epidermal and cortical tissue by bacterial colonies living both in the surface mucilage and in the cortical tissue of mature roots (Greaves and Darbyshire, 1972; Foster and Rovira, 1976).

Cortical cell senescence, however, will be dependant on plant genotype. Henry and Deacon (1981) demonstrated a greater rate of cortical cell senescence for winter wheat as compared to winter barley. Deacon and Mitchell (1985) reported much lower rates of cortical cell death, as measured by the percent of anucleate cells in the cortex for barley, oats, and rye plants than for wheat. Deacon and Lewis (1982) indicated that there was a greater rate of cortical senescence in wheat plants which showed a susceptibility to root rot.

The active excretion of carbon products from the root in the form of root cap mucilage or actively excreted root exudates represents a significant loss of carbonaceous material from the root system. Lee and Gaskin (1982) indicated that a large amount of root exudate material was recovered from solution culture experiments within 48 hours of applying a ^{14}C label. This is similar to results obtained by Minchin and McNaughton (1984), who used $^{11}CO_2$ to measure the short term exudation from non-sterile roots. Minchin and McNaugton found that within 200 minutes of application of $^{11}CO_2$, as much as 30% of the tracer which had been translocated to the roots was found in the rooting medium. Thirty percent of this material could not be accounted for by root respiration, and was assumed to be root exudate material. Lee and Gaskin (1982) maintained that the incorporation of ^{14}C into the root exudate shortly after labelling would tend to indicate an active

process of organic matter loss from the root system.

In an actively growing root, a significant amount of photosynthetic material is utilized in the production and maintenance of root cap secretory cells. The discharge of mucilage at the root tip forces outer root cap cells to be sloughed off into the surrounding medium. The material present in the sloughed off root cap cells would be expected to be accounted for in studies concerning the root cap mucigel or insoluble root exudates. Although the average lifespan of individual root cap cells varies considerably with plant species, Rougier (1981) indicated that individual root cap secretory cells could have an average replacement time from several hours to 1 to 2 days. In a study conducted on primary corn roots by Clowes and Woolston (1978), it was estimated that when the rooting density in solution culture ranged from 50 to 250 roots per litre, the number of sloughed off root cap cells ranged from 7000 to 3000 cells per root per day. The number of cells which were sloughed off by a given root was inversely related to the rooting density. Clowes (1976) calculated that an average of about twelve thousand new cells are produced by maize primary roots per day. Maize roots, however, seem to have a relatively high rate of mucilage production and secretory cell replacement (Rougier, 1981). In contrast, MacLeod (1976) indicated that the secondary roots of fababeans sloughed off between 420 and 636 cells per root per day. It is likely, however, that under natural conditions, the rate of turnover of root cap cells is greater than measured in these experiments. The mechanical stress that the growing root tip would encounter under natural soil conditions would tend to

increase the rate of loss of root cap cells from that measured in solution culture experiments.

F. Organic Matter Lost From Roots Grown Under Axenic Conditions

Because the production and loss of root material is limited in plants grown in axenic solution culture experiments, these conditions have little value in estimating the turnover of nitrogen from the root system of plants under natural conditions. This data may, however, give some indication of the nature of the compounds exuded by the roots under natural conditions.

1. Root Exudates

a. Quantitative Measurement

Early work conducted on water soluble root exudates accounts for only a small fraction of the total exuded material. Quantitative analysis of this material indicated that it consisted of less than 1% of the total carbon budget of the annual crop (Rovira, 1969a; Harmsen and Jager, 1963). Barber and Gunn (1974) indicated that the majority of early studies underestimated the amount of exuded material by failing to account for the influence of mechanical stress on the quantity of material exuded by the roots under natural environmental conditions.

The total amount of water soluble material exuded by plants grown in sterilized soil is much greater than that measured in solution culture. Utilizing a synthetic soil, Harmsen and Jager (1963) found

that the water soluble exuded compounds from vetch were equivalent to 1.6% and 2.9% of the total root carbon. Barber and Martin (1976) found that the amount of water soluble material exuded by wheat and barley roots grown in sterilized sand for 21 days represented 3% and 9%, respectively, of the carbon which was translocated to the roots during the study. This represented 15% and 29%, respectively, of the total amount of organic carbon which was lost from the roots during the 21 day period.

The amount of water soluble exudates exuded by the root may be increased during water stress. Whipps and Lynch (1983) found that for wheat and barley plants grown for 21 days in sterile sand under water stressed conditions, over 50% of the organic material exuded was in the form of water extractable exudates. They indicated that the water stress may have been sufficiently severe to result in damage to the integrity of the root system, which may have been responsible for the increase in the relative amount of water soluble material exuded.

b. Nitrogen Content

The composition of water diffusible organic material lost by roots grown in sterile solution cultures has been extensively studied. It is apparent that qualitatively, practically any diffusible compound found in the plant roots can be found in the water soluble exudates (Rovira, 1969a; Hale et al., 1971). Hale et al. (1971) calculated the amount of amino-nitrogen exuded from plants grown in solution culture. The amount of nitrogen lost in the amino acid form ranged from 0.2 to 3 μg per plant per week during the first 14 days of growth. The highest rate of amino acid exudate in axenic solution culture was recorded for

pea seedlings. Where the total organic matter content of the exudate material was measured, the nitrogen present in the amino acid fraction accounted for less than 1% of the total water soluble organic exudate (Balasubramanian and Rangaswami, 1969).

The mechanical stress encountered by the root under natural soil conditions has been found to result in a considerable increase in the loss of water soluble amino-nitrogen from the roots. Miller and Schmidt (1965) grew black beans in a sterile soil and measured the NH_4OAc extractable amino acids present in the soil after 24 days of growth. During the 24 day study, between 60 and 104 μg of nitrogen was lost per plant per week in the form of amino acids. Similarly, Boulter et al. (1966) reported up to a seven fold increase in the excretion of certain amino acids from the roots of pea seedlings when a quartz sand rooting medium was used. Although this represents a significant increase in the amount of amino acids exuded by the actively growing plant, the results would suggest that this still represents a relatively small portion of the total root nitrogen budget.

2. Root Mucigel

a. Quantitative Measurement

Although no specific information is available on the quantity of mucilage excreted from root cap cells, it likely comprises a significant proportion of the total amount of carbon exuded from the root system. Based on microscopic observation, Sametsevich (1965) estimated the total root mucilage organic matter content to be equivalent to the organic matter content of the seed. Bowen and Rovira

(1973) indicated that 80% of the exudate of wheat roots in sterile culture experiments would be accounted for in the mucilagenous material. These studies, however, are based on qualitative observations which limit their reliability.

b. Nitrogen Content

Most studies on the mucilagenous layer have only considered maize roots, due to the high rate of mucilage production found in maize. Because information on the mucilage produced by other crops is extremely limited, there is no reason to conclude that the results found for maize are applicable to other plant species.

The mucilage produced at the root tip region consists mainly of high molecular weight polysaccharides (Rougier, 1981). Various studies have indicated a significant amount of protein material present in the mucilage material. Comparisons between these studies, however, are difficult because of the different techniques employed in order to collect the root cap mucilage.

Floyd and Ohlrogge (1970) collected scrapings of the entire mucigel material present at the root tip region. They reported that the total protein content of the mucigel collected was between 0.5% and 5% of the total carbohydrate content. Chaboud (1983) attempted a "purification" of the root cap mucilage in order to separate the mucilage present at the root tip due to the excretion of material from root cap cells from material which was a result of cell lysis and cell degradation. This purified fraction was found to contain between 1% and 5% protein. Similarly, Bacic et al. (1986) found that the purified slime of maize was 94% carbohydrate and 6% protein. These studies seem to indicate

that there is a significant concentration of amino-nitrogen present in the root mucilage, although most studies indicate lower concentrations than might be expected from root tissue analysis. Because excretion mucilage is lost to the surrounding medium via the golgi apparatus, this could be a very selective excretory process.

III. Materials and Methods

A. Analytical Procedures

1. Soil Analysis

a. Total Nitrogen

Total nitrogen was determined using a modification of the Kjeldahl method described by Bremner and Shaw (1958) for the analysis of soil nitrogen, including nitrite and nitrate. Twenty five grams of air dried soil, containing approximately 20 mg of nitrogen was added to an 800 ml Kjeldahl digestion flask. The sample was then mixed with 20 ml of a 5% (w/v) $KMnO_4$ solution. After approximately 30 seconds, 40 ml of 9 M H_2SO_4 was added to the mixture. After 5 minutes, 1 drop of n-octyl alcohol and 3.00 g of 100 mesh reduced Fe were added to the mixture. The sample was allowed to stand for about 15 minutes and then heated gently for 45 minutes. After cooling, 2 Kjeltabs S, each containing 5 g K_2SO_4 and 0.005 g Se, and 20 ml of concentrated H_2SO_4 were added. The sample was digested for 1 hour, cooled, and then digested for a second hour after the addition of another 10 ml of concentrated H_2SO_4. The digest was made alkaline with the addition of 50% NaOH, and the liberated NH_4 was collected in 25 ml of 0.1 N H_2SO_4 using a simple distillation procedure. The amount of NH_4 present in the distillate was determined by back titrating to the methyl red/bromocresol green endpoint with 0.1 N NaOH.

b. Extractable Nitrate and Nitrite

Nitrate and nitrite nitrogen were extracted by shaking 2.5 g of air-dried soil for 30 minutes with 50 ml of 0.5 N $NaHCO_3$. The concentration was then determined using a modification of the automated procedure suggested by Kampshake et al. (1967). Nitrate is reduced to nitrite in alkaline solution, using Cu and hydrazine. Nitrite concentration is measured electrometrically by diazotization of the nitrite using sulfanilic acid in H_2SO_4 and 1-naphthylethylenediamine dihydrochloride as the coupling agent. Absorbance was measured at 520 nm.

c. Extractable Phosphorous

Available phosphorous was determined on the extract for nitrate analysis using the acid molybdate method suggested by Murphy and Riley (1962). A molybdophosphate complex is formed in acid medium, and reduced with ascorbic acid. The absorbance of the blue color developed is measured at 815 nm.

d. Exchangeable Potassium

Available K was determined by the method described by Pratt (1965). Potassium was extracted by mixing 2.5 g of air-dried soil with 25 ml of 1 N NH_4OAc for 30 minutes. The sample was filtered and the potassium concentration was determined using flame photometry with lithium as an internal standard.

e. Water Soluble Sulphate

Sulphate was extracted from the soil by mixing 50 ml of 0.001 M $CaCl_2$ with 25 g of soil for 30 minutes and the solution was filtered through Whatman No. 42 filter paper. Extractable sulphate was then

measured on a Technicon Auto Analyzer II system using the $BaCl_2$ turbidimetric method described by Lazrus et al. (1966).

f. Percent Organic Matter

Percent organic matter was determined by an oxidation method suggested by Walkley and Black (1934). Excess $K_2Cr_2O_7$ was reacted with the soil in the presence of H_2SO_4. The concentration of organic matter was determined by back titrating the excess $Cr_2O_7^{=}$ with $FeSO_4$ using a Fisher automatic titrimeter.

g. DPTA Extractable Cu, Fe, Mn, and Zn

The concentration of DPTA extractable micronutrients were determined using the method described by Lindsay and Norvell (1978). Diethylenetriaminepentaacetic acid was used to extract the micronutrients from the soil, and the concentration of the micronutrients present in the extract were determined using a Perkin-Elmer Model 560 Atomic Absorption Spectrophotometer.

h. Soil pH

Soil pH was determined on a soil paste prepared by mixing 50 g. of soil with 50 ml of water. The pH of the paste was measured using a standard glass calomel electrode.

i. Soil Salinity

Soil salinity was measured directly from the paste prepared for the pH measurement using a Radiometer conductivity meter.

j. Carbonate Content

The carbonate content of the soil was determined empirically by reacting the soil with dilute HCl and noting the degree of effervescence.

k. Particle Size Analysis

The percent silt and clay were determined using the method described by Tyner (1940). Organic matter was destroyed by reacting the soil with 30% H_2O_2. A 0.05% sodium hexametaphosphate solution was utilized to achieve soil dispersion and particle size was then determined using the pipette method. The sand fraction of the soil was separated using wet sieving of the soil sample.

l. Field Capacity

Field Capacity was determined by allowing a volume of water, less than that needed to saturate the total soil volume to pass through a soil column for 48 hours. A sample of soil was taken from the middle of the wetted portion of the soil column and the water content was determined by oven drying at 110°c.

2. Total Nitrogen in Plant Tissue

Total nitrogen was determined by the Kjeldahl method, as described by Jackson (1958), except employing a back titration to determine the concentration of liberated NH_4. Air dried plant material, 2 Kjeltabs S, each containing 5 g of K_2SO_4 and 0.005 g of Se, and 25 ml of H_2SO_4 were added to an 800 ml. Kjeldahl digestion flask. The sample was digested for 1.5 hours. The sample was then made alkaline by the addition of 10 N NaOH, and the NH_4 from the digestate was collected in 0.1 N H_2SO_4 using a simple distillation procedure. The concentration of NH_4 collected was determined by back titrating the unreacted H_2SO_4 to the methyl red/bromocresol green endpoint.

3. Percent ^{15}N in Soil and Plant Material

Percent ^{15}N was determined by a method similar to that described by Bremner (1965), except that the liquid N_2 trap, used to freeze out water vapor and gaseous contaminants resulting from the hypobromate reaction, was not employed. The distillate collected for total nitrogen analysis was acidified by the addition of about 1 ml of 0.1 N H_2SO_4 and the sample was evaporated until approximately 10 ml of solution remained. In cases where insufficient plant material was available to liberate at least 3 mg of N, the distillate was evaporated until 4 ml remained. Approximately 4 ml of the solution was placed under vacuum conditions, at approximately 0.003 torr pressure and N_2 gas was liberated from the solution by the addition of NaOBr. The N_2 gas produced was collected in a gas sample tube containing about 5 ml of concentrated H_2SO_4. To minimize the amount of air present in the residual gas in the vacuum apparatus, an Ar purge of the system was employed. The ^{15}N concentration of the gas was determined by mass spectrometry, using the 6602 model C micromass mass spectrometer. The ion current intensities of atomic mass units 28 ($^{14}N^{14}N$), 29 ($^{15}N^{14}N$), and 30 ($^{15}N^{15}N$) were monitored using a single collector scanning method, and the atom percent ^{15}N was determined based on these peak heights. Air samples were also analyzed for the possible presence of methylamine (31 amu), ethylamine (45 amu), and O_2 (32 amu), in order to check for possible contamination due to incomplete digestion of the soil sample, or due to possible air leaks in the vacuum apparatus. The following criteria were used to determine which of the 28, 29, and 30 ion intensity readings were to be used to determine the percent ^{15}N in

the sample.

In cases where the percent ^{15}N was less than 10%, the ratio of the 28/29 ion intensities were used in order to determine the percent ^{15}N in the sample. Although Bremner (1965) suggests the use of all three ion intensities between 5% and 95% ^{15}N, this is likely invalid if impurities are not removed from the sample, using a liquid N_2 trap. In the liberation of N_2 using NaOBr, a significant amount of N_2O gas is also released. This would ionize in the mass spectrometer to produce a small NO peak. It was found during analysis, that a significant 44 amu ion peak, corresponding to N_2O, was present. It was found that samples at or near ambient levels of ^{15}N had an abnormally high 30 amu peak, which was believed to be due to the contribution of NO. At a constant level of NO, this would generally have added much less than 1% to the size of the 30 amu peak at ^{15}N levels greater than 10% It was felt, however, that this may cause a significant error in the determination of the ^{15}N concentration at enrichment levels less than 10%.

At levels above 10%, a combination of the 28,29, and 30 peaks were used to determine the enrichment levels.

In some instances during the plant analysis, significant levels of O_2 in the sample indicated that there may have been an air leak present. In these cases, where the percent ^{15}N determined by the ratio of the 28/29 and 29/30 peaks were not similar, the trial was discarded and a new analysis was conducted. A second trial, however, was not always possible due to a limited supply of plant material at some harvests. In these instances, the 29/30 amu ratio was used exclusively, since this ratio would not be significantly influenced by

a small concentration of air in the sample.

B. Experimental Design

1. Pot Design

A diagramatic representation of the soil container used for the experiment is shown in figure 1. The pots consisted of 1 1. rectangular chambers, 7 cm by 7 cm by 19 cm in size. Pots were made of cardboard, with a water resistant wax interior. Two 1 1 containers were fused together, so as to form 1 pot, 7 cm by 14 cm by 19 cm in size, with 2 individual rooting chambers per pot. The wall separating the chambers was approximately 1.5 mm in width. The outside of the pot was covered with aluminum foil in order to restrict light penetration through the walls of the pot.

2. Soil Treatment

The soil used was a fine sandy loam, taken from the Almasippi soil association. The soil was extracted from a depth of between 10 and 25 cm below the surface, which consisted of the lower portion of the A horizon and the upper 10 cm of the C horizon. The soil extracted in this manner was low in average organic matter content (1.6% organic matter), as well as total nitrogen (0.091% total nitrogen). A listing of the particle size distribution and some of the chemical properties of the soil is given in tables 1 and 2. The soil was air dried at 30°c., sieved through a 2 mm mesh, and thoroughly mixed. Fifty ppm of P and 126 ppm of K were added to the soil by mixing 100 ml of a

Figure 1. A diagramatic representation of the soil container used for the split root experiment.

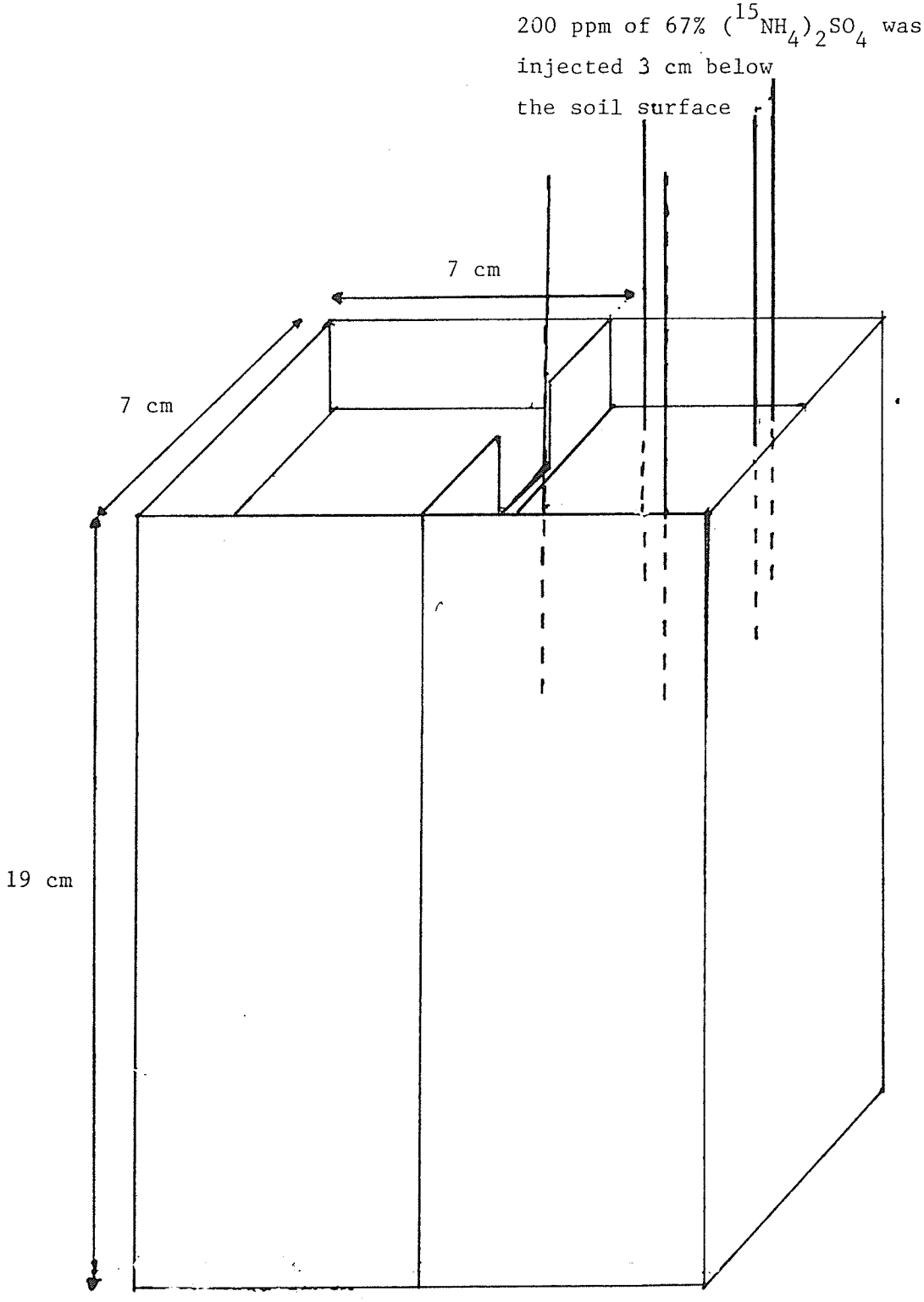

Table 1. Particle size distribution of the soil used for the growth chamber study.

Size Classification		Size Range (mm)	Percent of total soil
Sand	Coarse	0.5-1.0	0.4
	Medium	0.25-0.5	1.7
	Fine	0.1-0.25	61.9
	Very Fine	0.02-0.25	16.7
Silt		0.02-0.002	6.5
Clay		<0.002	12.7

Table 2. Chemical properties of the soil used for the growth chamber study.

Soil Characteristic	Value
pH	7.8
Salinity (mS cm^{-1})	0.3
Carbonate Content	high
Organic Matter (percent)	1.63
Total Nitrogen (percent)	0.091
Nitrate Nitrogen (ppm)	10.3
$NaHCO_3$ Extractable P "	2.4
NH_4OAc Exchangeable K "	110
Sulphate Sulphur "	4.4
DPTA Extractable Cu "	0.8
DPTA Extractable Fe "	16
DPTA Extractable Mn "	6.7
DPTA Extractable Zn "	2.8

solution, containing 0.281 g of K_2HPO_4 with 1 kg of air dried soil. The soil was then transferred to one of the pot chambers, so that each pot contained 1 kg of air dried soil.

3. Plant Treatment

a. Germination

Germination pots consisted of glass containers filled with silica sand. The pot base was covered with a 1 mm fiberglass mesh. The base of the pot was immersed in distilled water, and water availability at the surface of the germination chamber was maintained by capillary from the water bath, 30 cm below the surface of the pot. Century field pea seeds were surface sterilized by soaking for 20 minutes in 5% sodium hypochlorite, and then rinsed thoroughly with distilled water. Several seeds were removed and the average nitrogen content of the seeds before planting was determined. Approximately 400 seeds were planted in the germination chambers. Seeds were placed approximately 2 cm apart and at sufficient depth so that approximately 0.5 cm of sand covered the cotyledons. Seeds were planted with the longitudinal plane perpendicular to the soil surface so that the emerging radicle would grow parallel to the longitudinal plane. The germination chambers were placed on growth benches, and the seedlings were grown for 4 days at 20C. and an average photon flux density in the visible range of 650 μeinsteins per minute. At this point seedlings which had grown 2 to 3 cm above the soil surface and were in the latter stages of unhooking were removed from the soil.

b. Planting Procedure

After germination, the pea seedlings were prepared for transplanting into the soil system by a method similar to that described by Snow (1937). Fifty seedlings were selected where the radicle growth was relatively parallel to the longitudinal plane of the seed and little or no secondary root development had occurred. At this time, the primary root had grown an average of 6 cm. Roots were washed thoroughly in distilled water in order to remove all adhering sand particles. The primary root was then carefully sliced lengthwise so that two half root systems were separated from the root tip up to the epicotyl. Roots were then placed in a distilled water bath, approximately 2 cm in depth, for 24 hours under the conditions listed under "growing conditions". The seedlings were then removed from the water bath, and 45 seedlings were selected for planting.

The wall separating the pot soil chambers was cut away until the divisional wall was level with the soil surface. A 2 cm deep trench was dug in both soil chambers extending from the central divisional wall. 15 mg of Rhizobium Leguminosarium "C" culture, obtained from the Nitragin Company, Milwaulkee, Wisconsin, was added along each soil trench.

One seedling was planted in each pot. The seedlings were placed along the divisional wall so that one of the seed cotyledons was on each side of the wall. Thus, half of the primary root system was situated in each of the soil chambers shown in figure 1. The root was laid out along the soil trench, and was then covered with approximately 2 cm of soil. The soil was then brought to field capacity, and the

pots were placed in the growth chamber.

c. Addition of ^{15}N Fertilizer Source

Two days after planting, 36 of the 45 pots planted with seedlings were chosen for the study. A 2 gram sample of $(NH_4)_2SO_4$ containing 66.67% ^{15}N was dissolved in 10 ml of water. The nitrogen source was then injected 3 cm below the soil surface into one of the soil chambers in each pot. The nitrogen source was injected in 2 ml portions at 5 positions around the location of the root trench. Thus, the nitrogen source was located in a band near the seedling root within the soil chamber, but it should not have been in direct contact with the root.

d. Growing Conditions

Field peas were grown in a single growth chamber using a 16 hour photoperiod and with a day/night temperature of 22/17C. The relative humidity was maintained near 60%. Lighting was from Sylvannia cool-white florescent, supplemented with 10% incandescent light. The photosynthetically active photon flux density at the top of the canopy was maintained near 650 μeinsteins m^{-2} s^{-1}. Pots were watered daily in order to maintain the soil at approximately field capacity. Water content of the individual soil chambers was determined using the following procedure.

The water usage from both soil chambers of an individual pot was determined gravimetrically. The daily weight change was used in order to determine the amount of water to add to the entire pot.

In five of the pots, Soiltest MC-314 soil moisture cells, were placed in each of the soil chambers halfway down the soil column. The electrical resistance within the moisture cells was measured using a

Soiltest MC-300A soil moisture meter. The system had been previously calibrated in order to relate the water content of the soil used for the growth chamber experiment with the cell resistance. Using this system, the water content of each individual soil chamber for the 5 pots was determined once every 3 days for the duration of the experiment. The average difference in water content between the soil chamber where nitrogen had been added and the chamber where nitrogen was absent was determined for these pots, and this value was used to determine the approximate percent of the total amount of water to add to the individual soil chambers of the remaining pots.

The water infiltration rate was used in order to determine empirically if a given chamber contained a significantly different amount of soil moisture than was found to be average for that soil chamber. A small volume of the water being added to the pot was added to the surface of each soil chamber. The infiltration time of this water was measured, and if it was dramatically different than was found for other pots, the amount of water that was added to each soil chamber was adjusted accordingly.

In order to ensure that no plant nutrient, excepting nitrogen, was limiting to plant growth, a modified Hoglands nutrient solution lacking nitrogen was added weekly to the soil chambers. Tables 3 and 4 describe the composition of the nutrient solution. Fifty ml of the solution were added to each soil chamber after seedlings were transplanted into the soil. After this, 25 ml were added to each chamber weekly, except at 49, 56, and 63 days after planting, when the daily water consumption from the soil was sufficient to allow for the

Table 3. Nil nitrate nutrient solution.

Compound	Amount	Final Concentration
$CaSO_4 \cdot 2H_2O$	0.344 g l^{-1}	2 mM
K_2SO_4	0.174 g l^{-1}	1 mM
K_2HPO_4	0.174 g l^{-1}	1 mM
$MgSO_4 \cdot 7H_2O$	0.493 g l^{-1}	2 mM
18.7 mg l^{-1} Sequestrene 138Fe iron chelate (Ciba-Geigy)		
1 ml l^{-1} stock micronutrient solution		
Adjust pH to 6.8 $\pm$ 0.2 with 3N HCl		

Table 4. Micronutrient stock solution (As described by Johnson et al., 1957)

Compound	Amount	Concentration
KCl	3.728 g l^{-1}	50 mM
H_3BO_3	1.546 g l^{-1}	25 mM
$MnSO_4 \cdot H_2O$	0.845 g l^{-1}	5 mM
$ZnSO_4 \cdot 7H_2O$	0.575 g l^{-1}	2 mM
$CuSO_4 \cdot 5H_2O$	0.125 g l^{-1}	0.5 mM
$Na_2Mo_4 \cdot 2H_2O$	0.121 g l^{-1}	0.5 mM
$CoCl_2 \cdot 6H_2O$	0.951 mg l^{-1}	4 μM

addition of 50 ml of the nutrient solution per soil chamber.

e. Harvest procedure

During the course of the experiment, 3 of the 36 seedlings planted failed to grow, and these pots were discarded. Nine harvests were taken at 9,16,28,39,50,59,66,73, and 83 days after seedlings were transplanted into the soil. Three plants were removed in each of the first 3 harvests, and 4 plants were removed in each of the subsequent 6 harvests.

At harvest, the above ground portion of the plant was removed from the pot. Where pod development had occurred, pods were separated from the leaf and stem tissue. Plants were dried at 60C, weighed, and ground. The percent nitrogen and percent ^{15}N of the above ground plant matter and the pods were then determined.

After removal of the above ground plant material, soil chambers were separated. The soil chamber was then sliced open, the soil column was removed and placed on a 2 mm fiberglass mesh. The soil column was then suspended in a 4 l polyethylene container with 1.3 l of distilled water and the sample was gently shaken until most of the soil was removed from the root mat. The mesh was then raised above the water line, and 300 ml of distilled water was used to rinse the fiberglass mesh. The root mat was then removed, and carefully rinsed on a 2 mm. sieve until further rinsing did not remove any more adhering soil material. Any material present on the fiberglass mesh or on the 2 mm. sieve which could not be identified as part of the root system was deposited in the 4 l container. The root mat from each soil chamber was then individually dried at 60C, weighed, ground, and analyzed for

percent nitrogen and percent ^{15}N.

The soil solutions remaining in the 4 l containers were then treated with sufficient concentrated H_2SO_4 so that the pH of the solution measured 24 hours after the addition of the acid was between 4 and 4.5. The soil was dried at 75C. The soil was then ground to pass through a 2 mm sieve and analyzed for percent nitrogen and ^{15}N.

In order to obtain the percent nitrogen and percent ^{15}N of the soil previous to plant growth, 6 pots were placed in the growth chamber which had been treated as described in the experimental procedure, but in which no plants had been grown. Three chambers from these pots were removed at harvests taken 28, 50, 66, and 83 days after planting. The soil removed from these pots was treated as described above and the percent nitrogen and percent ^{15}N was determined on the treated soil.

IV. Results and Discussion

A. Early Seedling Growth and Development After Transplanting

Severe stress was induced in the young pea seedlings immediately after the root system was sliced. All seedlings demonstrated some leaf wilt immediately after planting. This generally persisted for approximately 2 days, at which time recovery was noted in most seedlings. Plant dry matter accumulation for each harvest is recorded in appendix 1. In the harvests taken at 9 days after transplanting, no more than 0.47g of total plant dry matter was recorded. Little or no above ground development was observed in the pea seedlings during the first week after the root system was disturbed. In the majority of trials, some above ground growth was observed at about 6 days after planting, or 7 days after the root system was split.

Of the 36 trials used for the experiment, 33 seedlings recovered from the imposed stress by the first harvest, 9 days after transplanting. Three of the seedlings died shortly after the root system was spliced and these replicates were removed from the experiment.

Water stress was likely the most significant problem limiting seedling development immediately after the root system had been spliced. Although the plant can regenerate lost or damaged root tissue, the splicing of the root system likely resulted in severe damage to the vascular cylinder. This would severely impede early water and nutrient transfer to the developing shoot tissue. It is

probable that early plant death in 3 trials, as well as the observed plant stress was due to water stress.

In order to limit seedling death after the roots had been spliced, care was taken to limit the amount water stress on the plant immediately after the splicing proceedure. During a preliminary analysis, 8 pea seedlings were treated as outlined in the proceedure, except that the seedlings were transplanted directly into the soil after the roots were spliced. Of the 8 seedlings, only 3 survived for 2 weeks after transplanting. Conversely, when 6 seedlings were left for 48 hours in a water solution culture before transplanting, 5 of the 6 seedlings which were transplanted were actively growing after 2 weeks. In the experiment reported, only 3 of the 36 plants failed to grow when plants were left in a water solution for 24 hours before transplanting.

B. Root Growth

Appendix 1 lists the amount of dry matter, the nitrogen and ^{15}N content of the root material for all harvests during the course of the experiment. Little or no root growth was observed in the soil amended with $(NH_4)_2SO_4$ in 7 of the 33 trials. For these 7 trials, the portion of the root system growing in the nitrogen amended soil was found to be heavily lignified and likely was relatively inactive at harvest. In 6 of the 7 trials, the limited root growth was accompanied with a significantly reduced uptake of the nitrogen 15 fertilizer source. Due to the lack of root development, the results of the 7 replicates were

not included in the analysis.

Figure 2 shows the root growth as a function of time for both soil chambers. During the early plant growth stages, the majority of the root growth occurred in the soil chamber in which no nitrogen fertilizer was added. Up to the onset of flowering, 40 days after transplanting, the rate of dry matter accumulation in the portion of the root system extracted from the unamended soil exceeded that of the amended soil. Little or no root development was observed in the portion of the root system growing in the nitrogen amended soil before the second harvest, 16 days after transplanting.

After flowering, the net rate of accumulation of dry matter in the root system was greater for the root growing in the soil amended with the nitrogen fertilizer source than for the root extracted from the unamended soil. The rate of accumulation of dry matter in the root material growing in the soil unamended with nitrogen fertilizer was seen to decline during the period of seed development and maturation, from 50 to 83 days after transplanting. From 73 to 83 days after transplanting, a net loss of dry matter was observed for this portion of the root system. This pattern was not reflected in the rate of production of dry matter in the root material harvested from the soil which was amended with nitrogen fertilizer. Between 59 and 83 days after transplanting, the rate of dry matter accumulation in the root material growing in the amended soil remained relatively constant with time.

A paired t test comparison between the dry weights of the root material extracted from the two soil chambers indicates that before

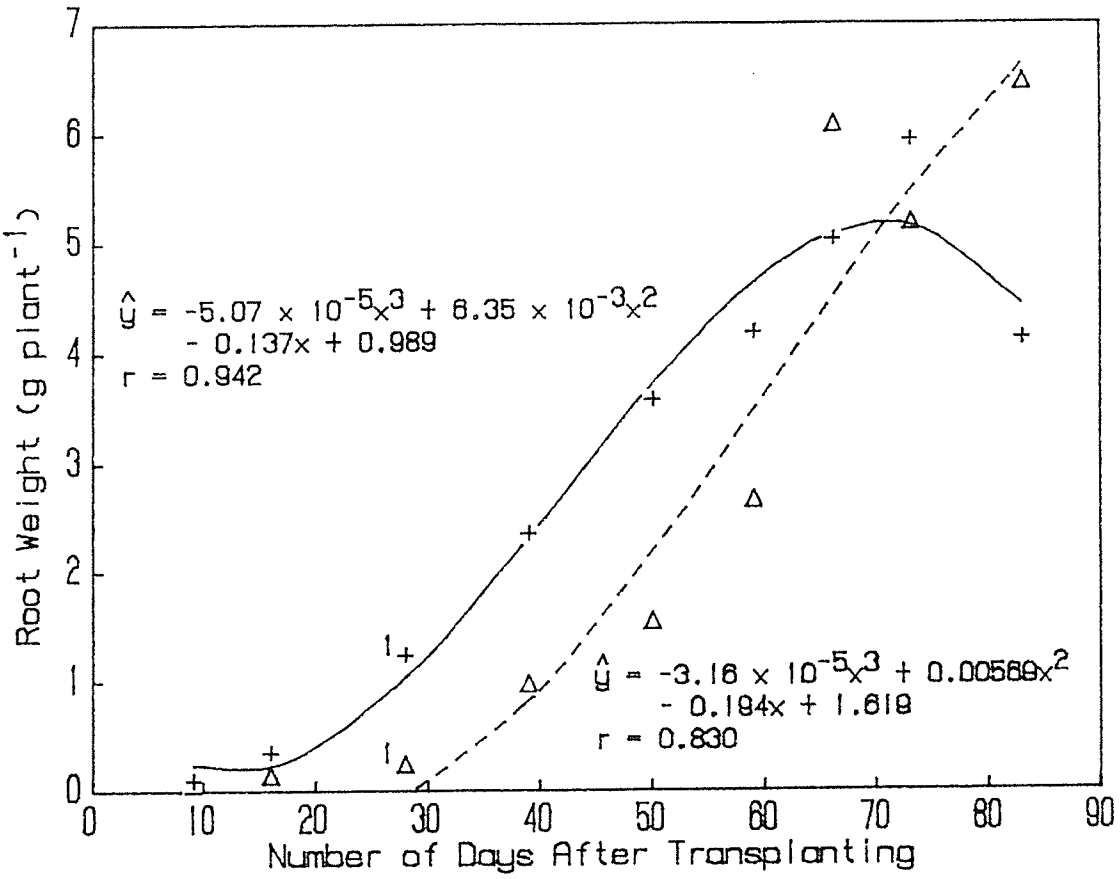

Figure 2. The dry matter weight of the root material harvested from the soil amended with nitrogen fertilizer (Δ), and the root material harvested from the soil with no nitrogen fertilizer added (+) as a function of time. Mean of 3 replicates.

[1]*mean of 2 replicates*

harvest 6, 59 days after transplanting, the amount of root material present in the unamended soil chamber was significantly greater than that of the nitrogen amended soil chamber at a probability of 0.05. There was no significant difference between the dry weights of the two root systems at a probability of 0.05 between harvest 6 and the final harvest.

The slower rate of root growth observed in the nitrogen amended soil before flowering was likely due to the concentration of free ammonium present in the $(NH_4)_2SO_4$ band near the root during the early stages of the experiment. Because 200 ppm $(NH_4)_2SO_4$ was added in a relatively narrow zone near the root, it is likely that during the early growth stages, the concentration of free ammonium in the band would have been inhibitory to extensive root growth. Bennett and Adams (1970) found a relationship between the concentration of aqueous ammonia and the inhibition of the growth of cotton roots in both nutrient and soil solution culture experiments. NH_3 concentrations of 0.22 mM were found to inhibit root growth in cotton seedlings. When southern peas were grown in solution culture using NH_4^+ as the nitrogen supply, there was a significant reduction in root production at bloom, podding, and maturity, than when $\geq$ 75% of the nitrogen supply was in the NH_4^+ form (Sasseville and Mills, 1979). Colliver and Welch (1970) found that corn root radical growth was extremely inhibited when the concentration of aqueous ammonia and ammonium in the soil was increased from 602 to 944 ppm (pH=8.1). Corn roots would not grow through soil layers enriched with 1,485 ppm NH_3 (pH=8.7).

C. Nitrogen Content of the Root System

Figure 3 compares the change in the nitrogen content of the root system as a function of time and soil treatment. The rate of nitrogen accumulation in the root system extracted from the soil amended with nitrogen fertilizer was significantly less than that of the root system extracted from the unamended soil during early plant growth. Although the percent nitrogen of the root tissue seemed to be relatively independent of the soil treatment, the lower rate of root dry matter production in the nitrogen amended soil resulted in a decreased total root nitrogen content until 73 days after transplanting.

During flowering and seed development, the net rate of nitrogen accumulation was greater for the root system growing in the soil amended with nitrogen fertilizer. By 73 days after transplanting, no significant difference was observed between the nitrogen content of the root system found in either soil treatment at a probability of 0.05.

D. Organic Matter and Nitrogen Distribution in the Plant material

The partitioning of nitrogen and organic matter in the plant is similar to that expected for an annual legume crop (e.g. Pate and Layzell, 1981). The change in the dry matter weight of the plant material with time is shown in figure 4. The net rate of dry matter production in both the shoot and the root material is seen to decline after the onset of pod production, 45 days after transplanting. Only the rate of dry matter production in the root growing in the soil

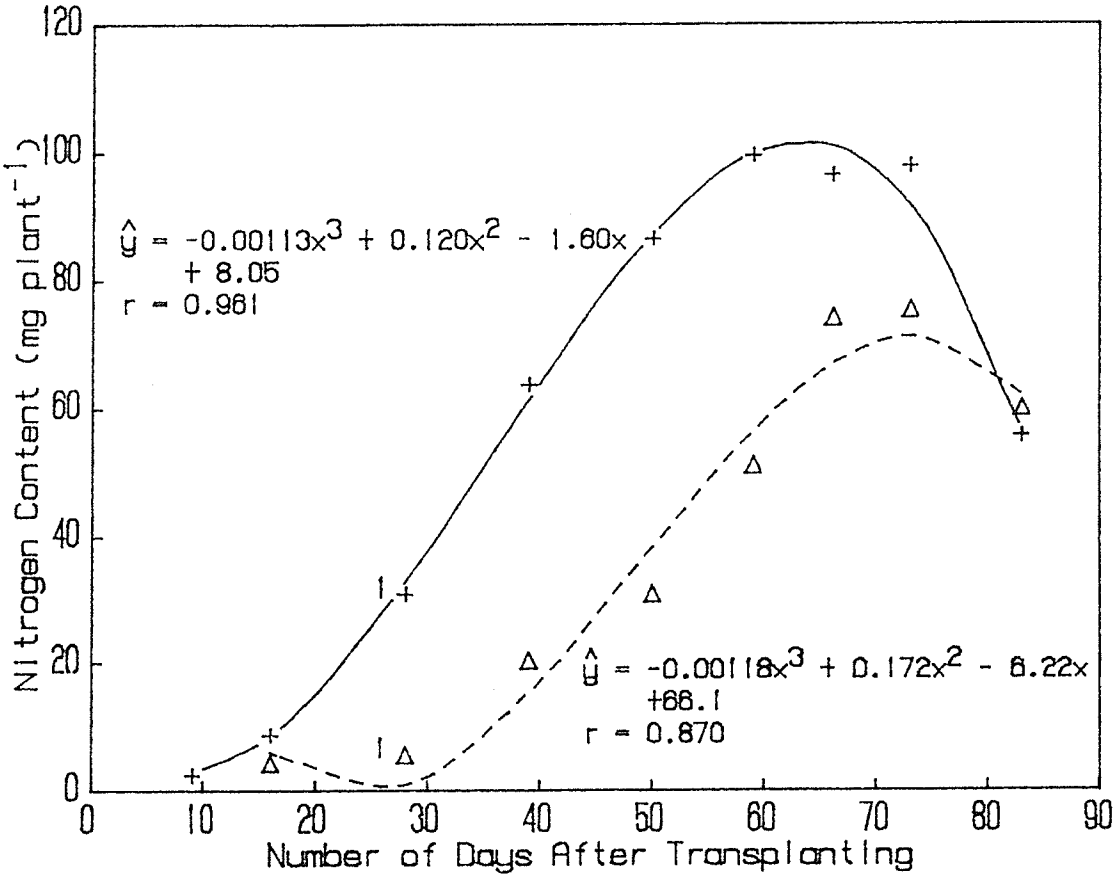

Figure 3. Total nitrogen content of the root material harvested from the soil amended with nitrogen fertilizer (Δ) and the soil with no nitrogen fertilizer added (+) as a function of time. Mean of 3 replicates.

[1]*mean of 2 replicates*

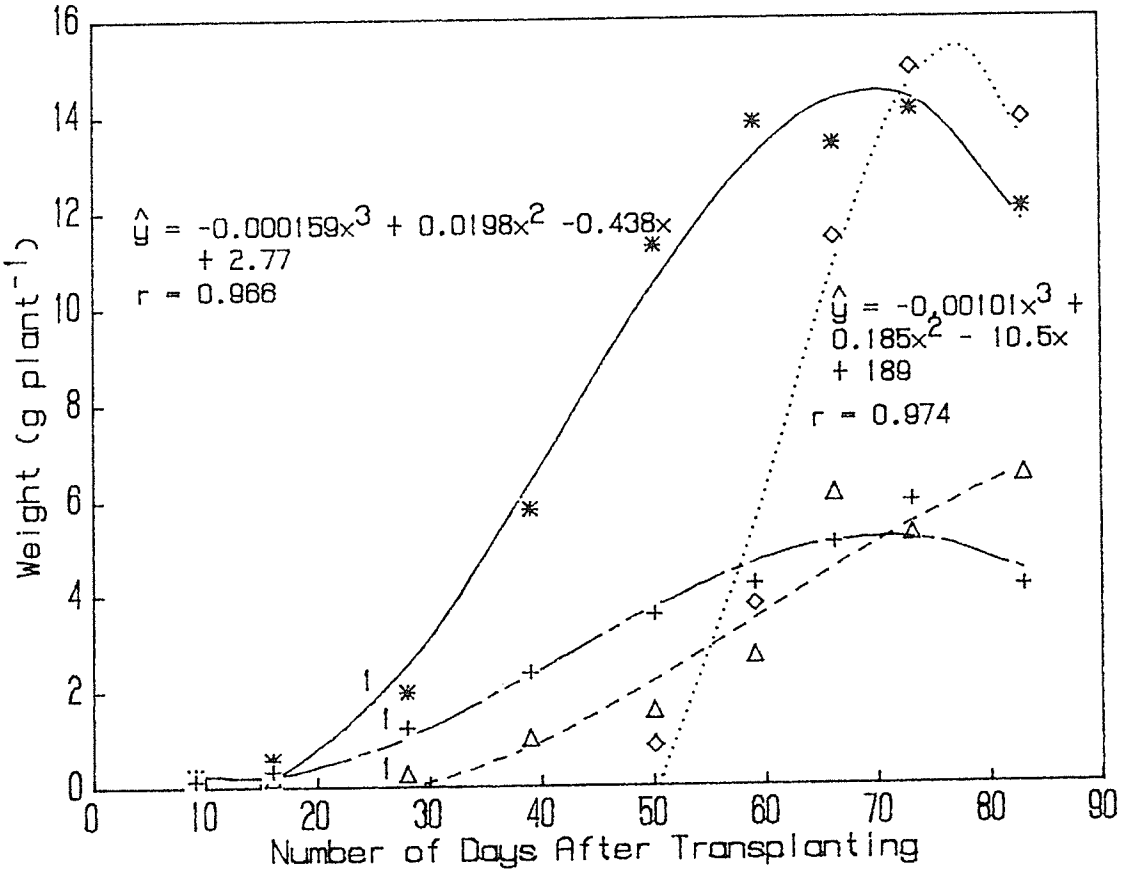

Figure 4. A comparison of the dry matter weight of the pods (◊), stem and leaf tissue (✳), the root material harvested from the soil amended with nitrogen fertilizer (+), and the root material harvested from the soil with no nitrogen fertilizer added (Δ) as a function of time. Mean of 3 replicates.

[1]*mean of 2 replicates*

amended with nitrogen fertilizer is seen to increase after this period. This is likely due to the detrimental effect of the nitrogen fertilizer source to root production during the early stages of growth. The ratio of the weight of above ground plant material to the root material is given in figure 5. The results show a general trend towards an increasing shoot to root ratio throughout the growing season. By maturity, 73% of the total dry matter of the plant material is accounted for in the above ground portion of the plant material.

The distribution of nitrogen in the plant material is significantly different from the dry matter production. Figure 6 shows the change in the nitrogen content of the plant material with time. Figure 7 lists the ratio of the nitrogen content of the above ground plant material to the root nitrogen content as a function of time. The pod material is acting as the major sink for nitrogen in the plant. At 16 days after transplanting, 43% of the total nitrogen content of the plant is found in the roots. After flowering and with the onset of pod production, there is a rapid increase in the ratio of the nitrogen content of the above ground plant material to that of the roots. By the final harvest only 20% of the total plant nitrogen content is accounted for in the root material. Sixty two percent of the total plant nitrogen budget at the final harvest is accounted for by the seeds and pods.

E. Nitrogen 15 Distribution in the Plant Material

The amount of the nitrogen 15 fertilizer source which is present in the total plant material is given in figure 8. The results indicate a

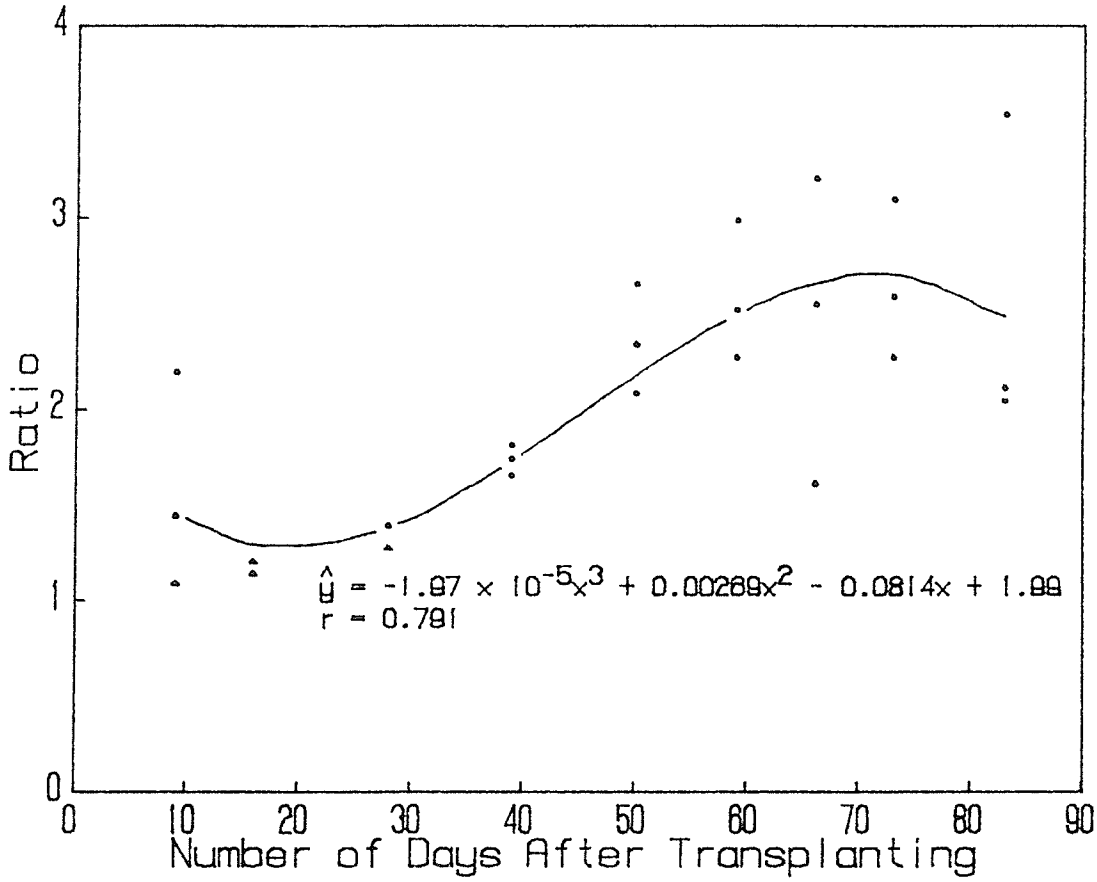

Figure 5. Ratio of the above ground plant material to the root material during the course of the experiment.

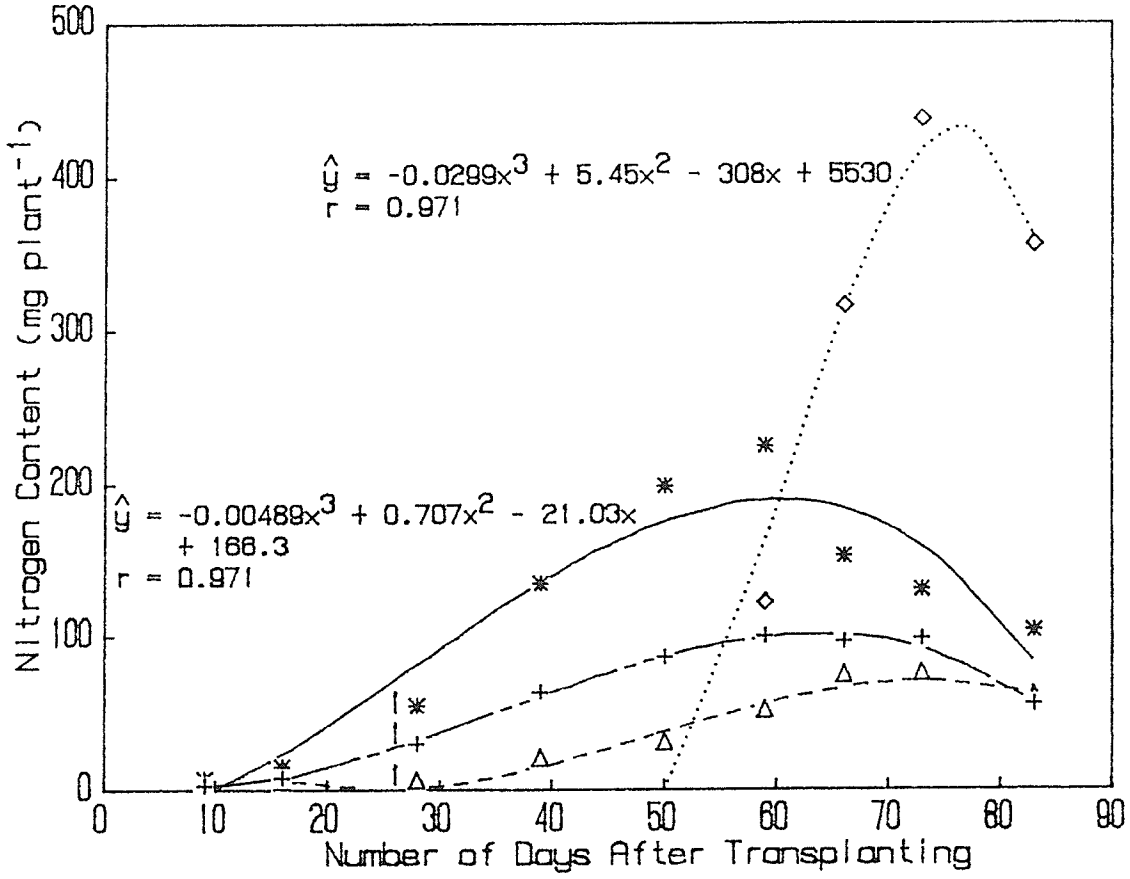

*Figure 6. A comparison of the total nitrogen content of the pods (◊), stem and leaf tissue (*), the root material harvested from the soil amended with nitrogen fertilizer (+), and the root material harvested from the soil with no nitrogen fertilizer added (Δ) as a function of time. Mean of 3 replicates.*

[1]*mean of 2 replicates*

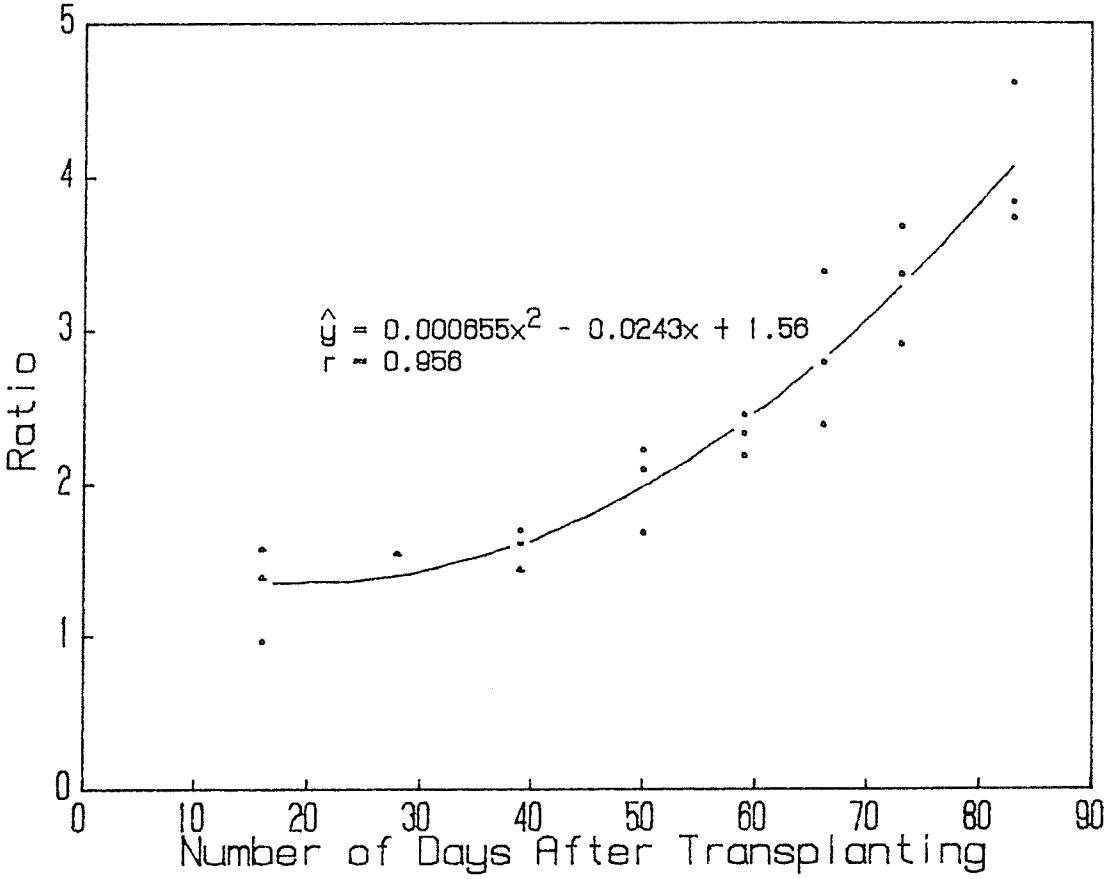

Figure 7. Ratio of the total nitrogen content of the above ground plant material to the total root nitrogen content as a function of time.

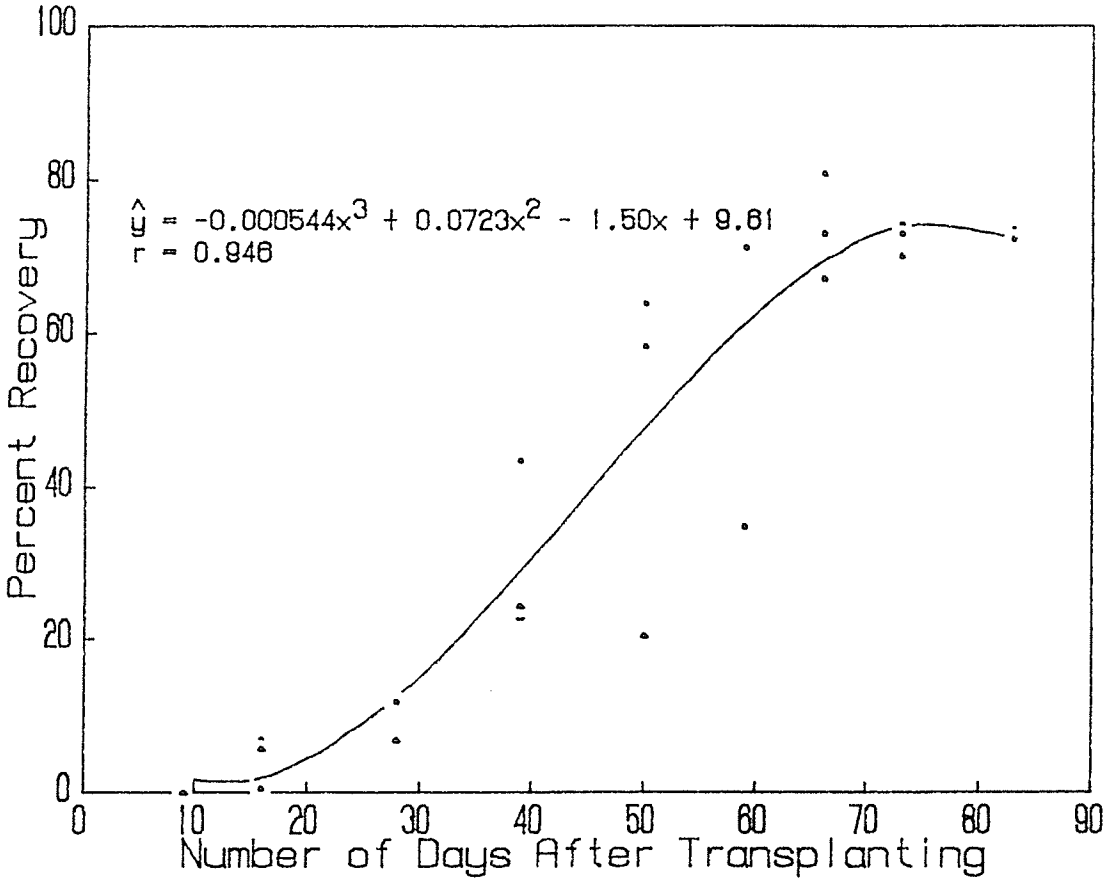

Figure 8. Percent of the added nitrogen which was recovered in the plant material as a function of time of harvest.

relatively good uptake of the fertilizer ^{15}N source. By the final harvest, 72% of the original fertilizer nitrogen source was calculated to be present in the plant material based on the percent ^{15}N enrichment found in the plant material. There seemed to be little fertilizer nitrogen uptake after harvest 6, 59 days after transplanting.

The change in the nitrogen 15 content of the plant material with time is given in figure 9. Although the root growing in the unamended soil generally contained a much greater total nitrogen content than that of the root growing in the nitrogen amended soil (figure 6), it contained significantly less total nitrogen 15 after harvest 5, 50 days after transplanting. The movement of the nitrogen 15 fertilizer source, however, was sufficient to result in high concentrations of nitrogen 15 in all plant tissue.

F. Nodulation

Where the $(NH_4)_2SO_4$ source had not been added, nodules were observed on the root surface by the second harvest, 16 days after transplanting. Nodules were present on the root surface in all subsequent harvests. From 16 to 59 days after transplanting, the inside of the nodules appeared red in colour. After harvest 7, 66 days after transplanting, the inside of all nodules examined were grey in colour. It is likely, therefore, that the nodules were inactive by 66 days after transplanting.

No nodules were present on the portion of the root system which had grown in the soil amended with $(NH_4)_2SO_4$ fertilizer until harvest 7, 66

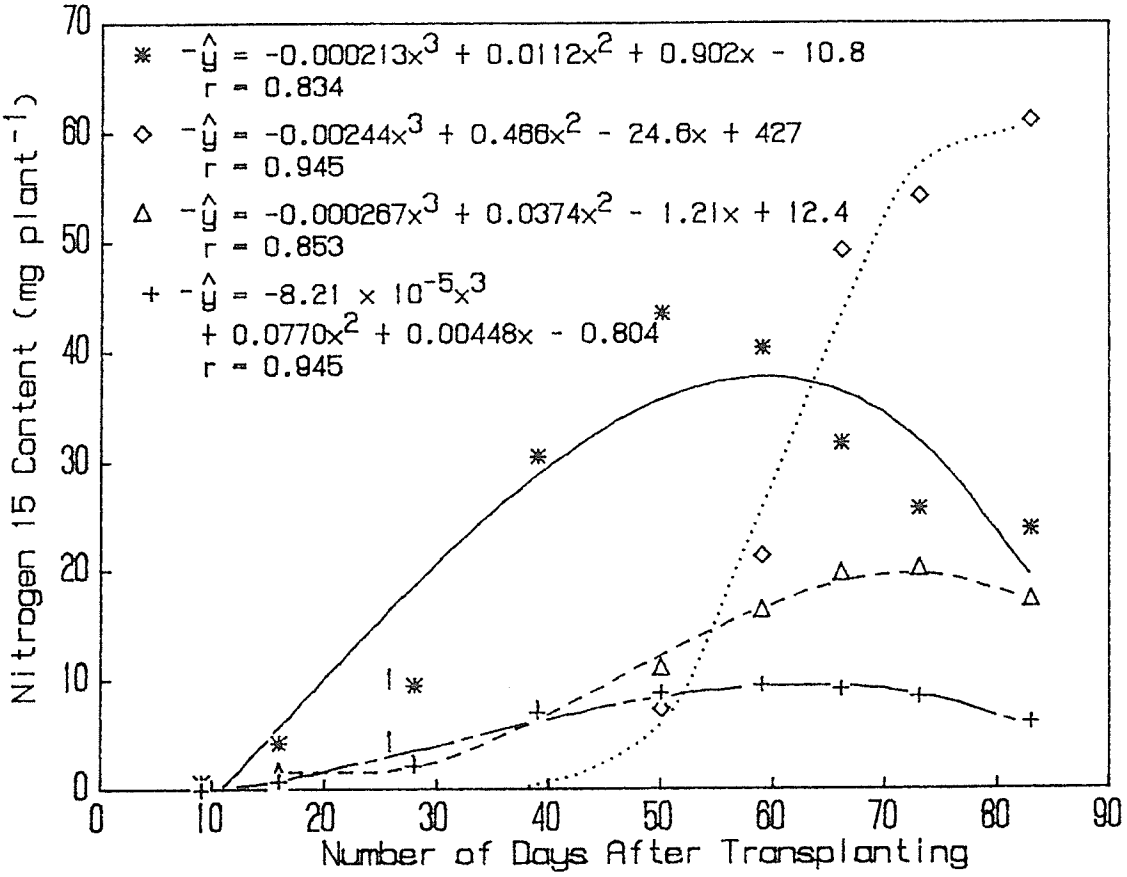

*Figure 9. A comparison of the total ^{15}N content of the pods (◇), stem and leaf tissue (*), the root material harvested from the soil amended with nitrogen fertilizer (+), and the root material harvested from the soil with no nitrogen fertilizer added (Δ) as a function of time. Mean of 3 replicates.*

[1]*mean of 2 replicates*

days after transplanting. Some nodulation was observed in one of three trials harvested at 66 days after transplanting. In the subsequent harvests, 73 and 83 days after transplanting, some nodulation was observed in 2 of 3 trials. All nodules appeared grey in colour, and were likely inactive at the time of harvest. No attempt was made to measure the amount of nodulation or the rate of nitrogen fixation in the root nodules.

The selective inhibition of nodulation in the nitrogen amended soil could be explained by the high concentration of free nitrogen around the root zone in the amended soil during the early stages of the experiment. Although it is known that nitrogen fixation can be inhibited by high concentrations of available nitrogen in the soil, it has been demonstrated that combined nitrogen can have a localized effect on early nodulation and nitrogen fixation. As early as 1917, Wilson used a split root system to demonstrate that a 4 mM nitrate solution inhibited nodulation of soybean roots directly exposed to the solution without affecting nodulation of roots growing in a nil nitrate solution (Wilson, 1917). When excised roots, growing in an external medium are fed with a nitrate source, it has been found that nitrate is less inhibitory to nitrogen fixation than when the nitrate source is in direct contact with the nodulating roots (Raggio et al., 1957,1965, Cartwright, 1967).

Early nodulation and nitrogen fixation in the root is likely controlled by the local carbon/nitrogen ratio or the availability of carbohydrates in the root tissue as an energy source for nitrogen fixation (Oghoghorie and Pate, 1971). Chen and Philips (1977) have

shown a relationship between the local root concentration of nitrate reductase and the nodule senescence of soybean roots. It is likely that local high concentrations of products of the nitrate reduction cycle result in a depression of N_2 reduction in the root and an inhibition of nodulation. In the present experiment, the added nitrogen source and the concentration of available nitrogen in the soil medium would not have been sufficient to supply the nitrogen measured in the plant tissue by the final harvest. It is likely, however, that a very high concentration of available nitrogen existed around the root growing in the nitrogen amended soil during initial stages of the experiment. While the early rate of root growth was greater in the unamended soil than in the nitrogen amended soil, the concentration of nitrogen 15 in the root material suggests that the added fertilizer nitrogen accounted for only a small portion of the total nitrogen budget of the root. This would be expected to result in a low concentration of combined nitrogen in the root, allowing for the diversion of photosynthate to produce nodulation and nitrogen fixation. The high concentration of available nitrogen present in the fertilizer band would be expected to inhibit nodulation in the root zone adjacent to the nitrogen fertilizer source.

G. Nitrogen 15 enrichment in the unamended soil

Figure 10 represents the change in the percent soil nitrogen 15 with time for the soil chamber which was not amended with nitrogen fertilizer. Except for harvests taken at 9 and 16 days after planting,

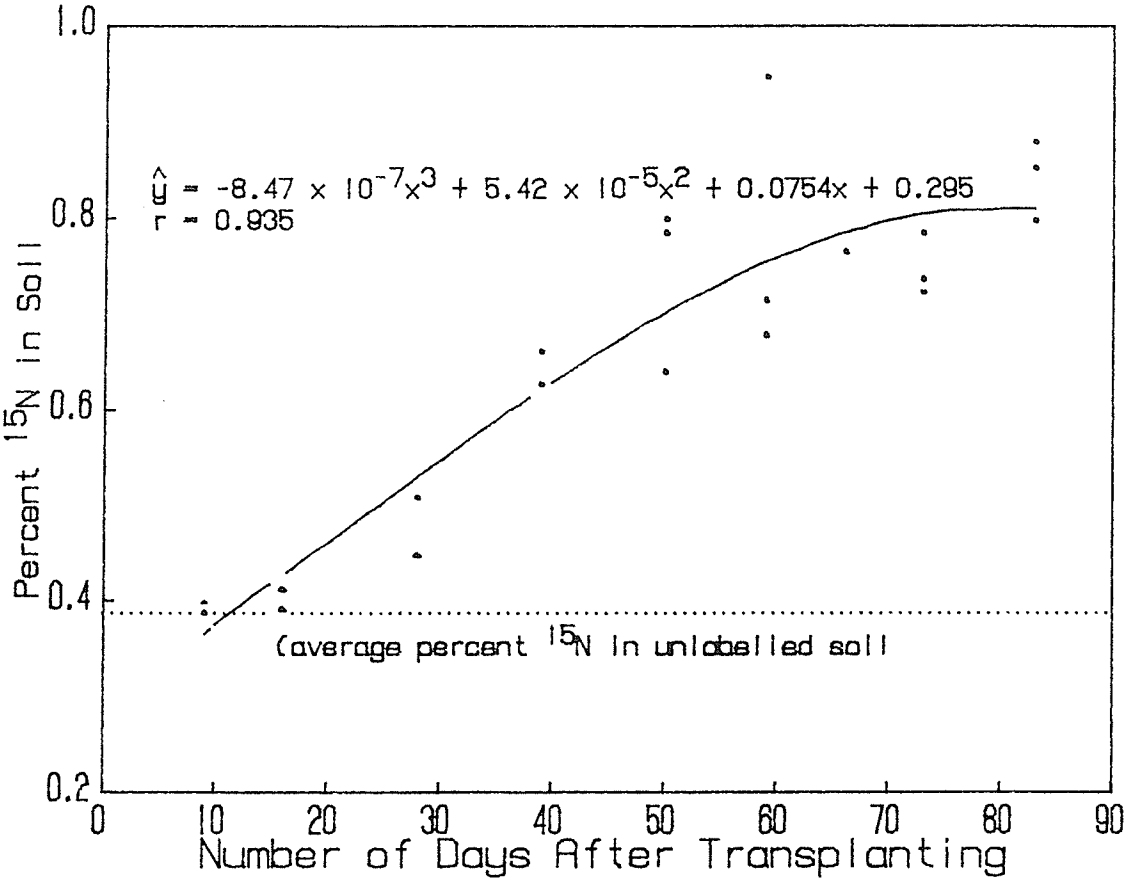

Figure 10. Percent ^{15}N of the soil which was unamended with nitrogen fertilizer as a function of the time of harvest.

the nitrogen 15 concentration of the soil is significantly greater than the background level of ^{15}N in the soil which can only be accounted for by the presence of nitrogenous material from the root system in the soil. At plant maturity, 83 days after transplanting the average percent nitrogen 15 in the unamended soil was 0.84%. This represents over a 2 fold increase in the percent nitrogen 15 in the soil.

At the harvest taken 9 days after transplanting, no significant increase in the nitrogen 15 content of the soil over the ambient soil nitrogen 15 level was recorded. Although the root splitting technique caused considerable damage to the root tissue, the two root halves were found to be healed and operating independently by the first harvest, 9 days after transplanting. Only a limited amount of root growth and translocation of nitrogen 15 into the root system occurred during the period from 0 to 9 days after transplanting. By 9 days after transplanting, there was an average of 2.6 mg. of nitrogen labelled with 1.9% nitrogen 15 in the root extracted from the unamended soil. This represents only about 2% of the maximum total nitrogen and nitrogen 15 content found in the root system growing in the unamended soil. It can be assumed, therefore that although the splitting of the root system resulted in considerable root damage, which may have caused a considerable increase in the rate of exudation of material from the root system, this represents an insignificant proportion of the measured cumulative exudation of nitrogen 15 from the root system.

H. Nitrogen 15 content of the root system in the unamended soil

Figure 11 shows the total nitrogen and ^{15}N content of the root as a function of time. There is a considerable variation in both the total nitrogen and total nitrogen 15 content of the root material found in the unamended soil with time. Variation in the ratio of the nitrogen 15 to the total nitrogen content of the root material, however, seems to remain relatively independent of time between days 16 and 83 of the experiment.

Considerable change in the percent nitrogen 15 of the root material growing in the unamended soil was observed between 0 and 16 days after transplanting. It can be assumed that until 2 days after transplanting, at which time the $(NH_4)_2SO_4$ source was added to the amended soil, the nitrogen 15 content of the root system was 0.387%, which represents the measured background level of nitrogen 15 in the analysis. At 9 days after transplanting, an average of 1.9% nitrogen 15 was found in the root system extracted from the unamended soil. Although this represents a considerable variation in the nitrogen 15 enrichment of the root system between days 0 and 16 of the experiment, there is very little total nitrogen accumulation in the root system during this period.

The relationship between the nitrogen 15 and nitrogen content of the root system growing in the unamended soil can be seen in figure 12. There is a strong linear correlation between the nitrogen 15 content of the root material and the total nitrogen content of the root material. Variation around the line seems to be independent of the time of

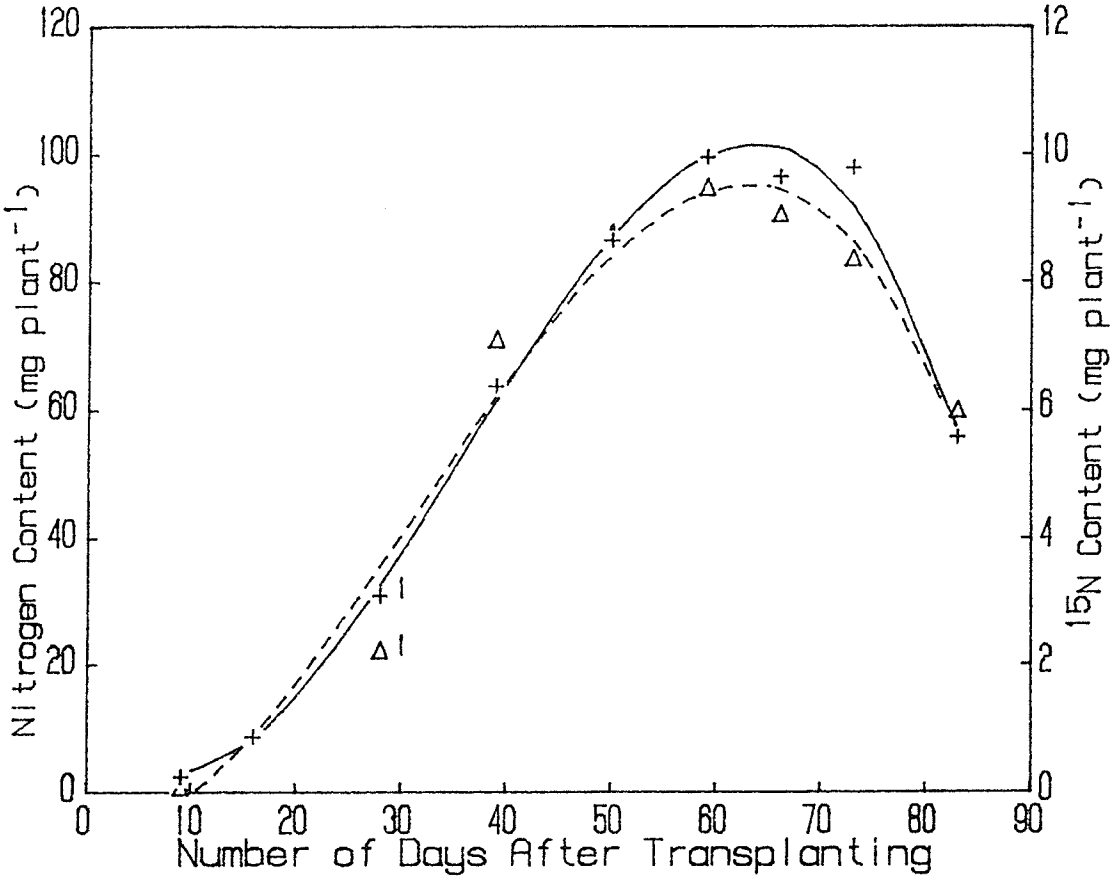

Figure 11. Total nitrogen (+) and ^{15}N (Δ) content of the root material grown in the soil with no nitrogen fertilizer added as a function of time. Mean of 3 replicates.

[1]*mean of 2 replicates*

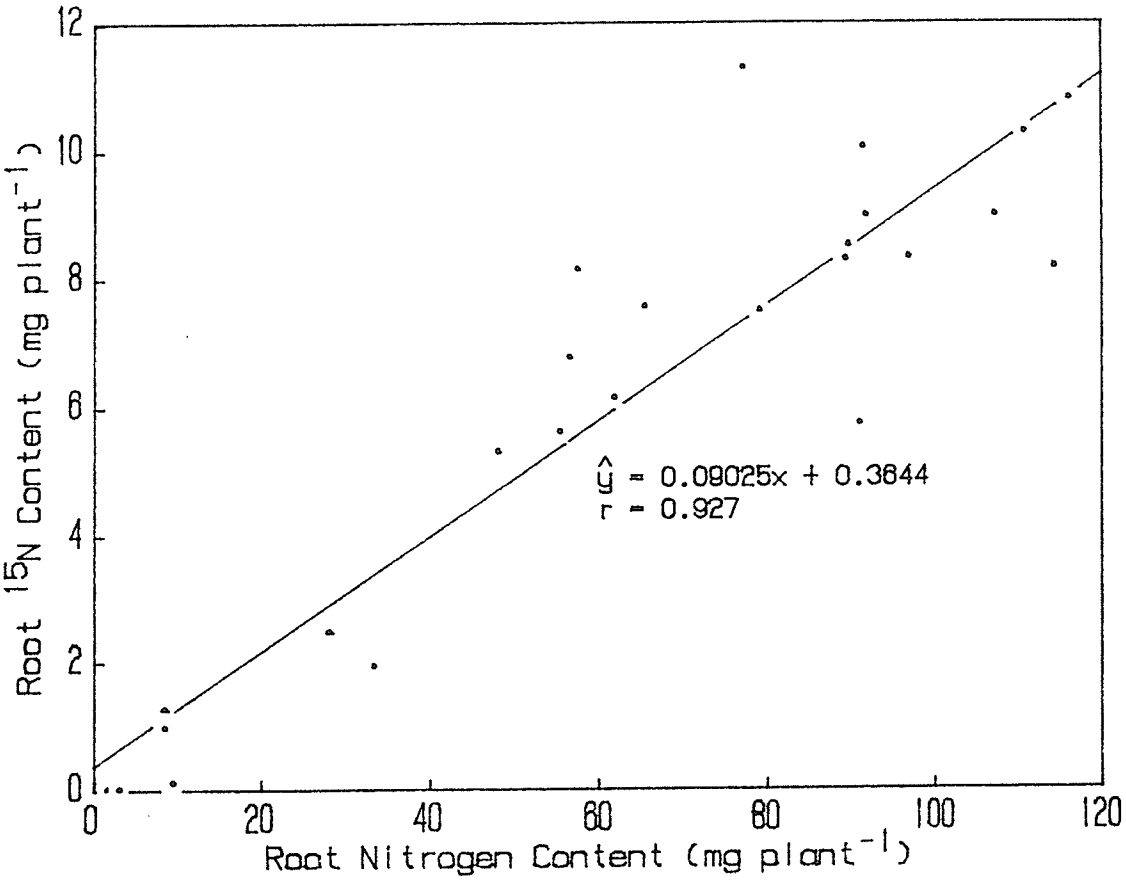

Figure 12. The nitrogen 15 content of the root material grown in the soil which had no nitrogen fertilizer addition as a function of the total nitrogen content of the root material.

harvest. Thus, there appears to be relatively little flucuation in the ratio of the nitrogen 15 to the total nitrogen content of the root material present in the unamended soil during the course of the experiment.

I. Nitrogen Lost From the Root System During Growth

The excess nitrogen 15 remaining in the soil after harvest, as diagramed in figure 10, can be assumed to originate from nitrogen 15 labelled material in the root system growing in that soil chamber. If the percent nitrogen 15 remains relatively constant with time, then the amount of root nitrogen remaining in the soil after harvest can be approximated by the formula:

$$\text{NDFR} = \text{total soil nitrogen } \frac{(\%^{15}\text{N excess in the soil})}{(\text{average } \%^{15}\text{N excess in the root})}$$

where NDFR is the nitrogen derived from the root.

The average percent nitrogen 15 in the root material can be calculated based on the change in the nitrogen and nitrogen 15 content of the root material over the duration of the experiment. The average percent nitrogen 15 in the root material from day 0 to any later time, t of the experiment is given by the ratio of the cumlative nitrogen 15 content and the cumulative nitrogen content in the root material from day 0 to the time, t. That is:

$$\frac{{}_0\int^{t}\text{RN}}{{}_0\int^{t}\text{RN15}} \times 100 = \text{average } \%^{15}\text{N in the root tissue.}$$

where:

RN = total nitrogen content of the root growing in the unamended

soil (mg plant^{-1})
RN15 = total nitrogen 15 content of the root growing in the unamended soil (mg plant^{-1}).

Based on the third order polynomial regression analysis given in figure 11, the predicted value of the nitrogen content of the root tissue growing in the unamended soil at any time between 9 and 83 days after transplanting is given by the formula,

$$RN = -0.00112n^3 + 0.120n^2 - 1.59n + 8.05$$
$$r = 0.961$$

where:

RN = total nitrogen content of the root growing in the unamended soil (mg plant^{-1})
n = number of days after transplanting
r = correalation coefficient.

Similarily, the predicted value of the nitrogen 15 content of the root tissue has been calculated to be,

$$RN15 = -8.21 \times 10^{-5}n^3 + 0.00770n^2 + 0.00448n - 0.804$$
$$r = 0.945$$

where:

RN15 = total nitrogen 15 content of the root growing in the unamended soil (mg plant^{-1}).

The cumulative nitrogen and nitrogen 15 contents of the root system, respectively, between day 9 and any time "t", can be calculated based on the integral of the polynomial regression line,

$$_9\int^t RN = -2.81 \times 10^{-4}n^4 + 0.0400n^3 - 0.786n^2 + 8.05n \Big|_9^t$$

and,

$$_9\int^t RN15 = -2.05 \times 10^{-5}n^4 + 0.00257n^3 + 0.00224n^2 - 0.804n \Big|_9^t.$$

Since the actual concentration of nitrogen and nitrogen 15 in the root tissue is unknown between 0 and 9 days after transplanting the cumulative nitrogen and nitrogen 15 content of the root system cannot

be estimated based on the above equations during this time interval. It is, however, known that there was no nitrogen or nitrogen 15 present in the root tissue at germination, 4 days before transplanting. It can also be assumed that until the addition of the ^{15}N fertilizer source, 2 days after transplanting, the percent nitrogen 15 in the root tissue was constant at the measured background concentration of nitrogen 15 of 0.387%. By 9 days after transplanting, it was found that there was a mean of 2.6 mg of nitrogen per root in the root growing in the unamended soil. The average percent nitrogen 15 in the root tissue at 9 days after transplanting was 1.9%. If it is assumed that,

1. the nitrogen accumulation in the root tissue was linear between 4 days before transplanting and 9 days after transplanting and,
2. the increase in percent ^{15}N excess of the root system was linear between 2 and 9 days after transplanting,

it is then possible to calculate the cumulative nitrogen and nitrogen 15 content of the root system from 0 to 83 days after transplanting.

The assumptions used to approximate the nitrogen and nitrogen 15 content of the root tissue between 0 and 9 days after transplanting would be expected to be associated with a large degree of error. This time period, however, represents a relatively small proportion of the total cumulative nitrogen and nitrogen 15 content of the root system over the entire course of the experiment. By the final harvest, 83 days after transplanting, the estimated cumulative nitrogen content of the root tissue from 0 to 9 days after transplanting represents only 0.3% of the total cumulative nitrogen content of the root tissue. Only 0.05% of the cumulative nitrogen 15 content is accounted for from 0 to

9 days after transplanting. Thus a relatively large error in the assumptions during this period of time would represent a small error in the calculated average percent nitrogen 15 content of the root tissue. The NDFR value for each harvest has been calculated based on the average percent nitrogen 15 of the root system and the percent nitrogen 15 excess found in the soil after harvest. Figure 13 compares the accumulation of nitrogen in the root tissue with the calculated concentration of root derived nitrogen remaining in the soil after harvest. Compared with the amount of nitrogen present in the mechanically isolated root material (solid line), the amount of root derived soil nitrogen (broken line) represents a considerable proportion of the total amount of root nitrogen. From day 16 to day 66 of the experiment, between 71% and 78% of the total root nitrogen content (the sum of the nitrogen content of the root system and the calculated root derived soil nitrogen) was accounted for in the root system extracted from the soil. Twenty two to 29% of the root derived nitrogen remained in the soil after harvest. By maturity, only 54% of the total root nitrogen was accounted for in the root material. There was no detectable nitrogen 15 enrichment in the soil for the harvests taken at 9 and 16 days after transplanting.

The observed decrease in the rate of nitrogen loss from the root system during seed maturation could be accounted for mainly by a decline in the percent nitrogen in the root tissue during the latter stages of plant growth. During the period of pod development and seed maturation, from 59 to 73 days after transplanting, there was little or no net accumulation of nitrogen in the root system. From 73 to 83 days

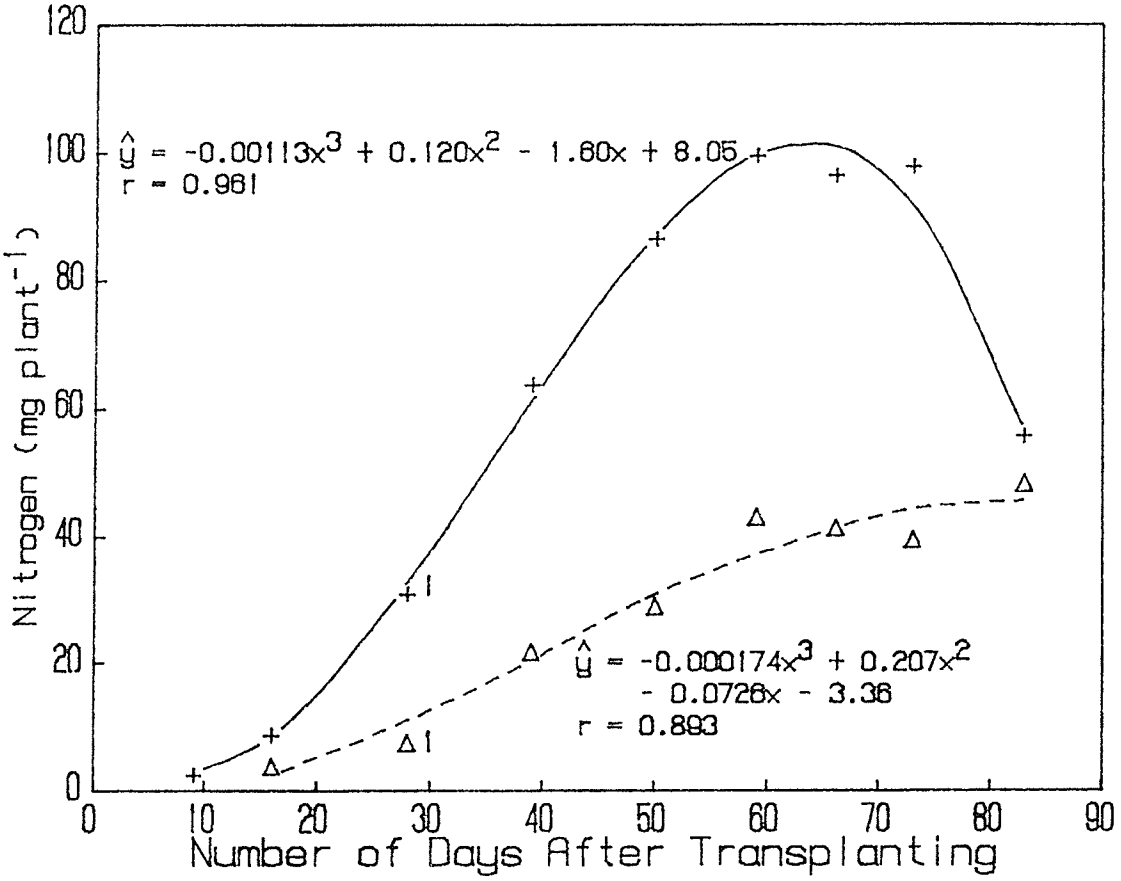

Figure 13. A comparison of the nitrogen content of the root material at the time of harvest (+) with the residual root derived nitrogen found in the soil at the time of harvest (Δ). Mean of 3 replicates.

[1]*mean of 2 replicates*

after transplanting, the rate of movement of nitrogen out of the root system was greater than the nitrogen input into the root system. This resulted in an observed decline in the nitrogen content of the root system during final maturation.

The decrease in the net accumulation of nitrogen in the root system is reflected in a decline in the rate of nitrogen incorporation into the soil from the root tissue. The polynomial regression analysis of the change in the residual root nitrogen found in the soil at harvest (figure 13) predicts that there is little change in the cumulative concentration of nitrogen lost from the root system to the soil from day 59 to the final harvest.

J. Total Amount of Nitrogen Lost From the Root System

The total amount of nitrogen lost from the entire plant root system cannot be directly calculated from the results. The proceedure employed does not allow for a direct estimate of the nitrogen lost from the portion of the plant root system growing in the soil amended with $(^{15}NH_4)_2SO_4$.

In order to quantify the rate of root nitrogen loss to the soil in relation to the total plant nitrogen content, it has been assumed that the rate of nitrogen loss from the root at any time "t" during the course of the experiment is proportional to the nitrogen present in the root system at the time "t". Thus the amount residual root nitrogen remaining in the soil at harvest at any time "t" can be calculated based on the equation,

$$NDFR_T = NDFR \times \frac{{}_0\int^t RN_T}{{}_0\int^t RN}$$

where:

$NDFR_T$ = residual soil nitrogen derived from the entire root system
NDFR = residual soil nitrogen derived from the portion of the root system in the soil with no fertilizer N addition.
RN = nitrogen content of the root material extracted from the unamended soil and,
RN_T = nitrogen content of the entire plant root system.

The calculation of the cumulative nitrogen content of the root material extracted from the unamended soil has been shown previously. The calculation of the cumulative nitrogen content of the entire plant root system can similarily be calculated based on the observed regression line for the entire plant root,

$$RN_T = -0.00199x^3 + 0.241x^2 - 5.33x + 38.5$$
$$r = 0.947$$

and the observed mean nitrogen content of the entire plant root system at 9 days after transplanting of 4.2 mg.

Figure 14 shows the change in the total content of plant nitrogen plus the calculated amount of nitrogen lost from the root system with time (short dashed line). Compared with the amount of nitrogen present in the entire plant system at harvest (solid line), it is apparent that the material lost from the root system represents a significant, although small proportion of the total plant nitrogen budget. The ratio of the measured root nitrogen content (long dashed line) to the measured plant nitrogen content decreases steadily throughout the growth period, ranging from 43% at day 16 to 20% at maturity. The ratio of the calculated loss of nitrogen from the root system to the measured plant nitrogen content follows a similar pattern, except near

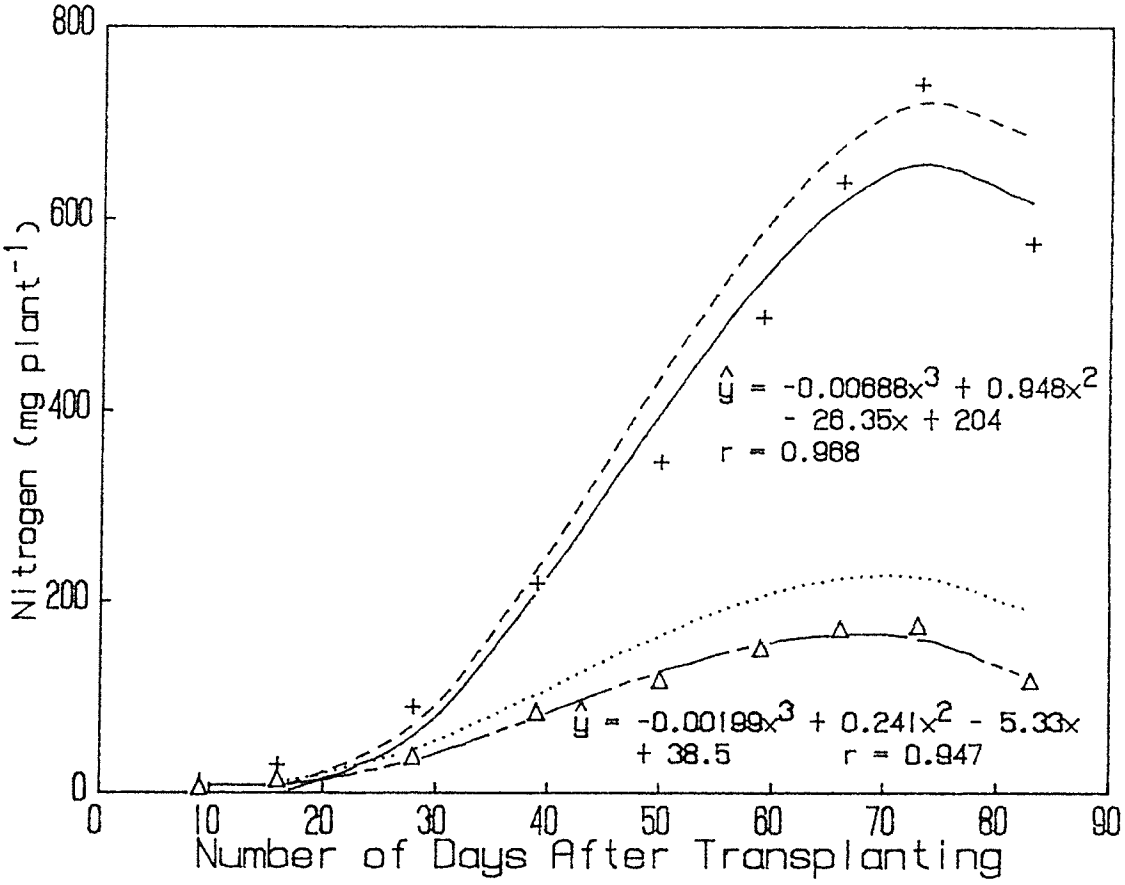

Figure 14. A comparison of the total measured root nitrogen content (— – — – —), the measured root nitrogen content plus the calculated amount of nitrogen lost from the root system (·····), the total measured plant nitrogen content (————————), and the measured plant nitrogen plus the calculated amount of nitrogen lost from the root system (- - - - - - - -).

plant maturity. At harvest 2, 16 days after transplanting, the calculated nitrogen loss from the root system is equivalent to 12% of the measured plant nitrogen content. By 73 days after transplanting, only 8.7% of the measured plant nitrogen content is present in the nitrogen lost from the root system due to the decrease in the significance of the root system as a sink for nitrogen. At maturity, 83 days after transplanting, the nitrogen loss from the root system is again equal to 12% of the measured plant nitrogen content due to an observed decrease in the measured plant nitrogen content between 73 and 83 days after transplanting.

The formula derived for the calculation of the nitrogen loss from entire root system is based on the assumption that the roots extracted from the two soil chambers exhibit a rate of nitrogen loss from the root system at any time, t proportional to the nitrogen in the respective roots. The validity of this assumption cannot be determined from the results. Because the $(NH_4)_2SO_4$ was added only to 1 soil chamber, it must be assumed that the two different soil chambers represent different soil treatments. It has been shown previously that the addition of the nitrogen fertilizer band initially depressed the rate of root growth, nodulation, the total nitrogen content of the root system. The effect of the change in soil treatments on the rate of loss of nitrogen from the root system is unknown.

V. Summary

The split root technique used in the experimental proceedure was found to be sensitive enough to measure changes in the soil nitrogen level resulting from material which was deposited from the root system of field peas during the growth cycle. Measurements of nitrogen lost from the root system of field peas could be made as early as 16 days after the seedlings were transplanted into the soil chambers, or 20 days after radicle emergence.

The technique used resulted in relatively uniform growth and uptake of the added ^{15}N source during the course of the experiment. The ratio of ^{15}N to total nitrogen in the plant root was maintained at a relatively steady state from 16 days after transplanting until final maturity. The method employed was therefore successful in maintaining a measureable nitrogen label in the root tissue from an early stage in the plant growth cycle until final maturity.

Using this technique, it was found that between 22% and 46% of the total measured nitrogen content of the below ground plant material remained in the soil after the root system was mechanically extracted from the soil. The proportion of nitrogenous material which remained in the soil after harvest was dependant on the time of harvest. During maturation, a decline in the total nitrogen content of the root system resulted in a rapid increase in the proportion of root nitrogen remaining in the soil after harvest.

The root system of the field peas was found to represent a relatively small sink for the total plant nitrogen after the initiation

of pod development. Although at 16 days after transplanting, 43% of the total measured plant nitrogen was accounted for in the extracted root sytem, only 20% of the plant nitrogen was present in the root system at final maturity. Thus, the loss of nitrogen from the root system of field peas represented a small, although significant sink for total plant nitrogen during the growth cycle. From 16 to 83 days after transplanting, between 8.7% and 12% of the total plant nitrogen was present in the soil after mechanical extraction of the root system.

VI. APPENDIX

Table A1. Air dry weight of plant material.

#Days After Planting[1]	Dry Matter Weight (g)				
	ROOT A[2]	ROOT B[3]	SHOOT	POD	TOTAL PLANT
9	0.025	0.116	0.15	0	0.29
	0.037	0.071	0.24	0	0.35
	0.073	0.119	0.28	0	0.47
16	0.053	0.360	0.47	0	0.88
	0.111	0.343	0.54	0	1.00
	0.238	0.348	0.66	0	1.24
28	*0.001	0.960	8.00	0	8.96
	0.359	1.141	13.02	0	14.52
	0.111	1.331	12.84	0	14.28
39	*0.164	2.418	4.59	0	7.17
	0.406	1.933	3.89	0	6.23
	1.753	3.220	8.63	0	13.60
	0.714	1.955	4.87	0	7.54
50	*0.019	4.076	6.86	0.79	11.74
	0.478	4.140	9.19	0.43	14.24
	2.250	3.128	13.05	1.18	19.61
	1.870	3.450	11.53	0.86	17.71
59	*0.018	6.875	7.66	5.78	20.34
	0.816	4.276	11.60	1.25	17.94
	3.834	4.575	13.62	5.50	27.53
	3.316	3.702	16.29	4.60	27.90
66	*0.112	5.676	7.79	7.85	21.43
	2.906	3.785	10.12	11.27	28.08
	5.966	4.853	15.47	12.03	38.32
	9.322	6.450	14.45	10.93	41.15
73	*0.135	5.247	8.41	9.97	23.76
	4.195	5.090	13.68	15.00	37.97
	6.539	5.276	14.42	16.11	42.35
	4.795	7.442	14.09	13.64	39.97
83	*0.149	3.945	9.31	8.78	22.18
	7.511	3.983	11.63	12.63	35.75
	2.574	4.601	11.67	13.69	32.54
	9.234	3.744	12.71	13.72	39.41

1. No. of days after transplanting seedlings into soil chambers.
2. Portion of plant root from the chamber with added $(^{15}NH_4)_2SO_4$.
3. Portion of plant root from the chamber with no added N.
*. trials in which Root A was likely inactive at harvest.

Table A2. Total nitrogen and ^{15}N content of plant material.

#Days After Planting[1]	ROOT A[2]		ROOT B[3]		SHOOT		POD		TOTAL PLANT	
	wt (mg)	%^{15}N	wt (mg)	%^{15}N	wt (mg)	%^{15}N	wt (mg)	%^{15}N	wt (mg)	%^{15}N
9	na	na	3.0	1.25	3.1	2.78			6.1	2.03
	na	na	1.8	1.53	7.7	4.15			9.5	3.66
	na	na	3.1	2.96	10.5	14.4			13.6	11.8
16	2.4	7.02	9.3	1.34	11.3	4.83			23.0	3.64
	3.7	42.9	8.4	11.8	18.9	30.3			31.0	26.8
	6.0	42.1	8.5	15.0	19.9	31.9			34.4	29.5
28	*na	na	23.9	1.72	214	4.52			238	4.24
	9.2	40.8	28.0	8.84	357	19.8			394	19.5
	1.3	38.7	33.2	5.77	369	14.7			404	14.0
39	2.5	23.0	66.4	2.38	107	4.39			176	3.90
	8.3	37.5	57.4	14.2	94	26.2			160	22.5
	36.0	36.1	79.0	9.46	195	22.5			310	20.8
	15.3	34.8	55.2	10.1	113	20.3			183	18.4
50	*na	na	79.6	8.25	105	12.0	27.6	11.4	212	10.5
	5.4	24.6	90.9	6.29	184	11.6	17.3	13.3	298	10.3
	52.5	36.0	91.6	9.81	204	23.1	38.7	28.7	387	22.3
	33.6	37.9	76.9	14.7	209	29.5	35.7	23.5	356	26.5
59	*na	na	110	4.41	90	6.27	160	3.96	361	4.68
	19.7	31.6	97	8.58	221	13.9	48.8	13.3	387	13.4
	68.8	31.0	111	9.29	215	18.9	176	19.0	570	18.5
	63.5	33.3	91	11.0	239	20.7	140	17.0	534	19.6
66	*1.9	31.4	96	6.67	95	11.7	229	11.4	422	10.5
	51.2	33.7	65	11.6	94	23.7	300	17.5	510	19.5
	78.8	24.6	107	8.38	181	18.4	335	17.4	702	17.1
	91.1	24.4	116	9.30	182	21.6	312	11.7	701	15.5
73	*2.9	5.19	95	6.01	96	10.3	323	6.53	517	7.13
	71.1	27.9	89	9.50	133	20.1	456	11.8	749	14.5
	87.5	24.8	89	9.28	120	19.1	472	11.0	768	13.6
	66.2	27.5	114	7.14	137	20.0	386	14.7	703	15.7
83	*2.7	22.5	56.5	7.83	72	13.7	263	10.7	394	10.9
	59.3	31.5	61.8	9.90	115	20.8	348	16.8	584	18.3
	46.0	32.1	48.0	11.1	83	25.1	350	19.0	527	20.3
	73.0	24.0	56.5	12.0	113	23.4	369	15.9	611	17.9

1,2,3,*.see footnotes table A1.1

Table A3. Nitrogen and percent ^{15}N content of the soil after removal of root material

#Days After Planting[1]	SOIL CHAMBER A[2]		SOIL CHAMBER B[3]	
	%N	$\%^{15}N$	%N	$\%^{15}N$
9	0.116	11.50	0.086	0.387
	0.114	10.49	0.081	0.383
	0.112	11.85	0.087	0.396
16	0.094	8.13	0.075	0.412
	0.085	7.26	0.080	0.390
	0.098	11.02	0.078	0.419
28	*0.091	9.40	0.085	0.437
	0.090	8.00	0.075	0.447
	0.091	8.85	0.079	0.508
39	*0.096	6.67	0.085	0.549
	0.111	7.01	0.086	0.627
	0.091	4.06	0.083	0.660
	0.095	5.03	0.087	0.624
50	*0.103	7.40	0.082	0.761
	0.102	7.10	0.070	0.639
	0.081	2.41	0.082	0.799
	0.084	2.56	0.079	0.783
59	*0.091	4.30	0.098	0.672
	0.096	2.85	0.102	0.713
	0.108	2.59	0.094	0.677
	0.099	2.40	0.109	0.946
66	*0.107	2.62	0.104	0.635
	0.084	2.16	0.103	0.764
	0.109	2.14	0.097	0.769
	0.072	2.14	0.103	0.767
73	*0.119	3.19	0.101	0.711
	0.113	2.28	0.099	0.735
	0.120	2.20	0.104	0.783
	0.099	2.11	0.097	0.721
83	*0.101	3.34	0.101	0.777
	0.080	2.36	0.096	0.796
	0.088	2.57	0.090	0.851
	0.092	2.48	0.105	0.879

1. No. of days after transplanting seedlings into the soil chamber
2. soil chamber ammended with 1.00g of 66.67% $(^{15}NH_4)_2SO_4$
3. soil chamber containing no added nitrogen source
*. trials in which root a was likely inactive at harvest

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